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Title: BINDING OF THE RESPIRATORY CHAIN INHIBITOR ANTIMYCIN TO THE MITOCHONDRIAL bc(1) COMPLEX: A NEW CRYSTAL STRUCTURE REVEALS AN ALTERED INTRAMOLECULAR HYDROGEN-BONDING PATTERN.

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Abstract: Antimycin A (antimycin), one of the first known and most potent inhibitors of the mitochondrial respiratory chain, binds to the quinone reduction site of the cytochrome bc1 complex. Structure-activity-relationship studies have shown that the N-formylamino-salicyl-amide group is responsible for most of the binding specificity, and suggested that a low pKa for the phenolic OH group and an intramolecular H-bond between that OH and the carbonyl O of the salicylamide linkage are important.
Two previous X-ray structures of antimycin bound to vertebrate bc1 complex gave conflicting results. A new structure reported here of the bovine mitochondrial bc1 complex at 2.28 Å resolution with antimycin bound, allows us for the first time to reliably describe the binding of antimycin and shows that the intramolecular hydrogen bond described in solution and in the small-molecule structure is replaced by one involving the NH rather than carbonyl O of the amide linkage, with rotation of the amide group relative to the aromatic ring. The phenolic OH and formylamino N form H-bonds with conserved Asp228 of cyt b, and the formylamino O

H-bonds via a water molecule to Lys227. A strong density the right size and shape for a diatomic molecule is found between the other side of the dilactone ring and the aA helix.

20 February 2005
Berkeley, CA, USA

Dear Editor,

On behalf of my colleagues and myself, I would like to submit the attached manuscript for publication as a regular paper in the Journal of Molecular Biology. It describes our work to date on two structures from the highest-resolution crystals available today of the cytochrome bc1 complex.

Both structures have stigmatellin at the Qo site; this seems to be a requirement for obtaining this crystal form. One of the crystals is from protein further preloaded with the potent respiratory inhibitor antimycin before crystallization; the other has only stigmatellin.

The antimycin is well-ordered so that the definition of the electron density of the antimycin and surrounding protein residues and water molecules is good enough to indicate the individual H-bonds and van-der-Waals interactions with a high degree of confidence. This structure allows us to understand the results of many years of empirical structure-activity-relationship research on antimycin and its analogues, and will be invaluable in designing improved drugs to act at this site and in understanding the mechanism of ubiquinone reduction that occurs naturally at this site.

Structures of the chicken, bovine, and yeast bc1 complex are already available, including two with antimycin bound. However we believe we are just now reaching the kind of definition that allows us to describe in detail the mode of binding, rather than just the site and approximate orientation of the inhibitor in the site. As evidence of the improved definition, three cis-prolines, modeled as "trans" in all previous structures, were obvious in our structures from an early stage of refinement. More importantly, we are able to correct a serious mistake in the conformation of bound antimycin, as well as minor errors in the interactions of antimycin with sidechains. In addition we begin an analysis of the flexibility of the protein in response to crystal packing, and fail to find any evidence of an expected long-range conformational change induced by antimycin.

This paper is intended to serve also as the primary citation for these two structures. Despite our decision to focus on the antimycin binding site and not to describe the entire structure, the paper has come out rather long. It is possible to further focus the paper by reducing or eliminating the discussion of other aspects of the structure, the structure determination, and/or the discussion of conformational changes (not) seen on binding antimycin.

My feeling is that since we are contradicting a previous paper on antimycin binding in this enzyme, some discussion of the overall structure is necessary to convince the reader that it is in fact more reliable. And since the "far-reaching conformational change" induced by antimycin is such an important part of the literature concerning this enzyme, we would be remiss if we described structures with and without antimycin and did not look for the conformational change. I would greatly appreciate advice from the editor and reviewers as to which parts would be distracting from the main point or less interesting to the readers.

Suggested reviewers:

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We have prepared a rather extensive set of supplementary materials to be made available online for the reviewer and later readers. The list is described in an appendix which I will include at the end of the manuscript as there is no provision for this item in the uploadable files. Of course we are receptive to comments about the suitability of these materials. The vrml files mentioned in the appendix may not be uploaded with this submission because they are not one of the explicitly allowed formats. These files need no processing: they are web-ready and can be viewed in a web browser after installing a plugin or helper application which is available (at least) for Windows, Linux, and Irix operating systems.

I thank you for your consideration,

Edward Berry, Ph.D.

*** Revision Notes**

Reviewers' comments:
Reviewer #2: This is a well written paper that describes two new crystal structures of stigmatellin-ligated bovine bc1 complex, one without ligand at the Qi site and a second with bound antimycin A. The data around the antimycin site are of high quality and allow the binding mode to be described accurately for the first time. The structure highlights errors in previous models of the key interactions of antimycin functional groups with surrounding protein structure and also shows for the first time the presence of a co-bound diatomic molecule in the antimycin pocket. Interestingly, no major conformational change differences were found between antimycin-bound and free structures, a finding that argues against previously proposed models in which large conformational changes are induced by changes within the Qi-site. The work is a valuable contribution to the understanding of the binding of antimycin and its mode of action.

I have one major and several relatively minor comments for author consideration:
Major Comment

The authors solve two structures, one with and one without antimycin A. However, the antimycin-free structure is not presented and, msot importantly, a number of key points about it are not specified. Specifically, is ubiquinone present in the Qi site and is each Qi site occupied or, as in the reported yeast structure with cyt c bound, is ubiquinone in only one of the two sites ? If ubiquinone is not present, then what replaces it ? It would be valuable to see an overlay of the Qi site residues in antimycin-bound and free states and far more useful than the figure 9 plot. Presumably, there must be significant changes of residues lining the pocket. The information on ubiquinone occupancy of the site and structure without antimycin seems essential for any evaluation of significance of (lack of) conformational effects of antimycin binding.

A short section has been added addressing these questions (p. 23, "comparison of antimycin and ubiquinone . . .), and the requested overlay is included as new Figure 7. We chose to superimpose with the structure Y21, which is essentially complete now, because the similar cell parameters minimize crystal packing distortion. Structure Y21 will be deposited and its PDB code inserted in the Accession section (actual code will be supplied with proof corrections)

Minor Points
- the paper is extremely long and parts of the descriptions of (page 10 onwards) overall structure, cyt b secondary structure and (page 27 onwards) stigmatellin binding confirm what is already published. If any shortening is warranted, then these parts could be condensed;

We feel the "overall structure" section is important, as this paper is to serve also as the primary citation for these structures, but it has been shortened a little. The description of secondary structure has been merged with that of heme b-hi and both parts condensed. Figure 3 showing secondary structure has been removed to the supplemental materials. The section on stigmatellin binding and the accompanying Table 6 have also been moved to supplemental materials. Mention of stigmatellin has been removed from the abstract, and a sentence added to the "overall structure" section. Old Figure 6 - the schematic of antimycin interactions - has been removed, as the same information is available in Table 3. Two paragraphs justifying the lack of a conformational change were removed from the long range conformational change section because the same ideas are presented in the discussion.

Page 16: The authors make the statement: Likewise if the water W5 ligating N? of His97 is an H-bond donor to the propionate and to a backbone carbonyl, then the imidazole N ?must be protonated; implying a positive charge on this imidazole as well. But, since the other nitrogen that ligates the metal is inevitably deprotonated, protonation of the N should mean that the histidine is net neutral.
The point is well taken, and this statement has been removed, as well as the one concerning charge on the other histidine.

In figure 1, numbering of atoms discussed in the text would be helpful
Selected atoms, including those mentioned in the text, have been labeled in Figure 1a according to the definition in PDB AMY and ANY. As mentioned in Footnote g, different naming conventions are in use.

In addition to these corrections, minor proofreading corrections have been made throughout and a number of sentences have been reworded or condensed for clarity. A file with differences highlighted can be supplied if desirable.

Running title: Structure of antimycin bound to cytochrome ***bc*$_1$**

BINDING OF THE RESPIRATORY CHAIN INHIBITOR ANTIMYCIN TO THE MITOCHONDRIAL *bc*$_1$ COMPLEX: A NEW CRYSTAL STRUCTURE REVEALS AN ALTERED INTRAMOLECULAR HYDROGEN-BONDING PATTERN.

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Antimycin A (antimycin), one of the first known and most potent inhibitors of the mitochondrial respiratory chain, binds to the quinone reduction site of the cytochrome *bc*$_1$ complex. Structure-activity-relationship studies have shown that the N-formylamino-salicylamide group is responsible for most of the binding specificity, and suggested that a low pK_a for the phenolic OH group and an intramolecular H-bond between that OH and the carbonyl O of the salicylamide linkage are important.

Two previous X-ray structures of antimycin bound to vertebrate *bc*$_1$ complex gave conflicting results. A new structure reported here of the bovine mitochondrial *bc*$_1$ complex at 2.28 Å resolution with antimycin bound, allows us for the first time to reliably describe the binding of antimycin and shows that the intramolecular hydrogen bond described in solution and in the small-molecule structure is replaced by one involving the NH rather than carbonyl O of the amide linkage, with rotation of the amide group relative to the aromatic ring. The phenolic OH and formylamino N form H-bonds with conserved Asp228 of cyt *b*, and the formylamino O H-bonds via a water molecule to

Lys227. A strong density the right size and shape for a diatomic molecule is found between the other side of the dilactone ring and the αA helix.

Keywords: cytochrome bc1; antimycin; respiratory chain; membrane protein complex; inhibitor binding site

Introduction

The cytochrome ***bc₁*** complex is an enzyme (E.C. 1.10.2.2, ubiquinol:cytochrome c oxidoreductase) that comprises the middle part of the mitochondrial respiratory chain. It is a multi-subunit membrane protein, with 10 or 11 protein chains in mitochondrial forms and 3 or more in bacterial complexes. It always contains the three redox subunits cytochrome ***b***, cytochrome ***c₁***, and the iron-sulfur protein (ISP); which contain respectively two hemes **B**, heme **C** and a Rieske-type Fe_2S_2 iron-sulfur cluster. It catalyzes reversible electron transfer from ubiquinol to cytochrome ***c*** coupled to proton translocation across the inner mitochondrial membrane, probably by a mechanism like the "protonmotive Q cycle"[1; 2; 3; 4].

The existence of inhibitors specifically binding to and inhibiting the two ubiquinone reaction sites was critical in the elucidation of the Q-cycle mechanism. One of the earliest known (for a review of early work see ref. [5]) and most potent (with a K_D on the order of 30 pM[5]) of these is antimycin A (antimycin)[b]. Antimycin binds specifically to the quinone reduction site ($\mathbf{Q_i}$ site) of the cytochrome ***bc₁*** complex. When bound, the UV-visible spectrum of the high-potential cytochrome ***b*** is shifted to the red and the

fluorescence of antimycin is quenched, leading to the conclusion[6] that antimycin binds near the heme b_H.

Together with British Anti-Lewisite (BAL) or alkyl-hydroxynapthoquinone (HNQ), antimycin enabled the demonstration of two independent pathways for reduction of cytochrome ***b*** by ubiquinol, one sensitive to antimycin and the other blocked by BAL treatment or HNQ, in the "double-kill" experiment[7].

When antimycin is bound, the bc_1 complex exhibits an unexpected inverse relation between the redox poise of the high potential chain (iron-sulfur protein and cytochrome c_1) and that of the ***b*** cytochromes, resulting in phenomena coined "oxidant-induced"[8] and "reductant-controlled"[9] reduction of ***b*** cytochromes. This is explained in the Q-cycle mechanism (Scheme 1) and in an earlier model[10] by having the ***b*** cytochromes and the high-potential chain connected to each of two sequential one-electron steps in the oxidation of quinol at the antimycin insensitive ($\mathbf{Q_o}$) site. In the Q-cycle scheme antimycin blocks the reaction at the $\mathbf{Q_i}$ site, at which cytochrome ***b*** equilibrates directly with the ubiquinone/ubiquinol couple, masking the oxidant-induced reduction in the absence of antimycin.

Generation of a semiquinone at the $\mathbf{Q_i}$ site is expected from the Q-cycle mechanism due to sequential one-electron reduction of quinone there by successive turnovers of the $\mathbf{Q_o}$-site reaction. A semiquinone signal has been observed by epr spectroscopy and it is eliminated by antimycin[11; 12], consistent with the predictions of the Q-cycle scheme. Thus antimycin could be considered a marker for the $\mathbf{Q_i}$ site of the Q-cycle mechanism.

Antimycin dramatically increases the stability of the bovine bc_1 complex in the presence of bile salt detergent taurocholate[13], inhibiting the "cleavage" reaction

quantitatively at stoichiometric concentrations. These and other observations[6; 14; 15; 16] led to the conclusion that antimycin-binding induces a far-reaching conformational change in the cytochrome ***bc1*** complex. Dithionite reduction of the complex results in a similar protection against cleavage[13], and affects the apparent cooperativity of antimycin binding[6] suggesting that redox state of cytochrome ***b*** may also be coupled to the conformational change. More recent indications of an antimycin-induced conformational change include an effect of antimycin on the sensitivity of the iron-sulfur protein to proteolytic cleavage[17], and an apparent effect of antimycin on "half-the sites reactivity" of the $\mathbf{Q_o}$ site[18].

One model[19] for the reaction mechanism of the ***bc1*** complex invokes conformational coupling between events at the $\mathbf{Q_i}$ site, where antimycin binds, and the $\mathbf{Q_o}$ site, where the bifurcated transfer of electrons from ubiquinol to cytochrome ***b*** and the iron-sulfur protein must be enforced to maintain proton-pumping efficiency. After different positions of the ISP ectodomain were observed in crystals and proposed to be involved in the catalytic cycle[20], it was suggested[21; 22] that such conformational coupling might prevent the ISP from returning to the $\mathbf{Q_o}$ site until the second electron had passed to heme $\boldsymbol{b_H}$ and the $\mathbf{Q_i}$ site, ensuring bifurcation.

Despite the circumstantial evidence for a conformational change involving the Qi site, no significant conformational change in the N-side or transmembrane domains of cytochrome ***b*** has been observed in the crystallographic structures. With the initial chicken ***bc1*** structures, we reported[23] an upper limit of 1A in the absence of stigmatellin (and 0.5 Å in its presence) for the maximum difference of C-α atom positions (residues 2-380) between crystals with and without antimycin. Gao et al.[24] reported rmsd 0.33 A

for all but one of the 378 residues modeled; comparison of structures 1ntk and 1ntz shows the largest deviations to be 2.2Å for residue 2 and 1.4 Å for residue 267.

In potentiometric titrations it has often been observed that the cytochrome b_{662} (b_{560} in bacteria) species attributed to heme $\boldsymbol{b_H}$ titrates heterogeneously[25; 26; 27; 28; 29; 30], with part showing a midpoint potential (E_m) around 150 mv and the rest around 50 mv (in bacterial chromatophores at pH 7). In the presence of antimycin only the low potential component is observed[30; 31]. In the presence of funiculosin there is only one component, with Em near that of the high potential component[30; 32]. If the system is poised so that $\boldsymbol{b_H}$ is partly reduced, addition of antimycin results in oxidation of cytochrome $\boldsymbol{b}$[25; 26]. These phenomena have been explained as due to the mechanism by which cyt $\boldsymbol{b_H}$ equilibrates with the Q pool via the $\mathbf{Q_i}$ site[26; 27; 31; 33], or alternatively as due to redox interaction between the cyt $\boldsymbol{b}$ heme and quinone species at the $\mathbf{Q_i}$ [29; 30] in which the redox state of one component affects the midpoint potential of the other. If the latter explanation is correct, the possibility that antimycin and funiculosin mimic different redox states of ubiquinone at the $\mathbf{Q_i}$ site seems attractive.

Thus there remains a large body of experimental observation concerning antimycin and the reaction at the $\mathbf{Q_i}$ site that is not very well explained at present. In the process of unraveling the details of the reaction, and in explaining these diverse observations, it would be very helpful to know how antimycin, funiculosin, and ubiquinone bind to the site. In addition, the $\mathbf{Q_i}$ site of fungal and other plant pathogens is an important target for crop protection agents [34; 35]. The site is of potential significance in treatment of human disease if species-specific inhibitors can be designed. Thus antimycin has been the subject of numerous structure-activity-relationship studies aimed at understanding the

mechanism of the enzyme and at developing powerful new crop protection agents[16; 36; 37; 38; 39; 40; 41].

Antimycin (Figure 1a) has a head group consisting of 3-formylamino salicylate, amidified to a dilactone ring consisting of L-threonine (whose amino group is amidified to the salicylate moiety) and a 2-alkyl, 3,4-dihydroxyvalerate. It is the 4-hydroxy group of the latter that participates in the dilactone, and the 3-OH is esterified by a branched carboxylic acid (acyl side chain). There is heterogeneity in the 2-alkyl group (alkyl side chain) and in the acyl side chain, which at least in antimycin A1 has recently been shown to consist mainly of 2-methyl butanoate[42] rather than isovalerate (3-methyl butanoate) as deduced earlier[43; 44]. High resolution chromatography has resolved commercial antimycin samples into as many as ten different compounds[45].

Early structure-activity-relationship studies have led to the conclusions that the N-formylamino-salicyl group is responsible for most of the binding specificity, and to the importance of a low pKa for the phenolic OH group[16]. The dilactone ring and substituents can be replaced by a long-chain fatty amine with retention of tight (μM) binding and inhibition. More recent studies have examined stereospecificity of the dilactone[37] and probed with substituents at various positions on the salicylamide group[38; 40]. Conclusions of the latter studies include the importance of the phenolic OH and formylamino groups and an intramolecular H-bond between the phenolic OH and the carbonyl O of the amide linkage by which the rest of the molecule is connected to the 3-formylaminosalicylic acid.

Antimycin was instrumental in locating the $\mathbf{Q_i}$ site in the first crystal structure of a $\boldsymbol{bc_1}$ complex[46], but no coordinates for antimycin were deposited in the Protein Data Bank

(PDB). Since then two structures have been made available with coordinates for antimycin, PDB entries 3BCC (chicken) and 1NTK (bovine). The low resolution of the former structure made it impossible to discern details required for a rigorous description of antimycin binding. Structure 1NTK was processed at higher resolution (2.6 Å), however the work presented here shows that it, too, has errors in the details of binding.

In this work we introduce two new crystal structures of the bovine mitochondrial bc_1 complex with stigmatellin at the $\mathbf{Q_o}$ site. PDB Entry 1PP9 (2.23 Å) has no $\mathbf{Q_i}$-site ligand added, while 1PPJ (2.28 Å) was co-crystallized with antimycin. This allows us for the first time to reliably describe the binding mode of antimycin at the level of detail required to begin to understand its diverse effects on the bc_1 complex.

Results

Resolution and quality of the structures

The diffraction was somewhat anisotropic, as judged by the "falloff" analysis in the program TRUNCATE and by anisotropic scaling during refinement in CNS which gave a B tensor with diagonal elements -15.3, 0.6, 14.7 $Å^2$ for 1PP9 and -12.5, 3.8, 8.6 $Å^2$ for 1PPJ. The data reduction and refinement programs we used have no provision for an ellipsoidal resolution cutoff, so to avoid losing any useful data in the well-ordered directions we used a resolution cutoff of 2.07 for 1PP9 and 2.0 for 1PPJ in the initial data reduction. In the final refinement for deposition and calculation of refinement statistics (Table 1b), a resolution limit of 2.1 Å was used for both structures. This should not be taken as the resolution of the structure, however, as the data in the outer shells were quite

weak. A more objective measure of the resolution of a diffraction dataset[47] is given by the "optical" resolution as calculated by the program SFCHECK[48] . However the optical resolution is defined differently (how close two features can be and still be resolved by the data, rather than as a d_{min} cutoff), so they are not directly comparable. A sparse random survey of structures deposited with data during 2002 showed (unpublished work of E. Tung) that in the range from 1.2 to 3.0 Å the optical resolution R_{opt} was related to reported resolution cutoff d_{min} by the expression ($R_{opt} = 0.42 + 0.59d_{min}$). The datasets for structures 1PP9 and 1PPJ have optical resolution 1.72 and 1.75. By the above relation this is the type of resolution to be expected from the average structure using a resolution cutoff of 2.23 or 2.28 Å.

While this is only marginally higher resolution than the best yeast or bovine ***bc*$_1$** structures previously available, we think the quality of the structures is significantly higher. This is due to the presence of a dimer in the asymmetric unit, which for the same solvent content doubles the number of unique reflections at a given resolution. Because non-crystallographic symmetry was quite good for most of the protein, the use of NCS-restraints resulted in effectively doubling the data/parameters ratio with consequent improvement in the refinement process. In addition, while making the final model we had the benefit of using all the previously deposited structures for comparison and evaluation, which we gratefully acknowledge.

At the current state of refinement (Table 1b) the free-R factor is approximately 0.40 in the shell around 2.1 Å for 1PP9, and below 0.4 at 2.0 Å for 1PPJ, suggesting the datasets actually contain some useful information to these resolutions. Analysis of the structures[c] by PROCHECK [49; 50] show them to be within the norm or better on all

measures as compared to 2.0 Å structures. These structures are the first cytochrome ***bc*$_1$** structures to achieve greater than 90% of the residues in the allowed regions (A, B, L) of the Ramachandran plot, as expected for real proteins based on analysis of structures solved at better than 2 Å with R-factors below 20%[50]. Overall real-space R-factors are 0.155 and 0.148, and real-space correlation coefficients are 0.909 and 0.921 for structures 1PP9 and 1PPJ, respectively (EDS website, http://fsrv1.bmc.uu.se). Representative electron density from well-ordered regions in crystal 1PPJ are shown in stereo pairs of Figure 2 as well as in the figures documenting the mode of antimycin binding (Figures 1, 5, and 6).

Still, the current structures are disordered in a few sections, and so for some features it will be best to look at structures from other crystal forms. In such areas where the structure is not completely determined by the data, the electron density has been interpreted liberally to provide our best guess of the actual arrangement. To avoid over-interpretation of the structure and possible erroneous conclusions concerning features not described in the text, it is important to compare all the available structures, and to examine the electron density on which the feature is based. To facilitate independent evaluation of structural features by others, the original data (structure factor amplitudes) for the structures have been deposited with the Protein Data Bank.

Overall Structure

The overall structure of the eukaryotic ***bc*$_1$** complex has been described previously[20; 46; 51; 52]. The transmembraneous region is made up of 26 transmembrane helices, with each monomer contributing 13: eight from cyt b and one each from subunits 7, 10 and 11 plus

the transmembrane anchor helices of the ISP and cyt c_1. The redox-active ectodomains of the ISP and cyt c_1 together with subunit 8 (acidic "hinge protein") make up the membrane extrinsic portion on the external or "P" side of the membrane, while the two largest subunits ("core" proteins[53]) and subunit 6 make up the extrinsic part on the "N" side. Subunit 11 is peripherally bound to the transmembrane domain[46] and readily dissociable after solubilization in DM. It is not present in this crystal form.

Table 2 lists the number of residues modeled for each subunit of each monomer of the two structures discussed here. It also defines the chain letters for the 10 subunits in each of two monomers: Chains A to J are subunits 1 to 10 of the "first" monomer, while N to W are the corresponding subunits in the second monomer. The hemes and iron-sulfur clusters are numbered starting at 501 in the same chain to which they are linked. Water molecules are numbered starting at 1, ligands present at the same place on both monomers are labeled starting at 2001 for monomer 1 and 3001 for monomer 2, and ligands without symmetry mates are numbered starting at 4001.

The 11-subunit bovine bc_1 complex contains 2166 residues per monomer (ref 54, Table 2), and the 10-subunit preparation used here has 2110 of these. Due to omission of disordered areas, the final structures contain about 2009 residues in each monomer, or 95% of the residues present. The model is lacking the first 17 residues of subunit 2, the first 14 residues of cytochrome b, the first 11 of subunit 6, the first 12 of subunit 8, about half of subunit 9, and smaller sections elsewhere. Monomer 1 of 1PPJ has fewer residues because it is lacking the first 29 residues of subunit 10, which were disordered. Poorly ordered residues that are likely to have mistakes in the current model include the

interdomain linkers of subunits 1 and 2 (A/N 223-230, B/O 230-233,) ; E:79-80 E:178-190; and F109-110.

The entire stigmatellin molecule is well ordered in the current structures, with all but two atoms (the methoxy carbon C5A and final carbon of the tail) covered by $2F_o$-F_c density at a contour level of 2.0 σ (Figure 2b). The stereochemistry of the 4 chiral centers and the planarity at the isoprenoid unit are clear, and are consistent with what is known from chemical investigations[55].

Modeling of the lipids and detergents in these structures is not yet complete, and will be described in a later paper. At present there are five phospholipids in 1PP9 and four in 1PPJ. One of the best ordered (residues 2007 and 3007; with phosphate H-bonding Tyr103 and Tyr104 of cyt b) is in the position of one of the lipids in the chicken ***bc1*** structures (e.g. 2BCC) and is also conserved in the yeast ***bc1*** complex (1KB9, 1P84). Phospholipids 2006 and 3006 correspond to the "interhelical lipid" described in the yeast ***bc1*** complex[52], at the coming-together of transmembrane helices from subunits 3, 4, 5, and 10. As described also by Iwata[56] there are two cardiolipin molecules in the bovine complex where one was modeled in yeast (1KB9).

Six hexyl glucoside (HG) molecules have been modeled in 1PP9, and nine in 1PPJ. For the most part these are poorly ordered and may be misidentified, however in 1PPJ there is one hexyl glucoside that is exquisitely defined by the density (Figure 2c). The hexose ring is pinned in a crystal contact between helix αM^d of chain A (at the level of 393-394) and the imidazole ring of O:His192 in a symmetry-related dimer, presumably accounting for the good order. In addition there are H-bonds from O2 of the sugar to A:Ser397 (shown) and from O6 to A:Glu394. This well-ordered detergent is seen in all

crystals examined so far that have cell edge a = 128 Å, but in the looser lattice of 1PP9 this contact does not occur and the detergent is disordered. In one crystal with cell edge **a** ~120 Å (not shown) this detergent is absent and O:His192 of the sym-related molecule packs directly against helix αM of chain A . Thus the detergent seems to be the "shim" which accounts for the frequent occurrence of the **a**=128 Å cell edge after partial dehydration of the crystals.

As expected in the presence of stigmatellin, the ISP is in the proximal or "b" position, with a hydrogen bond between His161 and stigmatellin, which is bound in cyt. b. The significance of this H-bond has been described in a recent note[57]. The methionine axial heme ligand in cyt c_1 has "R" chirality at the Sδ atom, as in the chicken 1BCC or yeast 1EZV structures (Figure 2a). The heme planes of heme b_H, heme b_L, and cytochrome c_1 are at angles of 25 - 26°, 5 - 6°, and 14 - 16° to the membrane normal, respectively. The orientation of the hemes about their pseudo-two fold axis is unambiguous and is the same as originally modeled in the chicken structure 1BCC. *Cis*-peptide linkages are present at the peptide bonds involving Pro222, Pro436 (cyt b) and Pro74 (cyt c_1) as the $(i+1)^{th}$ residue. The assignment is unambiguous for these three residues, and has been verified in cross-validated SigmaA-weighted F_o-F_c omit maps calculated for 1PPJ omitting residues in a sphere of radius 3 Å around the residue and calculated between 93.5 and 2.2 Å resolution[c]. Pro21 in subunit 2 is also modeled as a *cis* peptide, but the position of His20 is not well defined by the density so this is likely to be in error. No other cis-peptide linkages were found.

Heme-binding helix bundle and heme bH

As deduced from sequence analysis[58; 59; 60] and described in previous structures[20; 46; 52], cytochrome b is primarily α-helical, with eight transmembrane helices labeled A - H and four "surface" helices labeled **αa** (before Helix **A**), **αcd$_1$** and **αcd$_2$** (Between helices **C** and **D**), and **αef** (between helices **E** and **F**). There is one small β-sheet consisting of two antiparallel strands from the linker regions before helices **A** and **E**, which will be described below in connection with the antimycin site.

The two hemes are located within a four-helix bundle consisting of helices **A**, **B**, **C**, and **D**; with the high potential heme (heme ***b*$_H$**) toward the **N** side and low potential heme (heme **b$_L$**) toward the **P** side of the membrane. Both hemes have bis-histidyl ligation, with the histidine ligands provided by helices **B** and **D** (His83, 97, 182, and 196 in the bovine sequence). In addition there are four conserved glycines in helices **A** and **C** where the heme ring makes a close contact (Gly34, 48, 116, and 130).

Heme ***b*$_H$**, with axial ligands His97 and His196, is distinctly curved: pyrrole rings[e] **A** and **C** bend toward the His97 side while rings **B** and **D** are nearly in a straight line with the iron (forming the axis of curvature). Pyrrole rings **B** and **D** lie along the axis of the four-helix bundle with rings **A** and **C** on the sides, inserting between the helices that comprise the bundle. Ring **A**, exposed between helices **A** and **D,** contributes to the antimycin binding site (below).

As reported[20; 52] the propionate on the **A** ring is bent sharply back toward the axial ligand His97, making an ion-pair with the guanidino group of Arg-100 (bovine sequence numbering). We can see now (Figure 3) that this ion pair involves only one of the carboxylate oxygens and NH1 of the guanidino group of R100 (distance 2.8 Å), but that

the propionate in addition binds two very well-ordered water molecules (Figure 3 and Table 3). The same propionate oxygen that ion-pairs with R100 has a second bond (2.8 Å) to an entity modeled as water W4, whose other ligands are the other (**D**) propionate and Ser205 Oγ. The other oxygen of the **A** propionate is separated by 3.3 Å from the NH2 atom of Arg100, but makes a very strong (2.44 Å) bond with another stable water molecule **W5** which bridges between this propionate and the Nδ atom of the heme axial ligand His97. This water also makes bonds with the carbonyl oxygen of Trp30 and backbone N of C33 in the **A** helix. This rigid framework presumably serves to fix the plane of the heme-ligand histidine, and may be partly responsible for the heme curvature mentioned above. The sharply bent **A** propionate arm also forms one surface of the $\mathbf{Q_i}$ binding site (see below), and may be on the path for electron transfer between heme $\boldsymbol{b_H}$ and quinone at that site. The other propionate, on the **D** pyrrole ring of heme $\boldsymbol{b_H}$, H-bonds with one carboxylate oxygen to the side chains of Ser106 and Trp31, and with the other to the backbone nitrogen of Asn206 and to the water molecule W4 mentioned above. This arrangement of the propionates, Arg100, the two waters, and their ligands is the same in the presence or absence of antimycin, and is seen also in the yeast $\boldsymbol{bc_1}$ structures (e.g. 1P84), so it is likely to be a static arrangement. However if at some point in the reaction cycle the **A** propionate could be released to straighten out, it would put the carboxylate in the $\mathbf{Q_i}$ site, as a possible ligand for a quinone species there, as well as modulating the charge density near the heme iron and curvature of the macrocycle.

Ser205, one of the ligands for strongly ordered water W4, is replaced by Asn221 in *Rb. sphaeroides*. It has been proposed that the Oδ1 atom of Asn221 occupies the position

of W4 bridging between the two heme propionates, positioning the Nδ2 atom to serve as a ligand for quinone in the bacterial complex[33].

Molecular configuration of bound antimycin

The antimycin in structure 1PPJ is very well ordered, with average B-factors in the two monomers of 41.5 and 43.6 Å^2, barely above that for the backbone of cytochrome b (39.0, 40.9) and lower than the average B-factor for the structure (50.2 Å^2). The electron density is correspondingly good, and there is little ambiguity in the placement of any of the atoms except the tips of the alkyl and acyl side chains. The degree of order is greatest on the formylamino-salicylamide portion, which is well defined in $2F_o$-F_c maps contoured at 2.1 σ (Figure 1b), and decreases through the dilactone ring and into the alkyl and acyl side chains at the other end. At 1.5 σ (not shown) the acyl chain is visible through C3 and shows the methyl branch to be at the 2 position as reported[42] rather than the 3 position as previously believed, and at 0.9 σ (Figure 1c) there is weak density for C4 in one monomer, tentatively modeled in Figure 4b and 4c for completeness. The alkyl side chain has density through the fifth carbon when contoured at 0.9 σ (Figure 1c)

The dihedral angles of the formylamino group[38] are approximately 0° (Θ_1) and 180° (Θ_2), in agreement with values found in an energy-minimized structure[38]. Similar values were found in the small-molecule structure[42] and for the bound inhibitor in structure 1NTK. These angles put the formylamino group in the plane of the salicyl ring, directed away from the OH and carboxylate groups and toward Lys227 of cyt b (Figure 6). The observation[40] that a methyl group at position 4 but not at position 5 diminishes binding of

antimycin analogs is consistent because the methyl at position 4 (compound 17) would prevent the formylamino group from taking on this conformation[38].

The dihedral angle Θ_3 between the phenyl ring and carbonyl carbon of the salicylate moiety is approximately 180°; that is the amide group is rotated 180° relative to the salicyl ring from the small-molecule structure. This means that the internal H-bond between the phenolic OH and amide carbonyl oxygen, which was proposed to be important for inhibition[38], and which was observed in the small-molecule structure[42] (indicated in Figure 1a), is actually not present in the enzyme-bound form (Figure 1b and 1c). The observed orientation of the amide moiety with respect to the salicylate ring is contrary to that modeled in structure 1NTK. This and other discrepancies will be considered in the Discussion section.

The 9-membered dilactone ring of antimycin is puckered with alternating members directed up and down except Cβ of the threonine[f], which is between members facing up and down. The chirality of the chiral centers meshes with the puckering in such a way that the three bulky substituents as well as one methyl side chain (C5 of the valeric acid moiety) project equatorially, i.e. more or less in the plane of the dilactone ring, while the two carbonyl oxygens project perpendicular to the ring. The other methyl group (Cγ of threonine) projects at an intermediate angle. The planes of the ester and amide substituents and the salicyl ring are nearly perpendicular to the dilactone ring. This differs from the small-molecule structure, in which the plane of the salicyl ring and amide are approximately 45° from that of the dilactone ring (Compare Figures 1a and 1b; in both of which the salicylamide is in the plane of the picture).

The antimycin-binding site

Figure 4 shows the make-up of the antimycin binding site in different levels of dissection, and Table 4 lists contacts between antimycin and the protein. Briefly, the antimycin headgroup is found in a pocket which is bounded by helices α**A**, α**D**, α**E**, and α-**a**; as well as the edge of heme $\boldsymbol{b}_{\mathbf{H}}$ exposed between helices α**A** and α**D** of the four-helix bundle. Strong hydrogen bonds are formed directly with Asp228 in helix **E** and via ordered water molecules to Lys227 in Helix **E** and Ser35 in Helix **A**.

Figure 4a shows the underpinnings of the antimycin binding site in helices **A** and **E**. These helices cross at an angle, with Van der Waals contacts at the crossing between the side chains of Leu43 and Leu239 (not shown). The N-terminal (N-side[b]) ends of these helices are connected by β-bridges between residues in the sequence preceding the helices: residues 21, 23, and 25 in the region before helix **A** make backbone H-bonds with residues 221, 220, and 218 before helix **E**. In addition a strong hydrogen bond between the side-chains of Asp216 and Ser25 hold these two residues together. These β-bridges are represented by the antiparallel arrows in Figure 4, and together with helices **A** and **E** they bound a triangular volume that encloses the $\mathbf{Q_i}$ site. Another connection between the **A** and **E** helices is made by Lys227 in the **E** helix which H-bonds with the backbone oxygen of residue 27 and a highly ordered water molecule attached to helix **A**. These bonds are part of a more extensive H-bonding chain that is involved in antimycin binding but is present in both structures 1PPJ (with antimycin) and 1PP9 (without). This chain is shown in stereo in Figure 4a. Lys227 and the first water (**W1**) are bonded to each other and to the carbonyl oxygen of residue 27. **W1** is also bonded to Oγ1 of Asn32. Nγ2 of this residue H-bonds a second water (**W2**) which in turn bonds to Ser35Oγ and to the

carbonyl oxygen of Asp228, further linking the **A** and **E** helices. A third water (**W3**) also bonds with Ser35Oγ and additionally with the backbone **O** and **N** of residues 31 and 35, respectively (Figure 4a). As these latter two atoms would normally be involved in the α-helical H-bonding of helix **A**, **W3** can be seen as "intercalated" into the helix[g].

In addition to the A and E helices, both the **D** helix and the **α-a** surface helix contribute to the $\mathbf{Q_i}$ site. Helix **D** is omitted in Figures 4a and 4b for clarity (the view is from the position of helix **D**), but the linker polypeptide connecting helix **D** to helix **E** is shown. Ser205, early in the **D**/**E** linker is shown as ball-and-stick. This residue has been implicated in quinone binding at this site [24; 33; 52; 61], and will be discussed further below. The **α-a** surface helix, starting with residue 15 is shown. This turn borders on the Q_i site, and there is some indication that the carbonyl oxygens of residue 16 or 17 may H-bond with the sidechain of His201. Unfortunately this region is poorly ordered in both monomers of these crystals, and its contribution to the Qi site can better be seen in the yeast (e.g. 1KB9) or chicken (3BCC) structures.

In Figure 4b antimycin and heme $\boldsymbol{b_H}$ are added to the picture, and Figure 4c shows a spacefilling model of everything represented in 4b and also adds helix **D** as a thin ribbon. The H-bonding contacts of antimycin can be seen in Figure 4b and, in greater detail, in Figures 5 and 6. The only direct H-bonds with the protein involve conserved Asp228, the carboxylate of which binds to the phenolic OH and the formylamino NH. It seems reasonable to assume that Asp228 is deprotonated at the pH of the crystal, and serves as H-bond acceptor in both these bonds. The phenolic OH also has an H-bond with the intercalated water W3, as well as the intramolecular H-bond mentioned above with the amide NH. Following the reasoning above it must be the acceptor in both of these bonds,

as its one proton is being donated to Asp228. The formylamino oxygen H-bonds to water W1 discussed above. Otherwise the contacts appear all to be hydrophobic. Strikingly, no H-bond is made with the dilactone ring or its acyl side chain.

The substituents of the "**A**" pyrrole ring of heme $\boldsymbol{b_H}$ (the bent propionate described above and a methyl group) protrude from the 4-helix bundle between the **A** and **D** helices, forming part of the surface of the $\mathbf{Q_i}$ site. The space-filling model in Figure 4c illustrates the intimate contact between heme (orange) and antimycin (magenta), consistent with the electronic interactions required to explain the quenching of antimycin fluorescence and the spectral shift of cytochrome $\boldsymbol{b_H}$ on binding. The aromatic headgroup of antimycin is inserted into a cavity between the bent propionate and Phe220. The aromatic ring of Phe220 is not quite perpendicular to the salicyl ring, the actual angle being 77°. The axial methyl group of the dilactone ring interdigitates loosely with the methyl groups on pyrrole rings **A** and **B** of the heme.

Van der Waals contact with Ser205, a possible ubiquinone ligand

On the other side of the Formylamino-salicyl ring from Asp228, potential H-bonding partners are Ser205 and His201 (Figure 4b and c, Figure 6), both believed to be important in ubiquinone binding at the $\mathbf{Q_i}$ site[33; 52; 61; 62]. Ser205 makes Van der Waals contact with C5 of the salicylate ring, but there is no H-bonding partner on this area of antimycin.

In antimycin analogs lacking the 3-formylamino group, inhibition can be restored by 3- or 5-NO_2 groups, and to some extent by a 5-formylamino group[41]. This has been attributed to a requirement for an electron-withdrawing substituent to increase the acidity

of the phenolic OH[16], however, based on a more extensive set of analogs, Tokutake et al decided that electron-withdrawal did not correlate well with activity, and concluded specific interactions of both the formylamino and phenolic OH with the protein were involved. It seems likely that a nitro group in the 5- position would H-bond Ser205, while one in the 3- position would H-bond Asp228. Superposition of 3- or 5-nitrosalicylate on the salicylate moiety of antimycin in 1PPJ (not shown) results in too-close contacts (1.7 and 1.3 Å) of the 3-nitro group oxygen with Asp 228 and the 5-nitro group with Ser205. Small adjustments in the positions of these side chains, or slight repositioning of the ring, could allow a good fit.

Thus H-bonding may be as important as electron-withdrawal in explaining the effects of nitro- substituents. However in light of our assumption that the phenolic OH is the H-bond donor in the bond with Asp228, it has to be questioned whether the more acidic nitro- compounds, which might be deprotonated at neutral pH, could bind in the same way as antimycin. In fact the detailed inhibition pattern of nitrosalicylate compounds has been found to be quite different from antimycin and other 3-formylamino compounds, the nitrosalicylates being ineffective in eliciting either the "double-kill" behavior or oxidant-induced reduction seen with antimycin[40; 63].

His201 conformation

The most enigmatic part of the structure around the antimycin binding site is the critical conserved residue His201, which is believed to be a ligand for quinone[52; 61; 62] either directly[61] or indirectly through a water molecule[52]. When contoured at a 1.5σ

density level (Figure 6a) the imidazole ring seems well localized, but poorly shaped for this resolution. His201 here is modeled in a position close to rotamer 5^h, as in all vertebrate cyt ***bc*$_1$** structures currently available, and there is really no possibility of modeling it in with a significantly different value of chi-1.

The distance from His201Nε2 to the antimycin O is 4.20 Å, precluding any significant direct H-bonding. An extra bit of density has been modeled as water, with distances 2.81 Å from the water to the amide carbonyl O of antimycin and 2.63 Å to the Ne2 of His201. In fact the density is too close to the histidine to be a water molecule. Non-bonded interaction terms in the refinement process have pushed the modeled water outside of the density peak, and displaced His201 slightly from its best fit. When the water is moved to its density peak, the distances are 1.82 to the His201 and 3.07 to antimycin. When the water is removed and the model is subjected to further positional refinement, His201 moves only slightly closer to antimycin, distance 4.12 Å (not shown).

When contoured at a lower level (1.0σ, Figure 6b), the density around the imidazole spreads out in a triangular shape and merges with the peak assigned to water. This may result from a mixture of two alternative conformations: one in which the water is present at its density peak but His201 rotates back to be farther from it, and another in which the water is absent and His201 rotates forward toward the water position and bonds directly with antimycin.

The two conformations postulated here, and the presence or absence of a water molecule between the histidine and the $\mathbf{Q_i}$-site occupant, should not be confused with another interesting difference between available structures. In all the yeast structures presented to date, His202 (corresponding to His201 in the bovine sequence) is positioned

close to rotamer 3[h]. A water molecule bridges between the Nε2 nitrogen and the carbonyl oxygen of ubiquinone. In the chicken ***bc1*** structures with ubiquinone, His202 is close to rotamer 5, which allows a direct H-bond to ubiquinone. It seems quite reasonable that both these conformations are correct, depending perhaps on pH or ionic strength, and that the direct involvement of a water may be part of the mechanism for uptake of the protons involved in quinone reduction at the $\mathbf{Q_i}$ site. However that may be, all of the vertebrate ***bc1*** structures available today have His202 (201) in rotamer 5, and all the yeast structures have rotamer 3. At first glance the bovine structure 1NTZ (with quinone supplemented) seems to be an exception, as the H-bond between quinone and His201 is mediated by a water molecule as in the yeast structures. However superposition of the chicken and yeast structures shows that in 1NTZ, His201 is in the chicken position (rotamer 5) and the quinone is deeper in the pocket than in the yeast or chicken structures, making room for the mediating water.

Unknown molecule in hydrophobic site between dilactone and helix A

There is a strong density between the dilactone ring of antimycin and helix A which during most of the refinement of the structure was modeled as a water. Since there are no nearby H-bonding partners to account for stabilizing a water molecule in this position, it was removed before submitting the structure to the PDB. However this leaves a strong peak in difference Fourier maps, indicating that something is there, even if it is not a water. The peak is oblong and about the right size for a diatomic molecule. In consideration of the hydrophobic nature of the environment and the lack of H-bonding partners we think it may be a nonpolar gas such as nitrogen or oxygen. It is modeled as

O_2 in Figure 6, and labeled "unknown". The closest contacts in antimycin are O7 and C11 (3.7 and 4.0 Å). The closest contacts in helix **A** are the carbonyl **O** of ser35 (4.0 Å) and sidechains of Ile39 and Ile42 (4.0 - 4.2 Å).

Comparison of antimycin and ubiquinone binding positions; local conformational changes induced by antimycin

Disappointingly, the ubiquinone molecule in the crystals without antimycin (1PP9 and Y21) is much less well ordered than antimycin in 1PPJ. There is density in the position indicated for the ubiquinone ring by previous structures (1BCC and 1EZV), however the shape is not well-defined. It seems likely that the occupancy is significantly less than one, due to dissociation during the purification in detergent-containing, quinone-free buffers. Water or other molecules may have entered the unoccupied Qi sites, resulting in the poorly defined density of the crystallographic average. Current experiments are aimed at maintaining a high quinone occupancy by purification in detergent micelles doped with ubiquinone.

There is no strong indication of asymmetric ubiquinone occupancy such as reported[64; 65] for the yeast enzyme with one mole of cytochrome c bound per dimer: The peaks of the density attributed to ubiquinone in monomers 1 and 2 were 3.0 and 2.1σ in 1PP9, but 2.6 and 2.8 σ in Y21. The shapes were similar. Ubiquinone has been modeled into the density based on the previous structures, and refinement of the model did not lead to significant discrepancies from those structures. Thus the results obtained superimposing Y21 below are essentially the same as would be obtained superimposing the yeast or chicken structures. Another bovine structure (1NTZ) presents a slightly shifted position for ubiquinone.

Figure 7 shows superposition of the Qi sites of 1PPJ and Y21 based on a rigid core of cytochrome b, including the four-helix bundle[i]. The quinone ring does not superimpose with the aromatic salicylate ring of antimycin, rather it is centered on the carbonyl carbon of the amide

group. Carbonyl oxygen O1 of the quinone is positioned near the phenolic OH oxygen of antimycin (0.63 Å) making the same H-bonds to Asp228 and water W3 as that atom makes. The other carbonyl oxygen, O4, extends toward His201, reaching farther than the carbonyl oxygen of the antimycin amide and making a direct H-bond (or a water-mediated H-bond in the yeast structures) with that residue.

There is surprisingly little rearrangement in the protein backbone and even sidechains surrounding antimycin, as compared with for example the rearrangement of protein around the Qo site upon binding inhibitors[23; 66]. Gao et al.[24] also remarked on the structural rigidity of cyt b on antimycin binding, but reported significant conformational changes of cytochrome b residues Ser35, His201, Phe15, and Met194. Comparing structure 1PPJ with 1PP9 or Y21 (Figure 7) showed Ser35 to be identical and His201 not to have changed significantly. We see both conformations for Met194 in the presence or absence of antimycin. The side chain of Phe15 is completely disordered in all three structures.

Long-range Conformational changes induced by antimycin binding and by different crystal packing forces

As described in the introduction, there are several reasons to believe that antimycin binding triggers a long-range conformational change in the bc_1 complex. Cursory comparison of the structures described here gives no indication of such a change: the differences between crystals with and without antimycin are smaller than differences between crystals with the same Qo-site occupancy but different cell parameters, attributed to crystal packing distortions. The comprehensive comparison that would be required to put an upper limit on the magnitude of change that might be due to antimycin binding is beyond the scope of this work, however some preliminary quantitative

comparisons will be described which indicate that the change must be quite small. The conformational changes observed are also of interest to indicate the modes of flexibility of the protein, whether due to crystal packing forces or inhibitor binding.

In order to look for conformational changes induced by antimycin binding, we compared C-α positions of cytochrome ***b*** in structure 1PPJ with structures lacking antimycin (Figure 8 and Table 5). Structure 1PP9 lacks antimycin but has significantly different cell parameters (Table 1a) from 1PPJ, so differences may be due to different packing forces. A third structure of similar resolution (optical resolution 1.70) and lacking antimycin but with cell parameters similar to 1PPJ was compared to control for these changes.

In the structures compared here (all containing stigmatellin), a core domain of cytochrome b consisting of the four TMH of the four-helix bundle plus much of **cd_1-cd_2**, the ef-linker, and the F helix before the kink at 300 (αF1) could be superimposed with rmsd 0.133 Å or below and maximum deviation 0.28Å. The exact residues included in this core are listed in the legend to Figure 8, which shows the by-residue deviation, between 1PPJ (with antimycin) and two structures without antimycin, when cytochrome ***b*** is thus superimposed.

The N-terminus and the **de** linker, both involved in the **Q_i** site, were significantly different between 1PPJ and 1PP9, while the **ab** and **bc** linkers showed minor deviations. The mobile region in the **de** loop actually extends into the N-terminal part of helix **E**, as far as residue Lys227. Although significant, these movements are very slight, as can be seen in Figure 7.

Helix **E** starting at 228 could be included in the core domain but gave a slight but perhaps significant increase in rms deviation between the C chains of 1PPJ and 1PP9 (but not between P chains). To test the hypothesis that the **E** helix transmits a conformational signal to the P side upon antimycin binding at the $\mathbf{Q_i}$ site, helix **E** was excluded from the core region used for superposition, and treated as a separate domain.

With the cytochrome b chains thus superimposed, distance between corresponding atoms in pairs of chains were plotted in Figure 8. Any large effect of antimycin should be seen as differences between 1PPJ (with antimycin) and 1PP9 or Y21, and not between 1PP9 and Y21 (both without antimycin).

Such by-residue plots of atom deviation are limited in sensitivity by the inherent noise in the structure, the estimated standard deviation for atom positions being 0.3 - 0.5 Å for these structures (Table 1b). If it is assumed that sections of protein move as rigid bodies, much smaller movements can be detected because positions of all the atoms in the body contribute to determining its position and the "jitter" in individual atomic positions averages out. We tested two domains for such rigid body movement relative to the core domain used for superposition. One was the helix F_2+G+H region discussed below, which seems to be moving independently between 1PPJ and 1PP9, and the other was helix E, which shows significant deviations between these structures in the C chain (Figure 8a). To do this the operator best superimposing the domain in question was compared with the operator superimposing the core domain, giving an operator for the additional movement required to superimpose the domain in question after the core domain has been superimposed. This operator was then expressed as a rotation angle and as the largest movement of any atom in the domain upon application of the operator.

As a control to test the significances of the differences observed, the Y21 structure was re-solved twice starting with structures 1PPJ in one case and 1PP9 in the other. After positioning the models using the now-well-known intercrystal operators followed by rigid-body refinement and a few rounds of alternating positional minimization and restrained atomic B-factor refinement, the entire cyt b backbone (excluding the disordered region before residue 20[j]) was superimposable with maximum deviation 0.16 Å and RMSD 0.04. This implies that the small differences observed in this region between 1PPJ and 1PP9 are within the radius of convergence of positional refinement, and thus reflect real differences in the data and not results of accidental differences in the history of model-building.

The results from the comparison of interdomain operators are listed in Table 5. The **G** and **H** helices together with Helix **F** after the kink at residue 299 (**αF2**) form a separate domain which is rotated significantly with respect to the core domain described above: a rotation of 1.3° with maximum C-α displacement (at C378) of 0.81 Å in the case of C chains of 1PPJ and 1PP9). However the differences correspond more to differences in cell parameters and which of the two monomers in the dimer is being compared than to the presence or absence of antimycin, suggesting they result from different packing forces rather than an antimycin-induced change. The largest changes are seen comparing 1PP9 and 1PPJ, which differ in both presence of antimycin and cell parameters. Comparing the Y21 structure with 1PP9 (difference in cell parameters) and with 1PPJ (presence or absence of antimycin), 1PP9 shows the greatest movement of the F_2GH domain in both C and P chains, and the largest movement in helix E for chain C. While movement of the E helix in chain P was largest in 1PPJ, that movement was a barely

significant 0.51° rotation with maximal atomic displacement of 0.18 Å. This would appear to limit any antimycin-induced, long-range, static, conformational changes to a very subtle effect, at least in the presence of stigmatellin.

Ramachandran outliers

It is normal for well-refined structures even at high resolution to have 0.1-0.5% of residues in the "Forbidden" zone of the Ramachandran plot. However a Ramachandran outlier can also be an indication of a mis-built residue. Therefore we have examined the outliers in the two structures presented here to see how well the conformation presented is supported by the density.

Tyr155 of cytochrome b is a particularly interesting outlier that is well supported by the density. In bacterial ***bc*$_1$** complexes, there are two conserved glycines in the turn between helices **cd$_1$** and **cd$_2$**, corresponding to positions 155 and 157 in the bovine sequence. Gly157 is also conserved in the mitochondrial complexes, but surprisingly 155 tends to be an aromat- tyrosine in most vertebrates, phenylalanine in yeast. However the backbone phi, psi values for this residue are 66 to 68 and -39 to -42° in the bovine structures and 76.6, -75.0° in the yeast structure. These values lie outside the allowed region on the Ramachandran plot for any residue but glycine. Thus a mitochondrial progenitor has placed an aromat at a position in the fold where only glycine could be accommodated readily, and this strained aromat has been preserved through evolution. We will not speculate about the function, but note that the **αcd$_1$-cd$_2$** hairpin helix forms part of the **Q$_o$** site, and movement of this helix in response to **Q$_o$**-site occupancy or ISP position has been reported[23; 66].

The residue corresponding to Tyr155 is also a Ramachandran outlier in all available structures of the chicken or yeast ***bc*$_1$** complex, and in the bovine complex in tetragonal crystals (e.g. 1L0L). Outlier status is avoided in structures 1BE3 and 1BGY by flipping the plane of the preceding peptide (154-155) relative to all other structures, however this arrangement of the backbone is incompatible with the density in the crystals reported here. The backbone density for this residue is quite strong and unambiguous, however the density on the ring and OH has a peculiar shape. This side chain sticks out into the solvent from the turn of the **cd$_1$-cd$_2$** hairpin and makes no contacts with the rest of the protein, so it is not surprising if it is not well ordered. In chain P the tip of the side chain of Tyr155 makes a crystal contact (with chain B of a symmetry-related dimer). This contact varies with cell volume, resulting in the spike at residue 155 in Figure 8, a and c.

Residue Ala171 in chain B ("core II") also falls in the disallowed region of the Ramachandran plot. This is in a 3-10 helical turn at the end of helix α**D**^{d}. The electron density leaves little doubt as to the positions of the atoms, so we believe this also is a real outlier. It is an outlier in the yeast (1P84) and tetragonal bovine (1L0L) structures as well, but not in 1BGY, again as a result of flipping the peptide plane (B170-B171) in a way which is inconsistent with the density in our structures.

Residue Met71 in the "tether" region of the iron-sulfur protein differs in the two monomers. The conformation modeled in the first monomer (chain **E**) is an outlier in the structure deposited for 1PPJ (with antimycin), but not in 1PP9 or the Y21 structure, or in chain R of any structure. This residue appears to have several conformations and is not very well-ordered. It is likely subjected to considerable strain in some positions of the ISP extrinsic domain, which could provide the energy for an unfavorable backbone

conformation. This dynamic linker region is worthy of further study to decide if it is really an outlier in some of its conformations, and to better define the different conformations.

The other two Ramachandran outliers are found in poorly defined regions (A223,224 in the interdomain linker of the largest subunit) and probably represent errors in the model.

Discussion

A number of features of the ***bc*$_1$** complex revealed by the presented structures and not discerned in previous structures suggests that this is the most accurate structure of the ***bc*$_1$** complex available. In particular, the binding mode of the inhibitor antimycin is defined to a high level of accuracy. The new structure is consistent with results of structure-activity relationship studies, however it does not support one of the conclusions from those studies: that the intramolecular hydrogen bond between phenolic OH and carbonyl O described for the molecule in solution[38] and in the small-molecule crystal[42] is important for binding and inhibitory activity. On the contrary, the bound molecule has the carbonyl oxygen facing His201 with a long, weak, or water-mediated hydrogen bond while the amide nitrogen H-bonds to the phenolic hydroxyl oxygen. This is quite understandable because the phenolic OH group is H-bonding to aspartate and is likely to be the donor in that interaction. This would make an H-bond to the carbonyl oxygen impossible, as that atom can only be an H-bond acceptor. The amide nitrogen, on the other hand, has one proton that would be available for H-bond donation. Such a rearrangement of the H-bonding pattern upon binding is not surprising, in fact the possibility was suggested in the small-molecule structure report[42]. The importance of the intramolecular H-bond was inferred from the fact that an antimycin analog in which the amide is separated from the salicylate benzene ring by an extra carbon (compound B of ref [38]) and thus could not form the H-bond, was

10^4-fold less potent than an analogue with the amide directly connected as in antimycin. However this compound would be equally unable to form the intramolecular H-bond between amide nitrogen and phenolic oxygen that we observe in the bound inhibitor, so these experimental results are consistent with our structure. In fact it could be said that our structure supports the conclusion of that study regarding the importance of an internal H-bond between phenolic oxygen and the amide, but those experiments gave no hint that the amide is flipped; and the amide N, rather than O, is involved in the H-bond.

The structure of bound antimycin and the surrounding protein presented here differs in some significant details from that of the structure 1NTK[24]. Most importantly, the conformation of antimycin in the binding site is different. While both structures agree that the dilactone and formylaminosalicylate rings are rotated relative to each other as compared to the small-molecule structure, 1NTK keeps the dihedral between the amide moiety and the FSA fixed, preserving the intramolecular H-bond between the phenolic OH and the amide O. In 1PPJ there is 180° rotation about this dihedral relative to the small-molecule structure, breaking the intramolecular bond and forming a new one between the amide NH group and the phenolic O.

In addition two key residues Ser35 and Lys227 have their side chains modeled differently in the two structures, resulting in different roles for these residues in antimycin binding. Gao et al.[24] report from structure 1NTK that Ser35 forms H-bonds with the amide carbonyl O and a carbonyl O of the dilactone ring. In 1PPJ Ser35 is in the most stable rotamer, facing away from antimycin (Figure 5a) and H-bonds with two waters (W3, W2) and the carbonyl oxygen of residue 32, but makes no direct H-bond to antimycin. If it were changed to rotamer $2^{\mathbf{h}}$ it would be positioned to H-bond the carbonyl oxygen of the threonine moiety of the dilactone, as in the model of Gao et al. However the electron density (Figure 5a) gives no indication of Ser35 in rotamer 2, even at partial occupancy. On the contrary, as described above W3 mediates an H-bond between Ser35 and the phenolic OH of antimycin. Likewise Lys227Nζ makes a direct bond to the formylamino oxygen of antimycin in 1NTK, but in 1PPJ these atoms are 5.2 Å apart and

ordered water W1 binds between them (Figure 5b, 6). Neither structure has Lys227 in one of the five most common rotamers, however in 1PPJ it is in rotamer 26 (3% frequency) of the more extensive rotamer library described in ref [67].

Whenever different results are obtained from two different crystal forms of the same protein under different conditions, it has to be asked whether the different results correctly represent two different states of the protein (possibly corresponding to different steps along a reaction pathway) or whether the feature is actually invariant and one of the structures is in error. In the case of the orientation of the antimycin amide group it seems unlikely that both binding modes are possible. Unfortunately supporting data (structure factors) are not available for the 1NTK structure, which makes it impossible to test whether the data would have been equally consistent with our current model. However the density depicted in the stereodiagram of Figure 2A of ref [24] appears consistent with our structure, having an unaccounted-for protrusion about where we put the carbonyl oxygen, and having the modeled carbonyl oxygen at the edge of contoured density with no surrounding protrusion of the density.

While we want to emphasize that the structure of antimycin in 1PPJ is based on the electron density from x-ray diffraction by a crystal and not on chemical considerations or structure-activity relationships, the flipping of the salicylate amide moiety relative to the small-molecule structure seen here nicely explains why compound **D** of Reference [38], which is methylated on the amide N, is a poor inhibitor despite still having the internal H-bond (albeit weaker) between phenolic OH and carbonyl oxygen in solution. The amide of a secondary amine cannot be a hydrogen-bond donor, so the intramolecular H-bond that we see between phenolic OH and amide N cannot form, and the methyl group would clash with the phenolic OH preventing the molecule from taking this conformation. The

explanation is less obvious with the structure presented in 1NTK, as the amide nitrogen is oriented toward a spacious part of the pocket containing only water molecules that might be expected to be displaceable (although methylation would prevent H-bonding with the water).

As for the discrepancies regarding the roles of Ser35 and Lys128, it seems more possible that the different rotamers observed in the current 1PPJ structure as opposed to 1NTK represent different states depending on pH or ionic strength. However the failure of that structure to correctly orient the amide linkage weakens the argument for different conformations of these residues. Since electron density was not shown supporting the modeled rotamers, and data are not available for independent evaluation, we hesitate to propose alternate conformations for these residues at this time.

Ser35 is not required for antimycin binding, as *Rhodobacter* and *Paracoccus*, which have Val or Ile here, are inhibited by antimycin. However *Rhodospirillum rubrum* has Ser as in mitochondria, and is more sensitive (in whole cells) than *Rhodobacter* (pers. communication of Fevzi Daldal). Schnaufer et al.[68] found that mutation of Ser35 to Ile led to antimycin resistance in *L. tarentolae*. However the effect of Ser35Ile substitution might be expected due to steric effects, and is not necessarily indicative of a role of this residue in H-bonding to antimycin or stabilizing the waters involved in antimycin binding.

Despite evidence summarized in the introduction for a long-range conformational change induced by antimycin binding, no indication of such a change has been reported from the previous x-ray structures. Our analysis of the present structures also gives no indication of such a change, suggesting it must be a rather subtle change if it exists at all.

Much of the evidence for a conformational change is circumstantial, and perhaps amenable to alternative explanations. For example the effect of antimycin on the stability in bile salts may involve strong binding interactions between the inhibitor and the protein serving to hold together the different parts of the sequence contributing to the binding site more strongly than they would be held together in the absence of the inhibitor, perhaps tying down a loose end to prevent some kind of "unraveling" which may initiate the cleavage reaction.

Antimycin may affect conformational dynamics of the protein in solution or embedded in the lipid bilayer, allowing or preventing the visitation of certain conformational states while not affecting the resting state that we see in the crystal, and these transient states may be responsible for the observed effects. It must also be remembered that all structures being compared here have stigmatellin at the $\mathbf{Q_o}$ site, and it is possible that this tight-binding inhibitor locks the conformation of The $\mathbf{Q_o}$ region and prevents conformational changes that would otherwise have been induced by antimycin. A similar comparison made with the chicken $\boldsymbol{bc_1}$ crystals in the absence of stigmatellin did not show any clear antimycin-induced change[23], but the resolution was lower and refinement not very complete at that time.

Materials And Methods

Bovine hearts were obtained from a slaughterhouse or meat market and either used fresh or stored at -20°C or below before use in the mitochondrial preparation. "Sol-grade" dodecyl β-**D**-maltopyranoside (DM) and "anagrade" hexyl-β-**D**-glucopyranoside (HG) were purchased from Anatrace. Stigmatellin and polyethylene glycol (PEG) were from

Fluka. Crystallization screen kits mentioned below, as well as cryocrystallography supplies, were from Hampton Research.

Mitochondrial protein was determined by the Lowry method[69] with bovine serum albumin as a standard. Cytochrome ***bc*$_1$** concentration was determined from the difference in absorbance of the dithionite-reduced sample at 562 vs 600 nm, for which an extinction coefficient for the bovine enzyme of 70 $cm^{-1}mM^{-1}$ (E. Berry, unpublished; based on pyridine hemochrome analysis) was used.

Protein purification- Purification was as described[70], involving solubilization of mitochondria with 1.0 g DM per gram protein, anion exchange chromatography on DEAE Sepharose CL6B with a gradient from 260-500 mM NaCl (in 50 mM KPi buffer pH 7.5, 0.5 mM EDTA, 0.1 g/l DM) and size-exclusion chromatography on Sepharose CL-6B in "sizing buffer" (20 mM K-MOPS pH 7.2, 100 mM NaCl, 0.5 mM EDTA, 0.1 g/l DM). Pooled fractions from the last column were adjusted to 5 μM cyt ***bc*$_1$** by ultrafiltration or dilution in the same buffer. Stigmatellin and Antimycin A were added to 10 μM (2-fold molar excess) from 10 mM and 15 mM alcoholic stock solutions.

Before setting up crystallization droplets a final step (PEG fractionation) was carried out in which the inhibitor-loaded ***bc*$_1$** complex was mixed with successive portions of a precipitant solution containing 100 mM KMES pH 6.4, 100 g/L PEG 4k, and 0.5 mM EDTA. This procedure clearly separates two populations, a minor fraction ("aggregated material") which usually precipitates at around 0.3 volumes of precipitant and contains all of the contaminating cytochrome oxidase (present as supercomplex or micelles containing two separate complexes, and incompletely separated by the size-

exclusion column) from the major fraction which usually does not begin to precipitate until more than 0.6 volumes have been added. In the case of the antimycin-containing crystal, material precipitating between 0.29 and 0.76 volumes of precipitant was collected by centrifugation and redissolved in several times the original volume of the above-mentioned sizing buffer. To reduce NaCl and residual PEG from precrystallization, the buffer was exchanged by several cycles of dilution in final buffer (20 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 g/L UDM) and ultrafiltration on Amicon YM-100 membrane. It is difficult to dissolve the PEG-fractionation pellet directly in a small volume of final buffer- perhaps due to residual PEG it is necessary to have a higher ionic strength, provided by NaCl in the sizing buffer.

Crystallization- Crystallization was by sitting-drop vapor diffusion. Protein in the final buffer described above was mixed with 0.15 volume of 2.5 M HG, and then one volume (usually 10 μl) of this detergent-supplemented protein was mixed with 0.9 volume of major precipitant and 0.1 volume of minor precipitant/additive. The major precipitant consisted of 60 g/L PEG-3350, 100 g/L glycerol, 100 mM Na-cacodylate pH 6.7, 20 mM $MgCl_2$, and 3 mM NaN_3, and the minor precipitant/additive was Hampton Research's "Screen II #31", consisting of 200 g/L Jeffamine M600 in 0.1 M HEPES pH 7.5.

We calculate the final pH to be about 6.86, ignoring buffering by the protein itself. The ionic strength is 72 mM before vapor diffusion. The droplets were allowed to equilibrate by vapor diffusion against a reservoir containing the major precipitant.

Data collection- The crystals were mounted in a nylon loop on a magnetic pin (Hampton Research) and flash-cooled in liquid nitrogen for cryogenic data collection. The diffraction limit and the cell parameters were highly variable, and in some cases warming

the crystal to room temperature for 1-2 minutes and refreezing in the cold stream improved the diffraction dramatically. This seems to be related to the extent of dehydration of the crystals, and we are currently working on a way to optimize the diffraction by systematic dehydration.

The crystal from which structure 1PPJ was obtained was mounted in a loop and dipped in a mixture containing equal parts of the mother liquor and cryoprotectant (250 ml/L glycerol, 120 g/L PEG 4k, 10 mM K-MES pH 6.7, 3 mM Azide) before freezing in liquid nitrogen. After a preliminary exposure revealed diffraction to 4 Å and space group $P2_12_12_1$ with cell parameters 152 × 178 × 227, the pin was removed from the cold stream and set, base down, at room temperature, so the crystal in the loop was dehydrated by the downdraft produced by the cold copper pin. After 3 minutes the pin was returned to the cold stream for data collection, now with resolution limit 2.0 Å and cell parameters 128, 169, 232.

The crystal for structure 1PP9 (without antimycin) was not intentionally dehydrated, however it was one of only 2 crystals diffracting to around 2 Å from about 40 that were mounted from the same well. It is likely that these two crystals were exposed to air longer than the others before freezing. The cell parameters for 1PP9 are somewhat intermediate between the before and after parameters for 1PPJ, suggesting it is less dehydrated. The other crystal from that well diffracting to 2.0 Å had essentially the same cell parameters as 1PPJ. This is the crystal Y21, mentioned in the discussion of antimycin-induced structural changes.

Diffraction patterns were collected in 0.5° rotations. Even for the best diffracting crystals, the mosaic spread was large (1.0-1.5°). In order to reduce the data to 2.0 Å

without excessive overlap, it was necessary to assume a lower mosaic spread (0.6°) during spot integration. This results in sampling spot intensity near the maximum of the rocking curve ("profile peak sampling") but ignores tails of the measured reflection's rocking curve as well as overlap from tails of neighboring spots in reciprocal space. This together with radiation decay described below contributes to the higher than usual R-merge and R-sym values for these datasets.

Both crystals used in the present work were rod-shaped, with dimensions ~0.2 x 0.2 x 1.5 mm. This allowed collecting several different datasets from each crystal, at different positions along the long axis of the crystal. It was later determined that significant radiation damage occurred during data collection as indicated by increasing B-factor. In the case of the antimycin-containing crystal (1PPJ), the final dataset was constructed by merging early data from each individual dataset, with a cutoff when the B-factor for scaling against a particular reference was more than 15 $Å^2$ greater than that for the first exposures. The measurements from these selected frames from each data collection were individually scaled and merged in scalepack[71]. The resulting incomplete datasets were merged together using scalepack to make the final dataset. The statistic R_{merge} in Table 1 and in the PDB file header refers to the R-merge obtained at this second merging step. For structure 1PP9 data from a single collection was used and R_{merge} in Table 1 (R_{sym} in the PDB entry) refers to the initial merging of frames within the dataset.

To prepare for cross-validated (cv) refinement[72] a test set of reflections ("Free-R flags") was chosen from an ideally generated complete dataset to 1.8 Å, randomly selecting 5% of the reflections. This set of Free-R flags was used with every dataset from this crystal form to avoid biasing the cross-validation.

Structure determination.

Phasing- The first (low resolution) dataset from a crystal of this new orthorhombic form was solved by molecular replacement using PDB entry 1BE3 as model. The iron-sulfur proteins were repositioned as in entry 2BCC, and several regions that were observed not to fit the $2F_o$-F_c density map were rebuilt. As successively higher resolution datasets were collected, they were phased by molecular replacement using the best available previous structure from the same crystal form. Variation in cell parameters made rigid body refinement of the previous structure against the new data unreliable for positioning the molecule in the cell.

For each crystal, the model was refined by cycles of manual rebuilding using the graphics program O[73] alternating with rigid body, multi-rigid-body, positional, and restrained atomic B-factor refinement in CNS[74]. When significant improvement was achieved in one crystal, the appropriate changes were transferred to the other crystals by refining the improved model to convergence against the other datasets, comparing atomic positions with the previous models for those datasets, and examining the differences in the density to decide which model was appropriate on a crystal-by-crystal and residue-by-residue basis.

Non-crystallographic symmetry- The crystal contains a dimer of the bc_1 complex in the asymmetric unit. Initially non-crystallographic symmetry restraints were used for all protein atoms. During rebuilding to fit the electron density it became clear that certain residues did not obey NCS, and the restraints were released for those residues. For a while NCS restraints were eliminated, and the two monomers were refined and rebuilt

independently. The resulting structure was examined to locate areas that seemed to violate NCS, and restraints were re-applied everywhere else. The remaining NCS violations were examined to determine whether the electron density supported the violation. If not, the residue was rebuilt in both monomers to be consistent with NCS and the restraint was re-imposed. If the NCS violation appeared real, the surrounding was examined for explanations in the form of crystal contacts. Except in the case of clear NCS violations, application of NCS restraints invariably improved the R-free statistic. It is not known, however to what extent this is due to improvement in the ratio of (data + restraints) to parameters, and to what extent to communication between the test and working sets of reflections (bias) due to the NCS relationship.

In addition to specific violations of NCS, subtle distortions of the protein between the two monomers were present, presumably due to intrinsic flexibility of the protein and the different packing forces. To allow for this flexibility without greatly increasing the number of parameters being fit, the NCS-restrained residues were divided into about 49 NCS groups each of which was allowed its own NCS operator.

Solvent- Water molecules were added with the water_pick program of CNS, and refreshed periodically by removal of waters flagged by whatcheck as too far from protein and picking of new waters. As the density improved, some of the solvent molecules took on distinct oblong or trigonal shapes, and some of these were modeled as oxygen or azide and glycerol, respectively. Phospholipid and detergent molecules appeared in varying states of disorder, and some of the best-defined have been modeled.

Validation- The refined structures were subjected to validation using procheck[50], sfcheck[48] and whatcheck[75]. Residues flagged as unusual were examined and in many cases rebuilt, then the refinement was repeated before testing again. ARP/wARP version

6.0 was used to eliminate model bias and confirm the well-determined parts of the structure by automated rebuilding from free atoms refined by the ARP/wARP[76] process "automated model building starting from existing model". ARP/wARP was able to trace the protein in as few as 53 chains containing over 3700 residues, as compared to 20 chains containing ~4020 residues in the final models.

When these steps ceased to yield further improvement, the model was saved and then submitted to a final round of non-cross-validated refinement (positional and B-individual) using all the data, with all parameters the same as during the final cv refinement. No manual adjustment was performed on the final refined structure. Refinement statistics for deposition were obtained by the "xtal_pdbsubmission" routine of CNS using both the final cv-refined structure and the final structure refined against all the data. The coordinates of the latter structure and the data used in refinement were deposited in the PDB.

Protein Data Bank accession number

Structure factors and coordinates have been submitted to the Protein Data Bank under the accession numbers 1PP9 (without) and 1PPJ (with antimycin). The structure referred to as Y21 is being deposited with accesion number 1???.

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Figure Legends

Figure 1. The structure of antimycin (stereo views). *A*, From the small-molecule crystal structure ([42] ; coordinates from the Cambridge Structure Database, CCDC # 125007). Hydrogen atoms have been removed from the carbon atoms for clarity. ***B,*** from the structure 1PPJ, with the FSA ring and amide group in the plane of the picture. ***C,*** as ***B*** but rotated 75° to view the dilactone ring nearly face-on. The electron density in parts ***B*** and ***C*** is a $2F_o$-F_c map contoured at 2.1 σ (***B***) or 0.9 σ (***C***) from structure 1PPJ.

Figure 2. Representative density in well-ordered parts of the structure. **(a)**. Heme-ligand met160, showing chirality about the Sδ atom. **(b)**. Stigmatellin **(c)** hexyl glucoside molecule sandwiched in a crystal contact. The maps are $2F_o$-F_c, calculated from data between 15 and 2.1 Å, sharpened with B -20, and contoured at 2.3 σ **(a),** 2.0 σ (**b**), or 1.8 σ **(c)**; from structures 1PP9 (a) or 1PPJ (b), (c).

Figure 3. H-bonding around the high potential cyt b heme. **(a)**- Heme $\mathbf{b_H}$ viewed from helix B. Water W4 bridges between the two heme propionate side chains, while W5 bridges between a propionate and the heme axial ligand His97. Arg100 interacts directly with this "bent" propionate, and via unlabeled water molecule W6 with the carbonyl O of His97. W5 is also H-bonding with backbone atoms of helix A, which has been removed from this view for clarity. **(b).** - The same region viewed from the heme position, looking toward helices A and B. The heme is removed except the two propionate side chains. Intercalation of waters W3 and W5 in the helical backbone of Helix A can be

seen. Waters W1-3 are discussed in the text in connection with antimycin binding. The map is a $2F_o$-F_c map calculated from data between 15 and 2.1 Å, sharpened with B -20, and contoured at 1.8 σ **(a)** or 1.7 σ (**b**).

Figure 4. Structure of the Q_i site and interaction with bound antimycin. The $\mathbf{Q_i}$ site lies in a triangular volume formed by helices **B** and **E** crossing at an angle **(a)**. The N-side (N-terminal) ends of these helices are held together by β-type H-bonding between residues just preceding the helices (arrows in cartoon) which bounds the third side of the volume, and by Lys228 of helix E which H-bonds with a backbone O of residue 27, and to a water molecule bonded to that atom and to Asn32 Oγ1 of Helix A. These bonds are part of a more extensive H-bonding chain involving also Trp31, Ser35 and two other waters. In **(b)** antimycin (magenta bonds) and heme $\boldsymbol{b_H}$ (orange bonds) are added. The methyl and propionate substituents of the "A" ring of heme protrude from the four-helix bundle between helices **A** and **D** (helix **D** removed for clarity), forming part of the surface of the binding site. The formylaminosalicylate headgroup of antimycin inserts into the triangular volume described above, sandwiched between Phe220 of helix E and the heme propionate, and H-bonding with Asp228 and (via another water) Lys227. In **(c)** the protein elements shown in **(a)** are rendered as space-filling model to show the surface of the binding site. Antimycin (magenta carbons) and heme $\boldsymbol{b_H}$ (orange carbons) are also space-filled to show the intimate contact between these moieties and the snug fit of the antimycin headgroup in the protein. The binding pocket is completed by the α-a surface helix (shown here starting with residue 15) and the **D** transmembrane helix, left as a

ribbon for clarity. There may be H-bonds involving His201 in helix **D** with the amide carbonyl oxygen of antimycin and with a backbone oxygen in the α-a helix.

At the lower extreme of antimycin is the aromatic ring, viewed edge-on and inserted between the bent propionate of heme $\boldsymbol{b}_{\mathbf{H}}$ and Phe220 in helix **E**. The carbonyl oxygen of the amide linkage is directed toward the viewer, seen beneath His201 of helix **D**. Higher up, the alkyl side chain extends to the right into the lipid-filled cleft. At the top is the acyl group, with the ester carbonyl oxygen direct towards the viewer. Note the close contact with heme $\boldsymbol{b}_{\mathbf{H}}$, involving not only the aromatic ring of antimycin but also part of the dilactone ring.

Figure 5. Ser35 carbonyl O faces away from antimycin, and Lys227 interacts with antimycin through a water molecule. (a) Ser35 and vicinity: a $2F_o$-F_c map contoured at 1.5 σ. Asp228 has been removed for clarity, and W2 is not shown. (b) Lys227, Asp228, and vicinity: Antimycin is in the front and lower part of the figure, with its formylamino oxygen at the center. Water W1 bridges between the formylamino oxygen and Lys227Nζ at the top of the figure. W1 also makes H-bonds with Oγ1 of Ser32 (left) and the carbonyl oxygen of C27 (right). Also visible in this figure, two-point H-bonding of Asp228 to antimycin. In the background is Asn32 with H-bonds stabilizing W1 and W2, and a bond from the latter atom to Ser35. The intercalated water W3 is behind antimycin, barely visible through the salicyl ring, with H-bond to Ser35 indicated. The map is the same as in (a).

Figure 6. His201 and Ser205. The view is nearly the same as Figure 5b, showing antimycin, Lys227, Asp228 and water 1. Residues behind those have been removed for clarity, and the C-terminus of helix **D** containing His201 and Ser205 is shown. Two different density levels are used to elucidate the interaction of His201 with antimycin and the possible involvement of a water molecule. The maps are $2F_o$-F_c, contoured at 1.5 σ in (a) and 1.0 σ in (b). Also shown is an unknown molecule modeled as dioxygen (see text).

Figure 7. Comparison of Qi-site residues and ligands in structures 1PPJ and Y21. The two structures were superimposed based on cyt. b residues 32-51, 79-99, 113-145, 161-201, and 263-300. The backbone is shown for parts of transmembrane helices A (pink), D (red), and E (green), in color for 1PPJ and gray for Y21; as well as some of the linker region preceding helices A and D. Relevant side-chains are drawn with bonds and carbon atoms the same color as the backbone. Water molecules are shown as red spheres for 1PPJ and pink spheres for Y21. Antimycin from 1PPJ is shown as a purple ball-and stick figure with red oxygen atoms, while ubiquinone from structure Y21 is yellow. Note the relatively invariant positions of the backbone and side-chains, and the positioning of the ubiquinone ring over the amide moiety of antimycin.

Figure 8. Flexibility in cyt b induced by antimycin and/or crystal packing forces. Various cyt b structures were aligned based on the relatively rigid core consisting of residues 32-51, 79-99, 113-145, 161-201, and 263-300. Deviations of Cα position are plotted vs residue number for selected pairs of structures. For each pair the differences in chain C are shown in red while those for chain P are in blue. The green rectangles along the x axis

indicate the position of helices in the sequence. PDB entries 1PP9 (without) and 1PPJ (with antimycin) are the structures featured in this article, while structure Y21 is from a crystal with cell parameters nearly identical to those of 1PPJ. Thus comparison of cyt b from the same monomer between 1PPJ with Y21 (b) gives the best indication of antimycin-induced changes, while comparison of two monomers in the same crystal, or of 1PP9 with Y21 (c), should show only crystal-packing-induced changes. Comparison of 1PP9 with 1PPJ (a) superimposes both sets of changes.

Table captions.

Table Captions

Table 1. Statistics from the structure determination process.

Table 2. Model completeness by subunit. For each subunit is given the actual number of residues present in the complex based on sequence, and the number of residues modeled in each monomer of the two structures presented here. The chain letters assigned to each subunit in each monomer are also indicated. Major differences are due to the lack of subunit 11 in the crystals and disorder of the first 30 residues of subunit 10 in chain J of 1PPJ.

Table 3. Potential H-bonding partners for six highly-ordered water molecules in the region of heme b_H and the Q_i site.

Table 4. Residues surrounding Antimycin at the Q_i site. For each contact closer than 4 Å is given the residue type, number, and atom; the atom of antimycin, and the contact distance in each monomer. For distances greater than 3 Å, only the closest contact of each protein residue is given. Except for waters, all the residues contacting antimycin belong to cyt b (chains C and P). The "water" modeled between His201 and antimycin O3 does not account for the density as currently modeled (Figure 6).

Table 5. Relative motion of domains of cyt. b between three crystals:

Footnotes

[a]Present address:

Ecole Superieure de Biotechnologie de Strasbourg, UPR9050 CNRS, Boulevard Sebastien Brant. BP 10413, 67412 Illkirch, France

[b]Abbreviations used are: antimycin, antimycin A; cyt $\boldsymbol{bc_1}$, cytochrome $\boldsymbol{bc_1}$ complex; DM, dodecyl maltoside; UDM, undecyl maltoside; HG, β-hexyl-**D**-glucopyranoside; FSA, formylaminosalycylate moiety of antimycin; CC correlation coefficient; PDB, Protein Data Bank; $2F_o$-F_c map, a map from Fourier coefficients calculated as twice the measured amplitude minus amplitude calculated from the structure, and phases calculated from the structure. "N-side" and "P-side" refer to the normally negative matrix side and positive inter-membrane side of the inner mitochondrial membrane. Structures that have been deposited in the PDB are referred to by their capitalized 4-character accession codes (e.g. "1P84"). Their authors and literature references can be obtained from the entry at the PDB if not given here. heme $\boldsymbol{b_H}$ and heme $\boldsymbol{b_L}$ refer to the high and low-potential hemes of cytochrome ***b***. Heme B refers to Fe-protoporphyrin 9 irrespective of oxidation state. Heme C refers to a modified Heme B in which both vinyl groups of the porphyrin have been saturated by addition of cysteine Sγ, forming covalent links to the protein.

[c]A collection of supplementary materials for this article, available from the publisher (), includes the following items.

Procheck validation reports for structures 1PP9 and 1PPJ

sfcheck validation reports for structures 1PP9 and 1PPJ

Set of figures laid out for cross-eyed stereo viewing

Scheme and description of the Q-cycle mechanism

Secondary structure diagram for bovine cytochrome b

Table of interaction distances between stigmatellin and the protein

Expanded Figure 5 with 7 views, one per page:

Stereodiagram of space-filling model of cyt b helix A backbone with intercolated waters W3 and W5.

Stereo views of omit map density for antimycin in 1PPJ.

Stereo views of omit map density for critical residues in 1PPJ C:Ser35, C:Lys227, C:His221-Pro222 (cis-peptide), C:His345-Pro346 (cis-peptide), D:Gly73-Pro74 (cis-peptide).

List of standard rotamers referred to, defined by side-chain dihedral angles:

VRML views of Figures1, 5, and 6 with electron density to allow examination from any angle.

[d]Secondary structure nomenclature for subunits 1 and 2 is defined in Figure 5 of ref. [46].

[e]The pyrrole rings of heme referred to here as A, B, C, and D correspond to protoporphyrin rings conventionally labeled IV, I, II, and III.

[f]There are different conventions for naming the atoms in antimycin, so we have tried to specify atoms from a chemical standpoint rather than by name. The protein database maintains two versions of antimycin, AMY (from 3BCC) and ANY (introduced with

1PPJ). These have the same atom names, but in ANY two additional carbon atoms have been added to the end of the acyl chain to allow for the possibility that it is heptyl, and the methyl group on the acyl chain has been moved from the 3- to 2- position in accordance with recent results[42]. In the Cambridge Structure Database of small molecule structures there is (CCDC # 125007) from the work of Deisenhofer's group[42], which uses different atom names. The PDB entry 1NTK uses the atom names from the Cambridge Database but the residue name (AMY) from the Protein Bata Bank. The atom names here, where used in the text and in Figure 1 and Table 4, are from ANY of 1PPJ.

[g]In fact the helical bonding is interrupted, with normal α-helical bonding involving the **N** atom of residues after 35, 3/10 helical bonding involving the **O** of residues before 31, and no strong helical bonds of either sort involving **O** of residue 31 or 32 and **N** of residue 35. The distance from 31**O** to 35**N** is 5.1 Å, and to 34**N** 4.1 Å. The intercolated water W3 is well ordered, with B-factors 25 and 32 $Å^2$ in 1PPJ (but 30 and 46 $Å^2$ in 1PP9), well below average for the structure, and with density in $2F_o$-F_c maps 3.9 to 4.7 σ. The heme-propionate-to-axial-ligand-bridging water W5 mentioned in connection with heme $\boldsymbol{b_{HI}}$ can also be seen as intercolated, with a short H-bond to 33 O, but it is equally close to 33 N and 34 N, with neither being as close as 35 N is to W3 (Table 3).

[h]Rotamer numbers used here refer to the lists of rotamers provided with the molecular visualization/modeling program O[73], with the most frequently-occurring being in each case rotamer 1. Lysine rotamer 26 is an exception, coming from the more extensive collection of rotamers in reference[67]. The actual side-chain dihedrals of the rotamers referred to are as follows:

Ser rotamer 1: chi_1=63°; Ser 2: chi_1= -62°; His 3: chi_1 = -169°, chi2= 80°; His 5: chi1 = -59°, chi2 = 169°; Lys 26: chi_1 = -66°, chi_2= 180°, chi_3= 67°, chi_4=180°.

[i]The structure Y21 was chosen because it's cell is most nearly isomorphous with 1PPJ, whereas 1PP9 has significantly different cell parameters. However superimposing 1PP9, or for that matter the yeast or chicken structures, puts ubiquinone in essentially the same place.

[j]The N-terminus of cytochrome b up to residue 20 is modeled differently in 1PP9 and 1PPJ, to the extent that manual rebuilding would be required for convergence when refined against the same data. Density is not very clear here and it seems likely that multiple conformations exist for all three structures. This region was modeled differently in the bovine cyt ***bc*$_1$** structures from Uppsala (1BE3, 1BGY) as compared to those from Bethesda (e.g. 1L0L). The possibility has been raised[77] that the N-terminus including α-a helix serves to transmit a conformational signal between $\mathbf{Q_i}$ sites of the two monomers.

Table 1

Protein Database entry:	1PP9	1PPJ
Inhibitors co-crystallized:	stigmatellin	stigmatellin, antimycin
A. DATA REDUCTION		
Unit Cell Dimensions	139.12 × 171.06 × 227.20 Å	128.53 × 168.75 × 231.53 Å
Solvent content	58.2 %	54.7 %
V_M	2.97	2.74
X-ray wavelength [a]:	0.99200	0.97977, 1.0000, 1.1000, 1.1808
Unique Reflections	312369	285923
Resolution Range, Å [a] :	50 - 2.1 (2.18 - 2.10)	250. - 2.100 (2.15 - 2.10)
"Optical Resolution" [b]	1.72 Å	1.75 Å
Completeness :	97.2% (83%)	98.1% (94.3 %)
Data Redundancy:	5.9	5.630
R Merge on I :	0.12 (>1.0)	0.149 (0.879)
$\langle I/\sigma_I \rangle$	10.9 (1.037)	18.6890 (2.819)
B. REFINEMENT:		
Resolution	24.99 - 2.10 (2.15-2.10)	93.53 - 2.10 (2.15 - 2.10)
Data Cutoff (σ_F)	0.0	0.0
Completeness	97.3 (91.9)	97.7% (90.3%)
# Reflections	305496 (19066)	285060 (16565)
R Value	0.250 (0.40)	0.224 (0.33)
Free R Value	0.287 (0.40)	0.260 (0.38)
Number Of Atoms Used		
Protein Atoms	31493	31181
Heterogen Atoms	1005	962
Solvent Atoms	1461	1406
B Values		
From Wilson Plot	27.3 $Å^2$	33.50 $Å^2$
Mean atomic B Value	46.9 $Å^2$	50.20 $Å^2$
Anisotropic B_{11}, B_{22}, B_{33}	15.35, -0.55, -14.81 $Å^2$	12.34, -3.71, -8.63 $Å^2$
ESD,- (cross-validated)[c]		
From Luzzati Plot[c]	0.32 Å (0.39 Å)	0.28 Å (0.35 Å)
From Sigmaa[c]	0.43 Å (0.47 Å)	0.33 Å (0.38 Å)
Rms Deviations From Ideality		
Bond Lengths	0.007 Å	0.006 Å
Bond Angles	1.5°	1.4°
Dihedral Angles	21.8°	21.8°
Improper Angles	1.02°	0.94°
C. VALIDATION:		
Residues in "Most Favored" region of Ramachandran	92.1%	92.4%
Residues in Ramachandran "disallowed" region	0.2%	0.2%
Bad Contacts/100 residues	0.5	0.3
Overall G-factor (ProCheck):	0.4	0.4
Real-space R-value	0.155	0.148
Real-space Correlation Coeff.	0.909	0.921

[a] Statistics in the highest resolution shell are given in parentheses.
[b] Optical resolution is defined in references [47; 48].
[c] Estimated std. dev. of atomic coordinates. Cross-validated estimates of ESD are given in parentheses.

Table 2. Model completeness by subunit. For each subunit is given the actual number of residues present in the complex based on sequence, and the number of residues modeled in each monomer of the two structures presented here. The chain letters assigned to each subunit in each monomer are also indicated. Major differences are due to the lack of subunit 11 in the crystals and disorder of the first 30 residues of subunit 10 in chain J of 1PPJ.

Subunit		number of residues actual	modeled in structure: 1PP9 monomer #1	1PP9 monomer #2	1PPJ monomer #1	1PPJ monomer #2
1	"core"1	446	A442	N442	A 441	N 441
2	"core"2	439	B423	O424	B 424	O 423
3	cyt ***b***	379	C365	P370	C 365	P 365
4	cyt c_1	241	D241	Q241	D 241	Q 241
5	ISP	196	E196	R196	E 196	R 196
6		110	F 99	S 99	F 99	S 99
7		81	G 73	T 74	G 73	T 74
8	"hinge"	78	H 66	U 66	H 66	U 66
9	signal	78	I 42	V 42	I 43	V 43
10		62	J 62	W 62	J 32	W 61
11		56	K 0	X 0	K 0	X 0
sum		2166	2009	2016	1980	2009

Table 3

Label	Water res. (C, P)		H-bond Partner Res.	Num	Atom	Distance C	Distance P	B-value C	B-value P
W1	119	959	** Antimycin		O2	2.6	2.6	44	38
			** LYS	227	NZ	2.6	2.6		
			** SER	28	O	3.1	2.9		
			** ASN	32	OD1	3.2	3.3		
W2	1008	214	** SER	35	OG	2.7	3.0	29	32
			** ASN	32	ND2	2.8	2.8		
			** ASP	228	O	3.0	2.7		
W3	222	28	Antimycin		N1	3.2	3.2	25	32
			** Antimycin		O1	2.9	2.8		
			** TRP	31	O	2.7	2.7		
			ASN	32	O	3.2	3.2		
			** SER	35	N	2.8	2.8		
			** SER	35	OG	3.0	2.9		
W4	168	109	** SER	205	OG	2.5	2.5	27	31
			** HEM	502	O2A	2.8	2.8		
			** HEM	502	O1D	2.7	2.7		
W5	2	1	** HEM	502	O1A	2.5	2.6	33	30
			** TRP	30	O	2.8	2.7		
			** HIS	97	ND1	2.9	2.8		
			** PHE	33	N	3.1	3.3		
			** GLY	34	N	3.1	3.4		
			ARG	100	NH1	3.5	3.4		
			ARG	100	NH2	3.5	3.2		
W6	108	35	** HIS	97	O	2.6	2.7	31	32
			** ARG	100	NE	3.2	3.1		
			** ARG	100	NH2	3.2	3.0		
			** GLY	101	N	3.3	3.4		

** - potential hydrogen bond

Table 4

Helix A and Waters (W)					Helix D & E					Heme bH				
protein atom		Anti atom	distance(Å) C	P	protein atom		Anti atom	distance(Å) C	P	Heme atom		Anti atom	distance(Å) C	P
ALA17	O	C10	3.6	3.7						HEM502	CMA	N2	3.8	3.8
ILE27	CD1	O2	3.5	3.5	MET190	CG	C23	4.0	3.7	HEM502	CMA	C9	3.8	3.7
TRP31	O	N1	3.4	3.4	MET194	CG	O9	3.6	3.5	HEM502	CMA	O4	3.2	3.2
ASN32	OD1	C8	3.9	3.9	LEU197	CD1	O4	3.2	3.1	HEM502	CMA	C20	3.3	3.3
GLY34	O	C27	3.9	4.0	SER205	OG	C4	3.5	3.5	HEM502	CMA	O7	3.7	3.7
SER35	CA	O7	3.0	3.1						HEM502	CMA	C27	3.9	3.9
GLY38	CA	C27	3.8	3.8	PHE220	CE1	C1	3.3	3.4	HEM502	CAA	C5	3.8	3.9
LEU41	CD2	C25	3.9	3.7	TYR224	CD1	O2	3.3	3.3	HEM502	CAA	C6	3.8	3.9
					ASP228	OD1**	N1	2.8	2.8	HEM502	CAA	C7	3.9	4.0
W3	O **	O1	2.9	2.8	ASP228	OD2**	O1	2.6	2.6	HEM502	CBA	C1	3.8	3.8
W1	O **	O2	2.6	2.6						HEM502	CBA	C5	3.7	3.8
"W"	O	O3	2.6	2.8						HEM502	CBA	C6	3.6	3.6
W1203	O	C5	---	3.7										

Table 5

F_2,G,H helices vs cyt. b core

	------Chain C---------			-------Chain P-------		
	angle	max disp,	atom	angle	max disp,	atom
1PP9 vs 1PPJ	1.392°	0.8389	C378	1.169°	0.6904	P378
1PPJ vs Y21	0.437°	0.3072	C378	0.310°	0.2654	P377
1PP9 vs Y21	0.960°	0.5969	C378	0.944°	0.5979	P378

E helix after 227 vs cyt. b core

	------Chain C---------			-------Chain P-------		
	angle	max disp,	atom	angle	max disp,	atom
1PP9 vs 1PPJ	0.927°	0.3315	C245	0.503°	0.1145	P245
1PPJ vs Y21	0.301°	0.1486	C245	0.513°	0.1814	P230
1PP9 vs Y21	0.859°	0.2438	C245	0.349°	0.1600	P228

Figure 1

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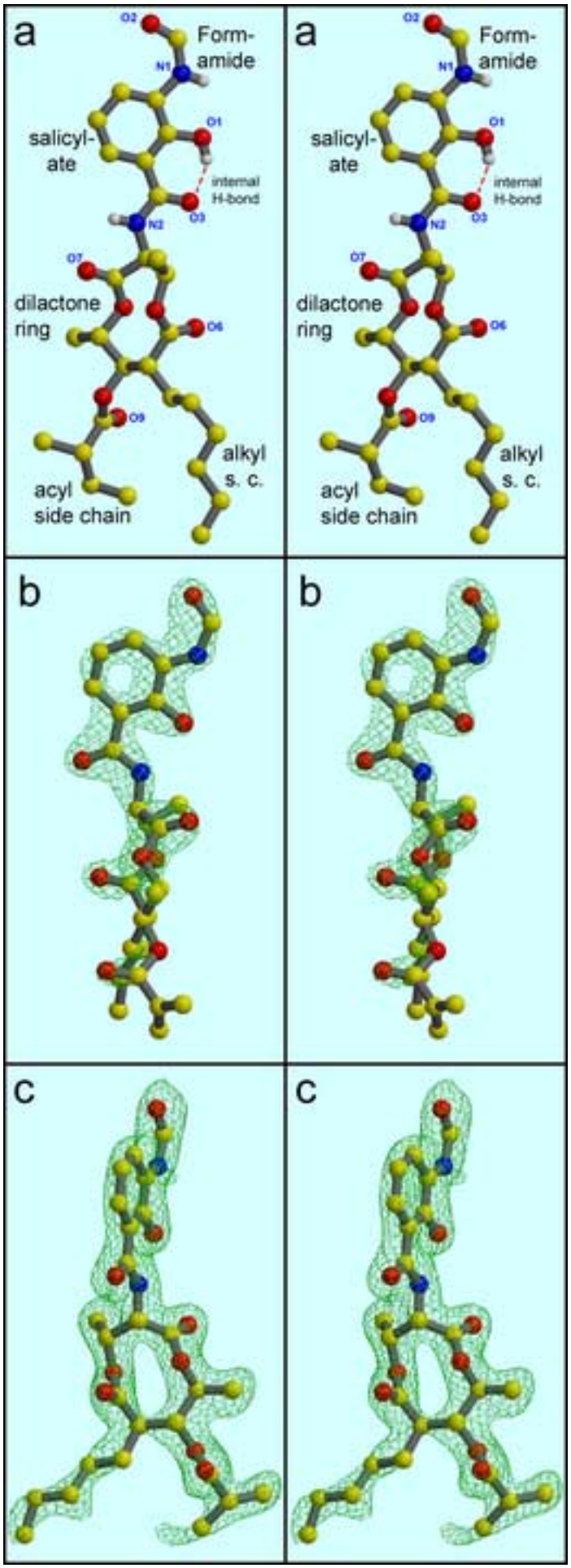

Figure 2

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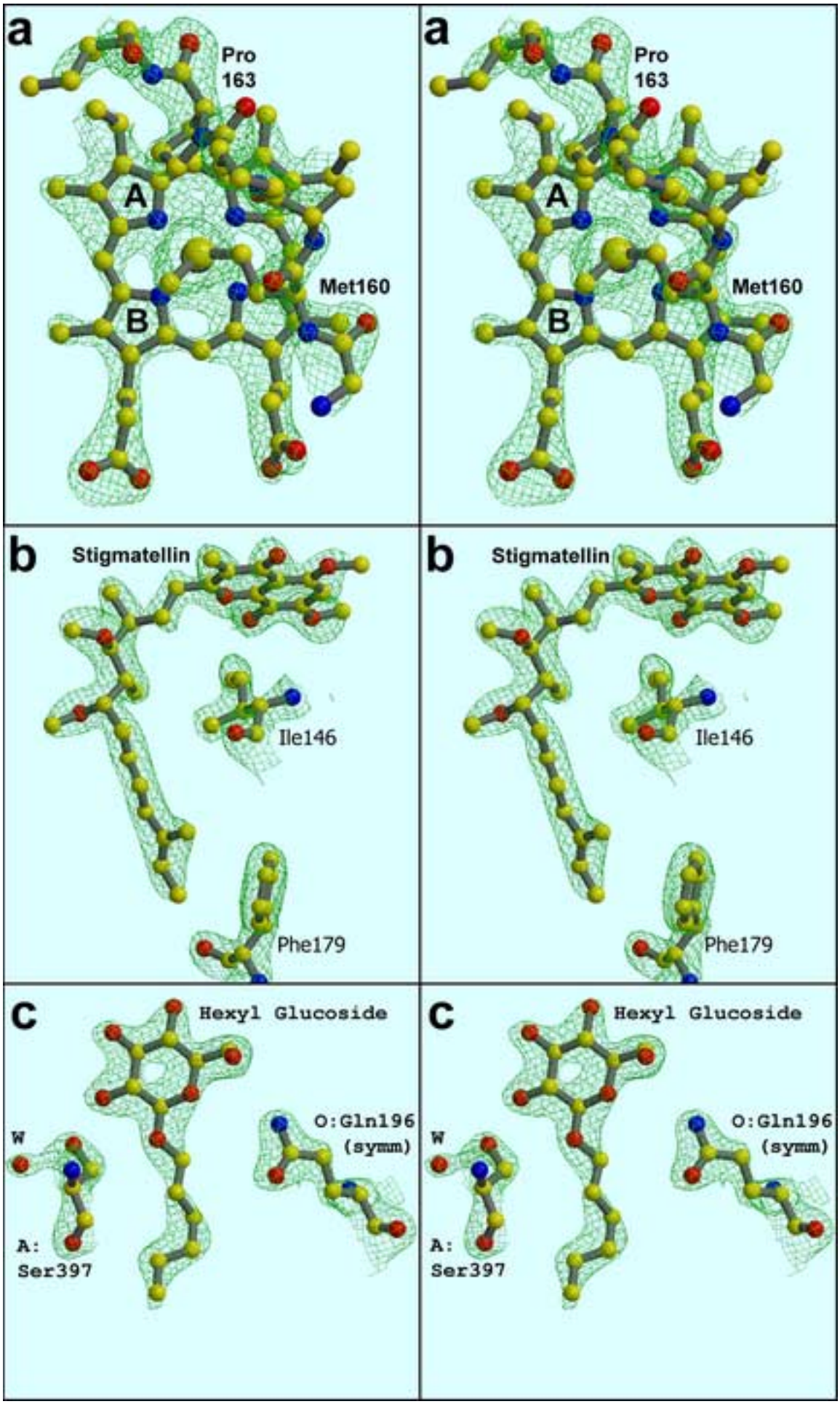

Figure 3

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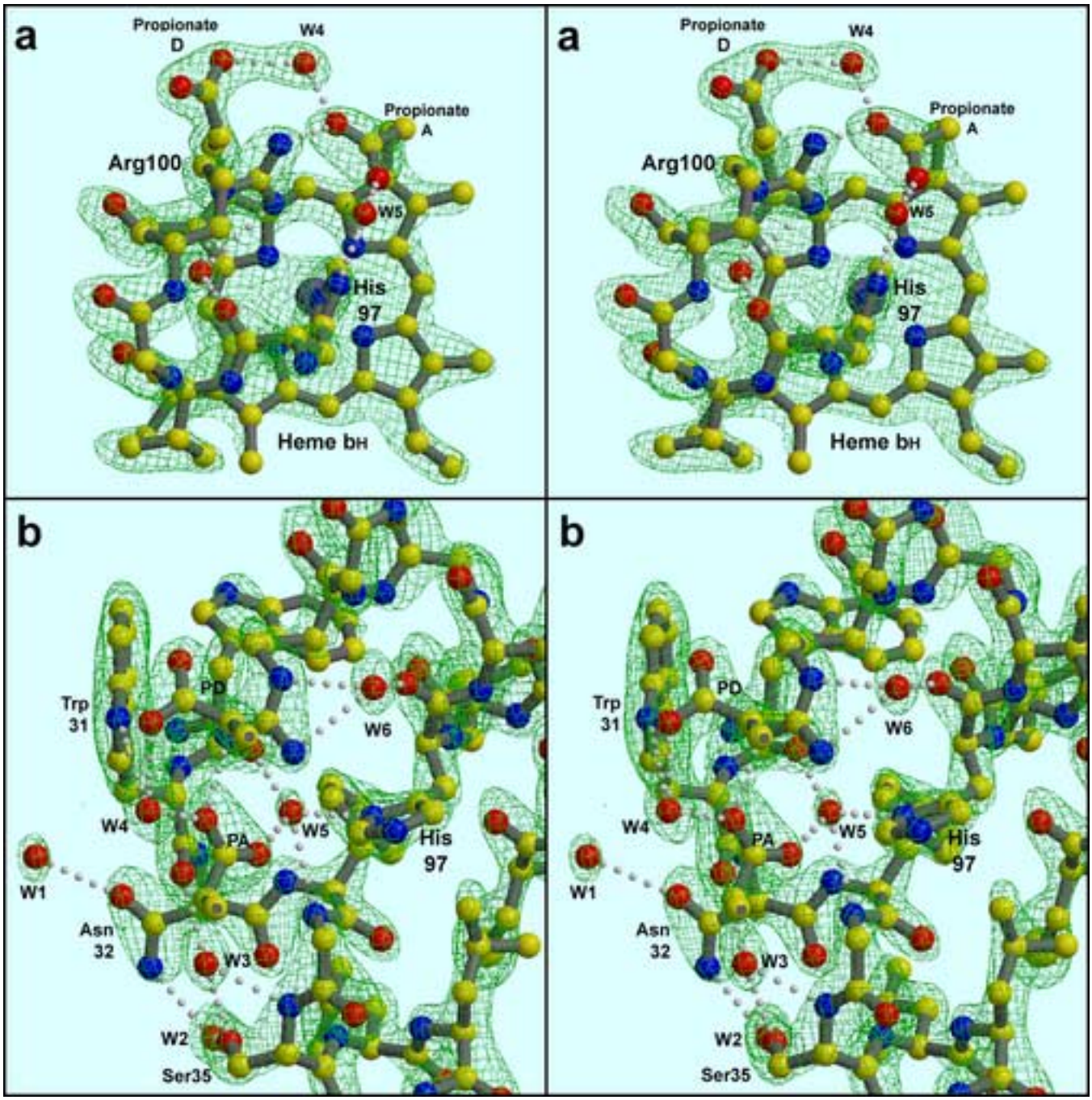

Figure 4

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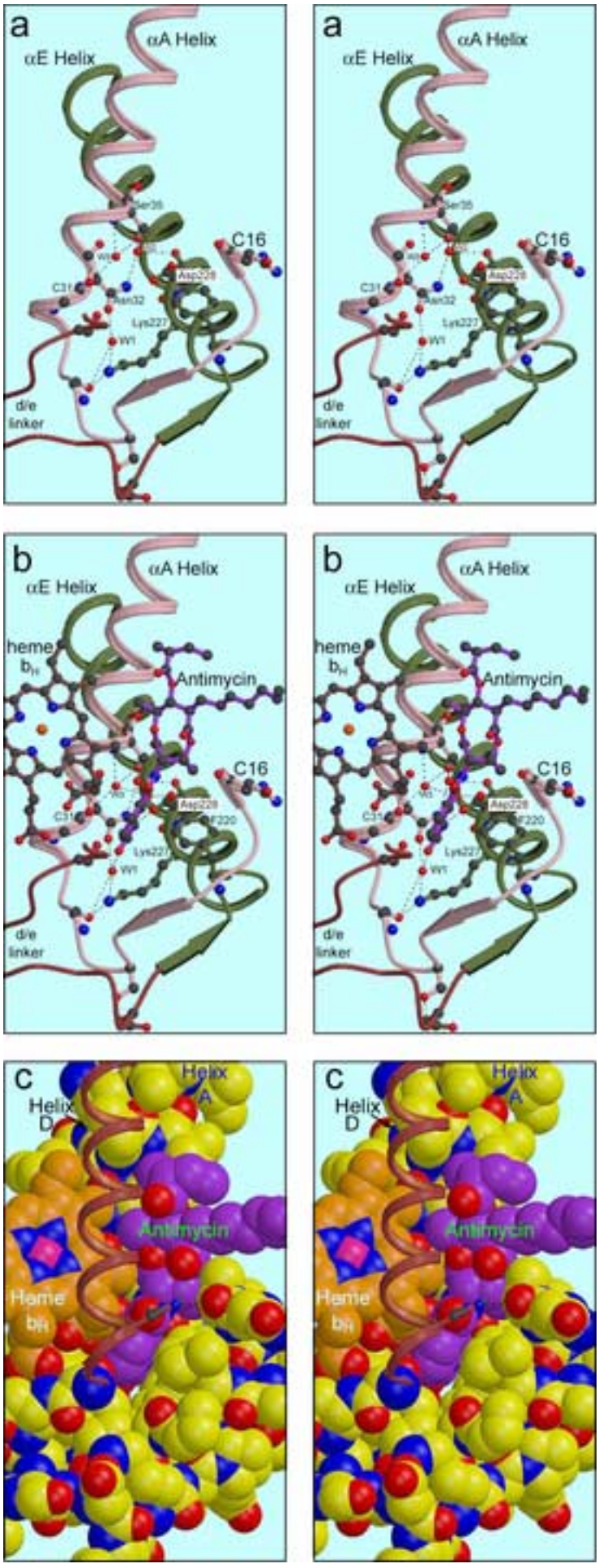

Figure 5

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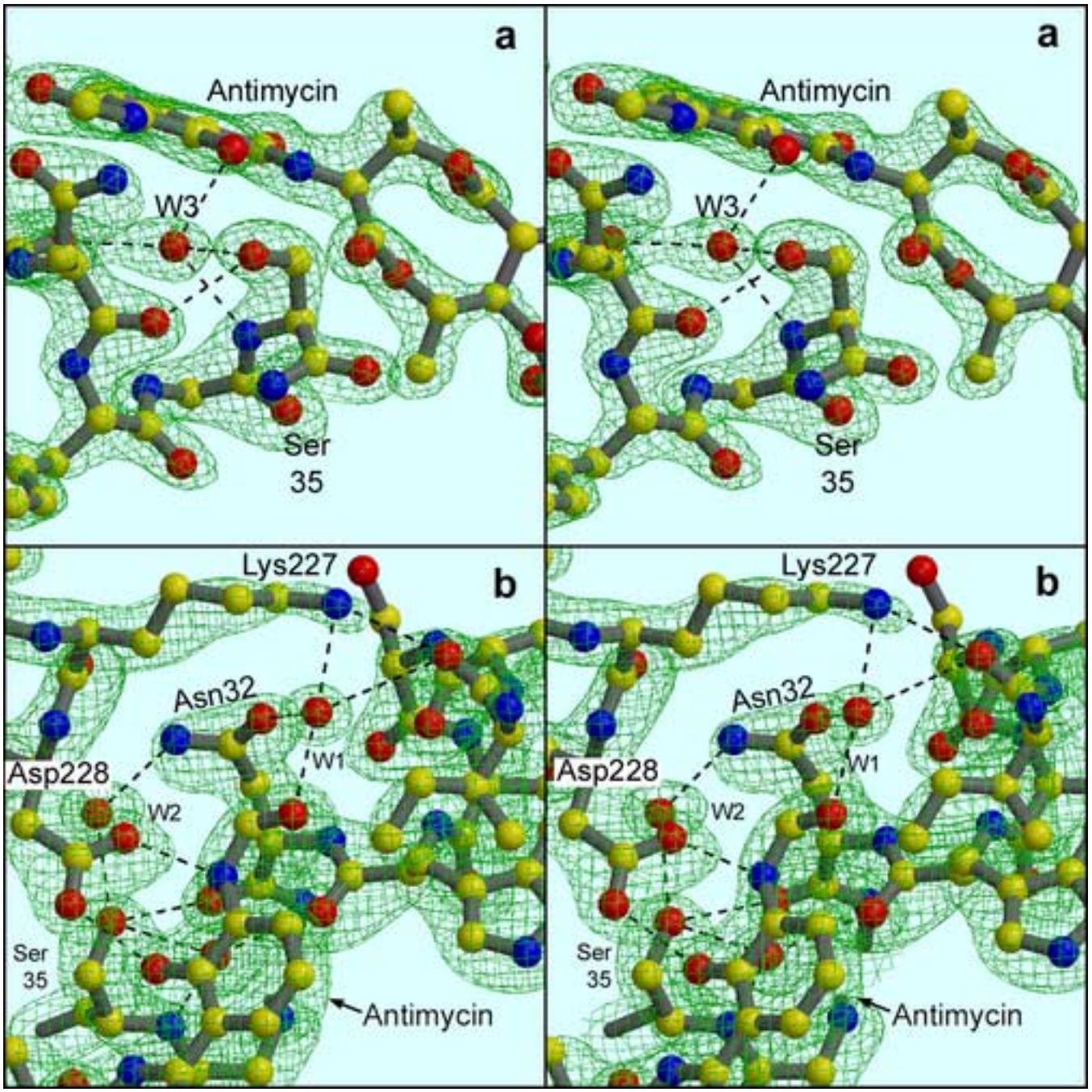

Figure 6

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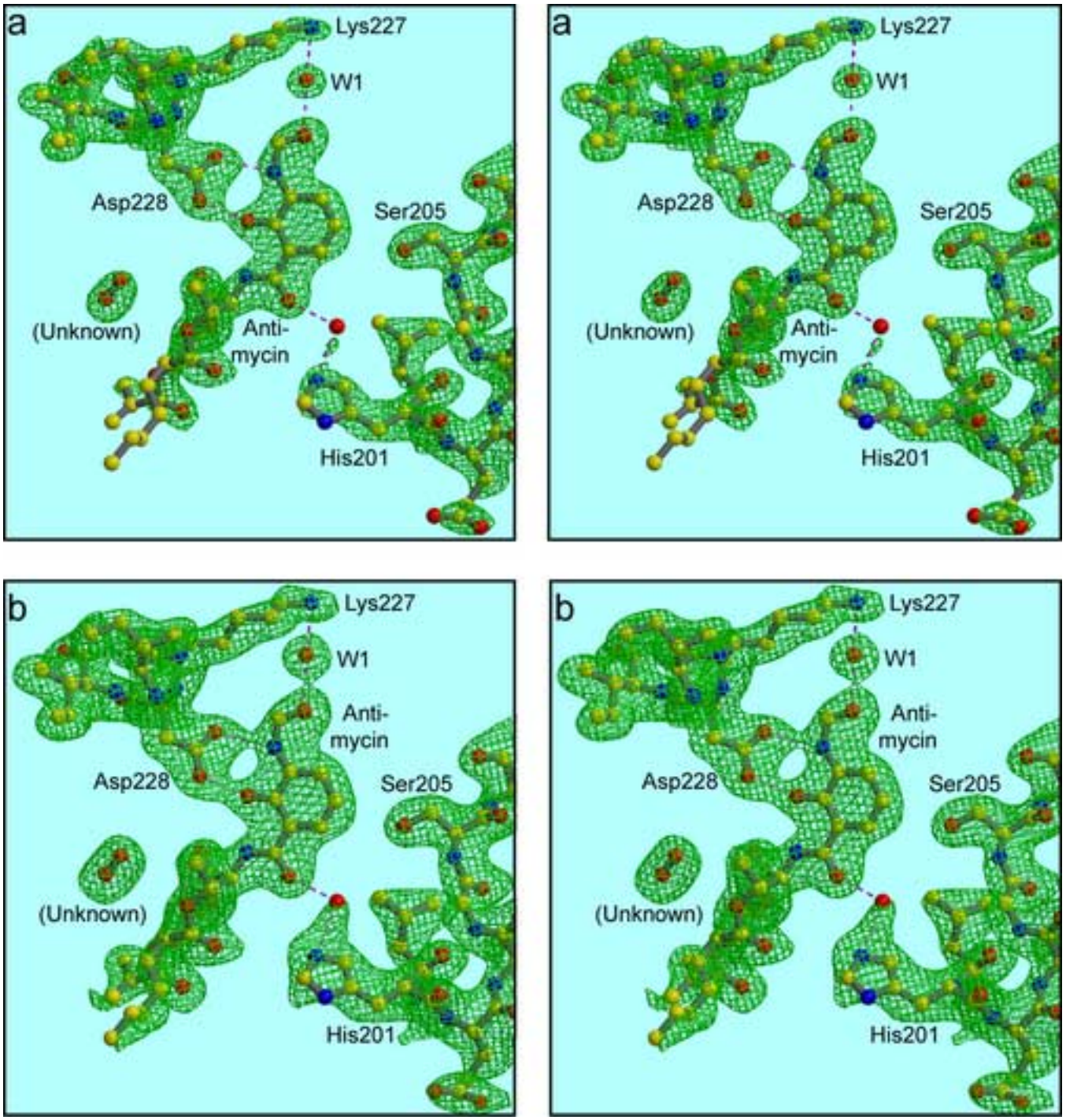

Figure 7

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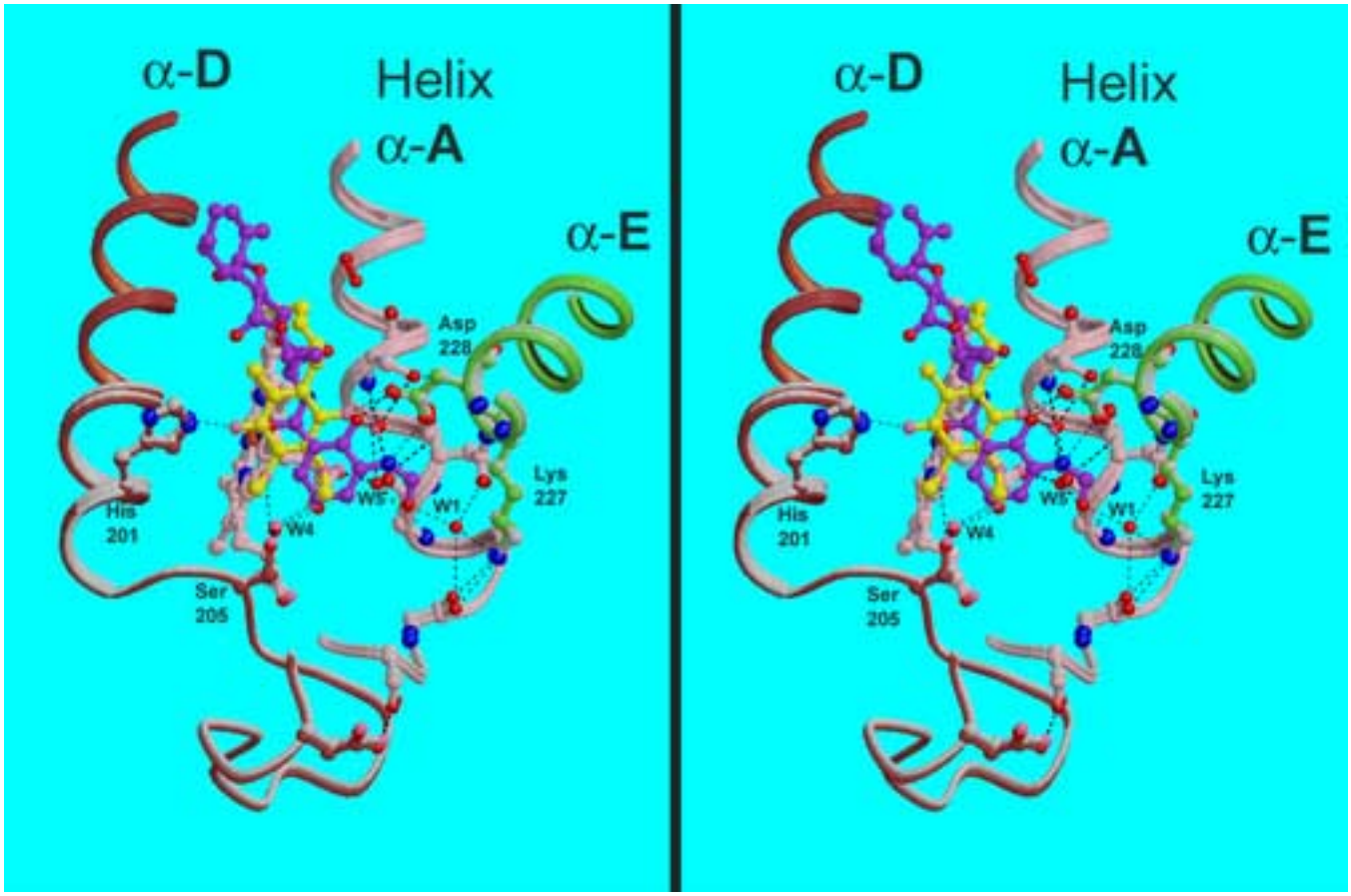
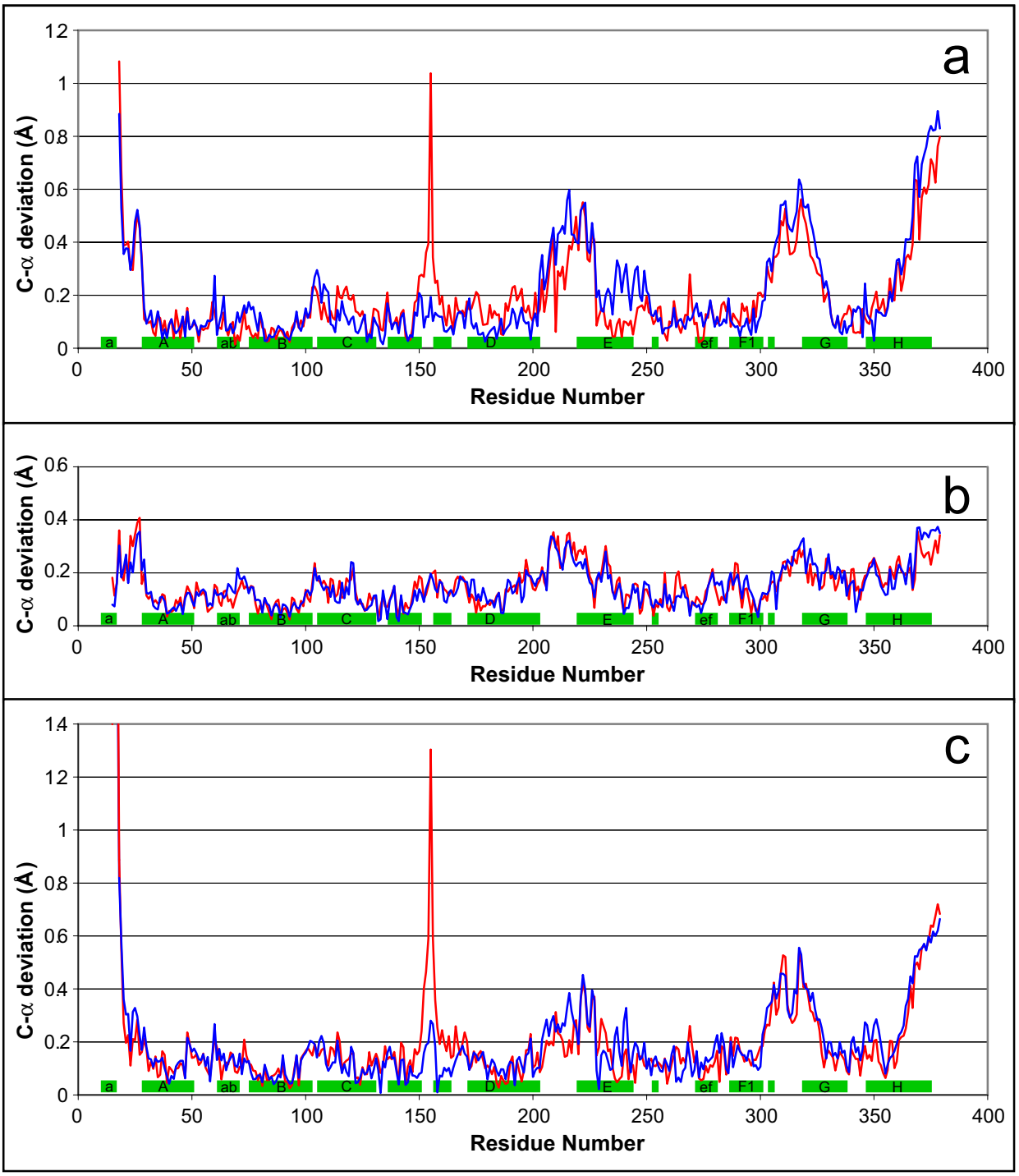


Figure 8


a
b
c
C-α deviation (Å)
Residue Number
0
50
100
150
200
250
300
350
400
a
A
ab
B
C
D
E
ef
F1
G
H

Supplementary materials for

BINDING OF THE RESPIRATORY CHAIN INHIBITOR ANTIMYCIN TO THE MITOCHONDRIAL *bc*$_1$ COMPLEX: A NEW CRYSTAL STRUCTURE REVEALS AN ALTERED INTRAMOLECULAR HYDROGEN-BONDING PATTERN.

Li-shar Huang, David Cobessi[a], Eric Y. Tung, and Edward A. Berry*

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1. Procheck validation reports for structures 1PP9 and 1PPJ
1pp9-procheck.pdf, 1ppj-prochreck.pdf

2. sfcheck validation reports for structures 1PP9 and 1PPJ
1pp9-sfcheck.pdf, 1ppj-sfcheck.pdf

3. Set of figures with stereo for cross-eyed viewing: **Figuresx.pdf**

4. Expanded Figure 5 with 7 views, one per page:
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Scheme S1. Q-cycle mechanism

Figure S1. Stereodiagram, space-filling model of cyt b helix A backbone with intercolated waters W3 and W5.

Figure S2. Stereo views of omit map density for antimycin in 1PPJ.

Figure S3 - S7. Stereo views of omit map density for critical residues in 1PPJ

S3. C:Ser35

S4. C:Lys227

S5. C:His221-Pro222 cis-peptide

S6. C:His345-Pro346 cis-peptide

S7. D:Gly73-Pro74 cis-peptide

Figure S8. Secondary structure of cytochrome b

Table S1. List of standard rotamers referred to, defined by side-chain dihedral angles:

Table S2. Interaction distances between stigmatellin and the protein

Description of stigmatellin in its binding site.

6. **VRML views** of the structure with electron density to allow examination from any angle.

These can be viewed in a web browser equipped with a VRML plugin or helper application such as:

For Windows: Cosmo-viewer or Cortona (www.parallelgraphics.com/products/cortona).

For Linux: FreeWRL or vrmlview (http://www.sim.no/download/).

For Irix: Cosmoviewer (available for download from the Silicon Graphics website).

R-stats vs resolution, 1PPJ

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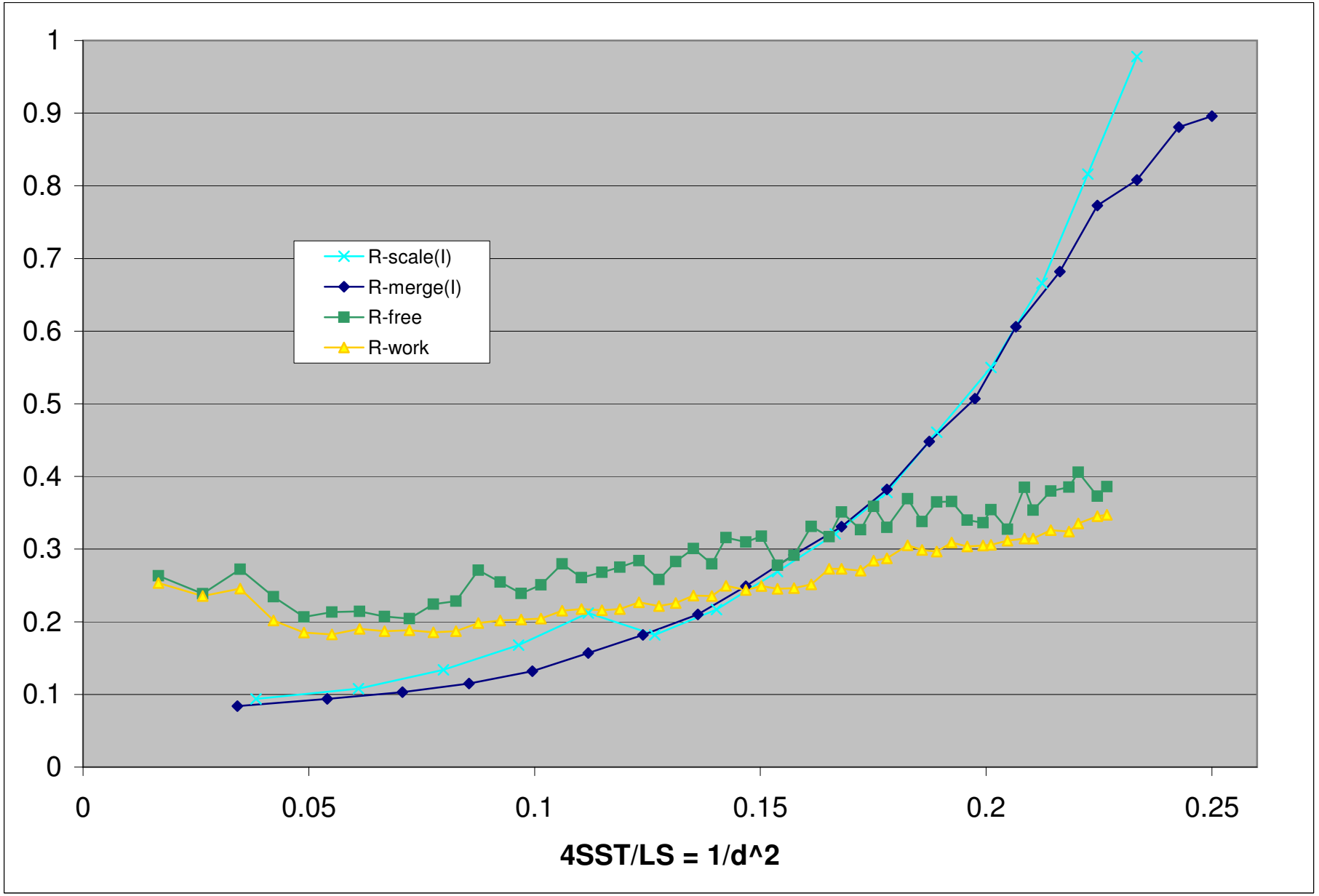
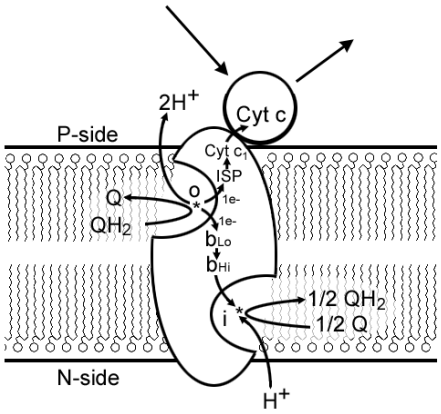


Supplemental Materials



Scheme S1. The protonmotive Q-cycle mechanism by which the cyt bc1 complex is believed to couple electron transfer to proton translocation. The horizontal band shaded with wavy lines represents the lipid bilayer, and the ellipse extending across the bilayer represents the bc_1 complex.

Oxidation of quinol or reduction of quinone results in release or uptake of protons. By arranging sequential oxidation and reduction steps to occur on opposite sides of the membrane, electron transport can be coupled to translocation of protons. If the ***bc₁*** complex simply oxidized quinone at the P-side of the membrane, one "scalar" proton would be released per electron passing through the complex to cytochrome ***c***. In the Q-cycle mechanism, quinol is oxidized at the P side of the membrane (in the Qo site, labeled "o"), but only one of the two electrons released is passed on to cytochrome ***c***. Thus two protons are released on the P side per electron passing through. The second electron is recycled back to the quinol pool by a reduction taking place in protonic equilibrium with the N-side aqueous phase (active site Q_i, labeled "i"), resulting in uptake of one proton per electron. This cycling of electrons from quinol back to quinol does not contribute to the driving force, but results in one proton being translocated from the N phase (normally low protonic potential) to the P phase (normally high protonic potential) and thus requires energy when the membrane is energized with the normal polarity. The energy is provided by the other electron, which passes on to cytochrome ***c*** and eventually to molecular oxygen in cytochrome oxidase. The overall stoichiometry is thus one proton translocated and one scalar proton released per electron, which is consistent with the experimentally determined stoichiometry of proton and charge translocation.

Notice that a single turnover of the Q_O site provides only one electron to the Qi site, while two electrons are required to reduce quinone to quinol. Although some early models proposed dismutation or input of an electron directly from a dehydrogenase to complete the reduction, it appears now that this site undergoes two non-equivalent single-electron reactions: first reducing quinone to a semiquinone and then, on the next turnover of the Qo site, reducing semiquinone to quinol. Thus the Qi site must bind semiquinone, quinone, and quinol at different stages of the catalytic cycle; and an inhibitor acting at the site might be expected to mimic any one of these three forms of the substrate.

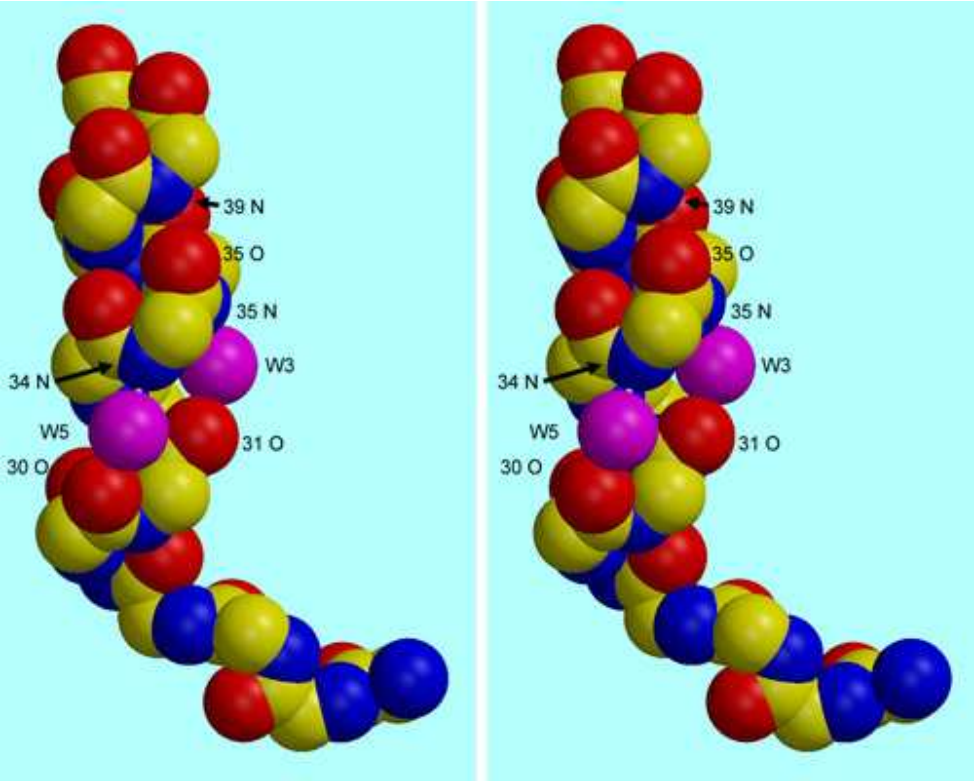


Figure S1. Helix-Intercalated Waters. Stereodiagram of the beginning of helix A of cyt b showing how the two "intercalated" waters fit into the secondary structure of the helix. Purple spheres labeled W3 and W5 are the waters, the other atoms are backbone atoms of cyt b (residues 25 to 39) with Molscript default colors (C, N, O yellow, blue, and red). Side-chain atoms have been removed for clarity. Notice the normal α-helical interaction between atoms 35 O and 39 N, and compare the relation of 31 O with 35 N or 30 O with 34 N.

Omit maps for critical features. Figures in the text show density maps made with phases calculated from the entire structure, as these phases are the most accurate if it is assumed the model is correct. Coefficients of 2Fo-Fc were used to minimize model bias, however there is still some model bias in such maps. As a more stringent test of the validity of critical parts of the model, Figures S2-S7 show features of the structure 1PPJ together with density from an omit map, in which those features were omitted to avoid model bias. Except in Figure S2, residues with atoms within 3 Å of the specified residues were also omitted. Before map calculation, the structure with omitted residues was subjected to simulated annealing from 1000K in steps of 25 K/cycle, followed by 100 steps of conjugate gradient minimization. No harmonic restraint was applied to the region around the omitted area during the refinement. The map coefficients were sigma-A weighted (2mFo-DFc).

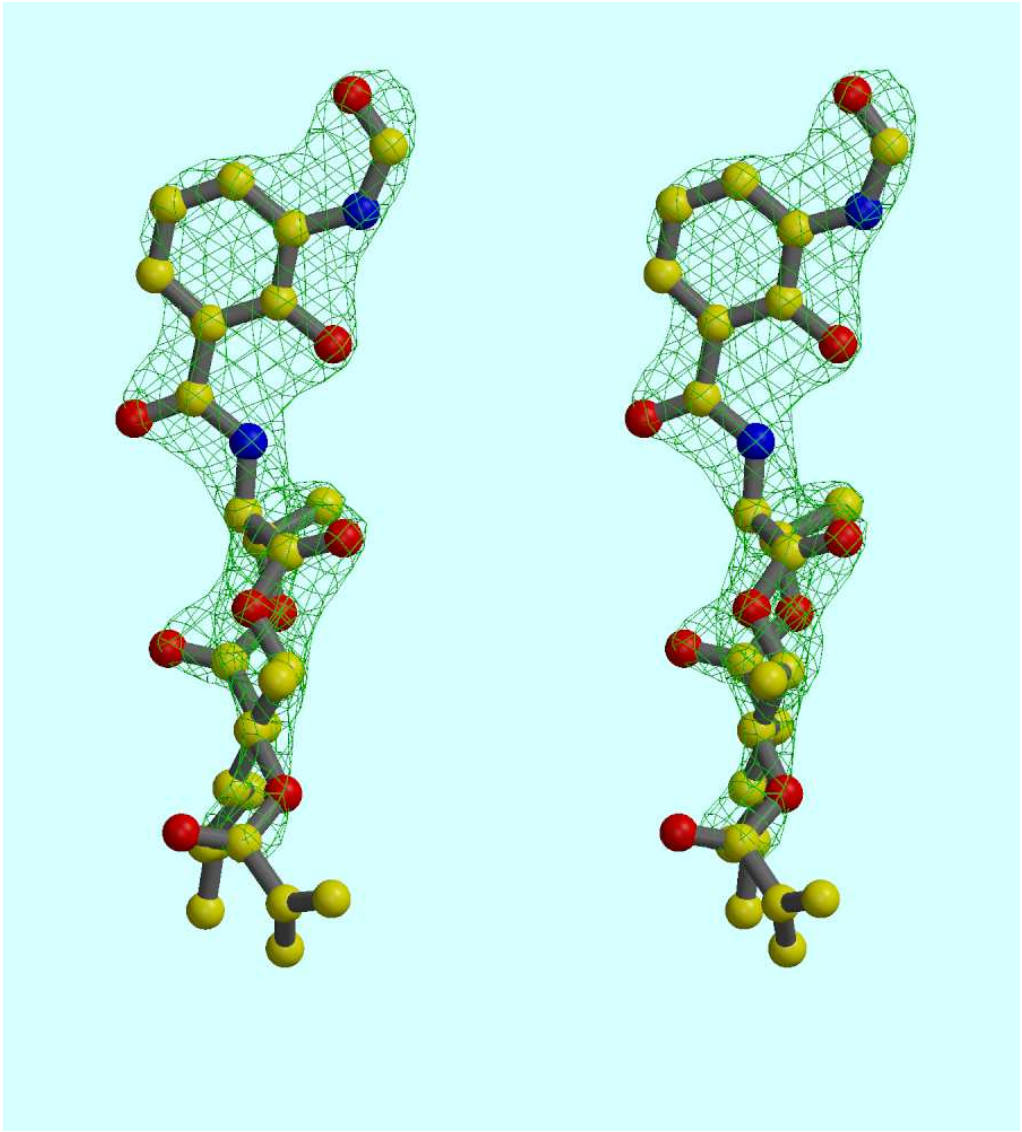


Figure S2. Stereo views of omit map density for antimycin in 1PPJ.
As Figure 1b except the map is a Sigma-A weighted simulated-annealing omit map with antimycin, Lys227, Asp228 and waters W1, W2, and W3 omitted from both monomers during annealing and map calculation. The map is calculated with coefficients 2mFo-DFc around antimycin bound in the C chain. Contour level 1.4 σ.

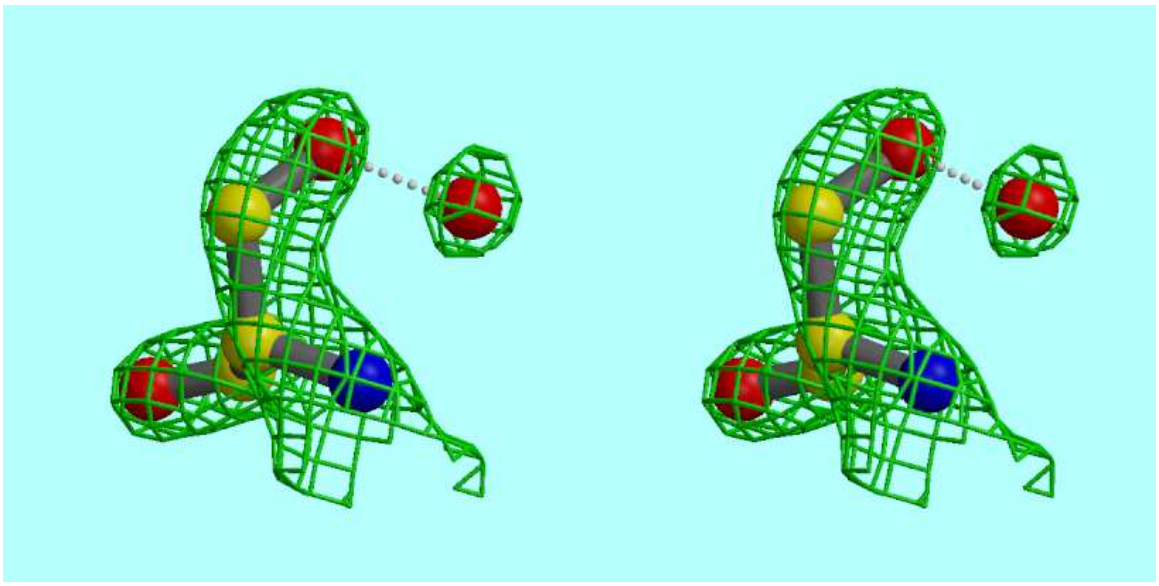


Figure S3. Omit map for C:Ser35. Map as in S2 except that C, P:Ser35 were omitted, together with residues within a 3Å sphere. Contour level 2.0 σ. The water molecule W is also shown.

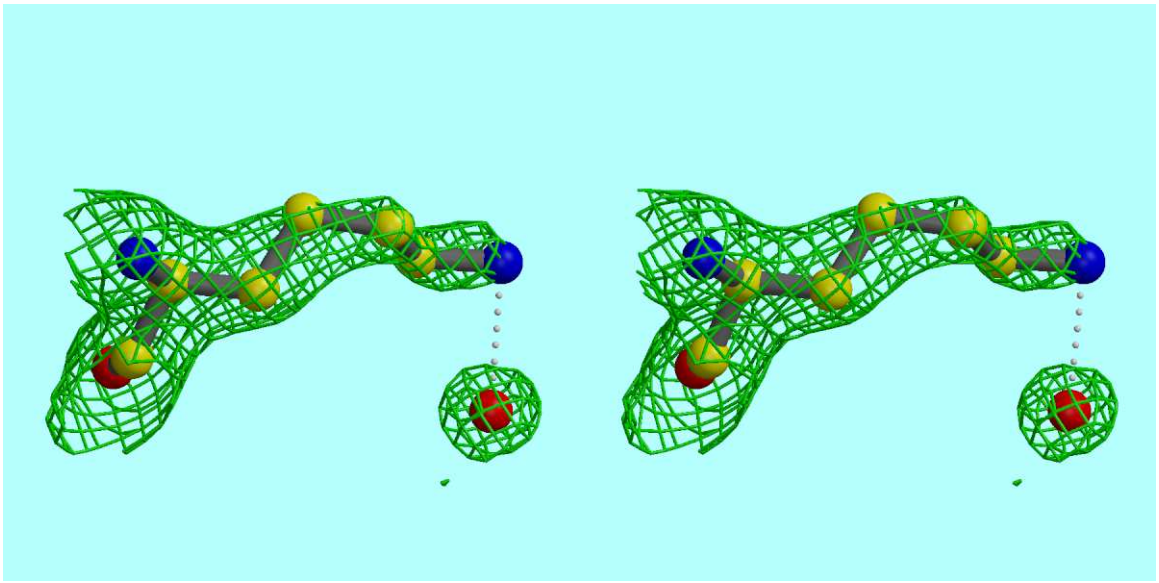


Figure S4. Omit map for P:Lys227. Map as in S3 except that C, P:Lys227 was omitted, together with residues within a ?Å sphere. Contour level 1.8 σ

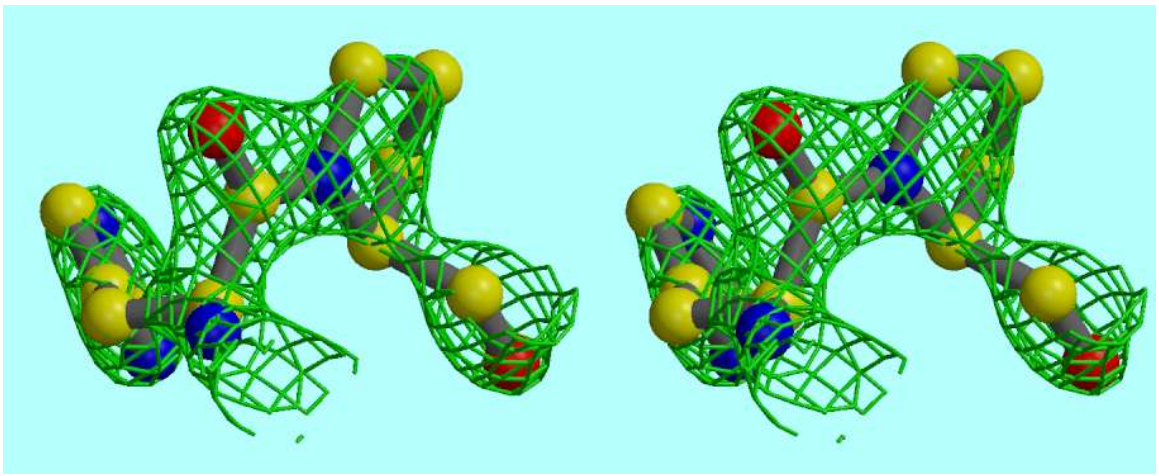


Figure S5. Omit map for C:His221-Pro222. Map calculated as in S3 except that C: and P:His221-Pro222 were omitted, Contour level 1.8 σ

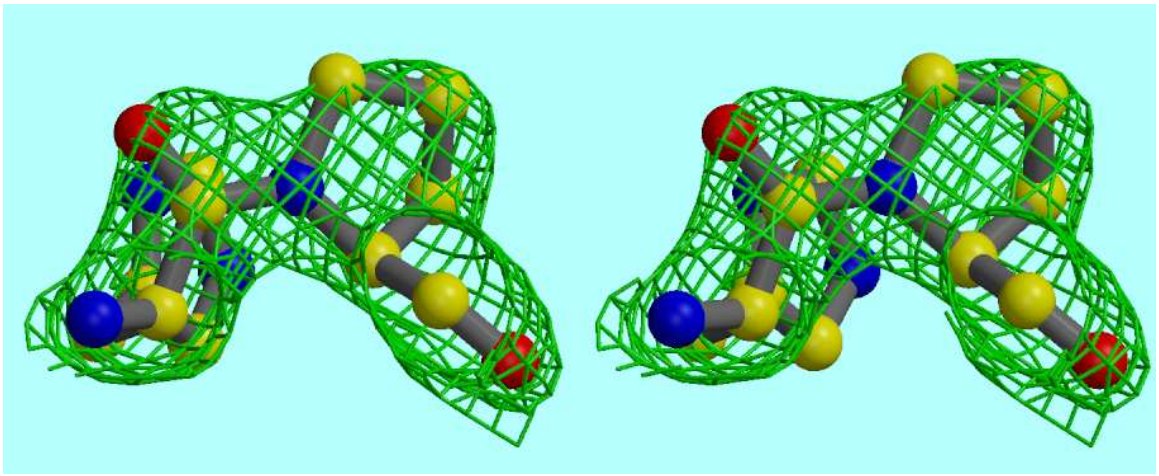


Figure S6. Omit map for C:His345-Pro346. Map calculated as in S3 except that C: and P:His345-Pro346 were omitted. Contour level 0.7 σ

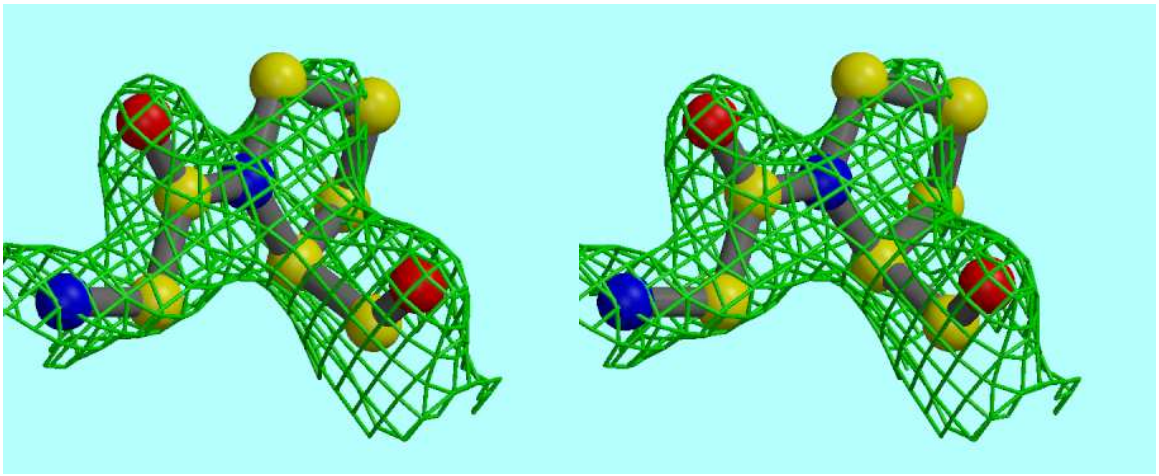


Figure S7. Omit map for D:Gly73-Pro74. Map calculated as in S3 except that D: and Q:Gly73-Pro74 were omitted. Contour level 1.6 σ

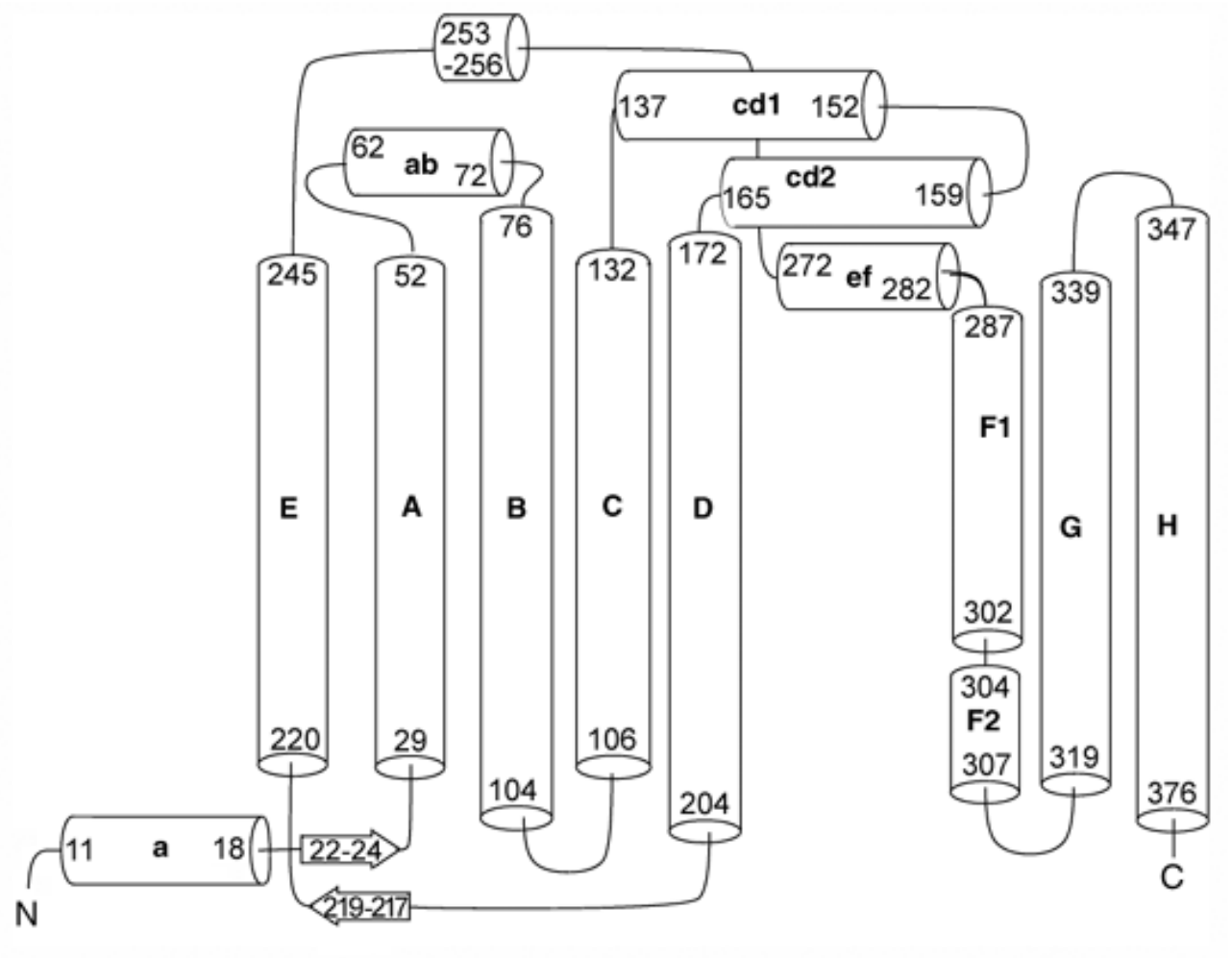


Figure S8. Secondary structure diagram of mitochondrial cytochrome b. (Modified from references [1; 2].) The residue-number assignments are based on analysis of the 1PPJ structure by the program DSSP [3]

Table S1. Definition of Rotamers referred to in the text.

residue	rotamer	Freq.[1]	chi1	chi2	chi3	chi4
Ser	1	45%	63			
	2	30%	-62			
His	3	16%	-169	80		
	5	8%	-59	169		
Lys	26[2]	3%	-66	180	67	180

[1]Freq: frequency with which the rotamer occurs in the population used to define the rotamers.

[2]The Lysine rotamer 26 seen in C:Lys227 is from the more extensive collection of ref. 77.

Stigmatellin binding- Stigmatellin binding has been reported from a number of cyt ***bc*$_1$** crystal structures [1; 4; 5] and coordinates have been deposited for two chicken ***bc*$_1$** structures (2BCC and 3BCC) and for all of the yeast ***bc*$_1$** structures (e.g. 1KB9). Coordinates have recently been deposited for a bovine structure containing stigmatellin (1SQX), however those coordinates are not available for comparison at the time of this writing. The two chicken structures had stigmatellin bound in opposite orientations. That of the later (2BCC) structure was confirmed by the higher-resolution yeast structures (1EZV and following), and by the structures described here.

The entire stigmatellin molecule is well ordered in the current structures, with all but two atoms (the methoxy carbon C5A and final carbon of the tail) covered by $2F_o$-F_c density at a contour level of 2.0 σ (Figure 2c). The stereochemistry of the 4 chiral centers and the planarity at the isoprenoid unit are clear, and are consistent with what is known from chemical investigations[6].

The current structures essentially confirm the binding mode reported in the yeast ***bc*$_1$** crystals except for some details of the "tail". The main polar interactions (Table 6) are an H-bond (2.80 Å) between the carbonyl oxygen O4 and His161 of the iron-sulfur protein and one (2.54 Å) between the ring OH group (O8) and the side chain of Glu271 in the conserved PEWY motif. As in the yeast structure there is a water molecule bridging between O8 and the second carboxylate oxygen of the PEWY glutamate. Another water molecule is bound between that second carboxylate oxygen, OH of the PEWY tyrosine, and the carbonyl oxygen of ALA127, stabilizing the position of the PEWY glutamate for its interaction with the $\mathbf{Q_o}$ site ligand and perhaps forming part of the pathway for release of protons upon oxidation of ubiquinol.

The other interactions (Table 6) appear to be hydrophobic and Van der Waals, although the interaction between Tyr278 and the O4 atom of stigmatellin may be a nonconventional H-bond as suggested by Palsdottir et al.[7] in the case of a related inhibitor. The tail of stigmatellin at the level of the first methoxy group passes through a hydrophobic orifice (between helices $\mathbf{cd_2}$, **C**, and **F**) into the bulk lipid phase, then bends sharply and lies against the outside surface. The hydrophobic orifice is bounded by residues Phe274, Met124, Ileu298, Ala277, Leu294, Leu149, Ile146, and Phe128 (going clockwise around when viewed from outside). On the outside, the tail lies on the surface between helices $\mathbf{cd_1}$ and **C**, contacting residues 128, 129, 146, 147, 178. The bent tail wraps around the side chain of Ile146 in the orifice, so that atoms Cγ1 and Cδ1 form part of the wall of the binding pocket for the head group, while Cγ2 forms part of the binding surface for the

external tail. Presumably the hydrophobic orifice is the entry point to the $\mathbf{Q_o}$ site for ubiquinone and hydrophobic inhibitors, and the size and shape of the orifice as much as the binding properties of the site determine what substrates and inhibitors can be accommodated.

Table S2. Residues surrounding stigmatellin at the Q_o site. For each residue is given the residue chain, type and number; the closest atom in the residue to stigmatellin, the closest atom of stigmatellin, and the distance between these two atoms. The residues are divided into the three areas discussed in the text.

Headgroup pocket:			Hydrophobic orifice:			Outside surface:		
protein residue	Stig. atom	dist-ance(Å)	protein residue	Stig. atom	dist-ance(Å)	protein residue	Stig. atom	dist-ance(Å)
R:HIS161 NE2	**O4	2.80	C:PHE274 CD1	C22	3.51	C:ALA125 N	O14	3.78
R:HIS161 NE2	O5	3.30	C:MET124 CB	O14	3.83	C:ILE146 CG2	C15	3.71
R:CYS160 CB	C5M	3.60	C:ILE146 CD1	C10	4.54	C:LEU121 O	C25	3.46
C:GLU271 OE1	**O8	2.54	C:ILE298 CG1	C23	4.09	C:PHE178 CE1	C21	4.00
HOH299 O	**O8	2.80	C:ALA277 CB	C22	3.82	C:MET129 CE	C21	3.68
C:TYR278 CD1	--O4	3.12	C:LEU294 CD2	O12	3.85	C:ILE164 CG2	C21	4.03
C:PRO270 CB	C8	3.55	C:LEU149 CD1	O12	4.69	C:PHE181 CD2	C21	4.21
C:VAL145 CG1	O5	3.46	C:PHE128 CE2	C18	3.82	C:THR147 OG1	C26	4.07
C:MET138 O	C7M	3.47				C:LEU150 CD1	C26	4.55
C:TRP141 C	C7M	4.51						
C:ILE268 CD1	C7M	3.99						
C:GLY142 CA	O7	3.64						
C:ILE146 CD1	O1	3.56						
C:LEU281 CB	C3M	4.50						

**Hydrogen bond

--Putative unconventional hydrogen bond[7].

1. Zhang, Z., Huang, L., Shulmeister, V. M., Chi, Y. I., Kim, K. K., Hung, L. W., Crofts, A. R., Berry, E. A. & Kim, S. H. (1998). Electron transfer by domain movement in cytochrome bc1. *Nature* 392, 677-84.
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3. Kabsch, W. & Sander, C. (1983). *BIOPOLYMERS* 22, 2577-2637.
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7. Palsdottir, H., Lojero, C. G., Trumpower, B. L. & Hunte, C. (2003). Structure of the Yeast Cytochrome bc1 Complex with a Hydroxyquinone Anion Qo Site Inhibitor Bound. *J Biol Chem* 278, 31303-31311.

Figures, Cross-eyed

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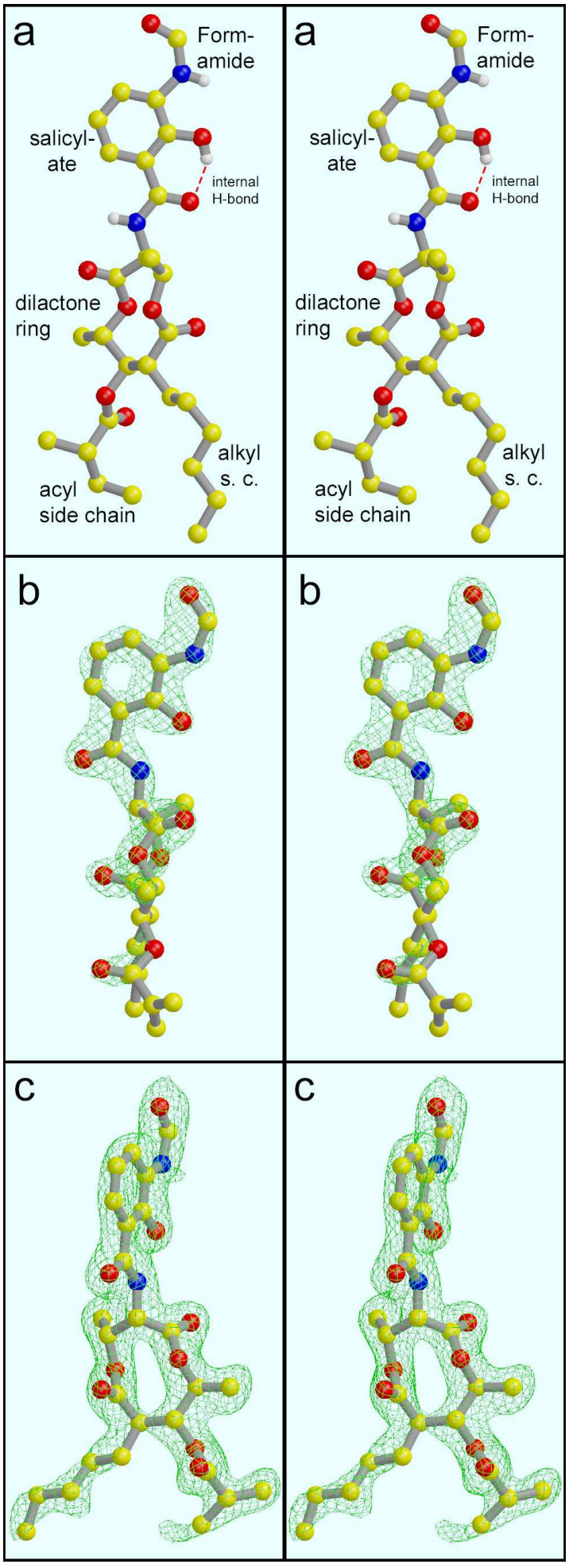


Figure 1
Huang et al.

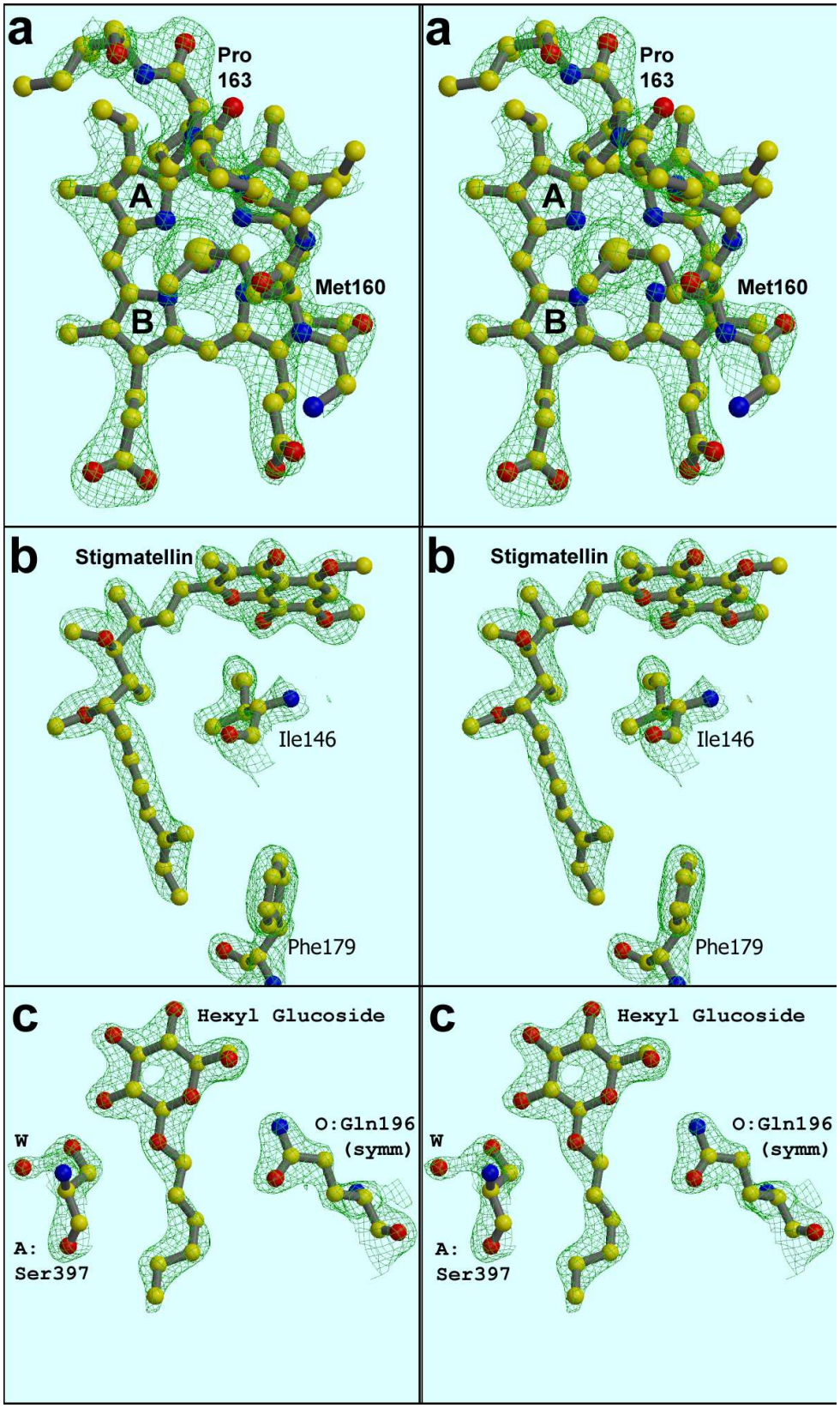


Figure 2
Huang et al.

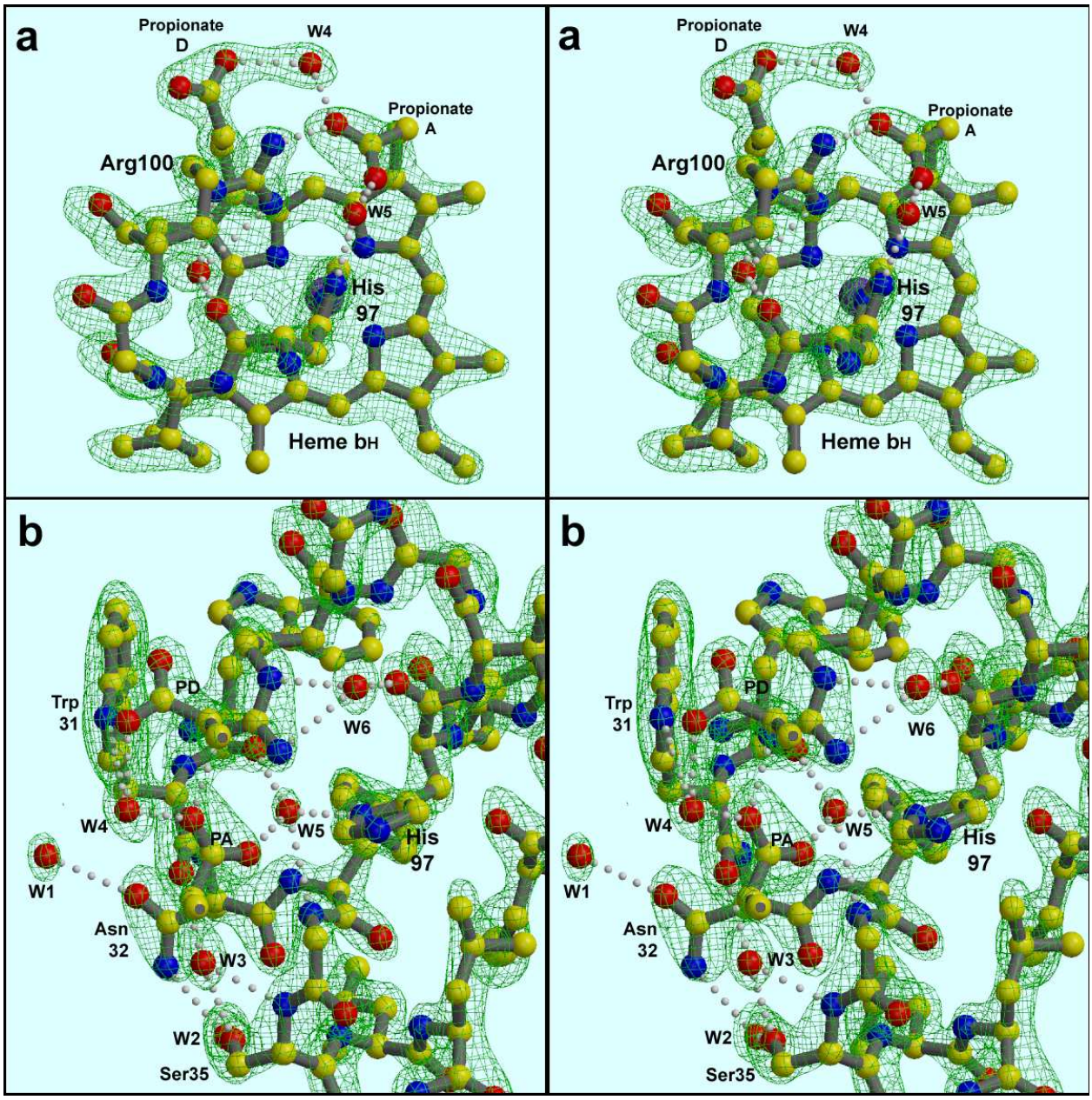


Figure 3
Huang et al.

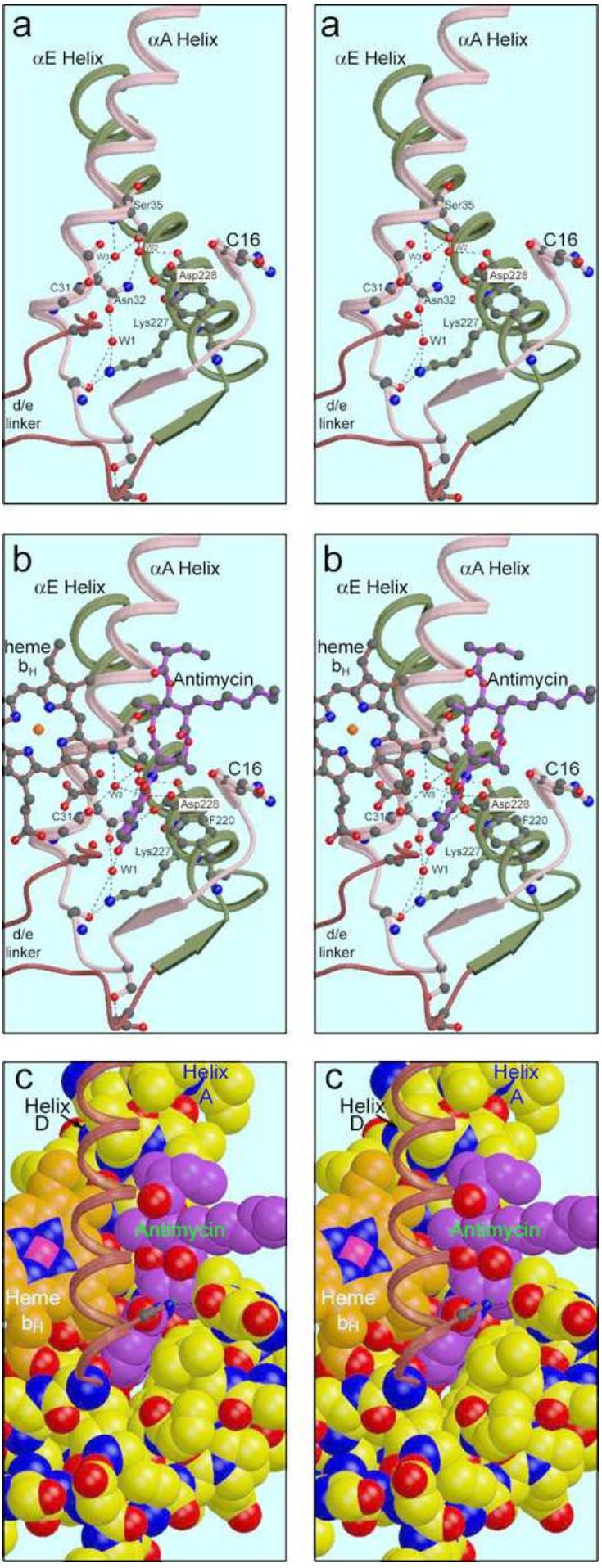


Figure 4.
Huang et al.

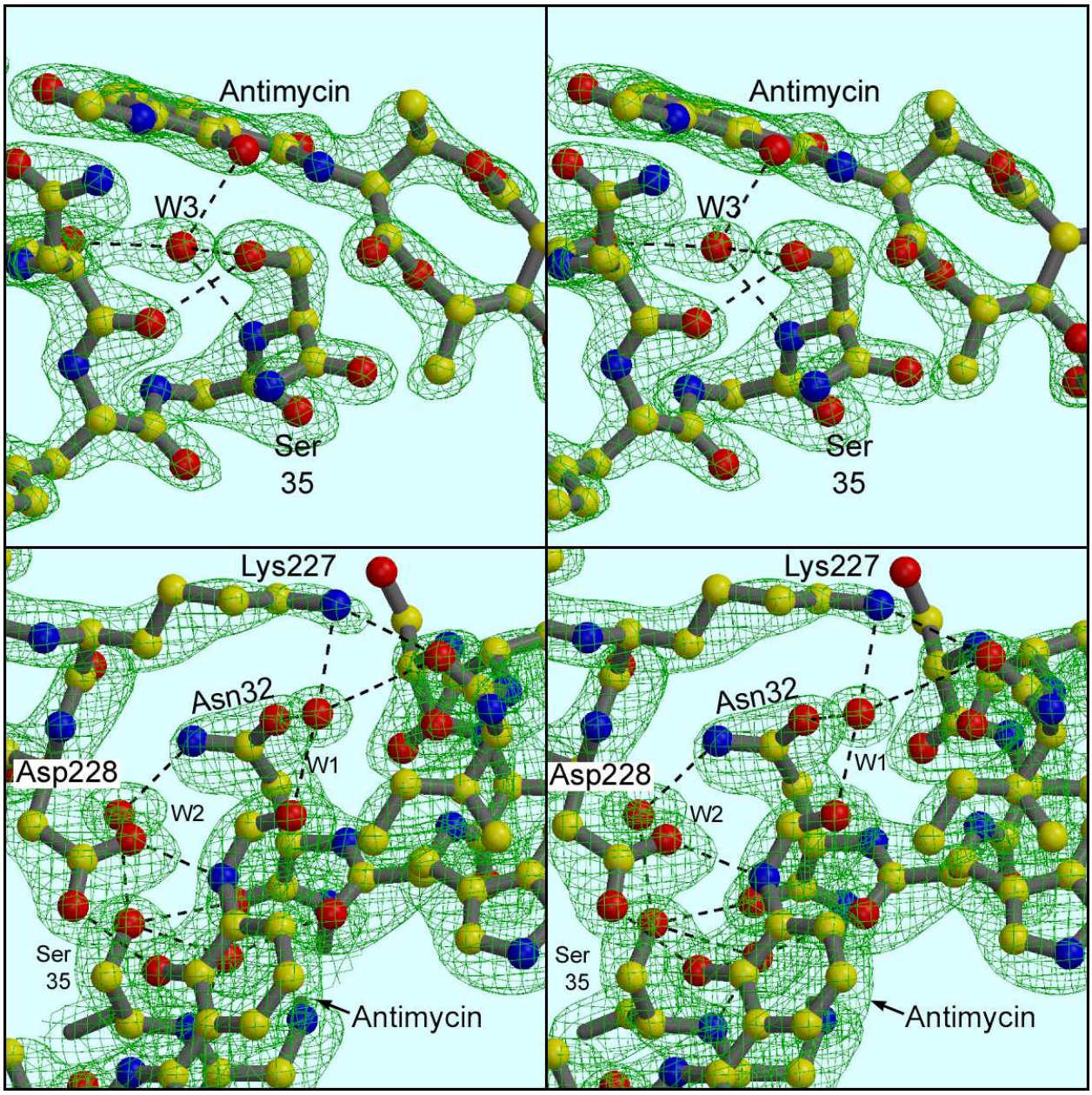


Figure 5
Huang et al.

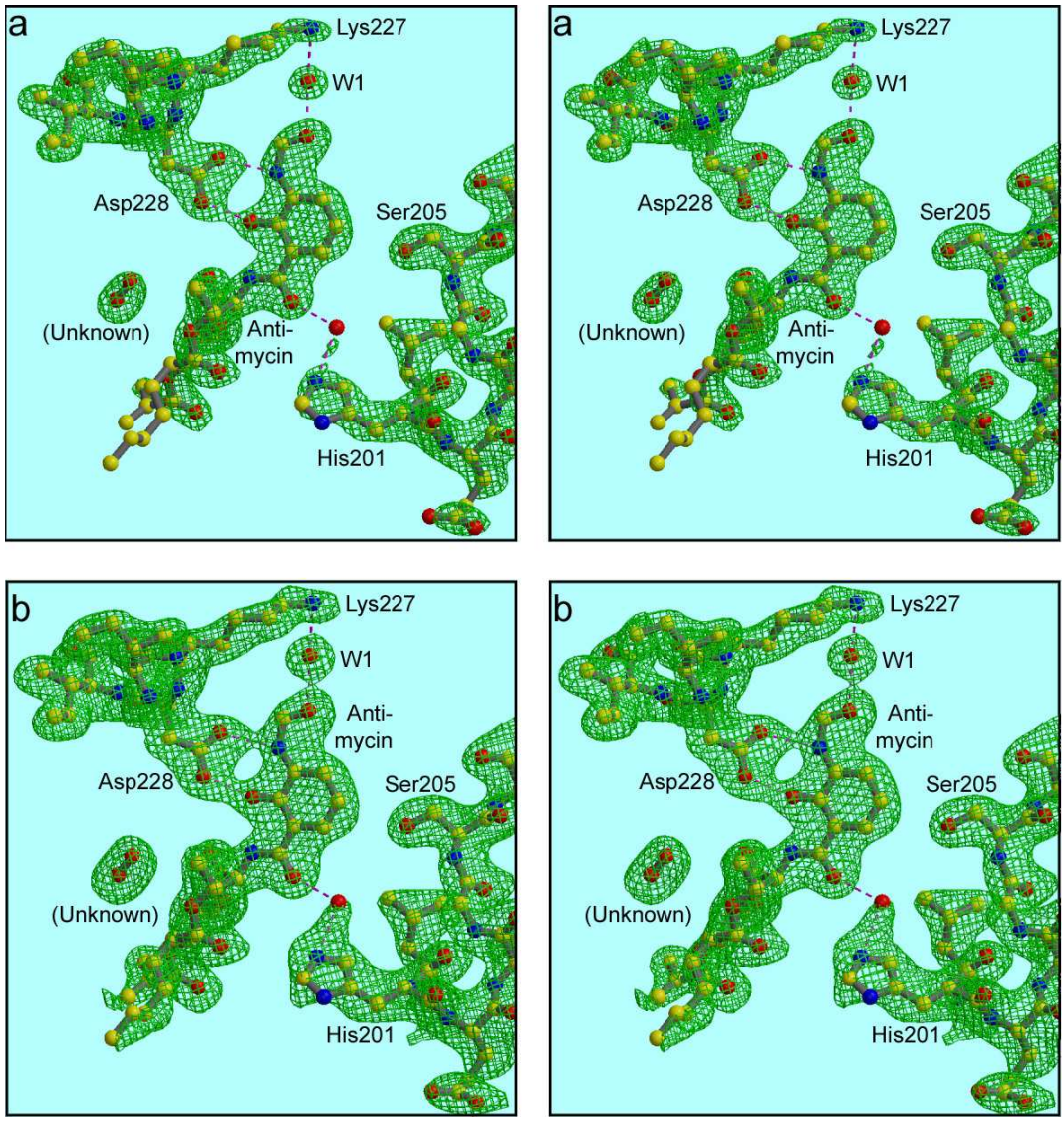


Figure 6
Huang et al.

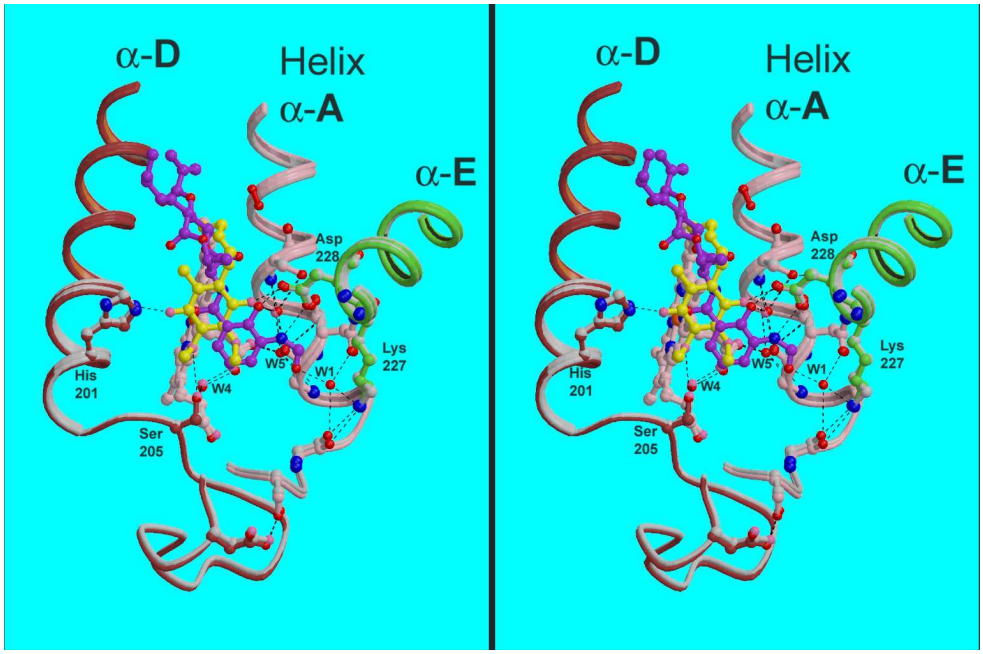


Figure 7
Huang et al.

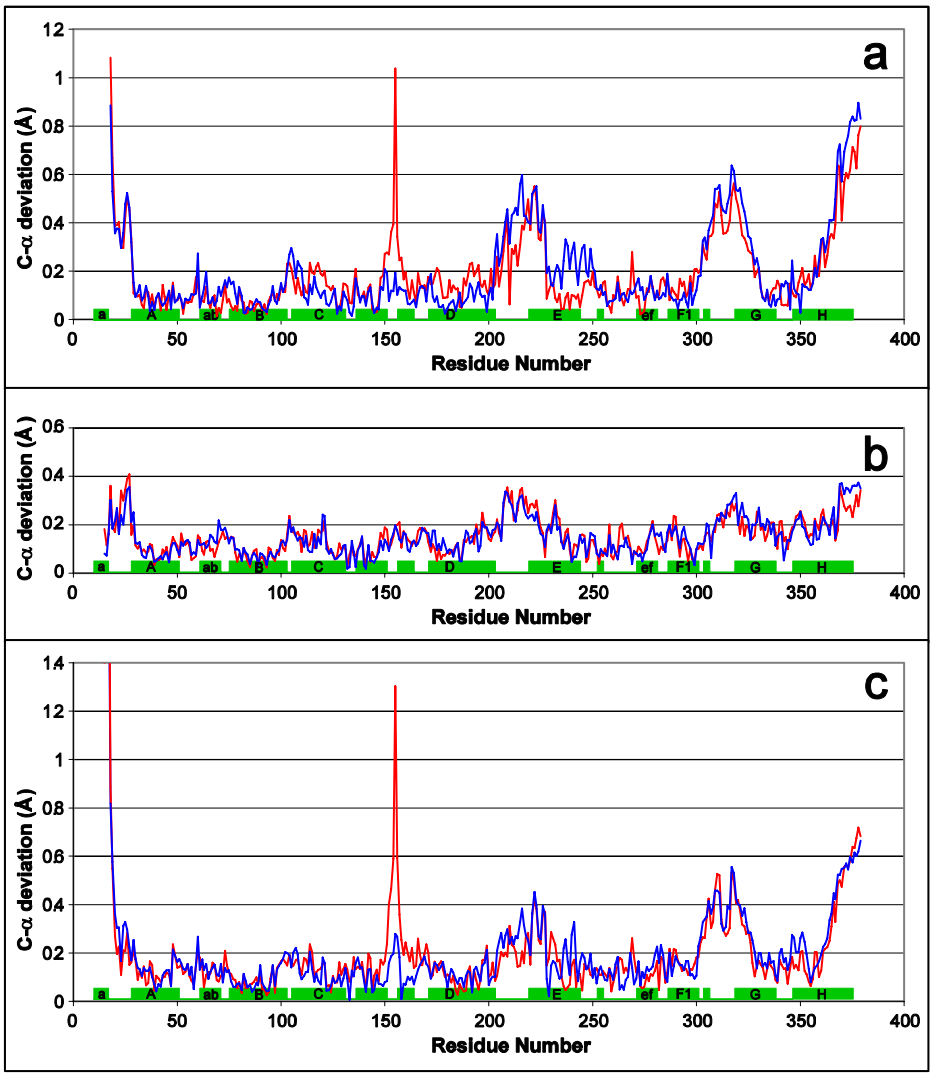
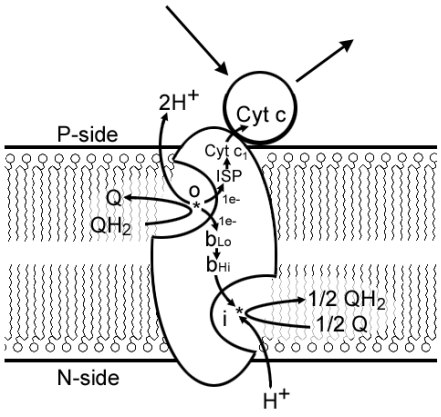


Figure 8
Huang et al.

Supplemental Materials



Scheme S1. The protonmotive Q-cycle mechanism by which the cyt bc1 complex is believed to couple electron transfer to proton translocation. The horizontal band shaded with wavy lines represents the lipid bilayer, and the ellipse extending across the bilayer represents the bc_1 complex.

Oxidation of quinol or reduction of quinone results in release or uptake of protons. By arranging sequential oxidation and reduction steps to occur on opposite sides of the membrane, electron transport can be coupled to translocation of protons. If the ***bc₁*** complex simply oxidized quinone at the P-side of the membrane, one "scalar" proton would be released per electron passing through the complex to cytochrome ***c***. In the Q-cycle mechanism, quinol is oxidized at the P side of the membrane (in the Qo site, labeled "o"), but only one of the two electrons released is passed on to cytochrome ***c***. Thus two protons are released on the P side per electron passing through. The second electron is recycled back to the quinol pool by a reduction taking place in protonic equilibrium with the N-side aqueous phase (active site Q_i, labeled "i"), resulting in uptake of one proton per electron. This cycling of electrons from quinol back to quinol does not contribute to the driving force, but results in one proton being translocated from the N phase (normally low protonic potential) to the P phase (normally high protonic potential) and thus requires energy when the membrane is energized with the normal polarity. The energy is provided by the other electron, which passes on to cytochrome ***c*** and eventually to molecular oxygen in cytochrome oxidase. The overall stoichiometry is thus one proton translocated and one scalar proton released per electron, which is consistent with the experimentally determined stoichiometry of proton and charge translocation.

Notice that a single turnover of the Q_O site provides only one electron to the Qi site, while two electrons are required to reduce quinone to quinol. Although some early models proposed dismutation or input of an electron directly from a dehydrogenase to complete the reduction, it appears now that this site undergoes two non-equivalent single-electron reactions: first reducing quinone to a semiquinone and then, on the next turnover of the Qo site, reducing semiquinone to quinol. Thus the Qi site must bind semiquinone, quinone, and quinol at different stages of the catalytic cycle; and an inhibitor acting at the site might be expected to mimic any one of these three forms of the substrate.

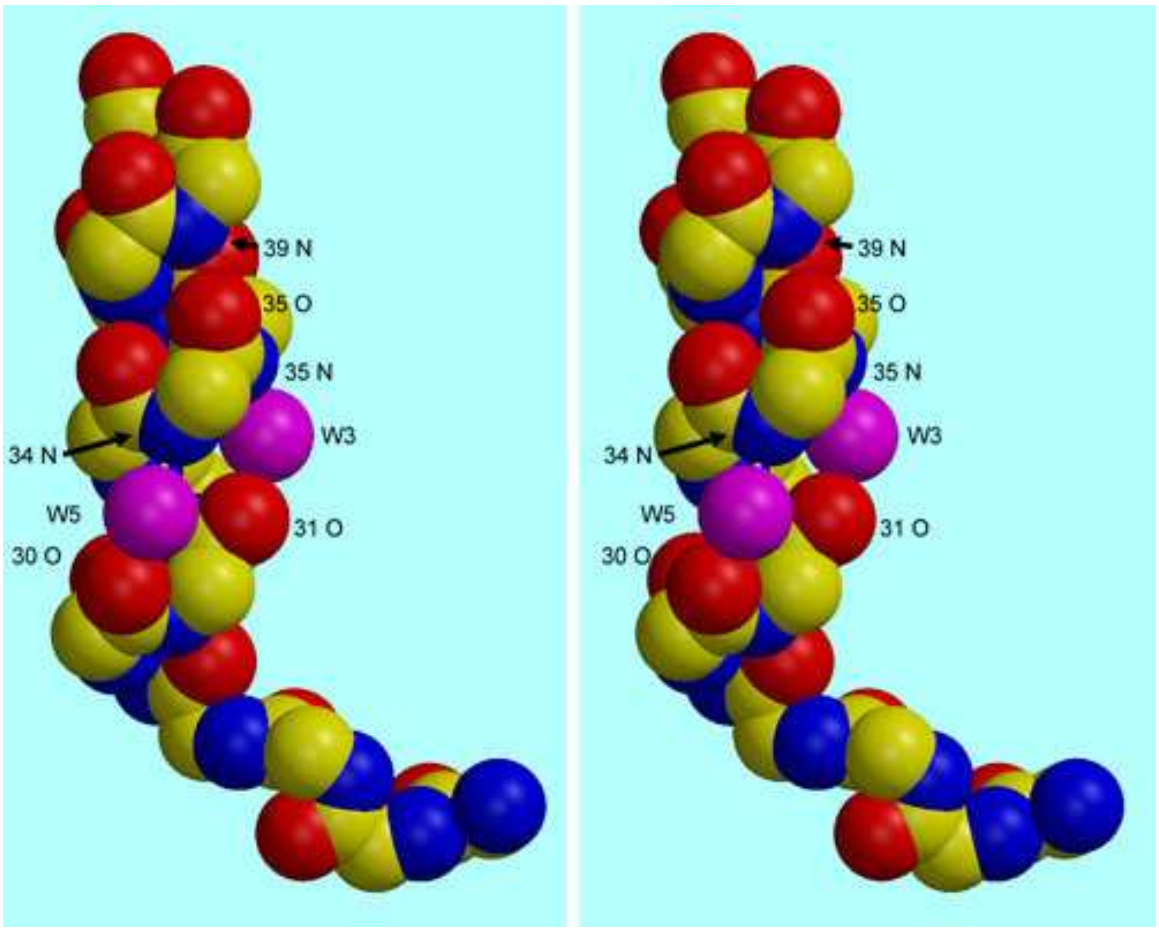


Figure S1. Helix-Intercolated Waters. Stereodiagram of the beginning of helix A of cyt b showing how the two "intercolated" waters fit into the secondary structure of the helix. Purple spheres labeled W3 and W5 are the waters, the other atoms are backbone atoms of cyt b (residues 25 to 39) with Molscript default colors (C, N, O yellow, blue, and red). Side-chain atoms have been removed for clarity. Notice the normal α-helical interaction between atoms 35 O and 39 N, and compare the relation of 31 O with 35 N or 30 O with 34 N.

Omit maps for critical features. Figures in the text show density maps made with phases calculated from the entire structure, as these phases are the most accurate if it is assumed the model is correct. Coefficients of 2Fo-Fc were used to minimize model bias, however there is still some model bias in such maps. As a more stringent test of the validity of critical parts of the model, Figures S2-S7 show features of the structure 1PPJ together with density from an omit map, in which those features were omitted to avoid model bias. Except in Figure S2, residues with atoms withing 3 Å of the specified residues were also omitted. Before map calculation, the structure with omitted residues was subjected to simulated anealing from 1000K in steps of 25 K/cycle, followed by 100 steps of conjugate gradient minimization. No harmonic restraint was applied to the region around the omitted area during the refinement. The map coefficients were sigma-A weighted (2mFo-DFc).

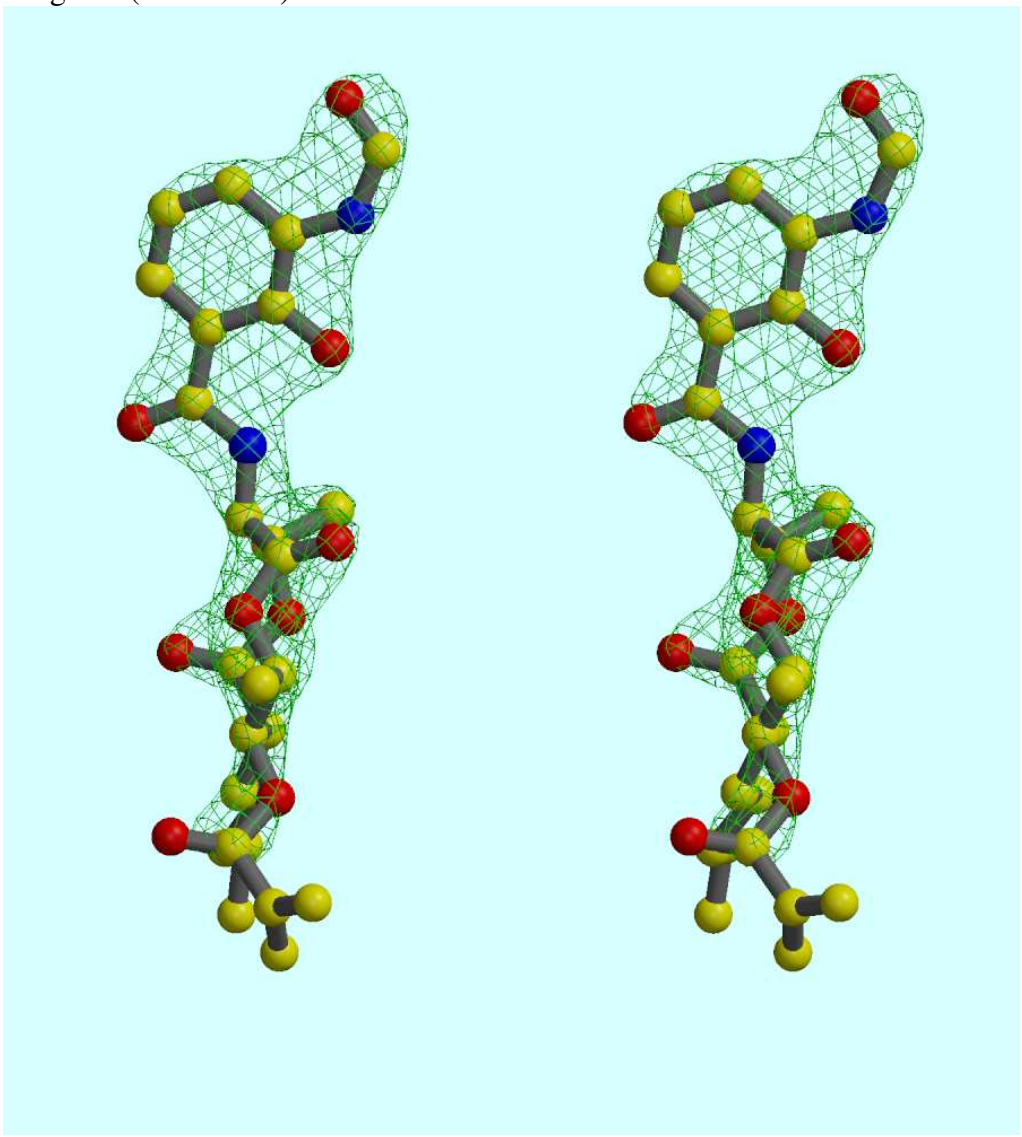


Figure S2. Stereo views of omit map density for antimycin in 1PPJ.
As Figure 1b except the map is a Sigma-A weighted simulated-anealing omit map with coefficients . Contour level 1.4 σ

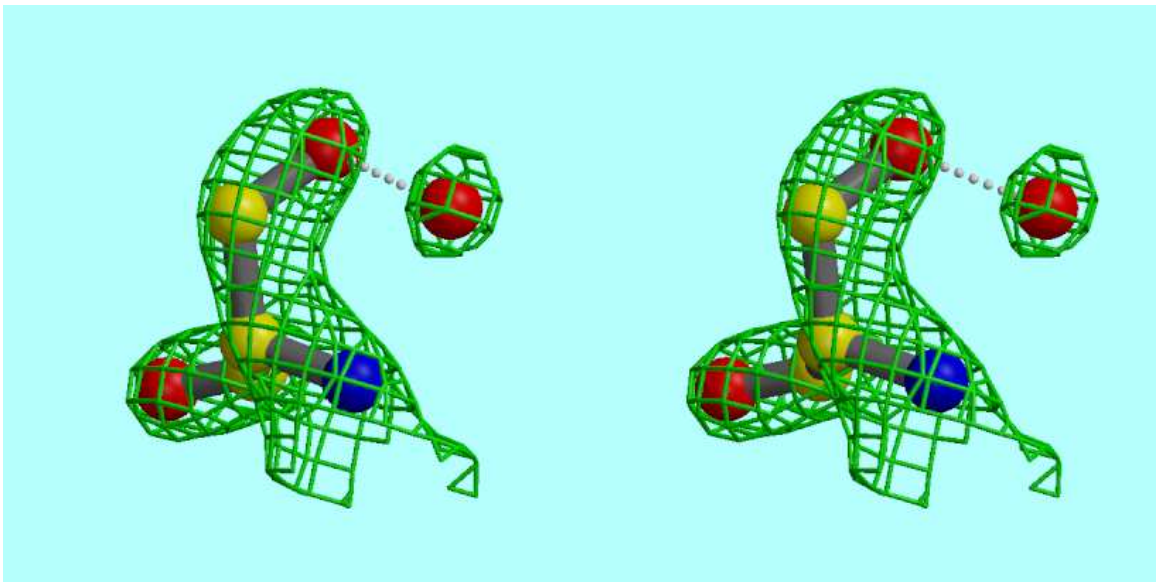


Figure S3. Omit map for C:Ser35. Map as in S2 except that C and P:Ser35 were omitted, together with residues within a 3Å sphere. Contour level 2.0 σ. The water molecule W is also included.

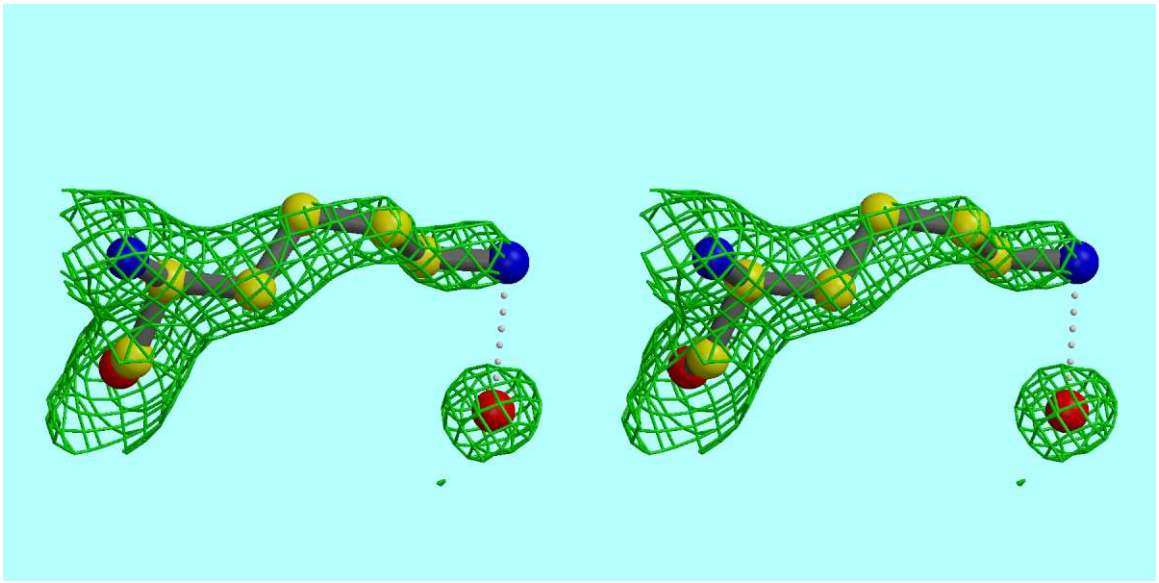


Figure S4. Omit map for P:Lys227. Map as in S3 except that C and P:Lys227 was omitted, together with residues within a ?Å sphere. Contour level 1.8 σ

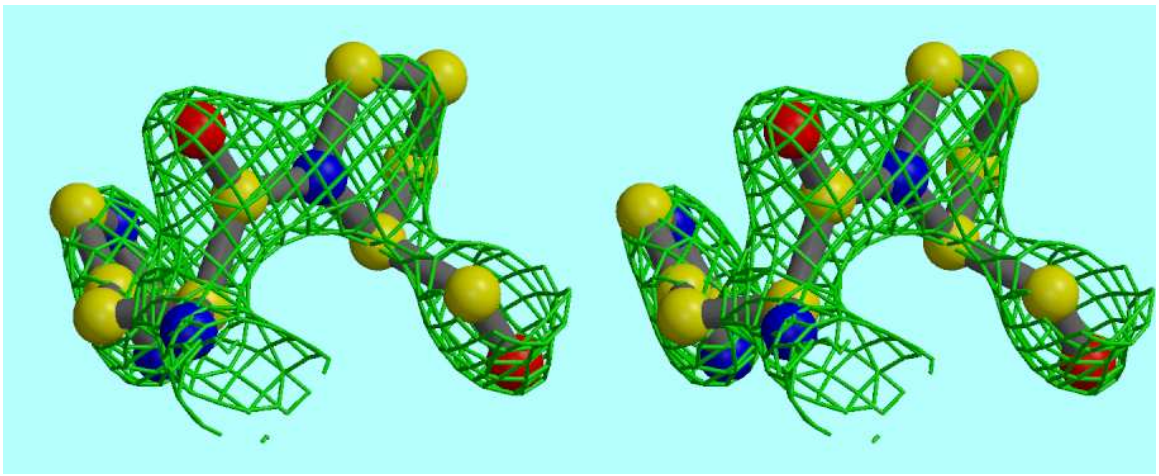


Figure S5. Omit map for C:His221-Pro222. Map calculated as in S3 except that C: and P:His221-Pro222 were omitted, Contour level 1.8 σ

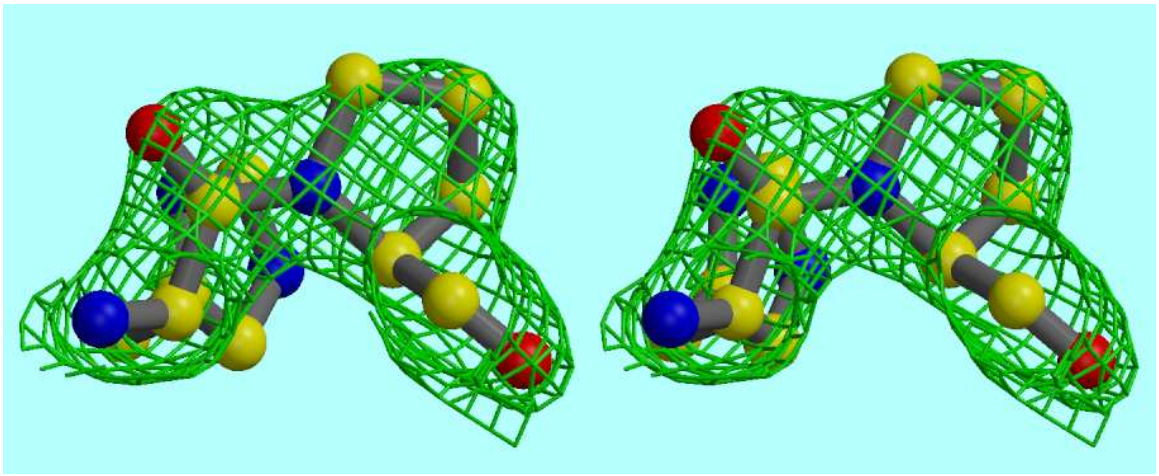


Figure S6. Omit map for C:His345-Pro346. Map calculated as in S3 except that C: and P:His345-Pro346 were omitted. Contour level 0.7 σ

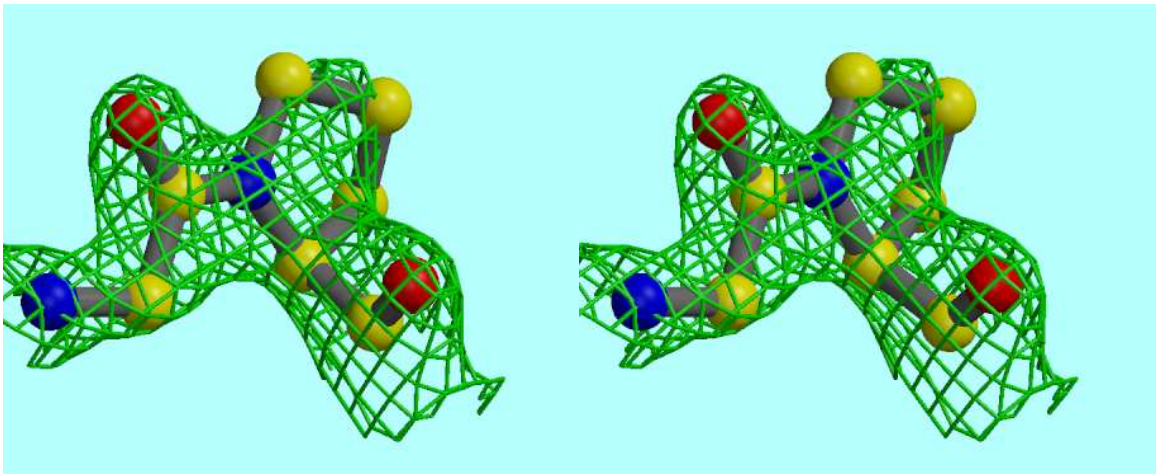


Figure S7. Omit map for D:Gly73-Pro74. Map calculated as in S3 except that D: and Q:Gly73-Pro74 were omitted. Contour level 1.6 σ

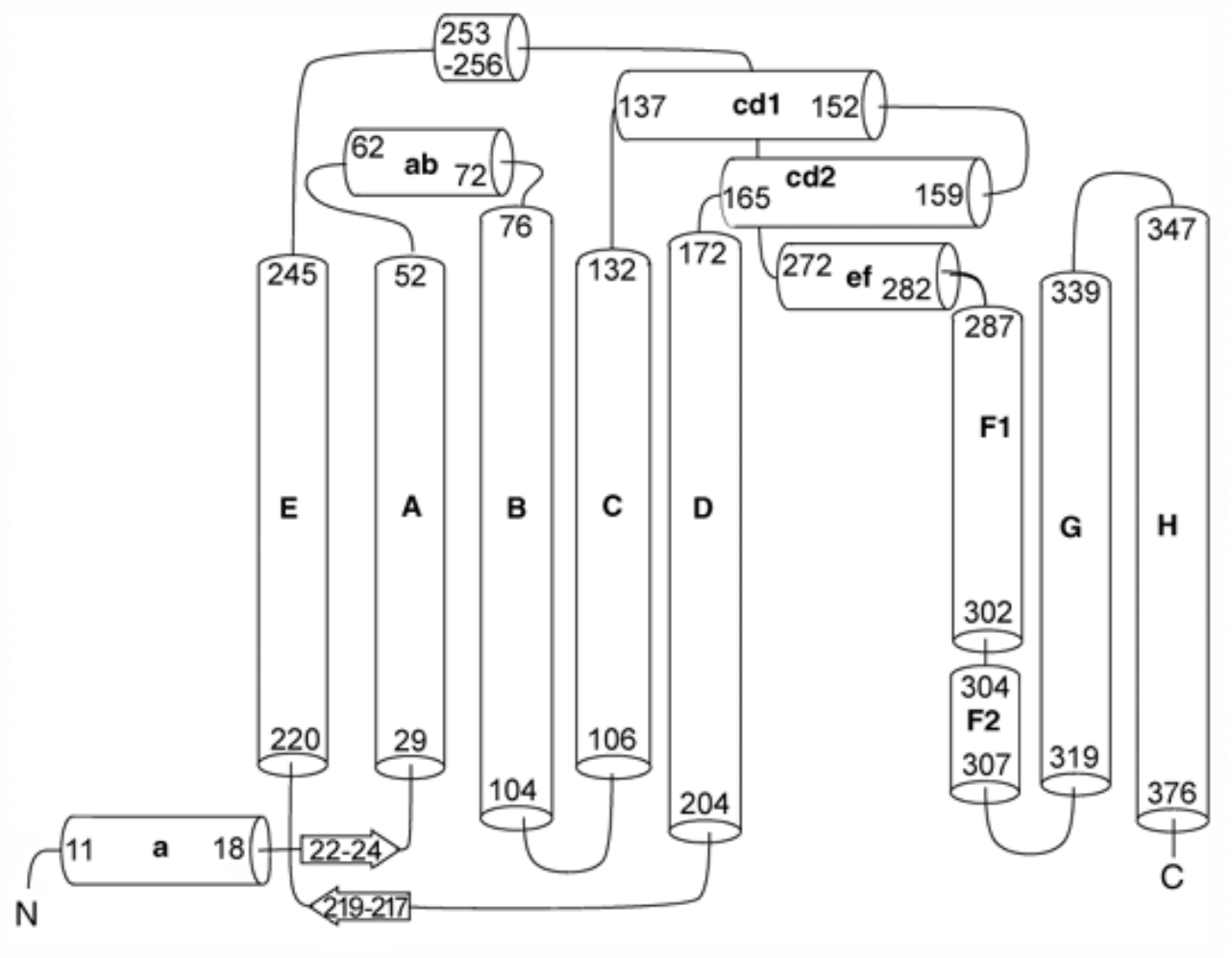


Figure S8. Secondary structure diagram of mitochondrial cytochrome b. (Modified from references [1; 2].) The residue-number assignments are based on analysis of the 1PPJ structure by the program DSSP [3]

Table S1. Definition of Rotamers referred to in the text.

residue	rotamer	Freq.[1]	chi1	chi2	chi3	chi4
Ser	1	45%	63			
	2	30%	-62			
His	3	16%	-169	80		
	5	8%	-59	169		
Lys	26[2]	3%	-66	180	67	180

[1]Freq: frequency with which the rotamer occurs in the population used to define the rotamers.

[2]The Lysine rotamer 26 seen in C:Lys227 is from the more extensive collection of ref. 77.

Stigmatellin binding- Stigmatellin binding has been reported from a number of cyt ***bc*$_1$** crystal structures [1; 4; 5] and coordinates have been deposited for two chicken ***bc*$_1$** structures (2BCC and 3BCC) and for all of the yeast ***bc*$_1$** structures (e.g. 1KB9). Coordinates have recently been deposited for a bovine structure containing stigmatellin (1SQX), however those coordinates are not available for comparison at the time of this writing. The two chicken structures had stigmatellin bound in opposite orientations. That of the later (2BCC) structure was confirmed by the higher-resolution yeast structures (1EZV and following), and by the structures described here.

The entire stigmatellin molecule is well ordered in the current structures, with all but two atoms (the methoxy carbon C5A and final carbon of the tail) covered by $2F_o$-F_c density at a contour level of 2.0 σ (Figure 2c). The stereochemistry of the 4 chiral centers and the planarity at the isoprenoid unit are clear, and are consistent with what is known from chemical investigations[6].

The current structures essentially confirm the binding mode reported in the yeast ***bc*$_1$** crystals except for some details of the "tail". The main polar interactions (Table 6) are an H-bond (2.80 Å) between the carbonyl oxygen O4 and His161 of the iron-sulfur protein and one (2.54 Å) between the ring OH group (O8) and the side chain of Glu271 in the conserved PEWY motif. As in the yeast structure there is a water molecule bridging between O8 and the second carboxylate oxygen of the PEWY glutamate. Another water molecule is bound between that second carboxylate oxygen, OH of the PEWY tyrosine, and the carbonyl oxygen of ALA127, stabilizing the position of the PEWY glutamate for its interaction with the $\mathbf{Q_o}$ site ligand and perhaps forming part of the pathway for release of protons upon oxidation of ubiquinol.

The other interactions (Table 6) appear to be hydrophobic and Van der Waals, although the interaction between Tyr278 and the O4 atom of stigmatellin may be a nonconventional H-bond as suggested by Palsdottir et al.[7] in the case of a related inhibitor. The tail of stigmatellin at the level of the first methoxy group passes through a hydrophobic orifice (between helices $\mathbf{cd_2}$, **C**, and **F**) into the bulk lipid phase, then bends sharply and lies against the outside surface. The hydrophobic orifice is bounded by residues Phe274, Met124, Ileu298, Ala277, Leu294, Leu149, Ile146, and Phe128 (going clockwise around when viewed from outside). On the outside, the tail lies on the surface between helices $\mathbf{cd_1}$ and **C**, contacting residues 128, 129, 146, 147, 178. The bent tail wraps around the side chain of Ile146 in the orifice, so that atoms Cγ1 and Cδ1 form part of the wall of the binding pocket for the head group, while Cγ2 forms part of the binding surface for the

external tail. Presumably the hydrophobic orifice is the entry point to the $\mathbf{Q_o}$ site for ubiquinone and hydrophobic inhibitors, and the size and shape of the orifice as much as the binding properties of the site determine what substrates and inhibitors can be accommodated.

Table S2. Residues surrounding stigmatellin at the Q_o site. For each residue is given the residue chain, type and number; the closest atom in the residue to stigmatellin, the closest atom of stigmatellin, and the distance between these two atoms. The residues are divided into the three areas discussed in the text.

Headgroup pocket:			Hydrophobic orifice:			Outside surface:		
protein residue	Stig. atom	dist-ance(Å)	protein residue	Stig. atom	dist-ance(Å)	protein residue	Stig. atom	dist-ance(Å)
R:HIS161 NE2	**O4	2.80	C:PHE274 CD1	C22	3.51	C:ALA125 N	O14	3.78
R:HIS161 NE2	O5	3.30	C:MET124 CB	O14	3.83	C:ILE146 CG2	C15	3.71
R:CYS160 CB	C5M	3.60	C:ILE146 CD1	C10	4.54	C:LEU121 O	C25	3.46
C:GLU271 OE1	**O8	2.54	C:ILE298 CG1	C23	4.09	C:PHE178 CE1	C21	4.00
HOH299 O	**O8	2.80	C:ALA277 CB	C22	3.82	C:MET129 CE	C21	3.68
C:TYR278 CD1	--O4	3.12	C:LEU294 CD2	O12	3.85	C:ILE164 CG2	C21	4.03
C:PRO270 CB	C8	3.55	C:LEU149 CD1	O12	4.69	C:PHE181 CD2	C21	4.21
C:VAL145 CG1	O5	3.46	C:PHE128 CE2	C18	3.82	C:THR147 OG1	C26	4.07
C:MET138 O	C7M	3.47				C:LEU150 CD1	C26	4.55
C:TRP141 C	C7M	4.51						
C:ILE268 CD1	C7M	3.99						
C:GLY142 CA	O7	3.64						
C:ILE146 CD1	O1	3.56						
C:LEU281 CB	C3M	4.50						

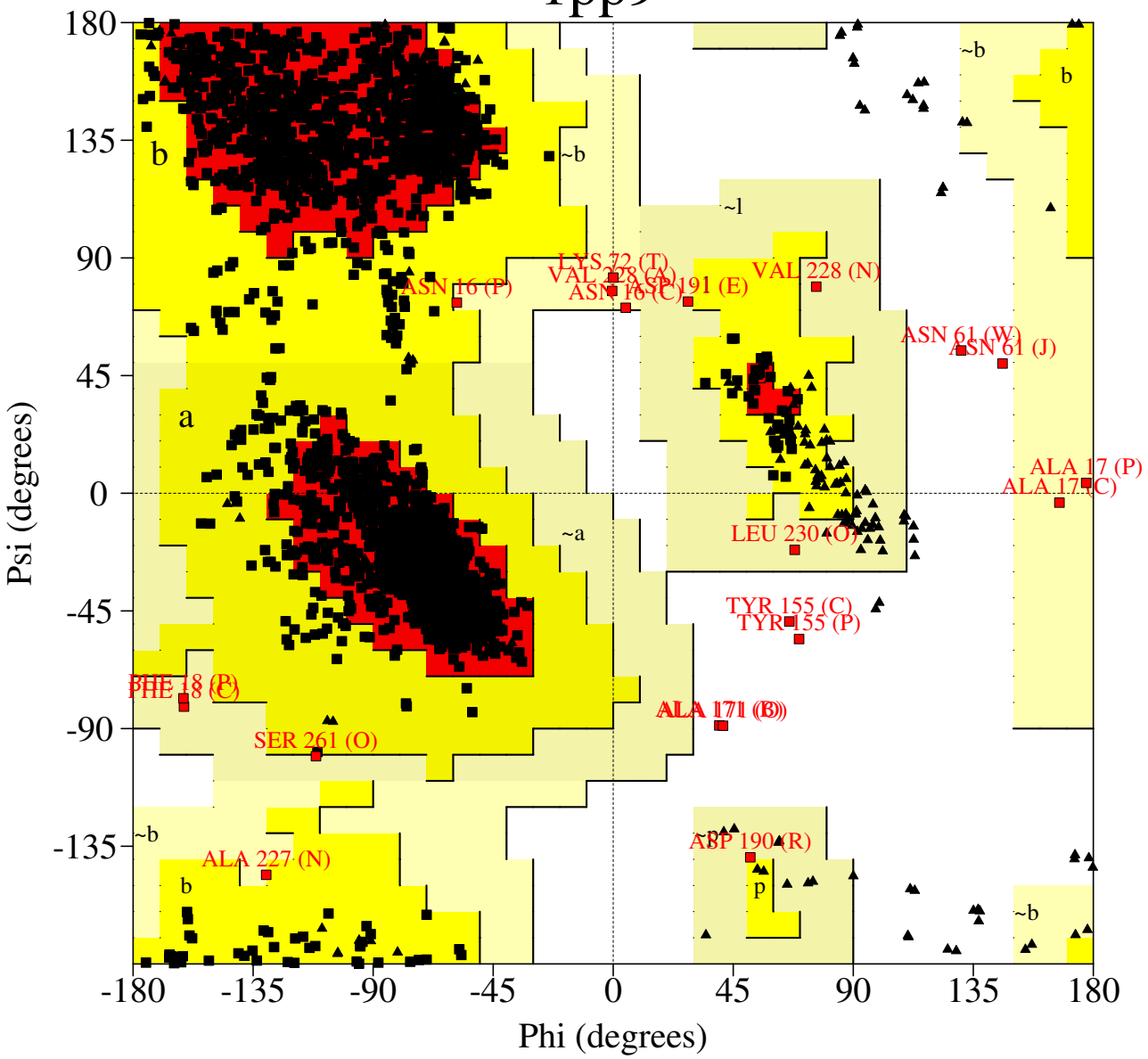
**Hydrogen bond

--Putative unconventional hydrogen bond[7].

1. Zhang, Z., Huang, L., Shulmeister, V. M., Chi, Y. I., Kim, K. K., Hung, L. W., Crofts, A. R., Berry, E. A. & Kim, S. H. (1998). Electron transfer by domain movement in cytochrome bc1. *Nature* 392, 677-84.
2. Berry, E. A., Guergova-Kuras, M., Huang, L. S. & Crofts, A. R. (2000). Structure and function of cytochrome bc complexes. *Annu Rev Biochem* 69, 1005-75.
3. Kabsch, W. & Sander, C. (1983). *BIOPOLYMERS* 22, 2577-2637.
4. Kim, H., Xia, D., Yu, C. A., Xia, J. Z., Kachurin, A. M., Zhang, L., Yu, L. & Deisenhofer, J. (1998). Inhibitor binding changes domain mobility in the iron-sulfur protein of the mitochondrial bc1 complex from bovine heart. *Proc Natl Acad Sci U S A* 95, 8026-33.
5. Hunte, C., Koepke, J., Lange, C., Rossmanith, T. & Michel, H. (2000). Structure at 2.3 A resolution of the cytochrome bc(1) complex from the yeast Saccharomyces cerevisiae co-crystallized with an antibody Fv fragment. *Structure Fold Des* 8, 669-84.
6. Enders, D., Geibel, G. & Osborne, S. (2000). Diastereo- and enantioselective total synthesis of stigmatellin A. *Chemistry* 6, 1302-9.
7. Palsdottir, H., Lojero, C. G., Trumpower, B. L. & Hunte, C. (2003). Structure of the Yeast Cytochrome bc1 Complex with a Hydroxyquinone Anion Qo Site Inhibitor Bound. *J Biol Chem* 278, 31303-31311.

Ramachandran Plot

1pp9



Plot statistics

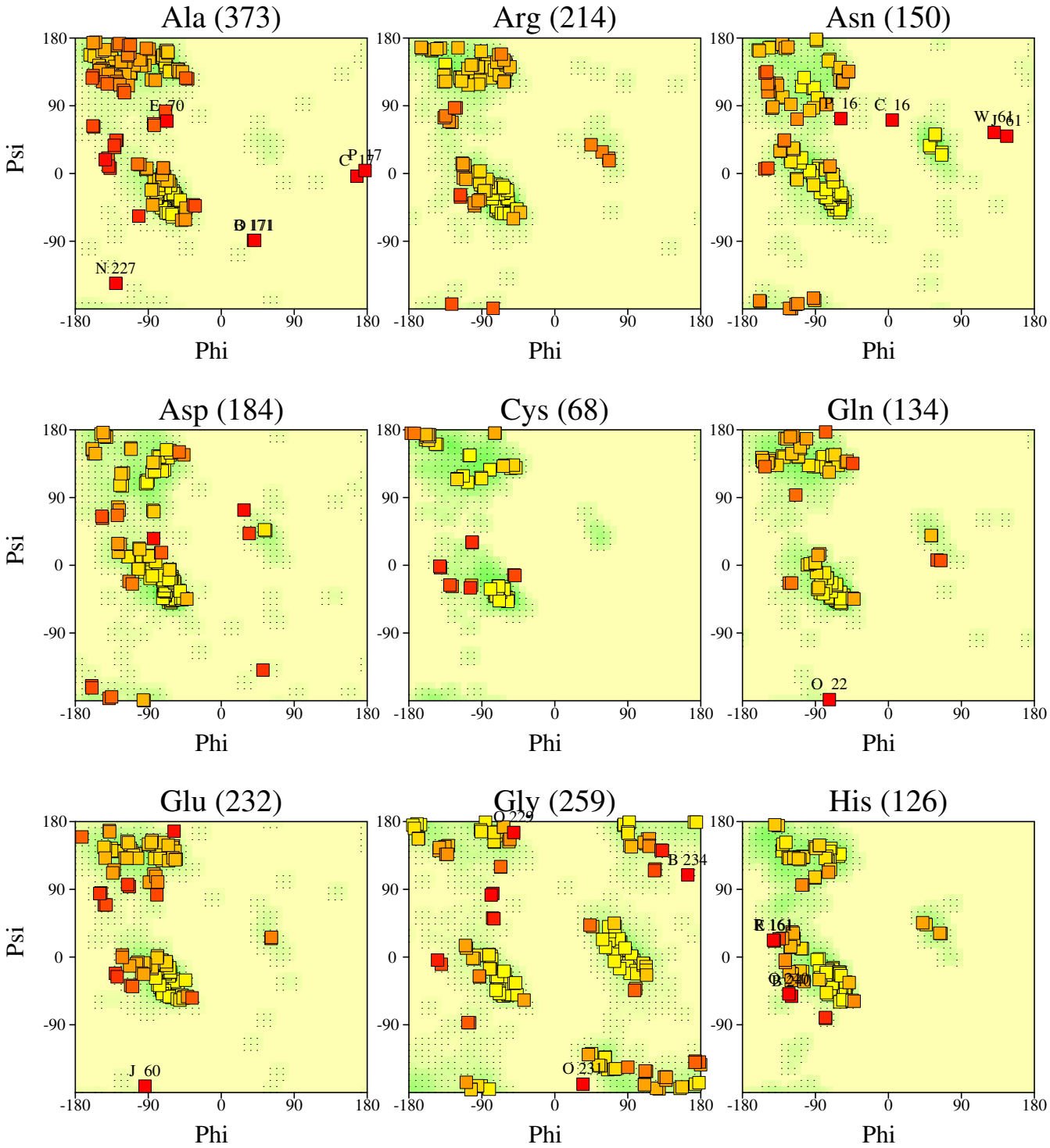
Residues in most favoured regions [A,B,L]	3231	92.1%
Residues in additional allowed regions [a,b,l,p]	256	7.3%
Residues in generously allowed regions [~a,~b,~l,~p]	14	0.4%
Residues in disallowed regions	6	0.2%
	----	------
Number of non-glycine and non-proline residues	3507	100.0%
Number of end-residues (excl. Gly and Pro)	49	
Number of glycine residues (shown as triangles)	266	
Number of proline residues	208	

Total number of residues	4030	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Ramachandran plots for all residue types

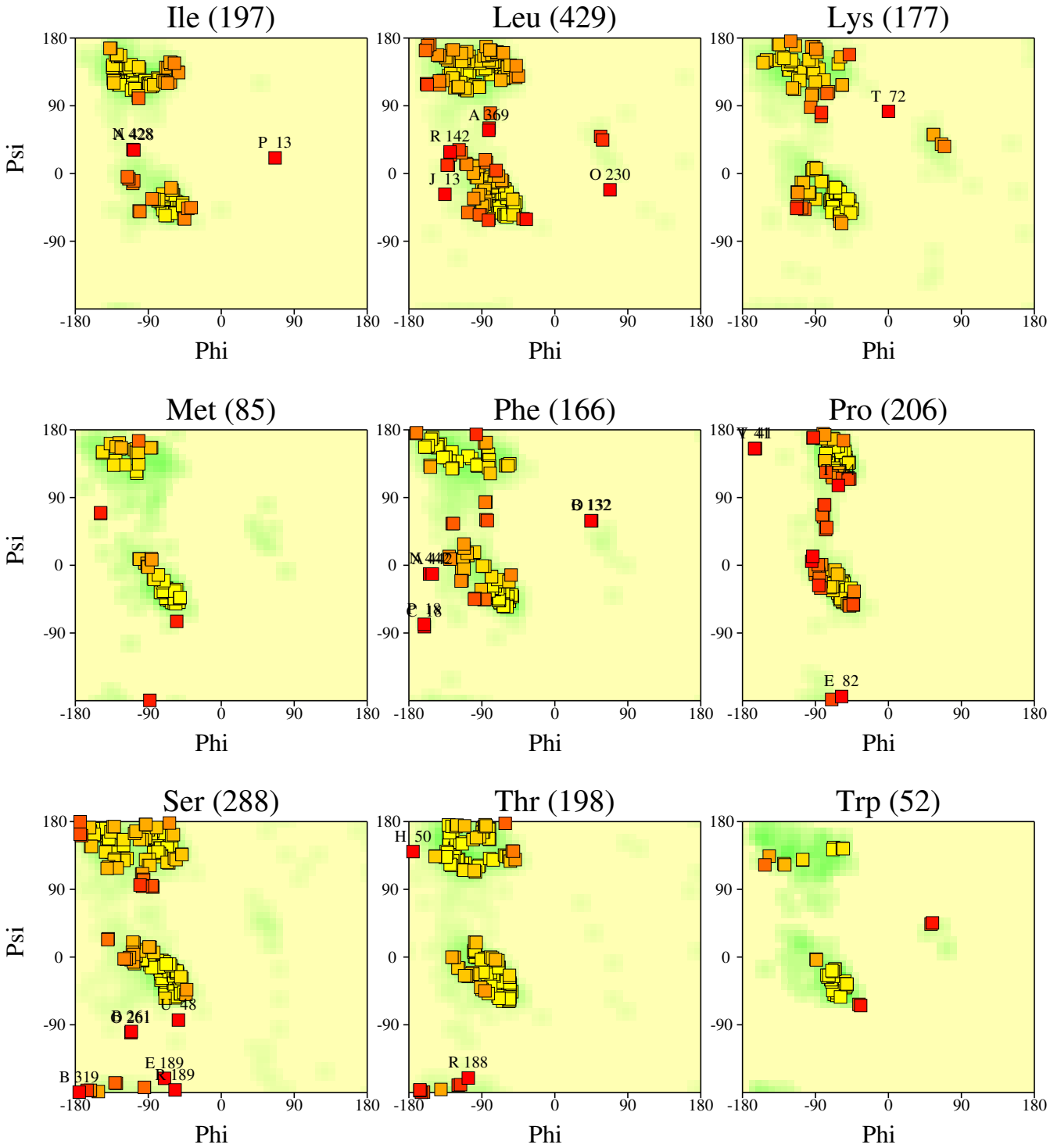
1pp9



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Ramachandran plots for all residue types

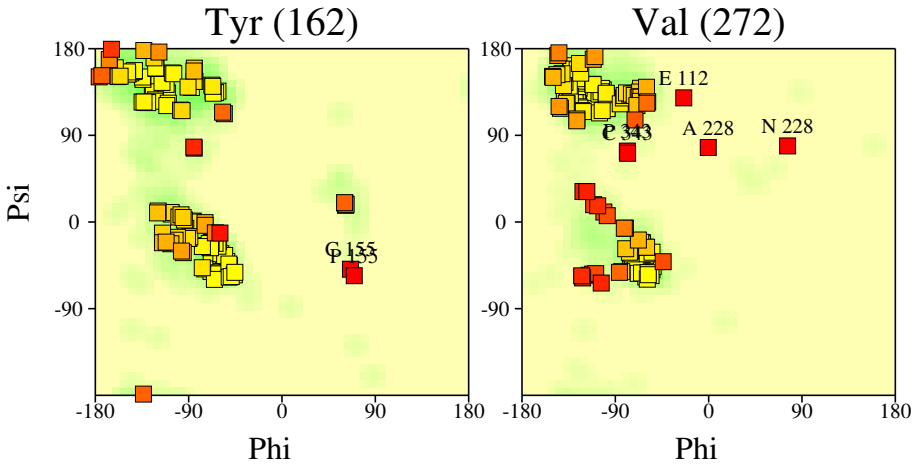
1pp9



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Ramachandran plots for all residue types

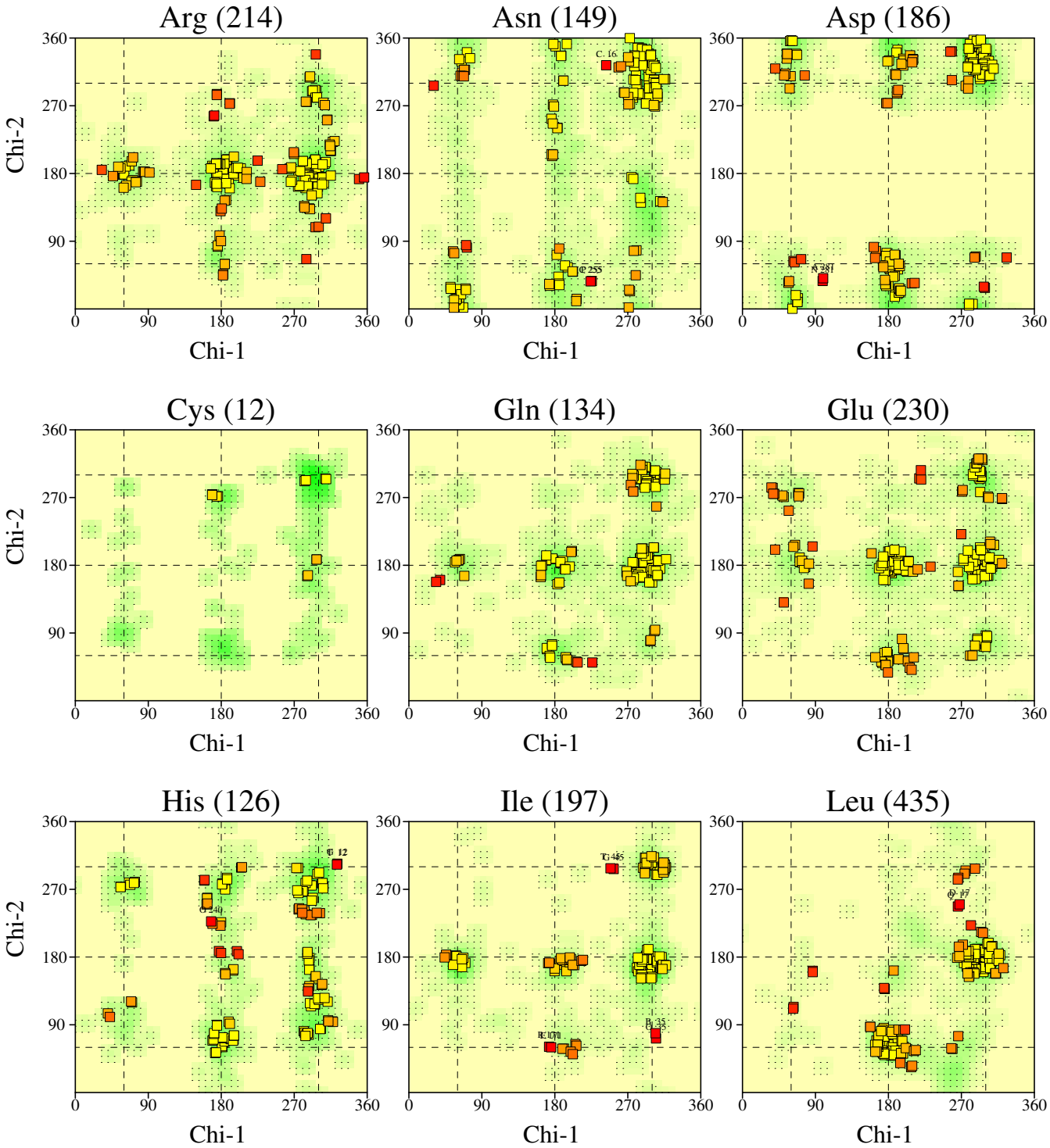
1pp9



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

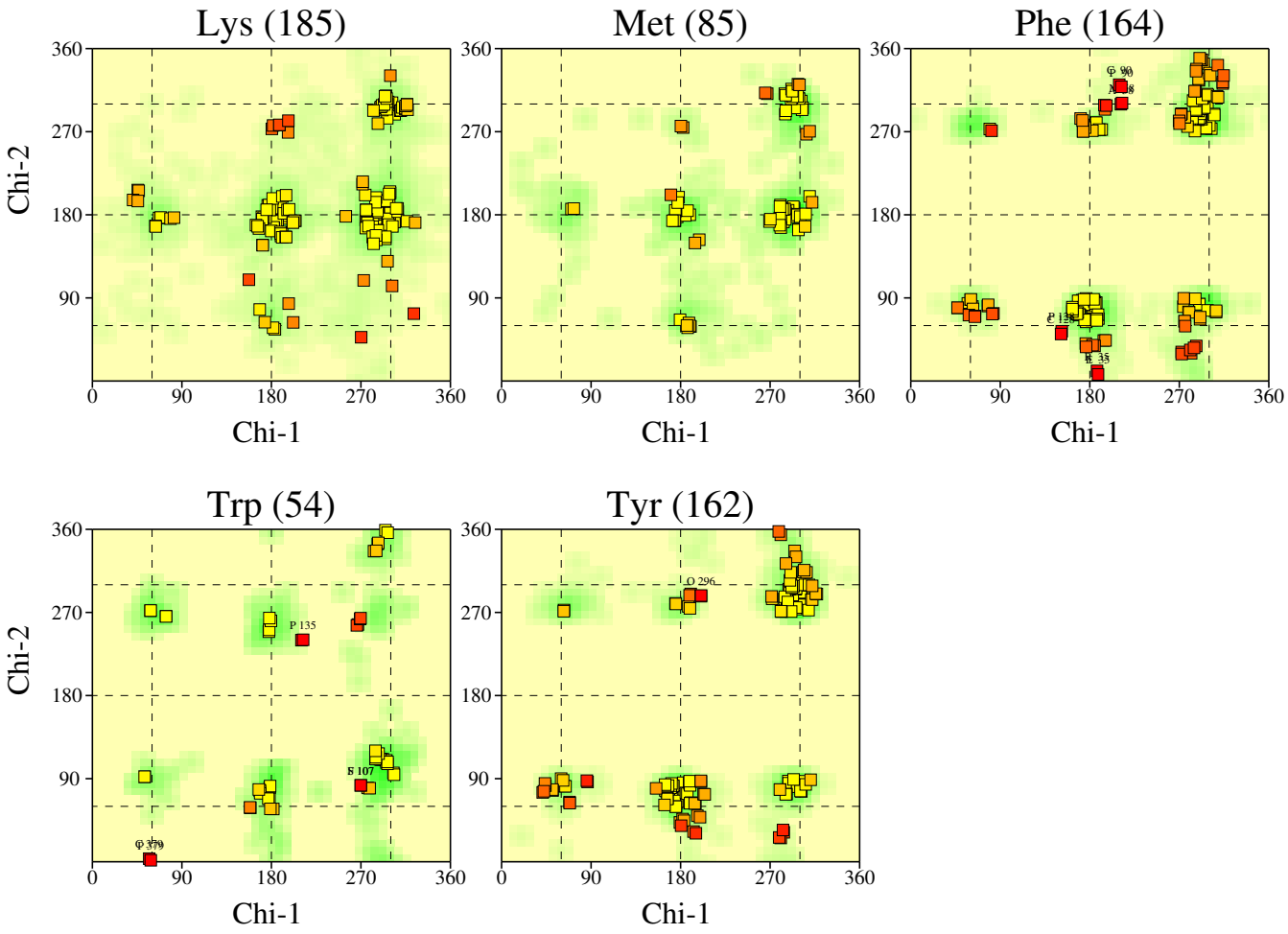
1pp9



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

1pp9



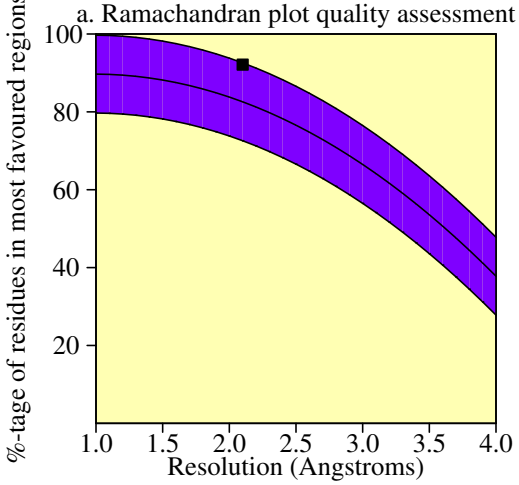
Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

PROCHECK

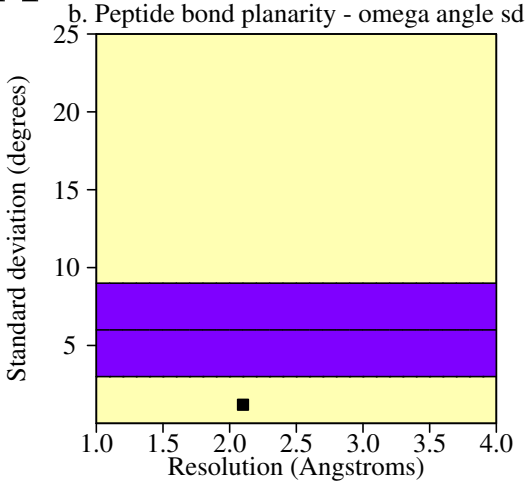
Main-chain parameters

1pp9

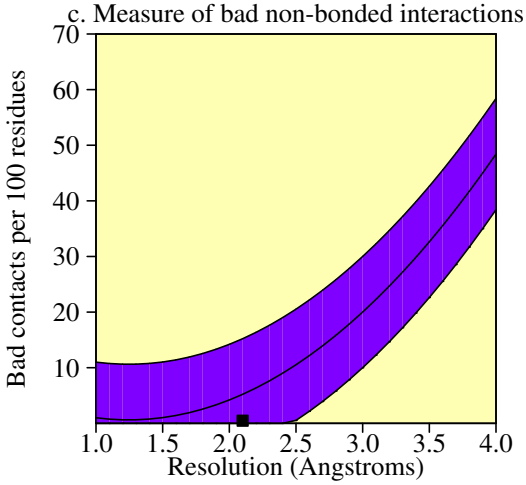
a. Ramachandran plot quality assessment



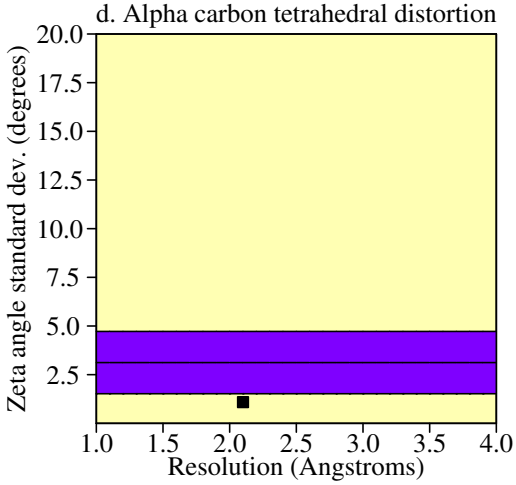
b. Peptide bond planarity - omega angle sd



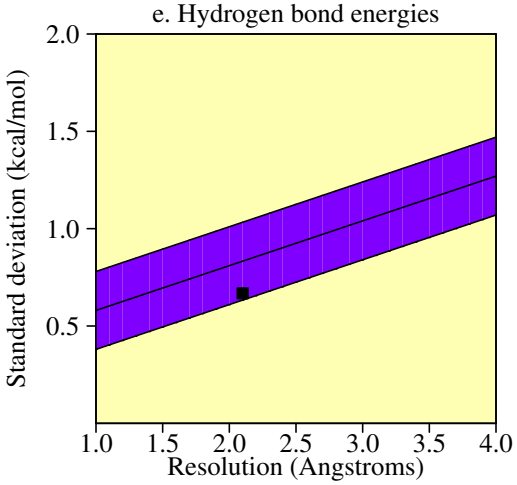
c. Measure of bad non-bonded interactions



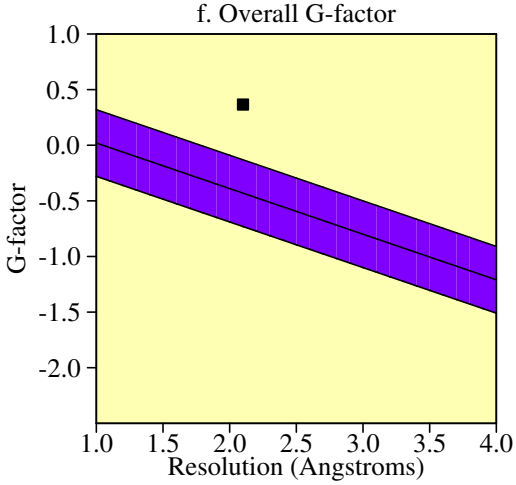
d. Alpha carbon tetrahedral distortion



e. Hydrogen bond energies



f. Overall G-factor



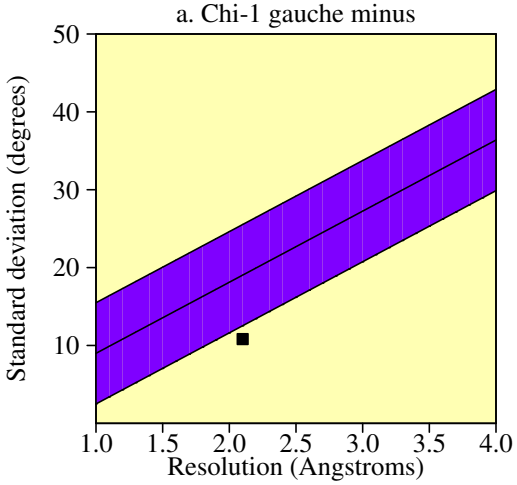
Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. %-tage residues in A, B, L	3507	92.1	82.6	10.0	1.0	Inside
b. Omega angle st dev	3985	1.2	6.0	3.0	-1.6	BETTER
c. Bad contacts / 100 residues	19	0.5	5.2	10.0	-0.5	Inside
d. Zeta angle st dev	3751	1.1	3.1	1.6	-1.3	BETTER
e. H-bond energy st dev	2682	0.7	0.8	0.2	-0.8	Inside
f. Overall G-factor	4030	0.4	-0.4	0.3	2.7	BETTER

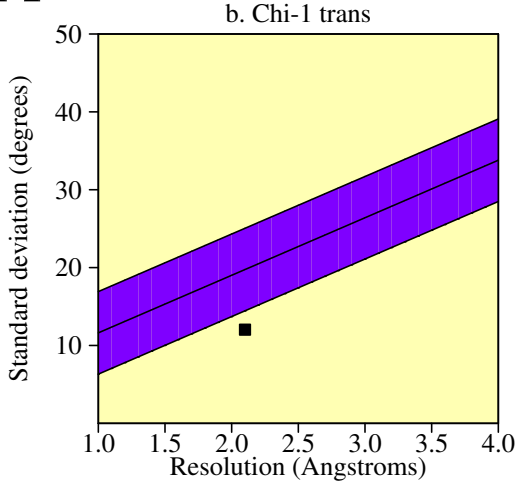
Side-chain parameters

1pp9

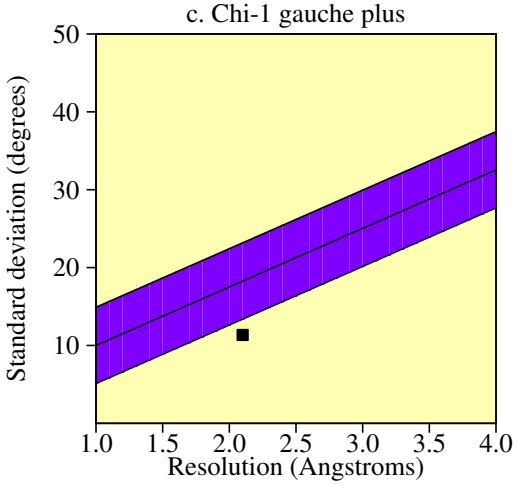
a. Chi-1 gauche minus



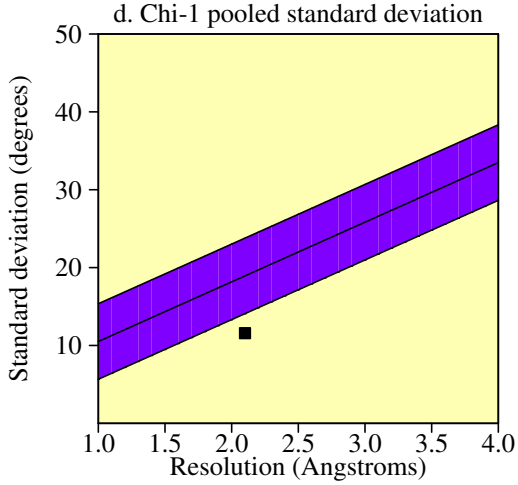
b. Chi-1 trans



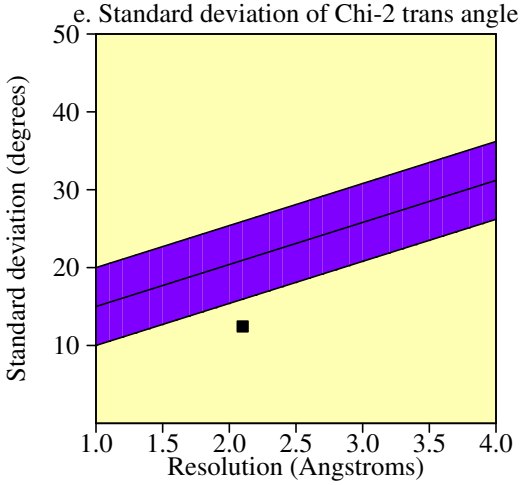
c. Chi-1 gauche plus

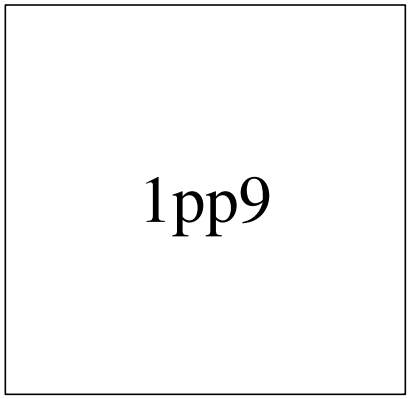


d. Chi-1 pooled standard deviation



e. Standard deviation of Chi-2 trans angle





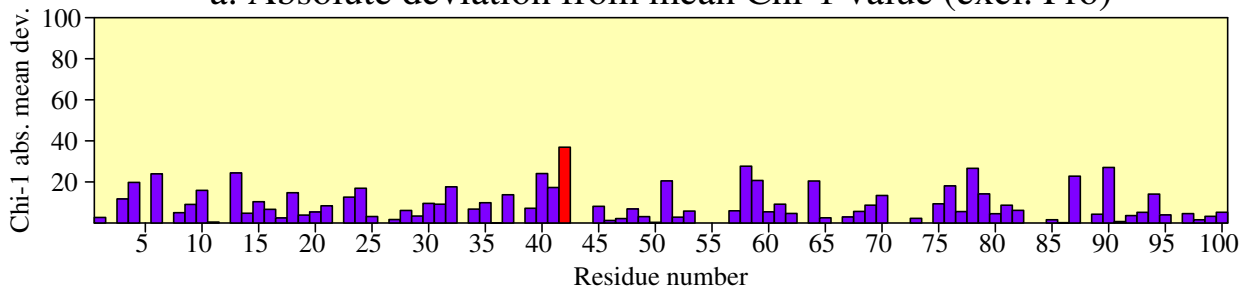
Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. Chi-1 gauche minus st dev	475	10.8	19.0	6.5	-1.3	BETTER
b. Chi-1 trans st dev	1047	12.0	19.7	5.3	-1.5	BETTER
c. Chi-1 gauche plus st dev	1642	11.3	18.3	4.9	-1.4	BETTER
d. Chi-1 pooled st dev	3164	11.6	18.9	4.8	-1.5	BETTER
e. Chi-2 trans st dev	1050	12.4	20.9	5.0	-1.7	BETTER

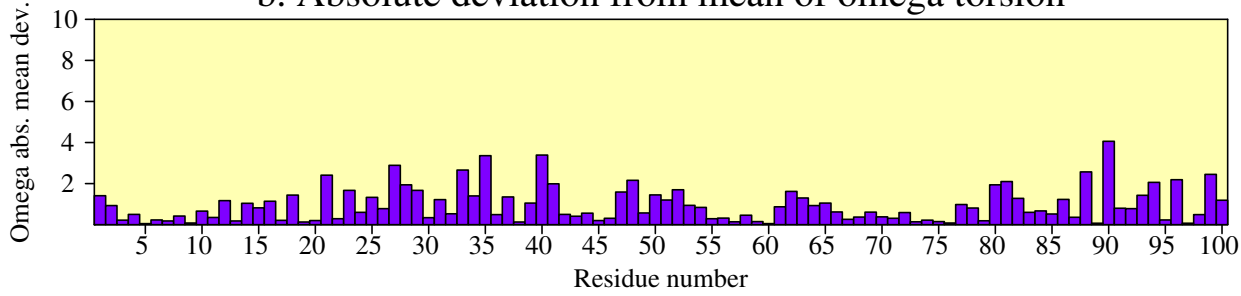
Residue properties

1pp9

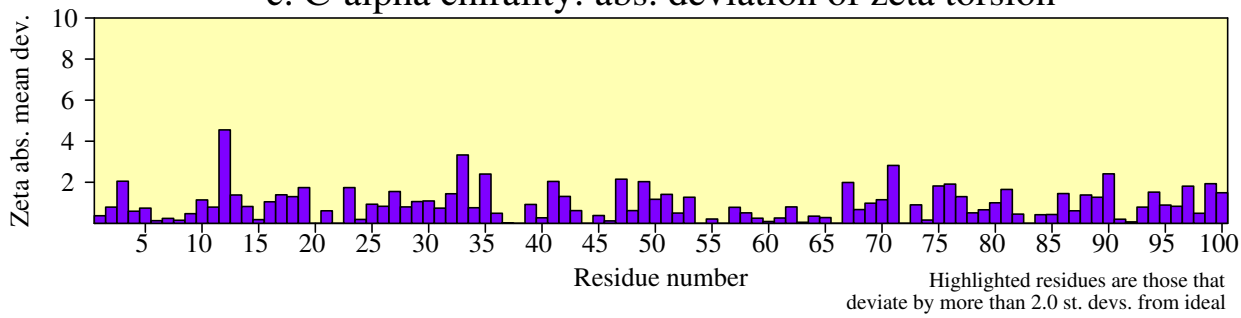
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

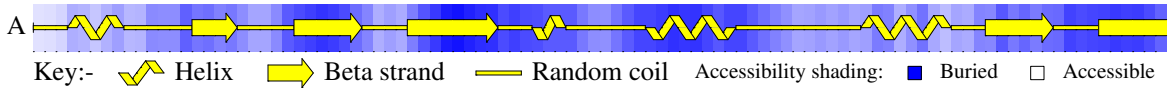


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



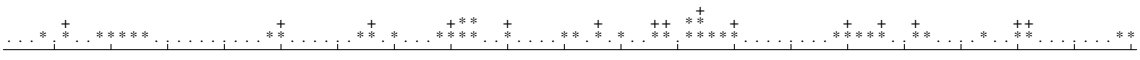
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

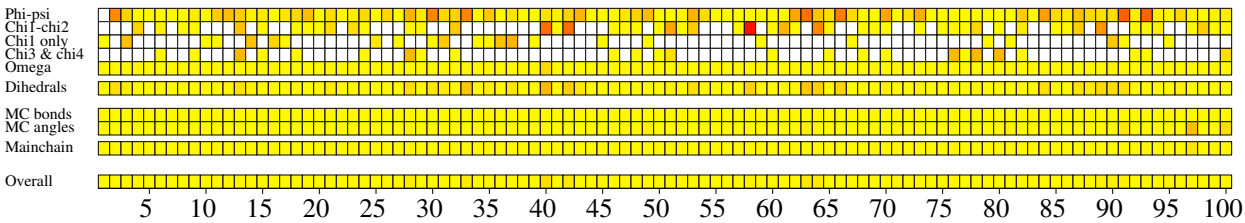
Most favoured Allowed Generous Disallowed

TATYAQALQSVPETQVSQLDNGLRVASEQSSQPTCTVGVWIDAGSRYESEKNNGAGYFVEHLAFKGTKNRPGNALEKEVESMGAHLNAYSTREHTAYYIK

f. Max. deviation (see listing)

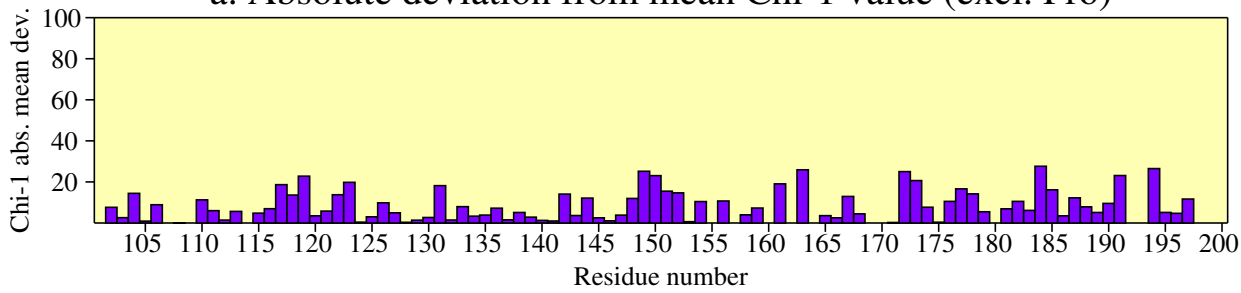


g. G-factors

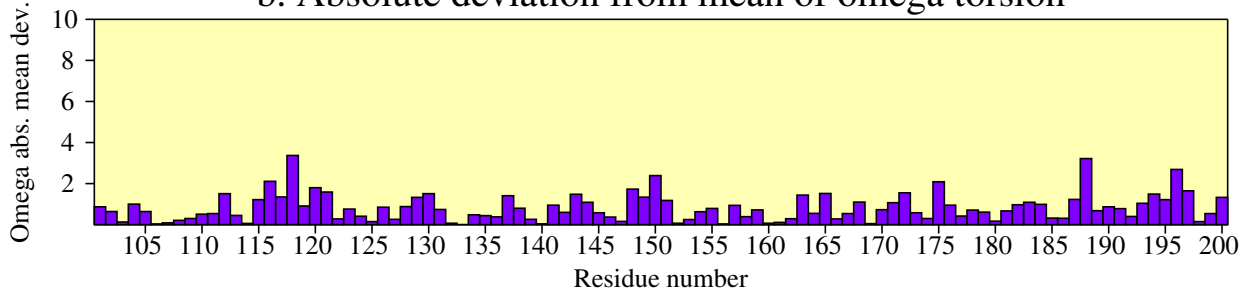


Residue properties
1pp9

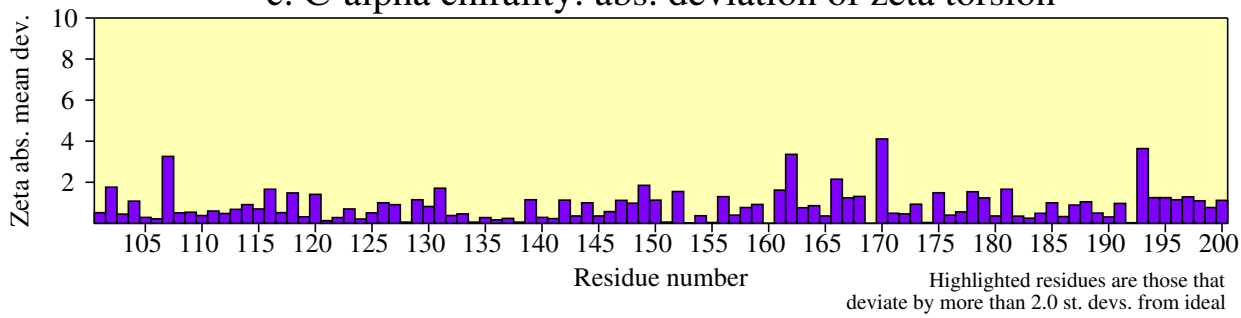
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

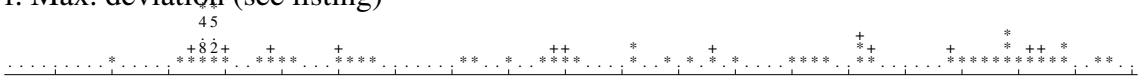
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

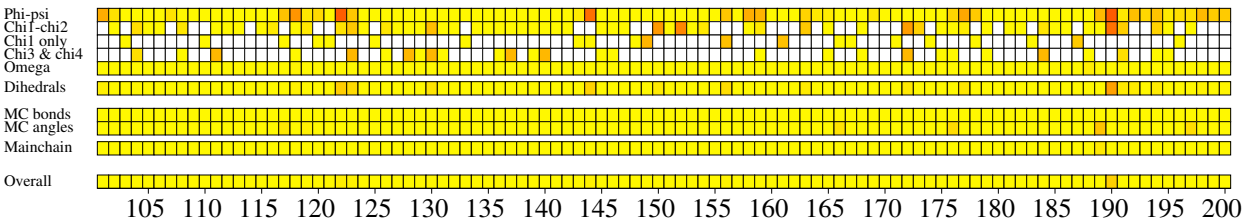
Most favoured Allowed Generous Disallowed

ALSKDLPKAVELLADIVQNCSLEDSQIEKERDVILQELQENDTSMRDVVFNYLHATAFQGTPLAQSVEGPSENVRKLSRADLTEYLSRHYKAPRMVLAAA

f. Max. deviation (see listing)



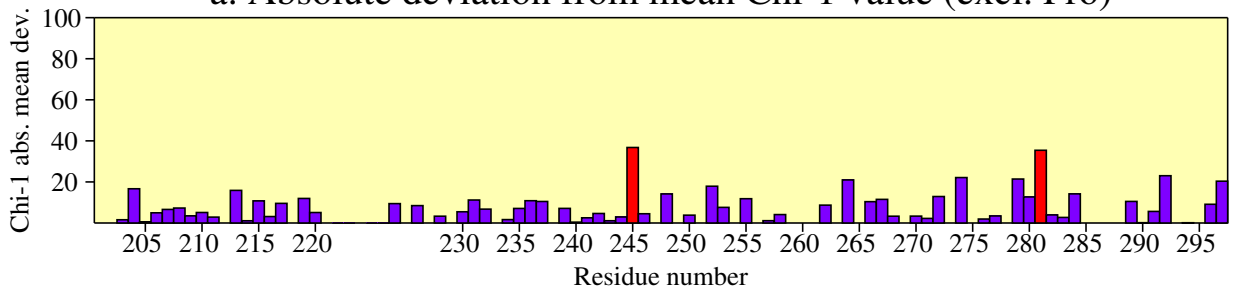
g. G-factors



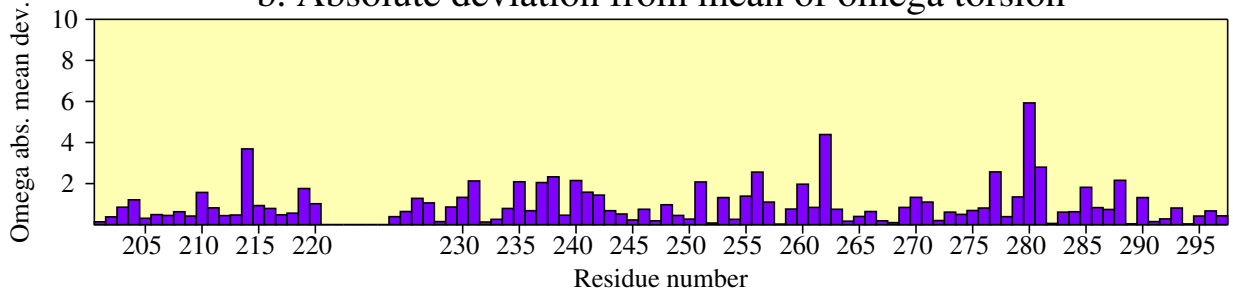
Residue properties

1pp9

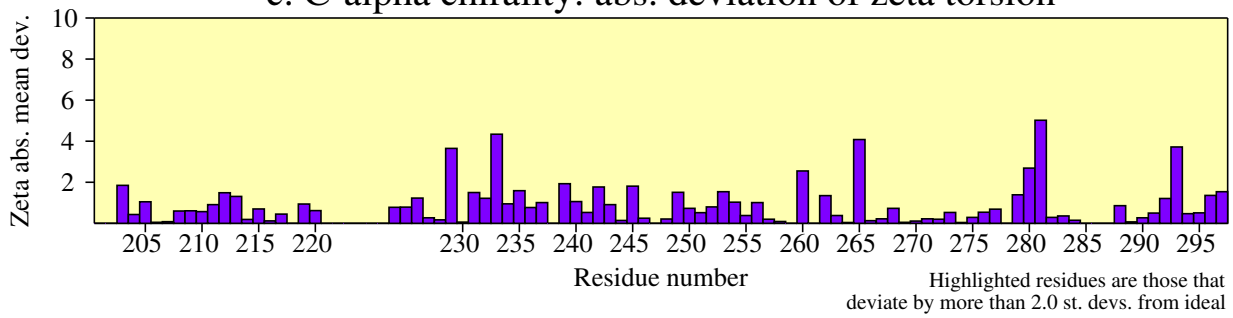
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

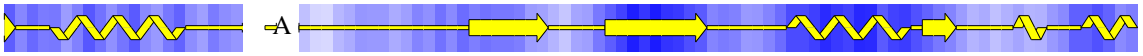


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

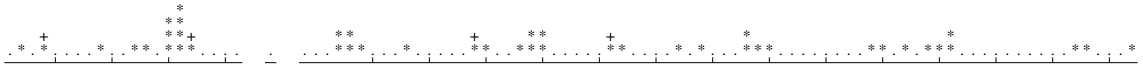


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

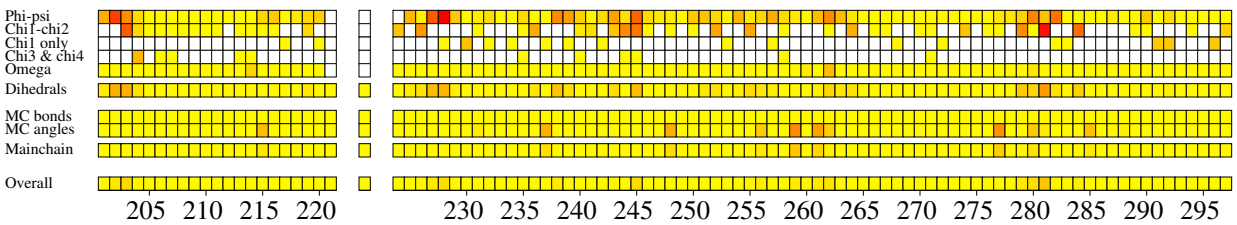
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

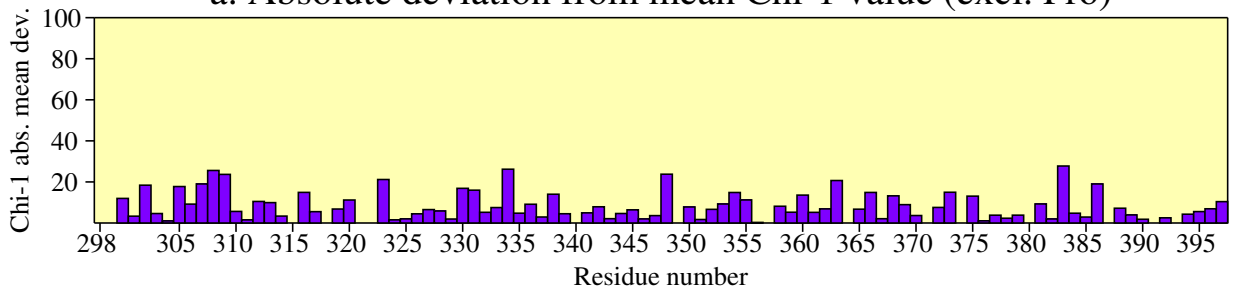


g. G-factors

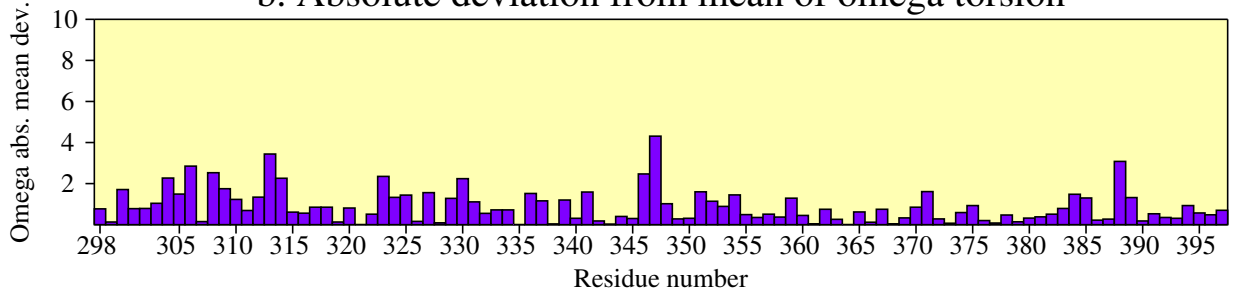


Residue properties
1pp9

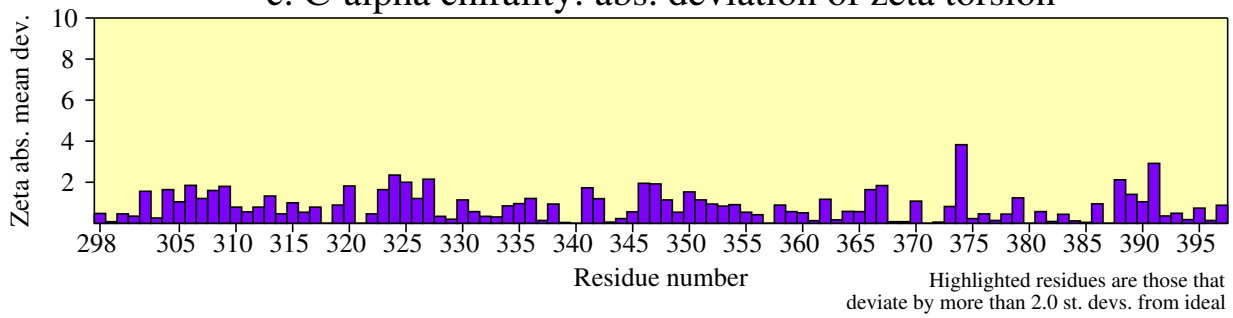
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

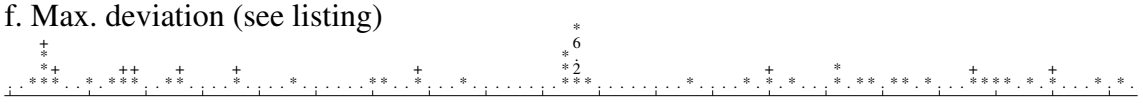
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

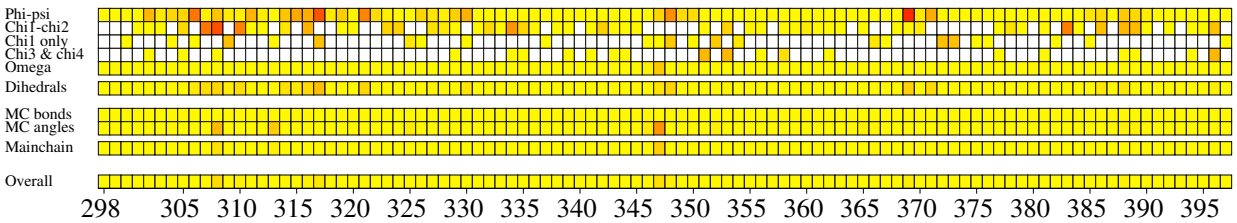
Most favoured Allowed Generous Disallowed

AATNKLCQSFQTFNICYADTGLLGAHFVCDHMSIDDMMFVLQGQWMRLCTSATESEVLRGKNLLRNALVSHLDGTTPVCEDIGRSLLTYGRRIPLAEWES

f. Max. deviation (see listing)

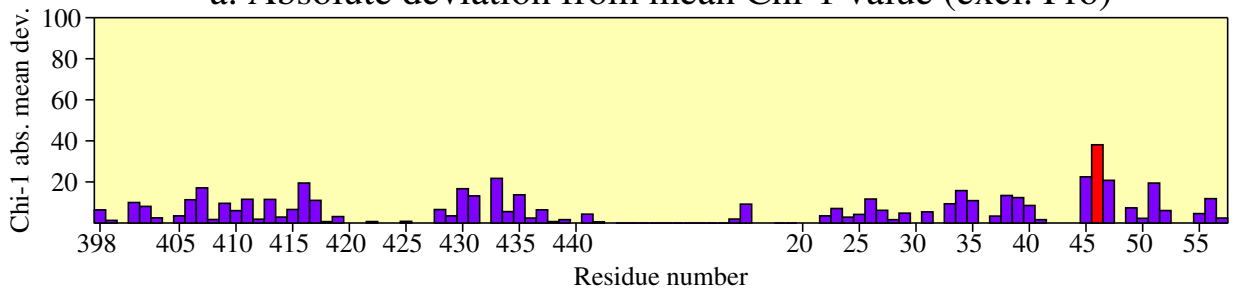


g. G-factors

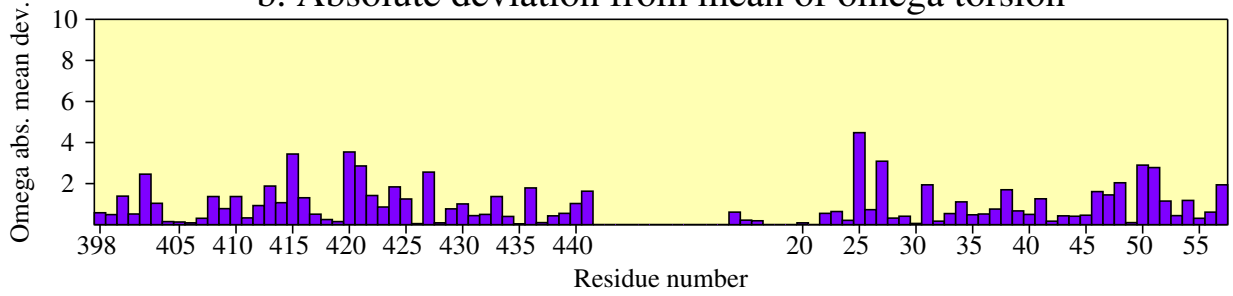


Residue properties
1pp9

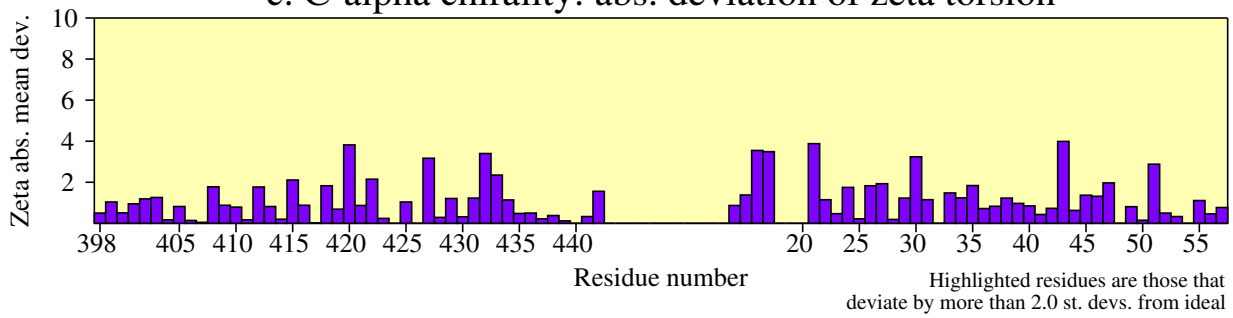
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

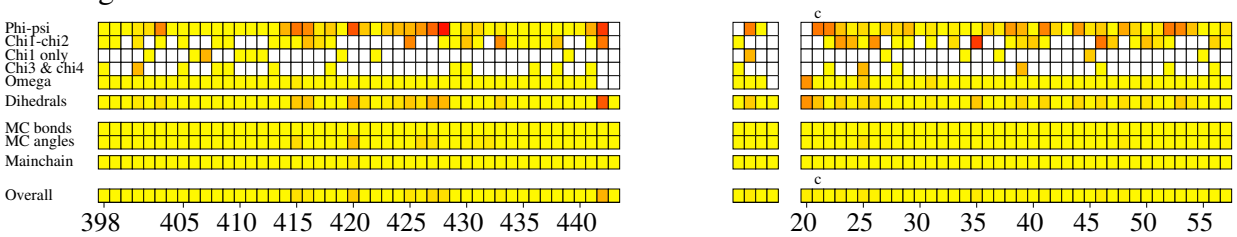
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

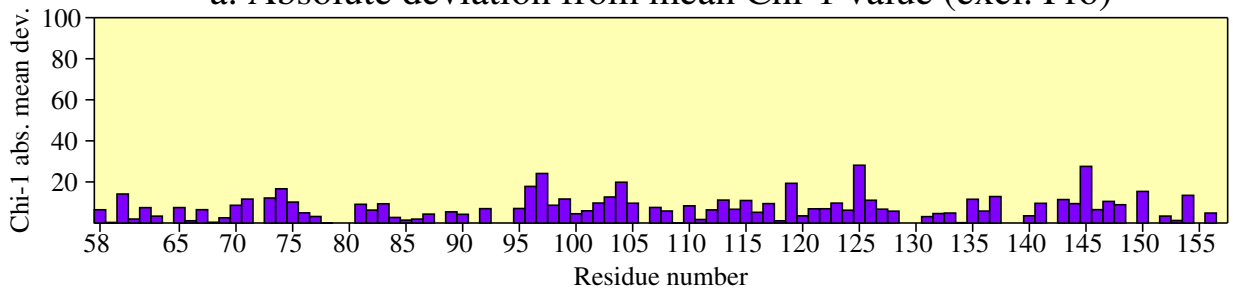
g. G-factors



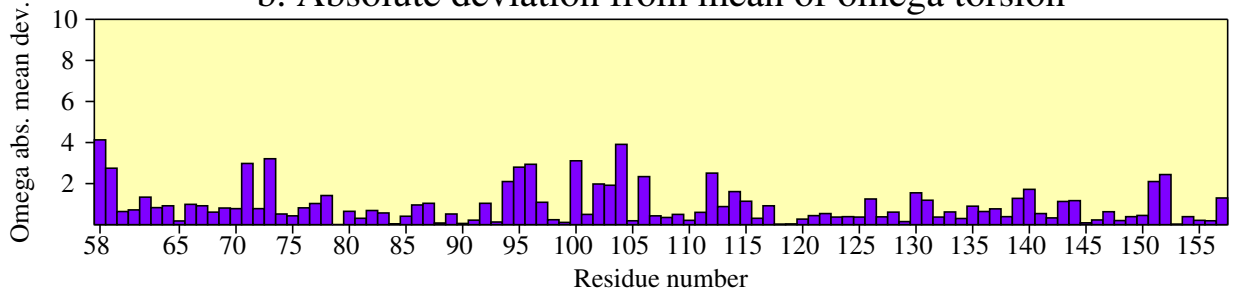
c = cis-peptide

Residue properties
1pp9

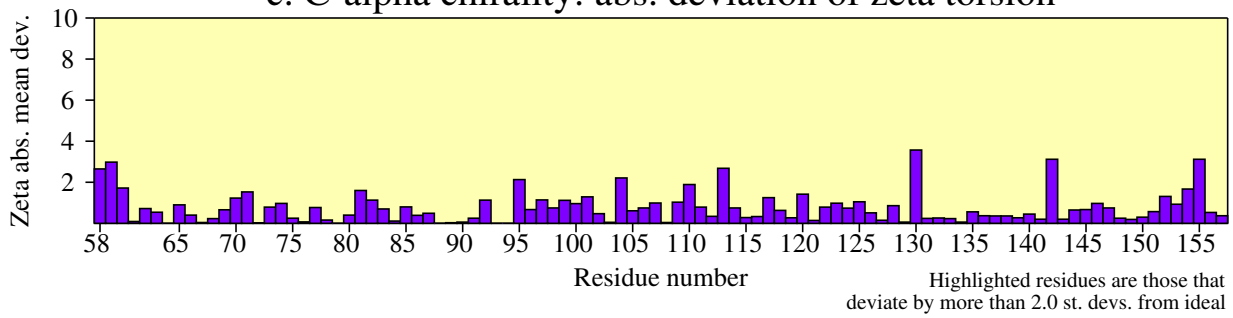
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

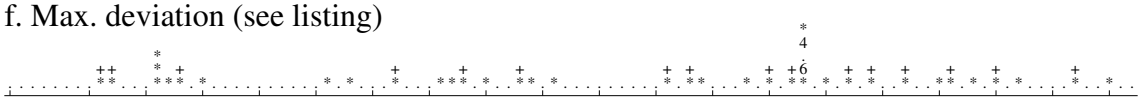
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

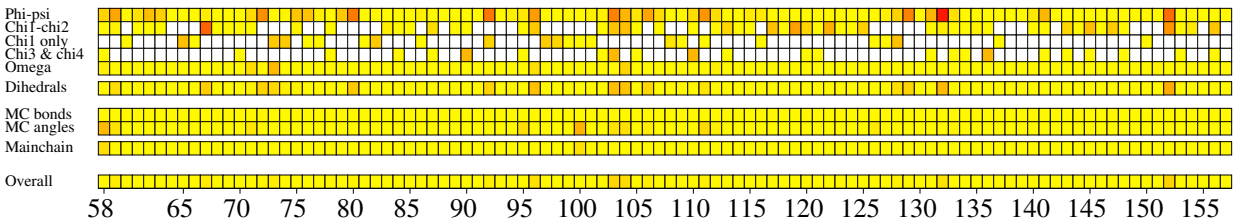
Most favoured Allowed Generous Disallowed

ENSNNLGTSHLLRLASSLTTKGASSFKITRGIEAVGGKLSVTSTRENMAYTVECLRDDVDILMEFLLNVTTAPEFRRWEVAALQPQLRIDKAVALQNPQA

f. Max. deviation (see listing)

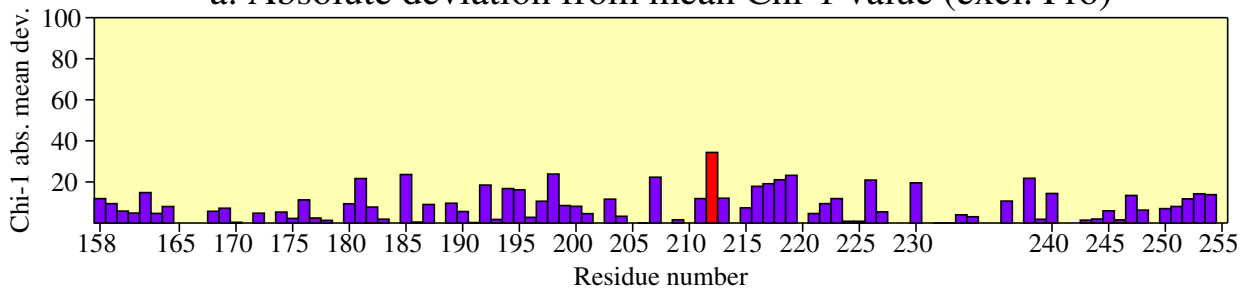


g. G-factors

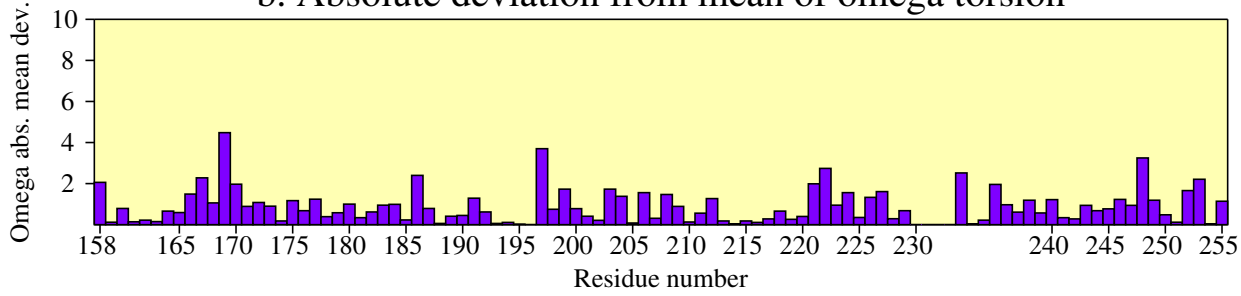


Residue properties
1pp9

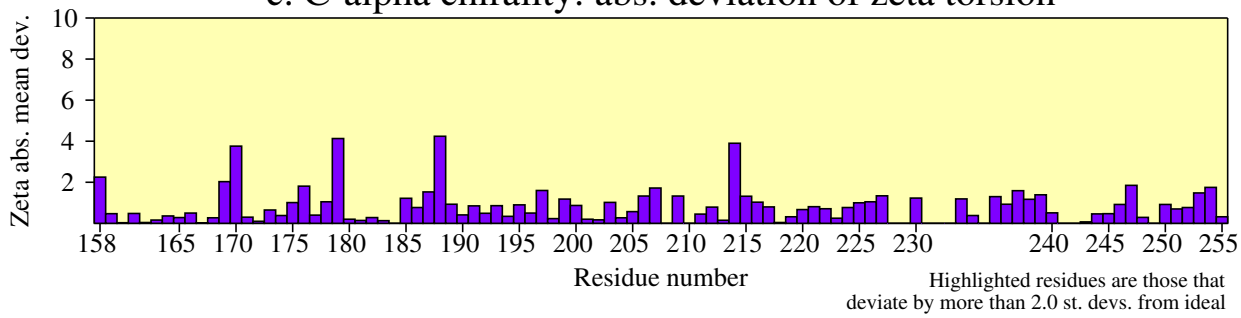
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

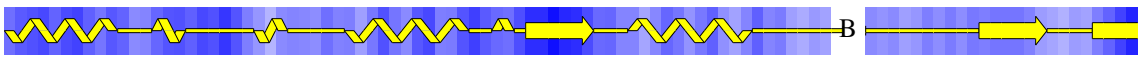


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

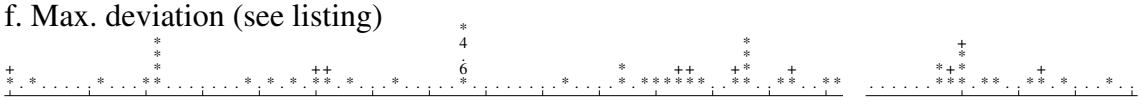


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

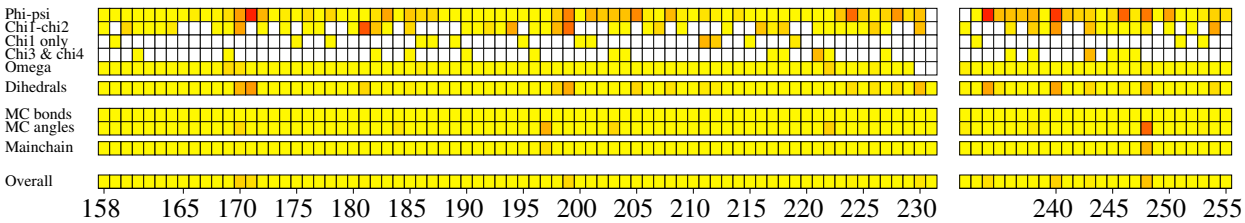
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



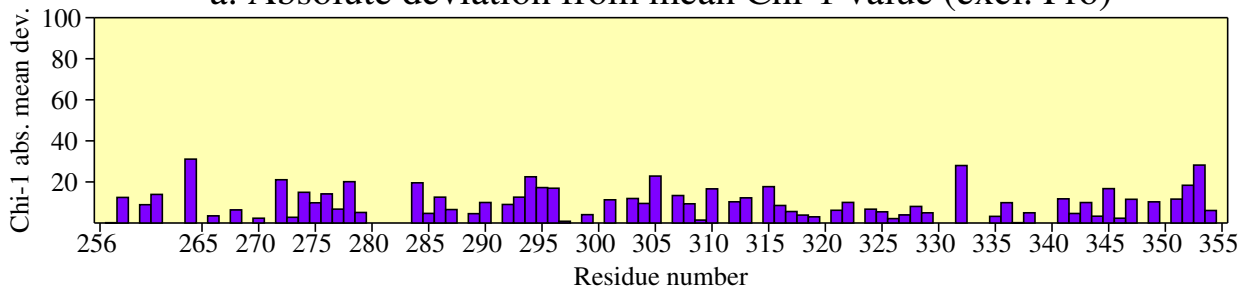
g. G-factors



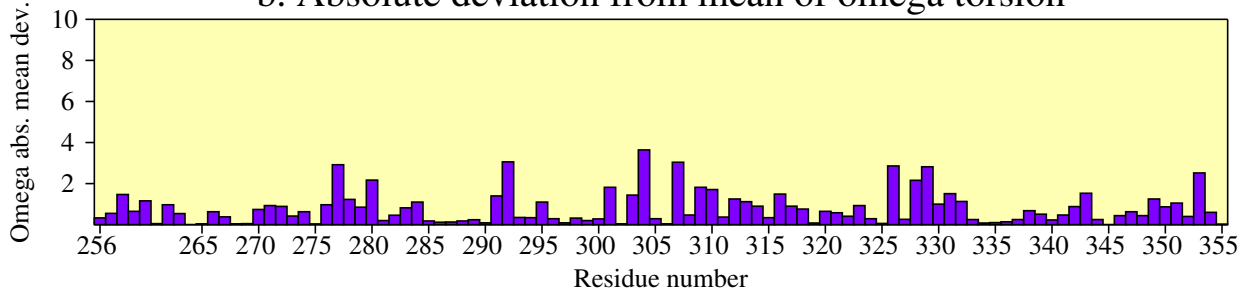
Residue properties

1pp9

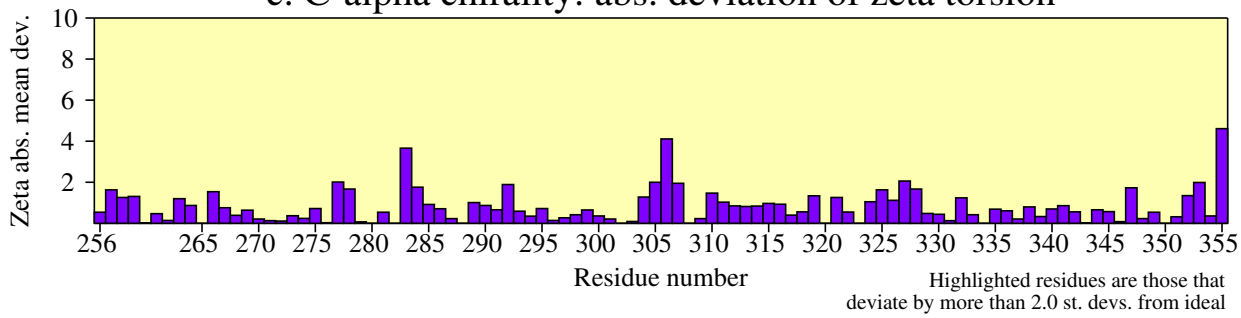
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

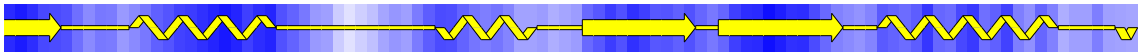


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

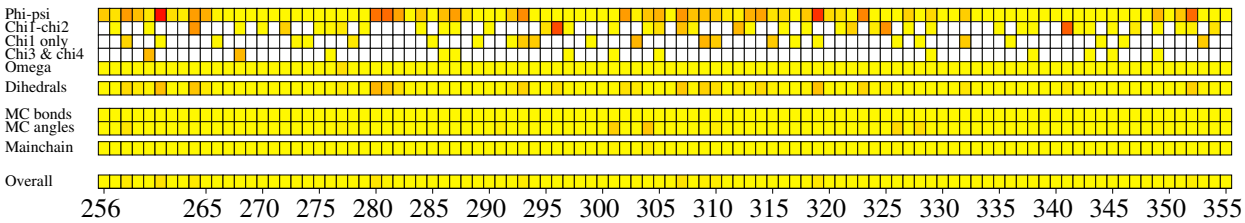
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

ALVAESAAIGSAEANAFSVLQHVLGAGPHVKRGSNATSSLYQAVAKGVHQPFDVSAFNASYSDSGLFGFYTISQAASAGDVIKAAYNQVKTIAQGNLSNP

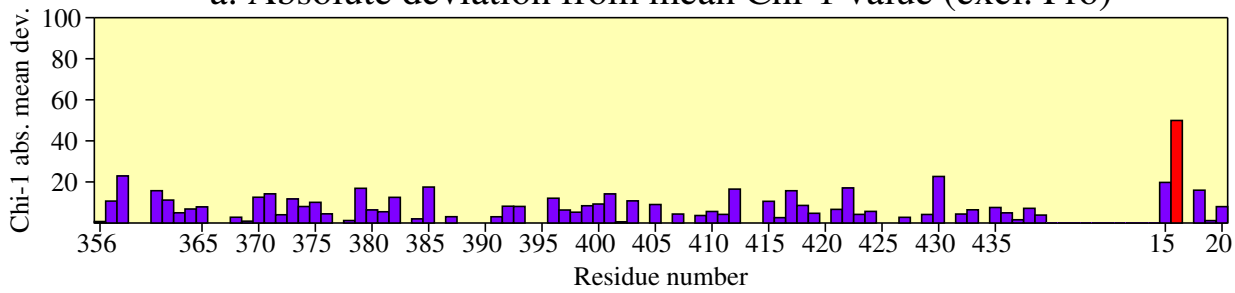
f. Max. deviation (see listing)

g. G-factors

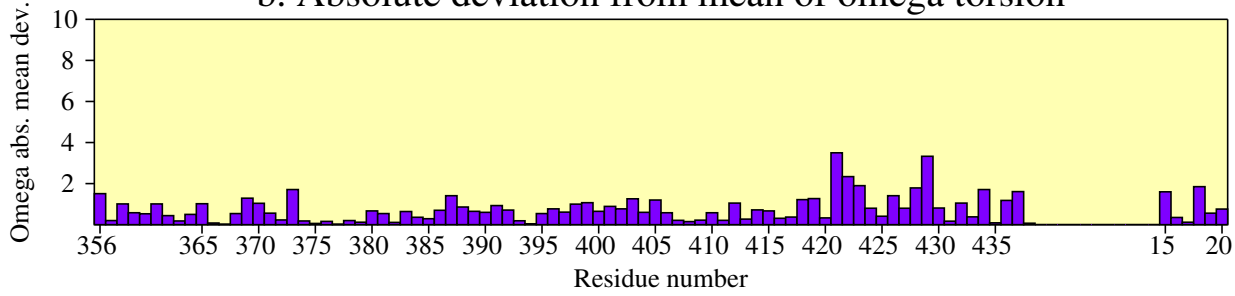


Residue properties
1pp9

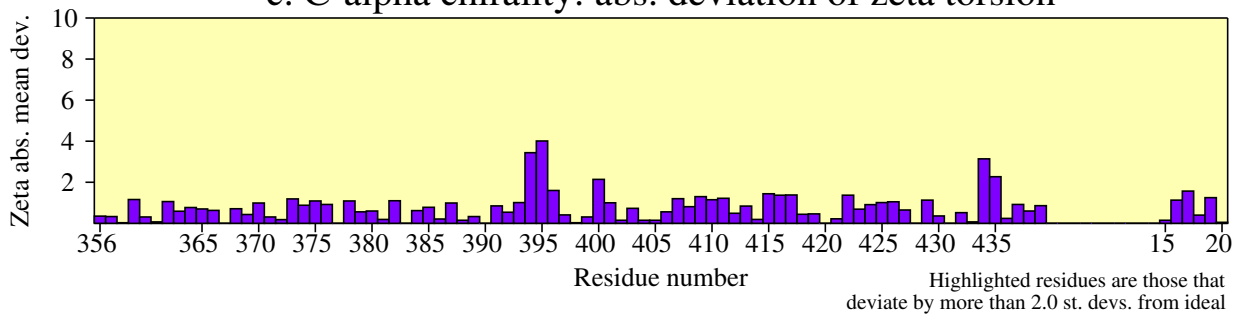
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



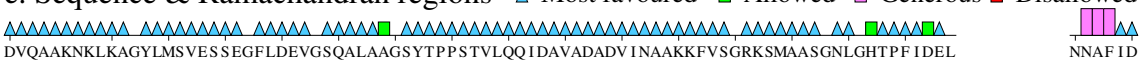
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

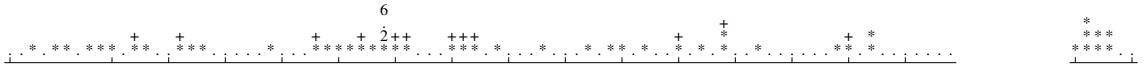
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

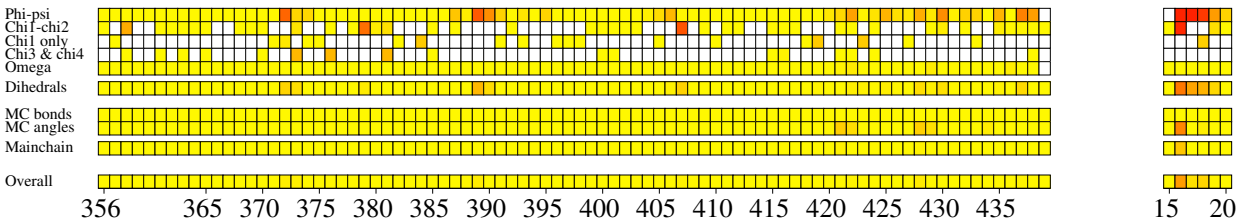
Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)

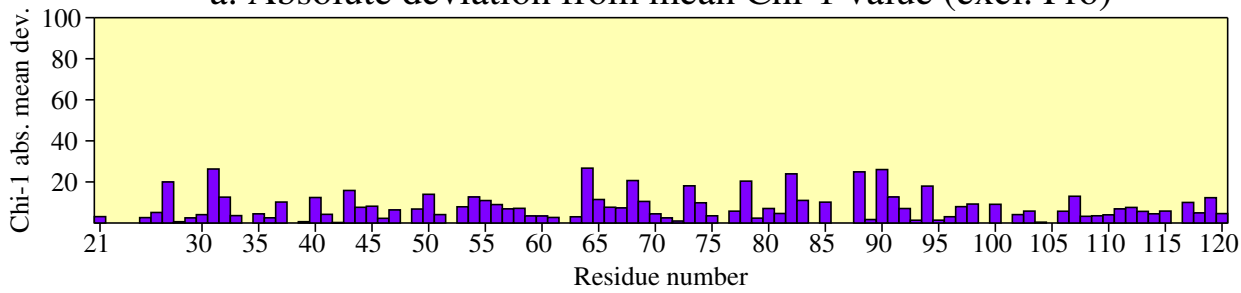


g. G-factors

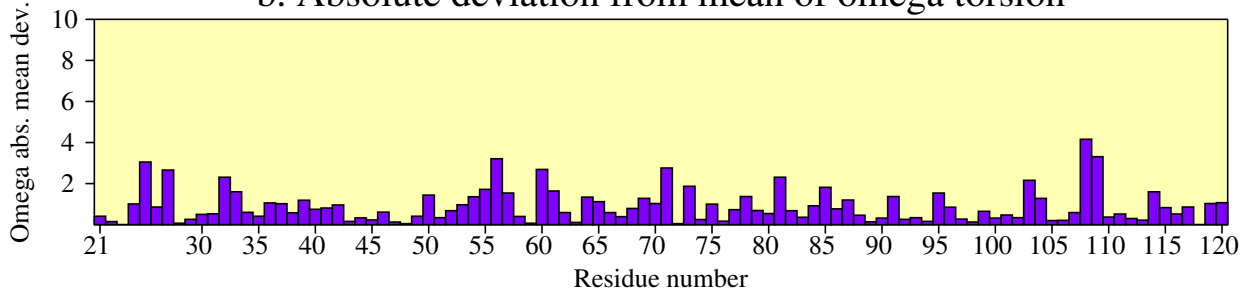


Residue properties
1pp9

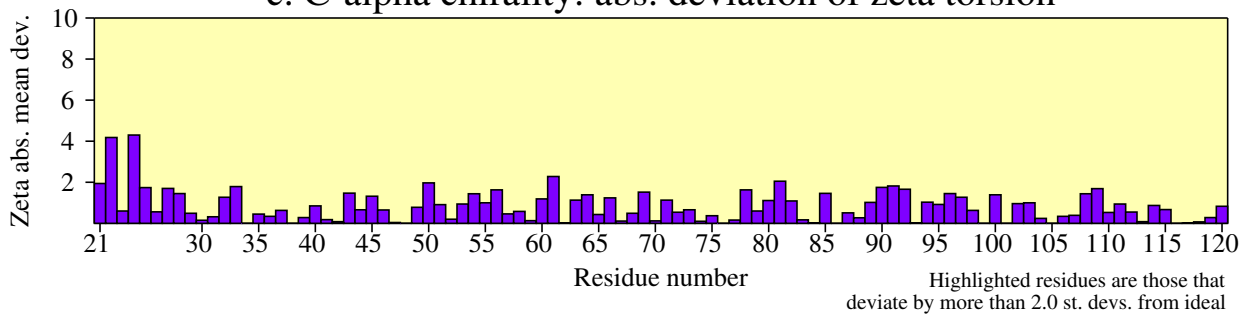
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

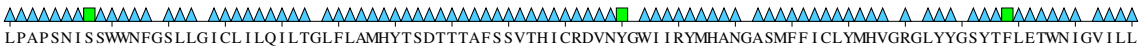


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

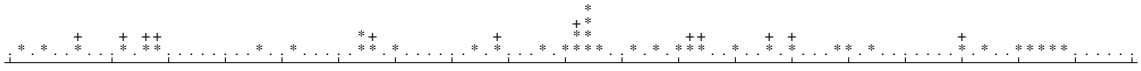
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

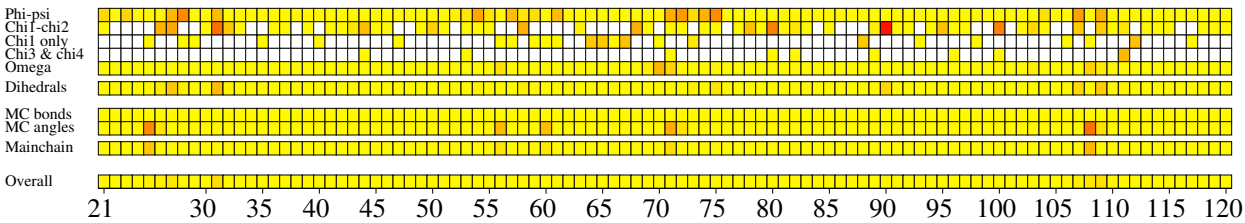


LPAPSNISSWWNFGSLLGICLILQILTGLFLAMHYTSDTTTAFSSVTHICRDVNYGWIIRYMHANGASMFFICLYMHVGRGLYYGSYTFLETWNIGVILL

f. Max. deviation (see listing)

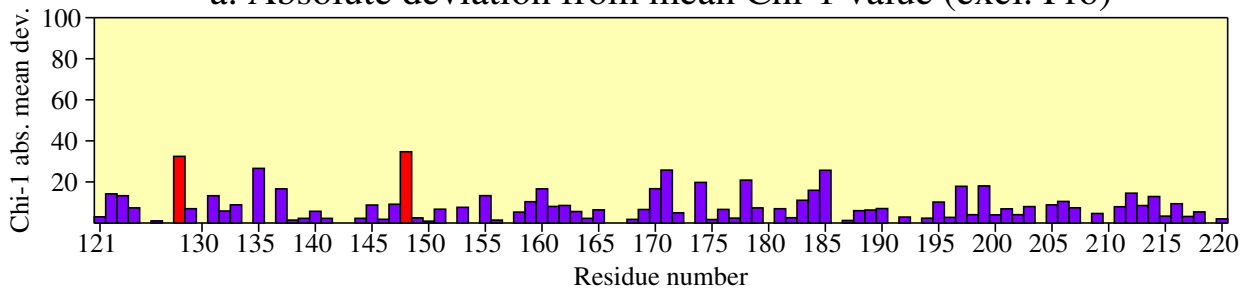


g. G-factors

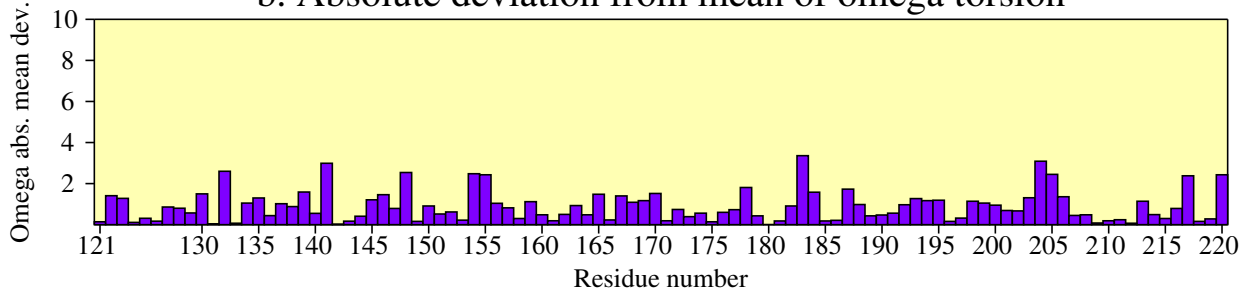


Residue properties
1pp9

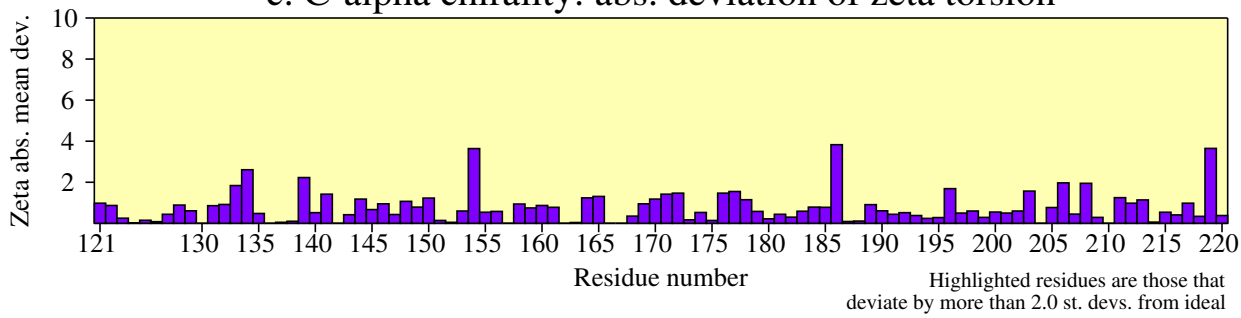
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

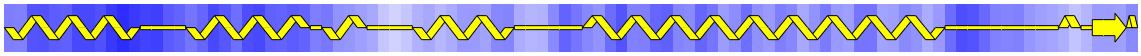


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



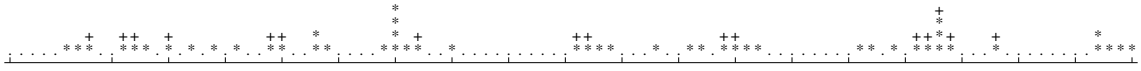
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

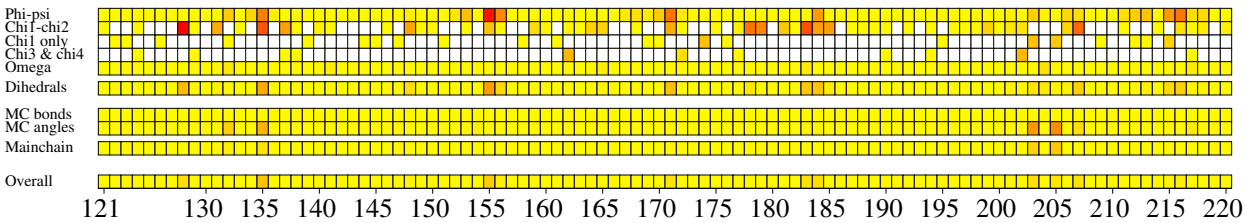
Most favoured Allowed Generous Disallowed

L T V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T N L V E W I W G G F S V D K A T L T R F F A F H F I L P F I I M A I A M V H L L F L H E T G S N N P T G I S S D V D K I P F

f. Max. deviation (see listing)



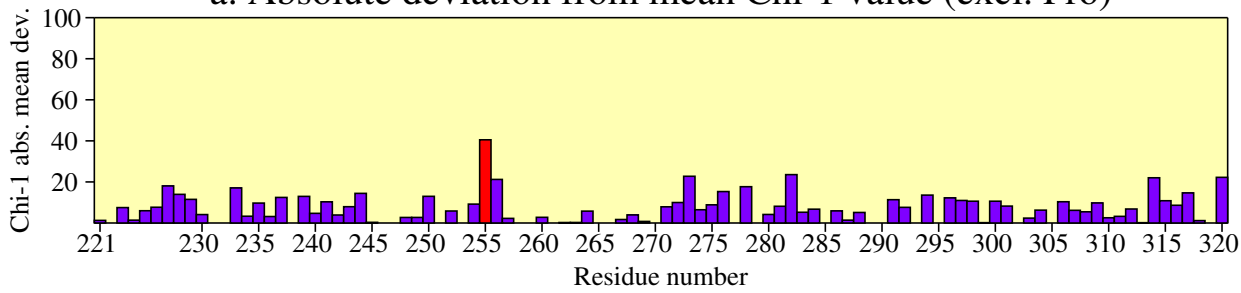
g. G-factors



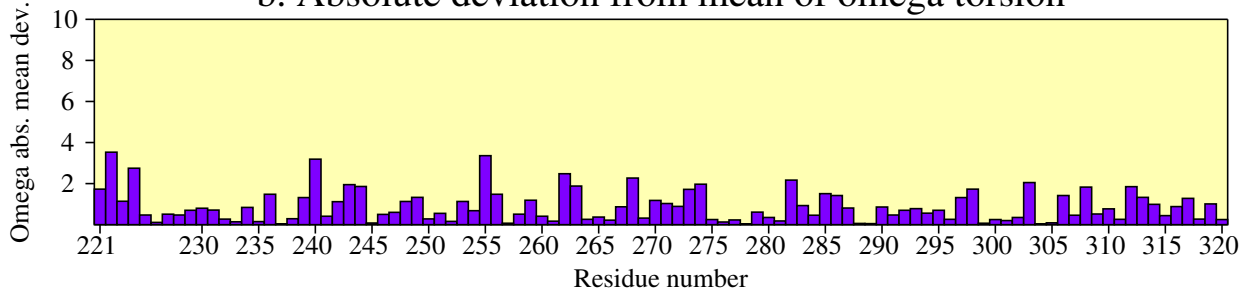
Residue properties

1pp9

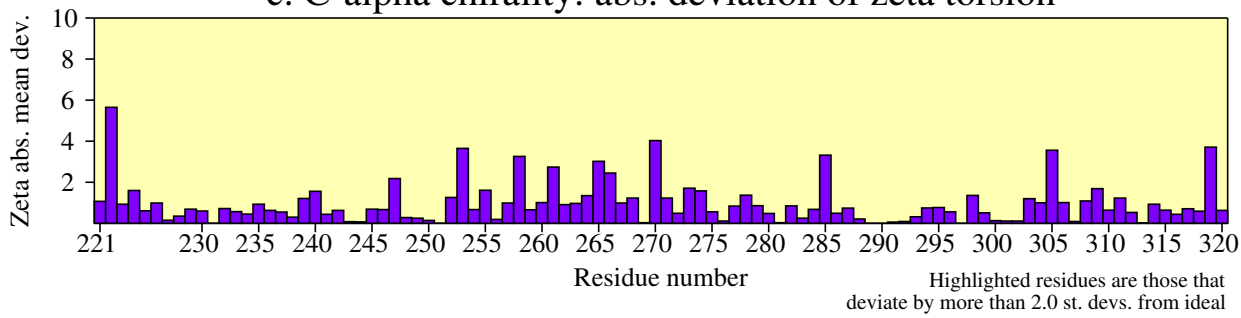
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



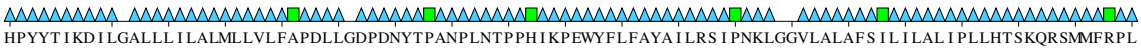
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

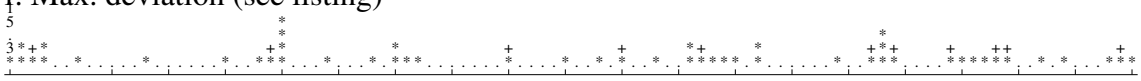
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

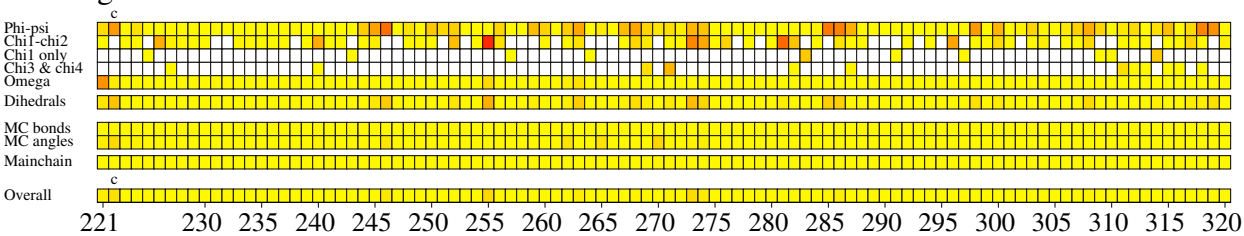


HPYYTIKDILGALLLILALMLLVLFAPDLLGDPDNYTPANPLNTPPHIKPEWYFLFAYAILRSIPNKLGGVLALAFSILILALIPLLHTSKQRSMMFRPL

f. Max. deviation (see listing)



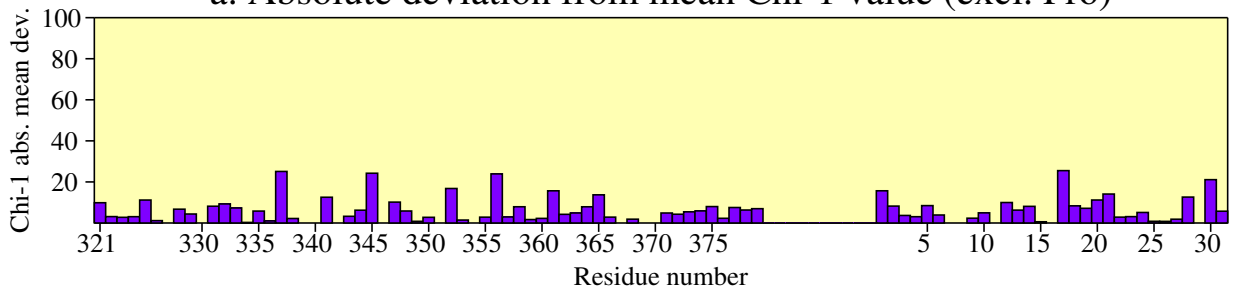
g. G-factors



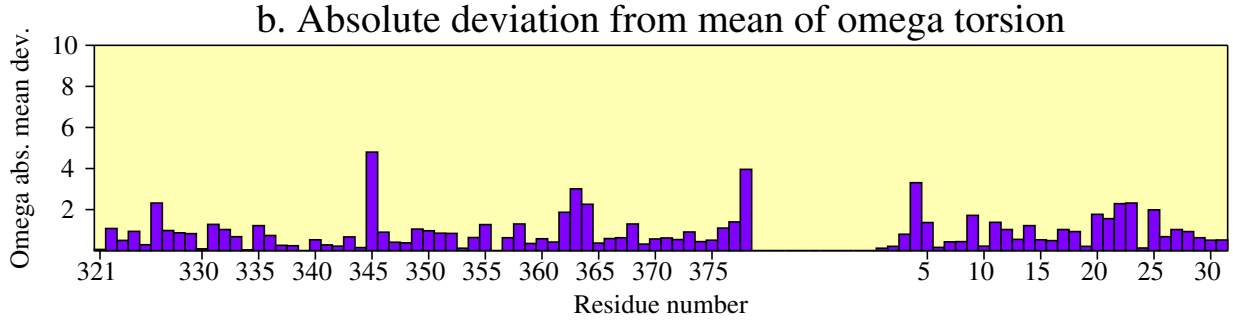
c = cis-peptide

Residue properties
1pp9

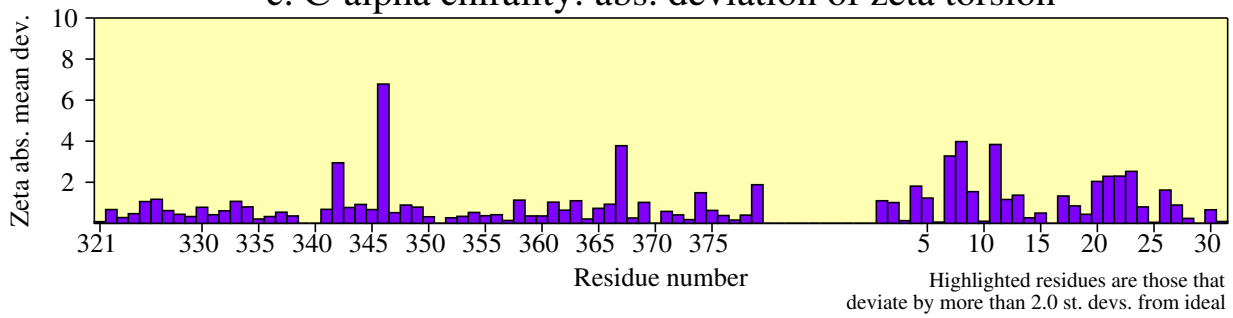
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



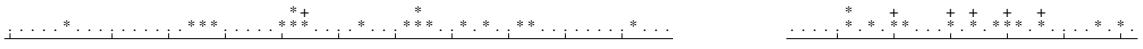
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

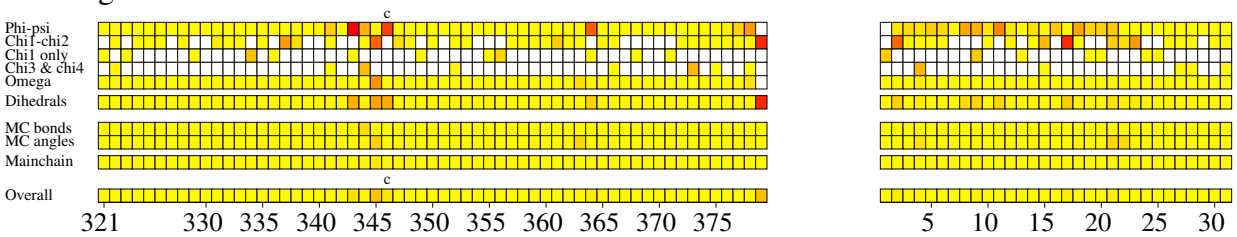
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



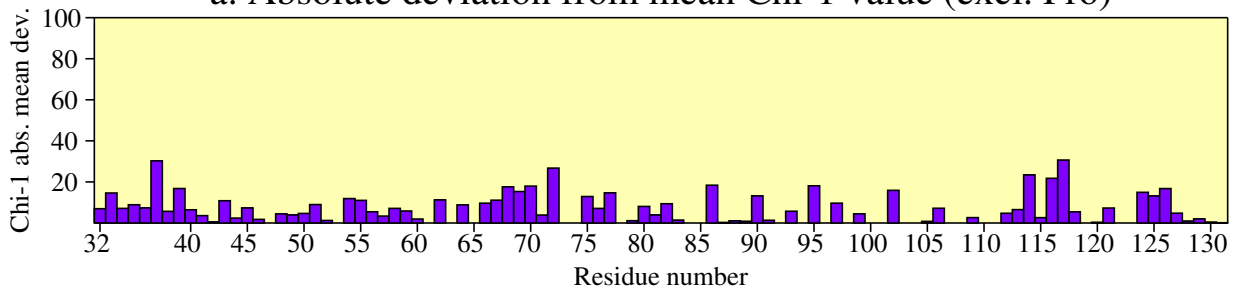
g. G-factors



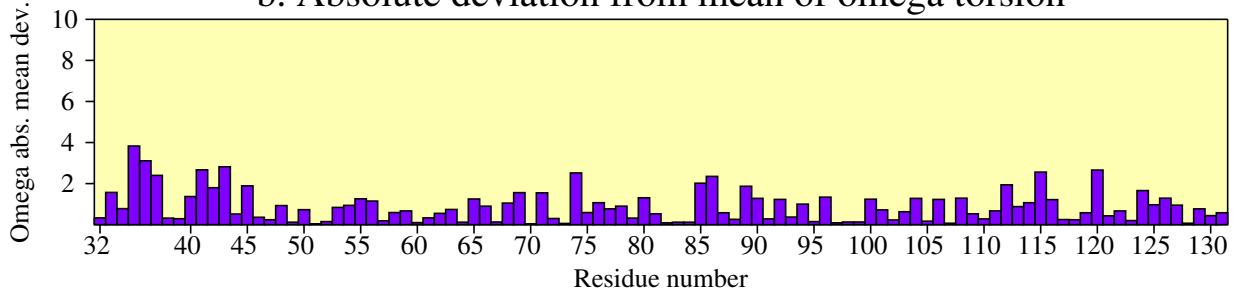
c = cis-peptide

Residue properties
1pp9

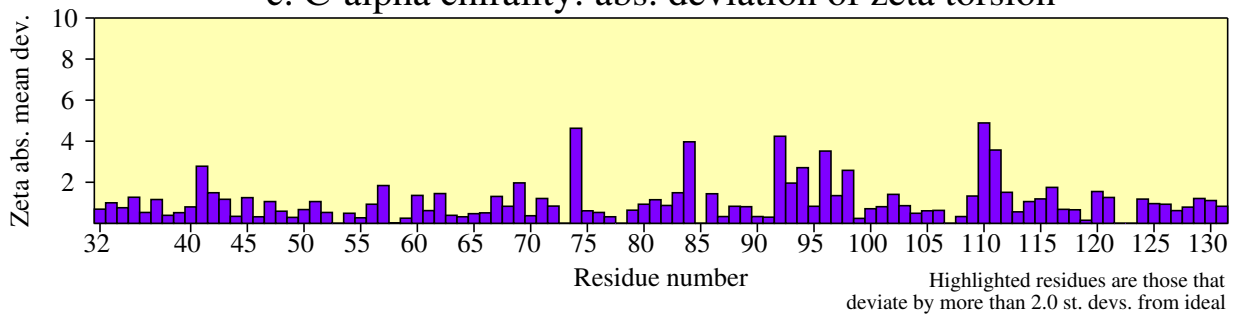
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

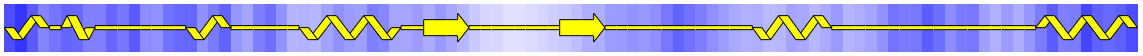


c. C-alpha chirality: abs. deviation of zeta torsion



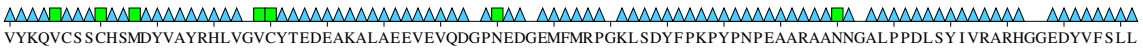
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



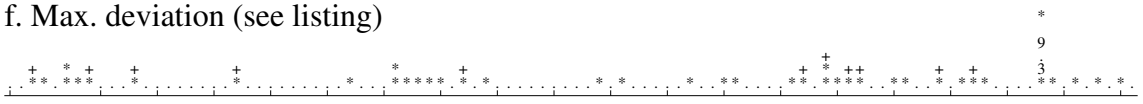
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

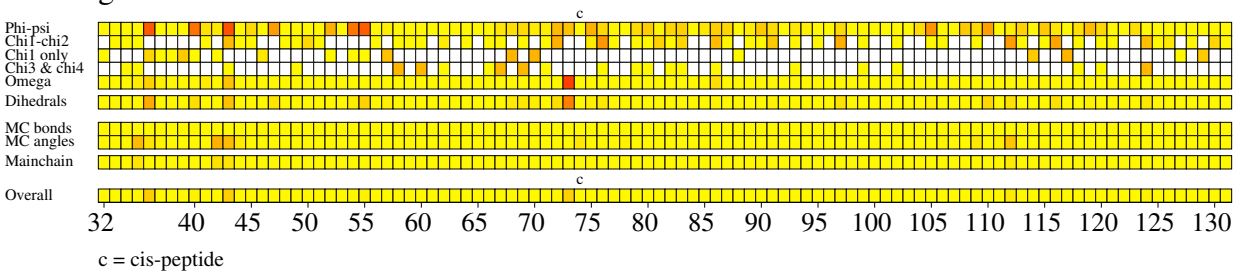


VYKQVCSSCHSMDYVAYRHLVGVCYTEDEAKALAEEVEVQDGPNEDGEMFMRPGKLSDYFPKPYPNPEAARAANNGALPPDLSYIVRARHGGEDYVFSLL

f. Max. deviation (see listing)



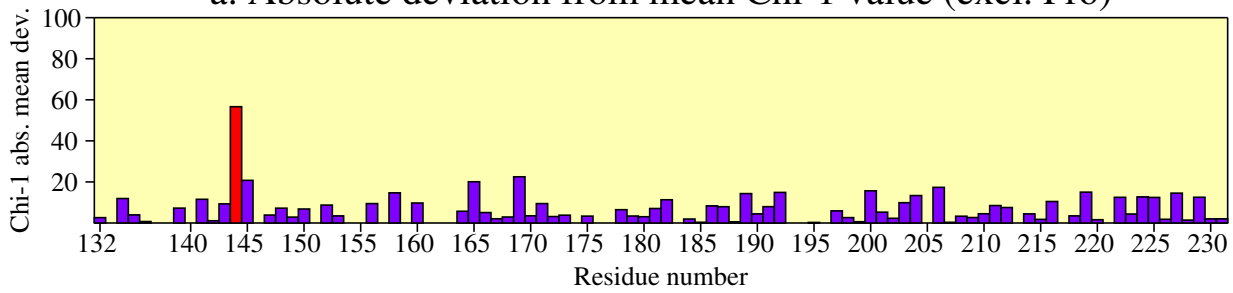
g. G-factors



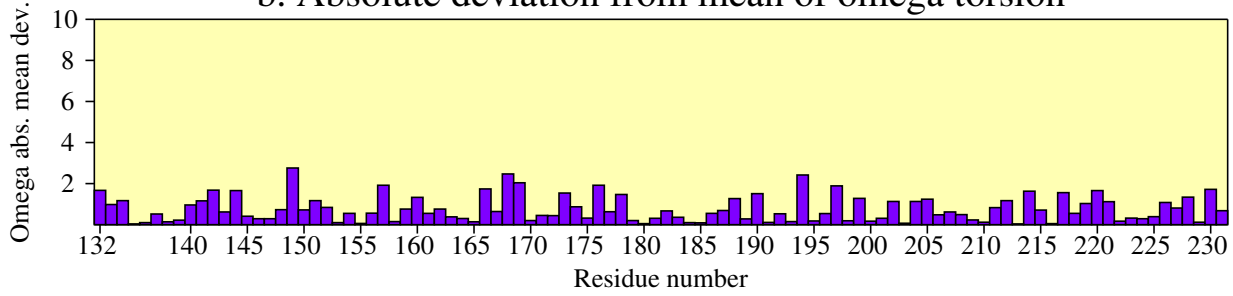
c = cis-peptide

Residue properties
1pp9

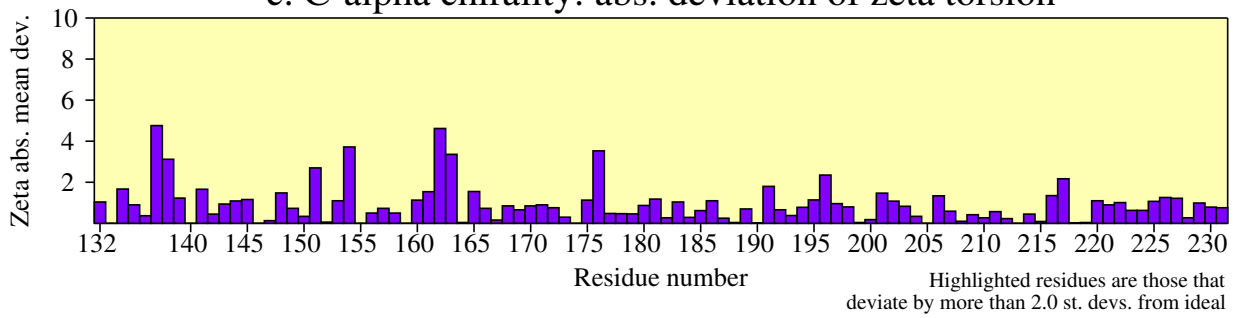
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

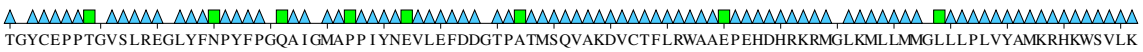


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

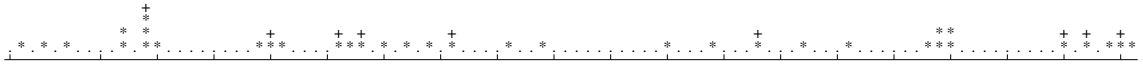
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

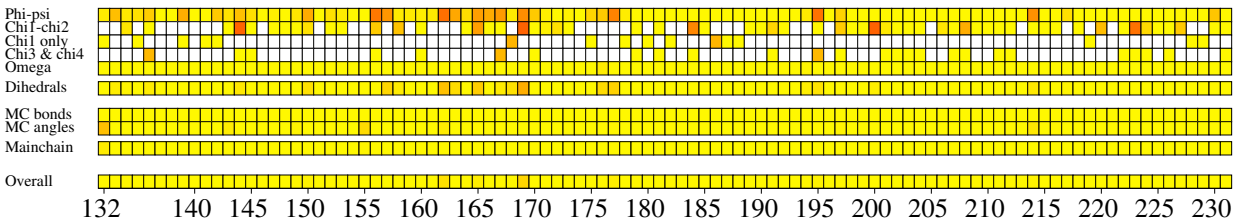


TGYCEPPTGVSLREGLYFNPYFPGQAIGMAPPIYNEVLEFDDGTPATMSQVAKDVCTFLRWAAEPEHDHRKRMGLKMLLMMGLLLPLVYAMKRHKWSVLK

f. Max. deviation (see listing)

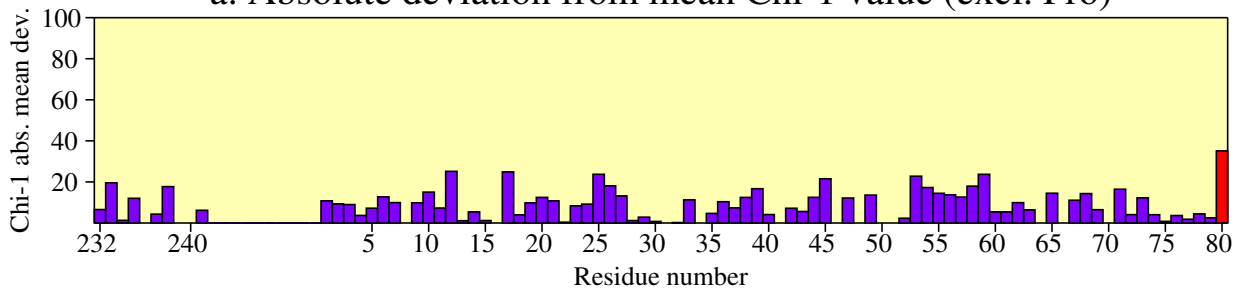


g. G-factors

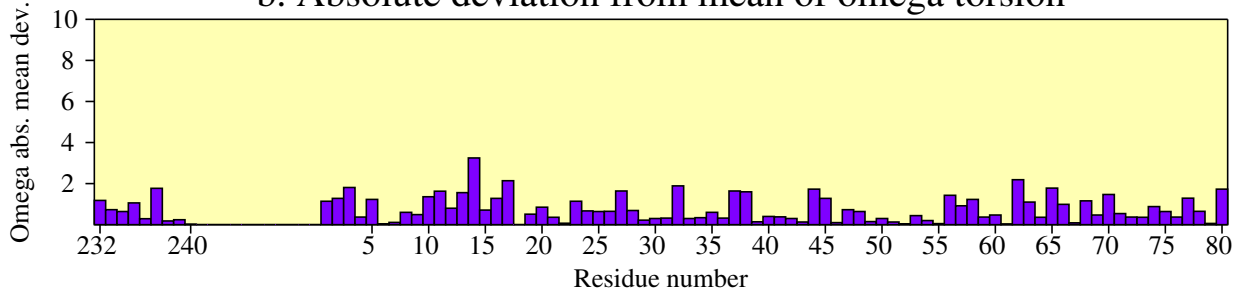


Residue properties
1pp9

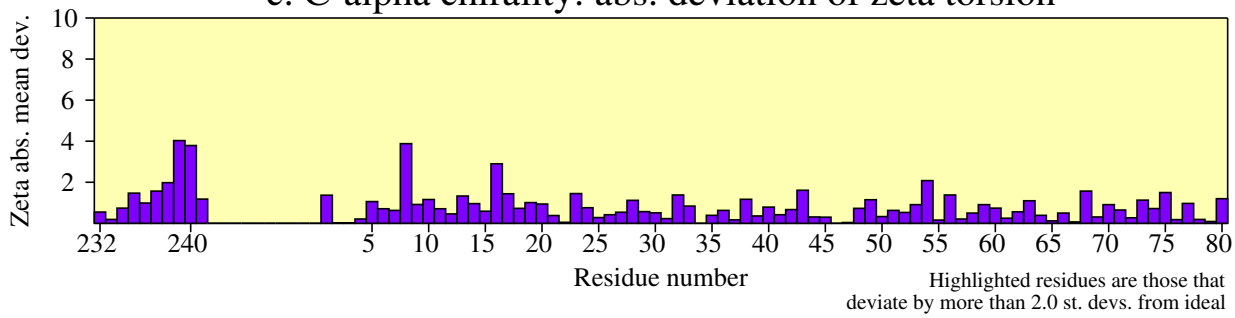
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



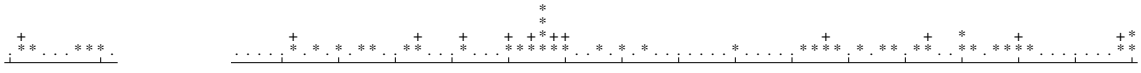
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

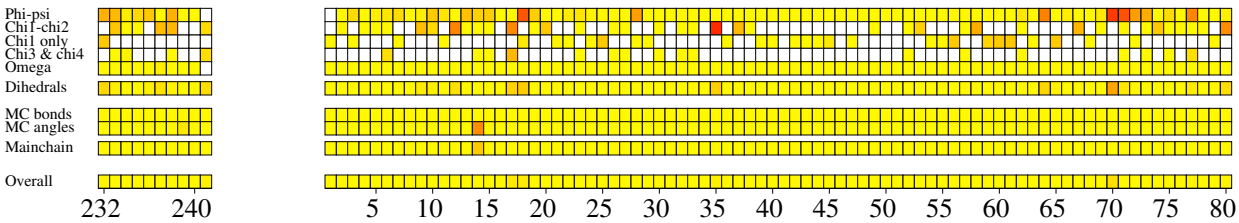
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

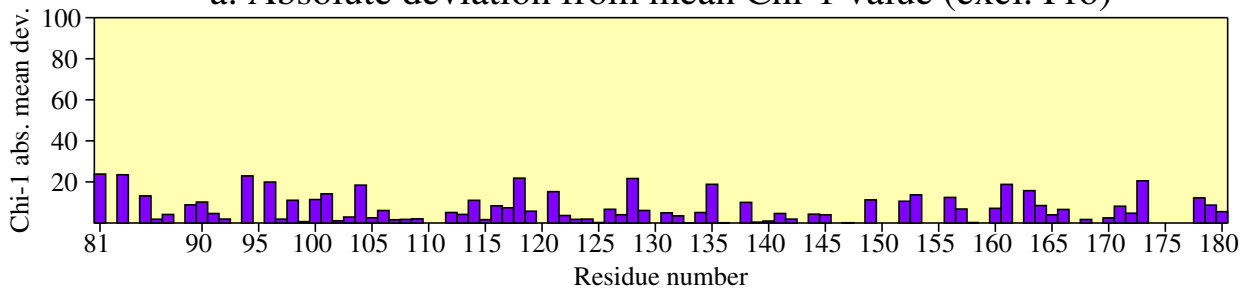


g. G-factors

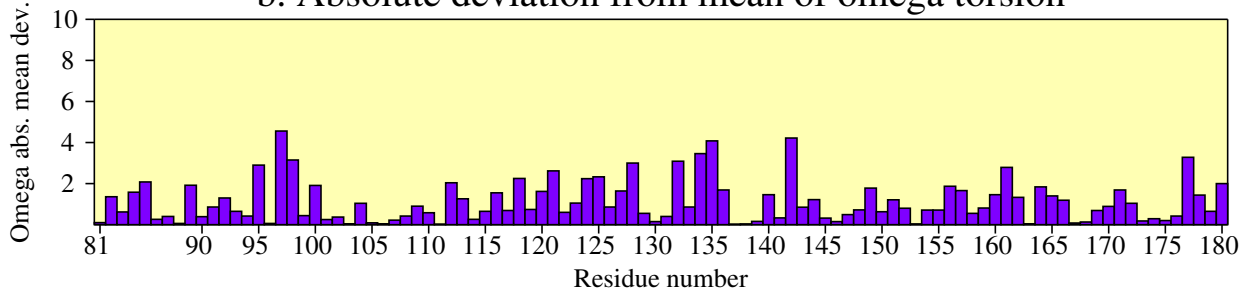


Residue properties
1pp9

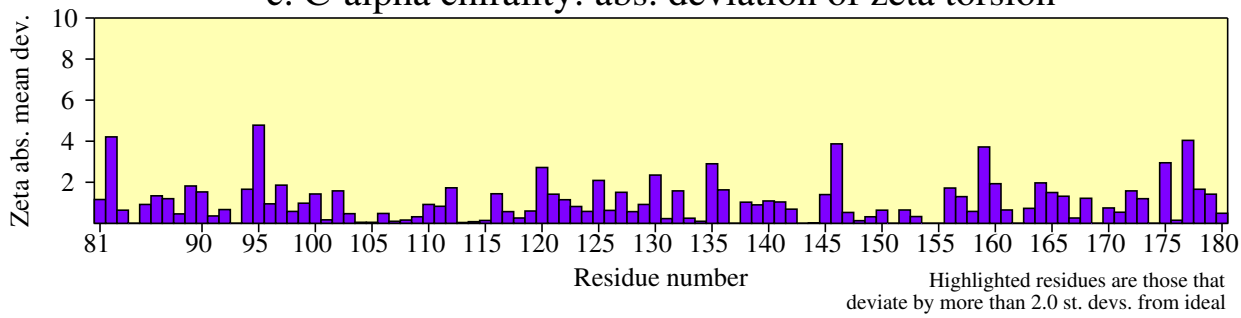
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

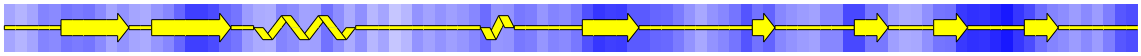


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



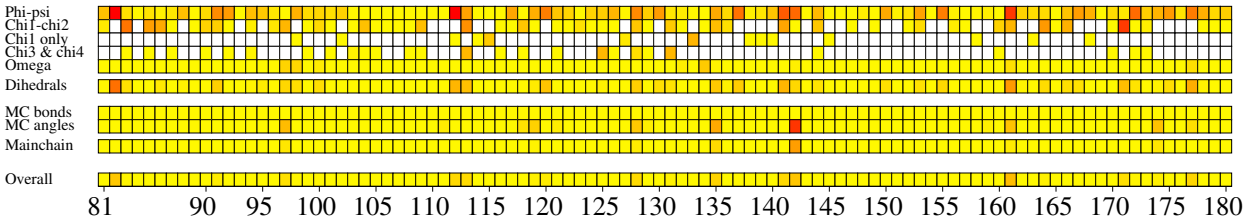
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

I P E G K N M A F K W R G K P L F V R H R T K K E I D Q E A A V E V S Q L R D P Q H D L E R V K K P E W V I L I G V C T H L G C V P I A N A G D F G G Y Y C P C H G S H Y D A S G R I R K G P A P L N L

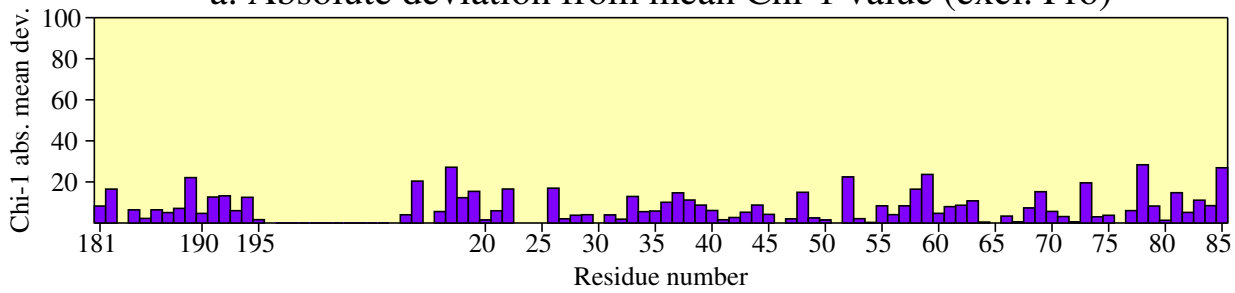
f. Max. deviation (see listing)

g. G-factors

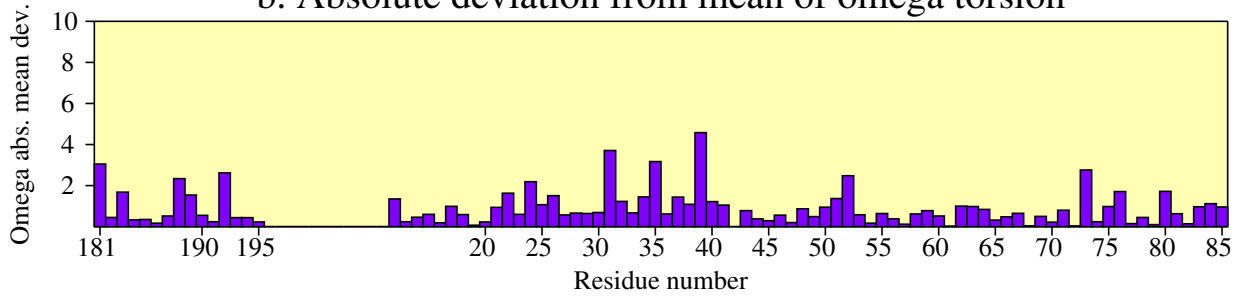


Residue properties
1pp9

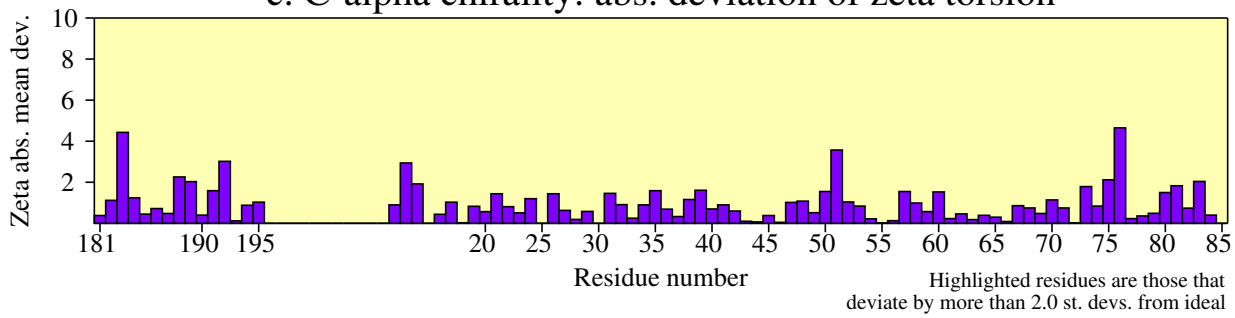
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



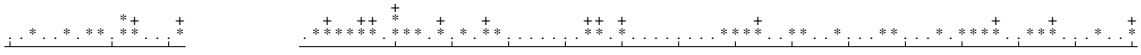
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

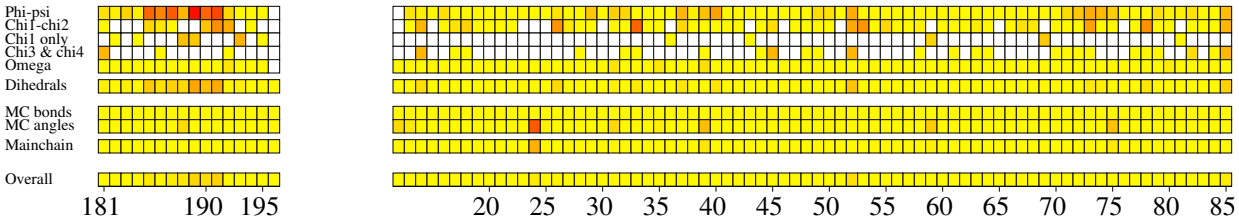
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

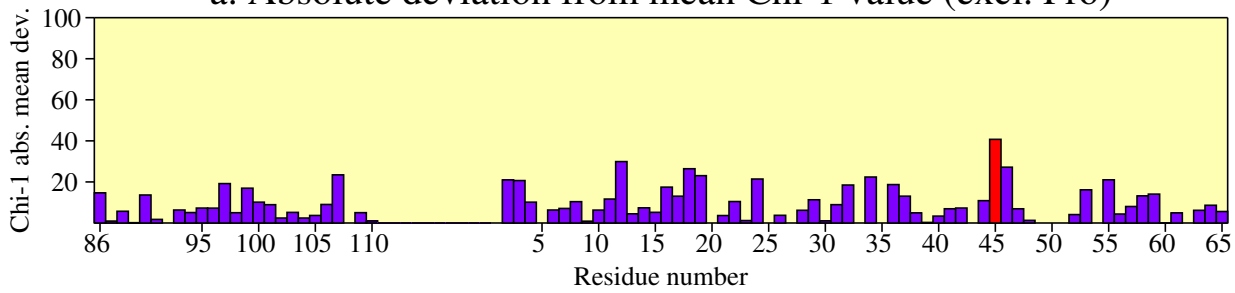


g. G-factors

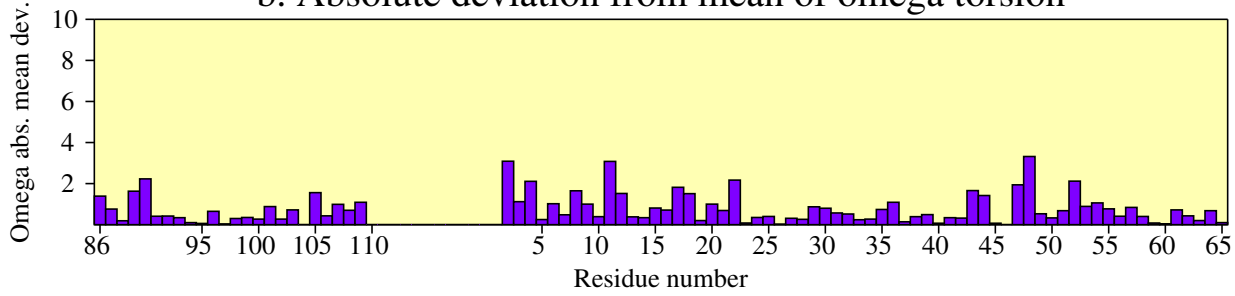


Residue properties
1pp9

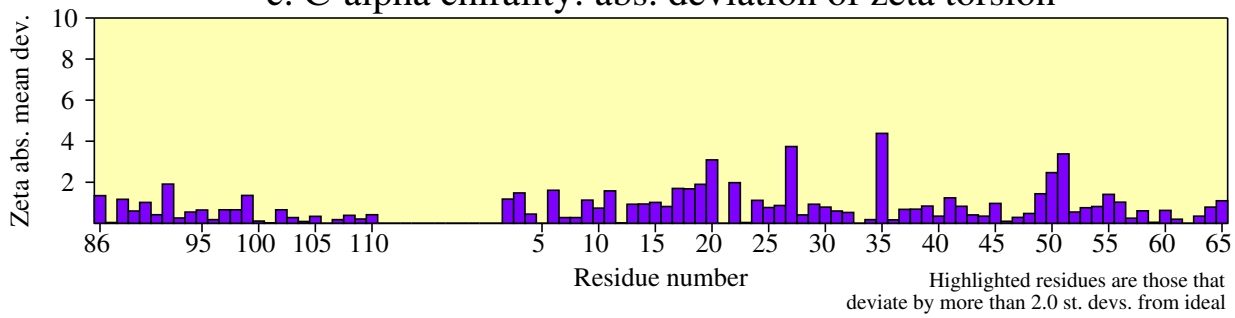
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

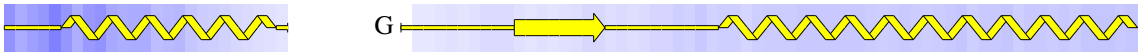


c. C-alpha chirality: abs. deviation of zeta torsion



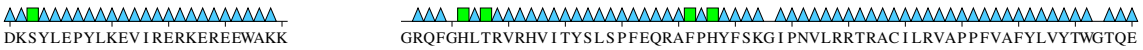
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



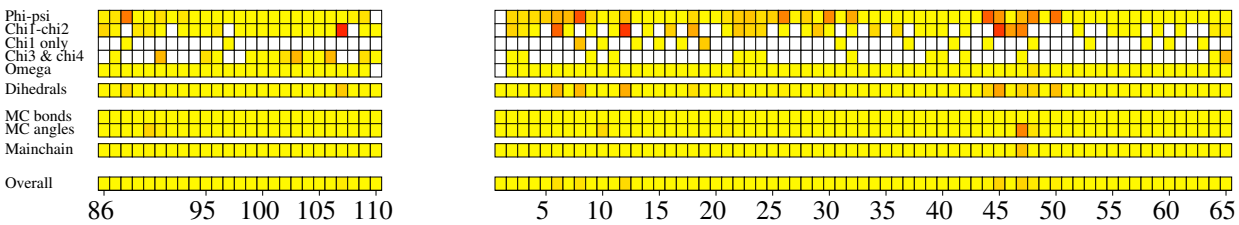
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed



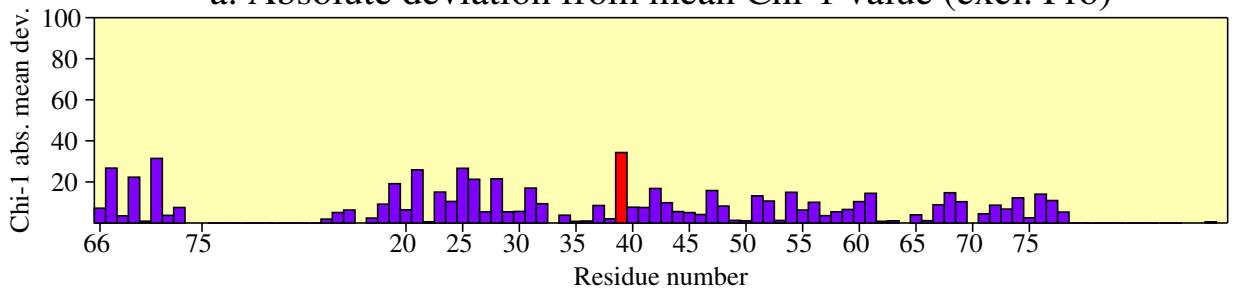
f. Max. deviation (see listing)

g. G-factors

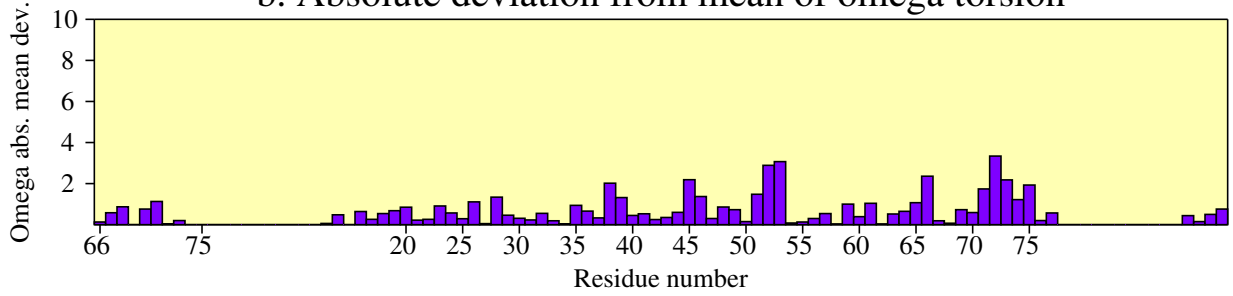


Residue properties
1pp9

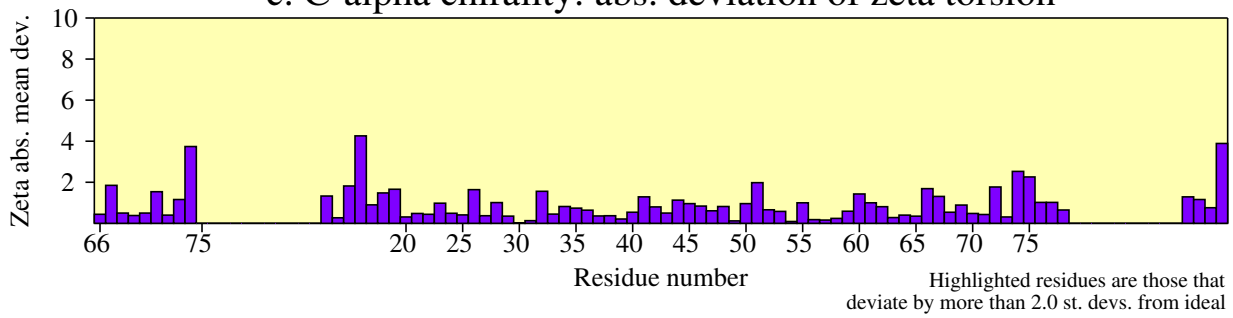
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

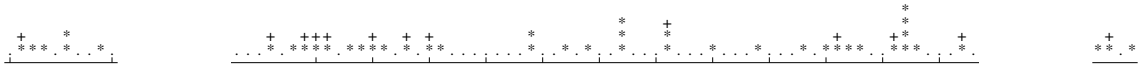


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

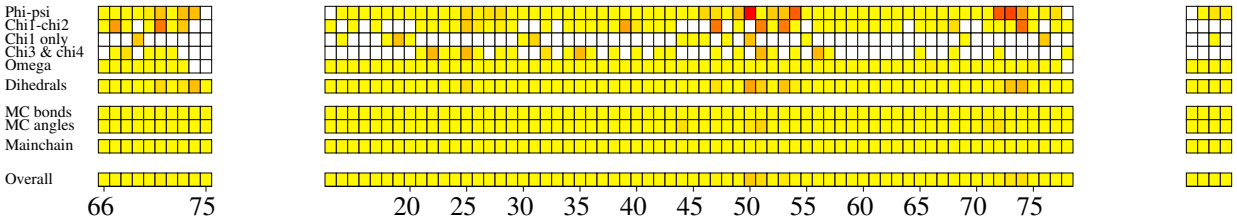
d. Secondary structure & estimated accessibility

e. Sequence & Ramachandran regions

f. Max. deviation (see listing)



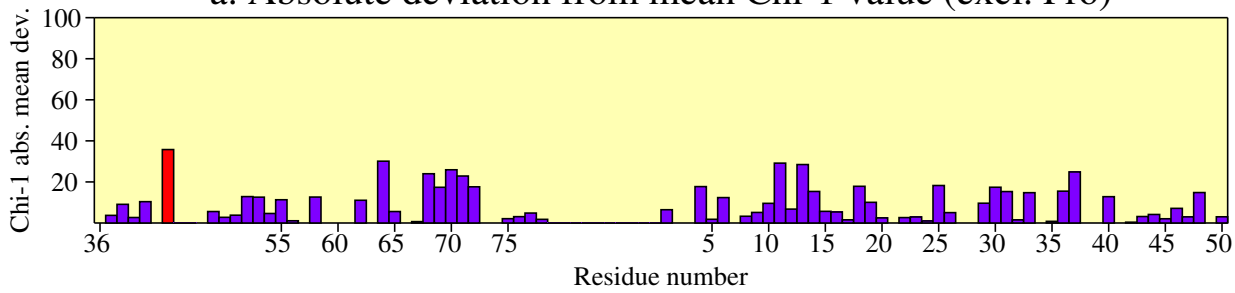
g. G-factors



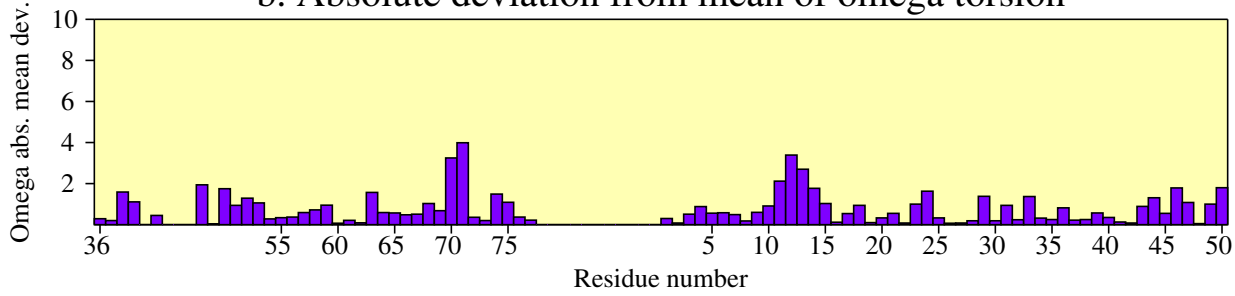
Residue properties

1pp9

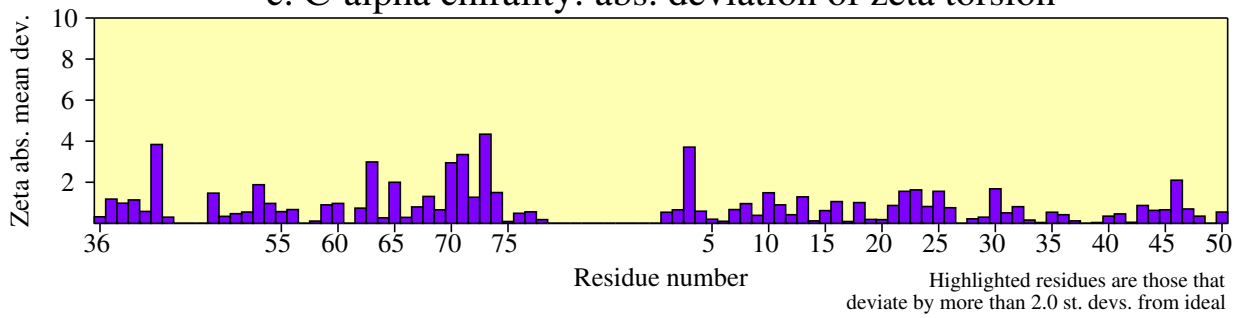
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

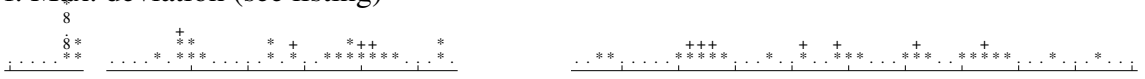
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

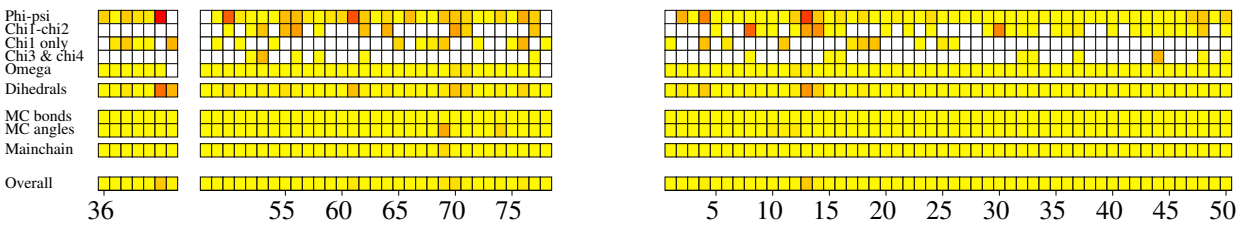
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

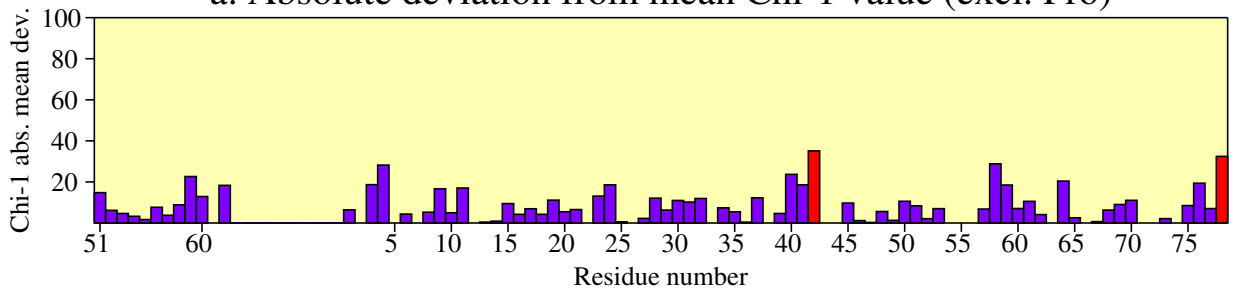


g. G-factors

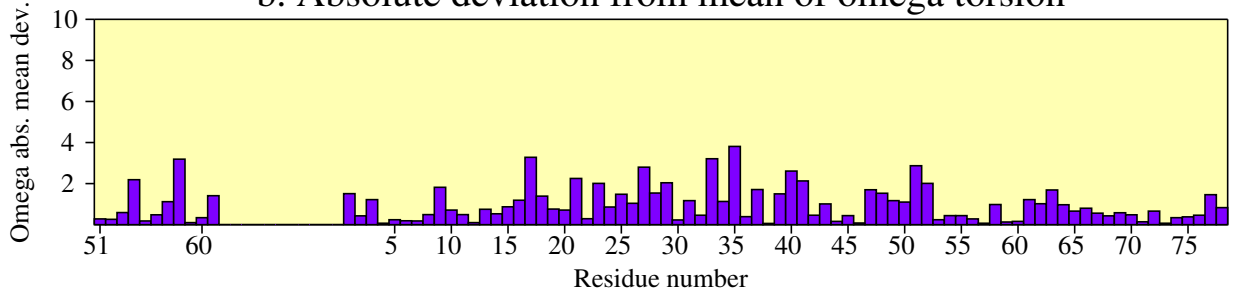


Residue properties
1pp9

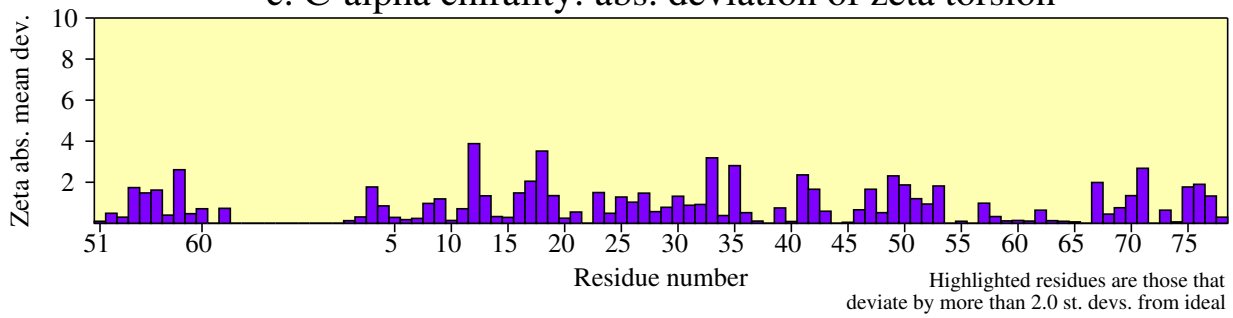
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



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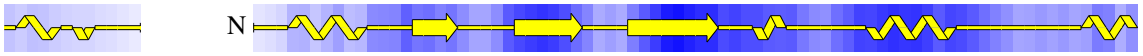
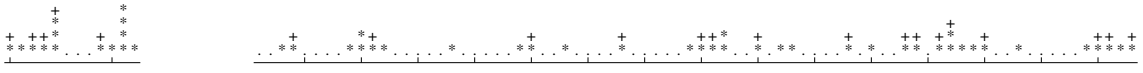
d. Secondary structure & estimated accessibility



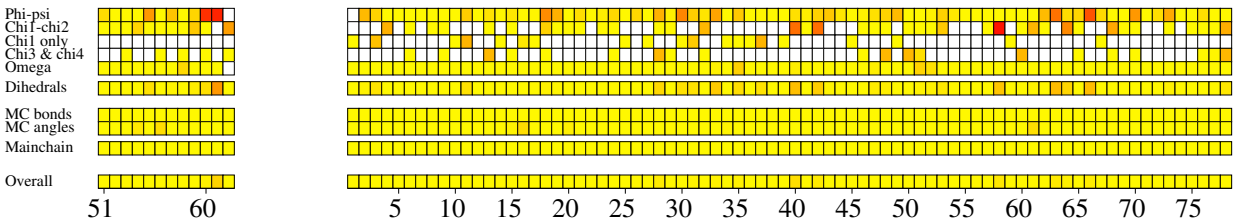
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

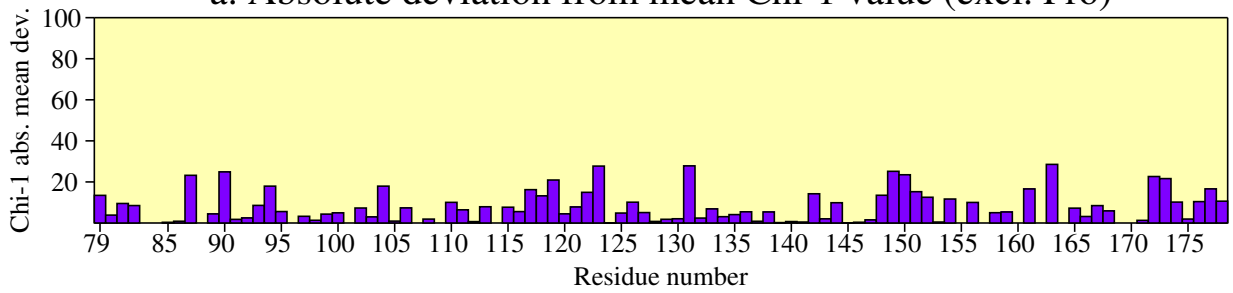


g. G-factors

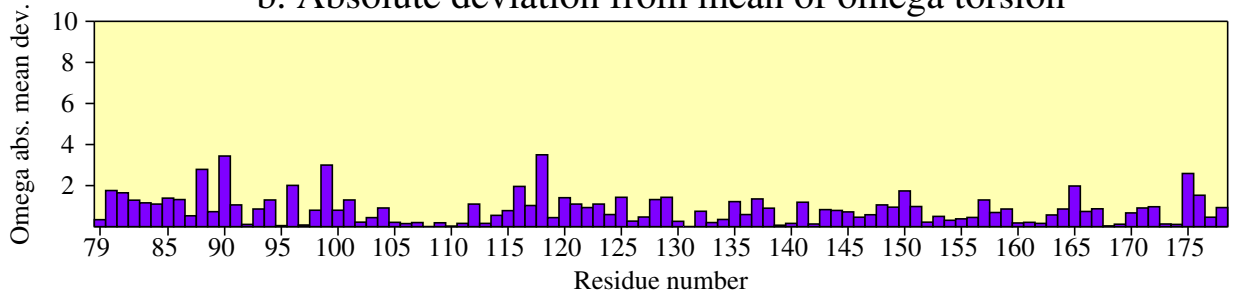


Residue properties
1pp9

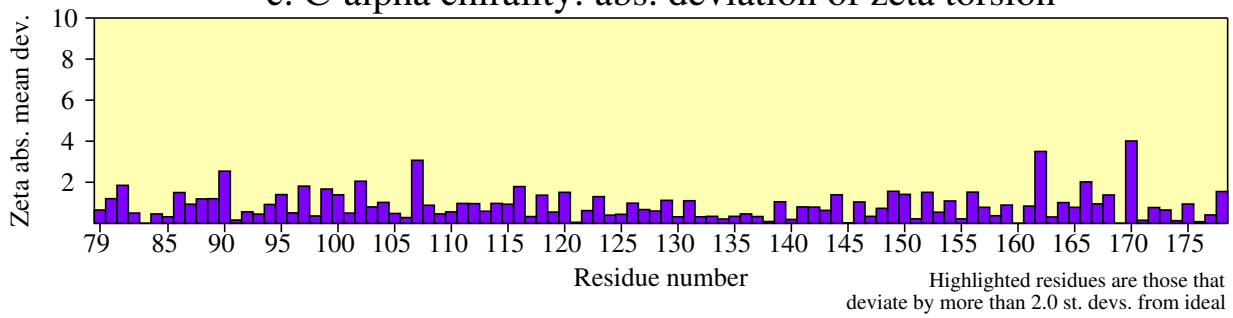
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

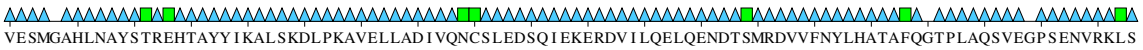


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

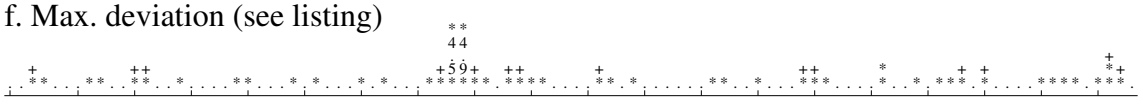
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

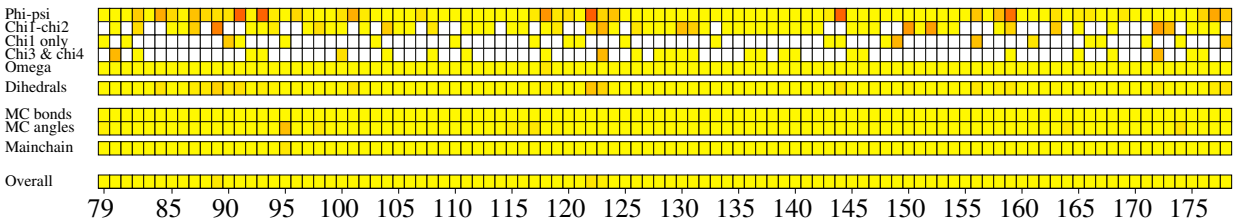
e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)



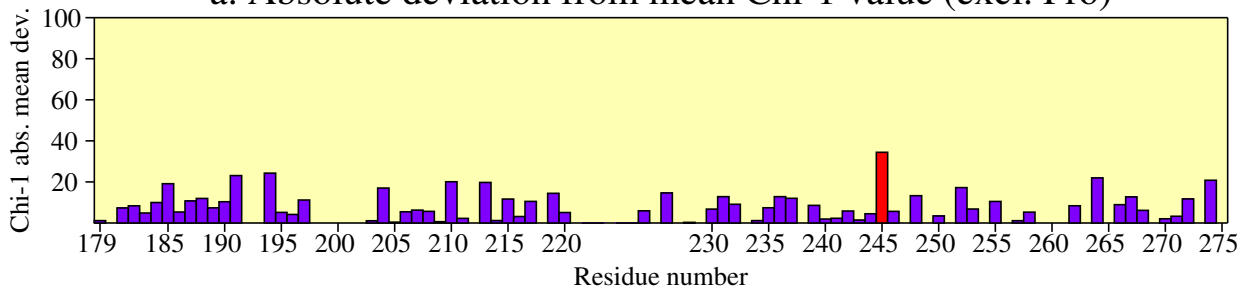
g. G-factors



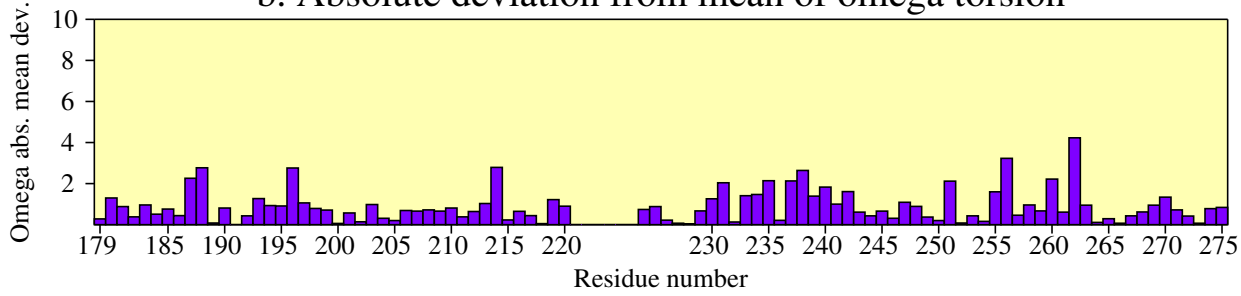
Residue properties

1pp9

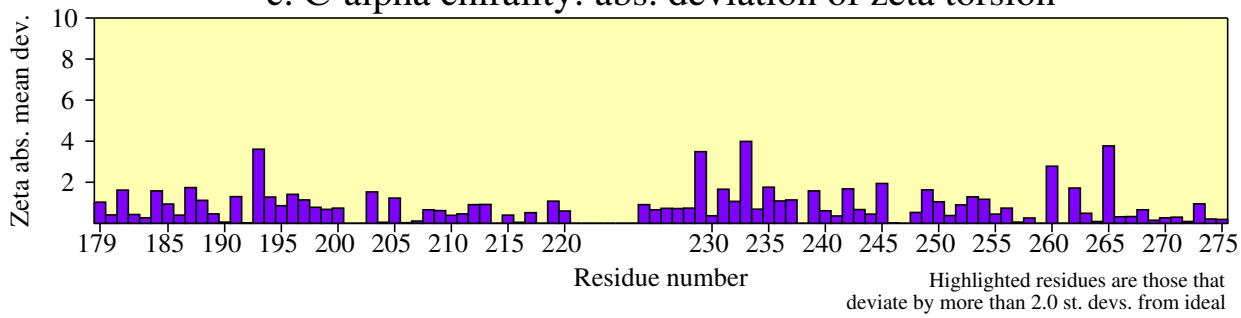
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

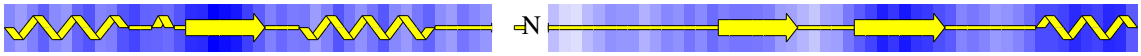


c. C-alpha chirality: abs. deviation of zeta torsion



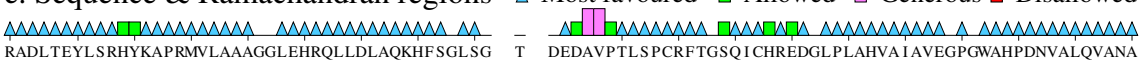
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

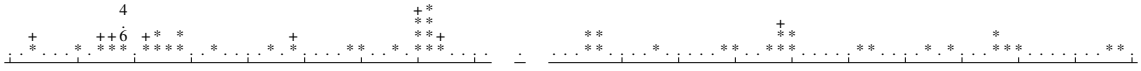


e. Sequence & Ramachandran regions

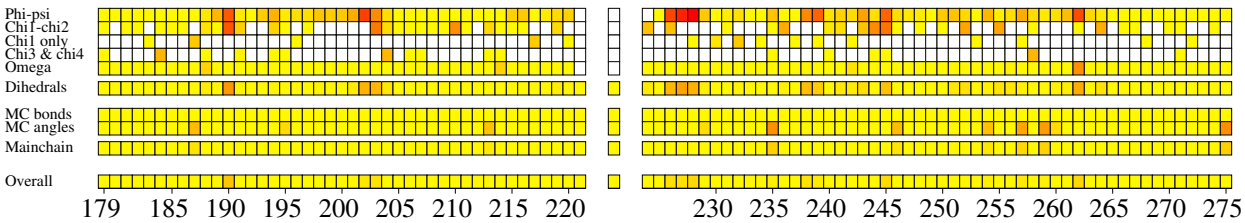
Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)

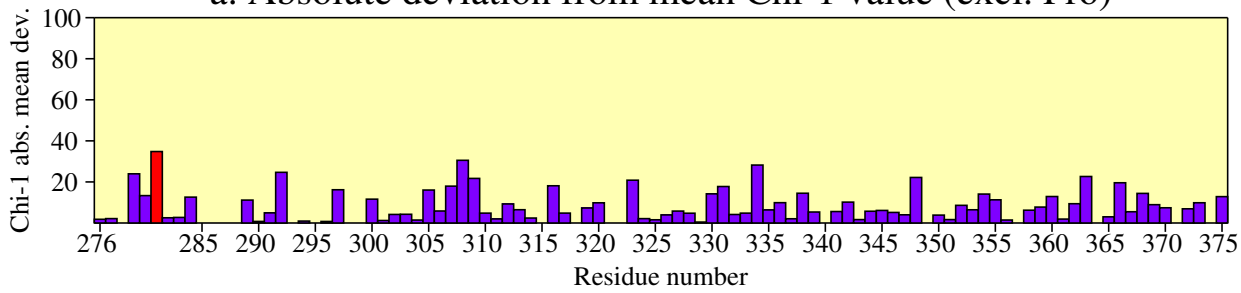


g. G-factors

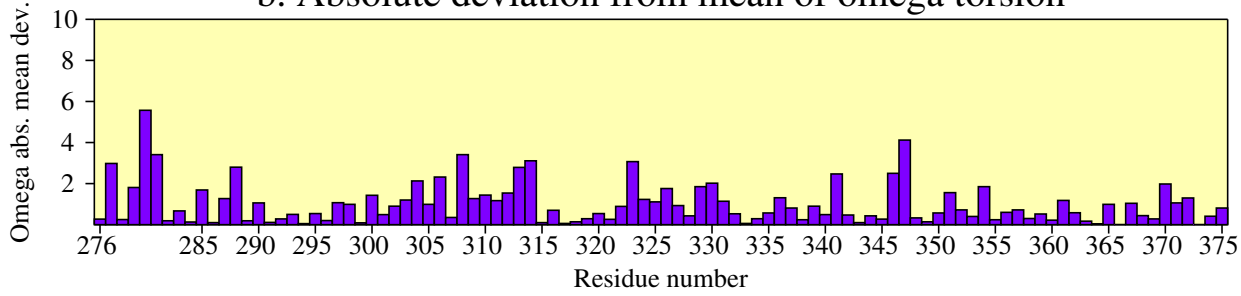


Residue properties
1pp9

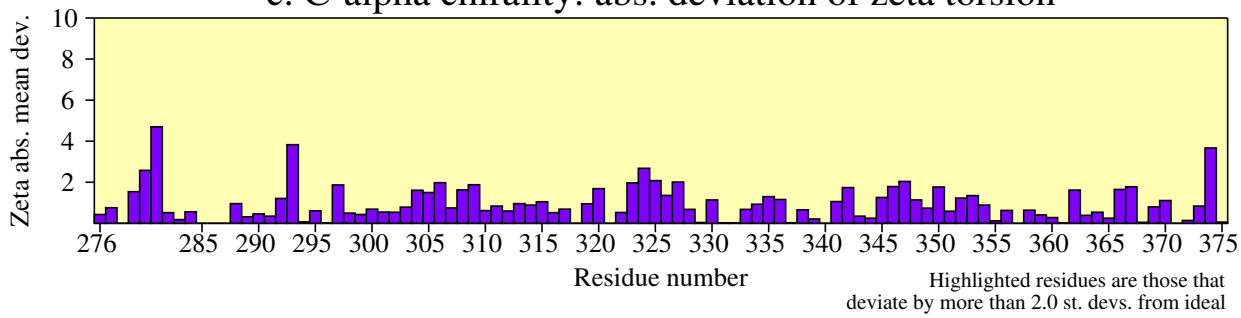
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

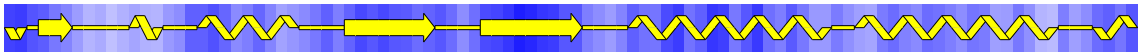


c. C-alpha chirality: abs. deviation of zeta torsion



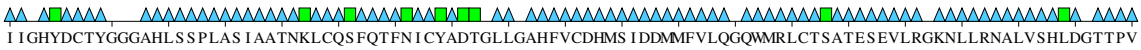
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



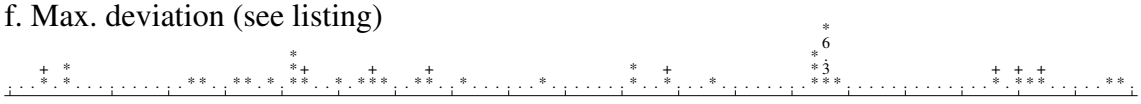
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

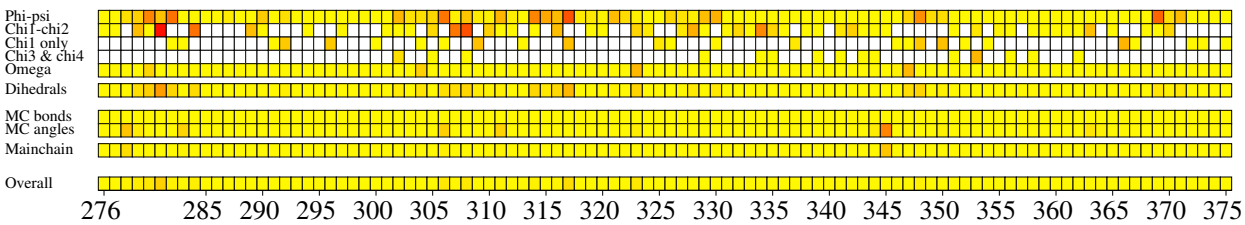


I I G H Y D C T Y G G G A H L S S P L A S I A A T N K L C Q S F Q T F N I C Y A D T G L L G A H F V C D H M S I D D M M F V L Q G Q W M R L C T S A T E S E V L R G K N L L R N A L V S H L D G T T P V

f. Max. deviation (see listing)



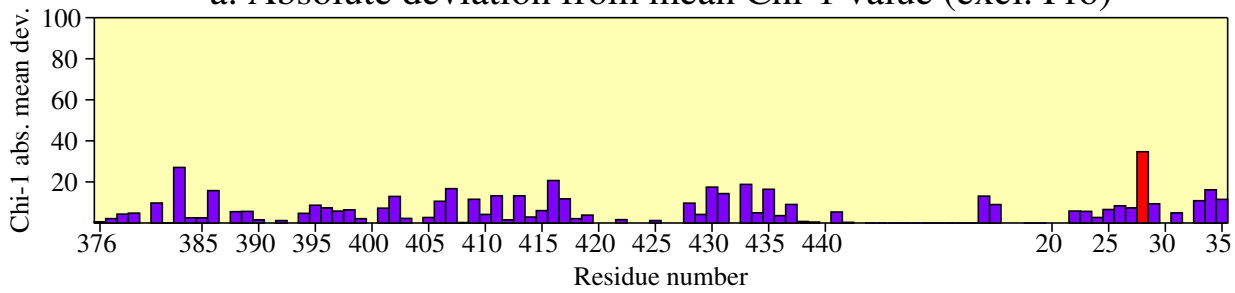
g. G-factors



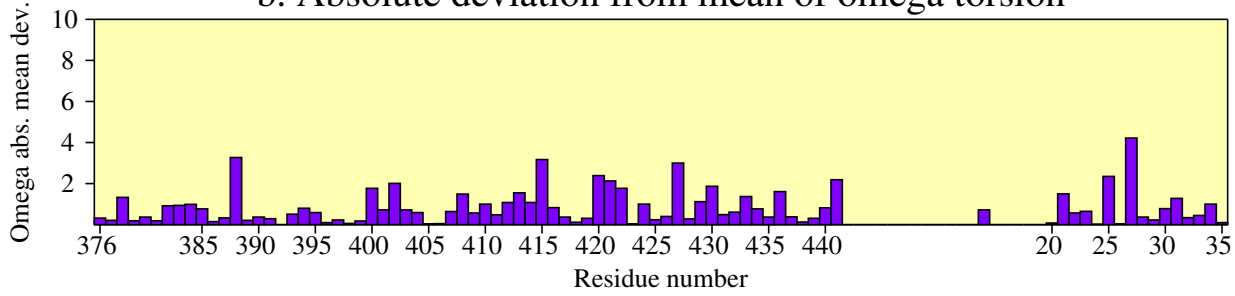
Residue properties

1pp9

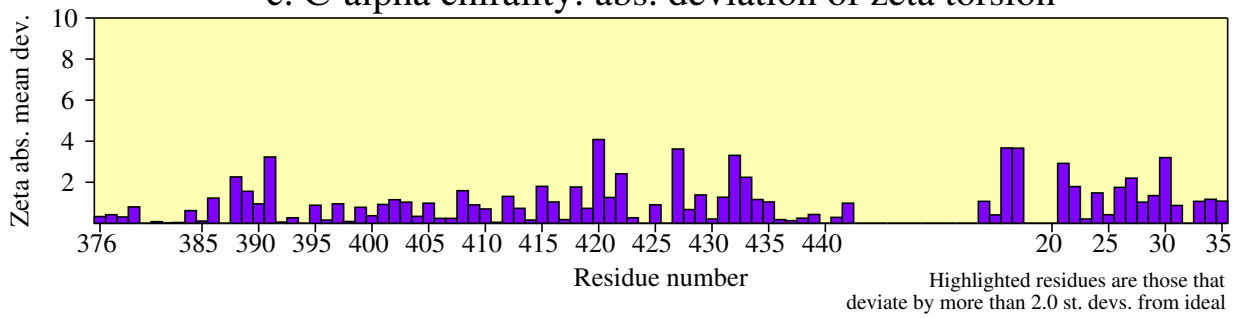
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

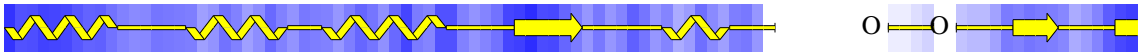


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

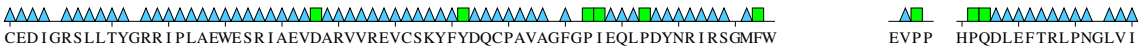
d. Secondary structure & estimated accessibility



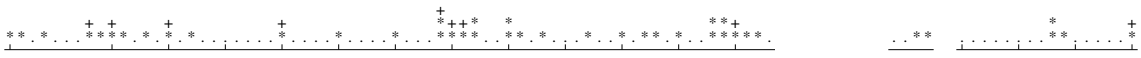
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

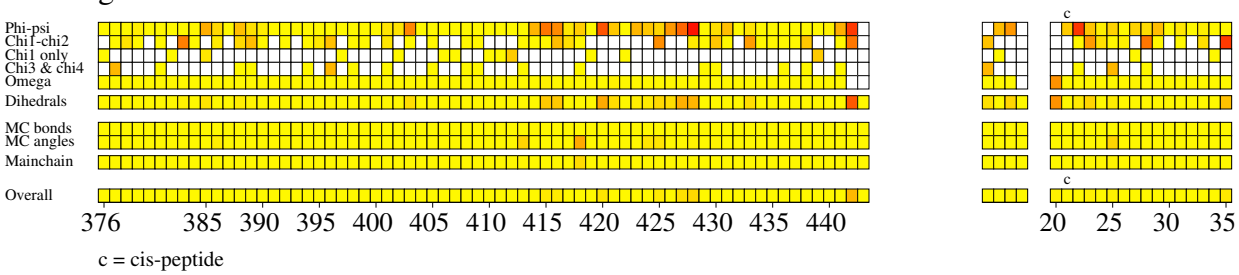
Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)



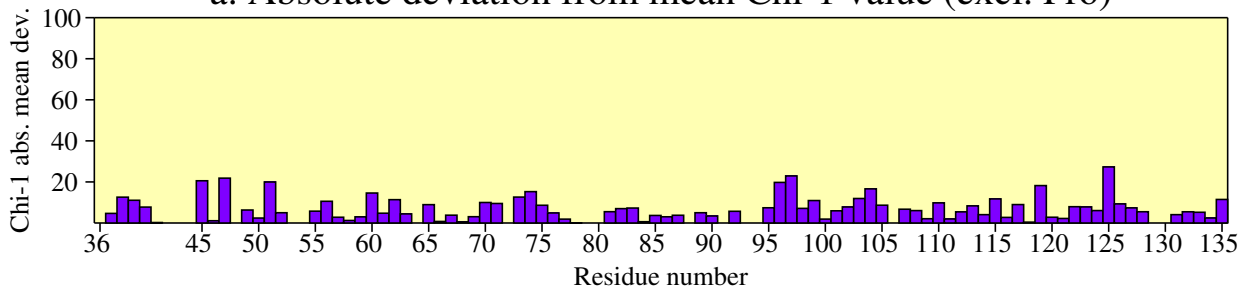
g. G-factors



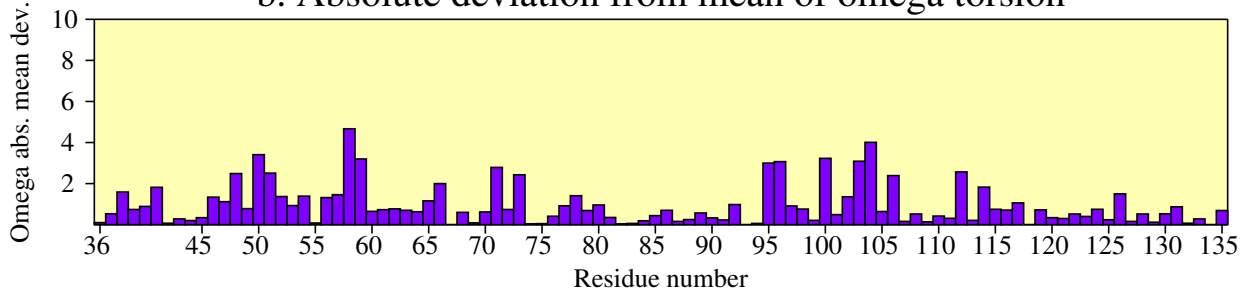
c = cis-peptide

Residue properties
1pp9

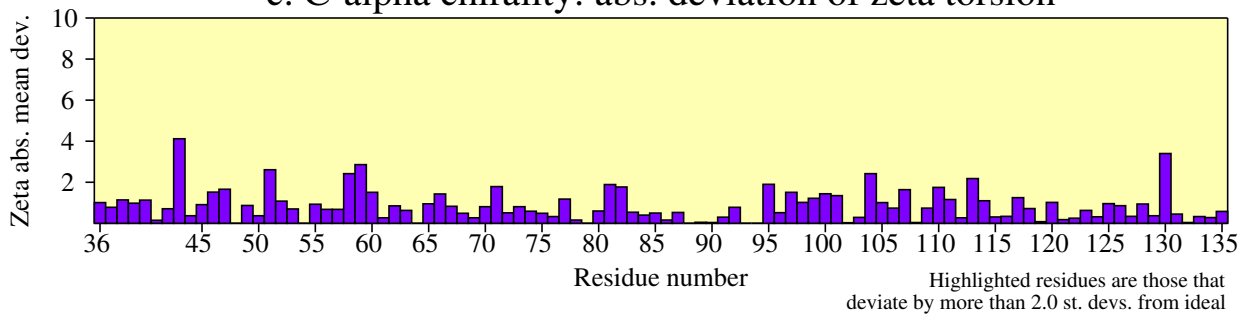
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

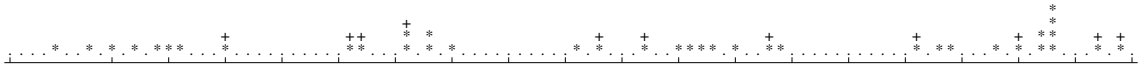
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

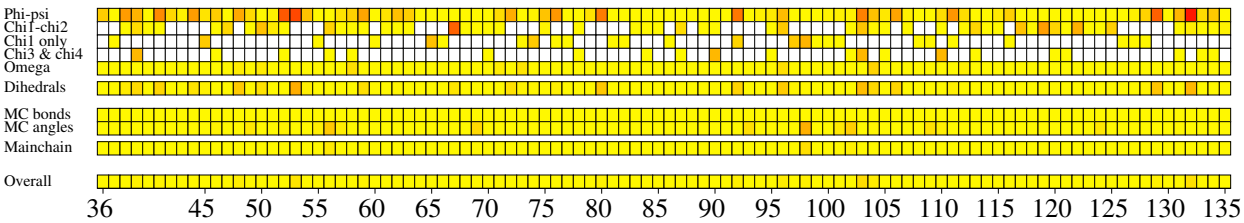
Most favoured Allowed Generous Disallowed

ASLENYAPASRIGLFIKAGSRYENSNNLGTSHLLRLASSLTTKGASSFKITRGIEAVGGKLSVTSTRENMAYTVECLRDDVDILMEFLLNVTTAPEFRRW

f. Max. deviation (see listing)

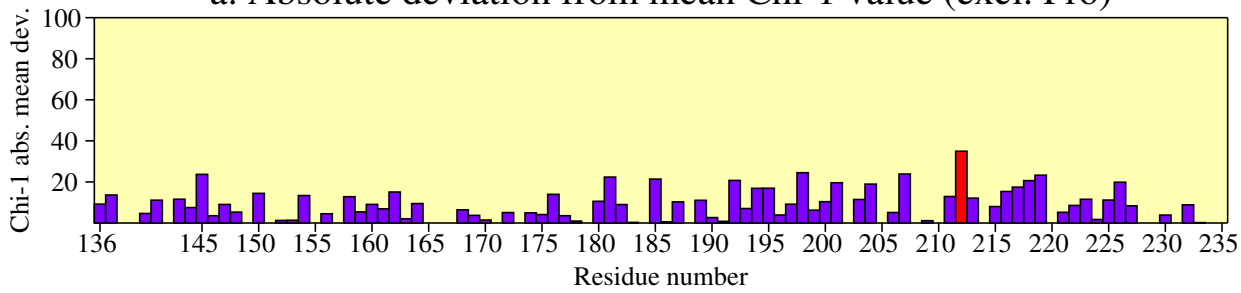


g. G-factors

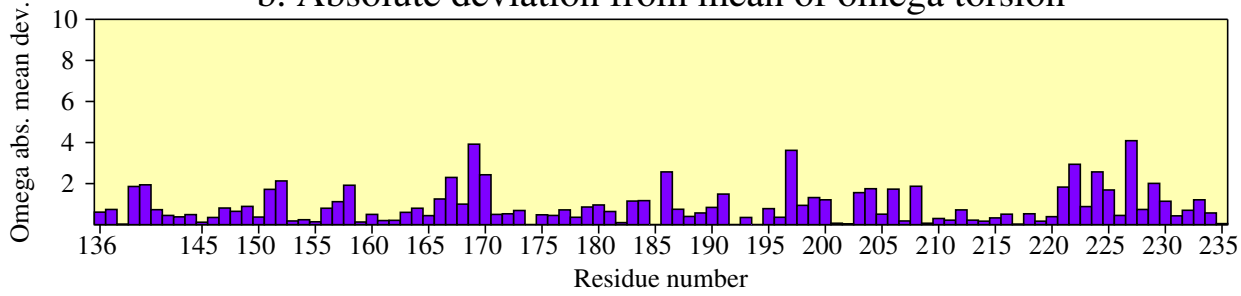


Residue properties
1pp9

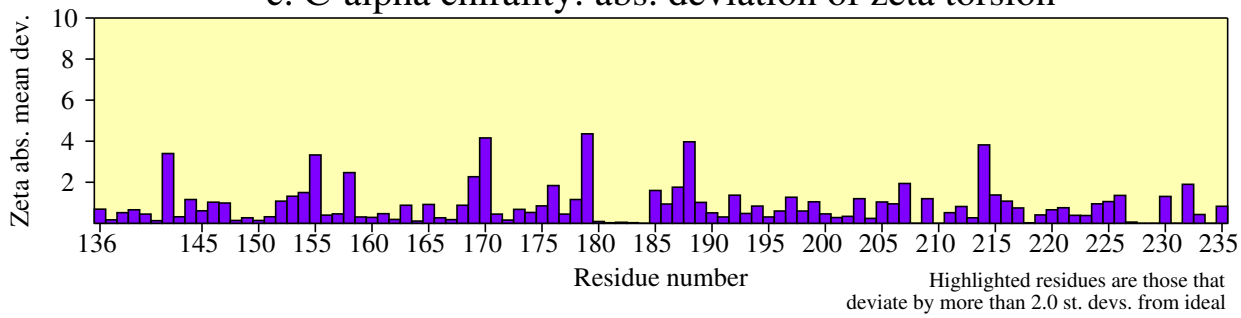
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

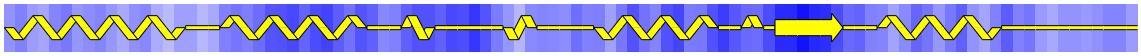


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



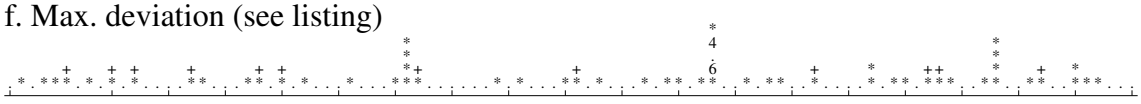
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

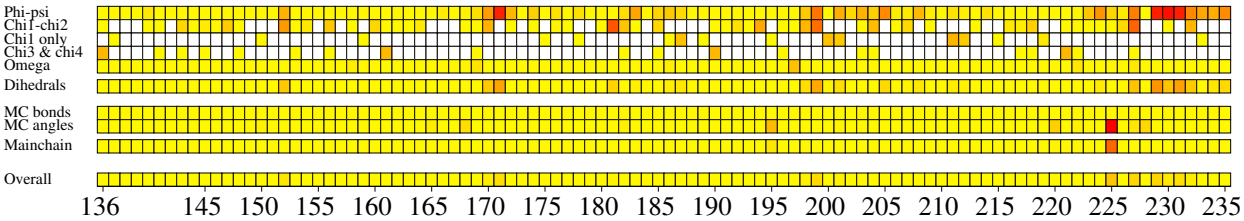
Most favoured Allowed Generous Disallowed

EVAALQPQLR IDKAVALQNPQAHV IENLHAAAYRNALANSLYCPDYR IGKVTPVELHDYVQNHFTSARMAL IGLGVSHPVLKQVAEQFLN IRGGLGLSGA

f. Max. deviation (see listing)

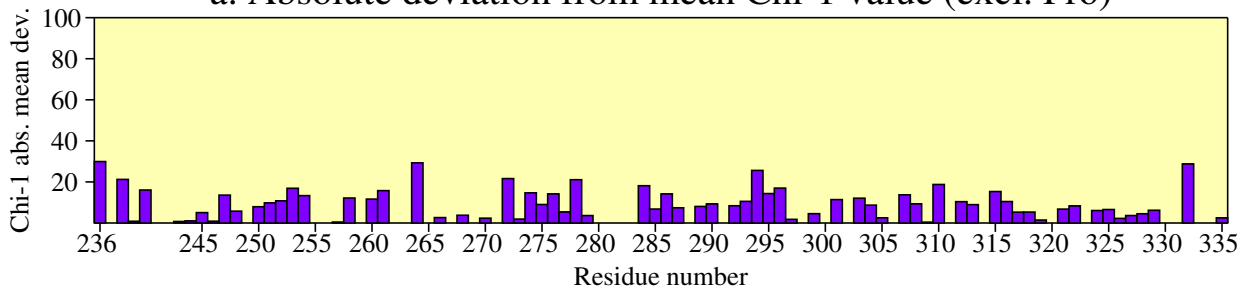


g. G-factors

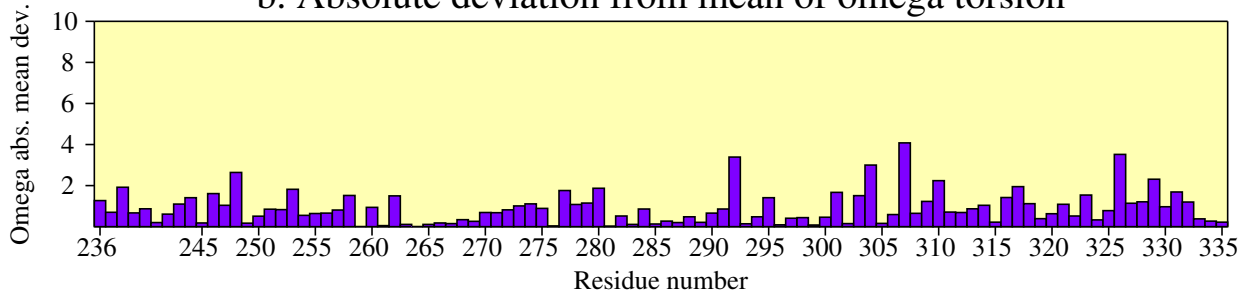


Residue properties
1pp9

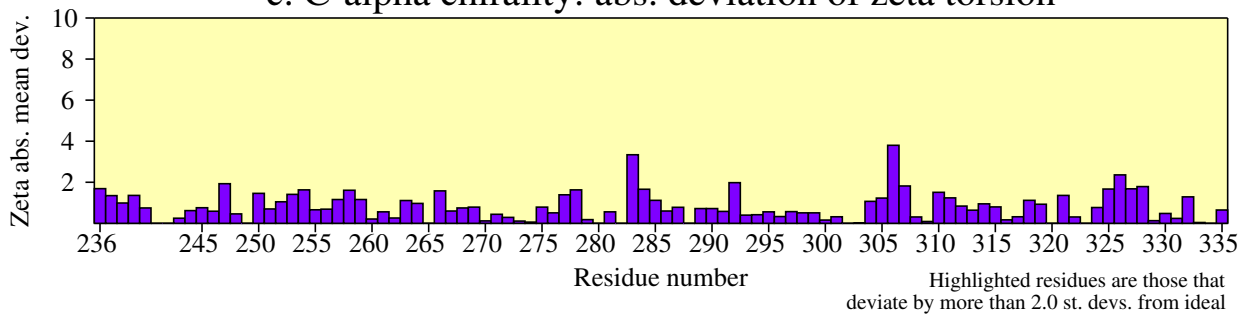
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

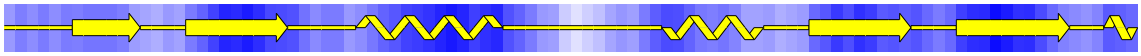


c. C-alpha chirality: abs. deviation of zeta torsion



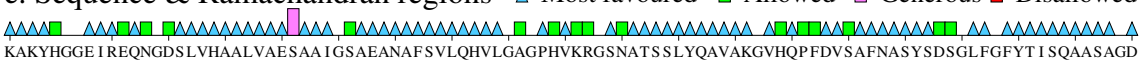
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



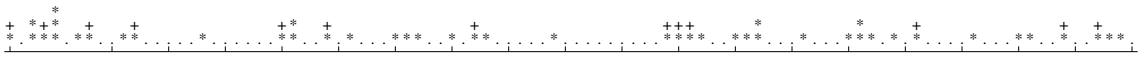
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

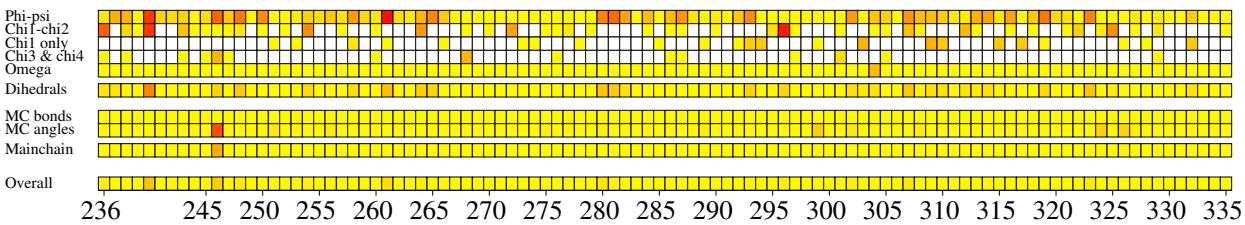


KAKYHGGE I REQNGDS L VHAALVAE S AA I GS AEANAF S VLQHVLGAGPHVKRGSNAT S S L YQAVAKGVHQP F DVS AF NAS Y S DSGL F GF Y T I SQAAS AGD

f. Max. deviation (see listing)

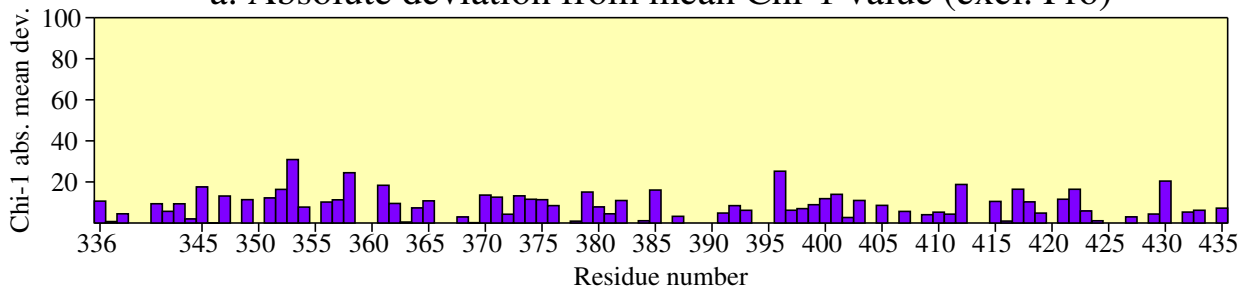


g. G-factors

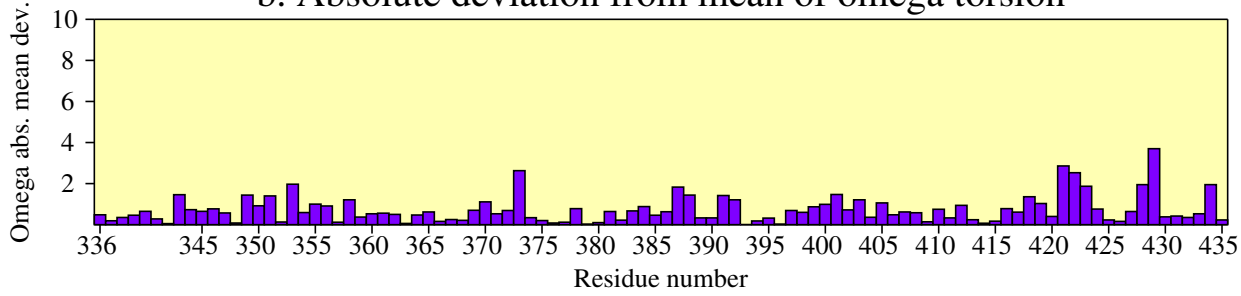


Residue properties
1pp9

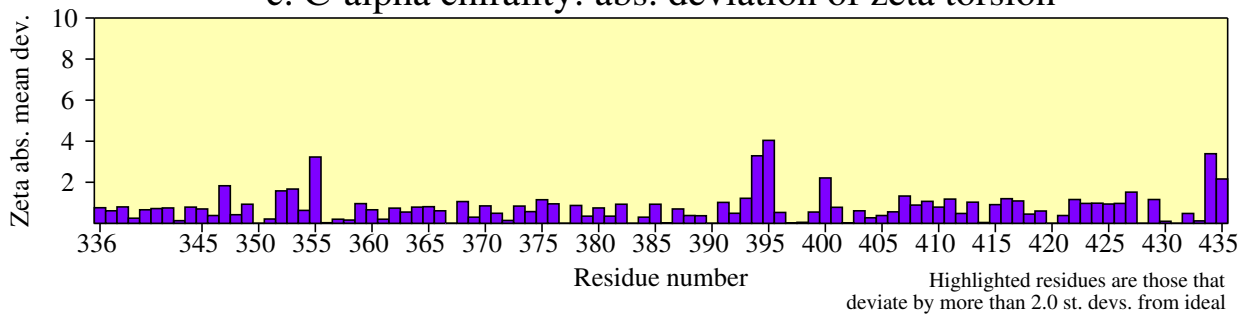
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

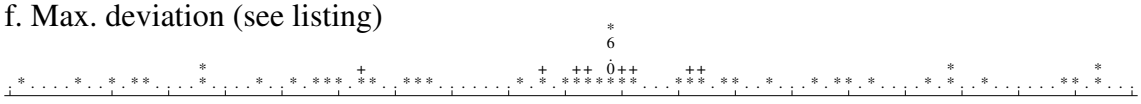
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

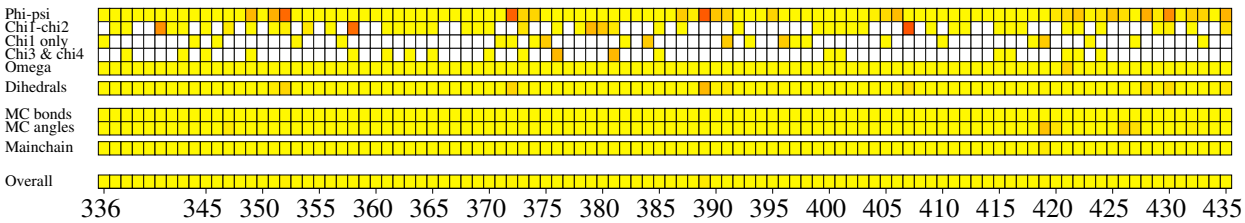
e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

V I K A A Y N Q V K T I A Q G N L S N P D V Q A A K N K L K A G Y L M S V E S S E G F L D E V G S Q A L A A G S Y T P P S T V L Q Q I D A V A D A D V I N A A K K F V S G R K S M A A S G N L G H T P F

f. Max. deviation (see listing)

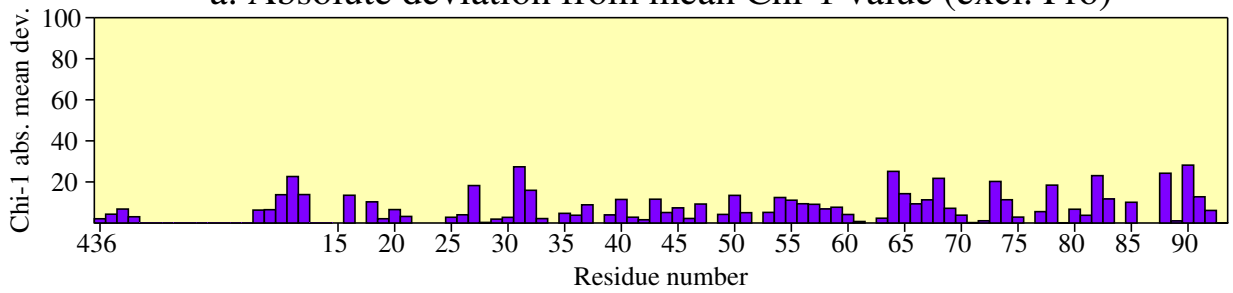


g. G-factors

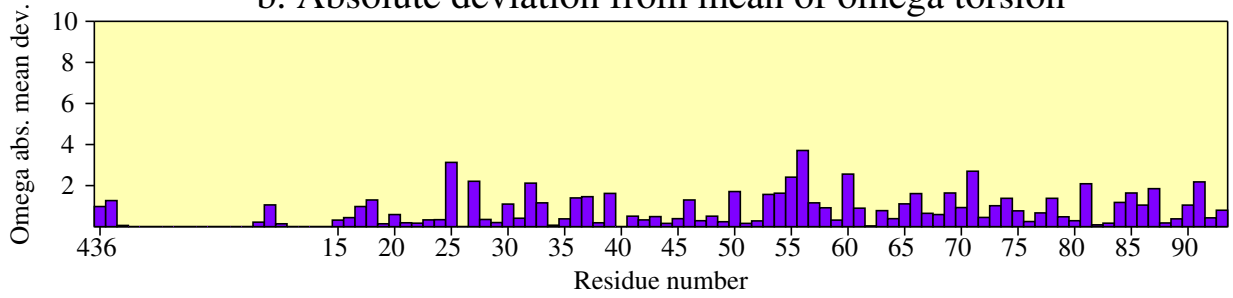


Residue properties
1pp9

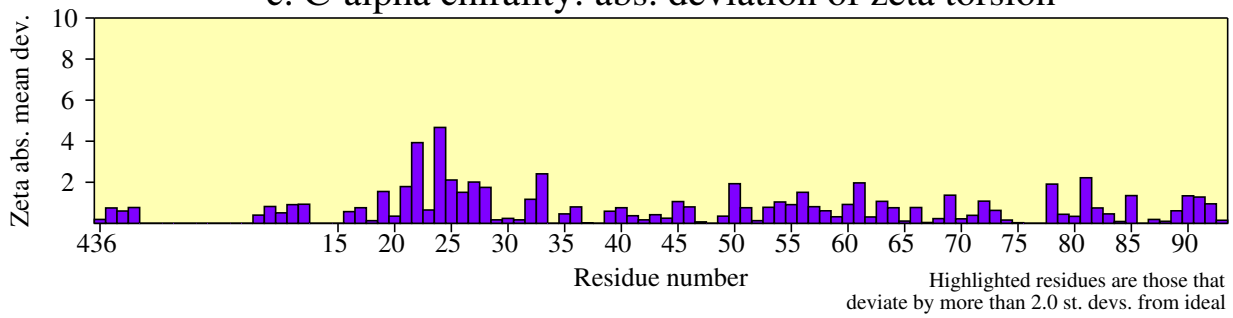
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

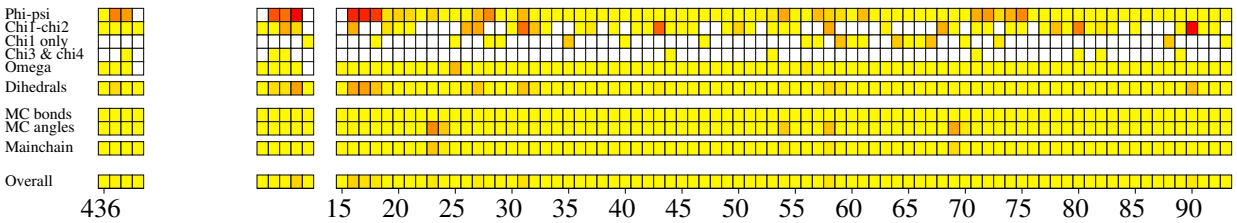
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

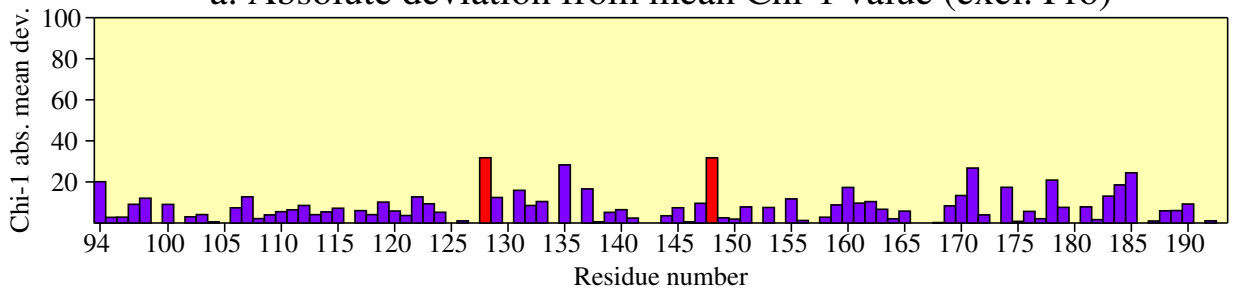
f. Max. deviation (see listing)

g. G-factors

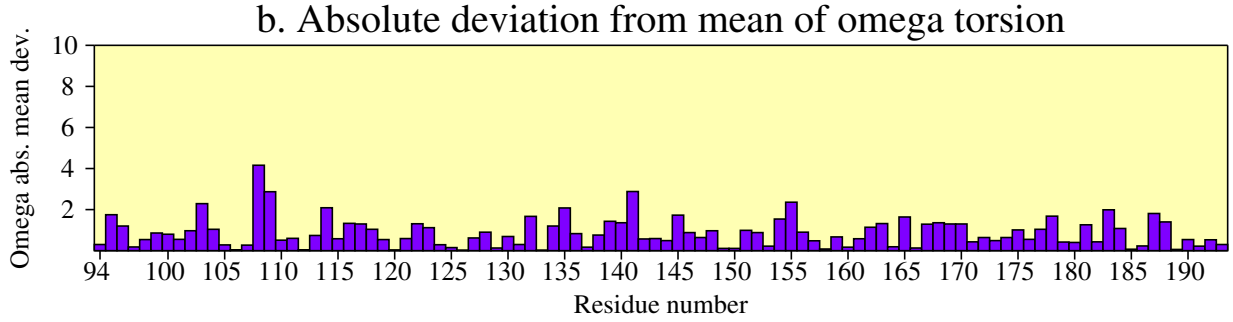


Residue properties
1pp9

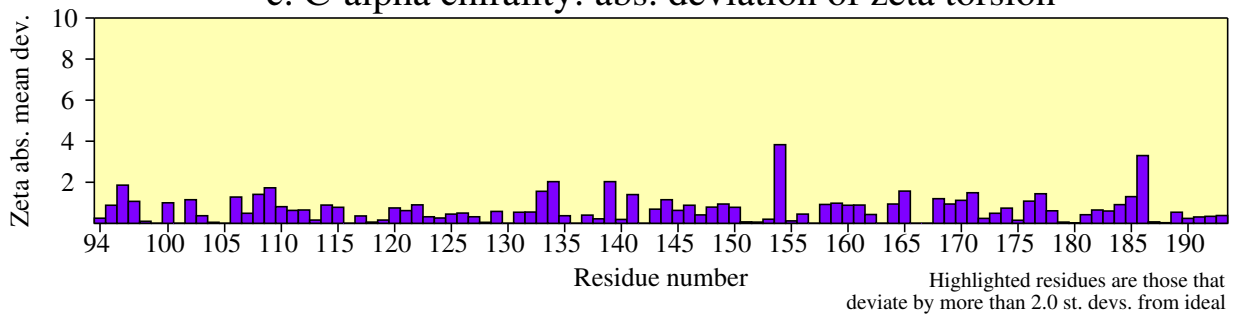
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

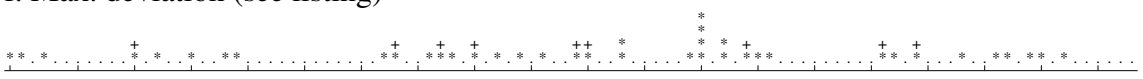
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

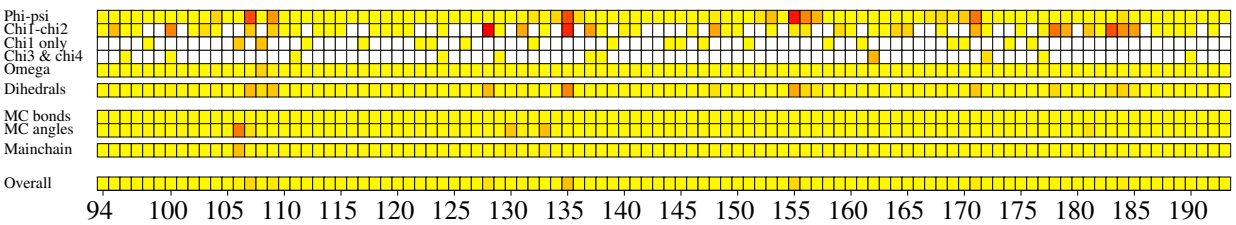
e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

LYMHVGRGLYYGSYTFLETWNIGVILLLTVMATAFMGYVLPWGQMSFWGATVITNLLSAIPYIGTNLVEWIWGGFSVDKATLTRFFAFHFILPFIIMAIA

f. Max. deviation (see listing)

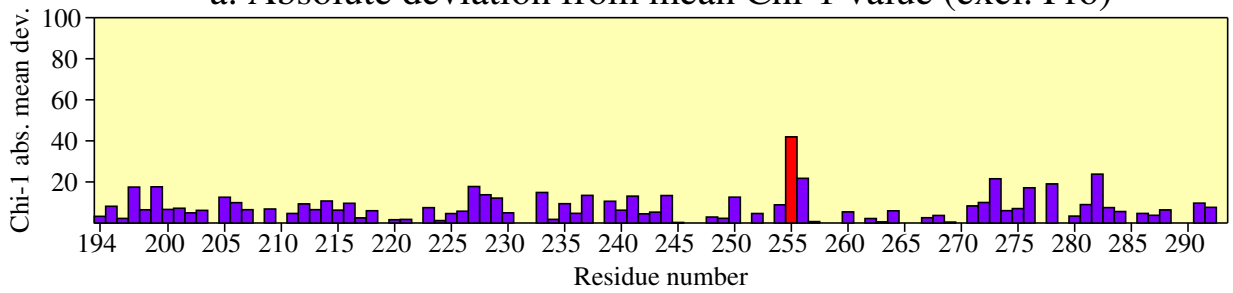


g. G-factors

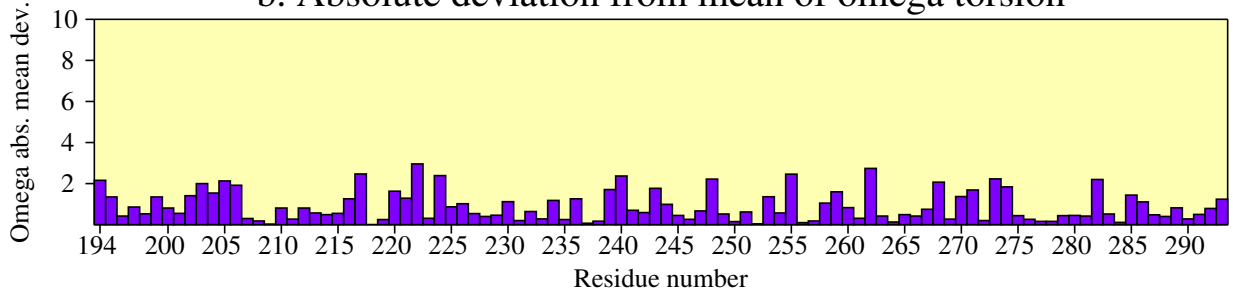


Residue properties
1pp9

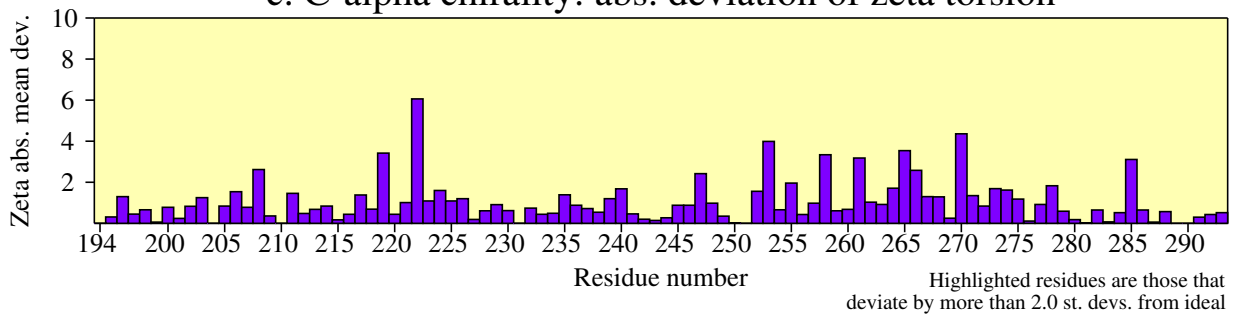
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



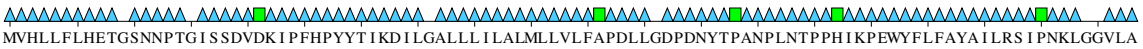
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

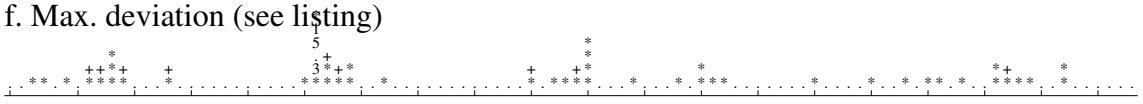
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

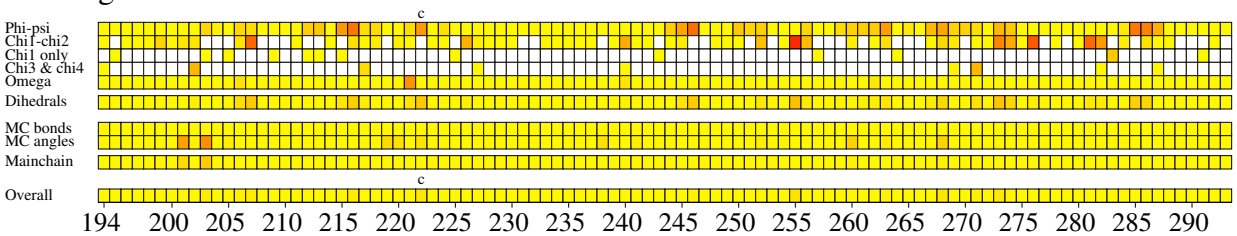


MVHLLFLHETGSNNPTGISSDVDKIPFHPYYTIKDILGALLLILALMLLVLFAPDLLGDPDNYTPANPLNTPPHIKPEWYFLFAYAILRSIPNKLGGVLA

f. Max. deviation (see listing)



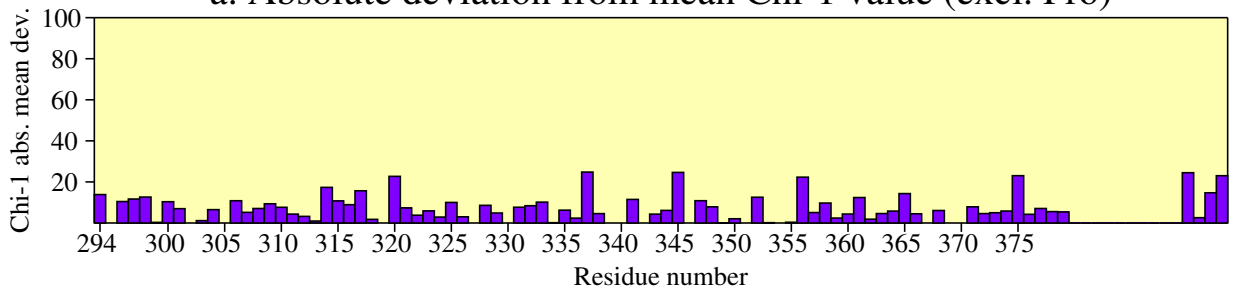
g. G-factors



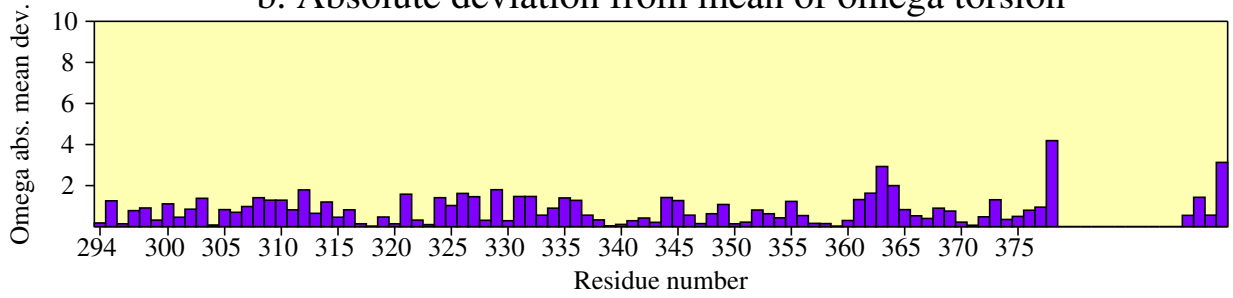
c = cis-peptide

Residue properties
1pp9

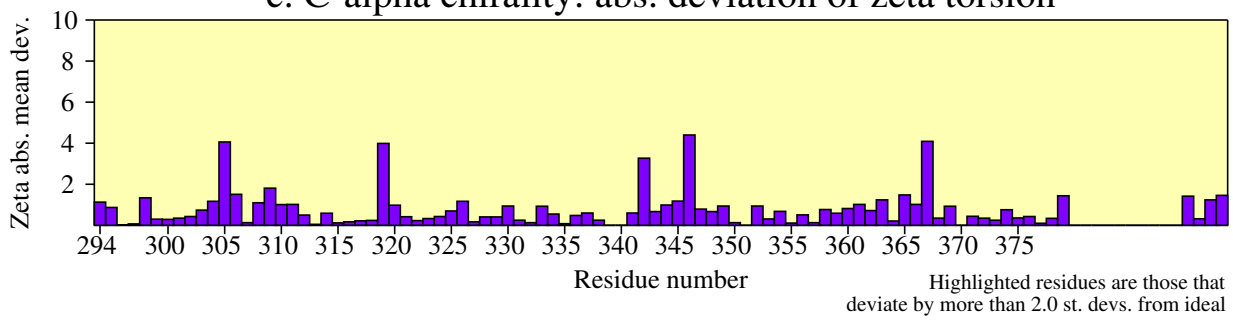
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

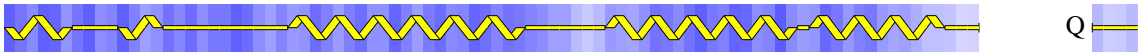


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

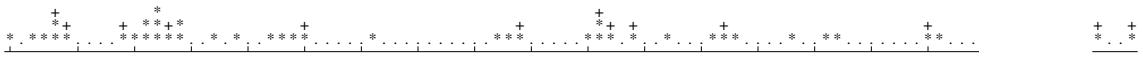
d. Secondary structure & estimated accessibility



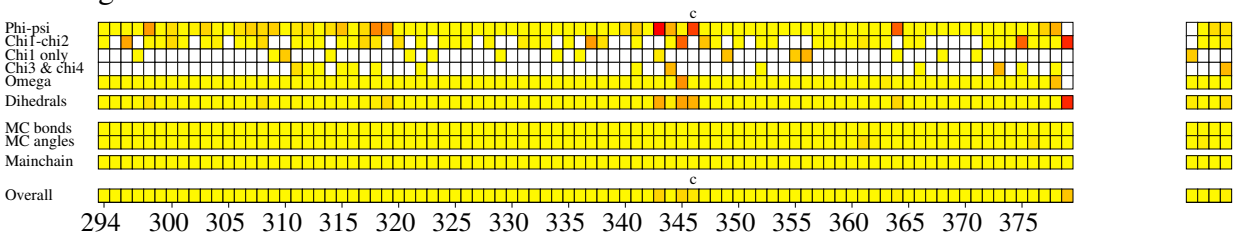
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



g. G-factors

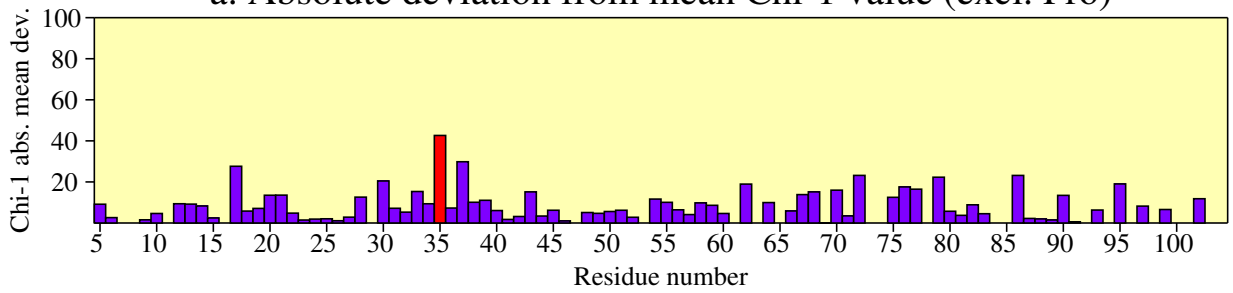


c = cis-peptide

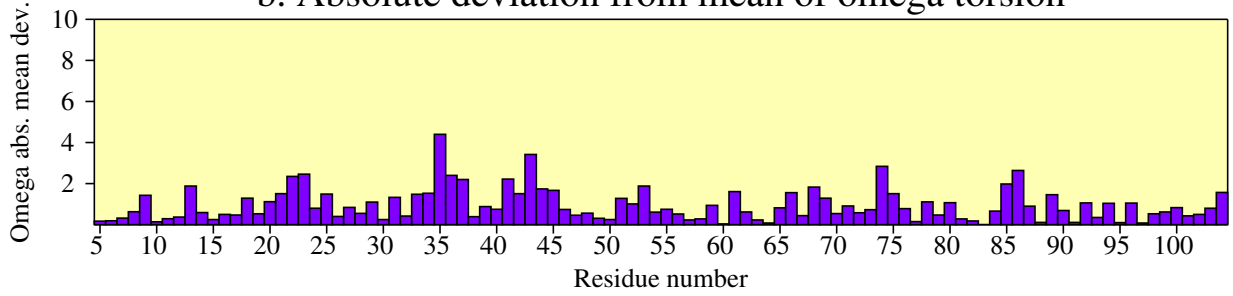
Residue properties

1pp9

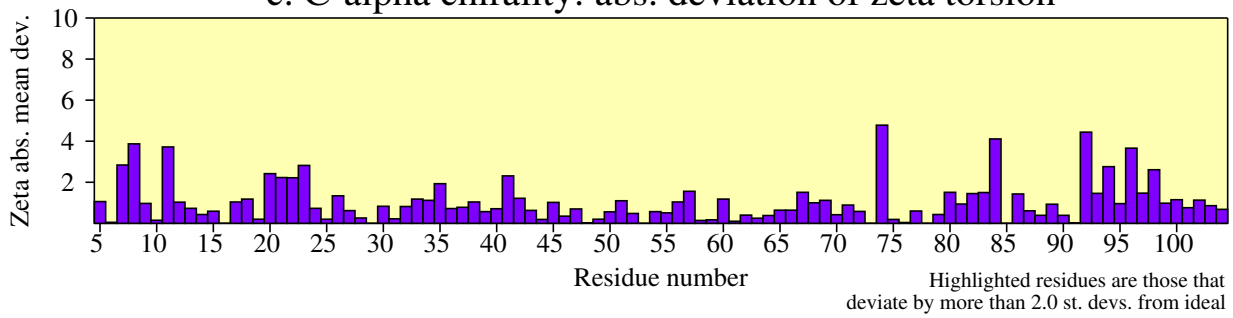
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

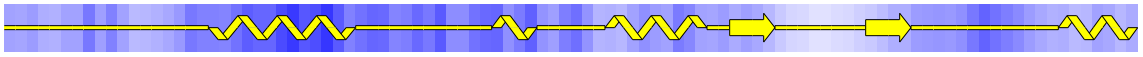


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



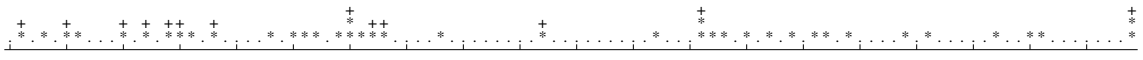
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

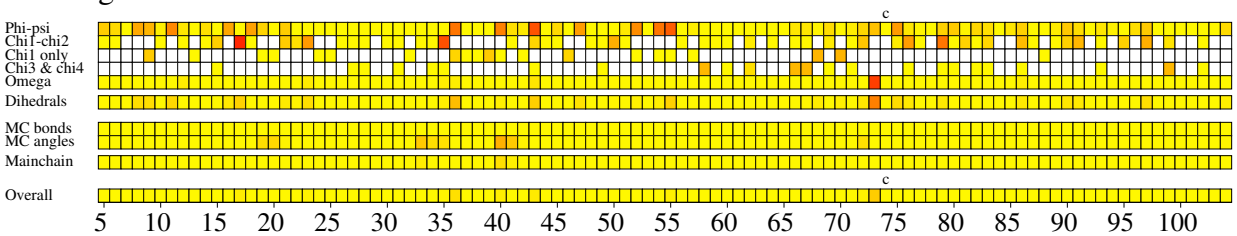
Most favoured Allowed Generous Disallowed

LHPPSYPWSHRGLLSSLDHTS IRRGFQVYKQVCSSCHSMDYVAYRHLVGVCYTEDEAKALAEEVEVQDGPNEDGEMFMRPGKLSDYFPKPYPNPEAARAA

f. Max. deviation (see listing)



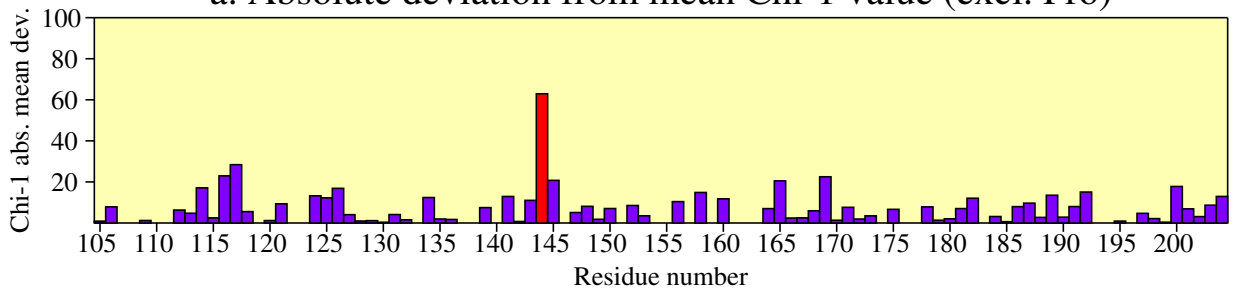
g. G-factors



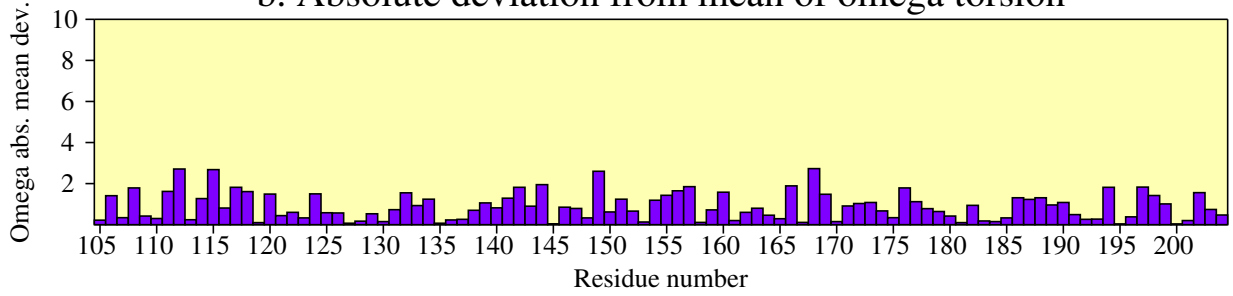
c = cis-peptide

Residue properties
1pp9

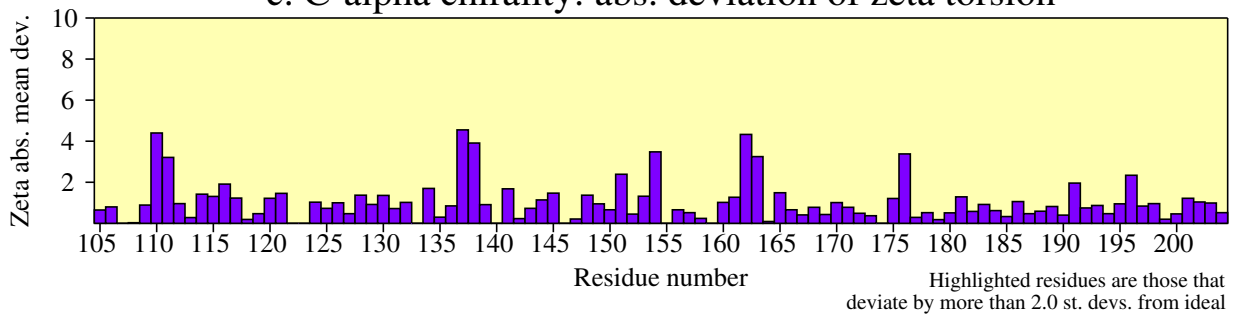
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

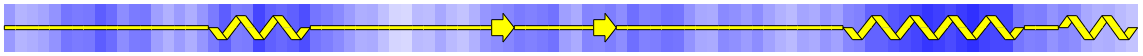


c. C-alpha chirality: abs. deviation of zeta torsion



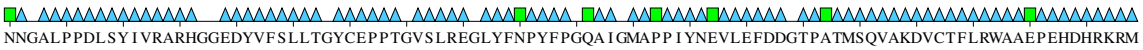
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



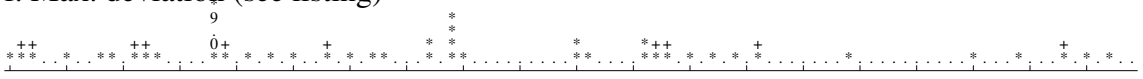
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

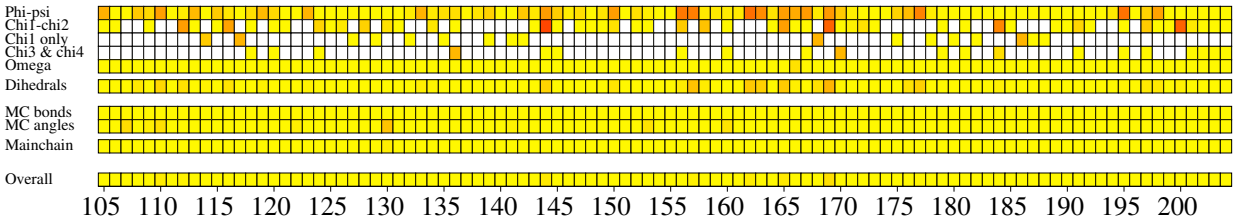


NNGALPPDLSYIVRARHGGEDYVFSLLTGYCEPPTGVSLREGLYFNPYFPGQAIGMAPPIYNEVLEFDDGTPATMSQVAKDVCTFLRWAAEPEHDHRKRM

f. Max. deviation (see listing)



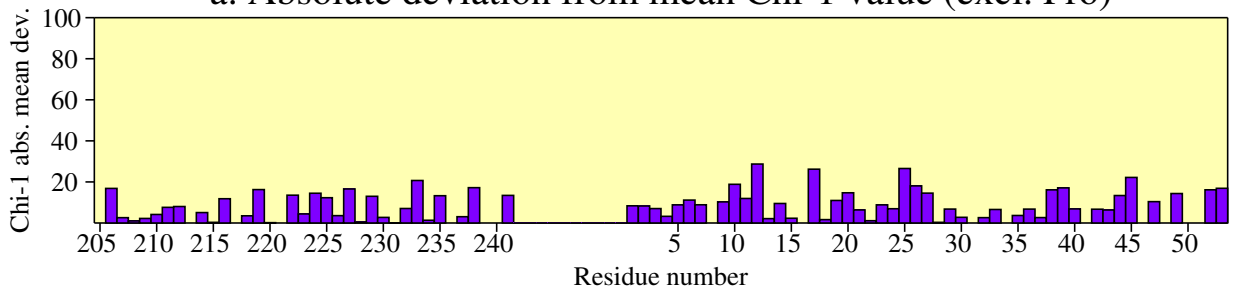
g. G-factors



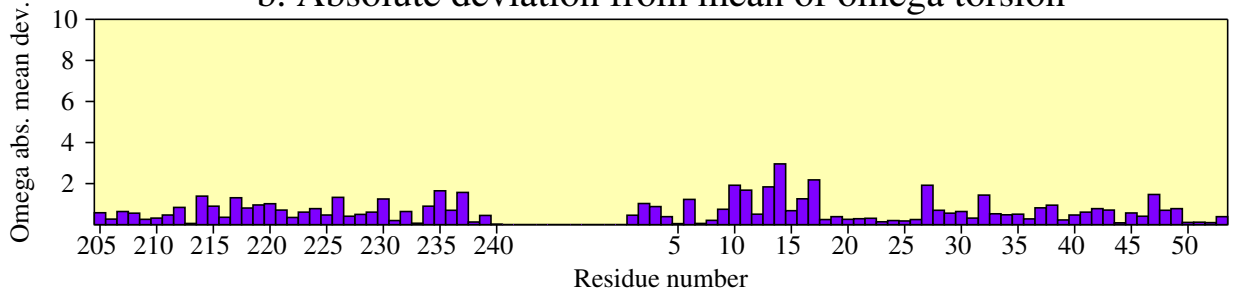
Residue properties

1pp9

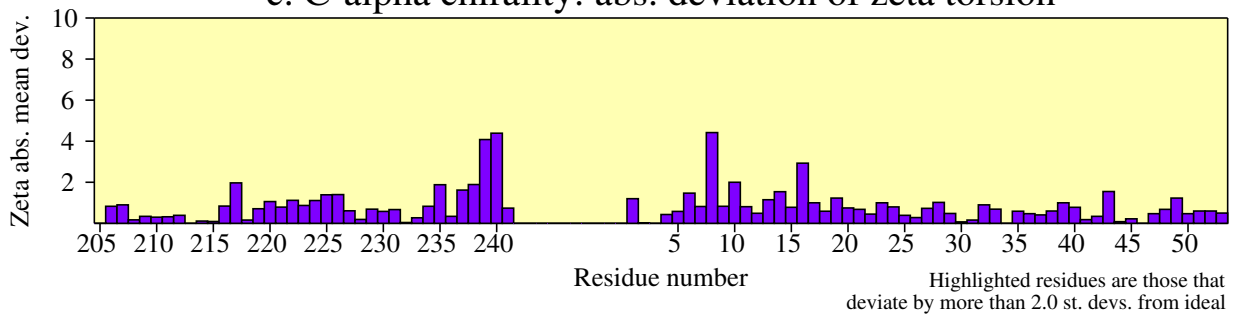
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

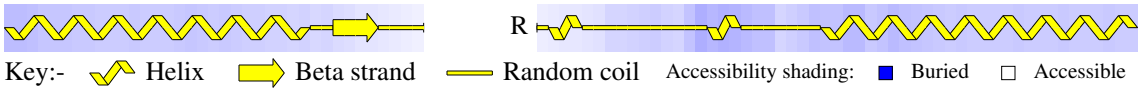


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

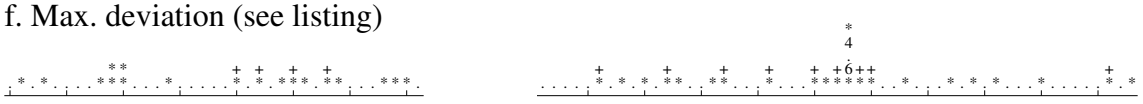
d. Secondary structure & estimated accessibility



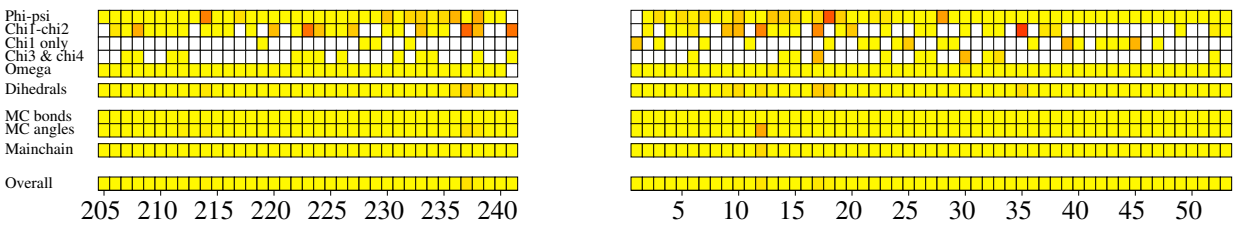
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

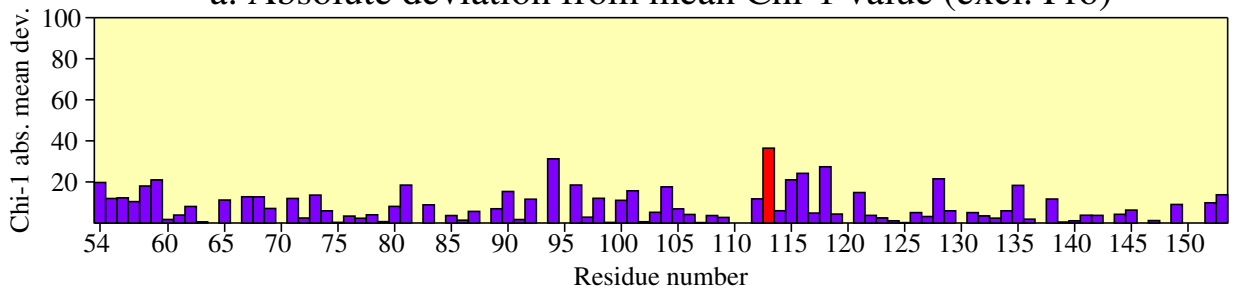


g. G-factors

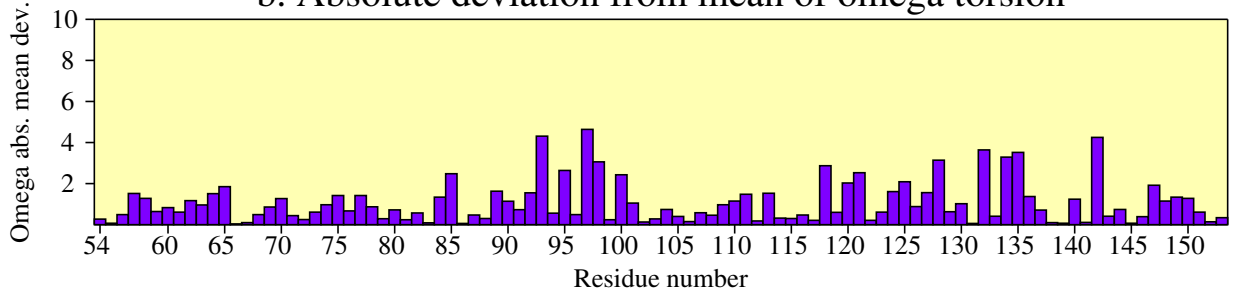


Residue properties
1pp9

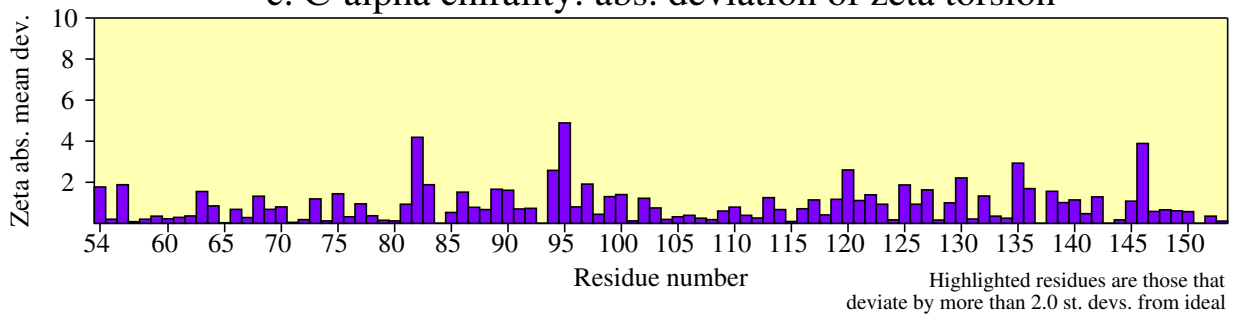
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

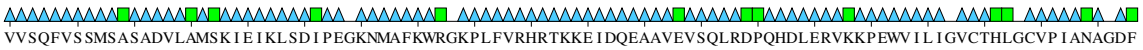


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

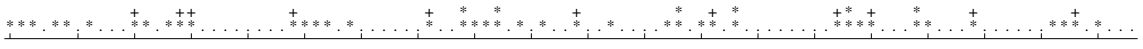
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

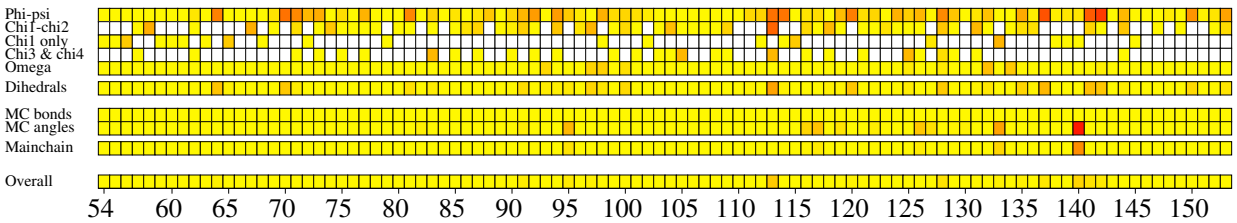


V V S Q F V S S M S A S A D V L A M S K I E I K L S D I P E G K N M A F K W R G K P L F V R H R T K K E I D Q E A A V E V S Q L R D P Q H D L E R V K K P E W V I L I G V C T H L G C V P I A N A G D F

f. Max. deviation (see listing)

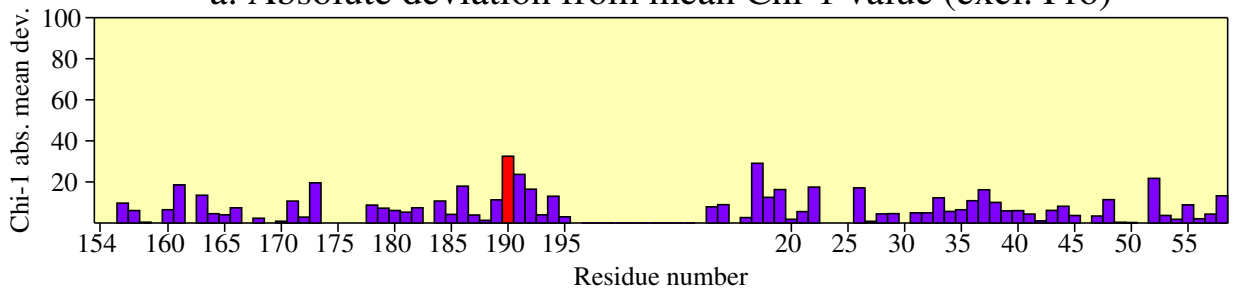


g. G-factors

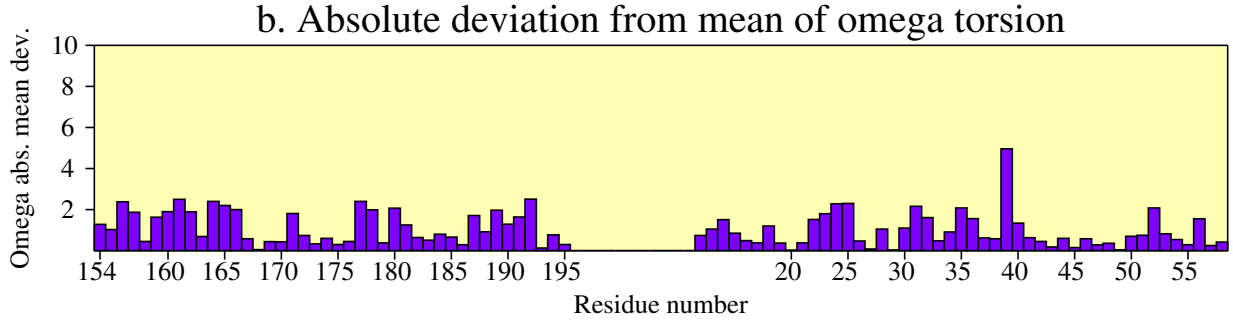


Residue properties
1pp9

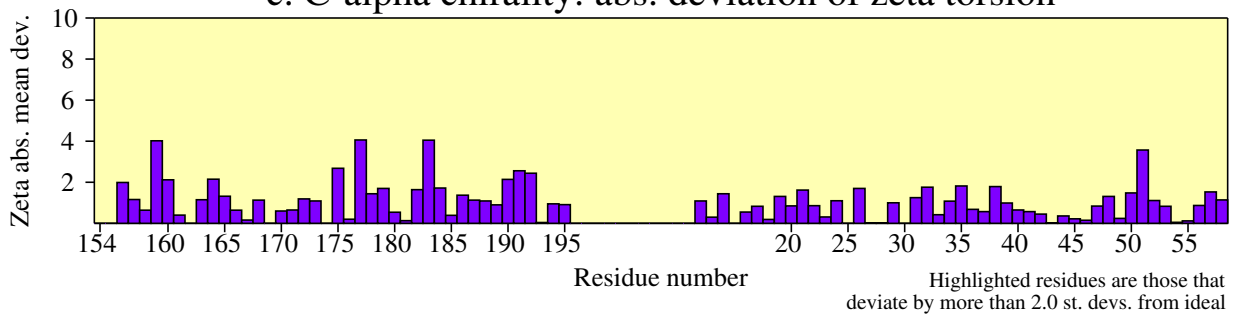
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

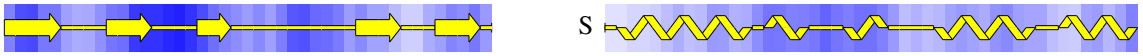


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

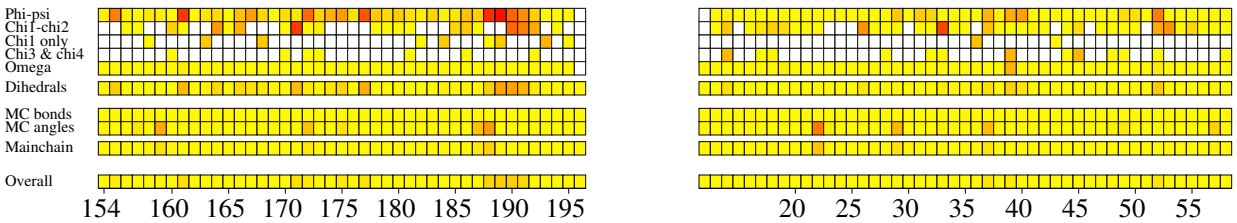


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

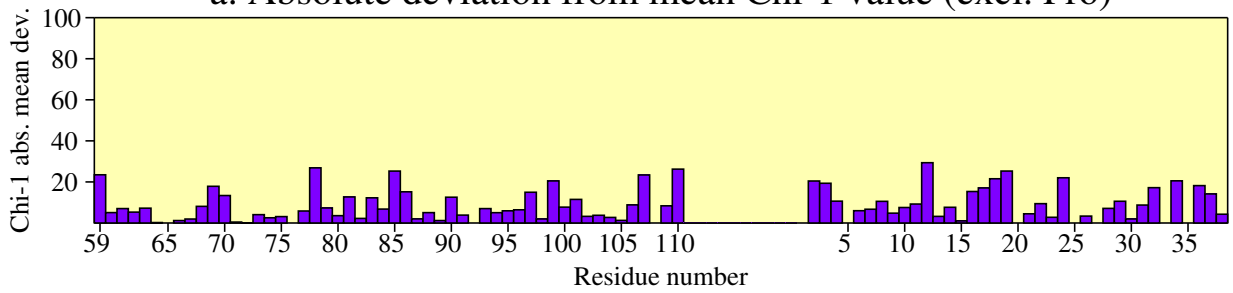
f. Max. deviation (see listing)

g. G-factors

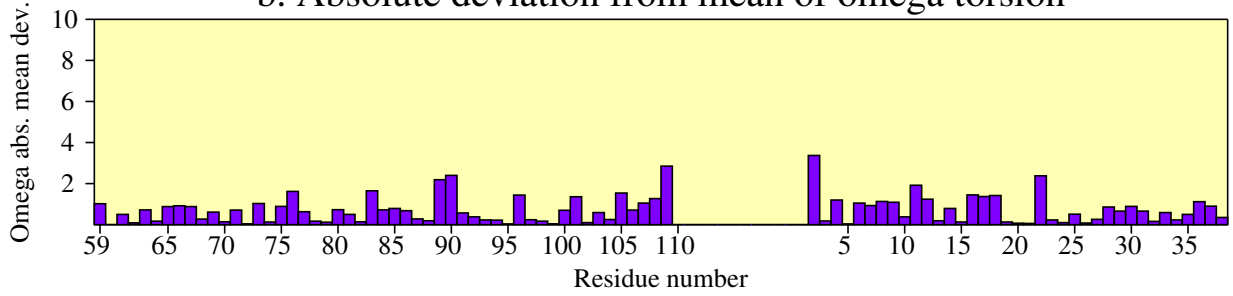


Residue properties
1pp9

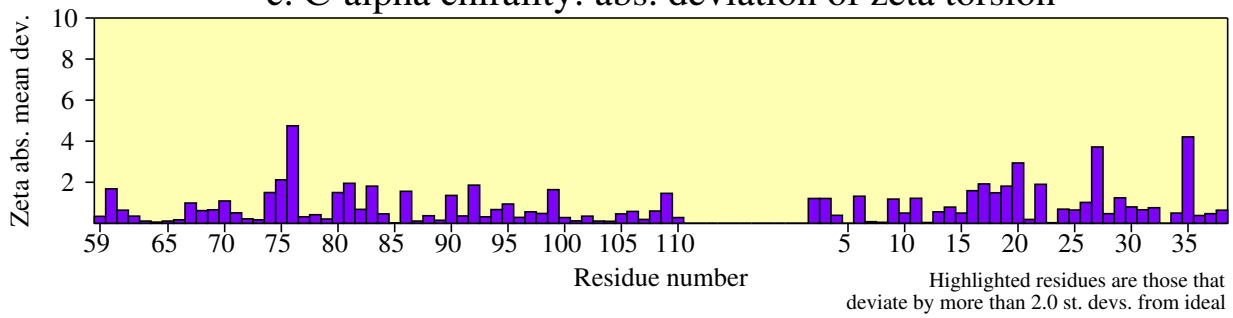
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

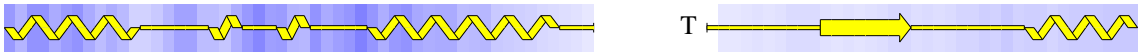


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

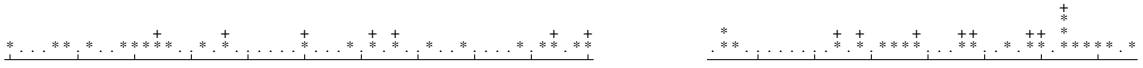
d. Secondary structure & estimated accessibility



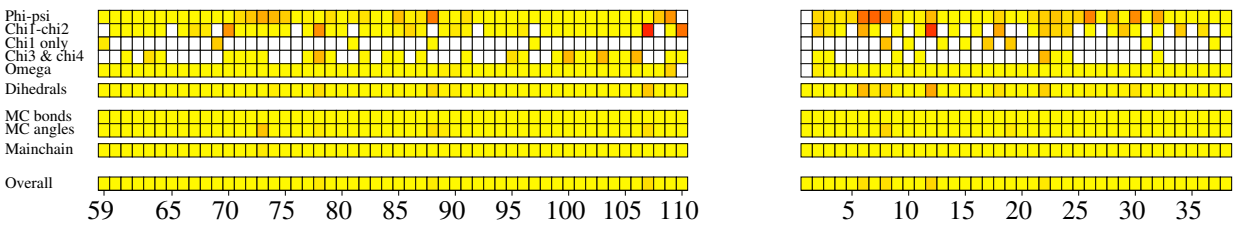
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



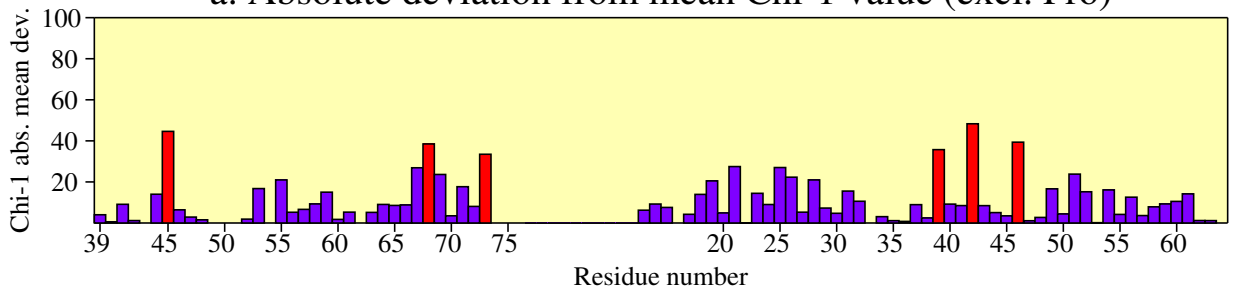
g. G-factors



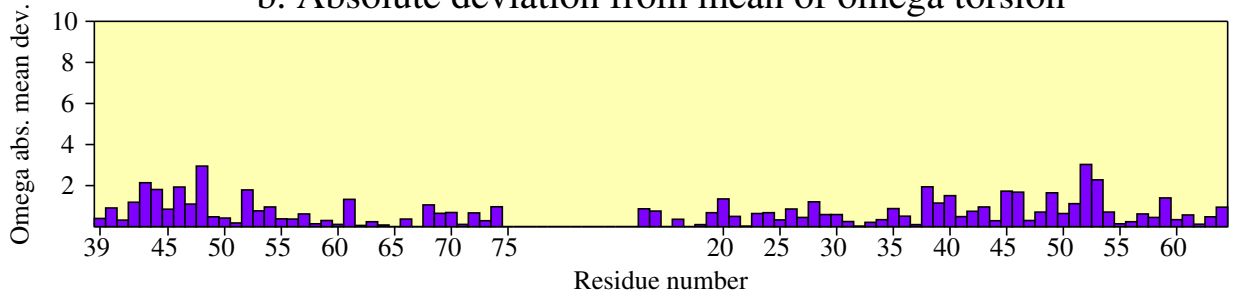
Residue properties

1pp9

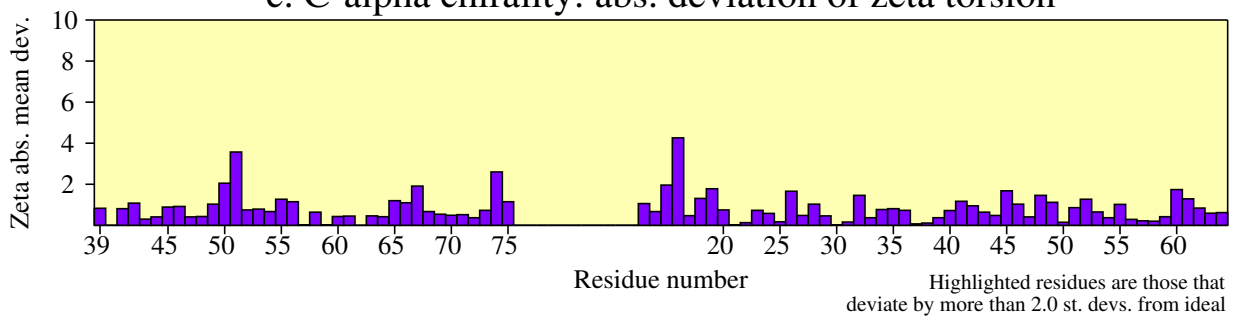
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

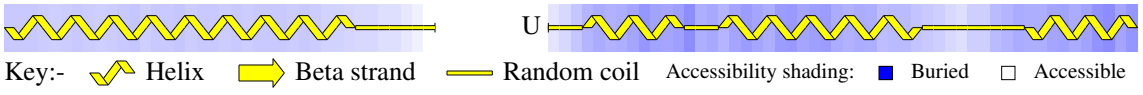


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

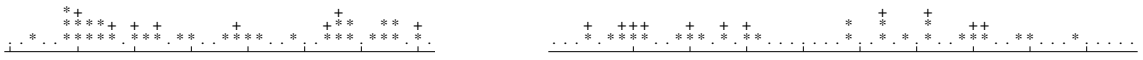
d. Secondary structure & estimated accessibility



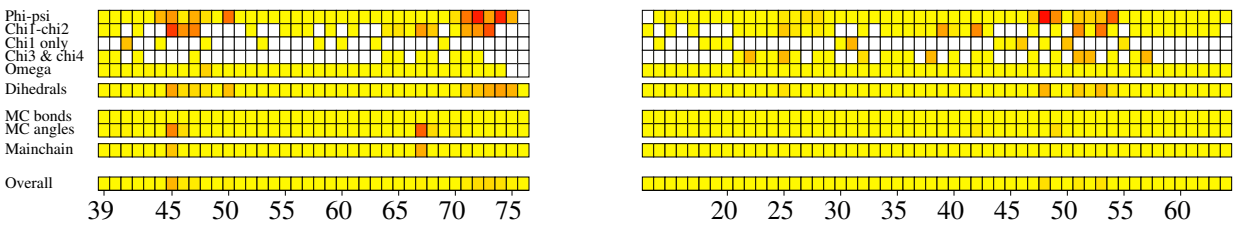
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



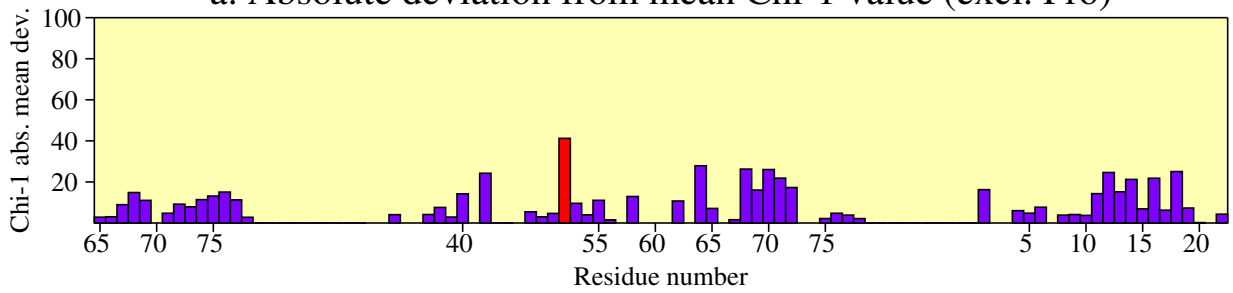
g. G-factors



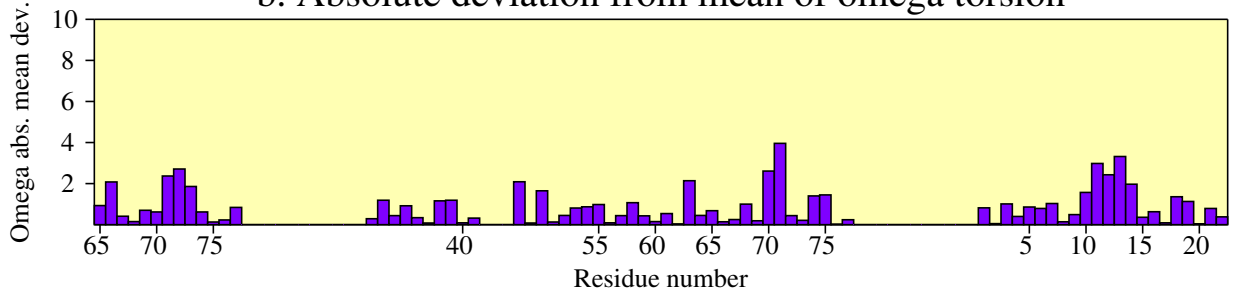
Residue properties

1pp9

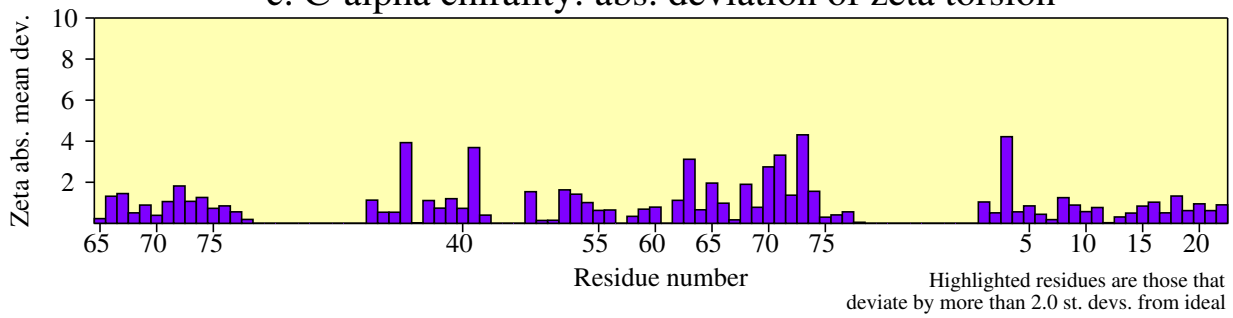
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

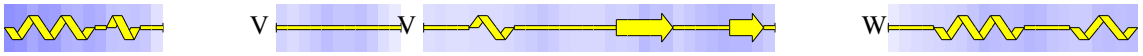


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

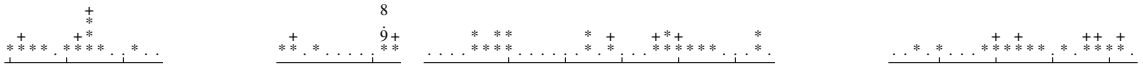


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

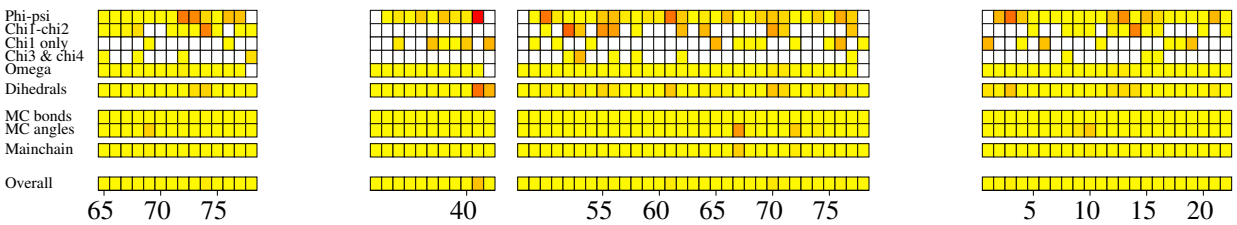
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



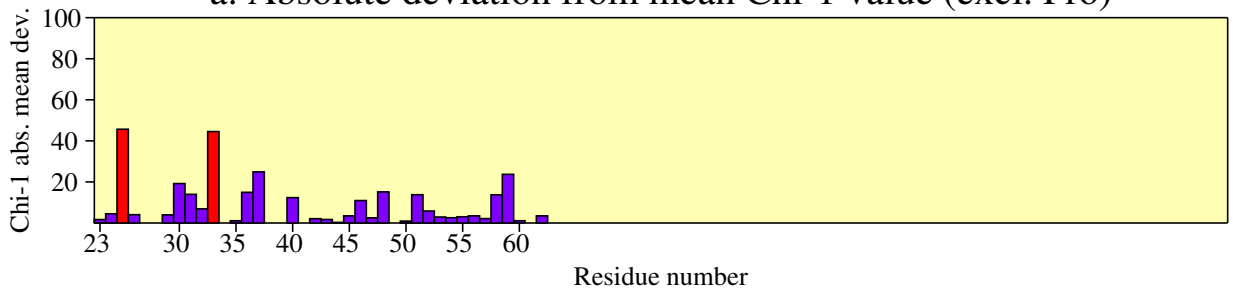
g. G-factors



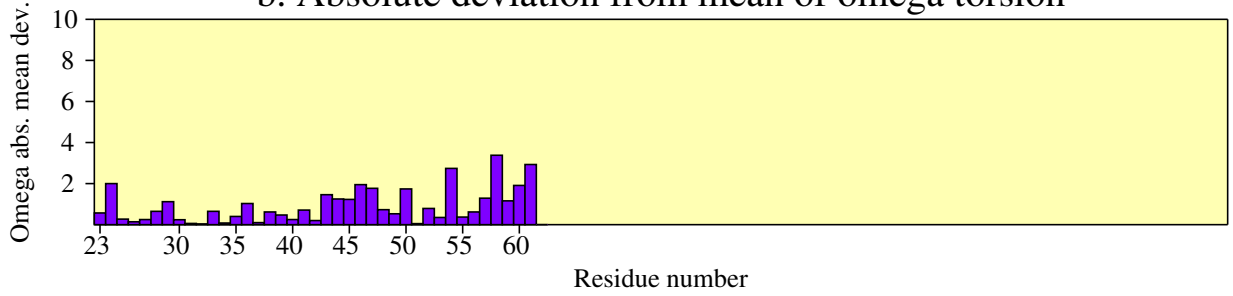
Residue properties

1pp9

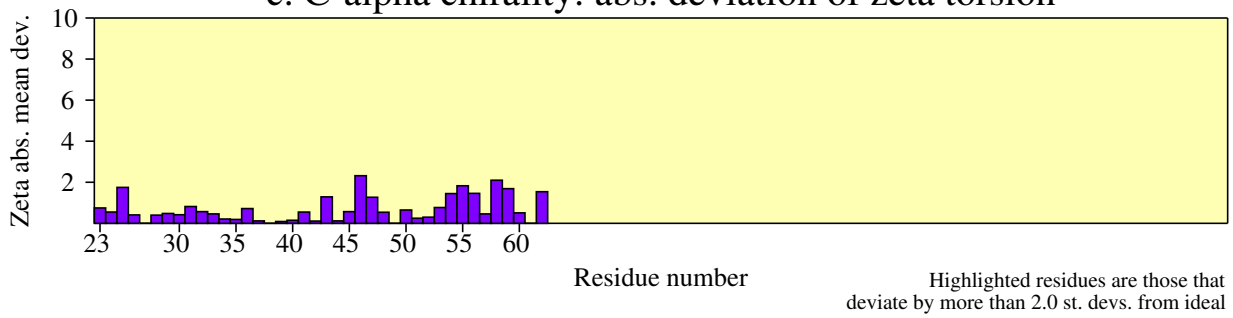
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



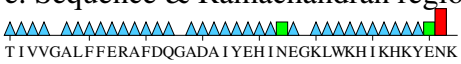
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

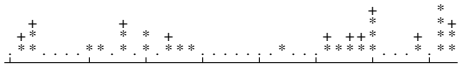
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

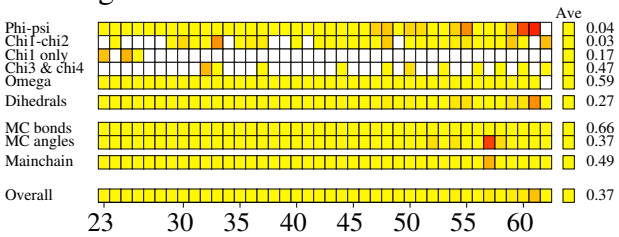


T I VVGALFFERAFDQGADA I YEH I NEGKLWKH I KHKYENK

f. Max. deviation (see listing)

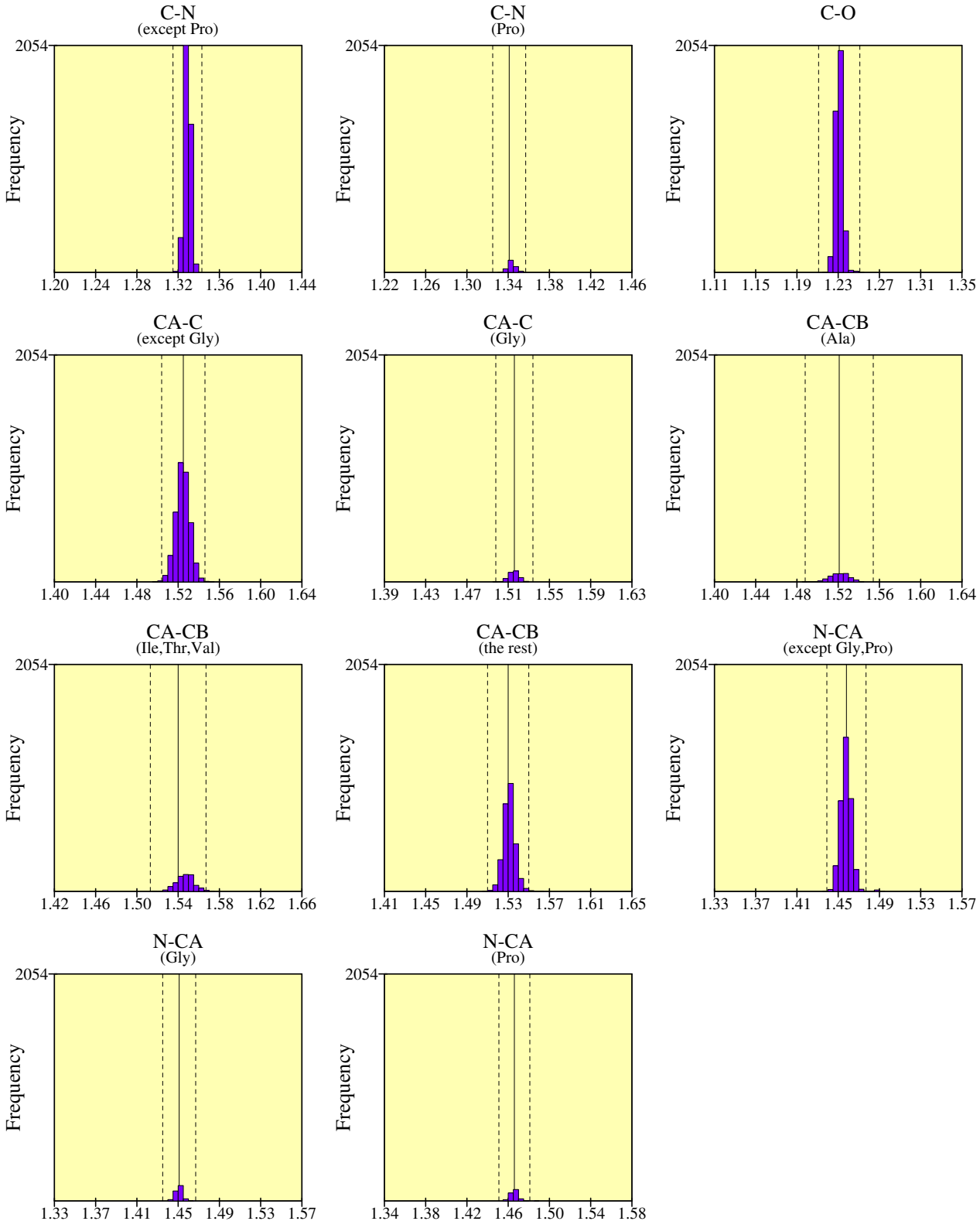


g. G-factors



Main-chain bond lengths

1pp9

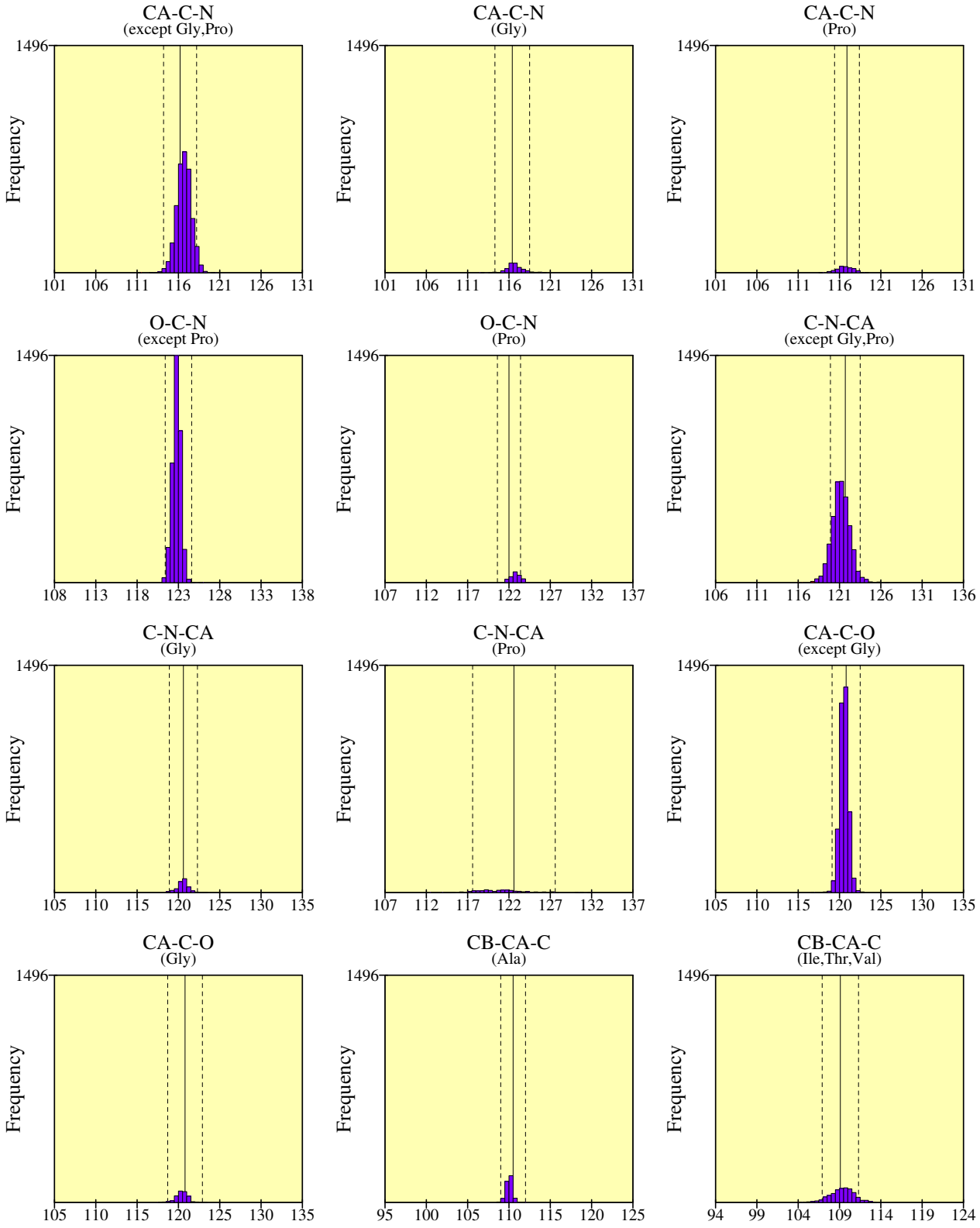


Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

Main-chain bond angles

1pp9



Black bars > 2.0 st. devs. from mean.

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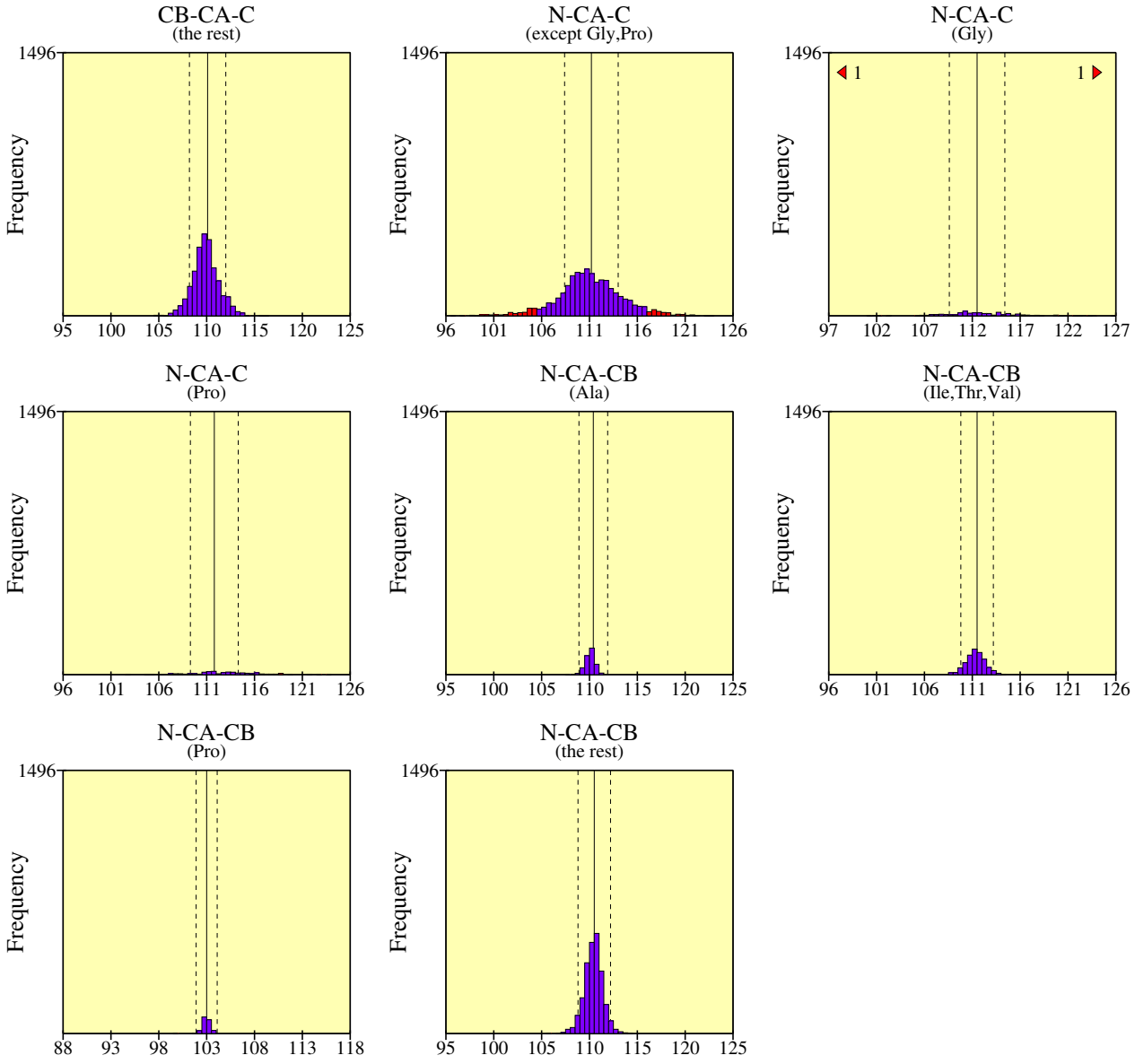
Main-chain bond angles

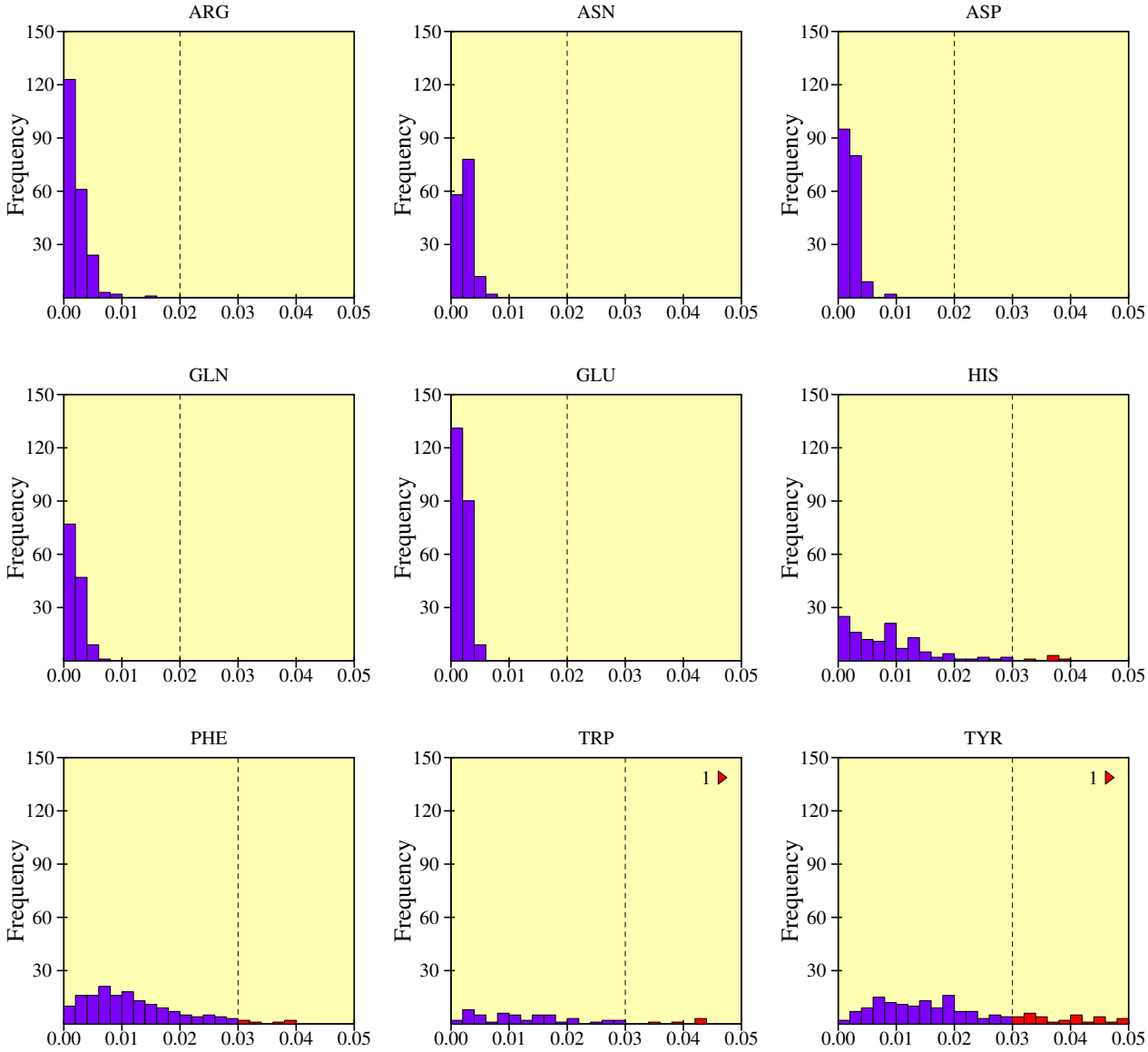
1pp9



Black bars > 2.0 st. devs. from mean. ◀ or ▶ signifies data points off the graph in the direction shown.
Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

RMS distances from planarity

1pp9

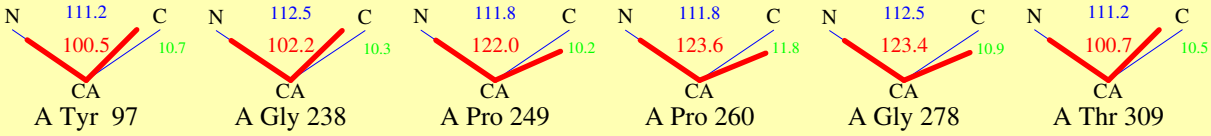


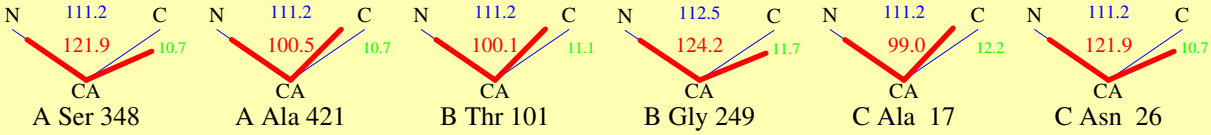
Histograms showing RMS distances of planar atoms from best-fit plane.
Black bars indicate large deviations from planarity: RMS dist > 0.03 for rings, and > 0.02 otherwise.

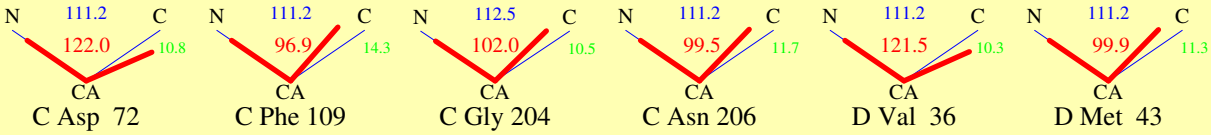
▸ signifies data points off the graph in the direction shown.

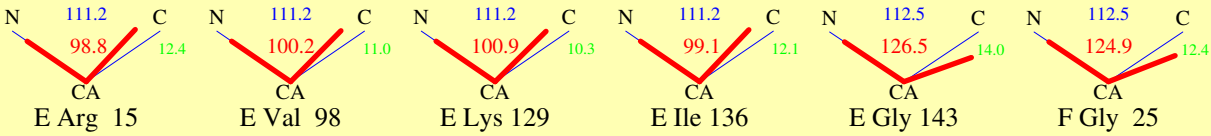
Distorted geometry
1pp9

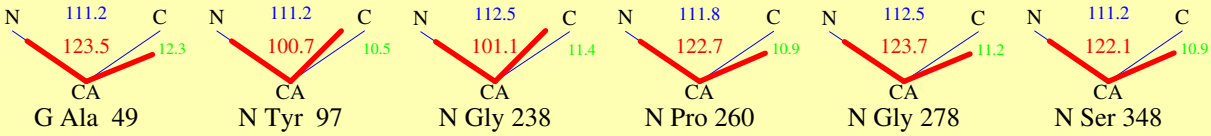
Main-chain bond angles

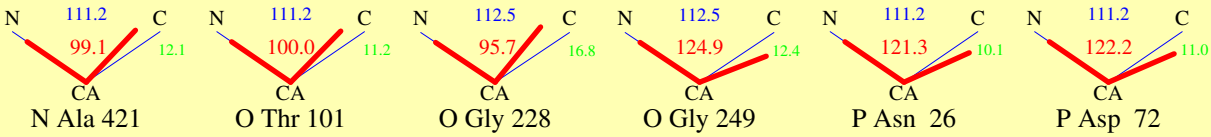


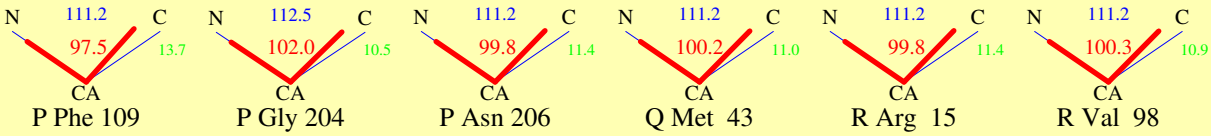


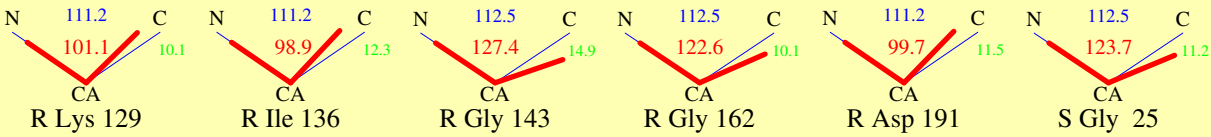






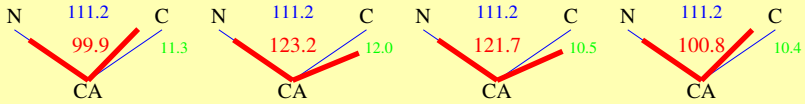






Distorted geometry
1pp9

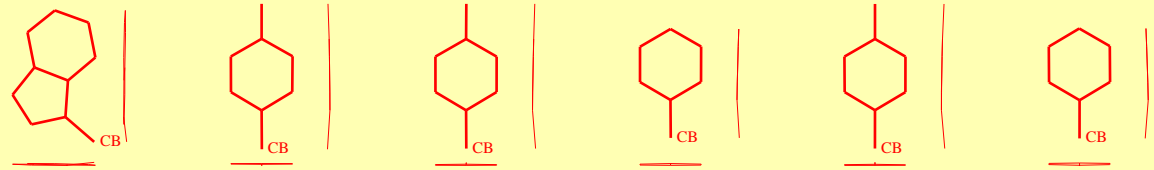
Main-chain bond angles (contd)



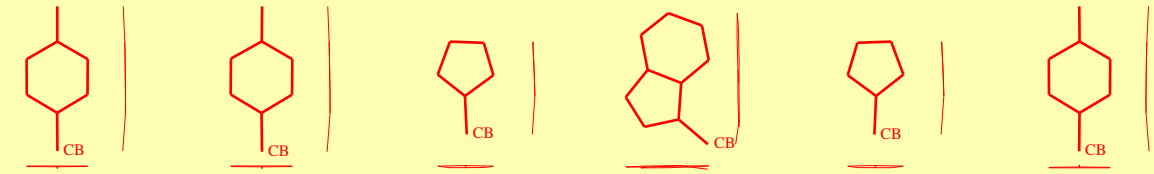
S Met 32 | T Ala 49 | T Arg 71 | W Asn 61

Bond angles differing by > 10.0 degrees from small-molec values. Values shown: "ideal", actual, diff.

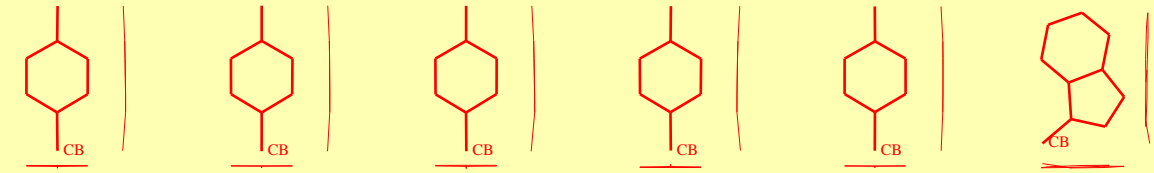
Planar groups



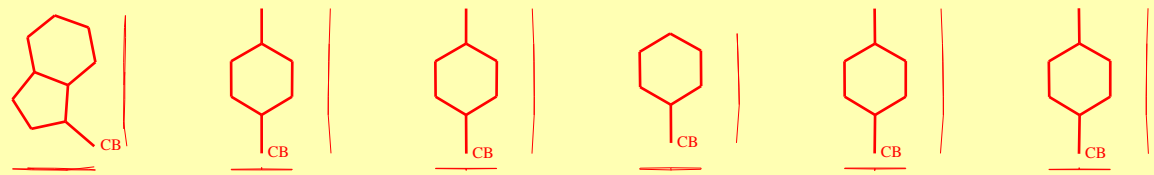
0.035 A Trp 40 | 0.033 A Tyr 314 | 0.040 A Tyr 416 | 0.031 B Phe 26 | 0.046 B Tyr 57 | 0.040 C Phe 33



0.040 C Tyr 75 | 0.041 C Tyr 81 | 0.038 C His 97 | 0.043 C Trp 141 | 0.040 C His 196 | 0.044 C Tyr 224



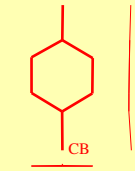
0.041 C Tyr 278 | 0.034 C Tyr 347 | 0.049 D Tyr 33 | 0.050 D Tyr 90 | 0.031 D Tyr 115 | 0.051 D Trp 192



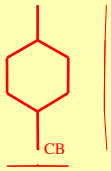
0.039 F Trp 19 | 0.037 F Tyr 20 | 0.034 F Tyr 55 | 0.038 F Phe 60 | 0.041 J Tyr 10 | 0.031 N Tyr 4

Distorted geometry
1pp9

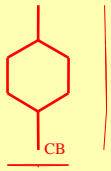
Planar groups (contd)



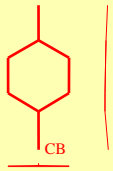
0.034
N Tyr 280



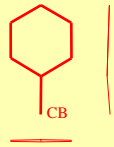
0.035
N Tyr 314



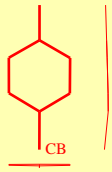
0.035
N Tyr 416



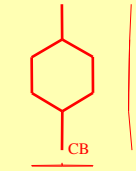
0.046
O Tyr 57



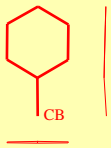
0.039
P Phe 33



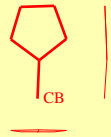
0.049
P Tyr 75



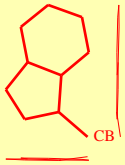
0.046
P Tyr 81



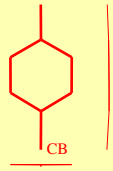
0.031
P Phe 91



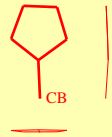
0.036
P His 97



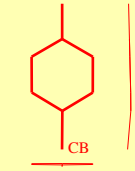
0.043
P Trp 141



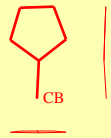
0.032
P Tyr 155



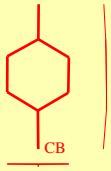
0.038
P His 196



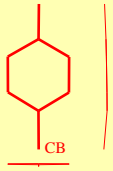
0.042
P Tyr 224



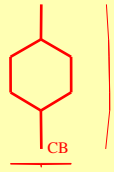
0.033
P His 267



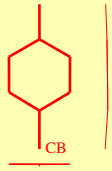
0.045
P Tyr 278



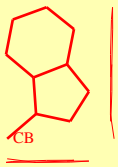
0.044
Q Tyr 33



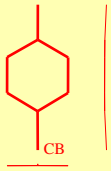
0.056
Q Tyr 90



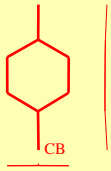
0.036
Q Tyr 152



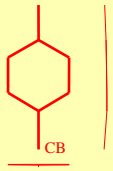
0.043
Q Trp 192



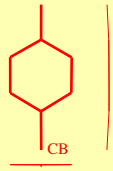
0.030
R Tyr 37



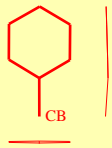
0.034
R Tyr 185



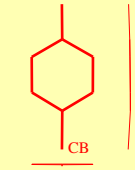
0.039
S Tyr 20



0.034
S Tyr 55



0.034
S Phe 60

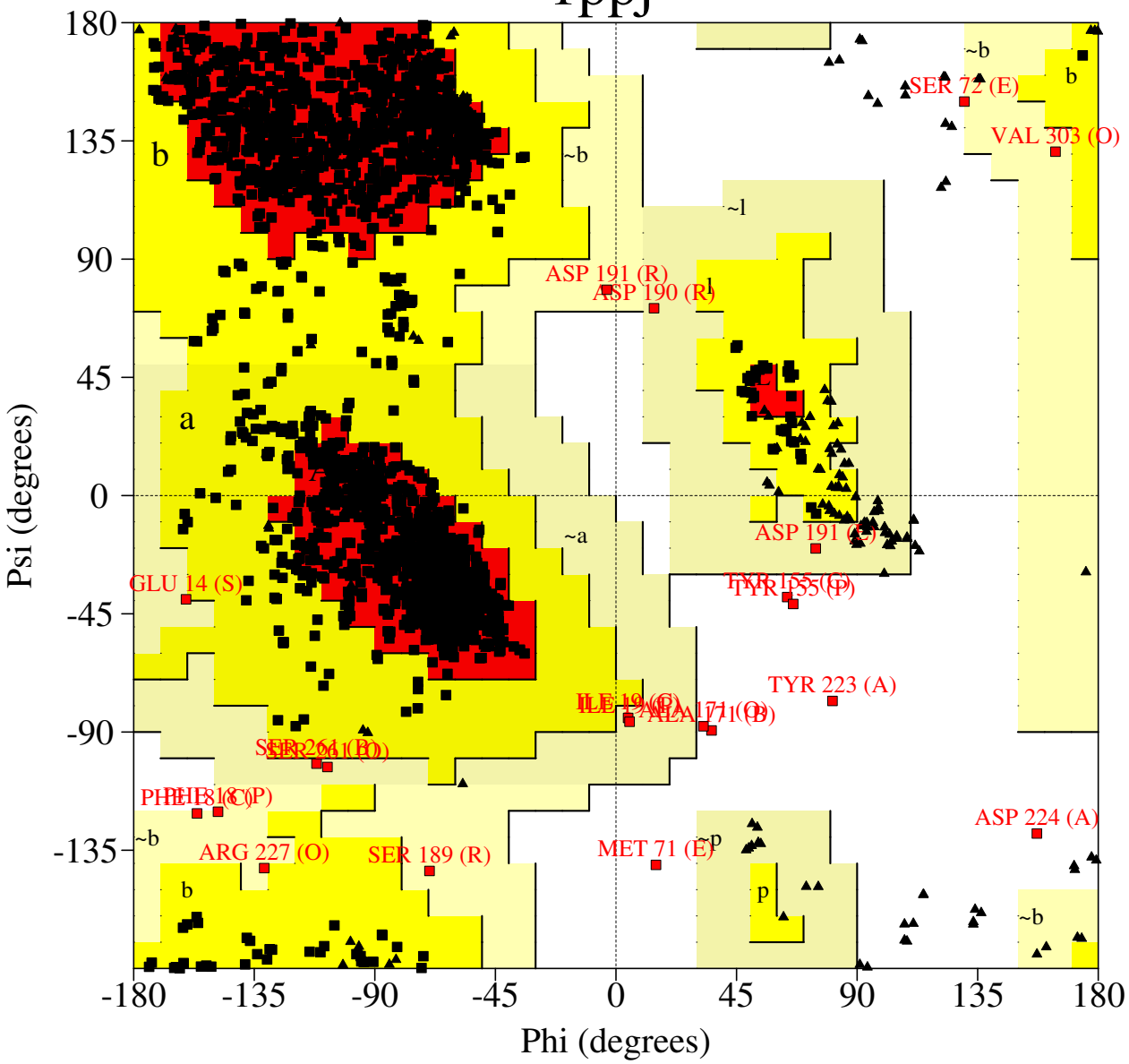


0.031
W Tyr 10

Sidechains with RMS dist. from planarity > 0.03A for rings, or > 0.02A otherwise. Value shown is RMS dist.

Ramachandran Plot

1ppj



Plot statistics

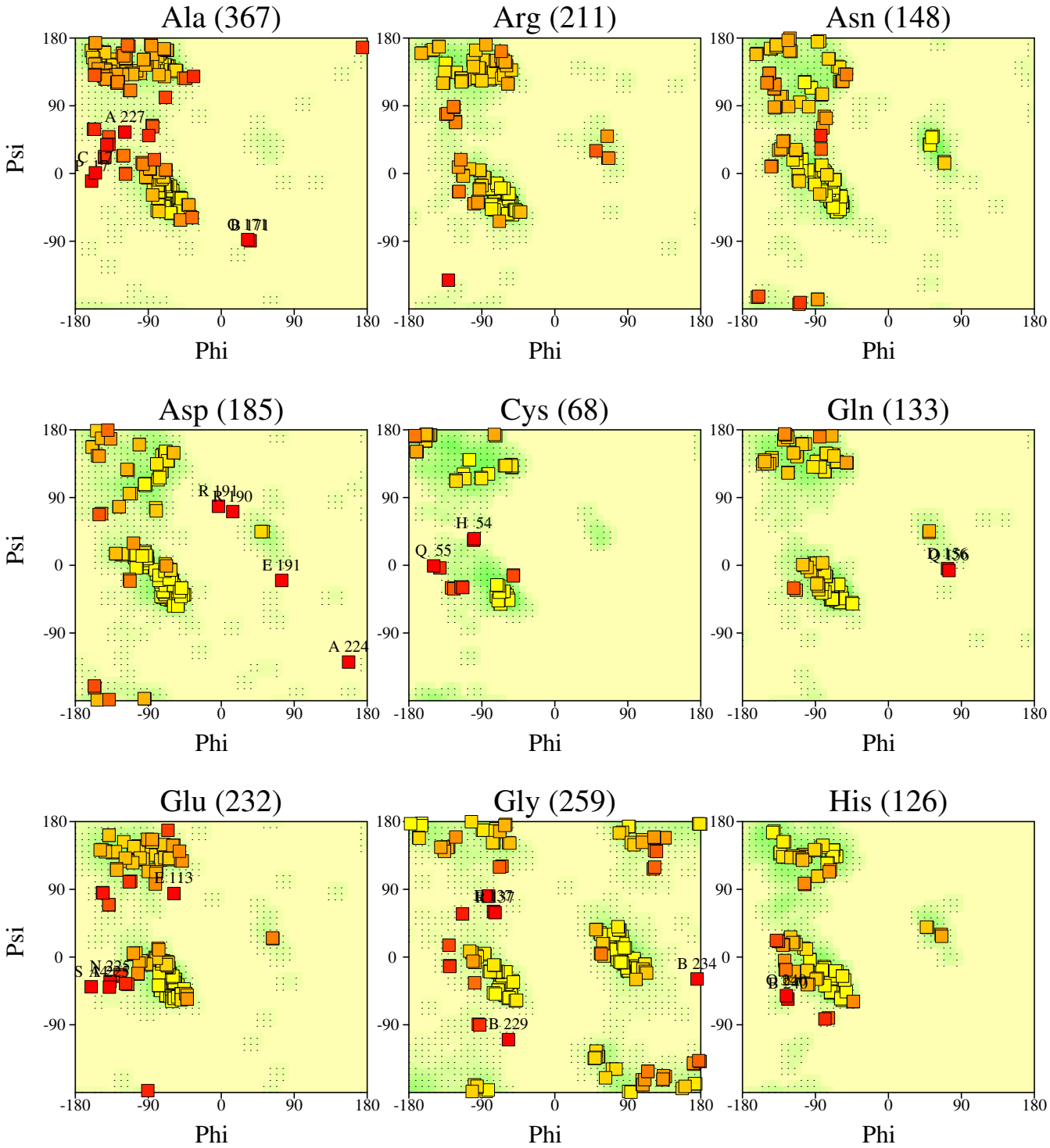
Residues in most favoured regions [A,B,L]	3213	92.4%
Residues in additional allowed regions [a,b,l,p]	243	7.0%
Residues in generously allowed regions [~a,~b,~l,~p]	14	0.4%
Residues in disallowed regions	7	0.2%
	----	------
Number of non-glycine and non-proline residues	3477	100.0%
Number of end-residues (excl. Gly and Pro)	48	
Number of glycine residues (shown as triangles)	265	
Number of proline residues	207	

Total number of residues	3997	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Ramachandran plots for all residue types

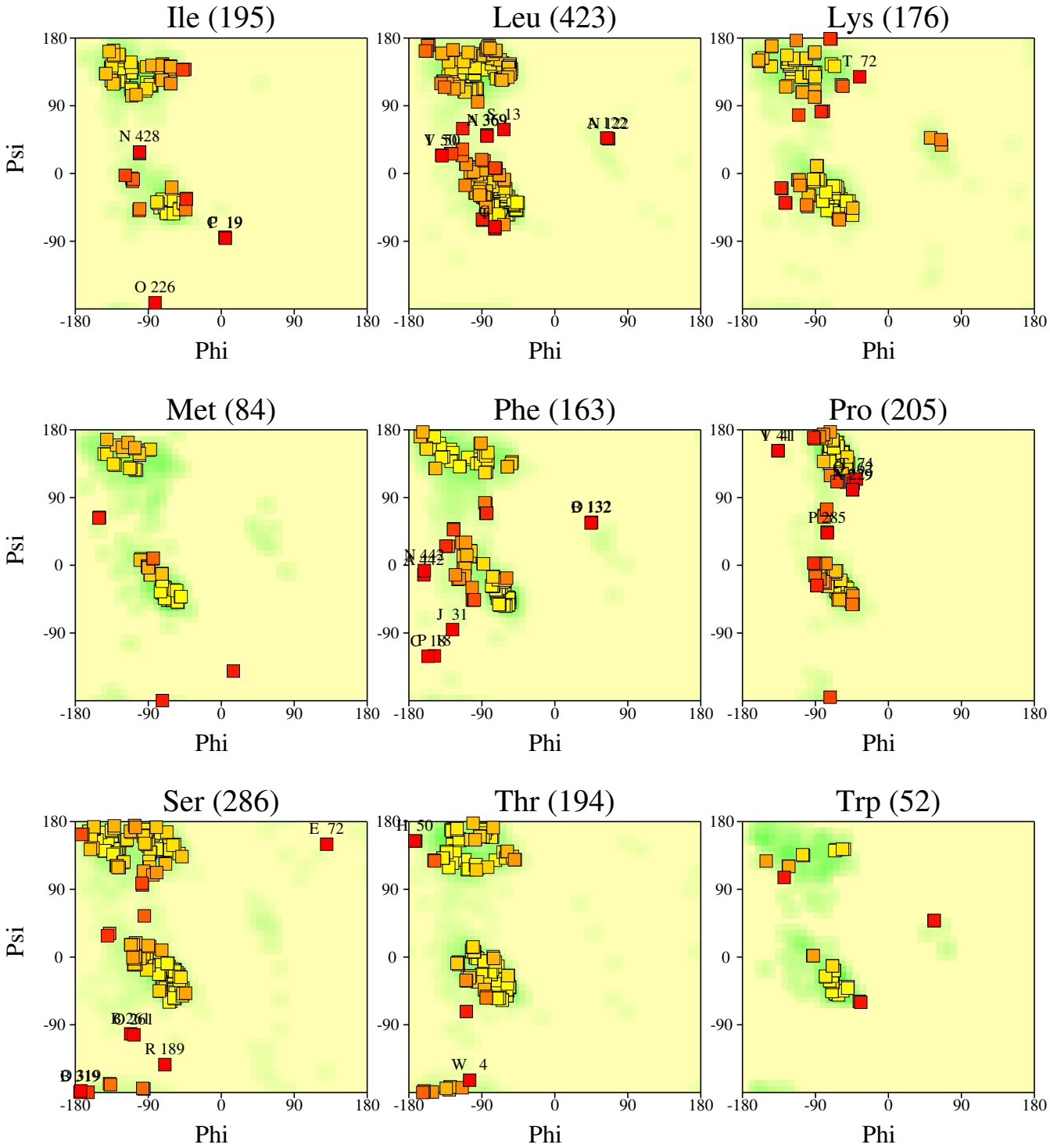
1ppj



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Ramachandran plots for all residue types

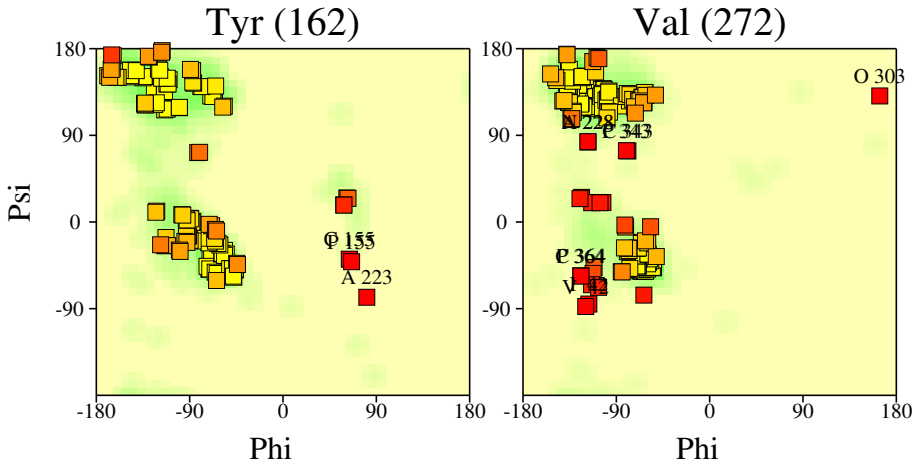
1ppj



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Ramachandran plots for all residue types

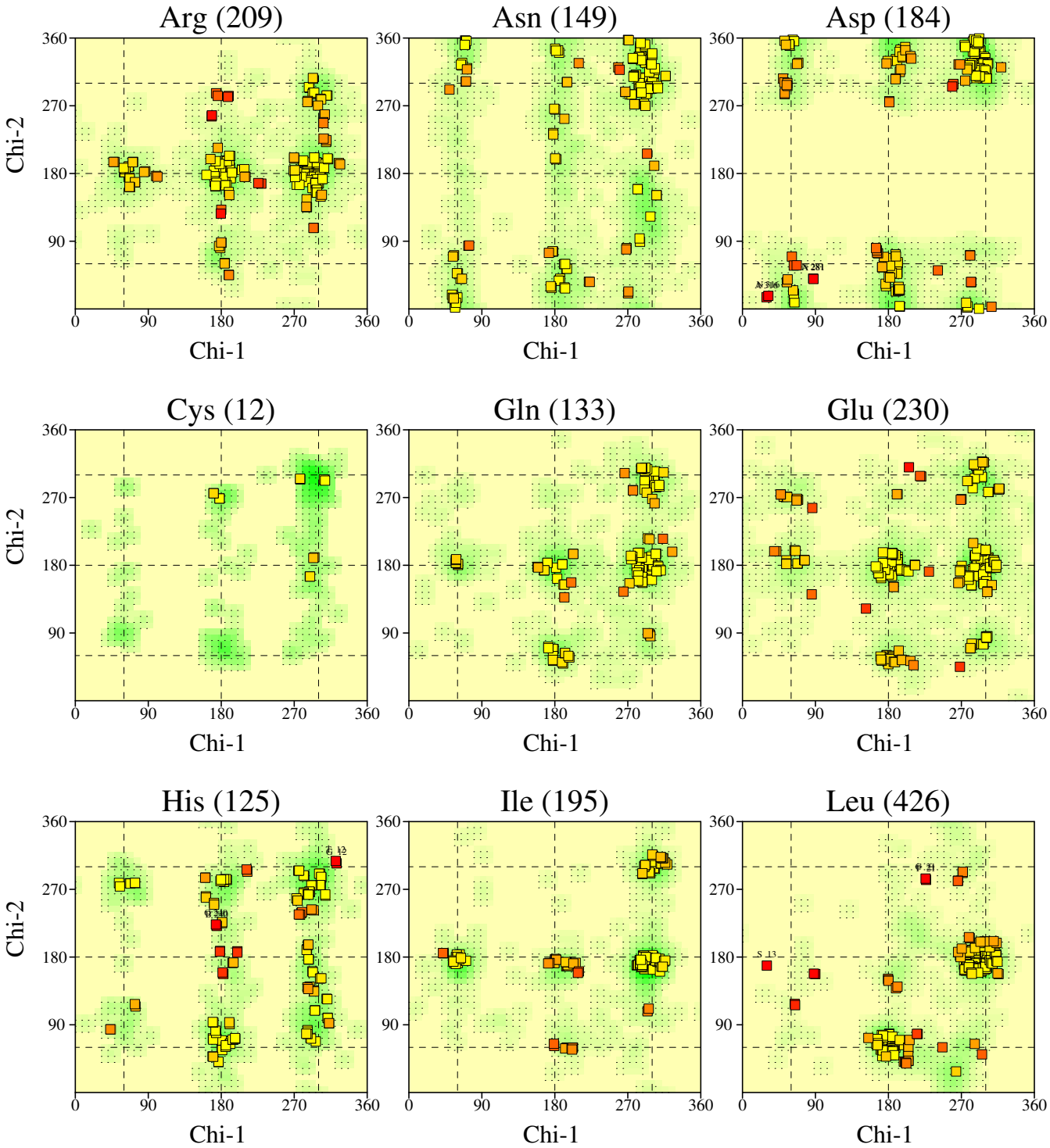
1ppj



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

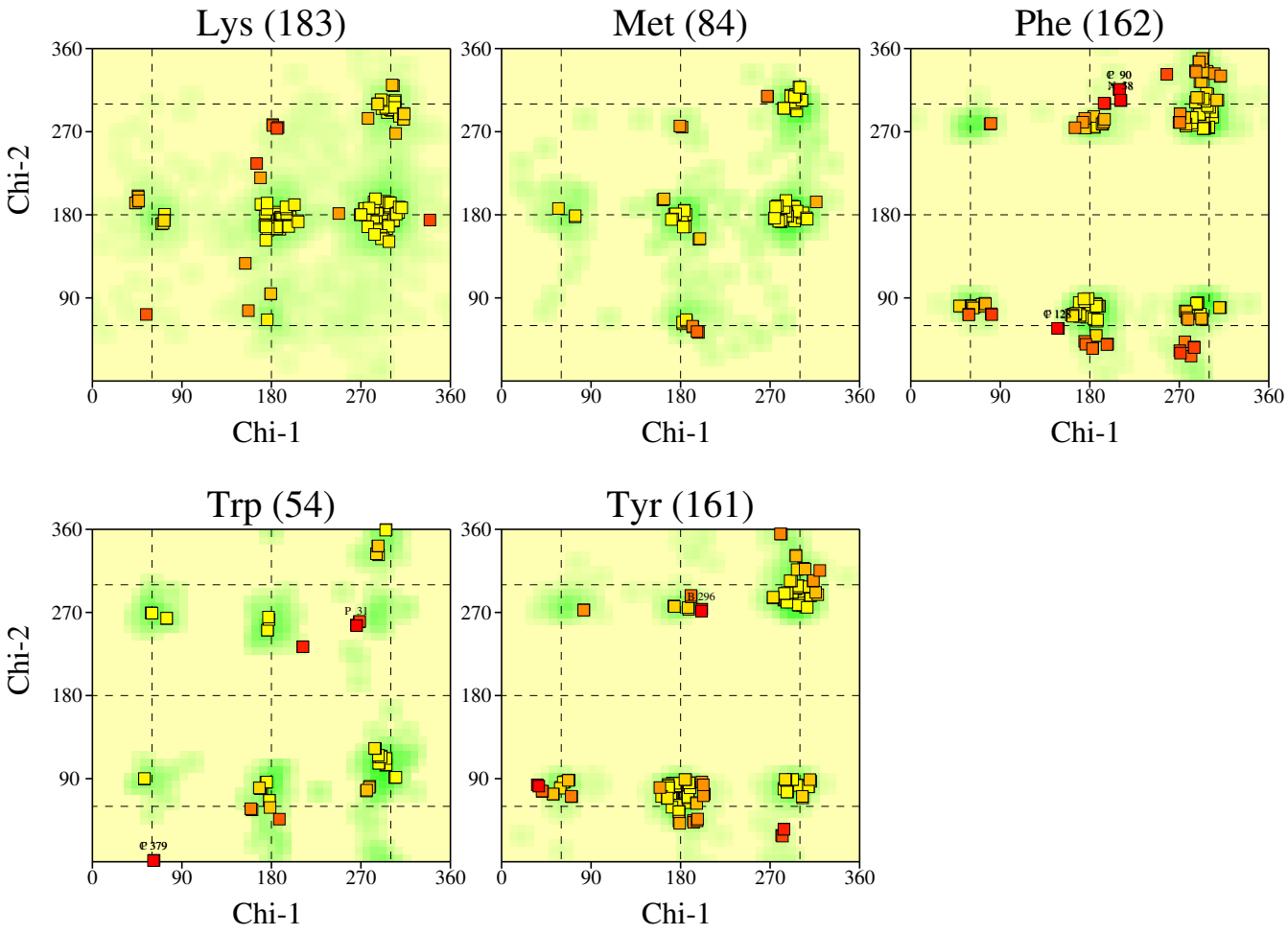
1ppj



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

1ppj



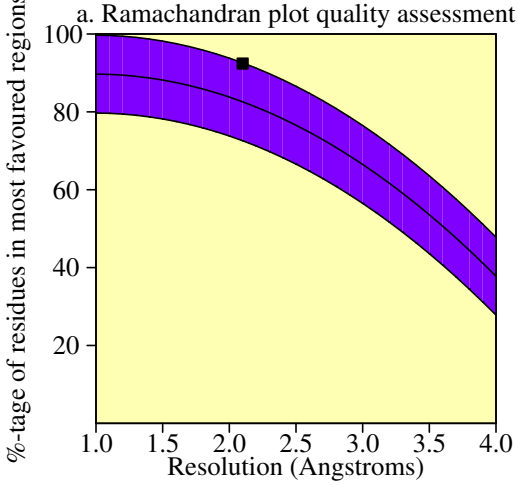
Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -3.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

PROCHECK

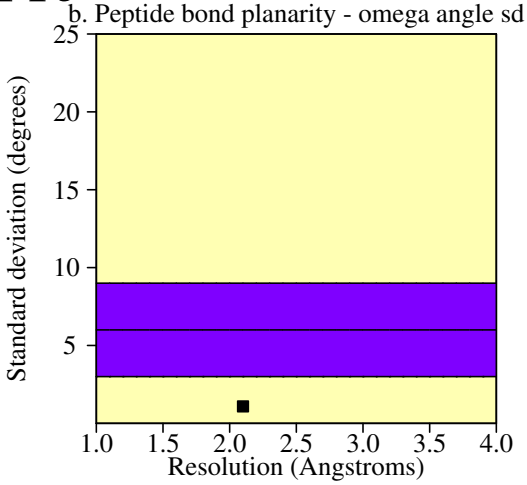
Main-chain parameters

1ppj

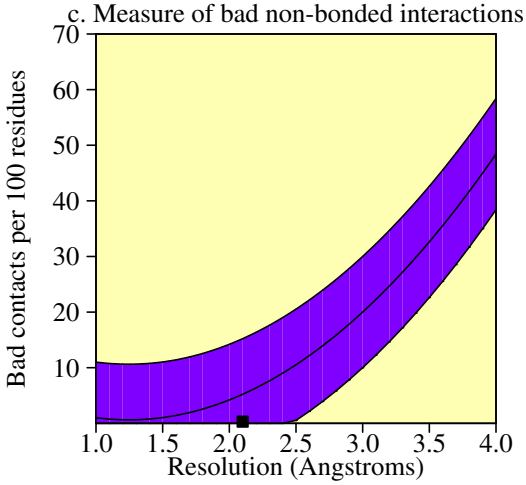
a. Ramachandran plot quality assessment



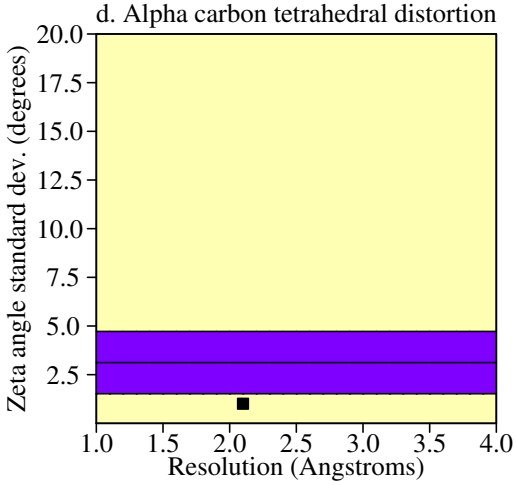
b. Peptide bond planarity - omega angle sd



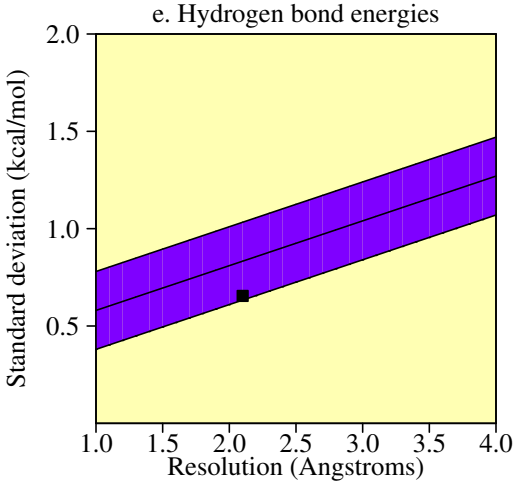
c. Measure of bad non-bonded interactions



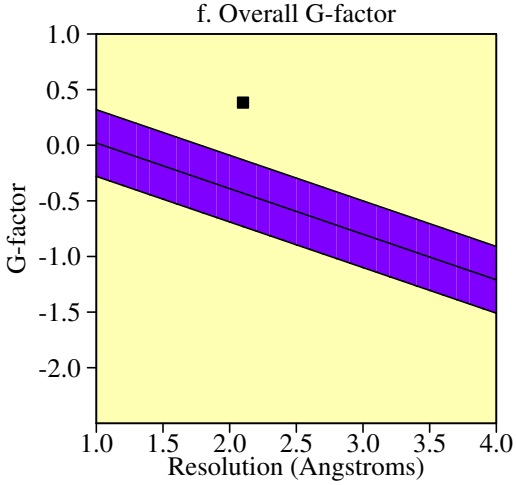
d. Alpha carbon tetrahedral distortion



e. Hydrogen bond energies



f. Overall G-factor



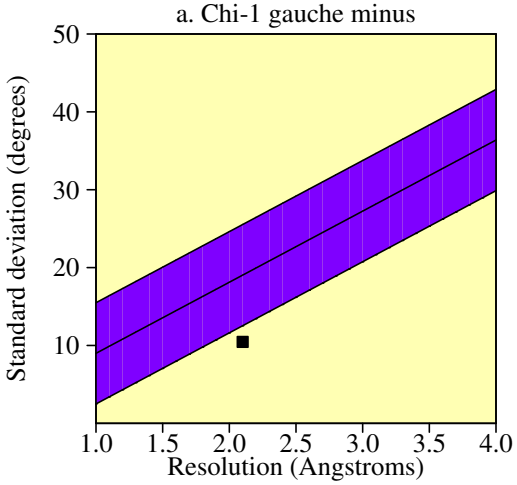
Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. %-tage residues in A, B, L	3477	92.4	82.6	10.0	1.0	Inside
b. Omega angle st dev	3950	1.1	6.0	3.0	-1.6	BETTER
c. Bad contacts / 100 residues	12	0.3	5.2	10.0	-0.5	Inside
d. Zeta angle st dev	3715	1.0	3.1	1.6	-1.3	BETTER
e. H-bond energy st dev	2644	0.7	0.8	0.2	-0.9	Inside
f. Overall G-factor	3997	0.4	-0.4	0.3	2.7	BETTER

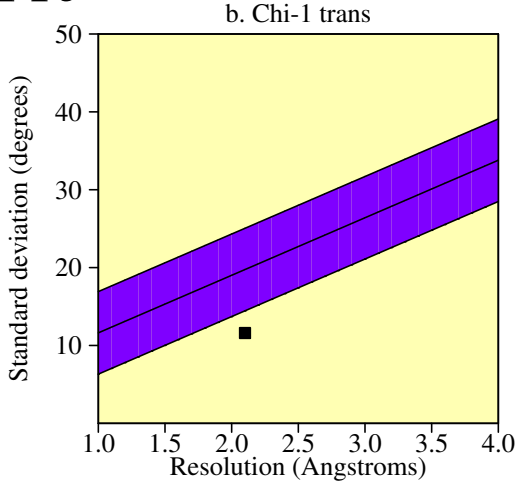
Side-chain parameters

1ppj

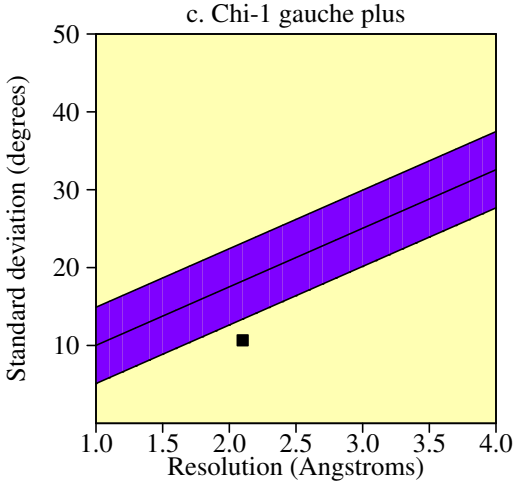
a. Chi-1 gauche minus



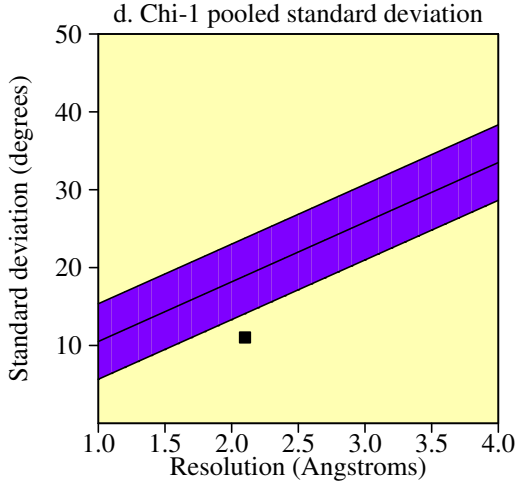
b. Chi-1 trans



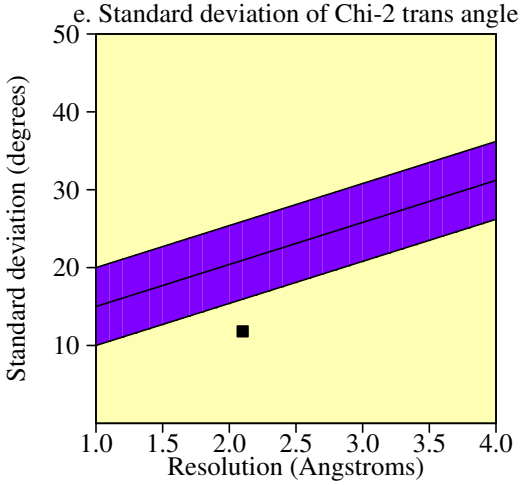
c. Chi-1 gauche plus

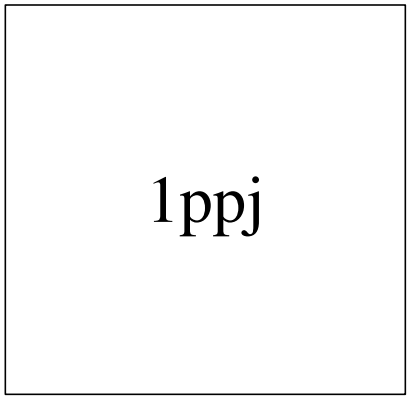


d. Chi-1 pooled standard deviation



e. Standard deviation of Chi-2 trans angle





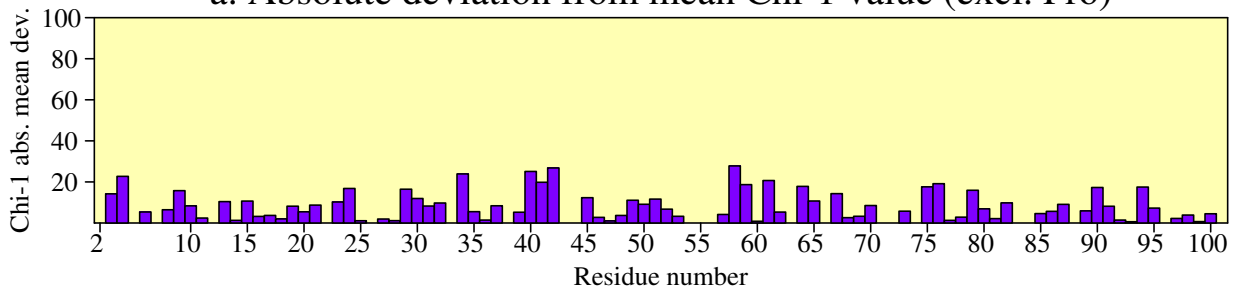
Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. Chi-1 gauche minus st dev	488	10.5	19.0	6.5	-1.3	BETTER
b. Chi-1 trans st dev	1019	11.6	19.7	5.3	-1.5	BETTER
c. Chi-1 gauche plus st dev	1620	10.7	18.3	4.9	-1.6	BETTER
d. Chi-1 pooled st dev	3127	11.0	18.9	4.8	-1.6	BETTER
e. Chi-2 trans st dev	1049	11.8	20.9	5.0	-1.8	BETTER

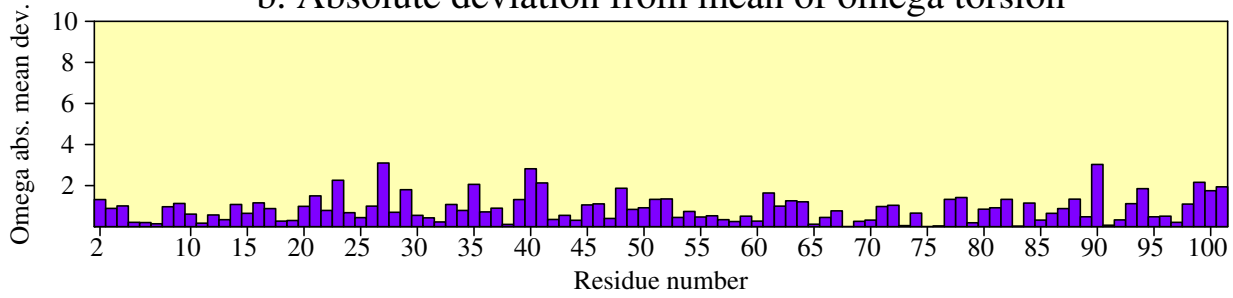
Residue properties

1ppj

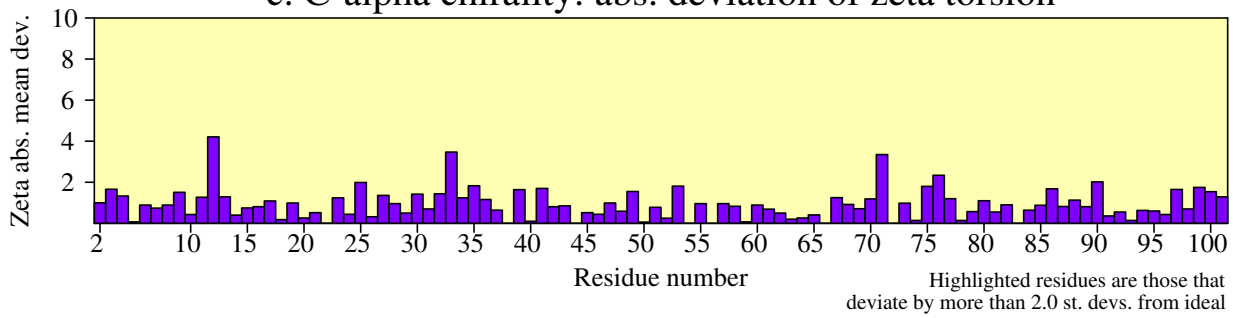
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

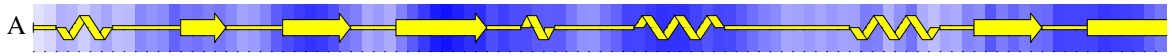


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

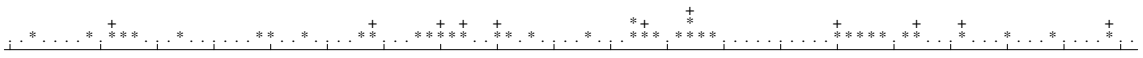


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

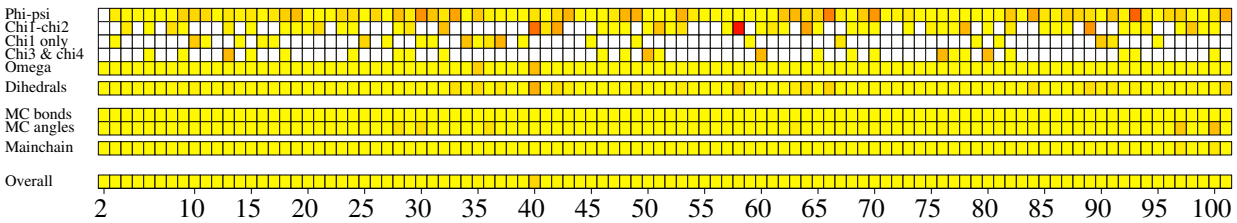
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



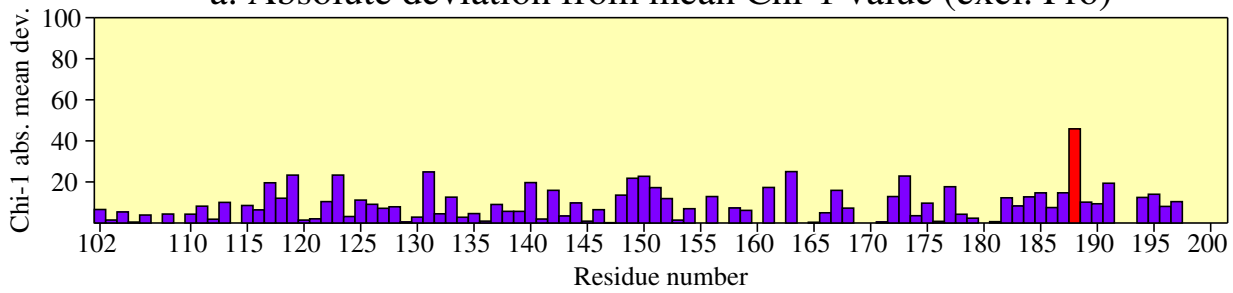
g. G-factors



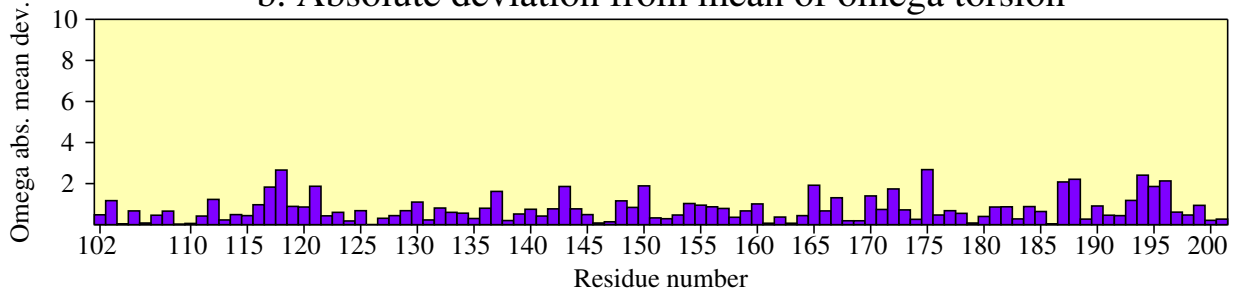
Residue properties

1ppj

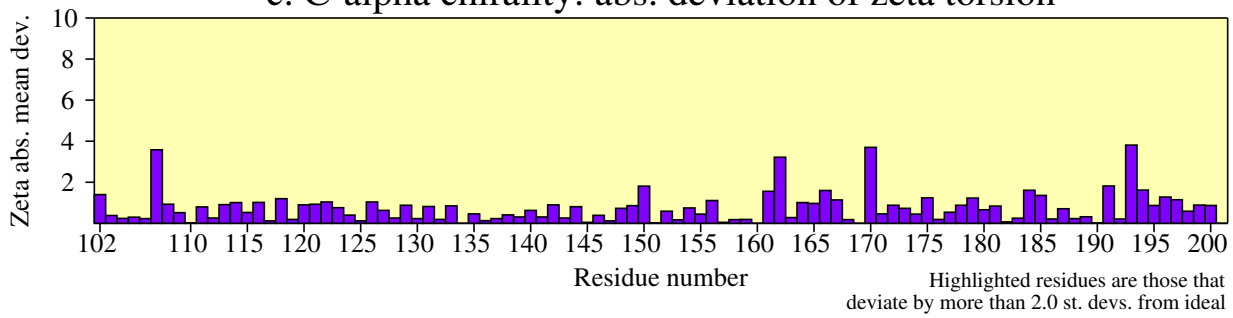
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



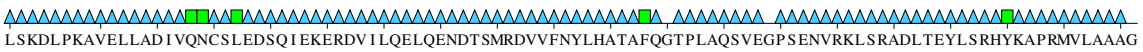
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

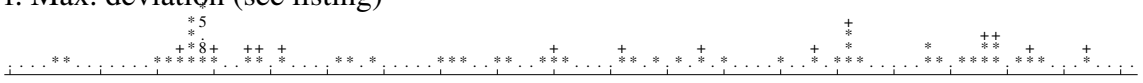
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

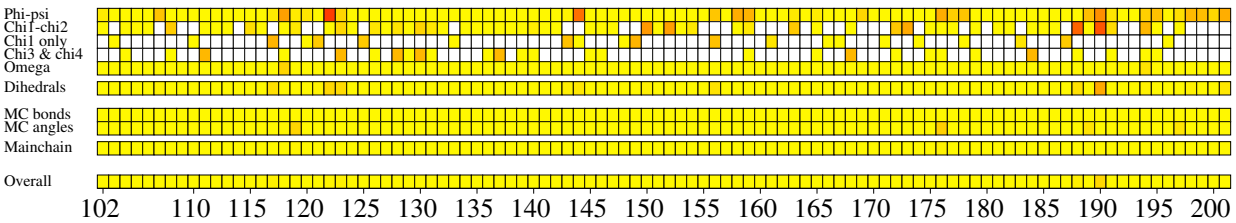


LSKDLPKAVELLADIVQNCSLEDSQIEKERDVILQELQENDTSMRDVVFNYLHATAFQGTPLAQSVEGPSENVRKLSRADLTEYLSRHYKAPRMVLAAAG

f. Max. deviation (see listing)

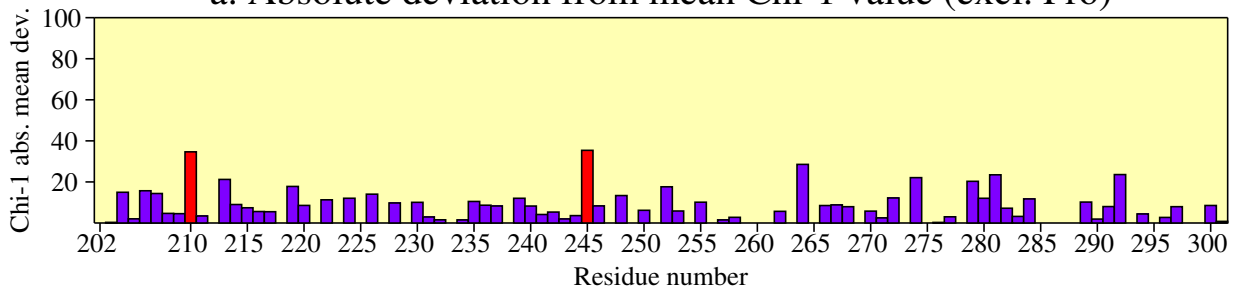


g. G-factors

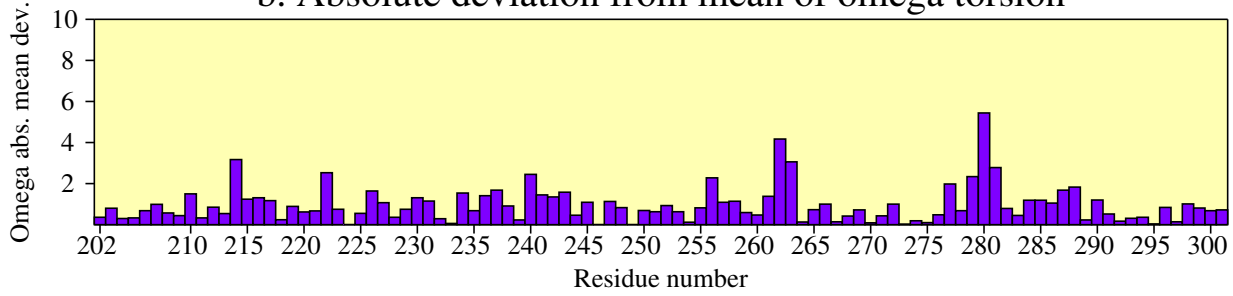


Residue properties
1ppj

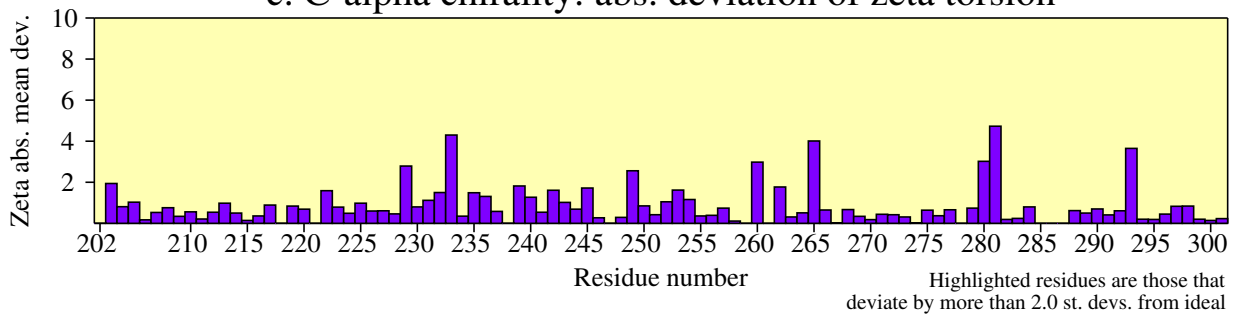
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

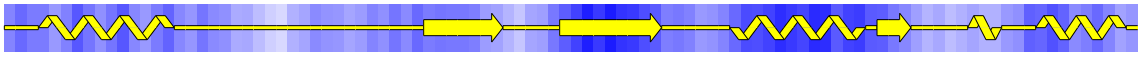


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



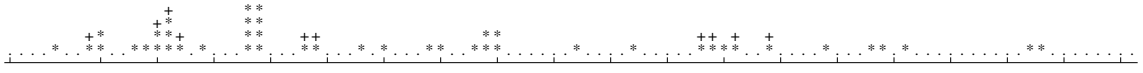
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

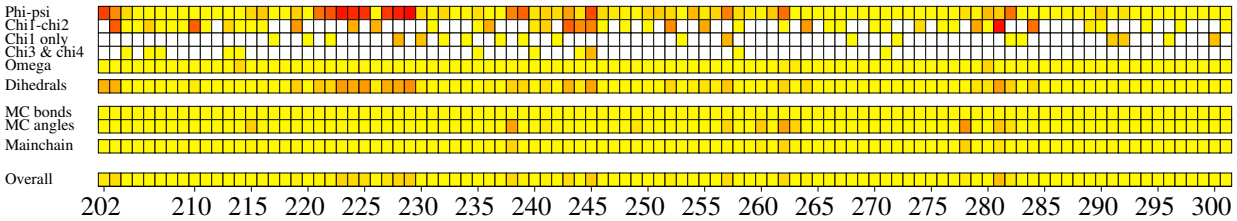
Most favoured Allowed Generous Disallowed

GLEHRQLLDLAQKHFSGLSGTYDEDAVPTLSPCRFTGSQICHREDGLPLAHVAIAVEGPGWAHPDNVALQVANAIIGHYDCTYGGGAHLSSPLASIAATN

f. Max. deviation (see listing)

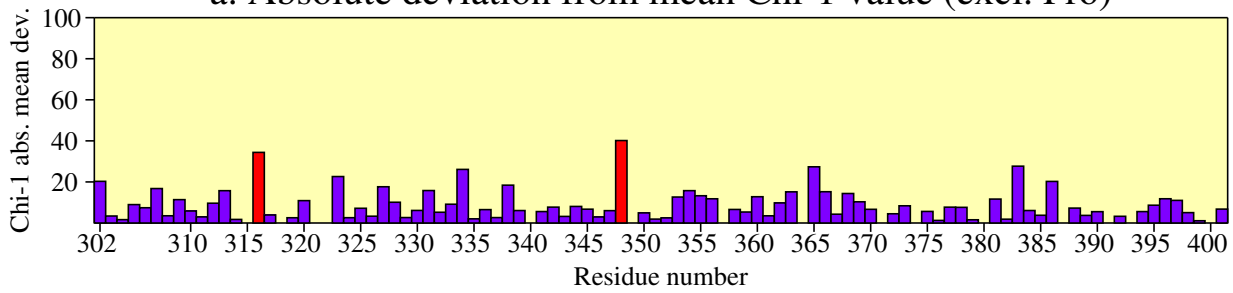


g. G-factors

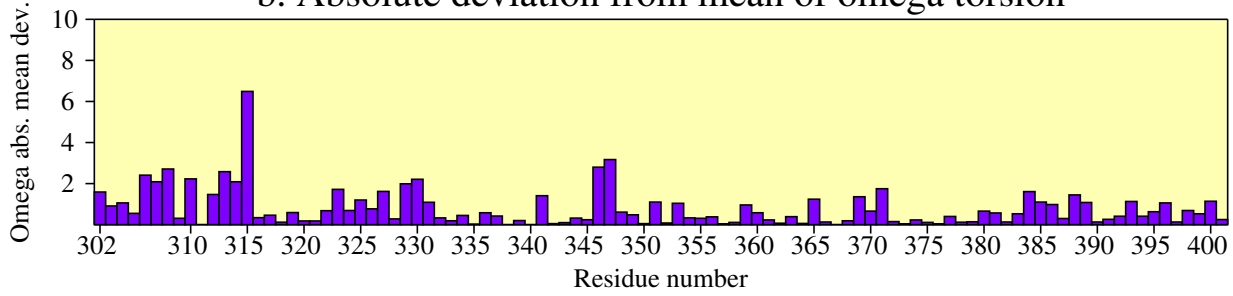


Residue properties
1ppj

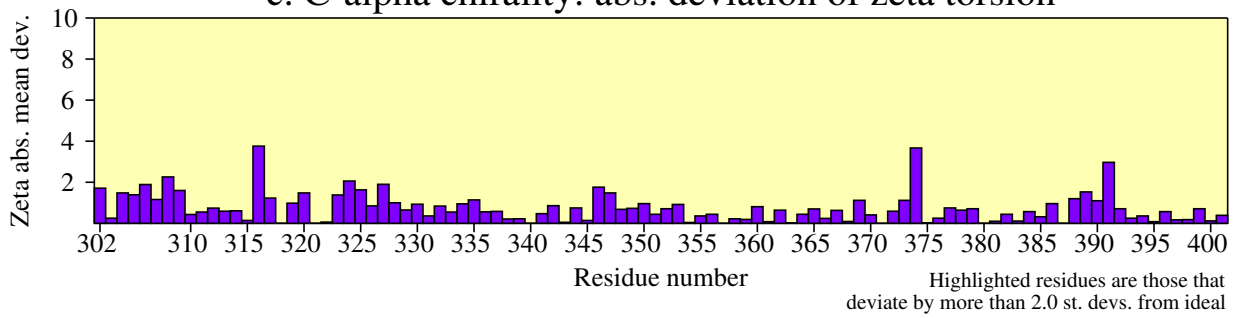
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

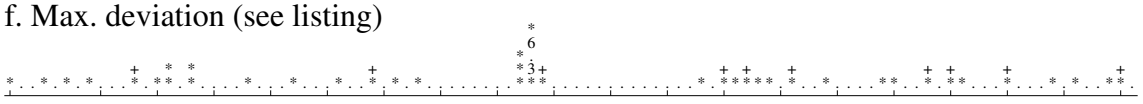
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

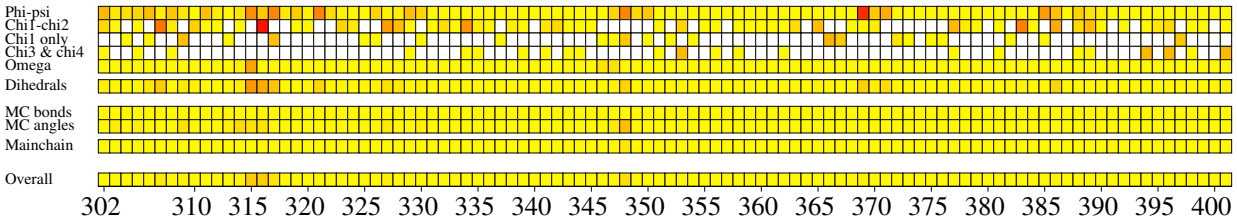
Most favoured Allowed Generous Disallowed

KLCQSFQTFNICYADTGLLGAHFVCDHMSIDDMMFVLQGQWMRLCTSATESEVLRGKNLLRNALVSHLDGTTPVCEDIGRSLLTYGRRIPLAEWESRIAE

f. Max. deviation (see listing)

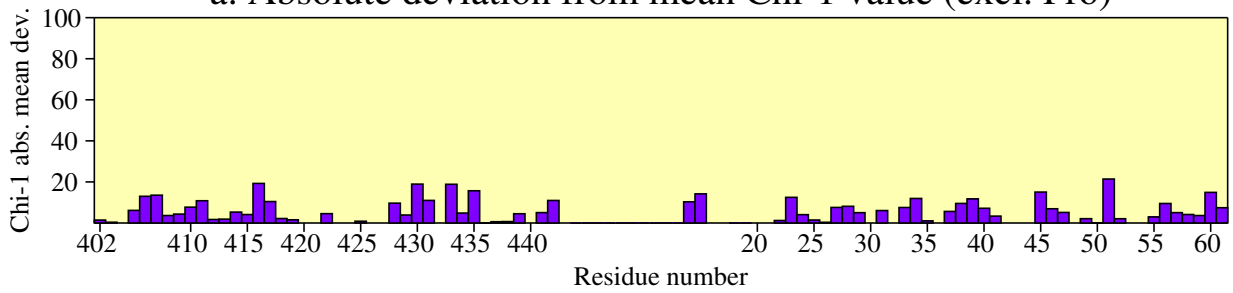


g. G-factors

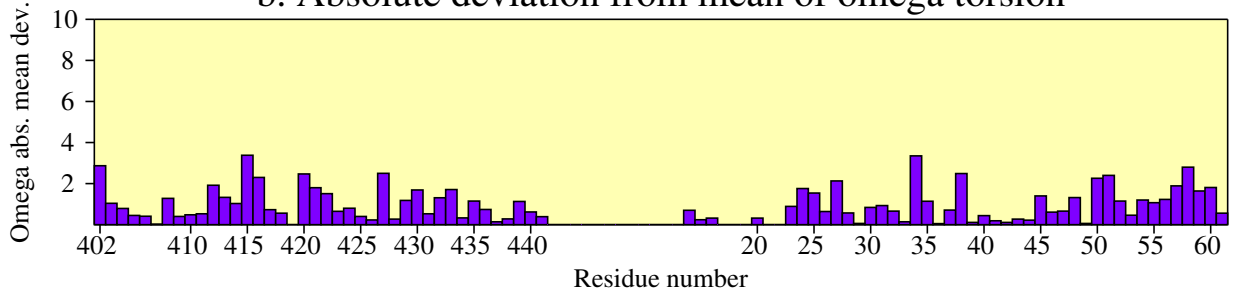


Residue properties
1ppj

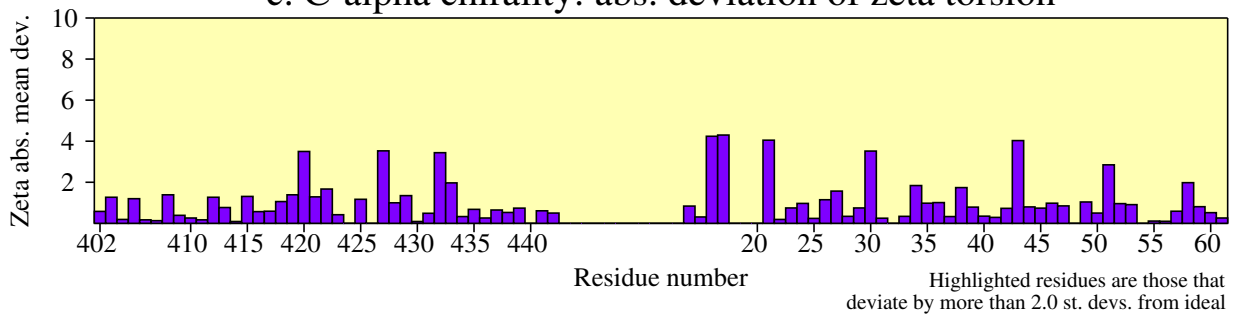
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

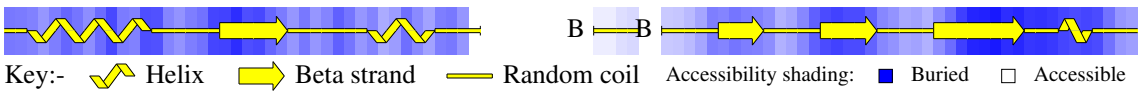


c. C-alpha chirality: abs. deviation of zeta torsion



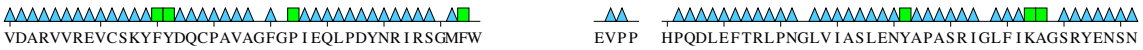
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

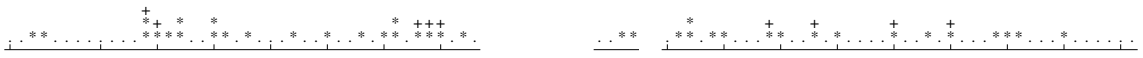


e. Sequence & Ramachandran regions

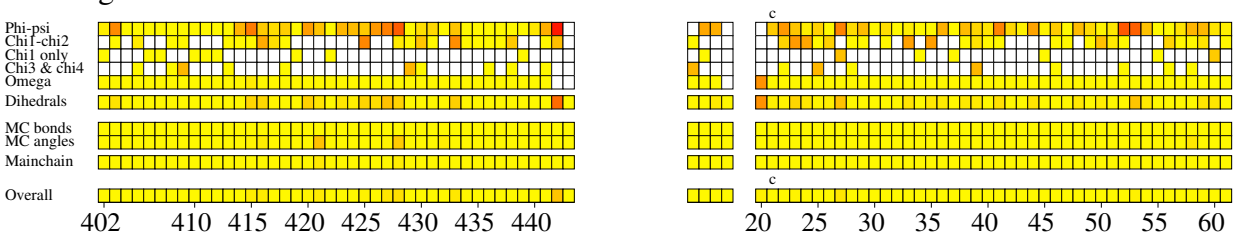
Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)



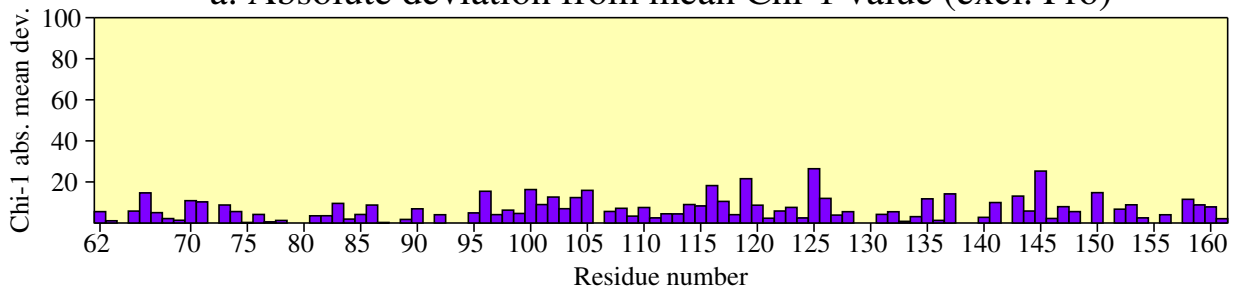
g. G-factors



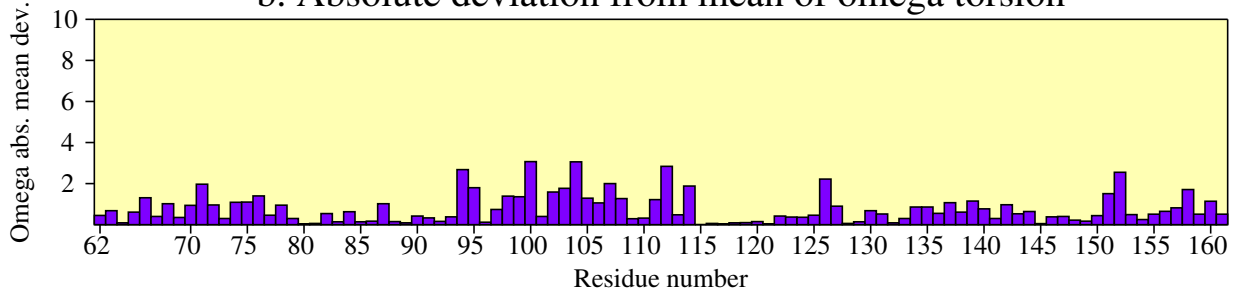
c = cis-peptide

Residue properties
1ppj

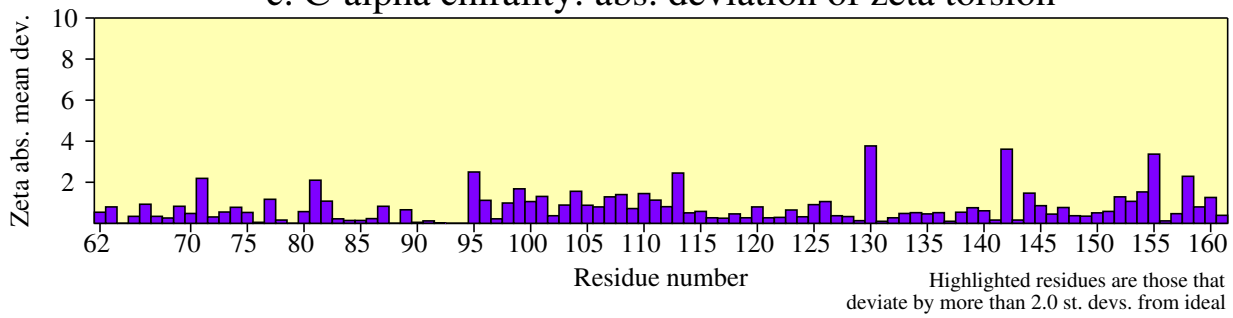
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

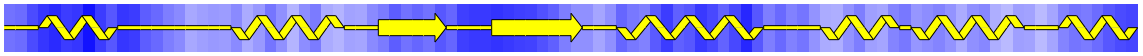


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



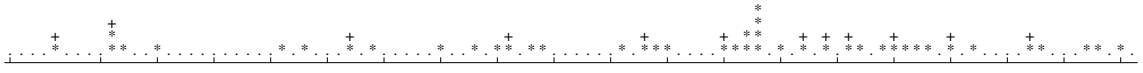
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

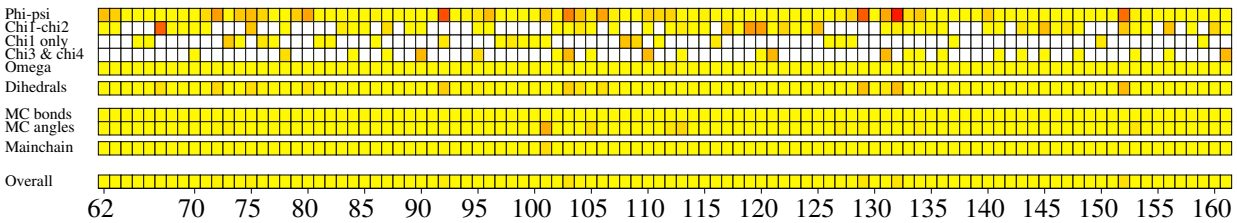
Most favoured Allowed Generous Disallowed

N L G T S H L L R L A S S L T T K G A S S F K I T R G I E A V G G K L S V T S T R E N M A Y T V E C L R D D V D I L M E F L L N V T T A P E F R R W E V A A L Q P Q L R I D K A V A L Q N P Q A H V I E

f. Max. deviation (see listing)



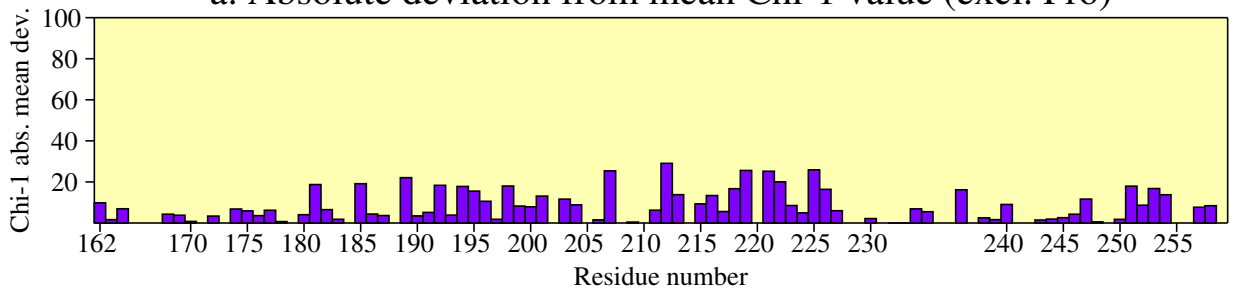
g. G-factors



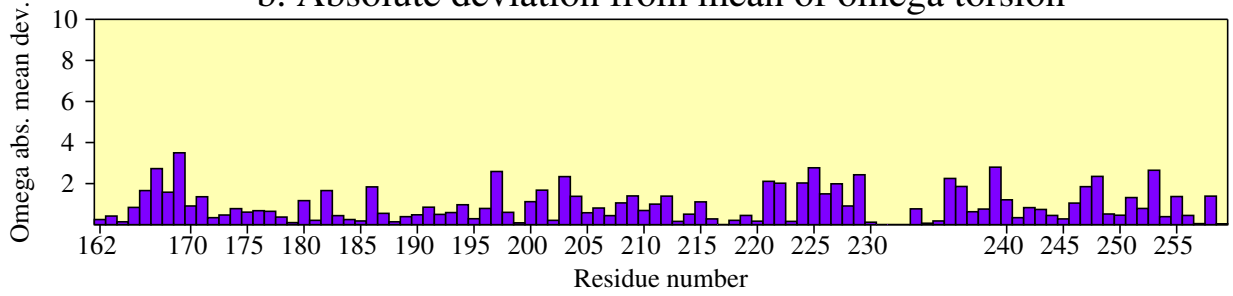
Residue properties

1ppj

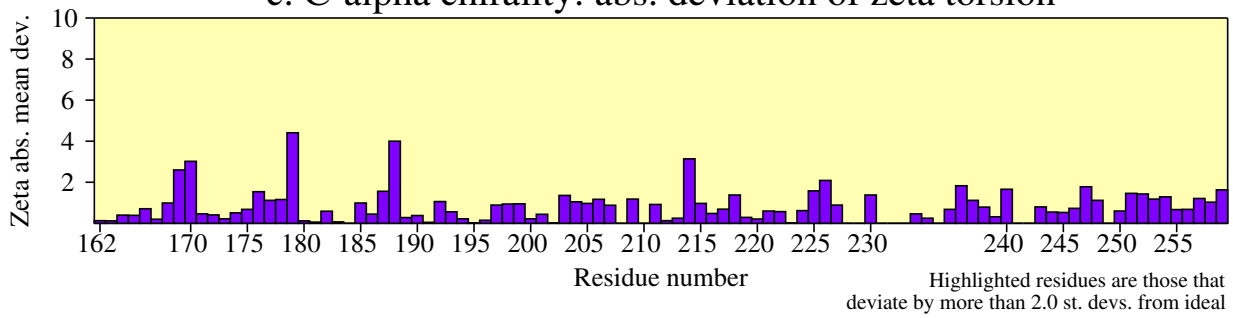
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

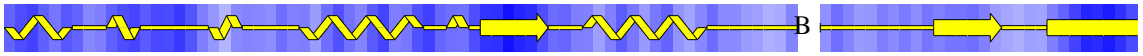


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

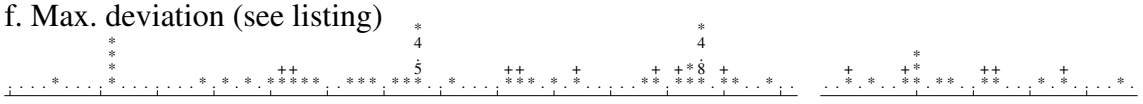


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

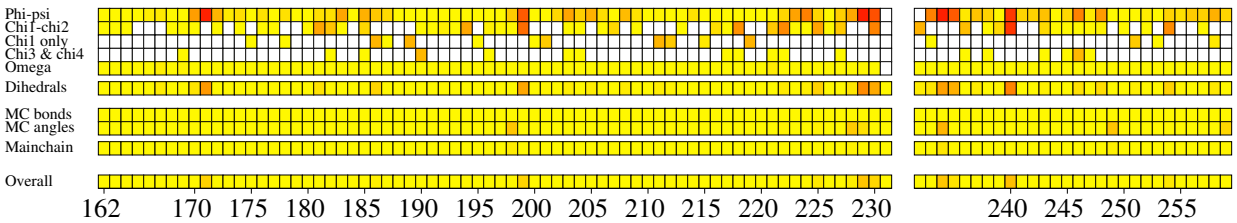
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

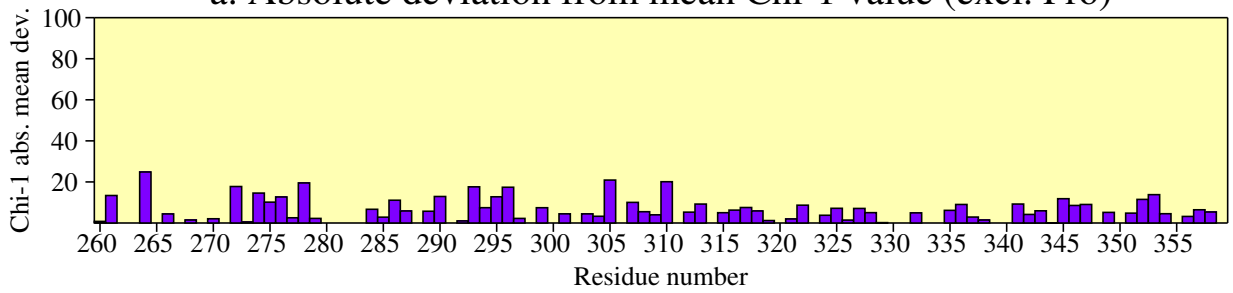


g. G-factors

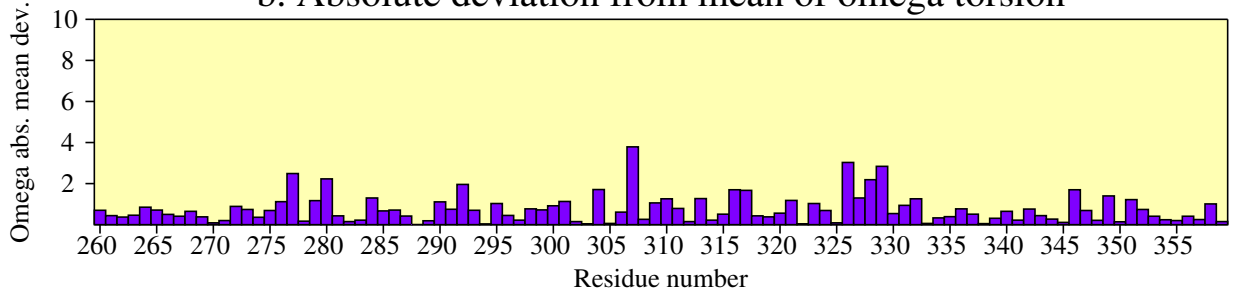


Residue properties
1ppj

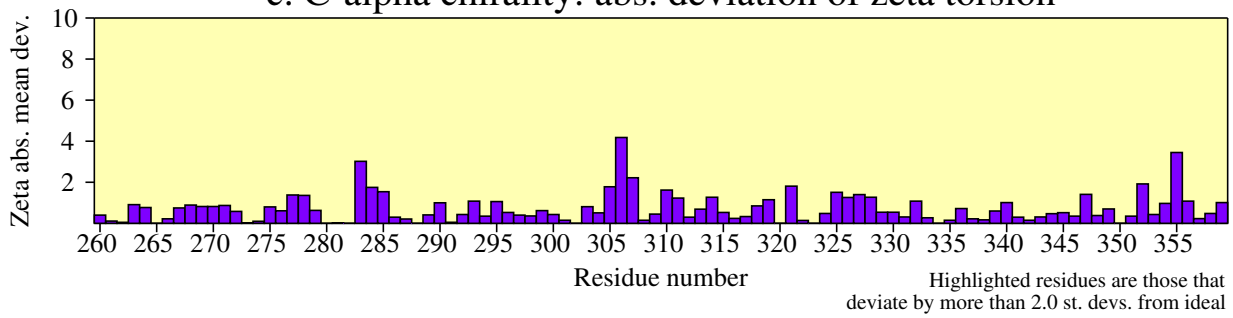
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



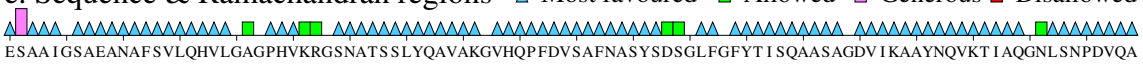
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

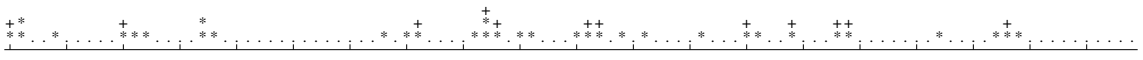
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

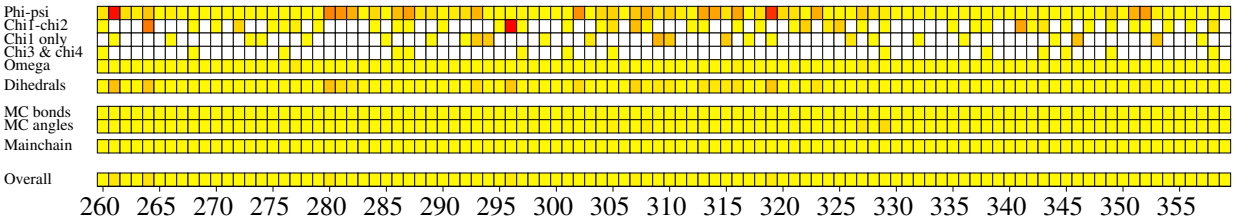


ESAAIGSAEANAFSVLQHVLGAGPHVKRGSNATSSLYQAVAKGVHQPFDVSAFNASYSDSGLFGFYTISQAASAGDVIKAAYNQVKTIAQGNLSNPDVQA

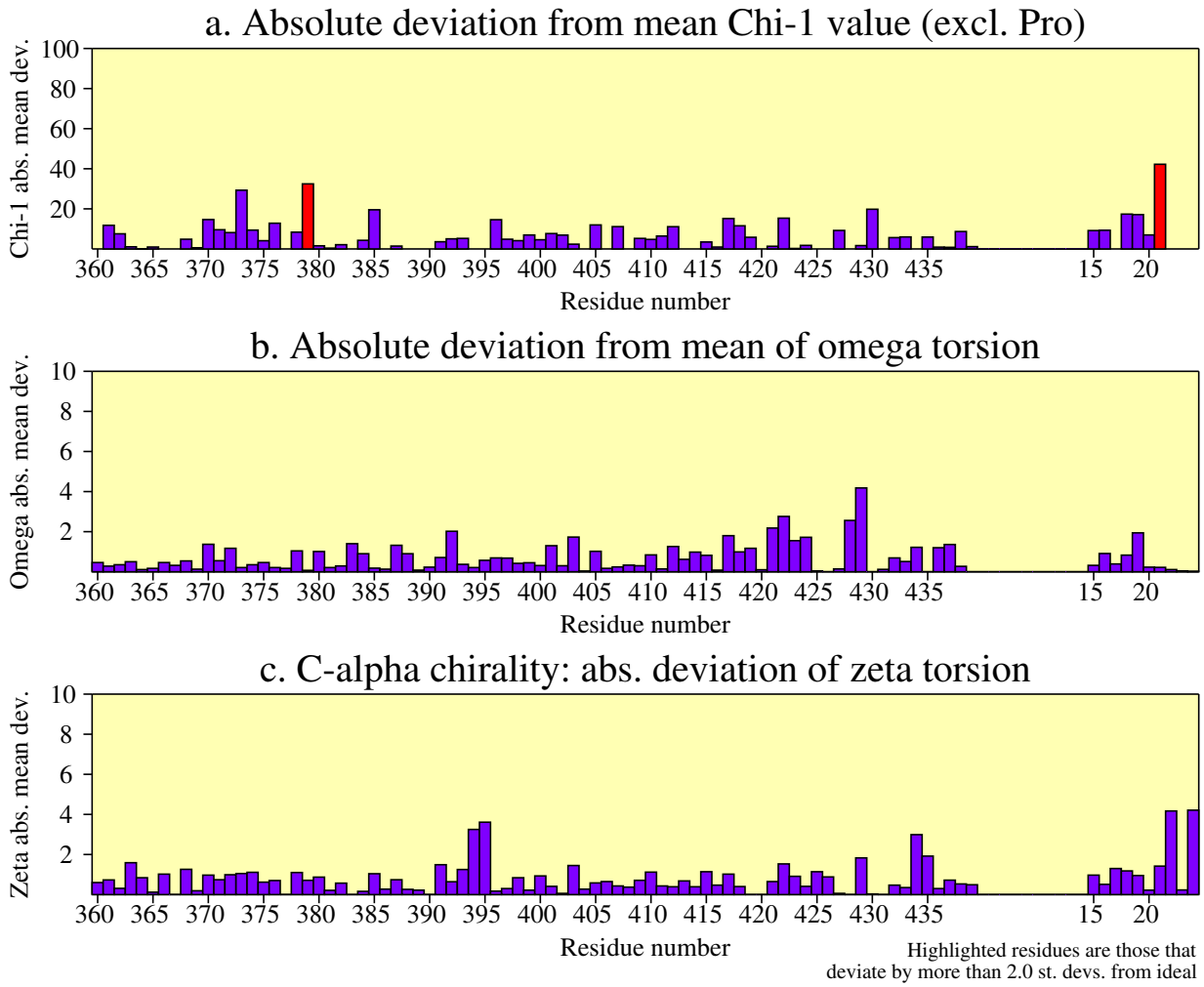
f. Max. deviation (see listing)



g. G-factors

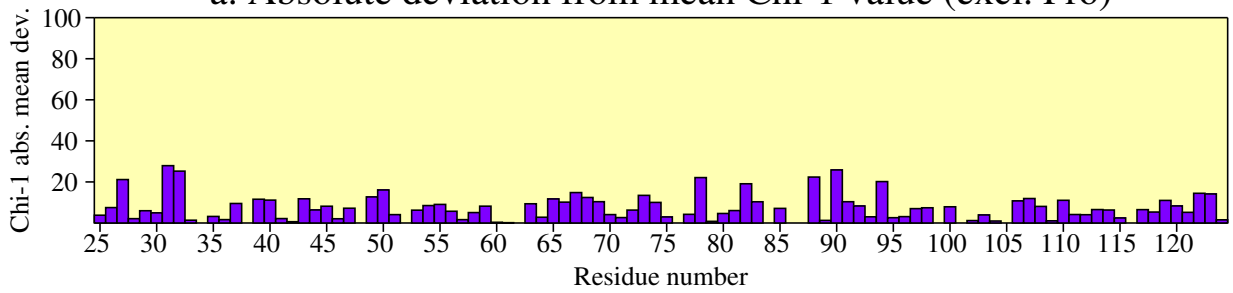


Residue properties
1ppj

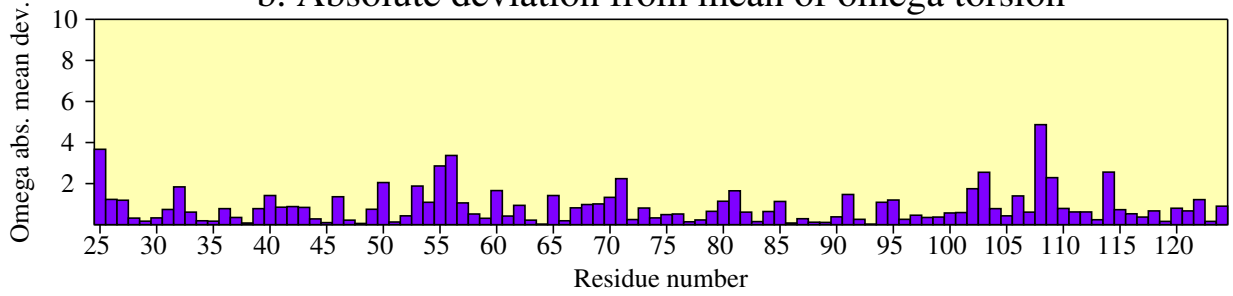


Residue properties
1ppj

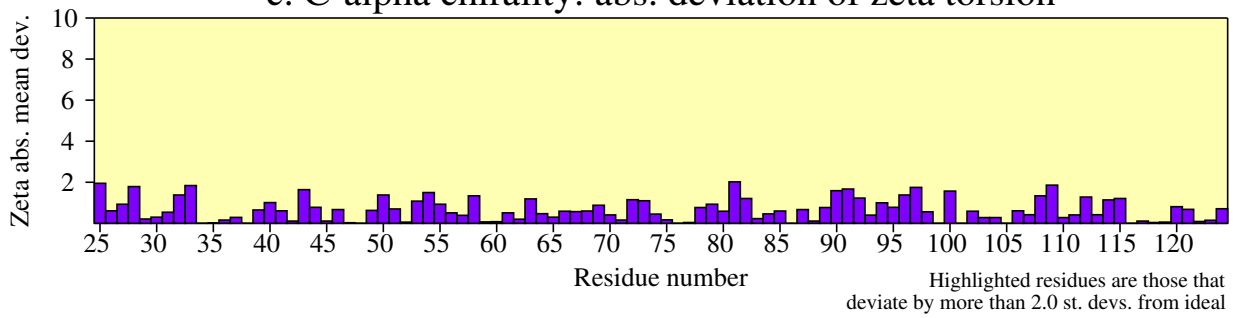
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

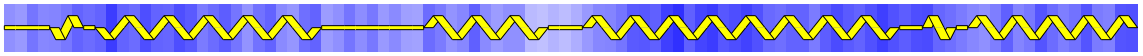


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



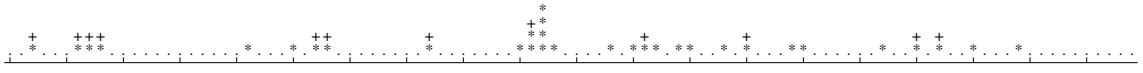
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

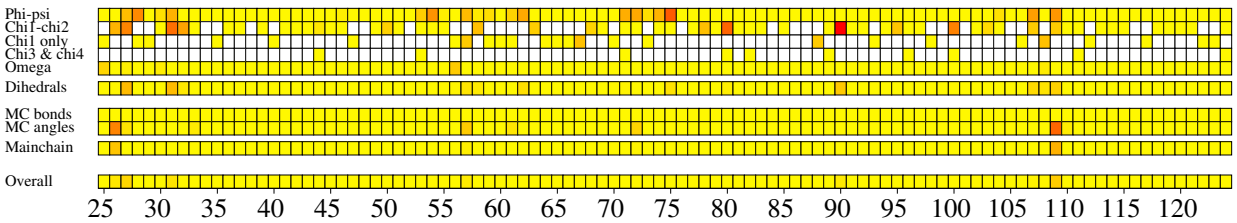
Most favoured Allowed Generous Disallowed

SNISSWWNFGSLLGICLILQILTGLFLAMHYTSDTTTAFSSVTHICRDVNYGWIIRYMHANGASMFFICLYMHVGRGLYYGSYTFLETWNIGVILLLTVM

f. Max. deviation (see listing)

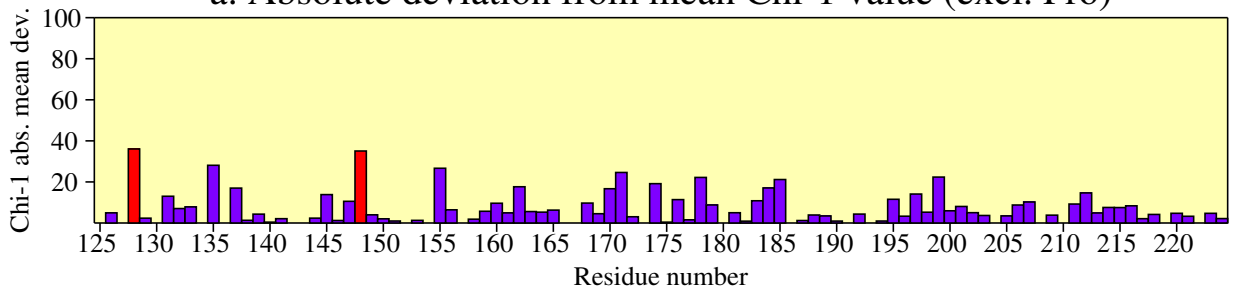


g. G-factors

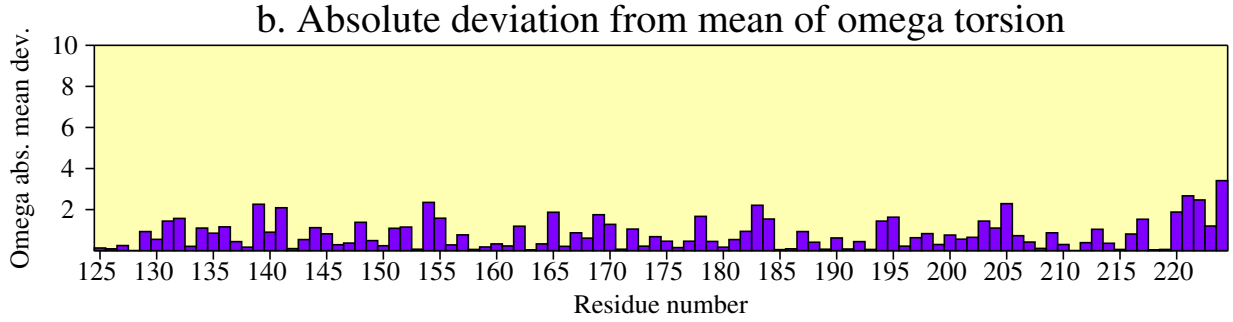


Residue properties
1ppj

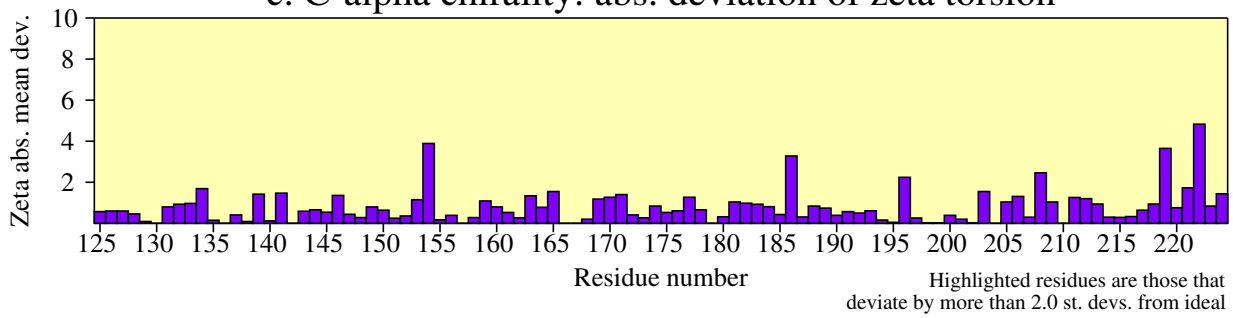
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

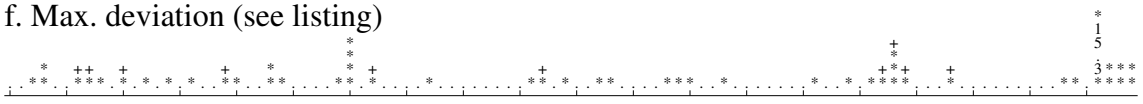
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

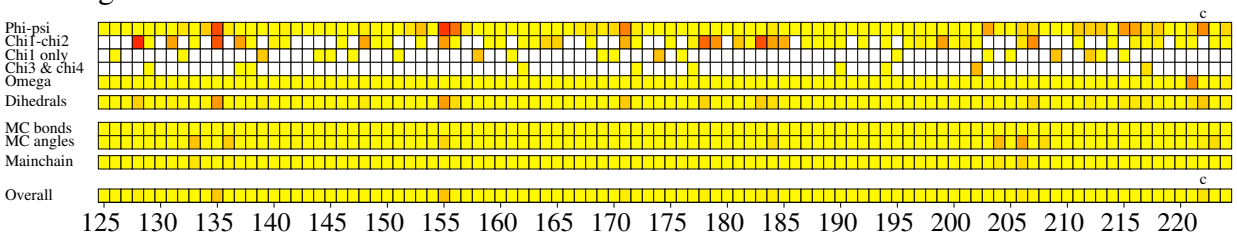
Most favoured Allowed Generous Disallowed

ATAFMGYVLPWGQMSFWGATVITNLLSAIPYIGTNLVEWIWGGFSVDKATLTRFFAFHFILPFIIMAIAMVHLLFLHETGSNNPTGISSDVDKIPFHPYY

f. Max. deviation (see listing)



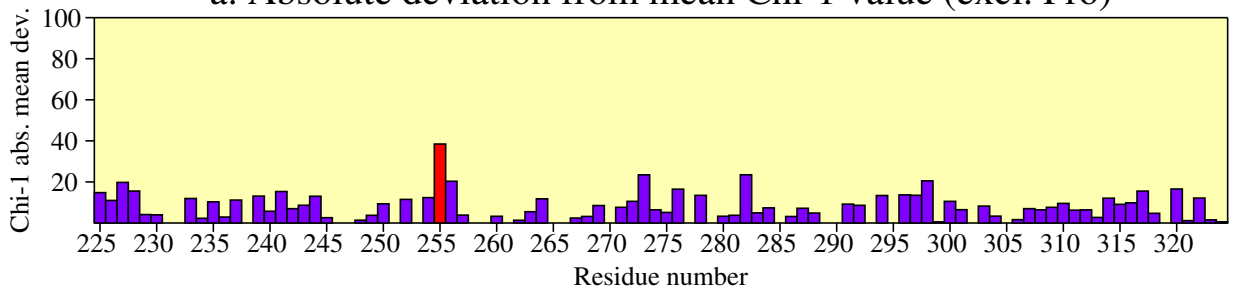
g. G-factors



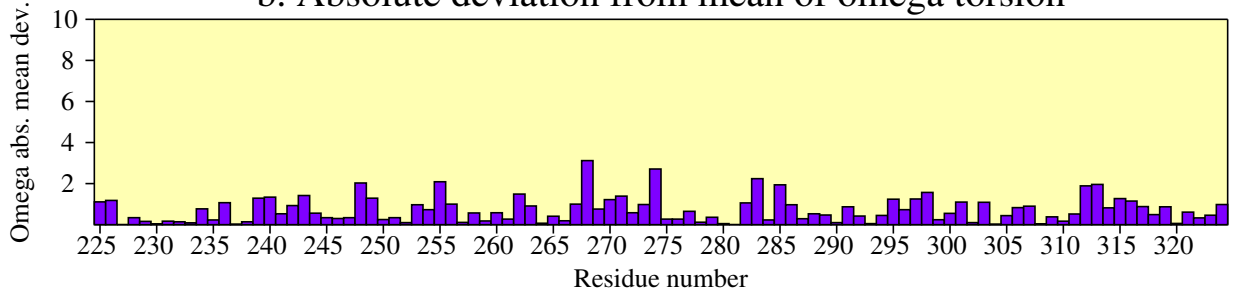
c = cis-peptide

Residue properties
1ppj

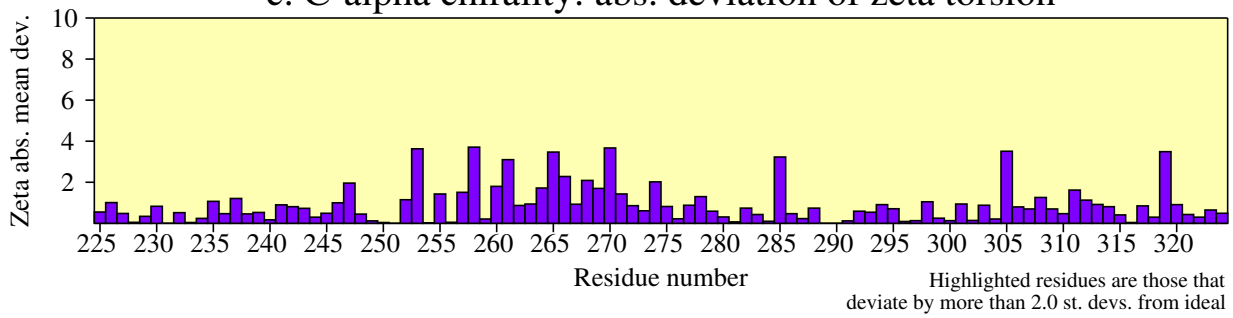
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

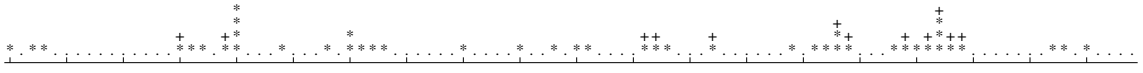
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

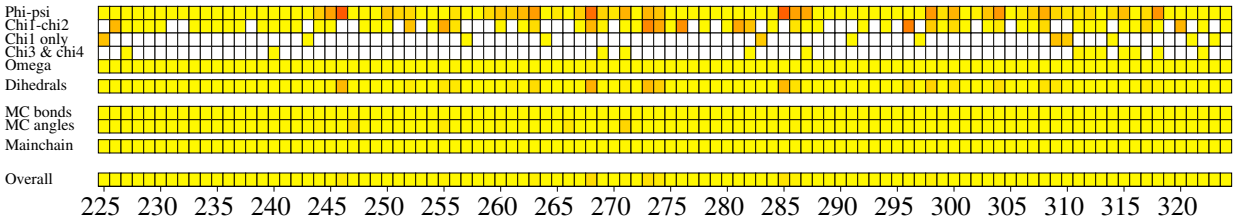
Most favoured Allowed Generous Disallowed

T I K D I L G A L L L I L A L M L L V L F A P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I L R S I P N K L G G V L A L A F S I L I L A L I P L L H T S K Q R S M M F R P L S Q C L

f. Max. deviation (see listing)

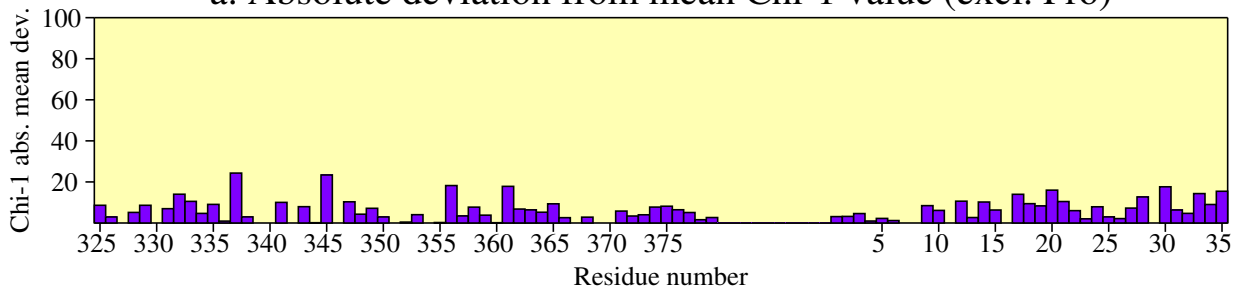


g. G-factors

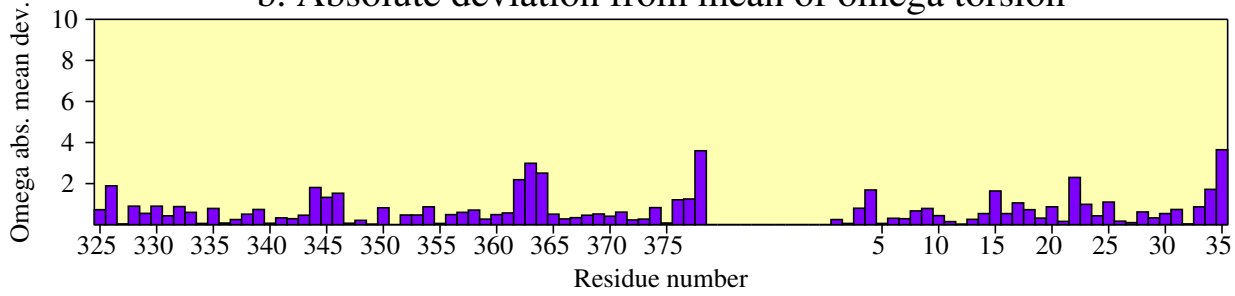


Residue properties
1ppj

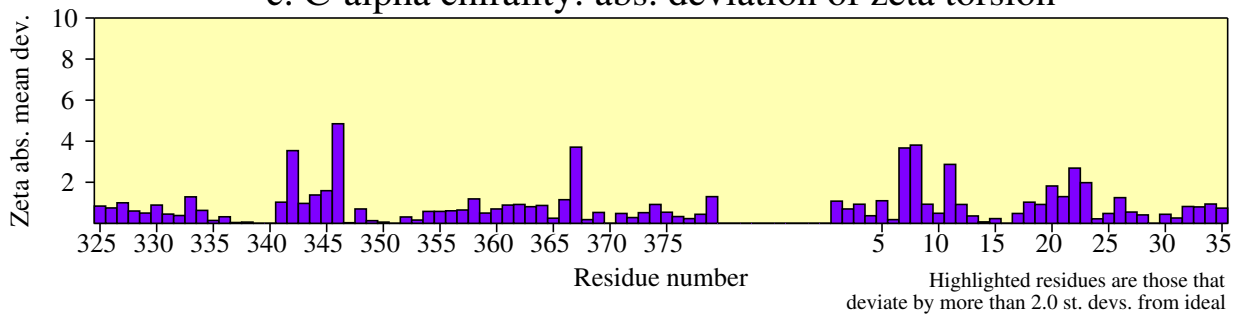
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

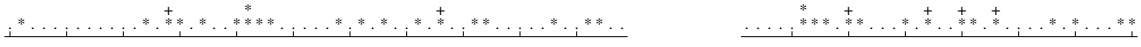


d. Secondary structure & estimated accessibility

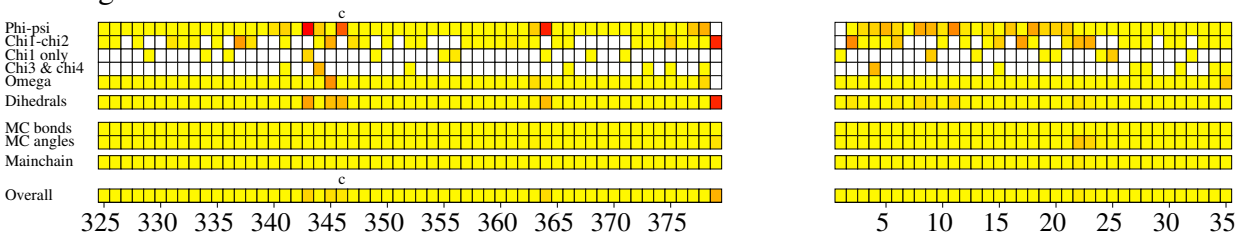
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)



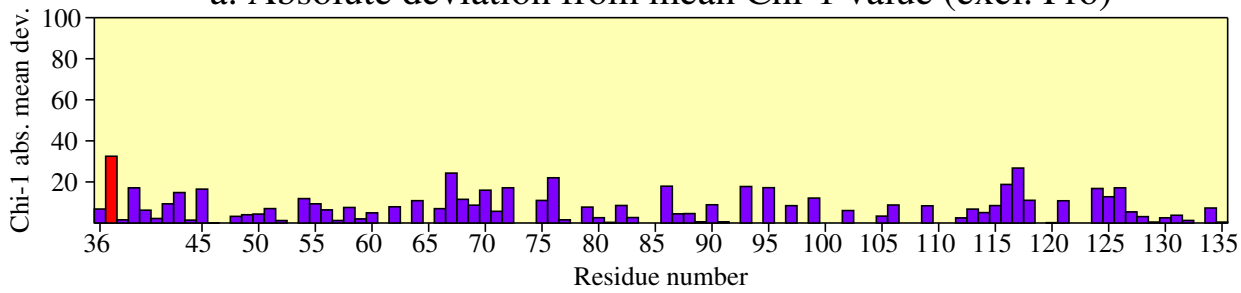
g. G-factors



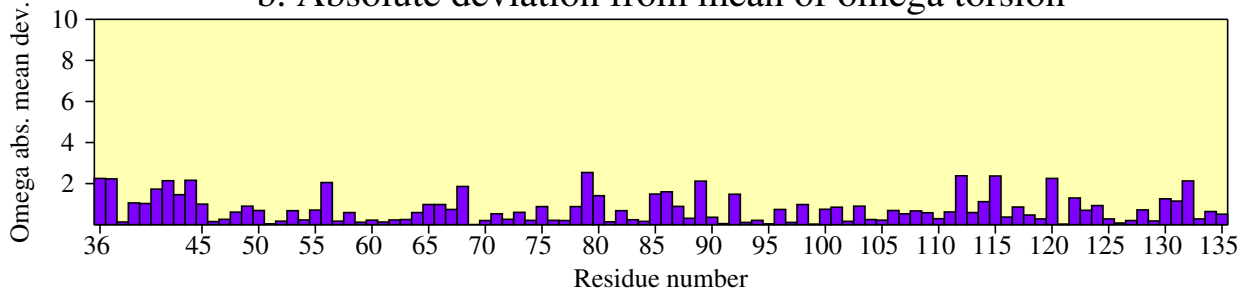
c = cis-peptide

Residue properties
1ppj

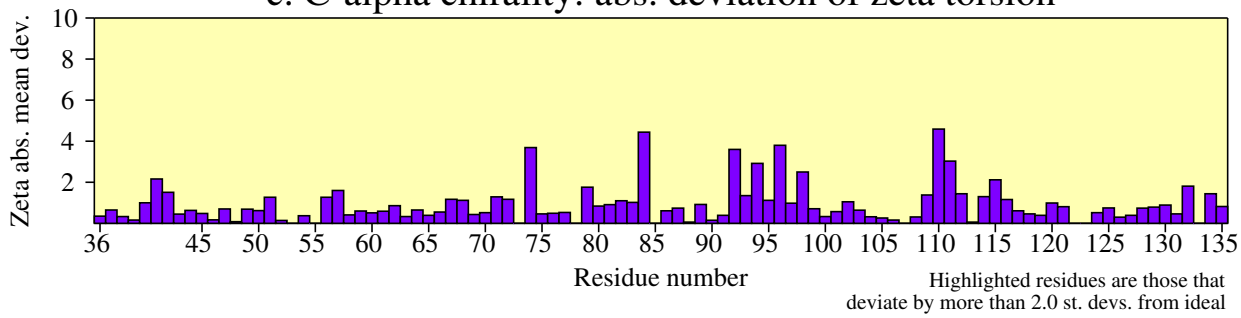
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



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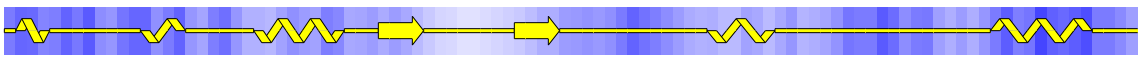
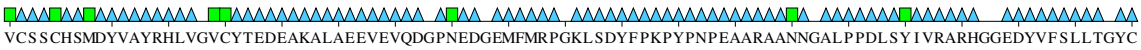
d. Secondary structure & estimated accessibility



Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

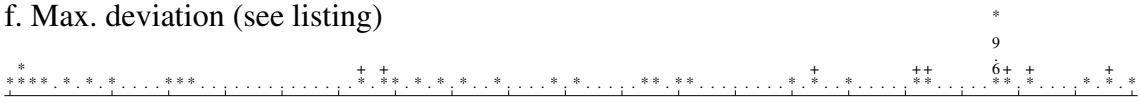
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

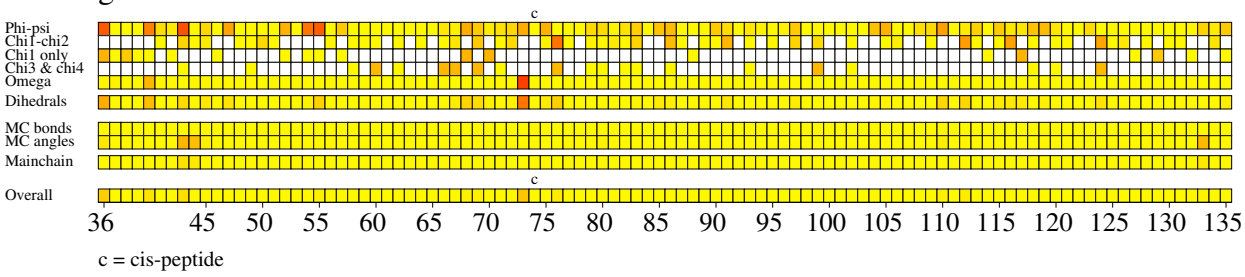


VCSSCHSMDYVAYRHLVGVCYTEDEAKALAEEVEVQDGPNEDGEMFMRPGKLSDYFPKPYPNPEAARAANNGALPPDLSYIVRARHGGEDYVFSLLTGYC

f. Max. deviation (see listing)



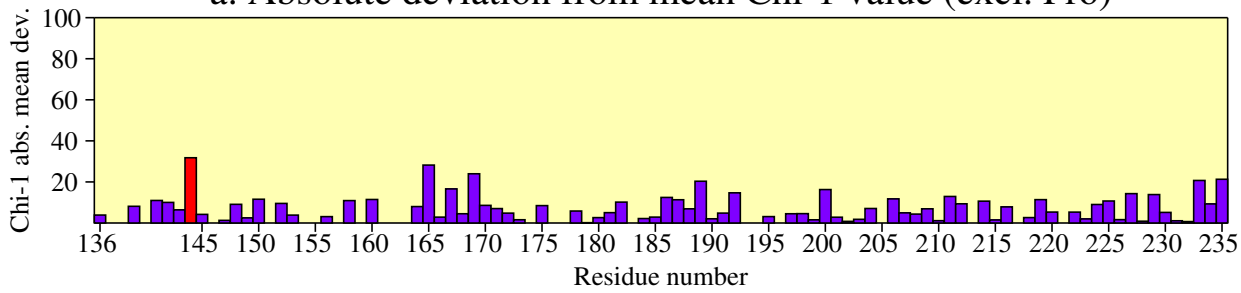
g. G-factors



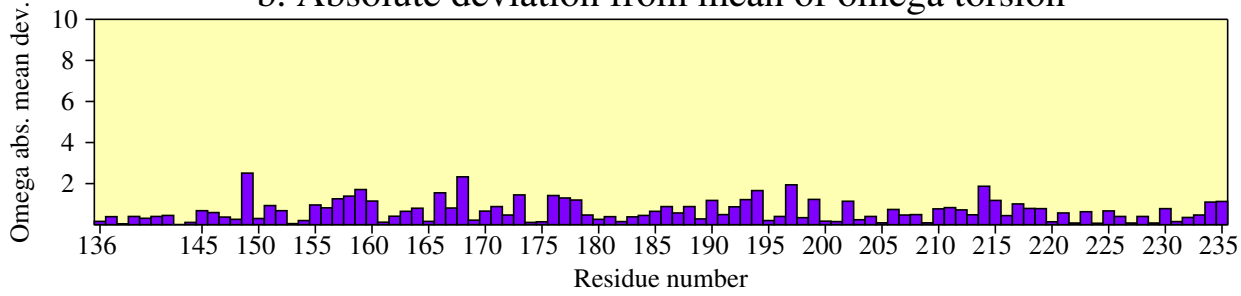
c = cis-peptide

Residue properties
1ppj

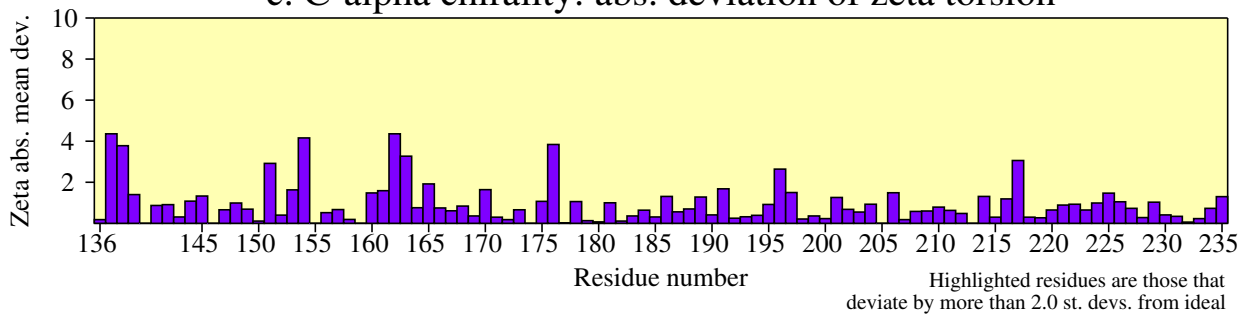
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



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d. Secondary structure & estimated accessibility

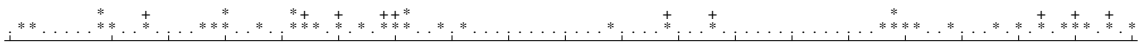
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

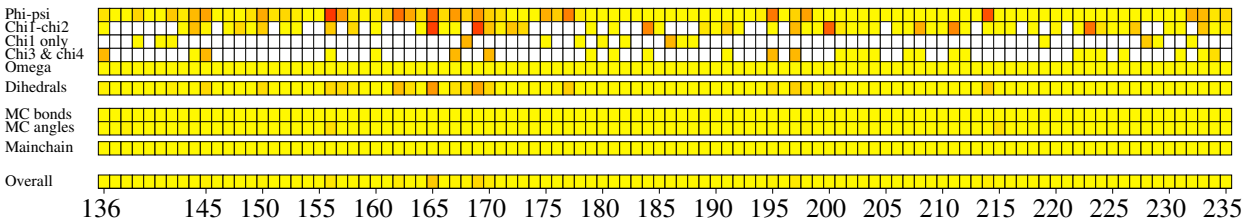
Most favoured Allowed Generous Disallowed

EPPTGVSLREGLYFNPYFPGQAIGMAPPIYNEVLEFDDGTPATMSQVAKDVCTFLRWAAEPEHDHRKRMGLKMLLMMGLLLPLVYAMKRHKWSVLKSRKL

f. Max. deviation (see listing)



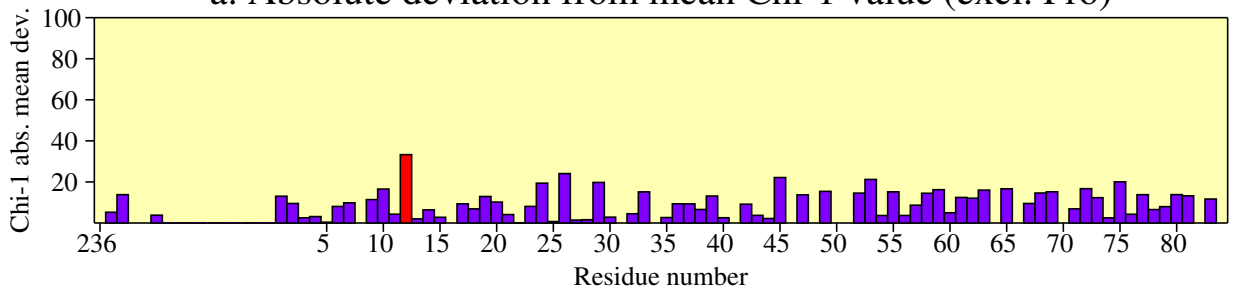
g. G-factors



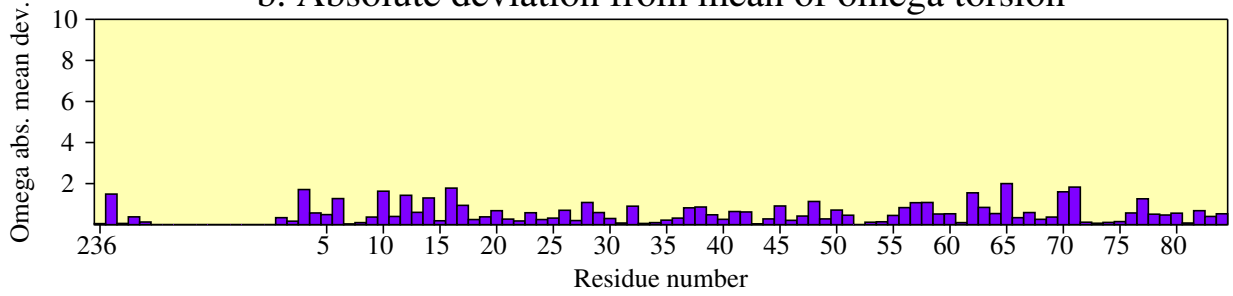
Residue properties

1ppj

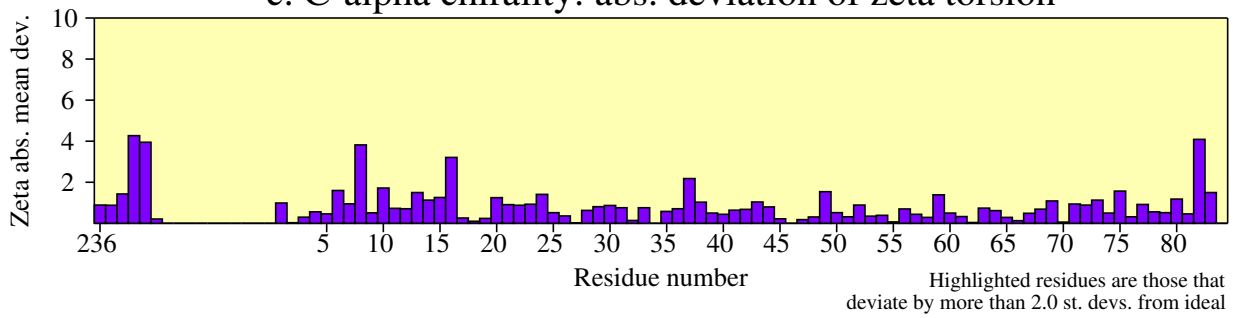
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

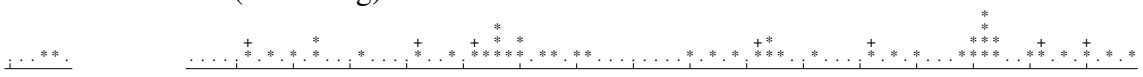
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

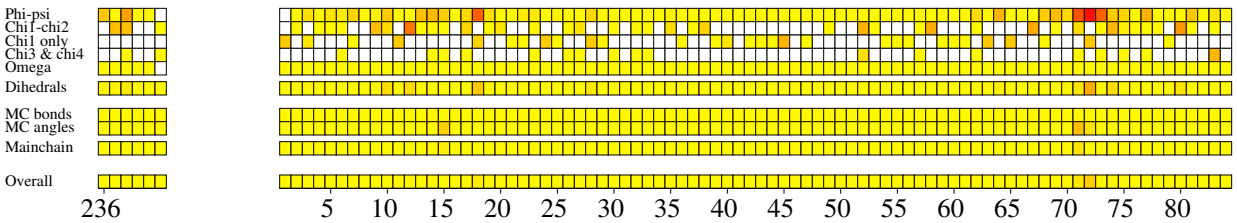
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

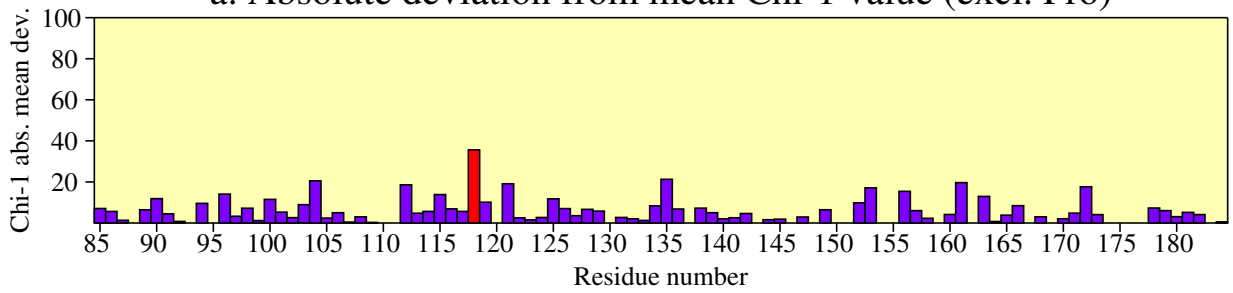


g. G-factors

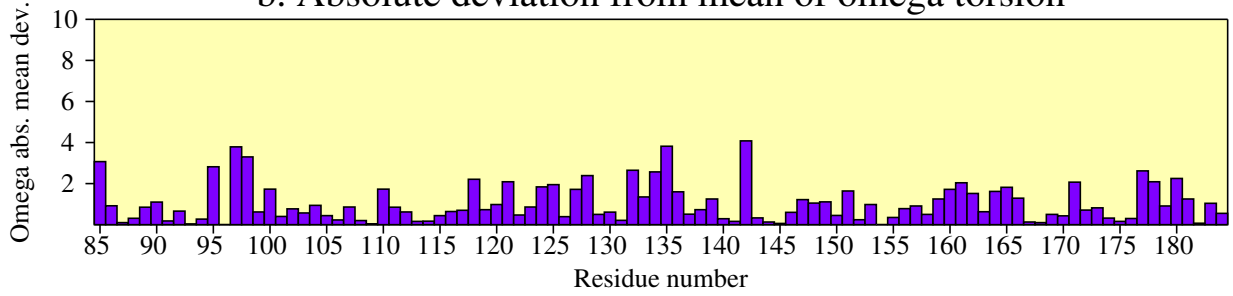


Residue properties
1ppj

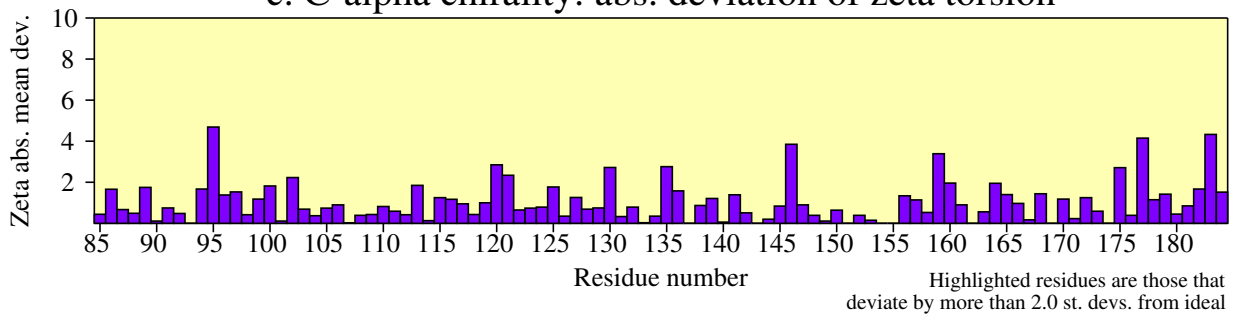
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

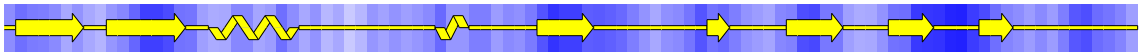


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



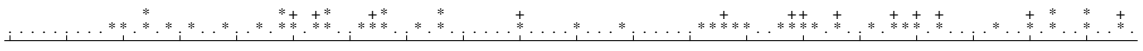
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

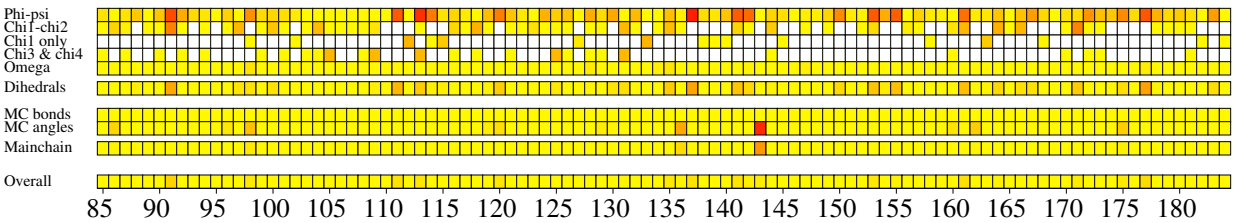
Most favoured Allowed Generous Disallowed

KNMAFKWRGKPLFVRHRTKKEIDQEAAVEVSQLRDPQHDLERVKKPEWVILIGVCTHLGCVPIANAGDFGGYYCPCHGSHYDASGRIRKGPAPLNLEVPS

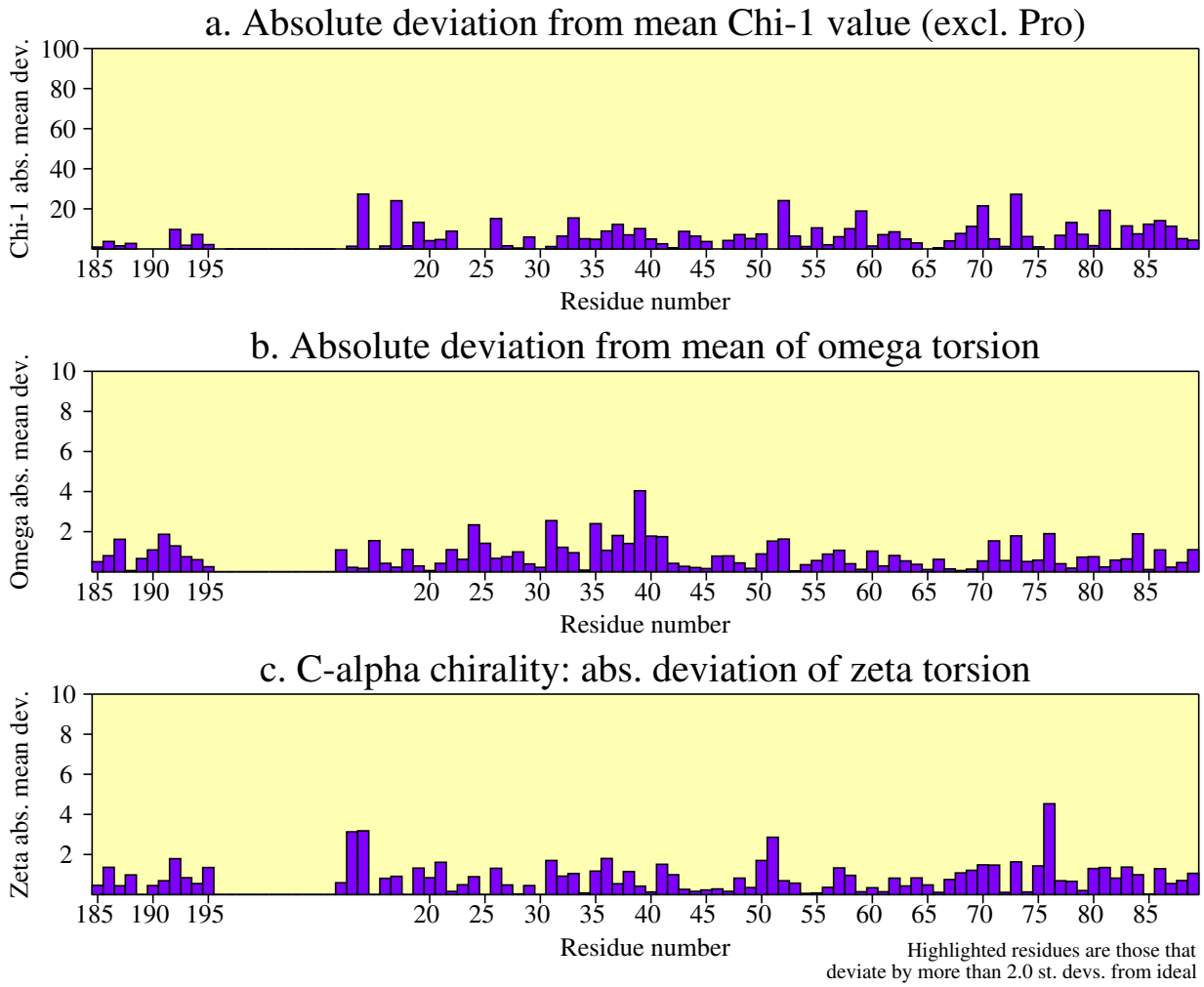
f. Max. deviation (see listing)



g. G-factors

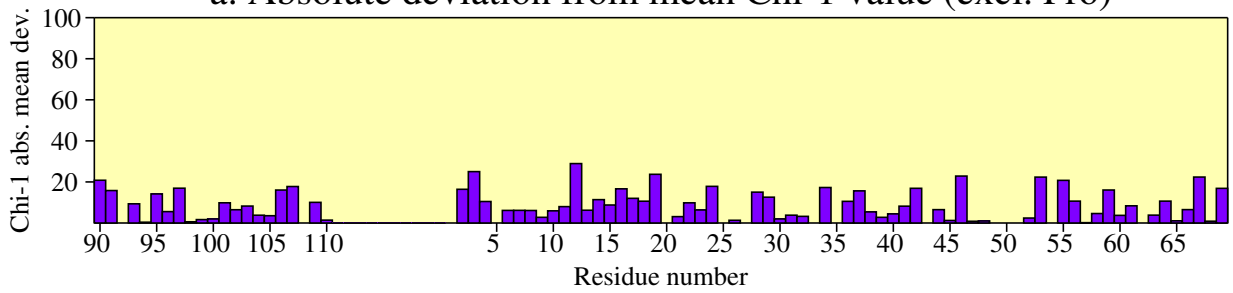


Residue properties
1ppj

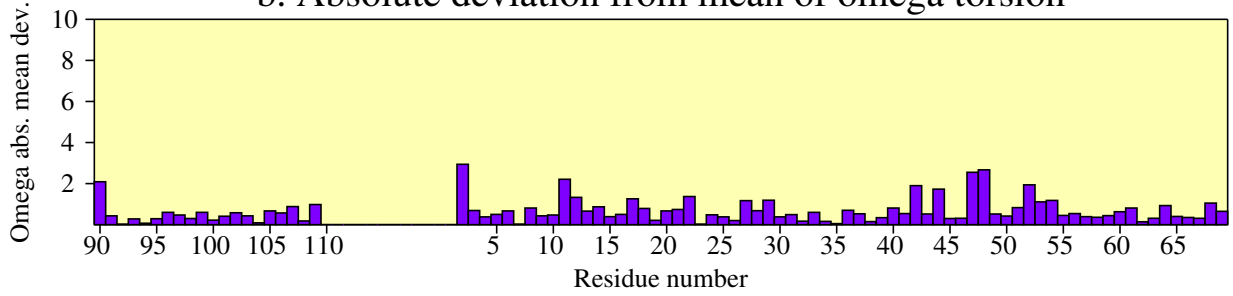


Residue properties
1ppj

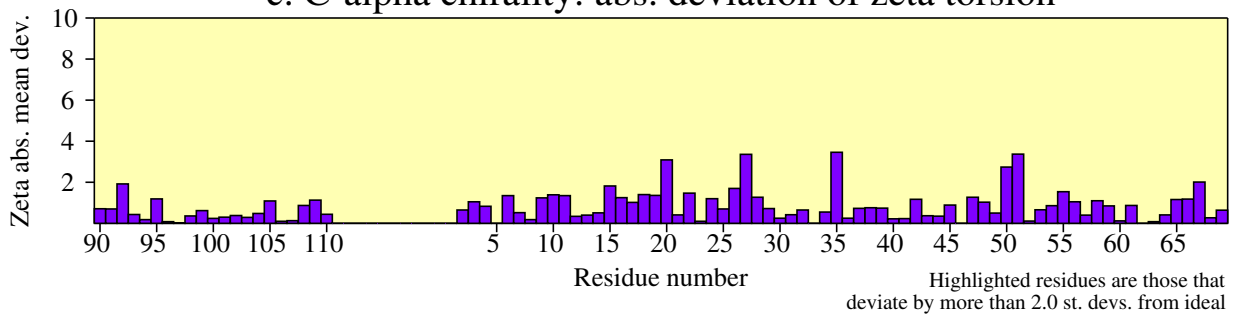
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

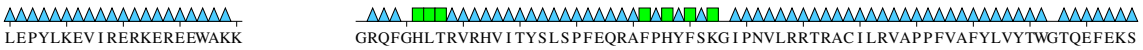


Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

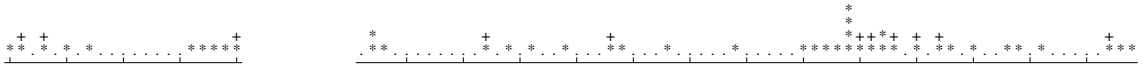
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

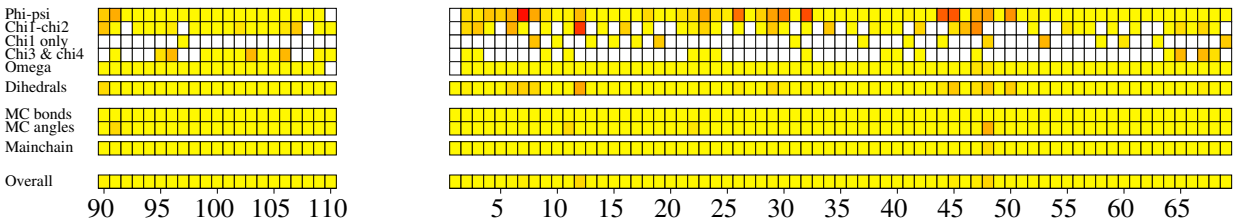
e. Sequence & Ramachandran regions Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)



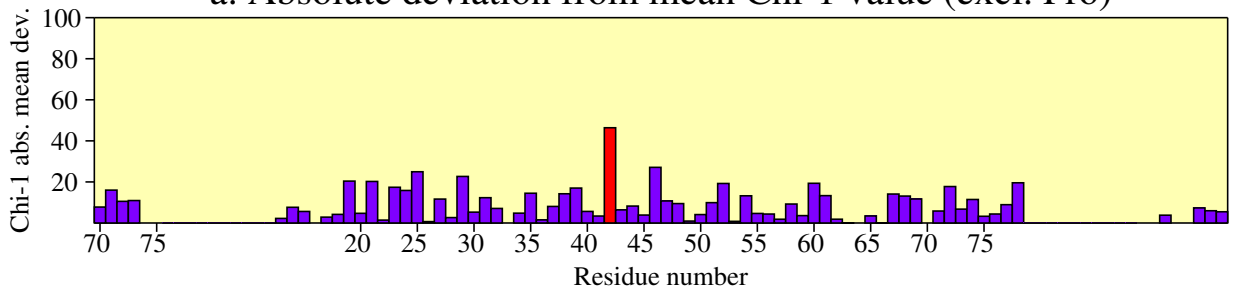
g. G-factors



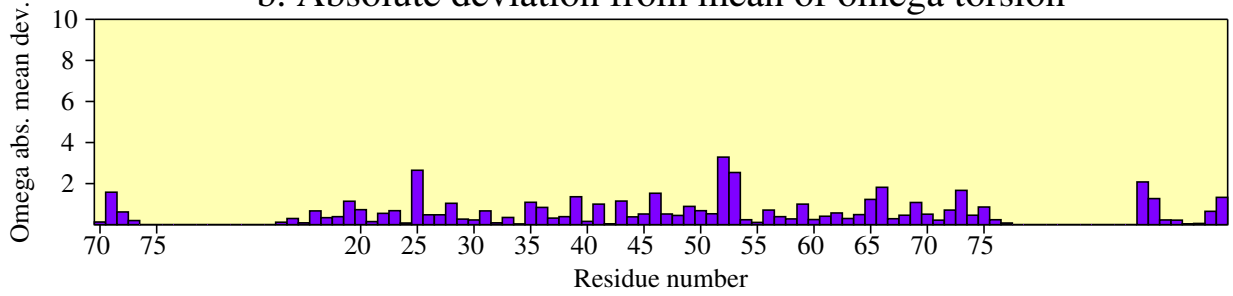
Residue properties

1ppj

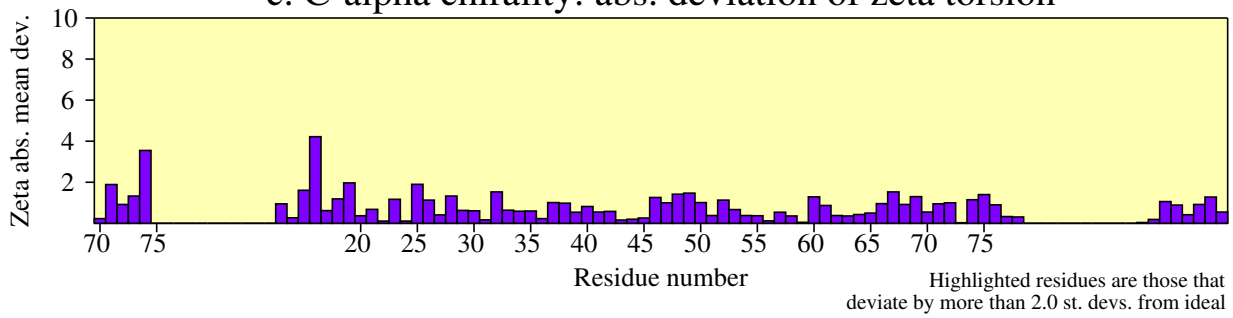
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

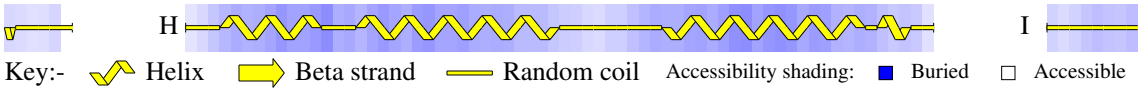


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

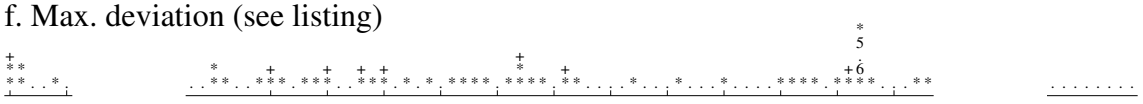
d. Secondary structure & estimated accessibility



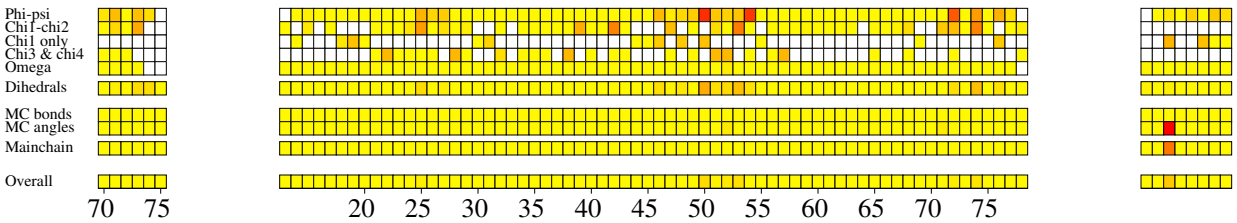
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

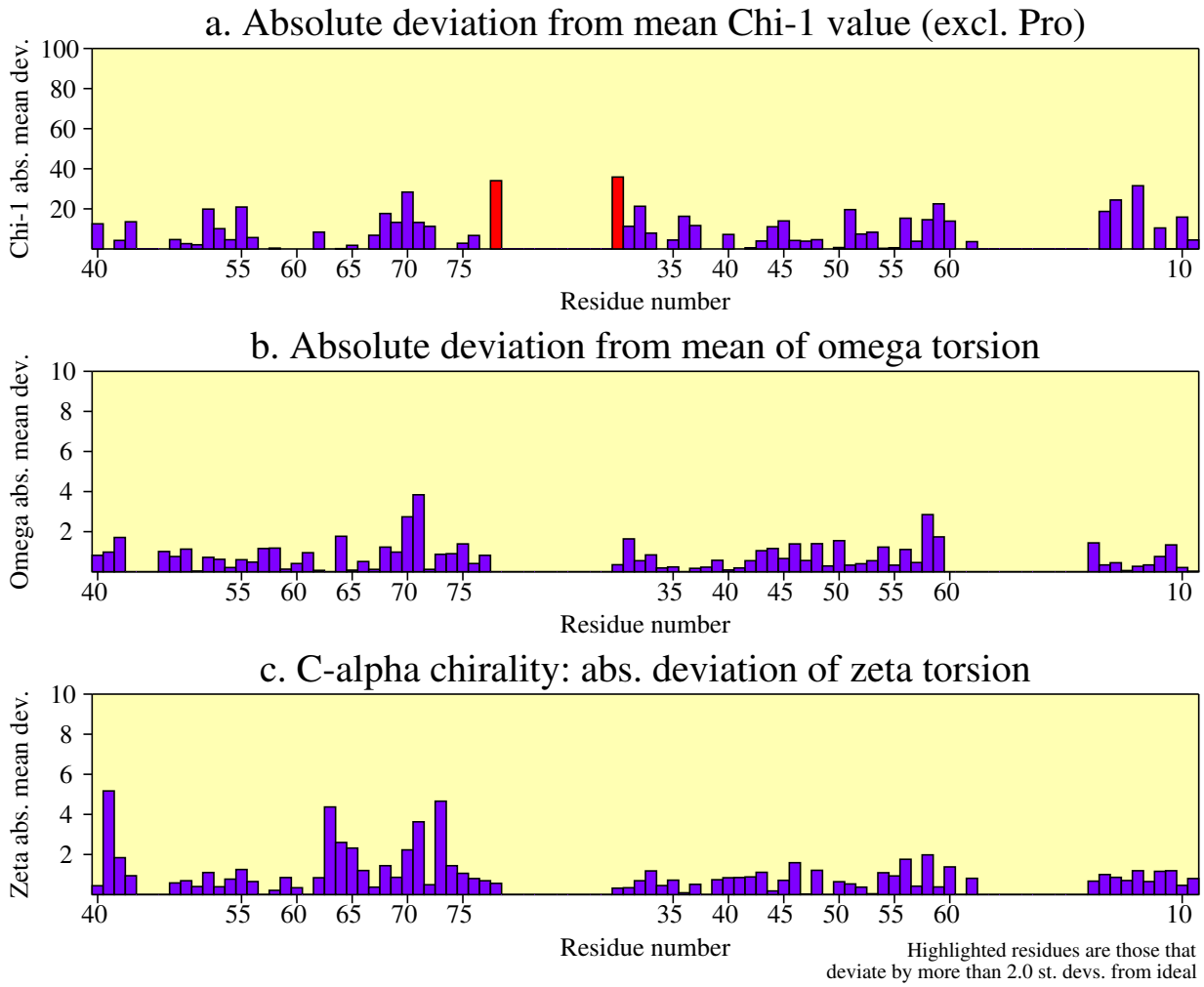
f. Max. deviation (see listing)



g. G-factors

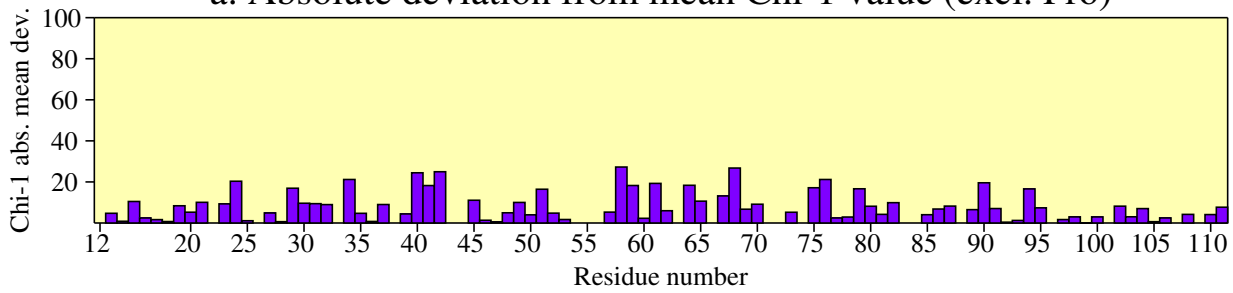


Residue properties
1ppj

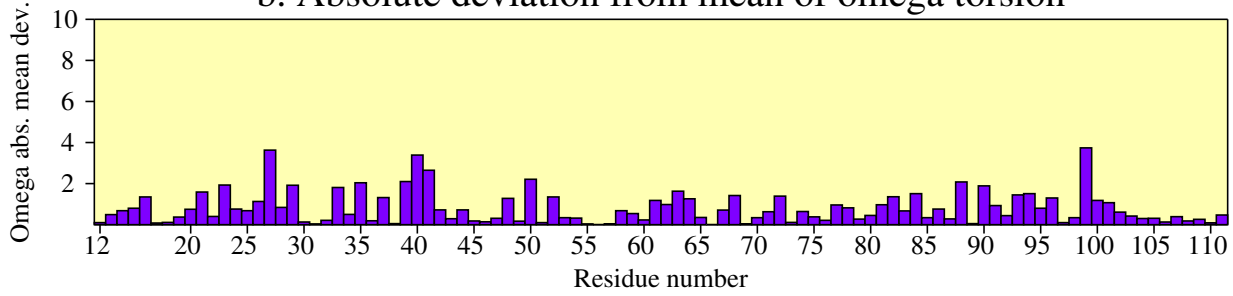


Residue properties
1ppj

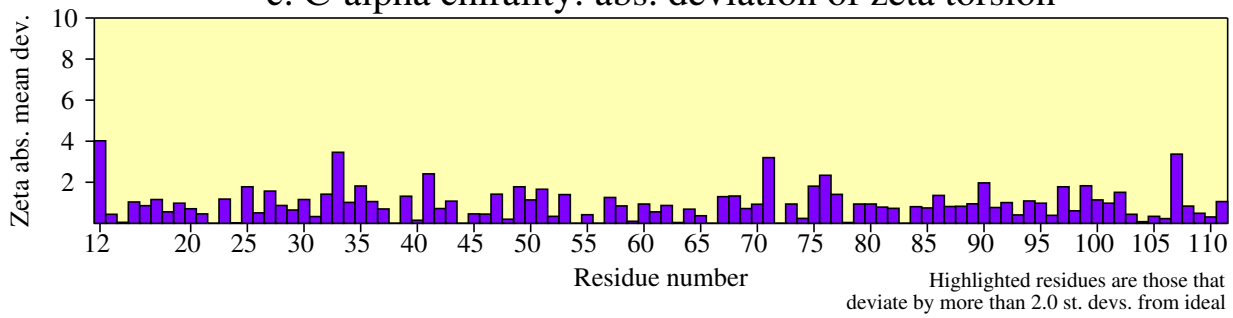
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

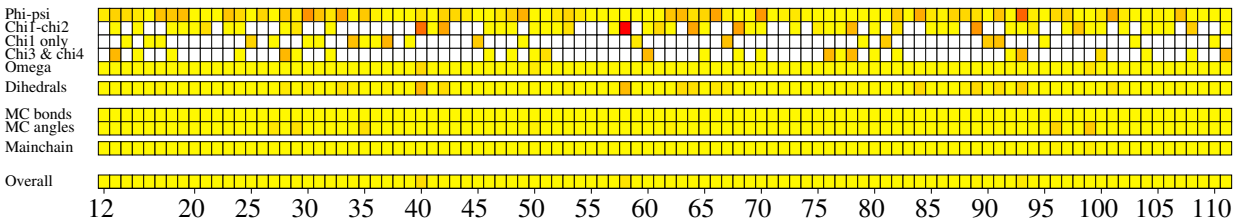
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

PETQVSQLDNGLRVASEQSSQPTCTVGVWIDAGSRYESEKNNGAGYFVEHLAFKGTKNRPGNALEKEVESMGAHLNAYSTREHTAYYIKALSKDLPKAVE

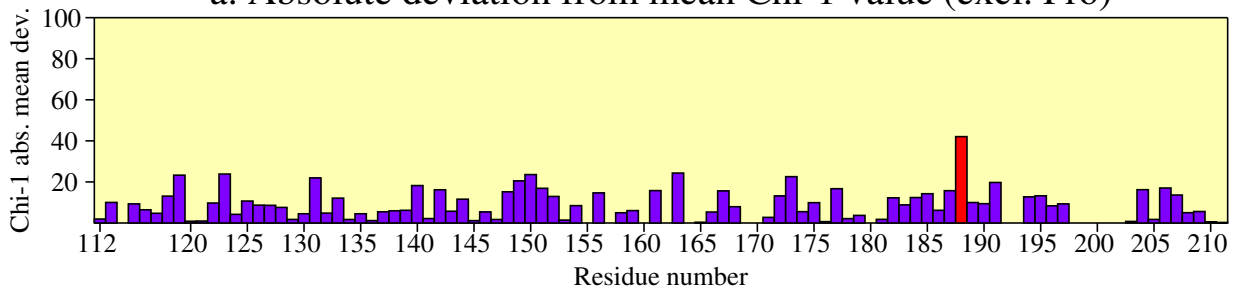
f. Max. deviation (see listing)

g. G-factors

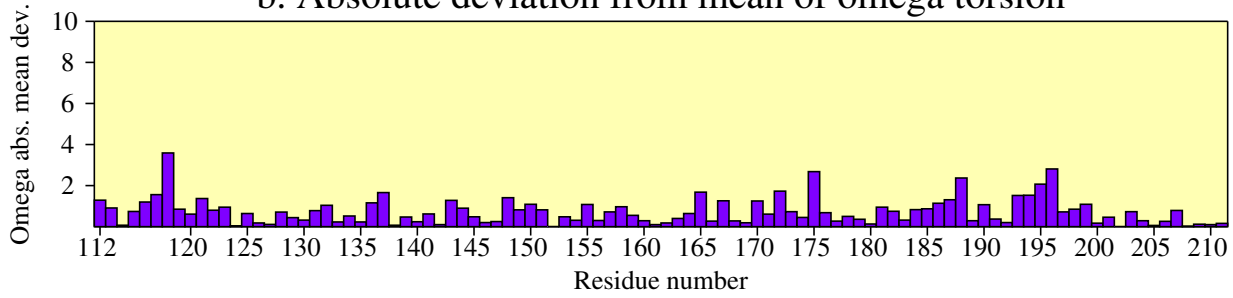


Residue properties
1ppj

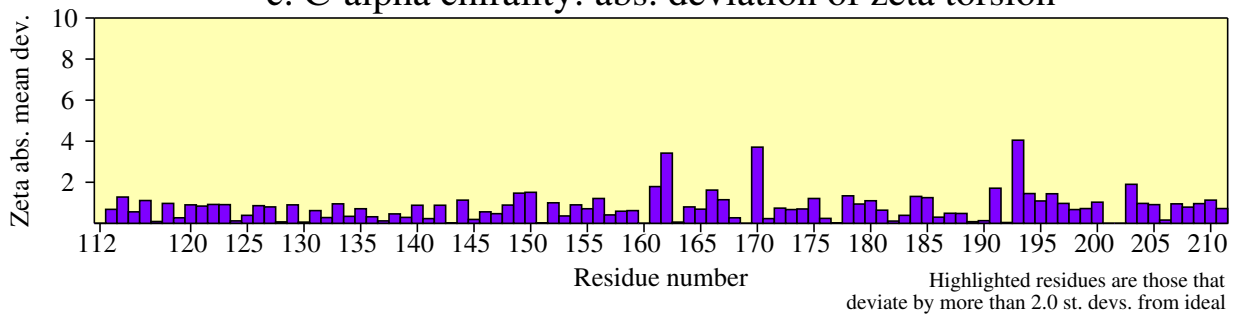
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



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d. Secondary structure & estimated accessibility

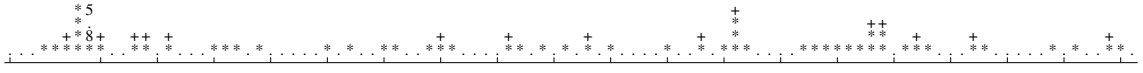
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

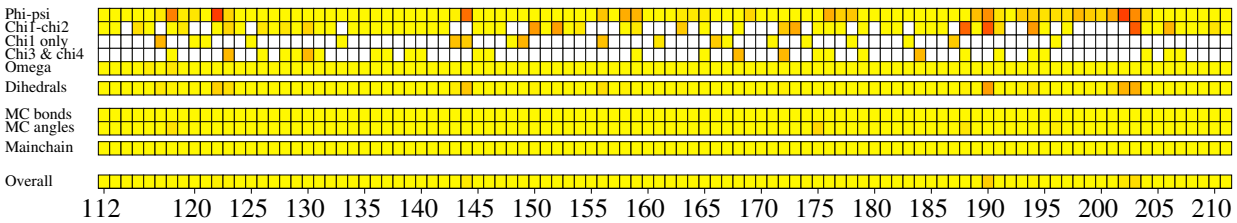
Most favoured Allowed Generous Disallowed

LLADIVQNCSLEDSQIEKERDVILQELQENDTSMRDVVFNYLHATAFQGTPLAQSVEGPSENVRKLSRADLTEYLSRHYKAPRMVLAAAGGLEHRQLLDL

f. Max$_\alpha$ deviation (see listing)



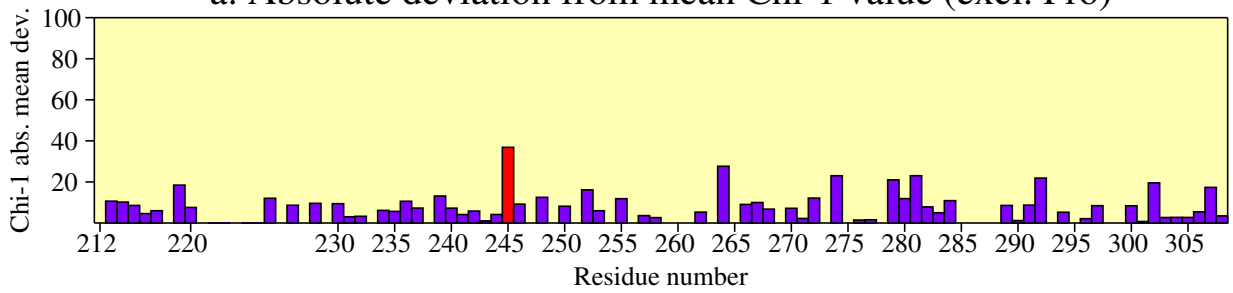
g. G-factors



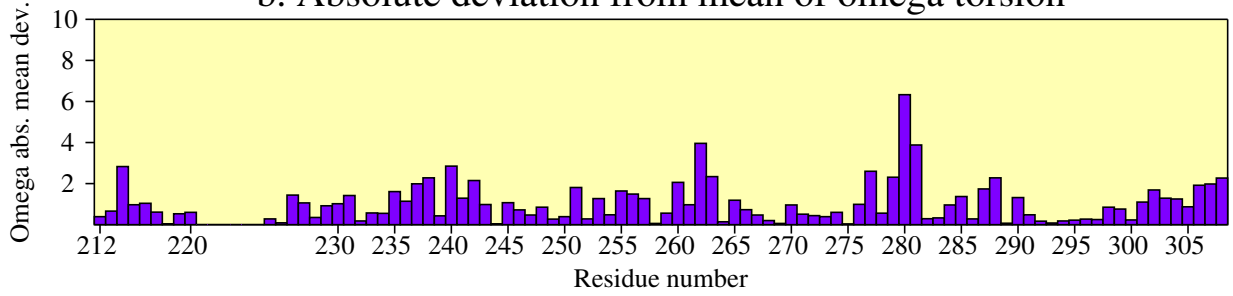
Residue properties

1ppj

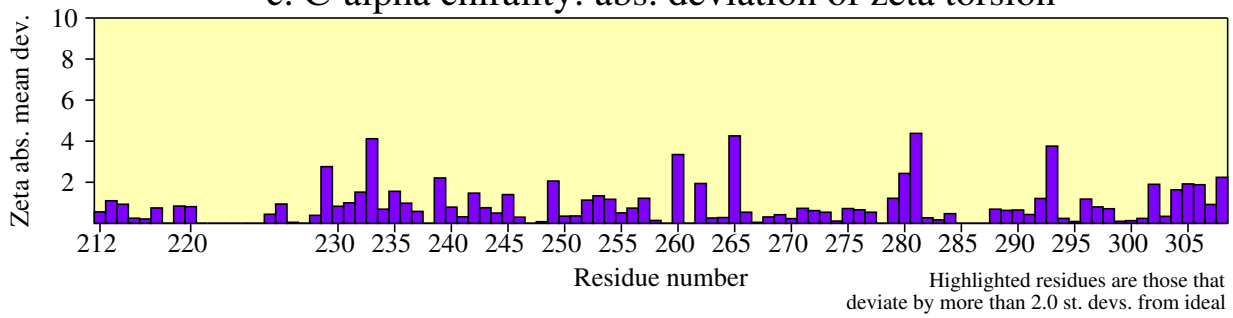
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



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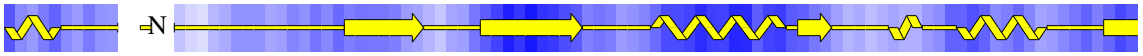
d. Secondary structure & estimated accessibility

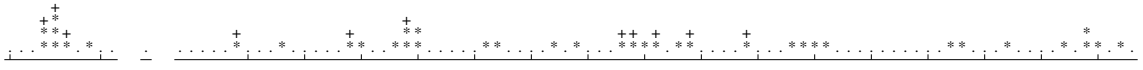


Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

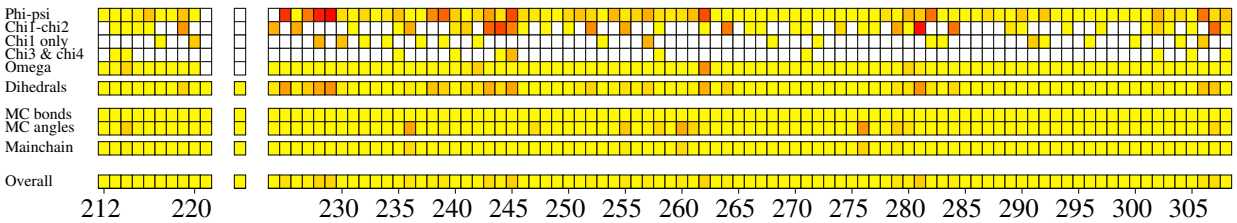
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

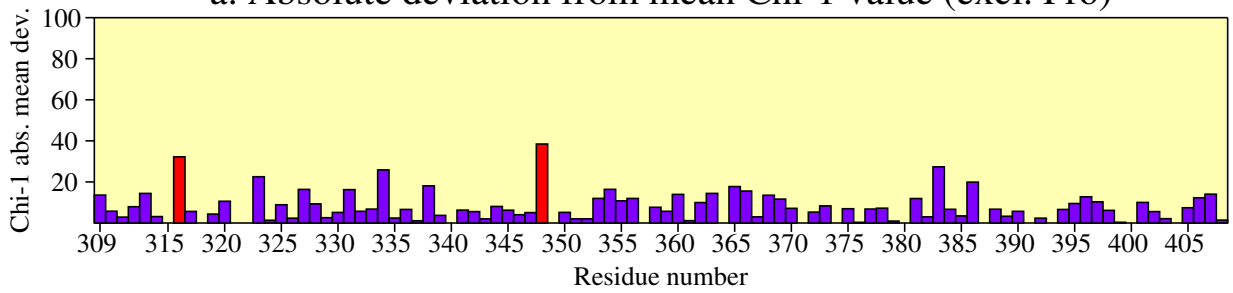


g. G-factors

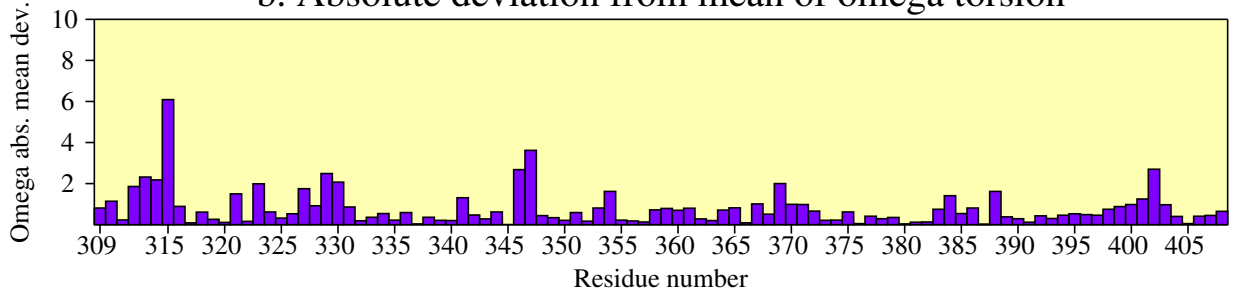


Residue properties
1ppj

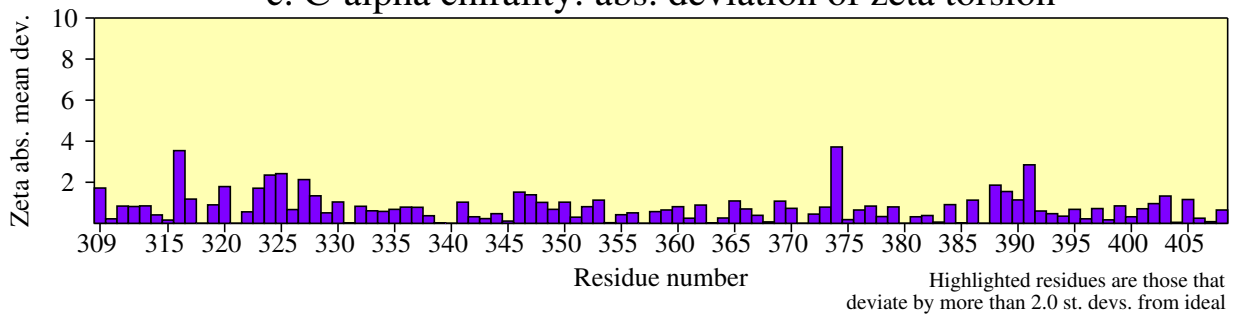
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

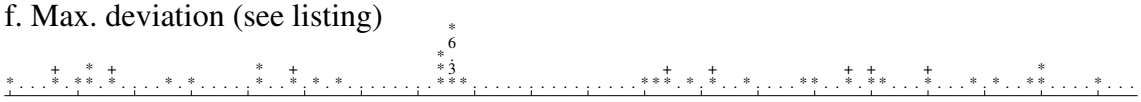
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

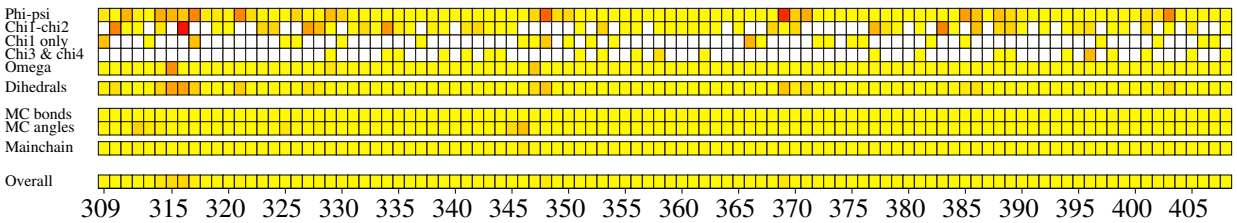
Most favoured Allowed Generous Disallowed

TFNICYADTGLLGAHFVCDHMSIDDMMFVLQGQWMRLCTSATESEVLRGKNLLRNALVSHLDGTTPVCEDIGRSLLTYGRRIPLAEWESRIAEVDARVVR

f. Max. deviation (see listing)



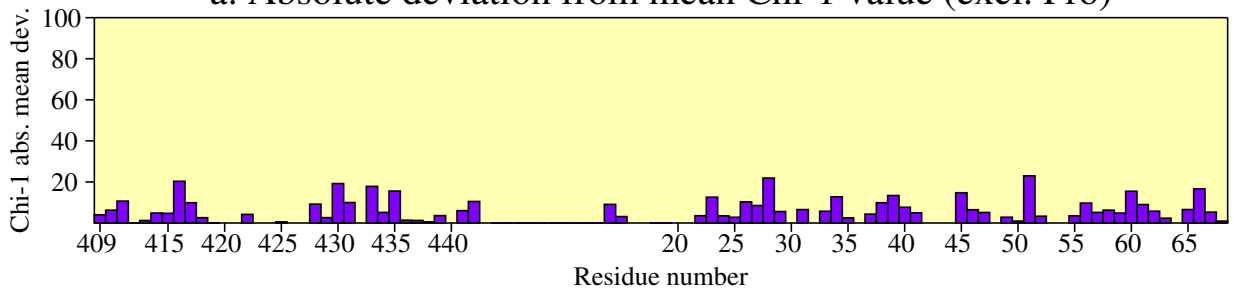
g. G-factors



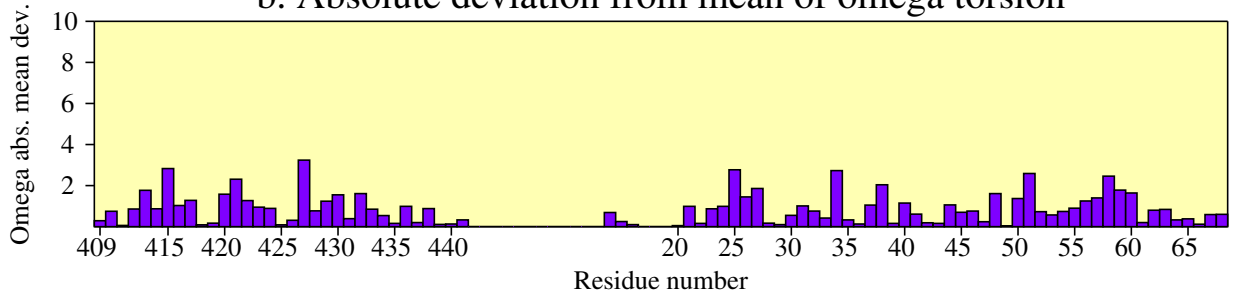
Residue properties

1ppj

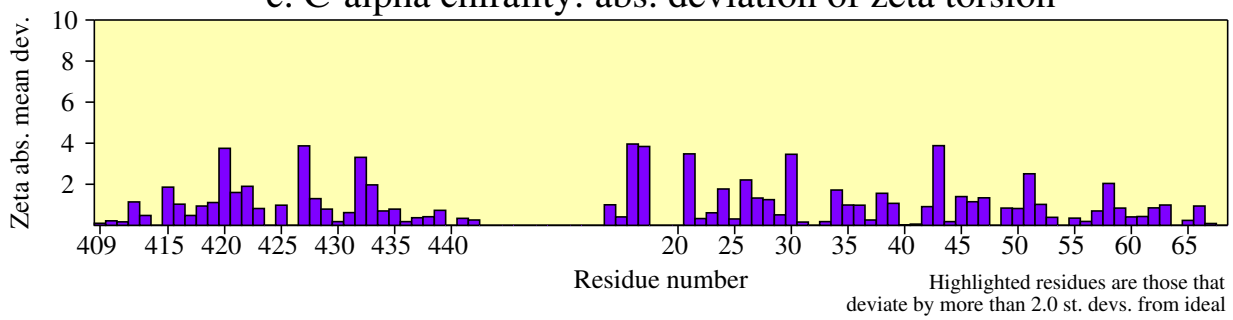
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

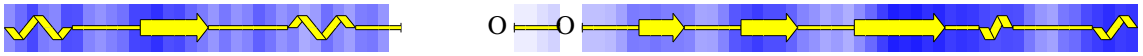


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

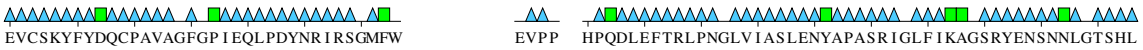
d. Secondary structure & estimated accessibility



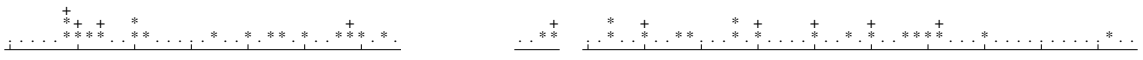
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

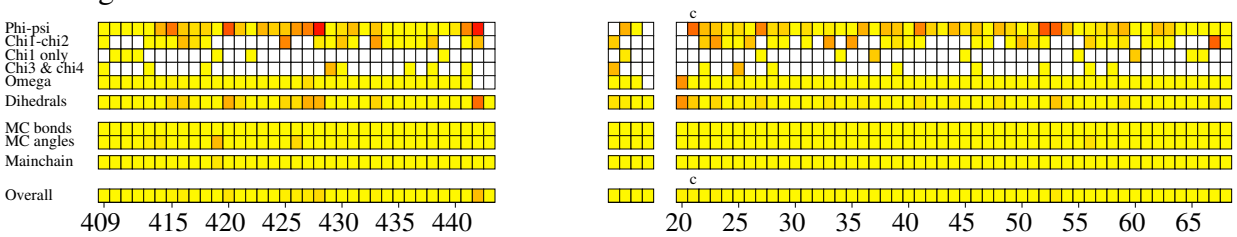
Most favoured Allowed Generous Disallowed



f. Max. deviation (see listing)



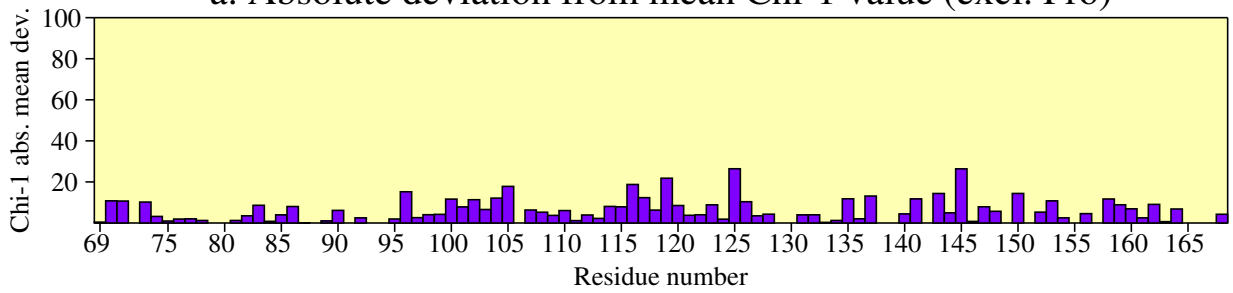
g. G-factors



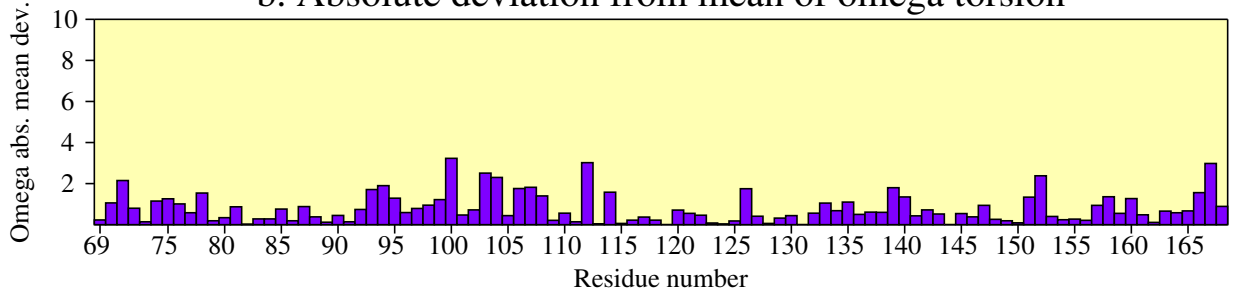
c = cis-peptide

Residue properties
1ppj

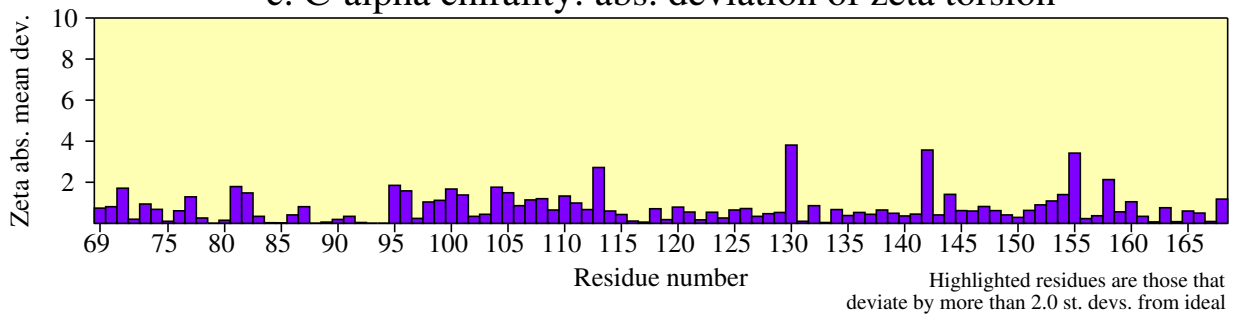
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

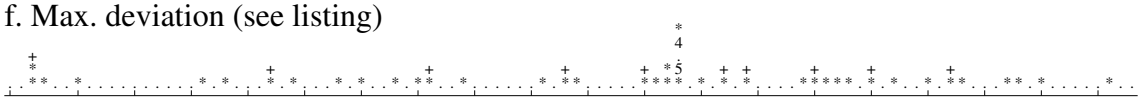
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

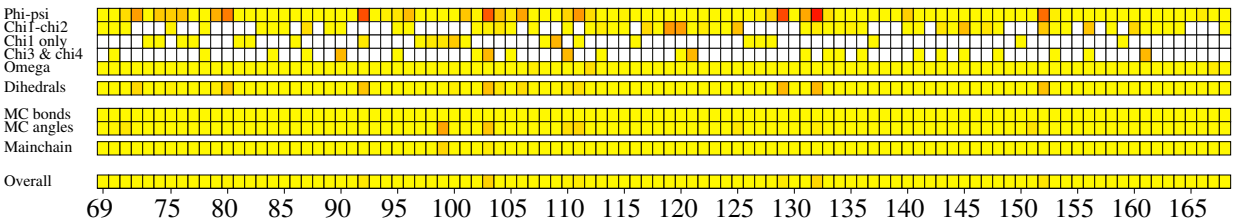
Most favoured Allowed Generous Disallowed

L R L A S S L T T K G A S S F K I T R G I E A V G G K L S V T S T R E N M A Y T V E C L R D D V D I L M E F L L N V T T A P E F R R W E V A A L Q P Q L R I D K A V A L Q N P Q A H V I E N L H A A A Y

f. Max. deviation (see listing)

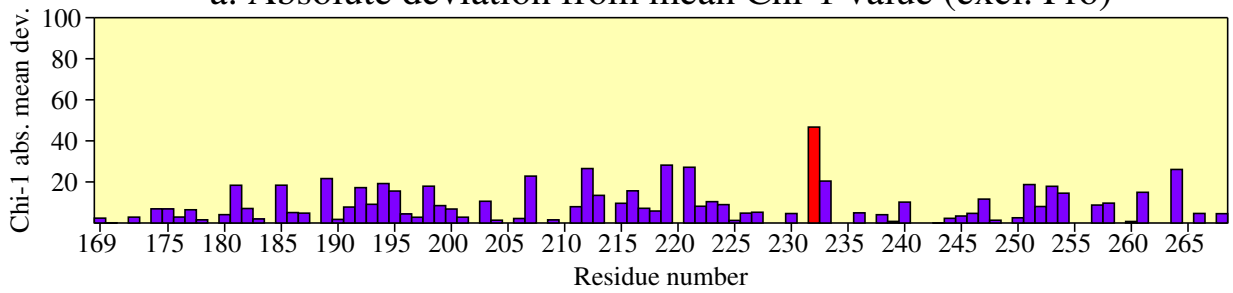


g. G-factors

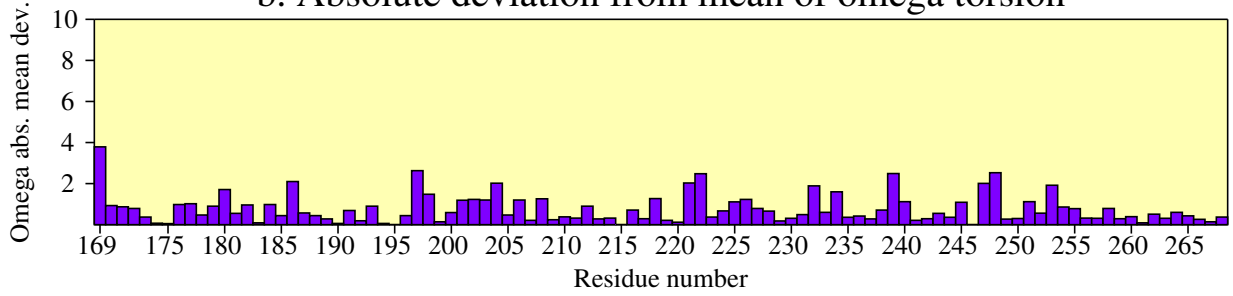


Residue properties
1ppj

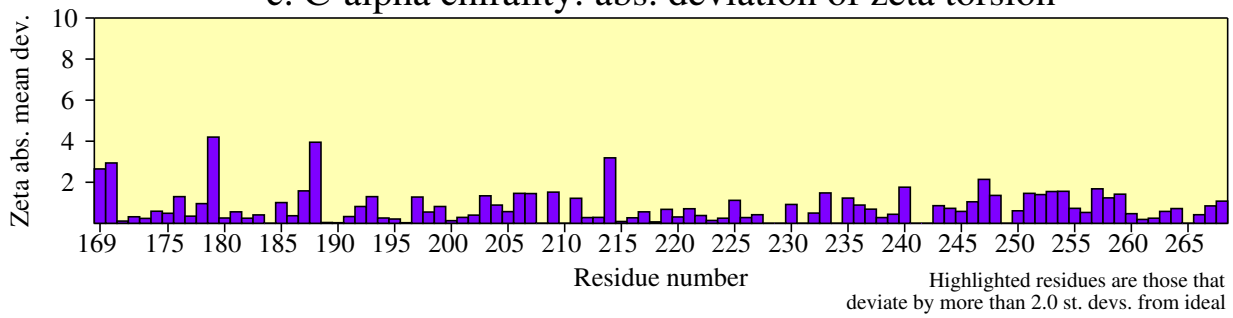
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



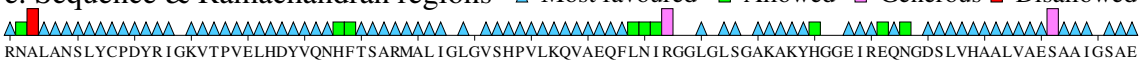
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

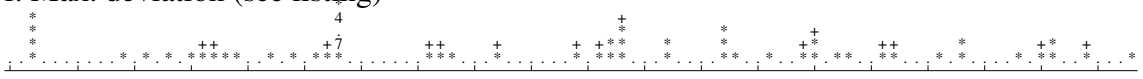
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

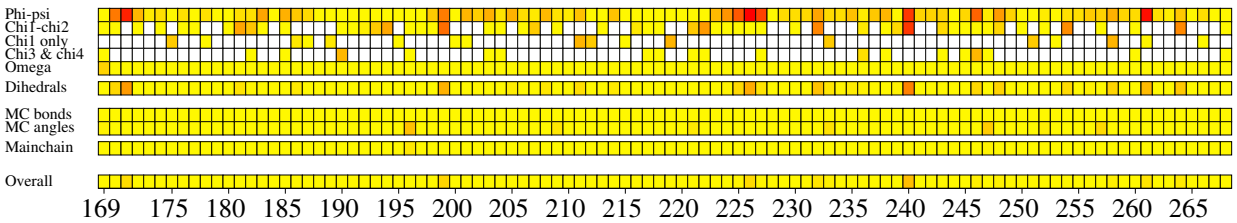


RNALANSLYCPDYRIGKVTPVELHDYVQNHFTSARMALIGLGVSHPVLKQVAEQFLNIRGGLGLSGAKAKYHGGEIREQNGDSLVHAALVAESAAIGSAE

f. Max. deviation (see listing)



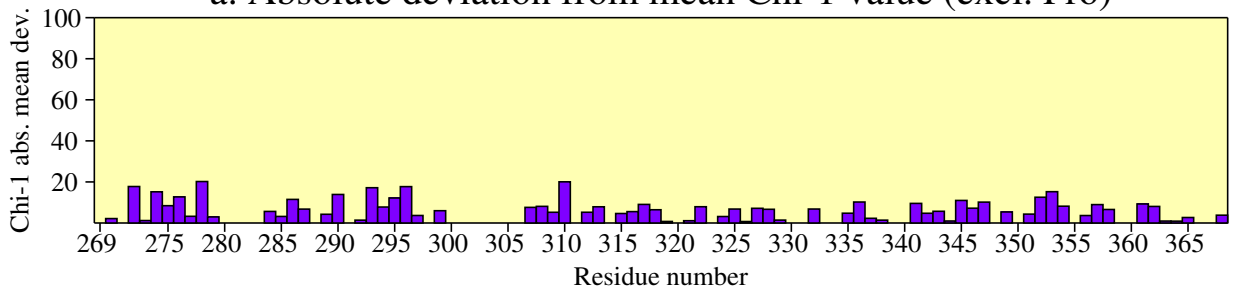
g. G-factors



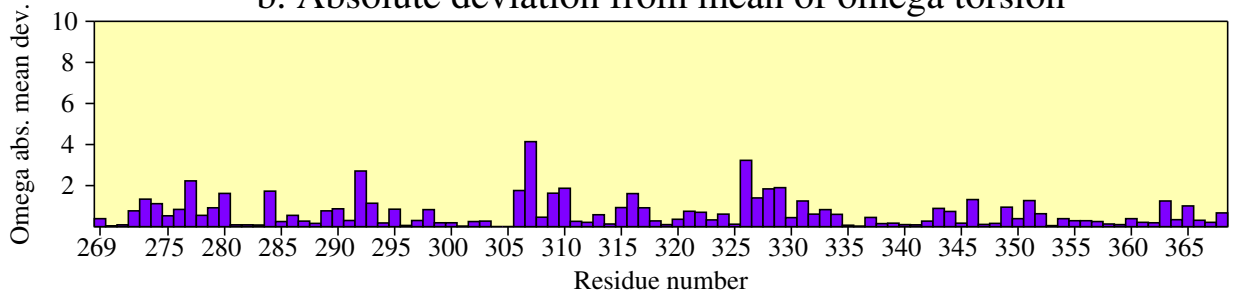
Residue properties

1ppj

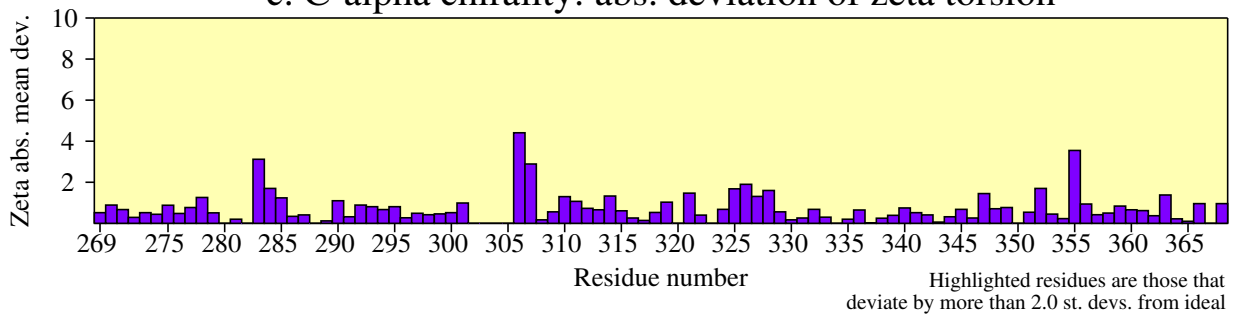
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

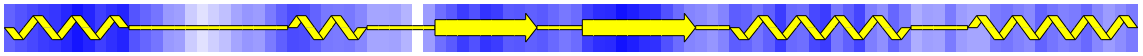


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



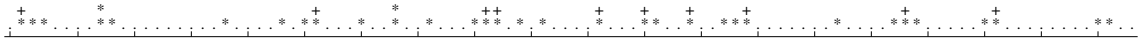
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

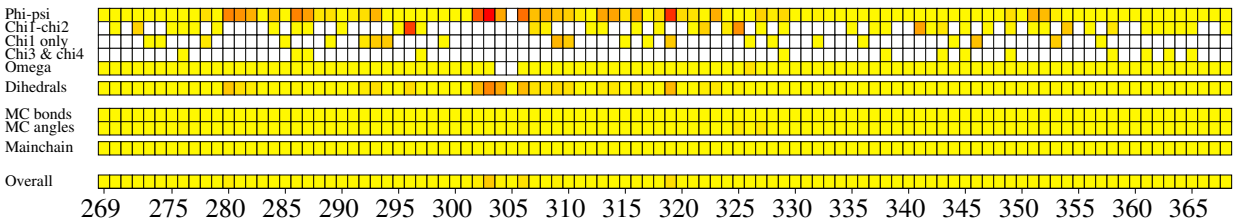
Most favoured Allowed Generous Disallowed

ANAFSVLQHVLGAGPHVKRGSNATSSLYQAVAKGVHQPFDVSAFNASYSDSGLFGFYTISQAASAGDVIKAAYNQVKTIAQGNLSNPDVQAAKNKLKAGY

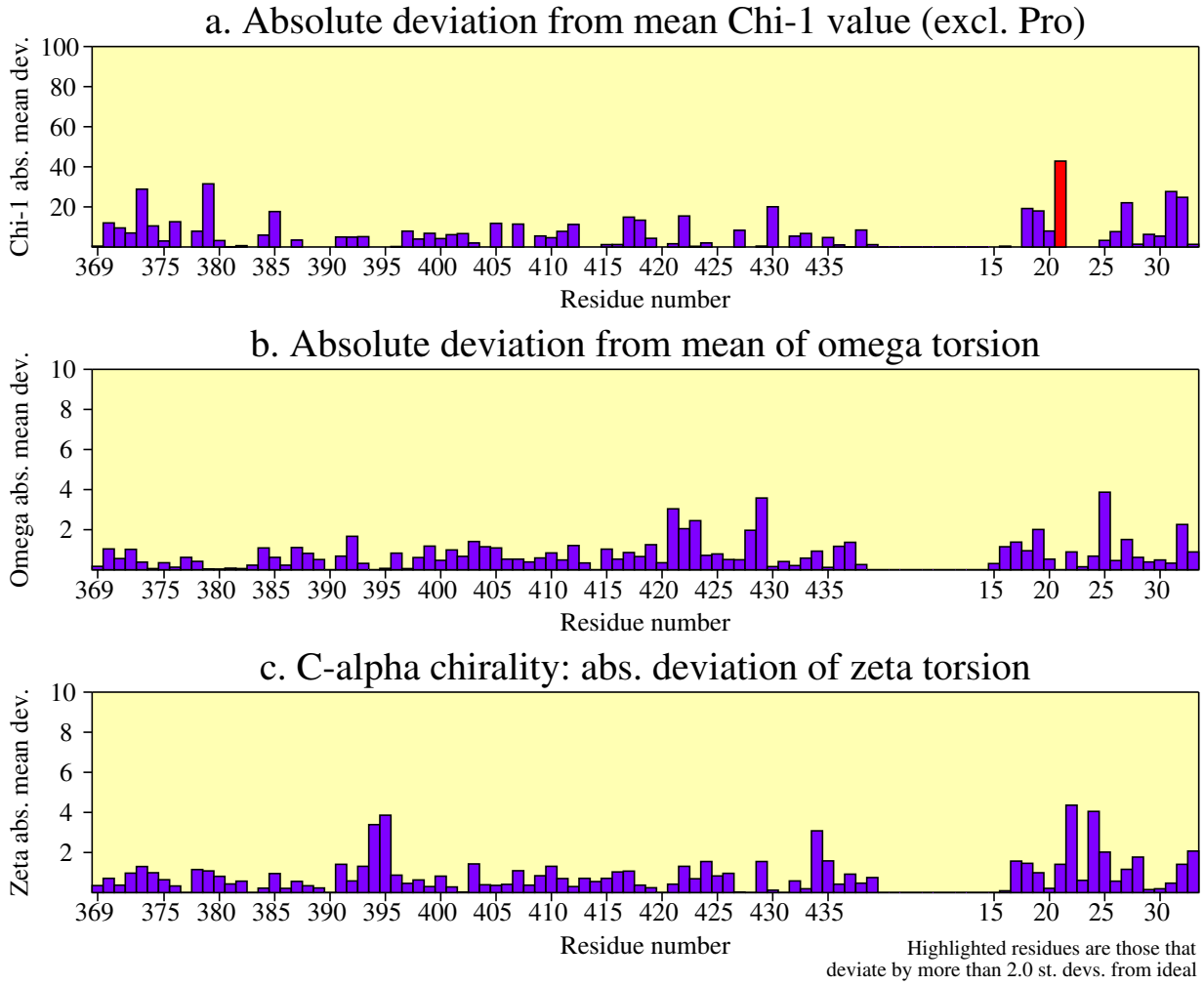
f. Max. deviation (see listing)

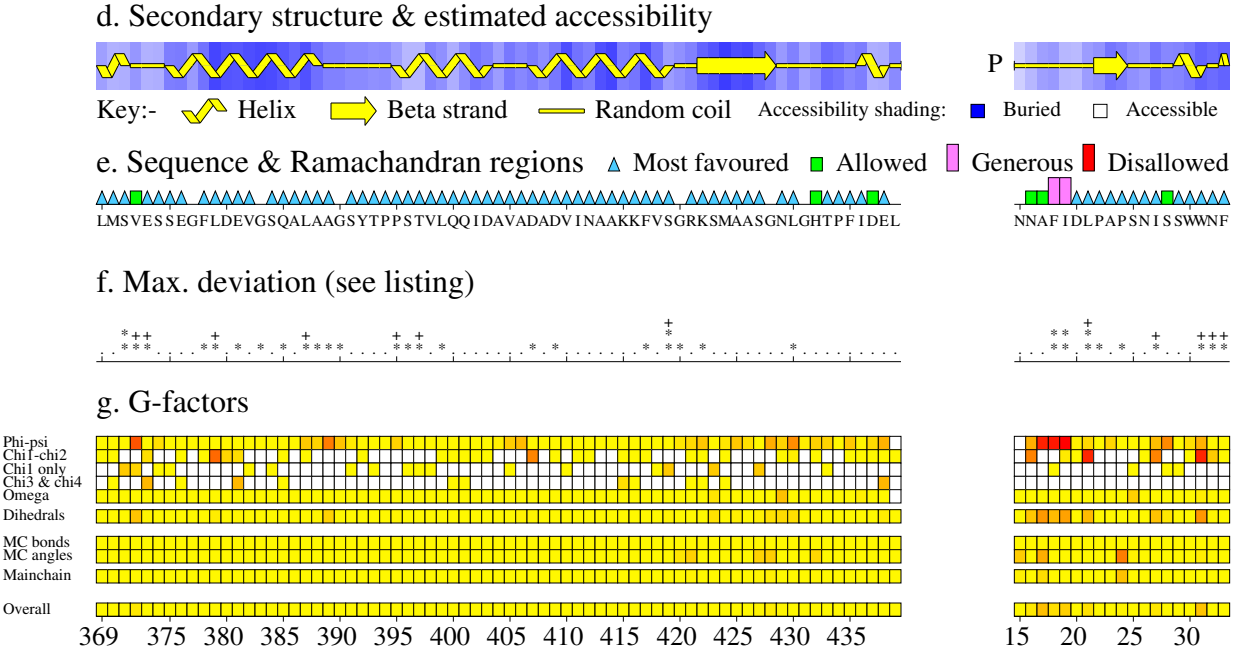


g. G-factors



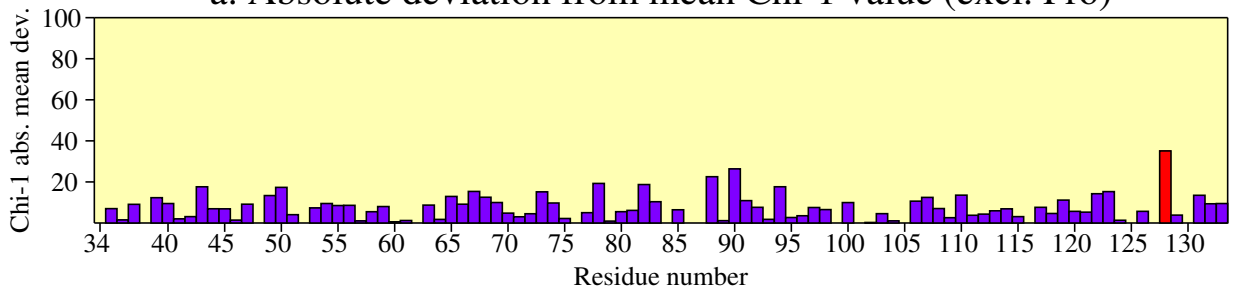
Residue properties
1ppj



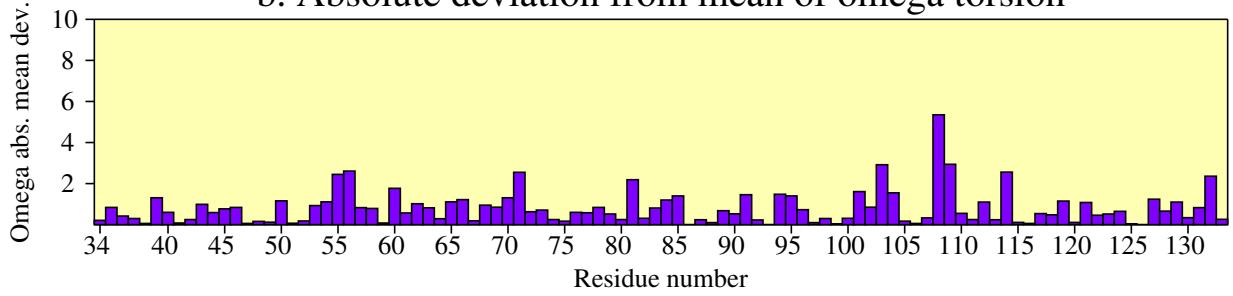


Residue properties
1ppj

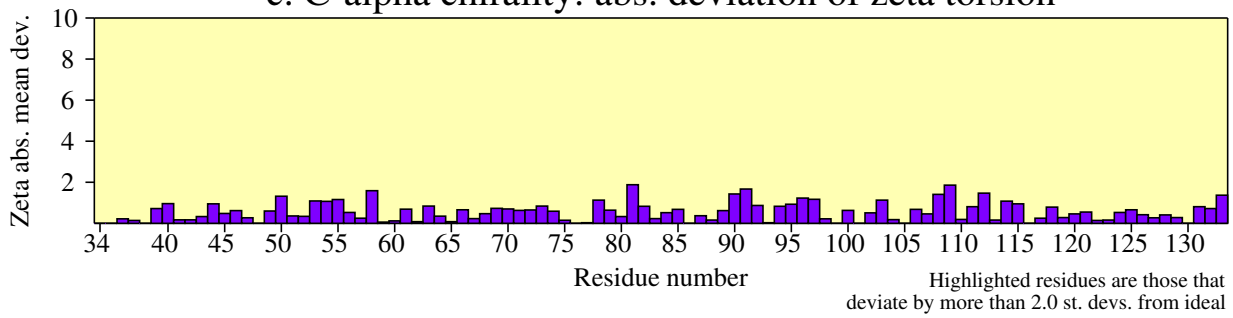
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

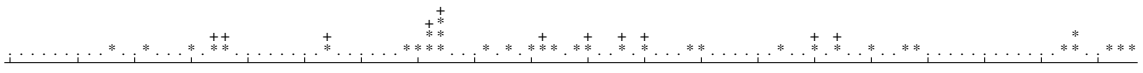
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

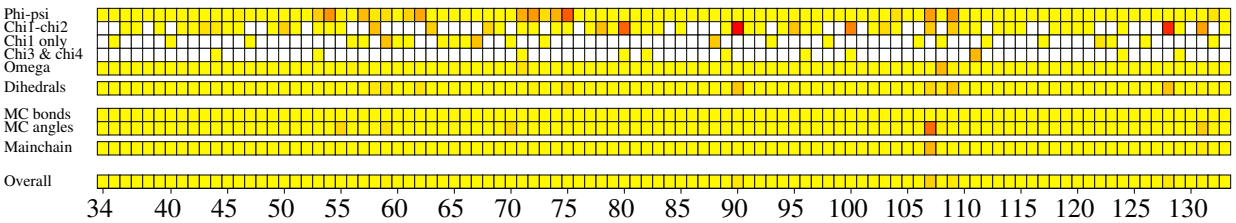
Most favoured Allowed Generous Disallowed

GSLLGICLILQILTGLFLAMHYTSDTTTAFSSVTHICRDVNYGWIIRYMHANGASMFFICLYMHVGRGLYYGSYTFLETWNIGVILLLTVMATAFMGYVL

f. Max. deviation (see listing)



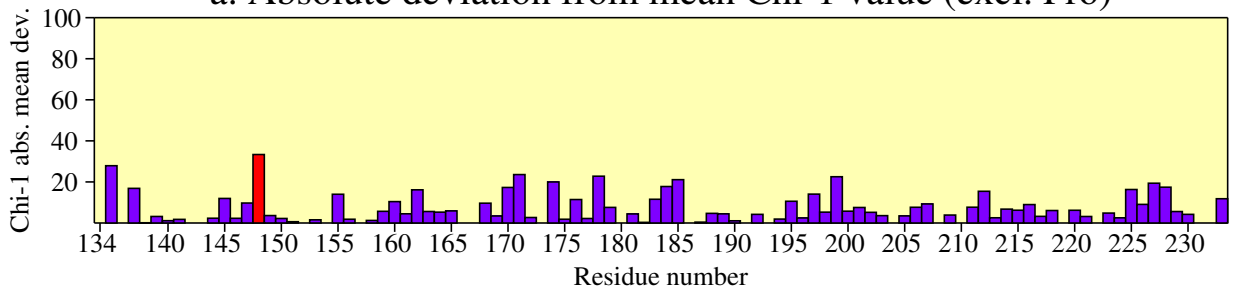
g. G-factors



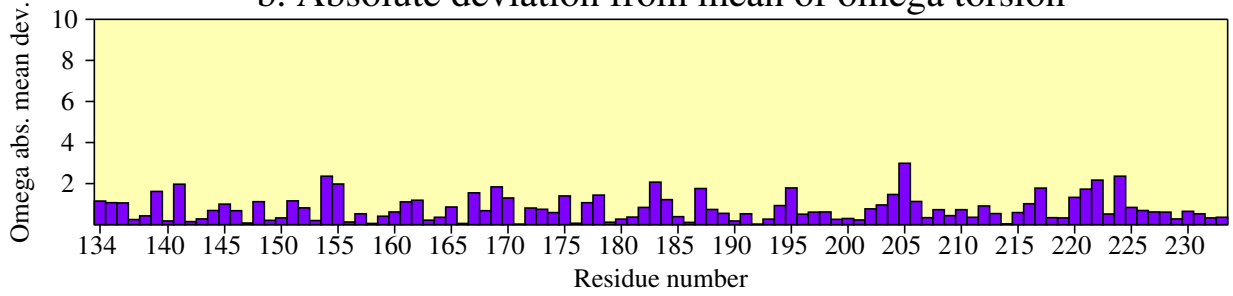
Residue properties

1ppj

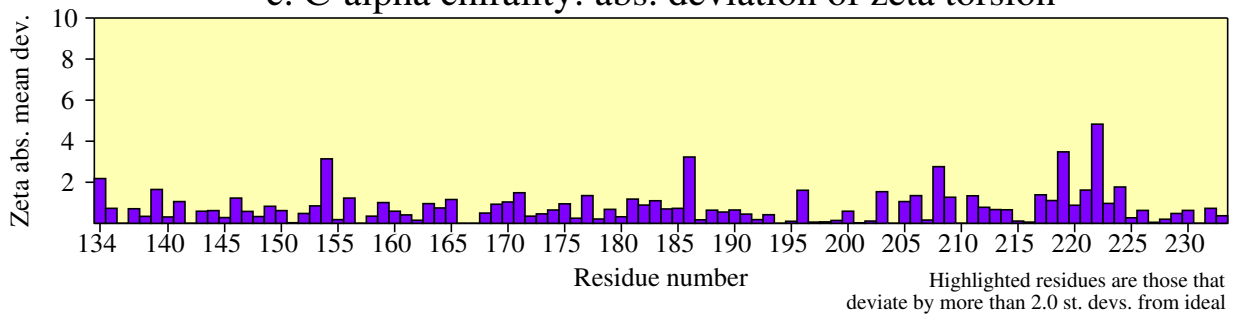
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



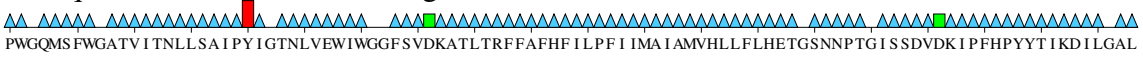
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

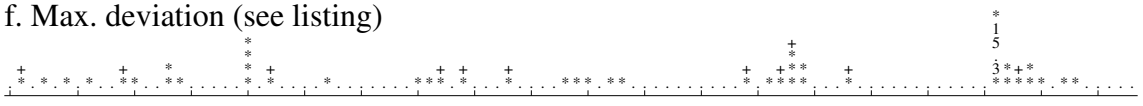
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

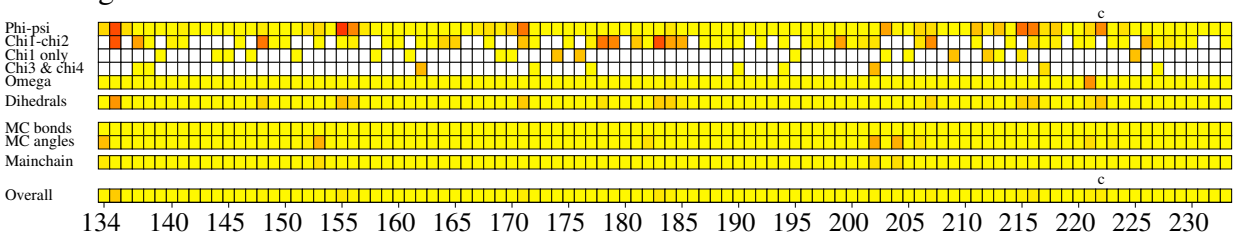


PWGQMSFWGATVITNLLSAIPYIGTNLVEWIWGGFSVDKATLTRFFAFHFILPFIIMAIAMVHLLFLHETGSNNPTGISSDVDKIPFHPYYTIKDILGAL

f. Max. deviation (see listing)



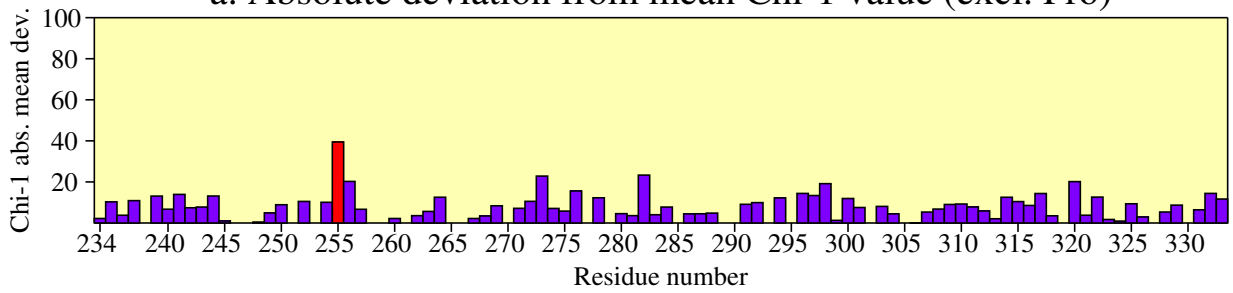
g. G-factors



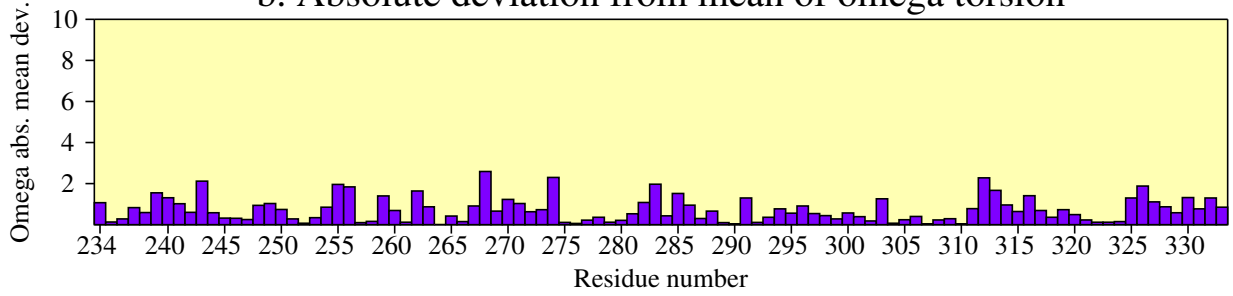
c = cis-peptide

Residue properties
1ppj

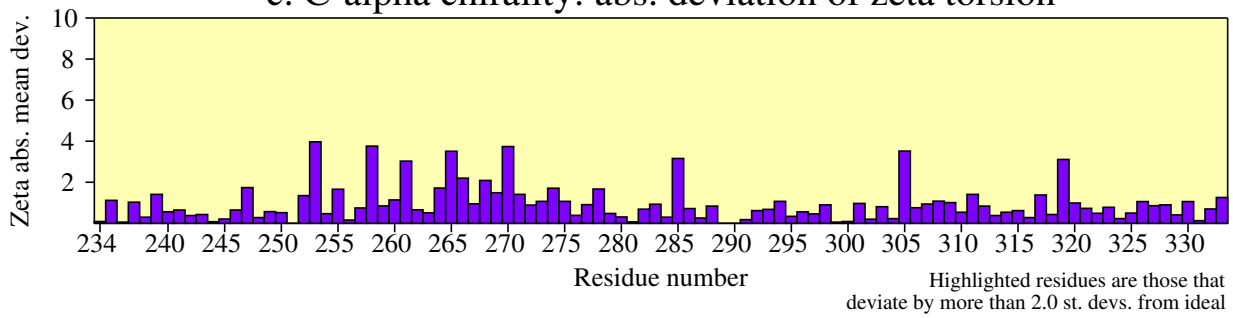
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



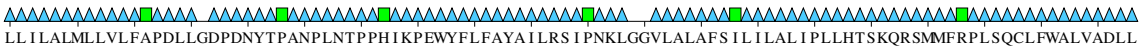
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

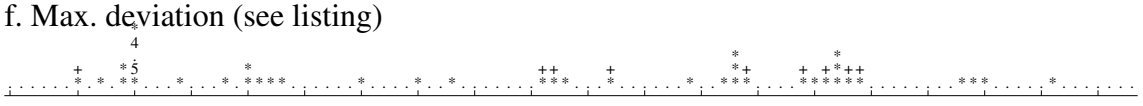
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

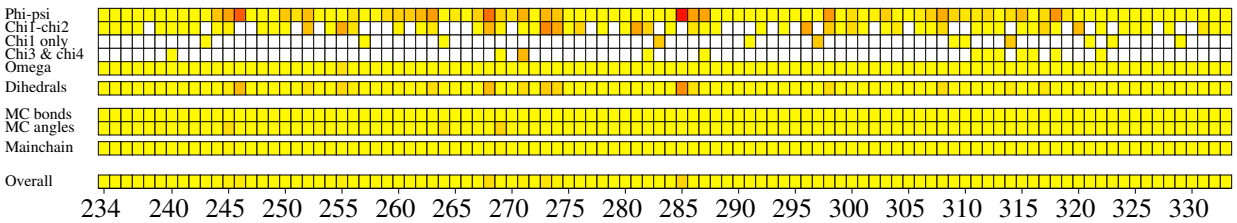


L L I L A L M L L V L F A P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I L R S I P N K L G G V L A L A F S I L I L A L I P L L H T S K Q R S M M F R P L S Q C L F W A L V A D L L

f. Max. deviation (see listing)

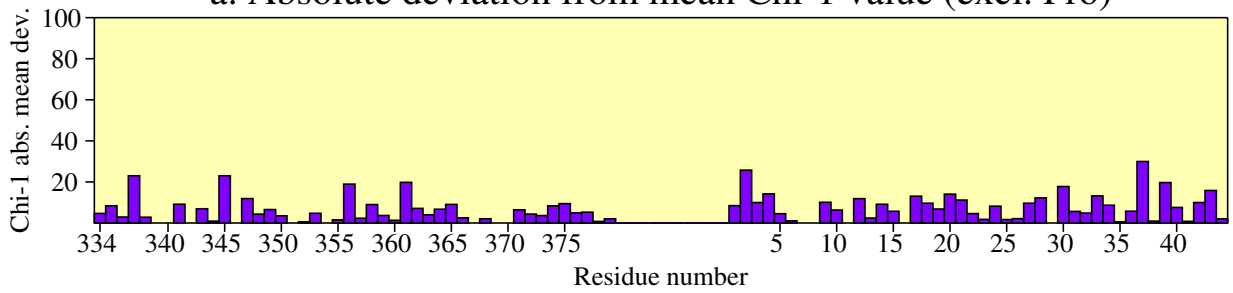


g. G-factors

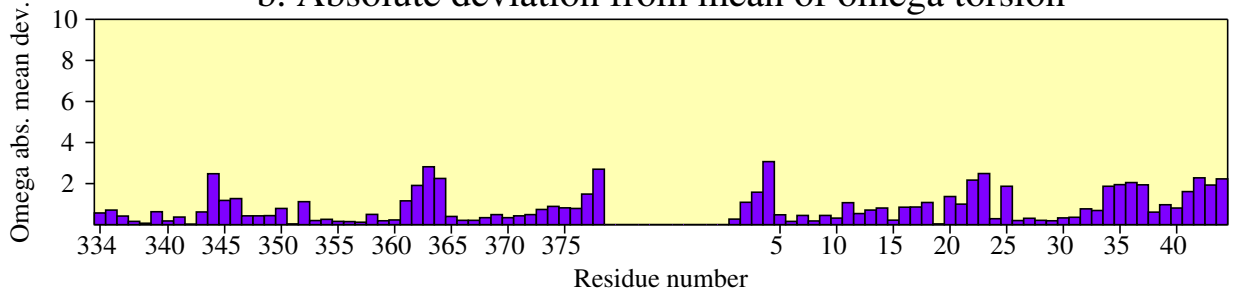


Residue properties
1ppj

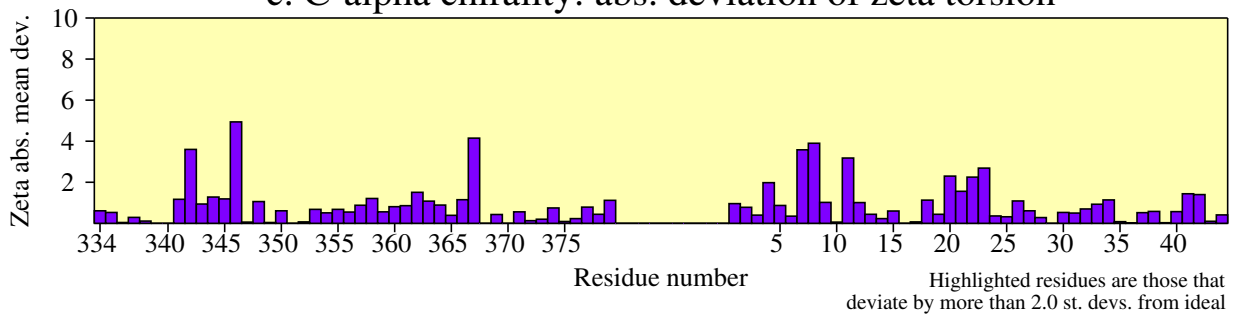
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

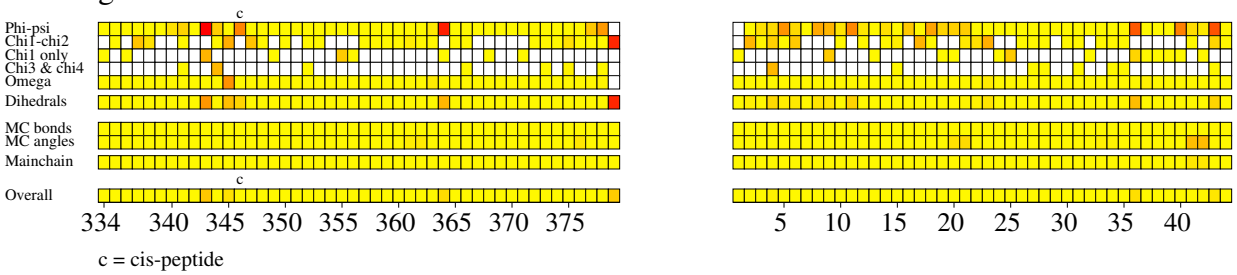
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

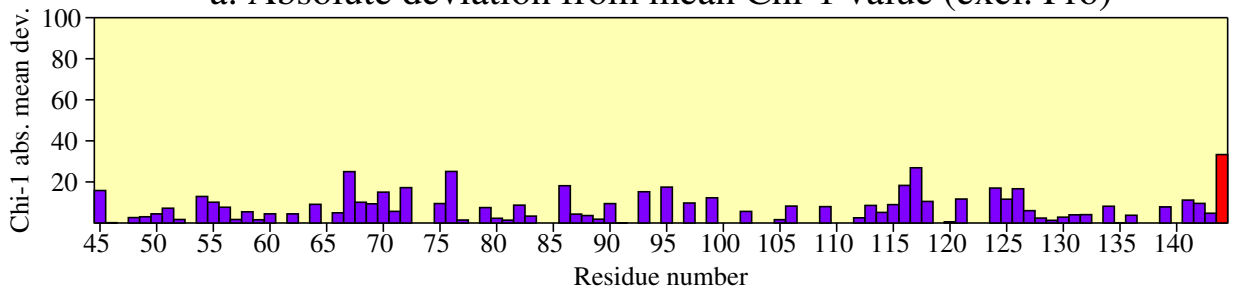
g. G-factors



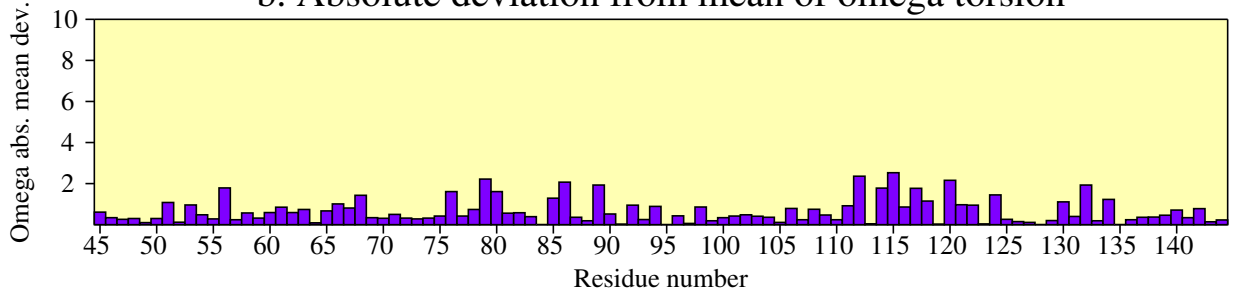
c = cis-peptide

Residue properties
1ppj

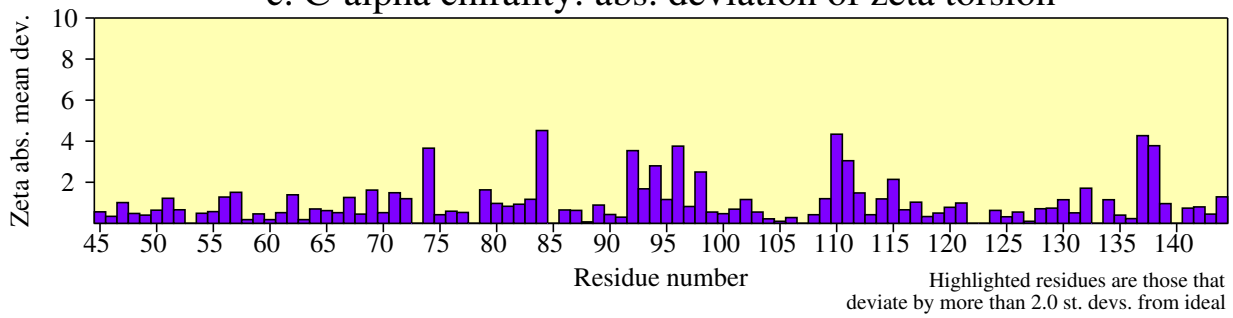
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

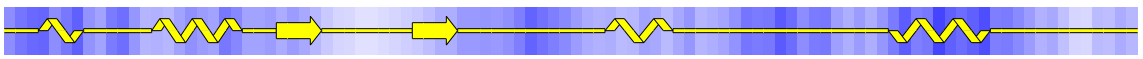


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



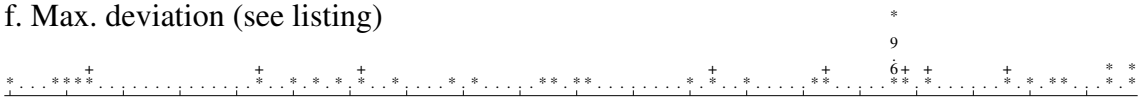
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

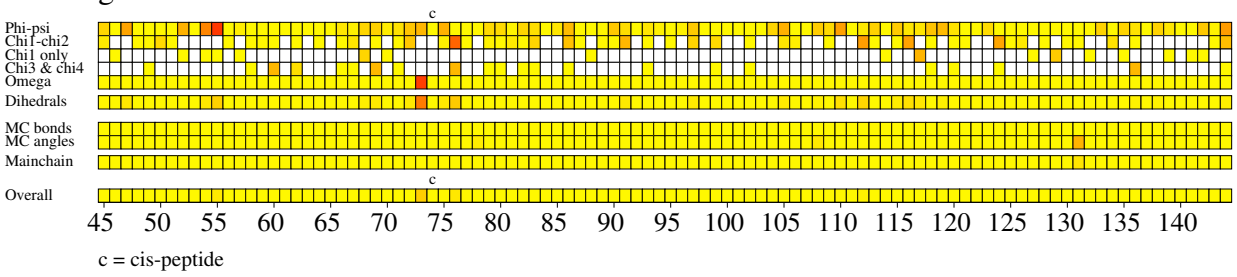
Most favoured Allowed Generous Disallowed

YVAYRHLVGVCYTEDEAKALAEEVEVQDGPNEDGEMFMRPGKLSDYFPKPYPNPEAARAANNGALPPDLSYIVRARHGGEDYVFSLLTGYCEPPTGVSLR

f. Max. deviation (see listing)



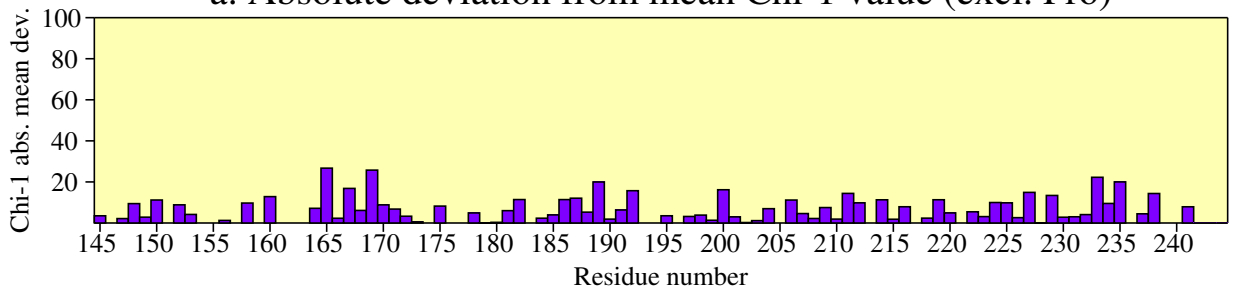
g. G-factors



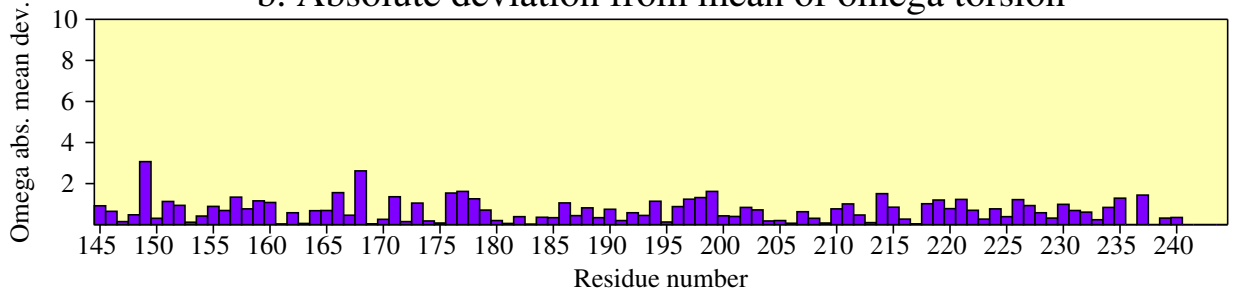
c = cis-peptide

Residue properties
1ppj

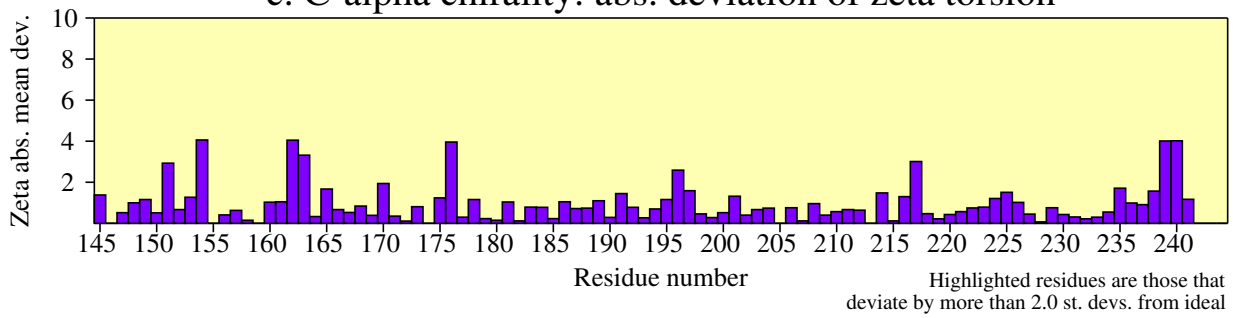
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

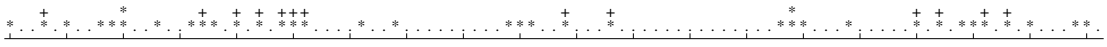
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

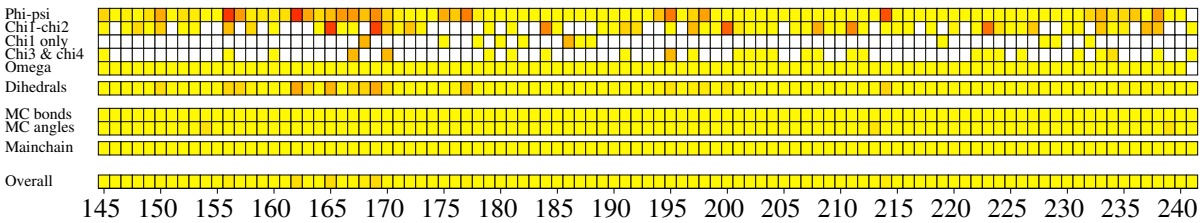
Most favoured Allowed Generous Disallowed

EGLYFNPYFPGQAIGMAPPIYNEVLEFDDGTPATMSQVAKDVCTFLRWAAEPEHDHRKRMGLKMLLMMGLLLPLVYAMKRHKWSVLKSRKLAYRPPK

f. Max. deviation (see listing)

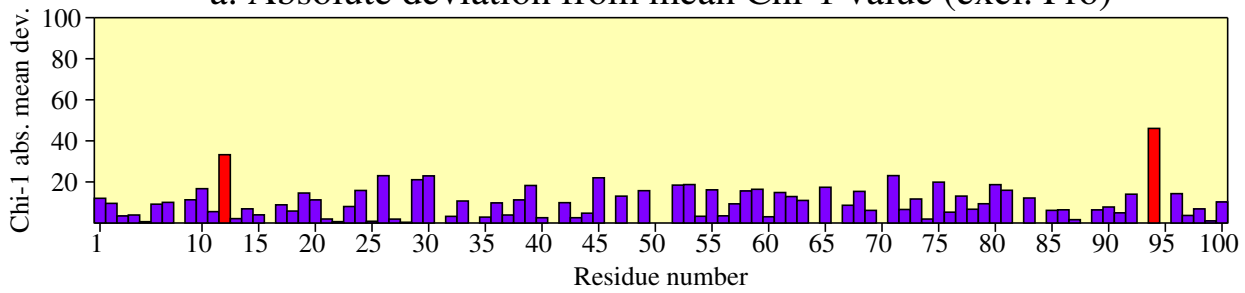


g. G-factors

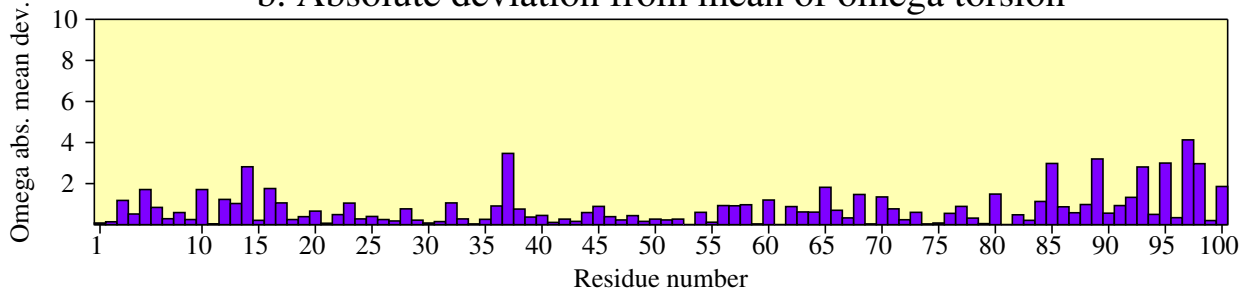


Residue properties
1ppj

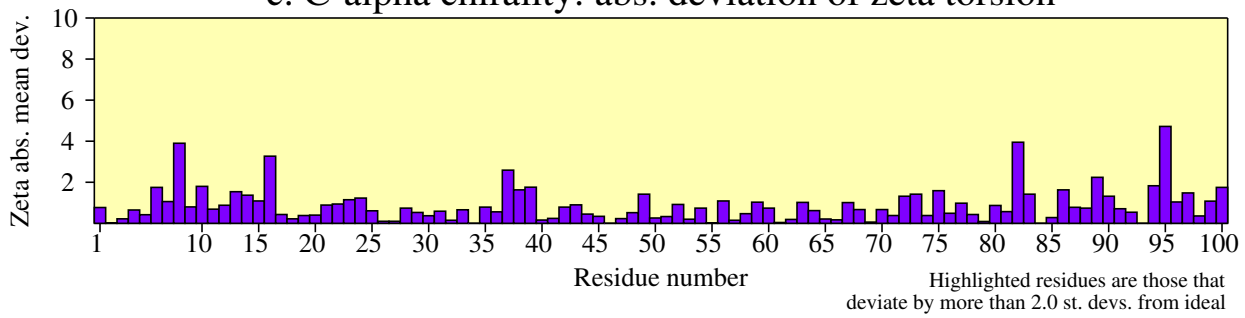
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

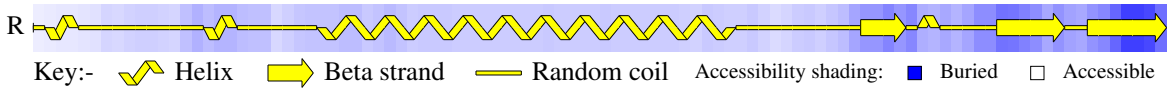


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

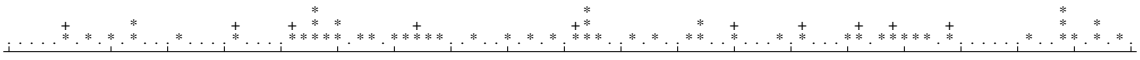
d. Secondary structure & estimated accessibility



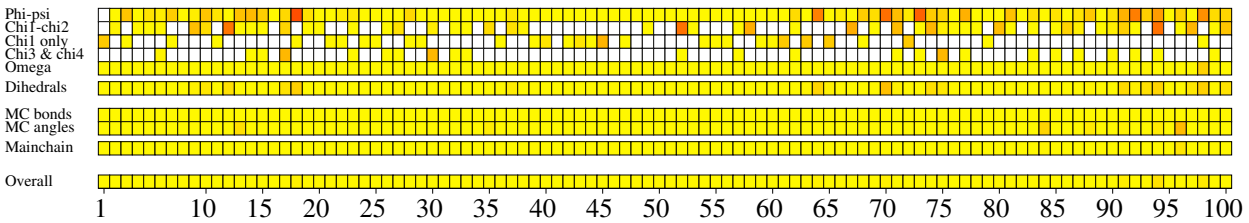
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

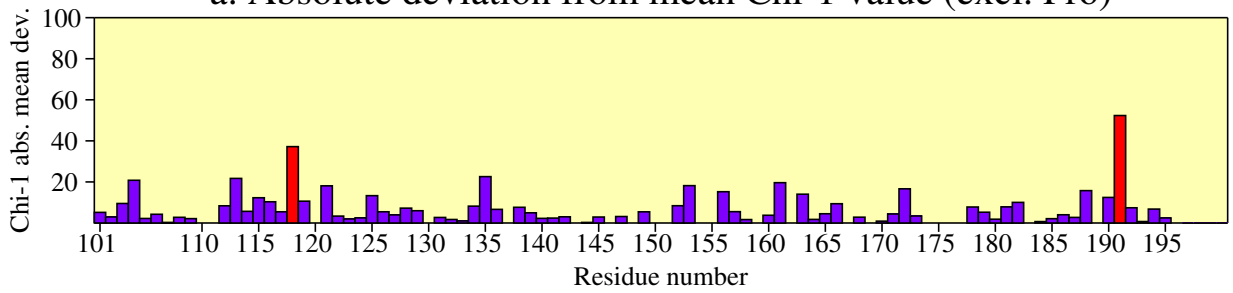


g. G-factors

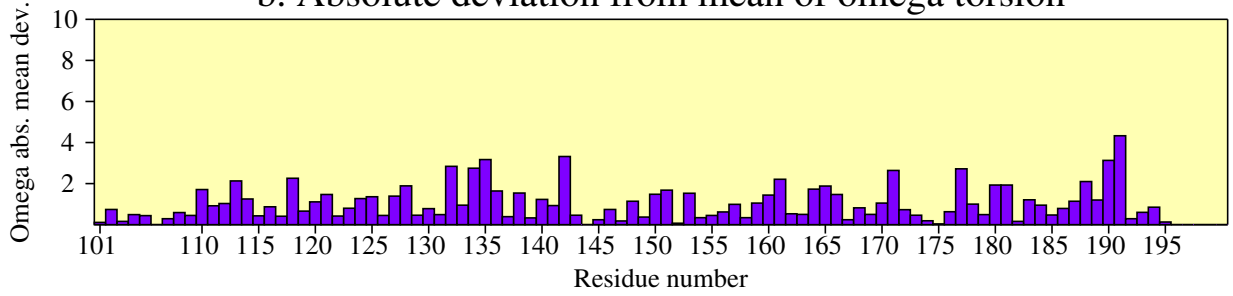


Residue properties
1ppj

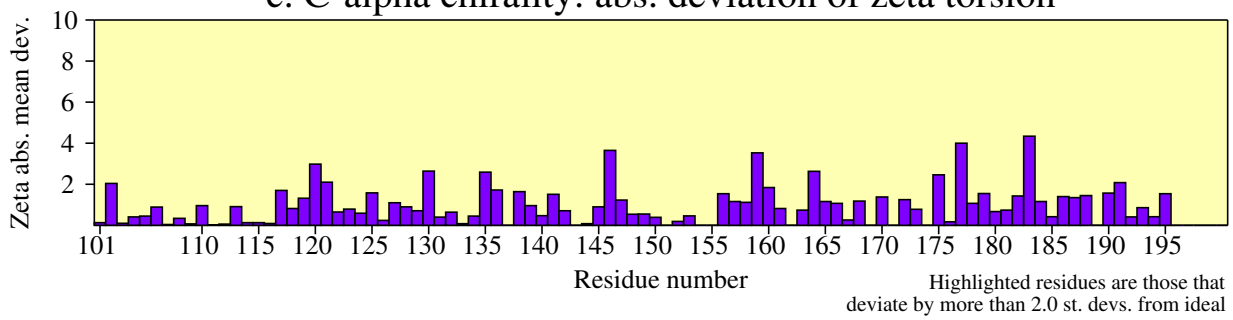
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

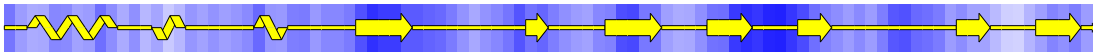


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility



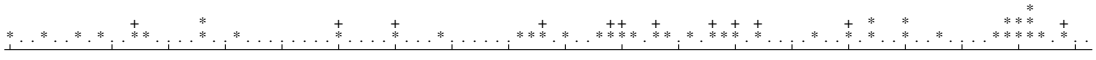
Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

e. Sequence & Ramachandran regions

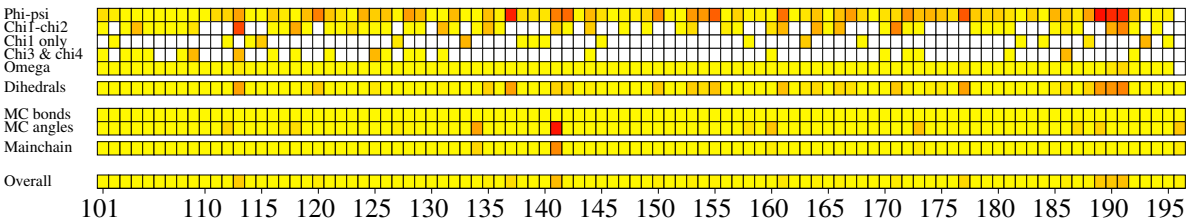
Most favoured Allowed Generous Disallowed

RTKKE I DQEAAVEVSQLRDPQHDLERVKKPEWV I L I GVCTHLGCVP I ANAGDFGGYYCPCHGSHYDASGR I RKGPAPLNLEVPSYEFTSDDMV I VG

f. Max. deviation (see listing)

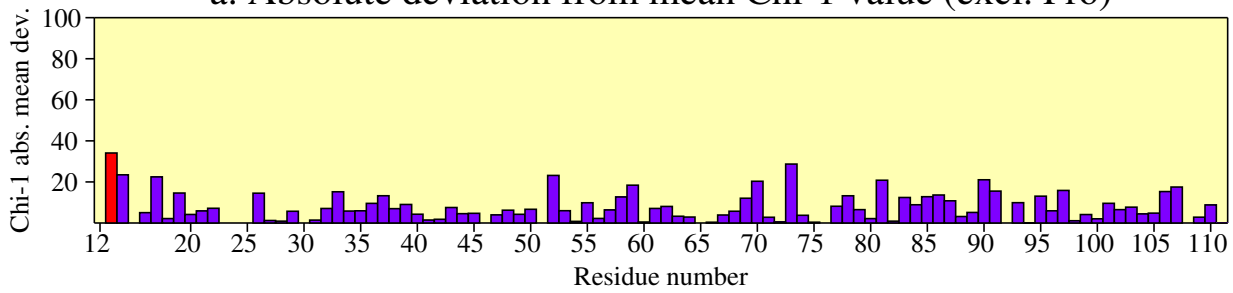


g. G-factors

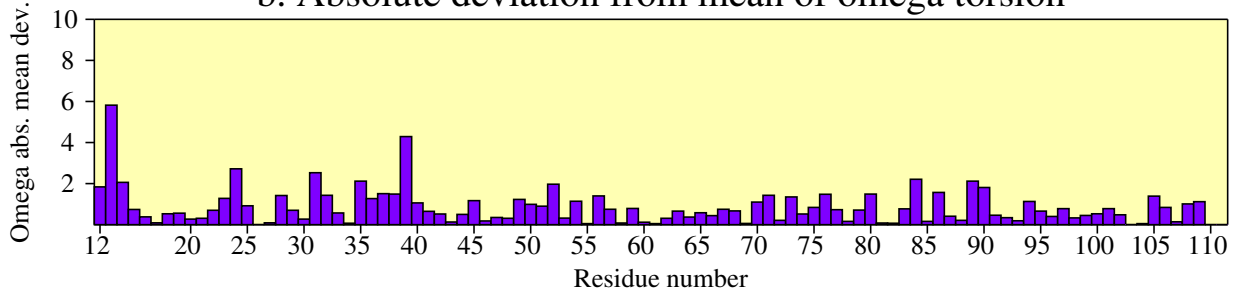


Residue properties
1ppj

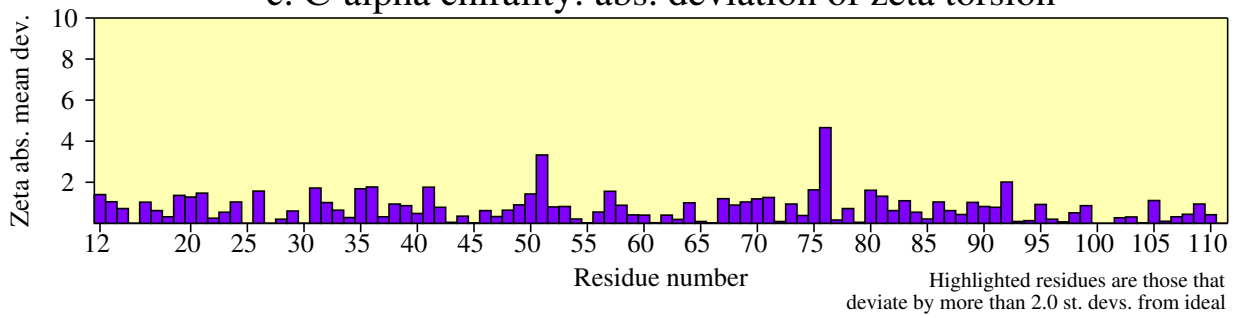
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



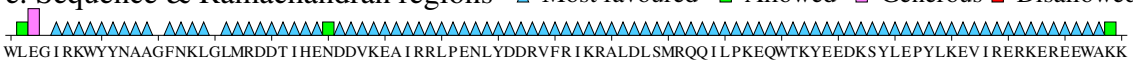
Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Buried Accessible

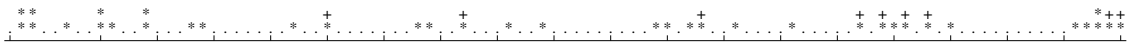
e. Sequence & Ramachandran regions

Most favoured Allowed Generous Disallowed

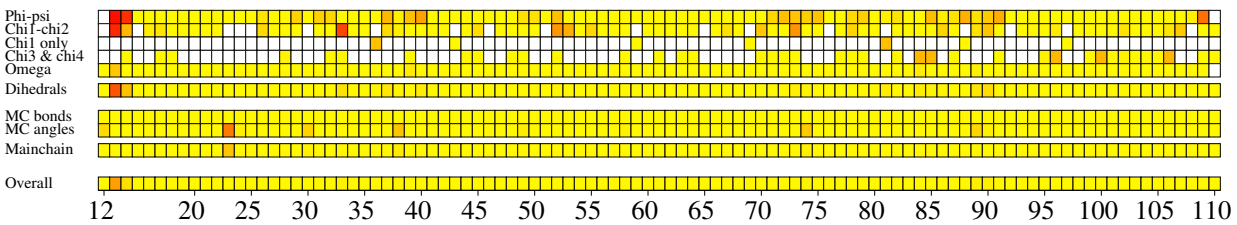


WLEGIRKWYYNAAGFNKLGLMRDDTIHENDDVKEAIRRLPENLYDDRVFRIKRALDLSMRQQILPKEQWTKYEEDKSYLEPYLKEVIRERKEREEWAKK

f. Max. deviation (see listing)

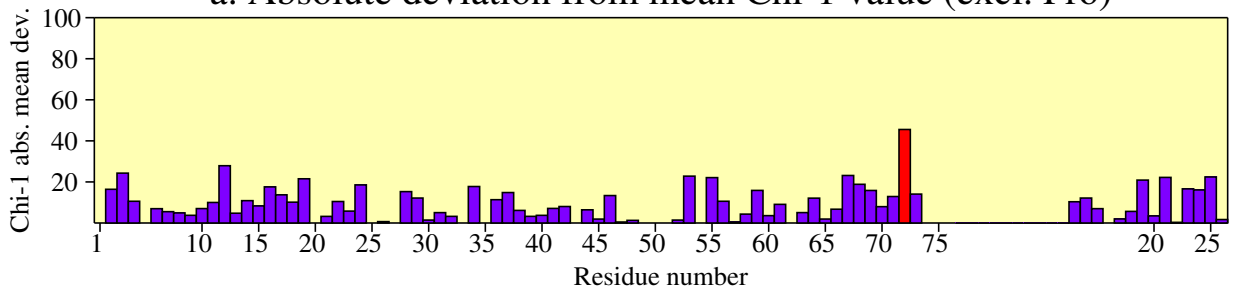


g. G-factors

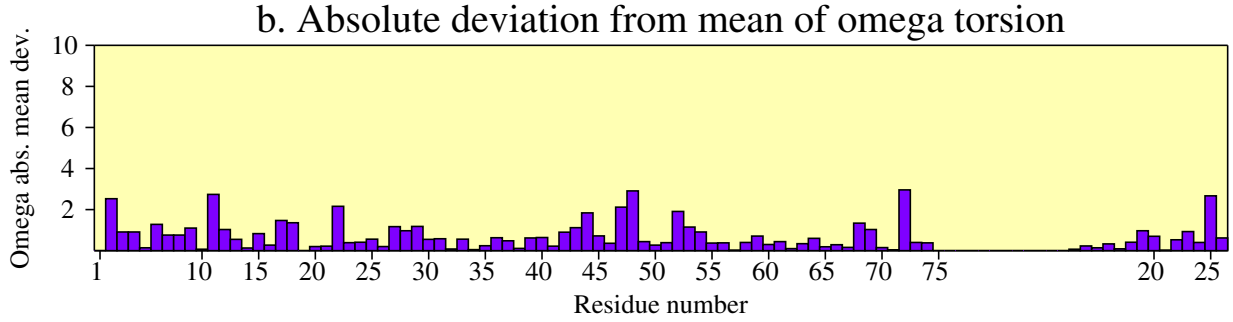


Residue properties
1ppj

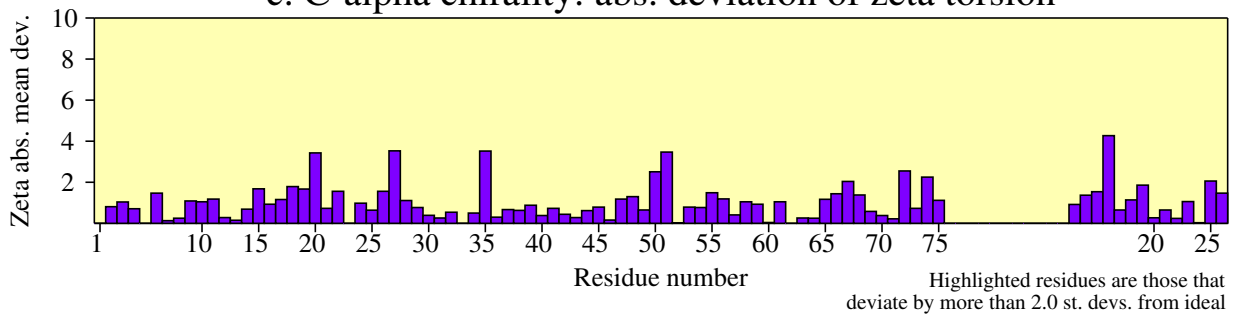
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion

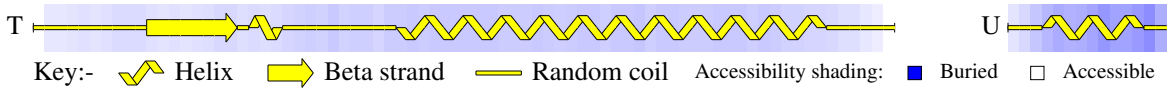


c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

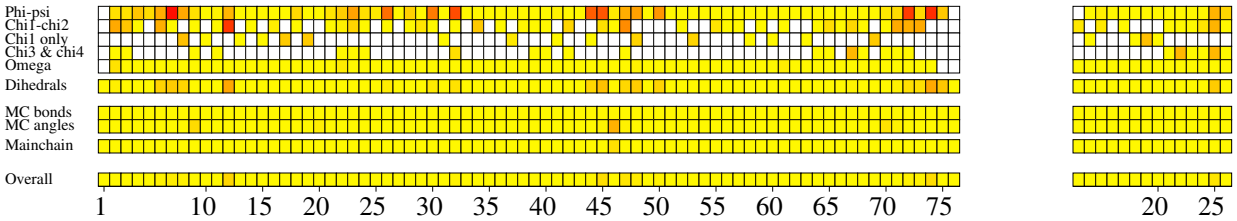


e. Sequence & Ramachandran regions

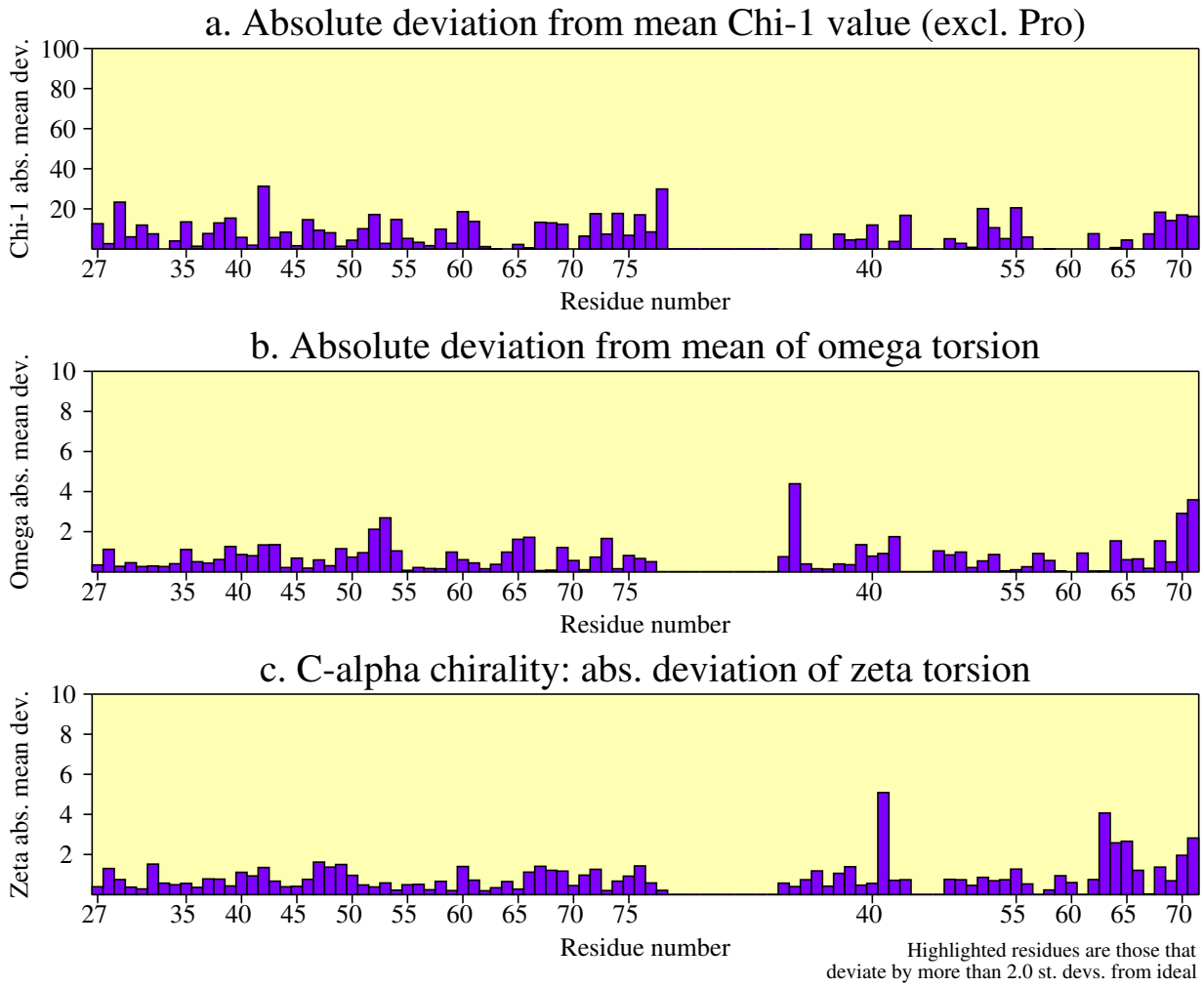
Most favoured Allowed Generous Disallowed

f. Max. deviation (see listing)

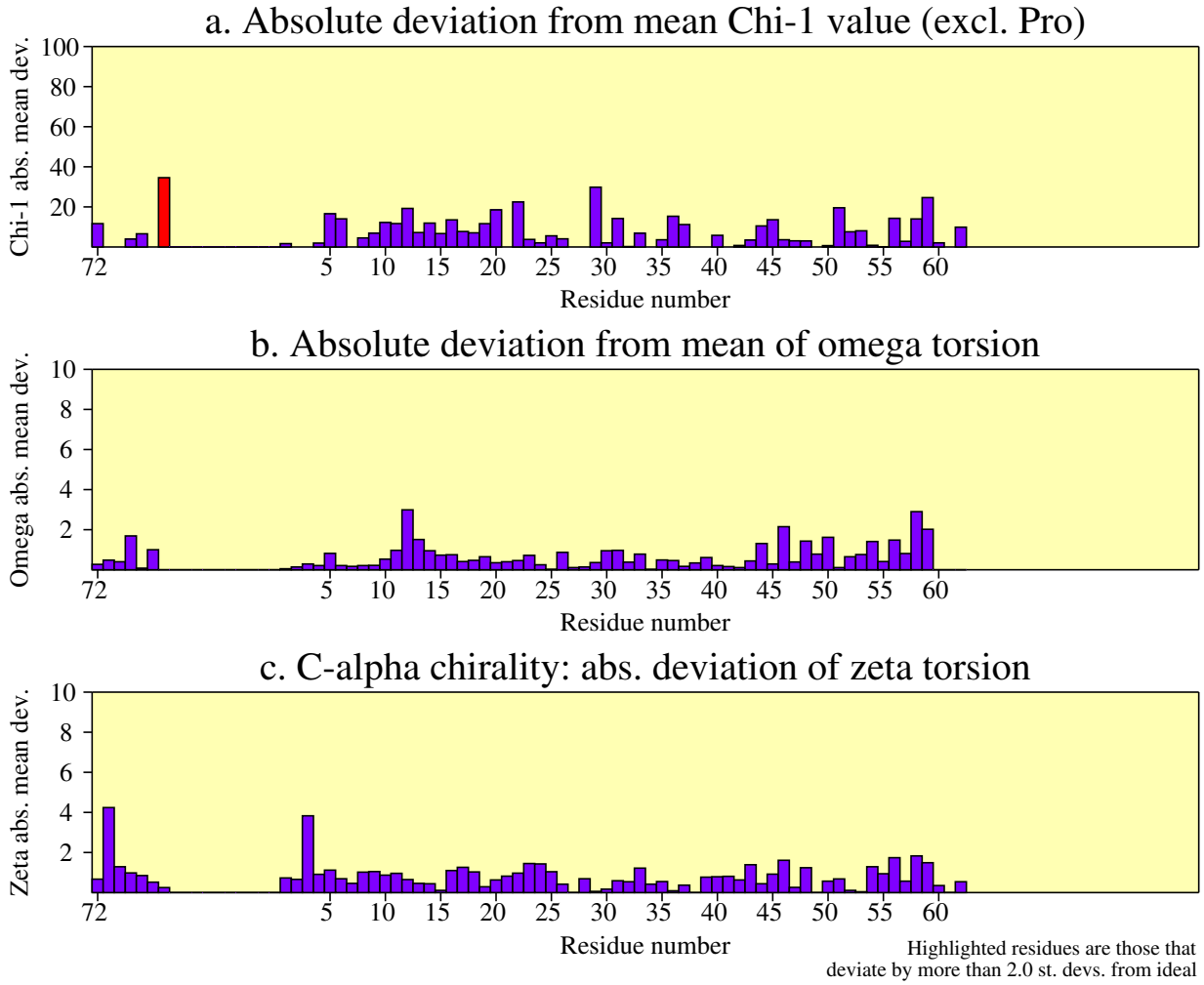
g. G-factors



Residue properties
1ppj

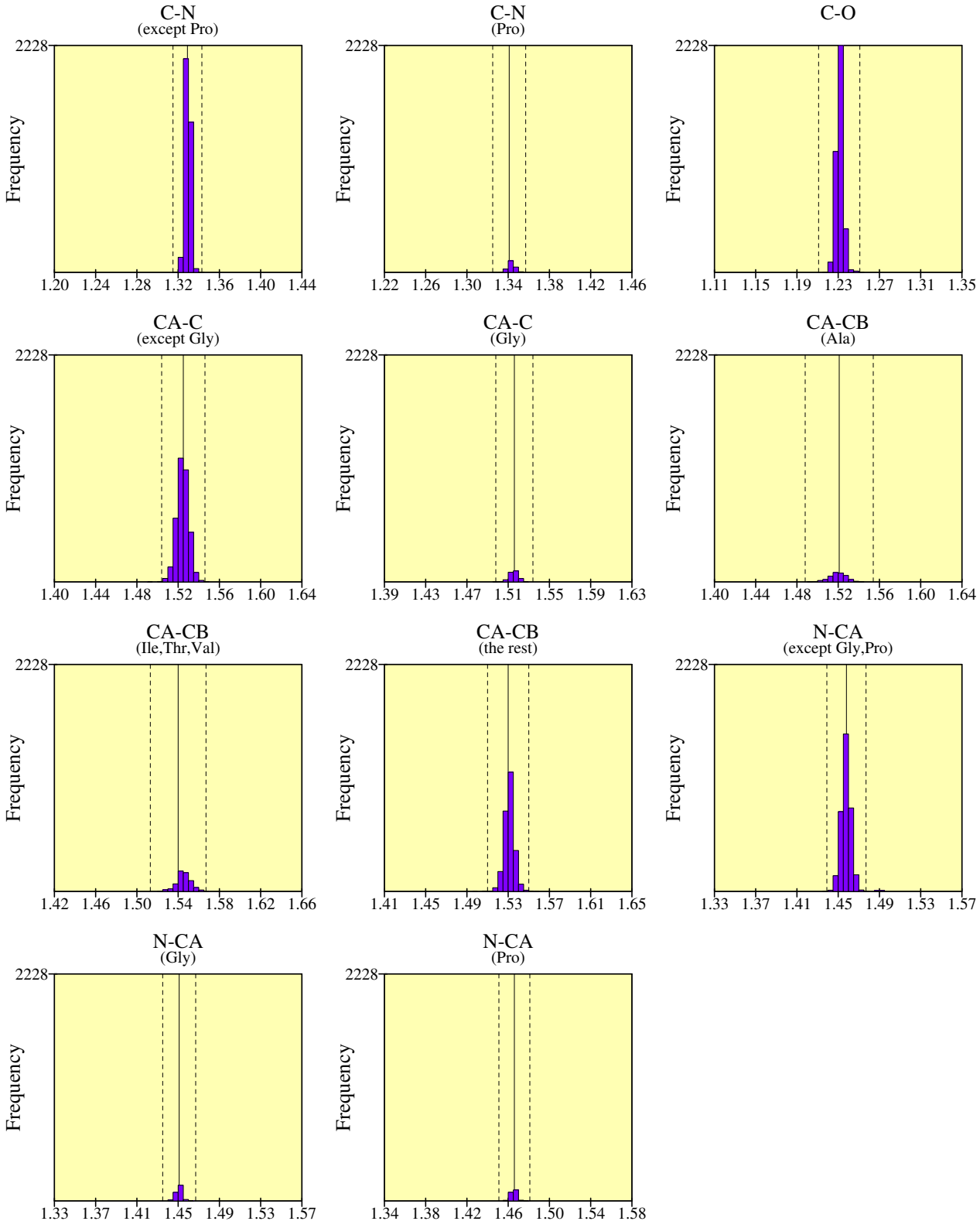


Residue properties
1ppj



Main-chain bond lengths

1ppj

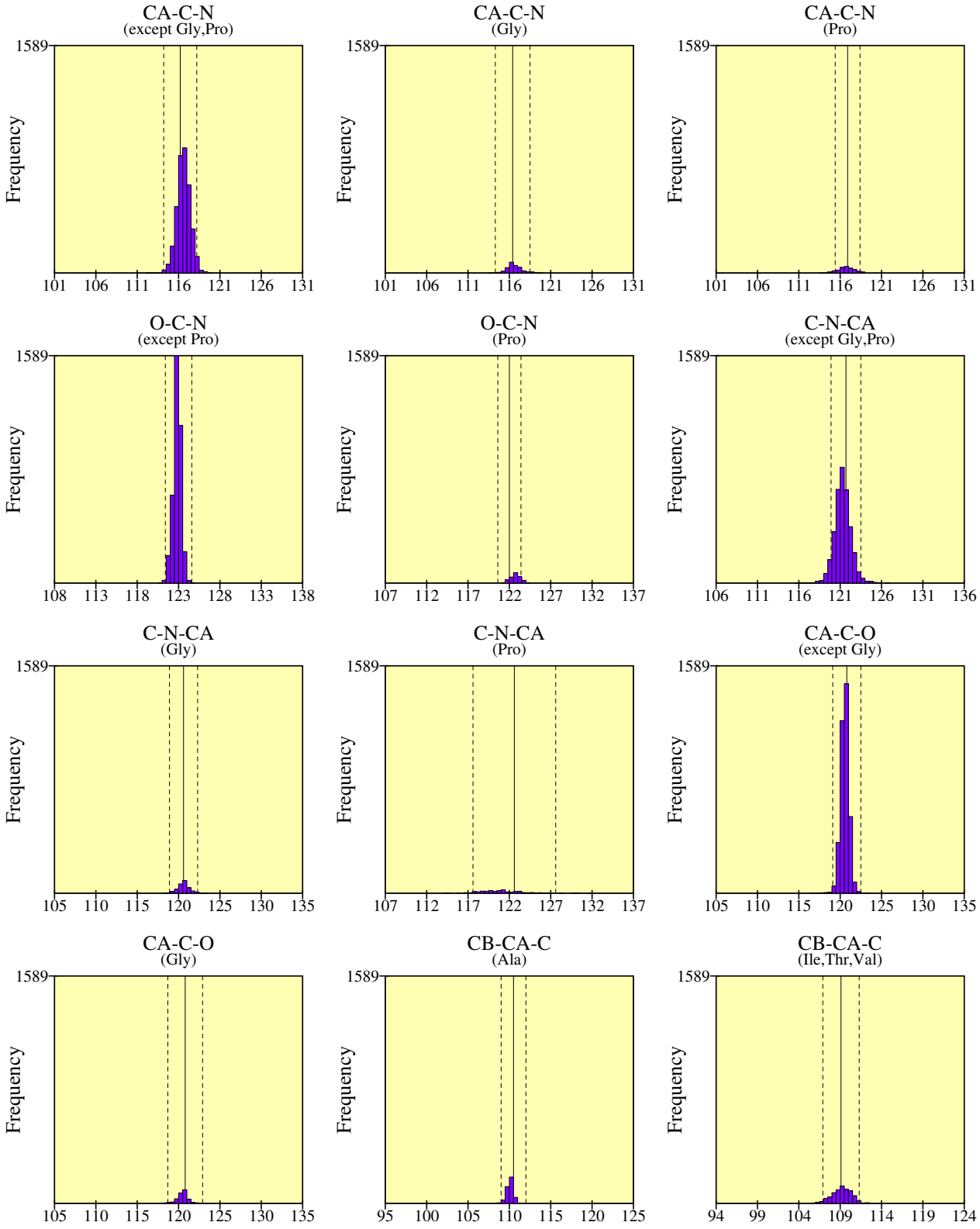


Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

Main-chain bond angles

1ppj

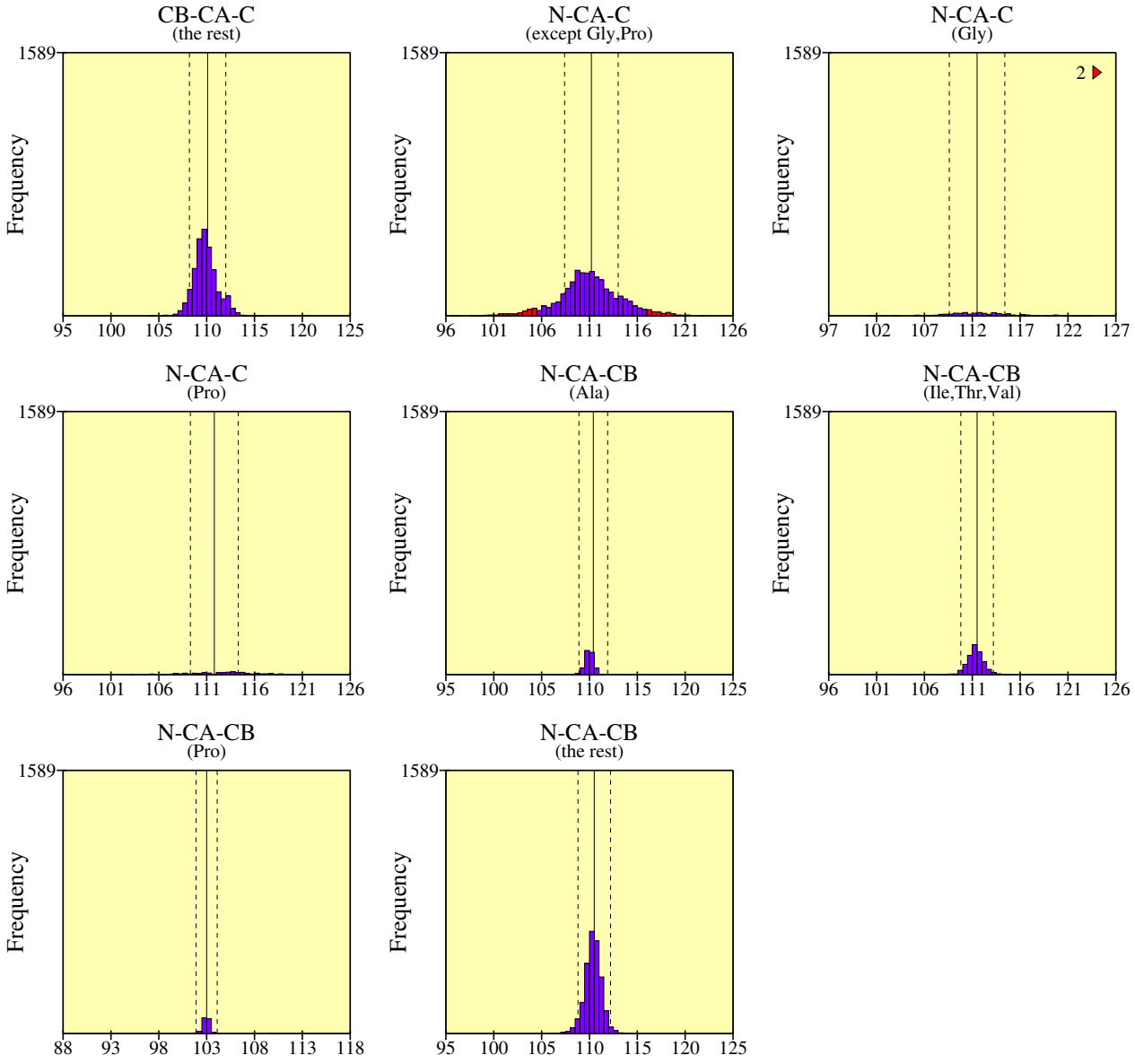


Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

Main-chain bond angles

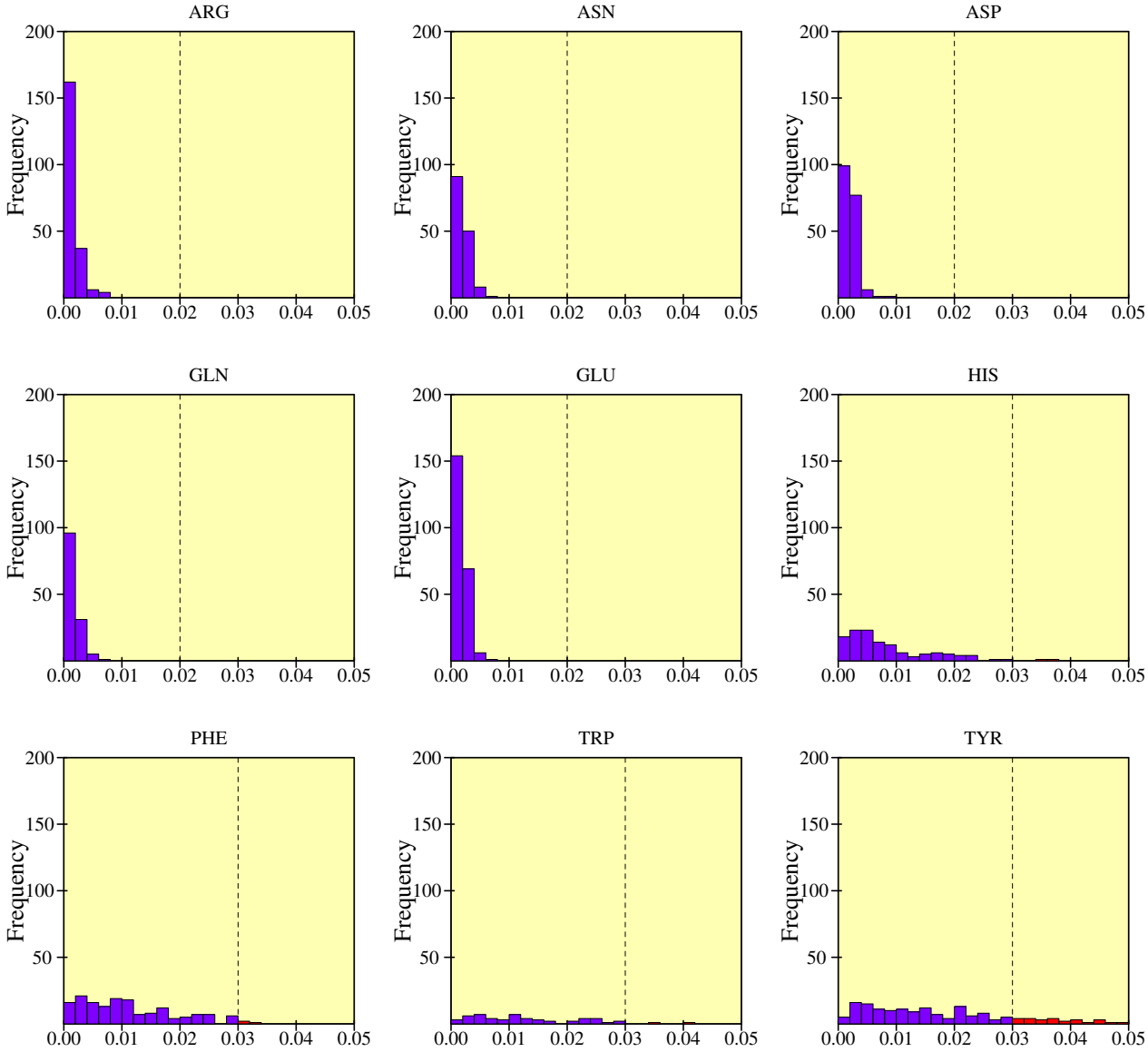
1ppj



Black bars > 2.0 st. devs. from mean. ◀ or ▶ signifies data points off the graph in the direction shown.
Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

RMS distances from planarity

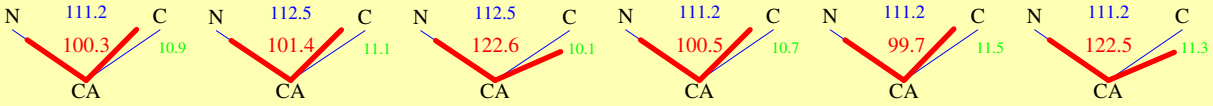
1ppj



Histograms showing RMS distances of planar atoms from best-fit plane.
Black bars indicate large deviations from planarity: RMS dist > 0.03 for rings, and > 0.02 otherwise.

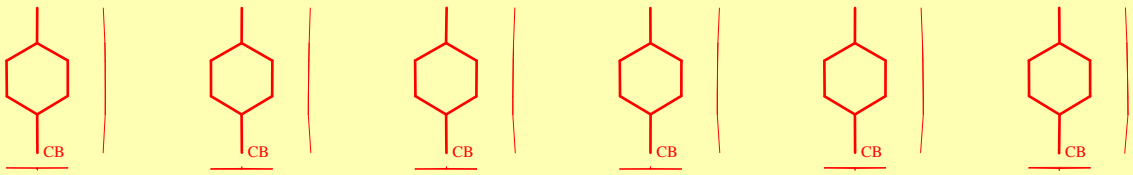
Distorted geometry
1ppj

Main-chain bond angles



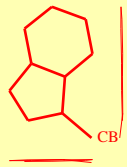
Bond angles differing by > 10.0 degrees from small-molec values. Values shown: "ideal", actual, diff.

Planar groups

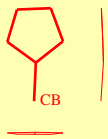


Distorted geometry
1ppj

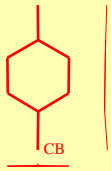
Planar groups (contd)



0.036
C Trp 141



0.036
C His 196



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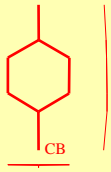
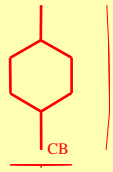
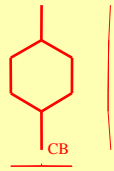
C Tyr 224



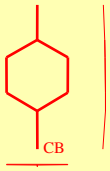
0.049
C Tyr 278



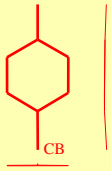
0.047
D Tyr 33



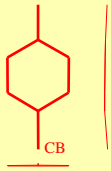
0.040
D Tyr 90



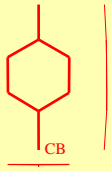
0.037
D Tyr 148



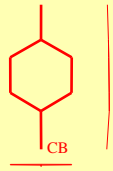
0.032
D Tyr 152



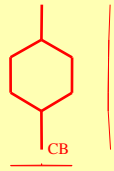
0.042
F Tyr 20



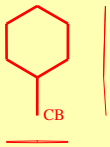
0.038
F Tyr 55



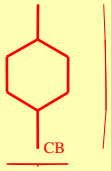
0.034
N Tyr 416



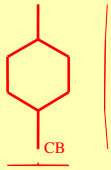
0.033
O Tyr 177



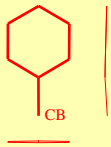
0.032
P Phe 33



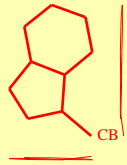
0.042
P Tyr 75



0.045
P Tyr 81



0.031
P Phe 91



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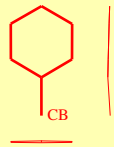
P Trp 141



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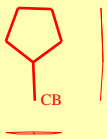
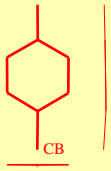
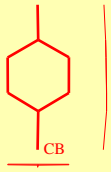
P Phe 168



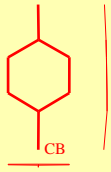
0.036
P His 196



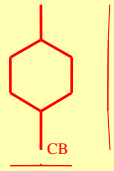
0.031
P Tyr 224



0.046
P Tyr 278



0.045
Q Tyr 33



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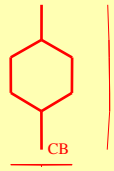

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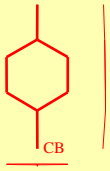
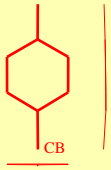
Q Tyr 90



0.035
Q Tyr 148



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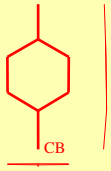


0.040
S Tyr 20

Sidechains with RMS dist. from planarity > 0.03A for rings, or > 0.02A otherwise. Value shown is RMS dist.

Structure Factor Check
1PP9

Title: BOVINE CYTOCHROME BC1 COMPLEX WITH STIGMATELLIN BOUND
Date: 16−JUN−03
PDB code: 1PP9

Crystal

Cell parameters:
a: 139.12 Å b: 171.05 Å c: 227.20 Å
α: 90.00° β: 90.00° γ: 90.00°

Space group: P 21 21 21

Model

33959 atoms (1437 water molecules)

Number of chains:	51
Volume not occupied by model:	48.5 %
<B> (for atomic model):	46.6 Å ^{2}
σ(B):	15.40 Å ^{2}
Matthews coefficient:	2.97
Corresponding solvent % :	58.24

Refinement

Program:	CNS 1.1
Nominal resolution range:	25.0 − 2.10 Å
Reported R−factor:	0.250
Number of reflections used:	305496
Reported Rfree:	0.29
Sigma cut−off:	N.A.

Structure Factors

Input

Nominal resolution range:		40.0 − 2.08 Å
Reflections in file:		308206
Unique reflections	above 0:	308206
	above 1σ:	294636
	above 3σ:	173856

SFCHECK

Nominal resolution range: 40.0 − 2.10 Å
(max. from input data, min. from author)

Used reflections:	305655
Reflections out of resolution:	2551
Completeness:	97.4 %
R_stand(F) = <σ(F)>/<F> :	0.063

Anisotropic distribution of Structure Factors
ratio of eigen values: 1.0000 0.7621 0.5479

B_overall (by Patterson):	36.2Å ^{2}
Optical resolution:	1.72 Å
Expected opt. resol. for complete data set:	1.72 Å
Estimated minimal error:	0.036 Å

Model vs. Structure Factors

R−factor for all reflections:	0.272
Correlation factor:	0.924
R−factor:	0.271

for F > 2.0σ
nom. resolution range: 24.98 − 2.10Å
reflections used: 292406

Rfree:	0.290
Nfree:	14590
R−factor without free−refl.:	0.270
Non free−reflections:	277816
<u> (error in coords by Luzzati plot):	0.355 Å
Estimated maximal error:	0.131 Å
DPI:	0.200 Å

Scaling

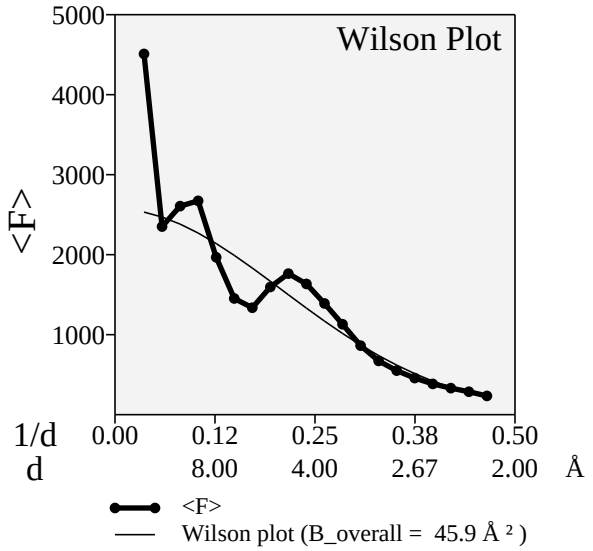
Scale:	1.092
Bdiff:	−5.07

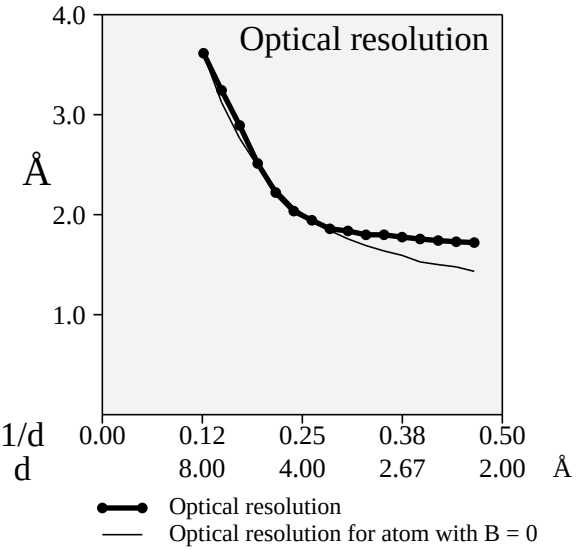
Anisothermal Scaling (Beta):
5.4493 1.4983 −2.1055 0.0000 0.0000 0.0000

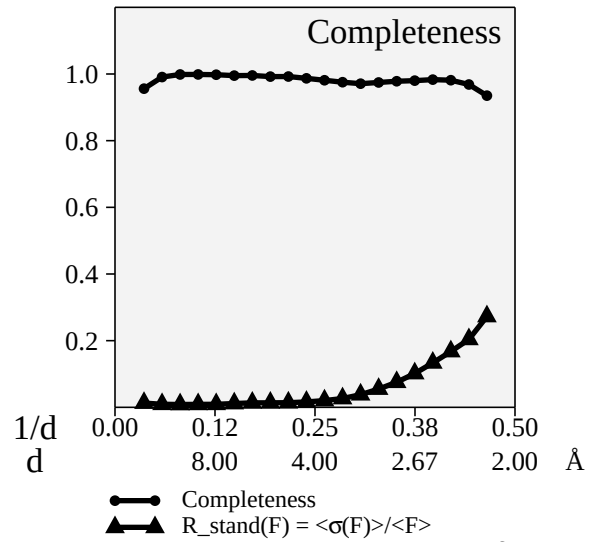
Solvent correction − Ks,Bs: 0.753 250.038

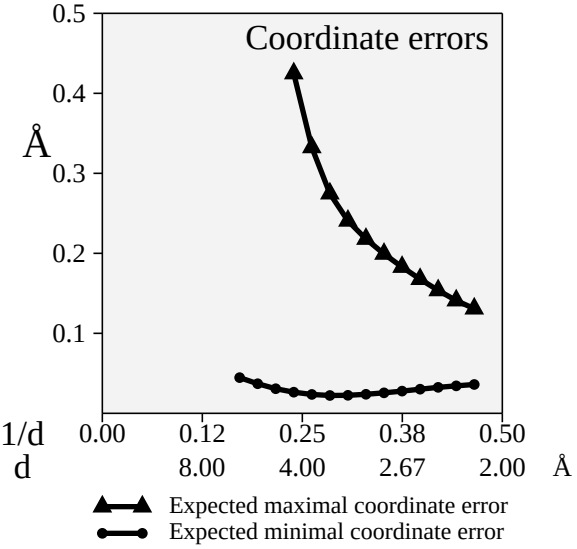
Structure Factor Check

1PP9

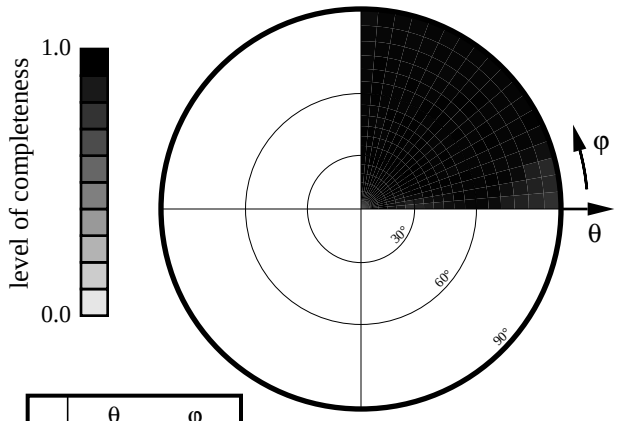






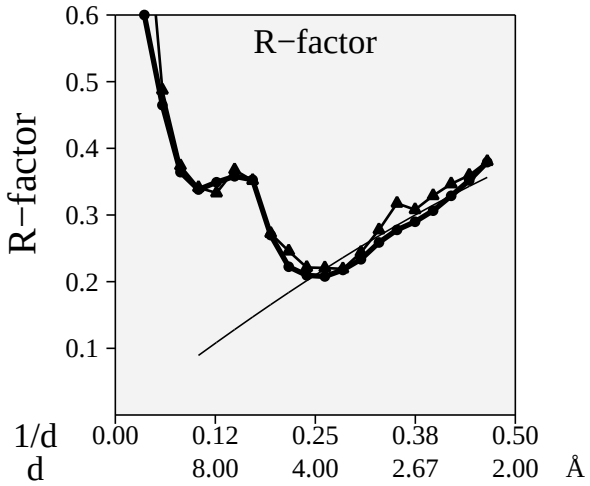


Stereographic projection of
the averaged radial completeness



	θ	φ
h	90.00	0.00
k	90.00	90.00
l	0.00	0.00

polar coordinates of the
crystallographical axes

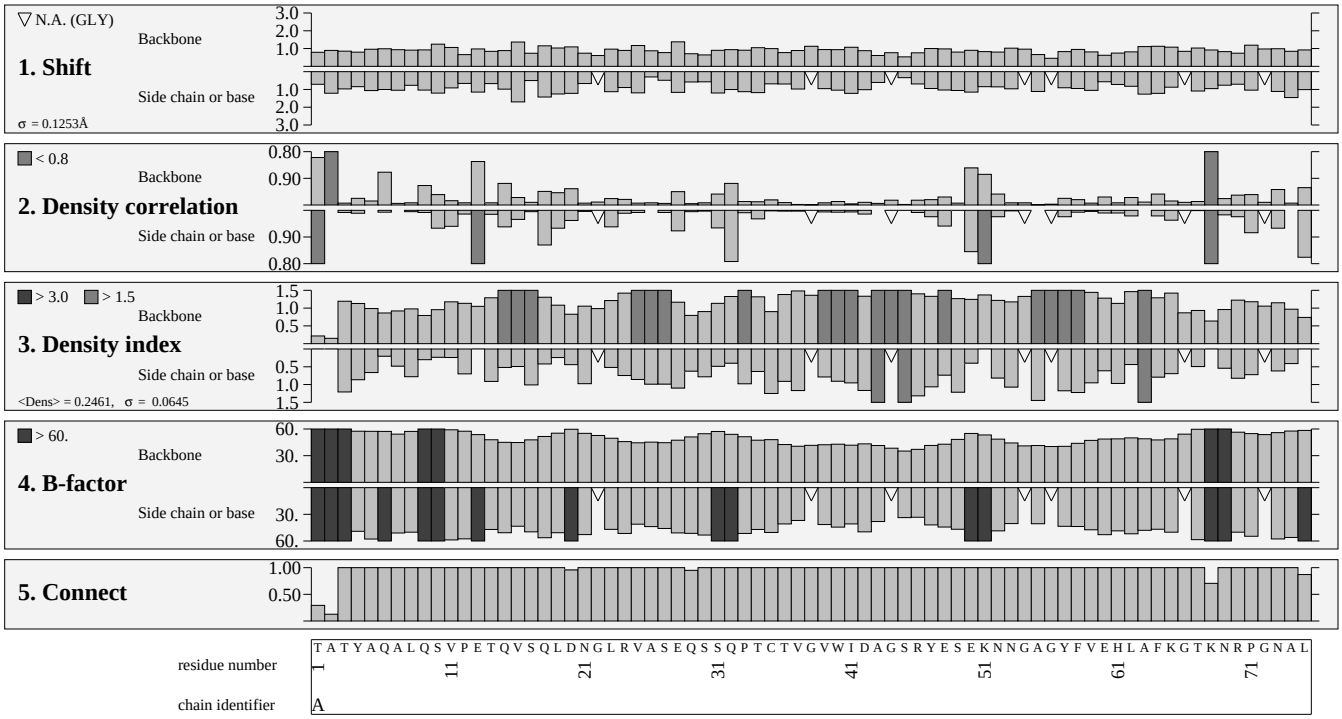


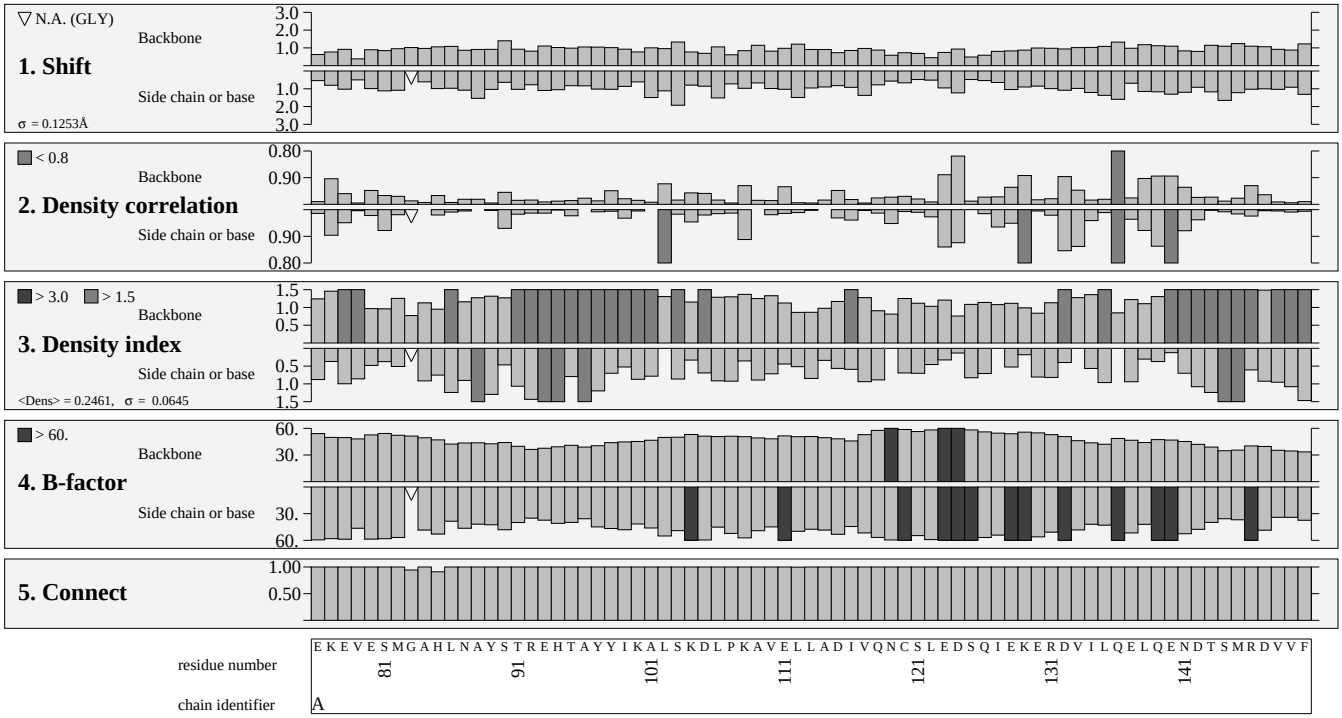
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation



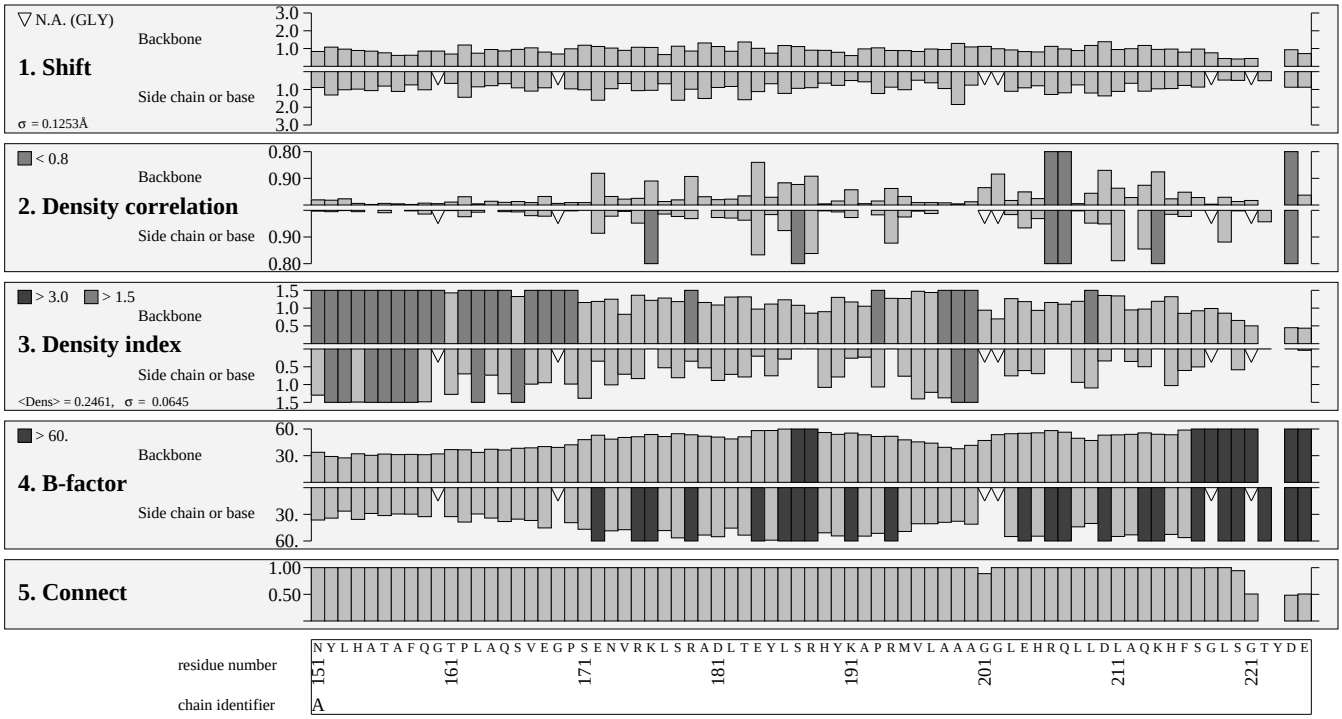


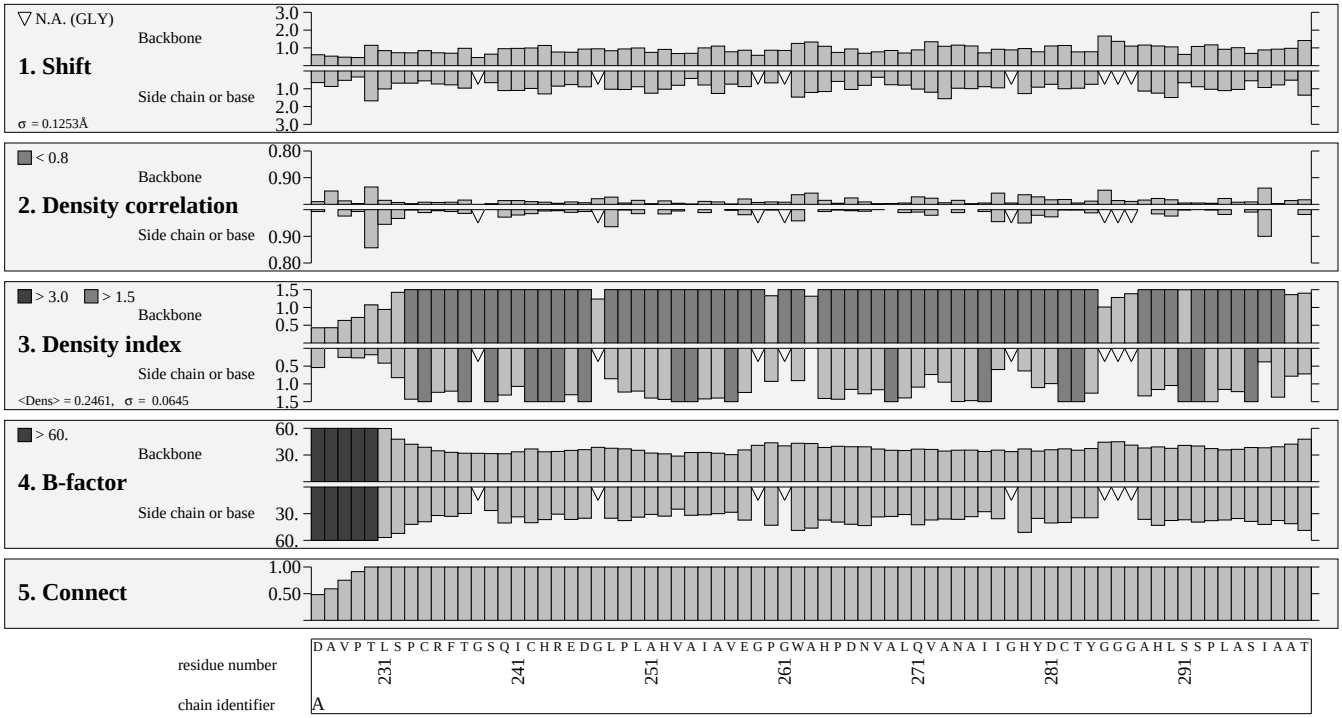
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (2)



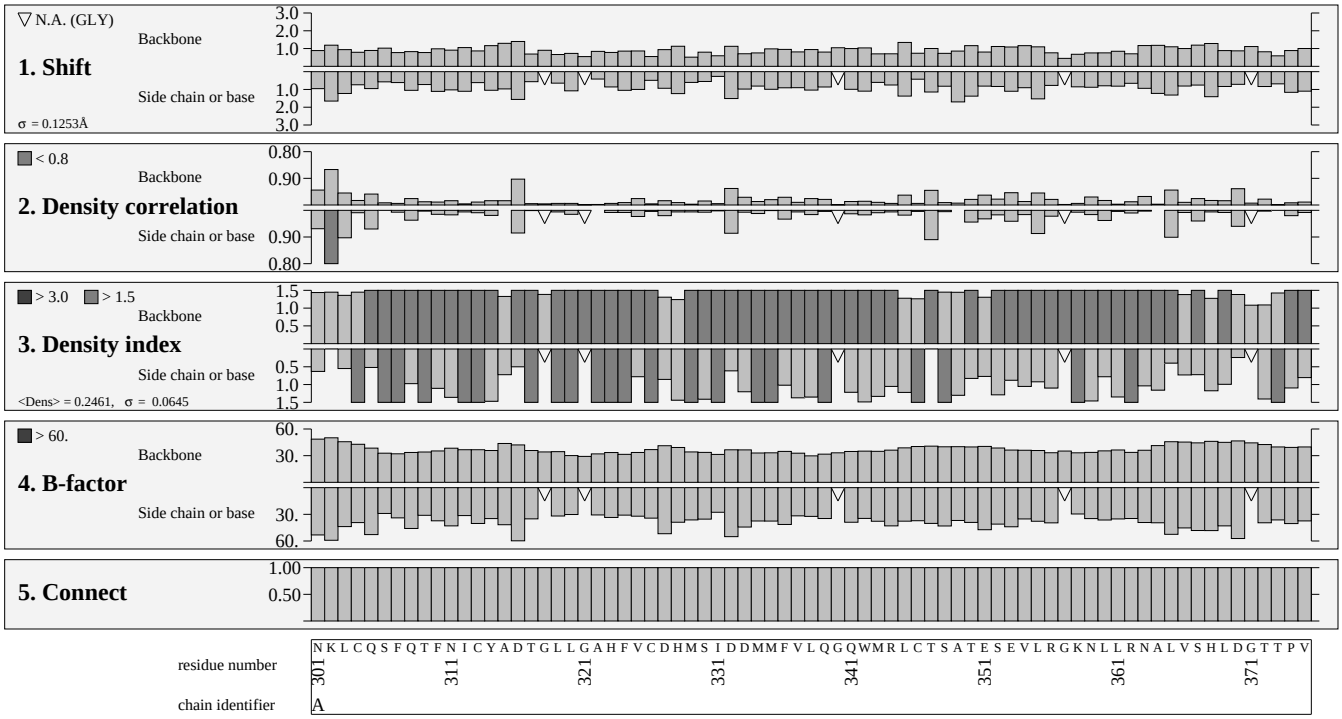


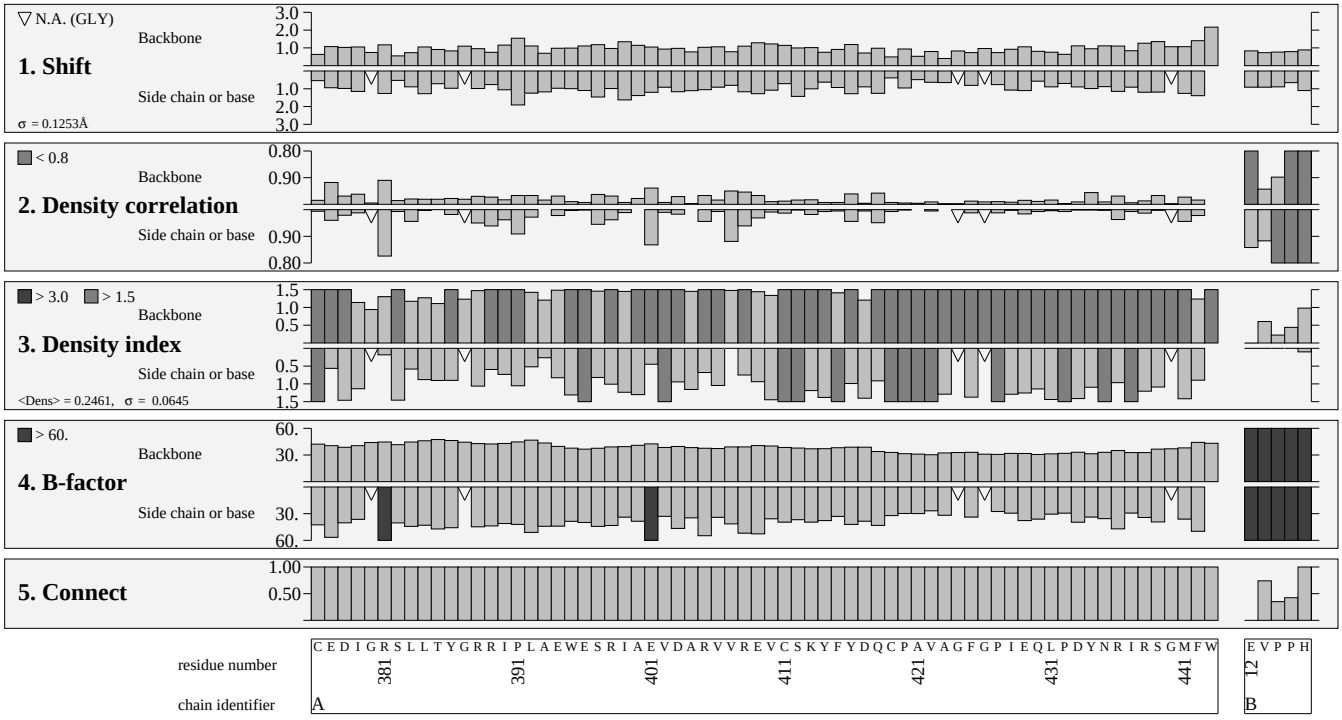
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (3)



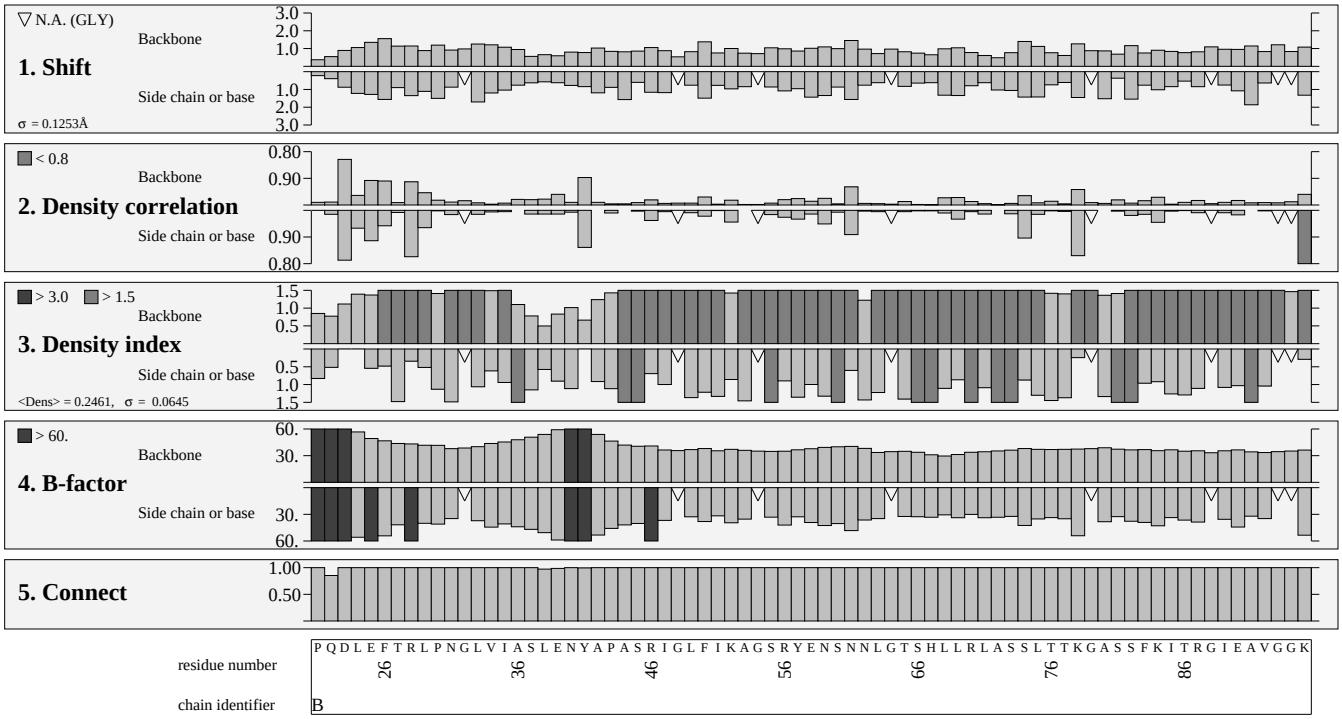


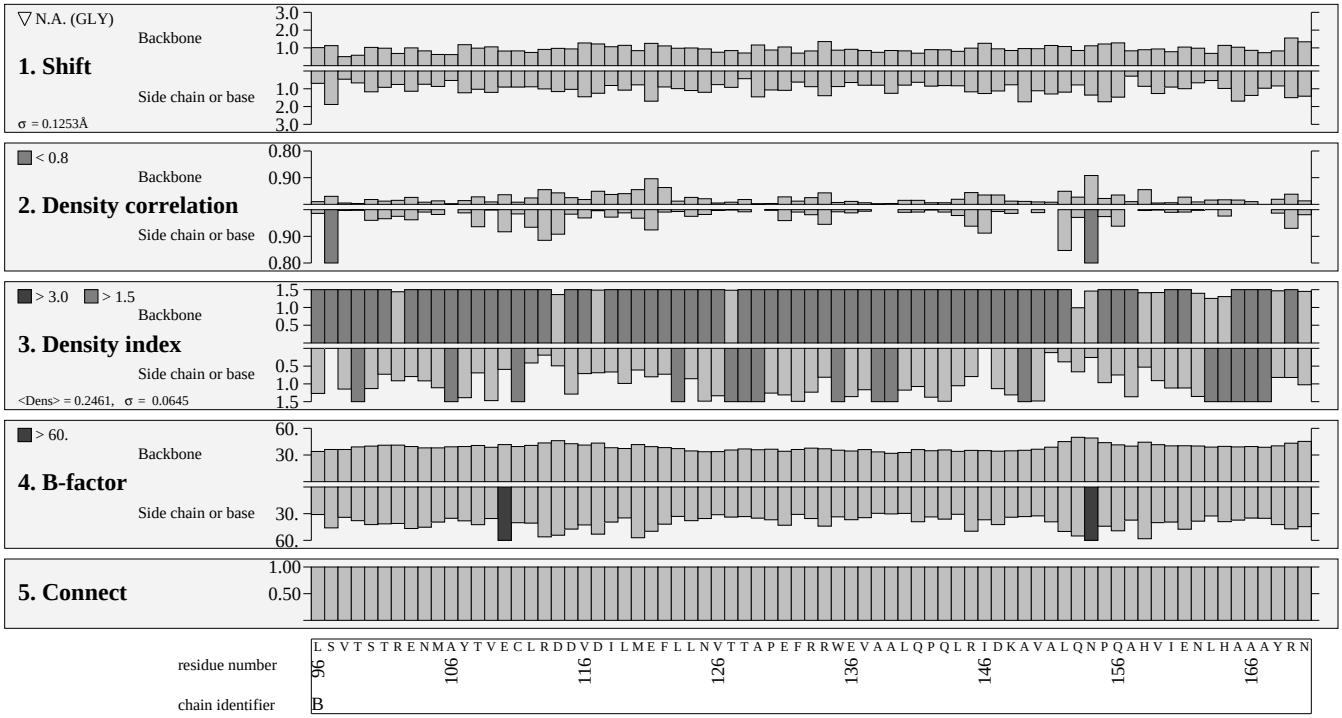
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (4)



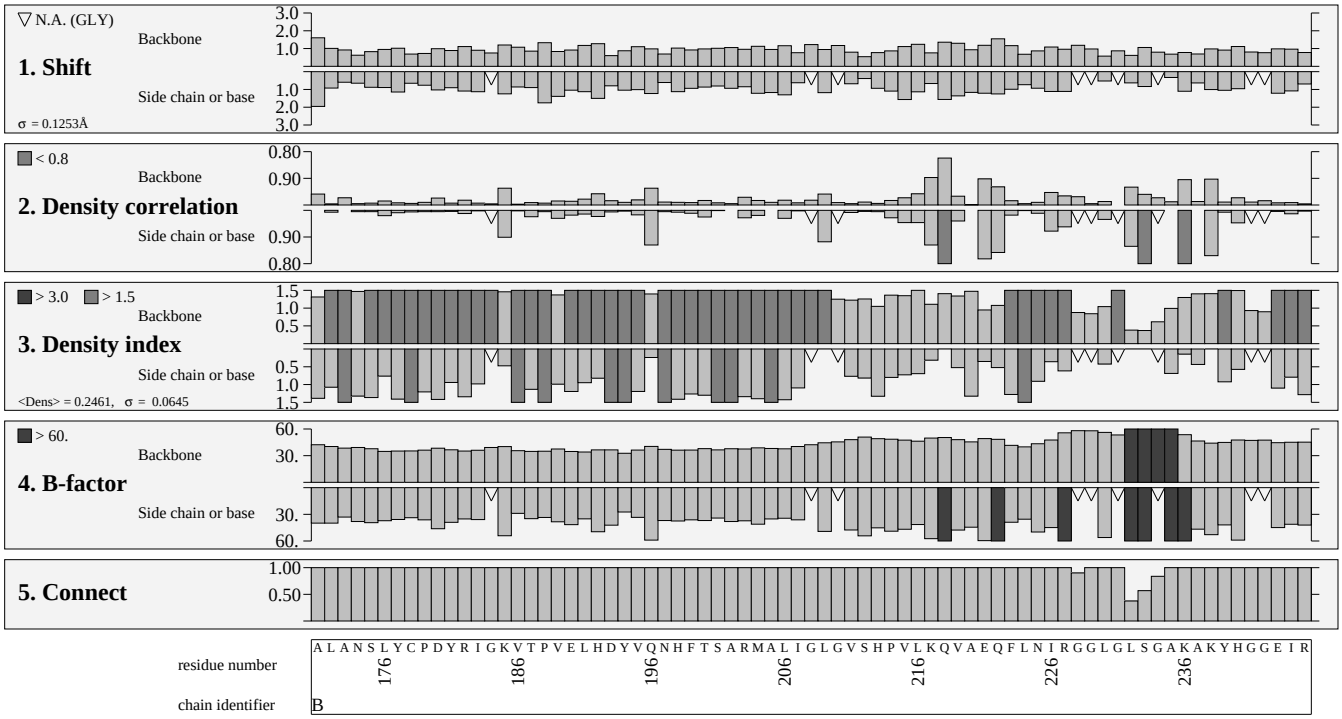


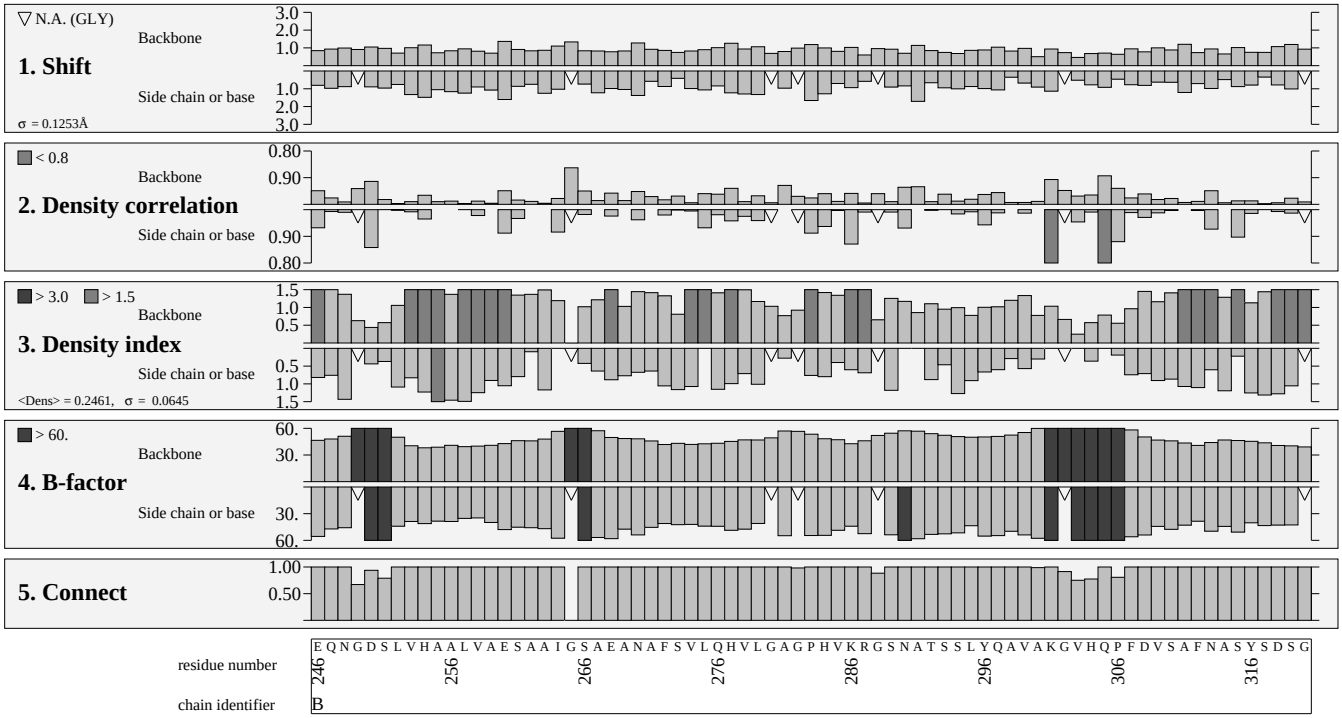
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (5)



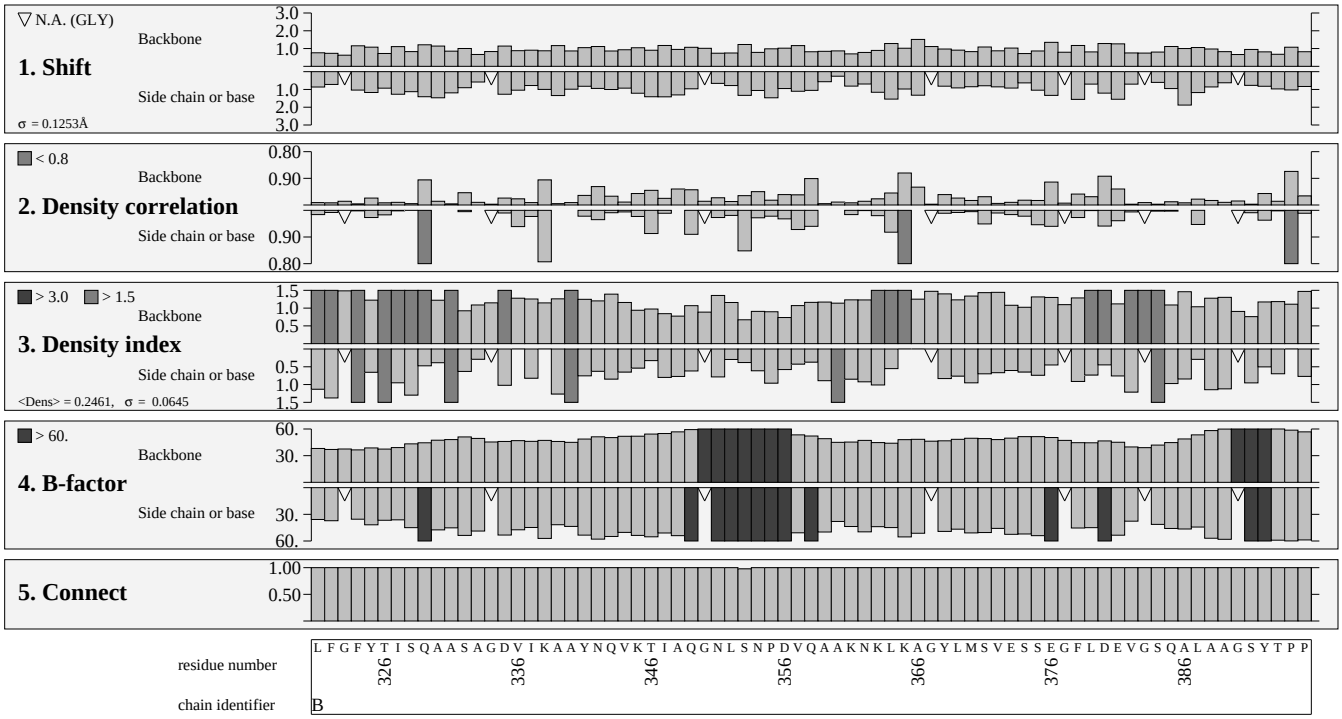


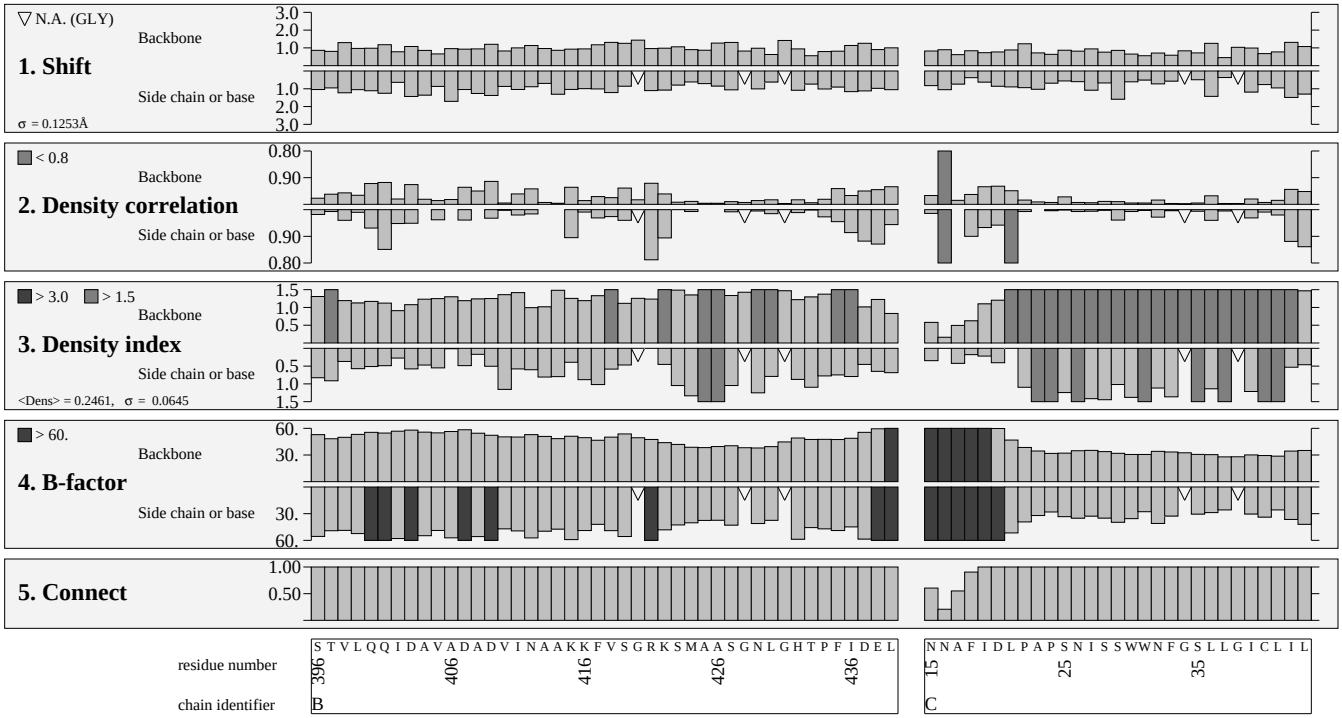
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (6)



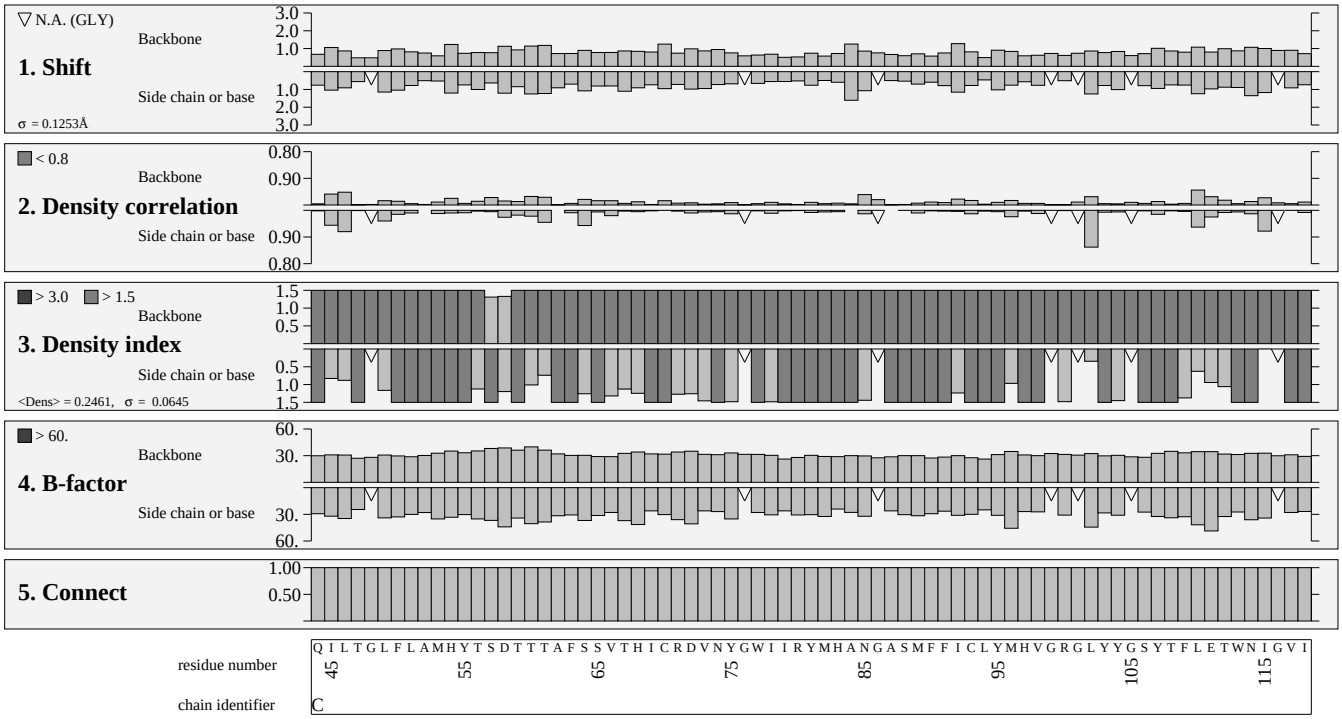


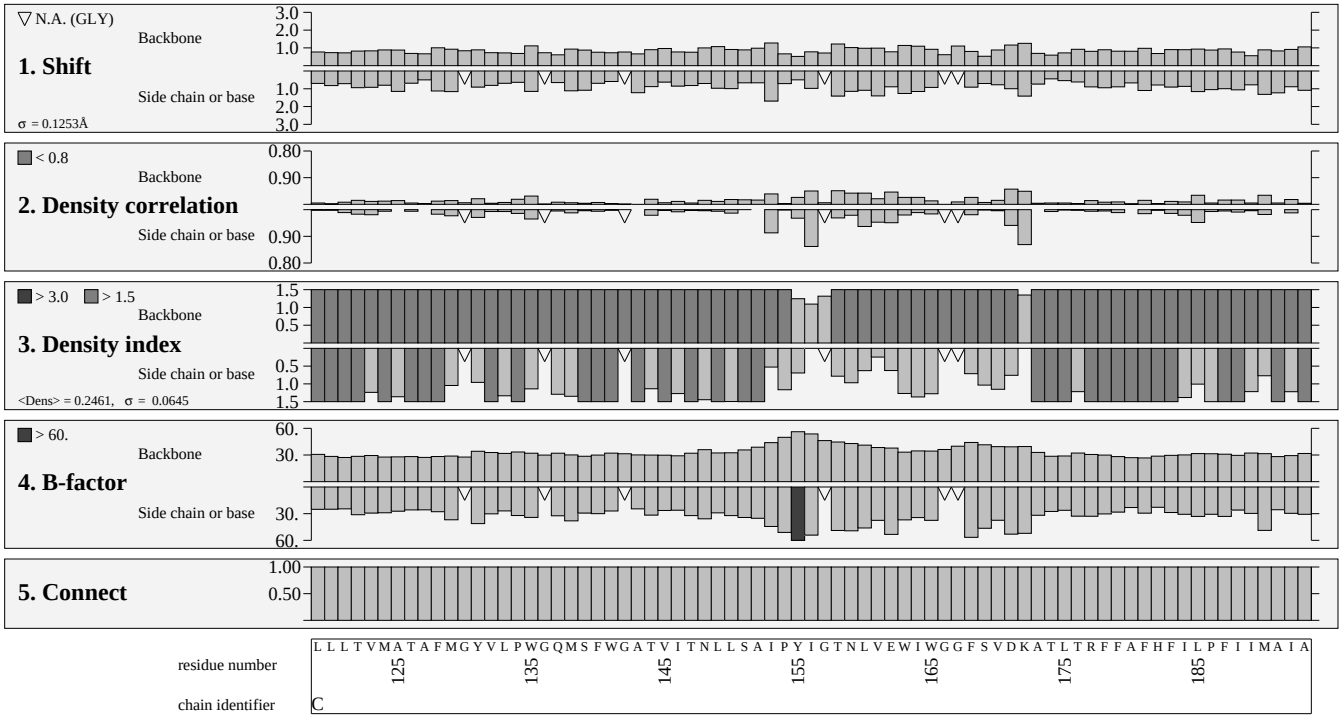
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (7)



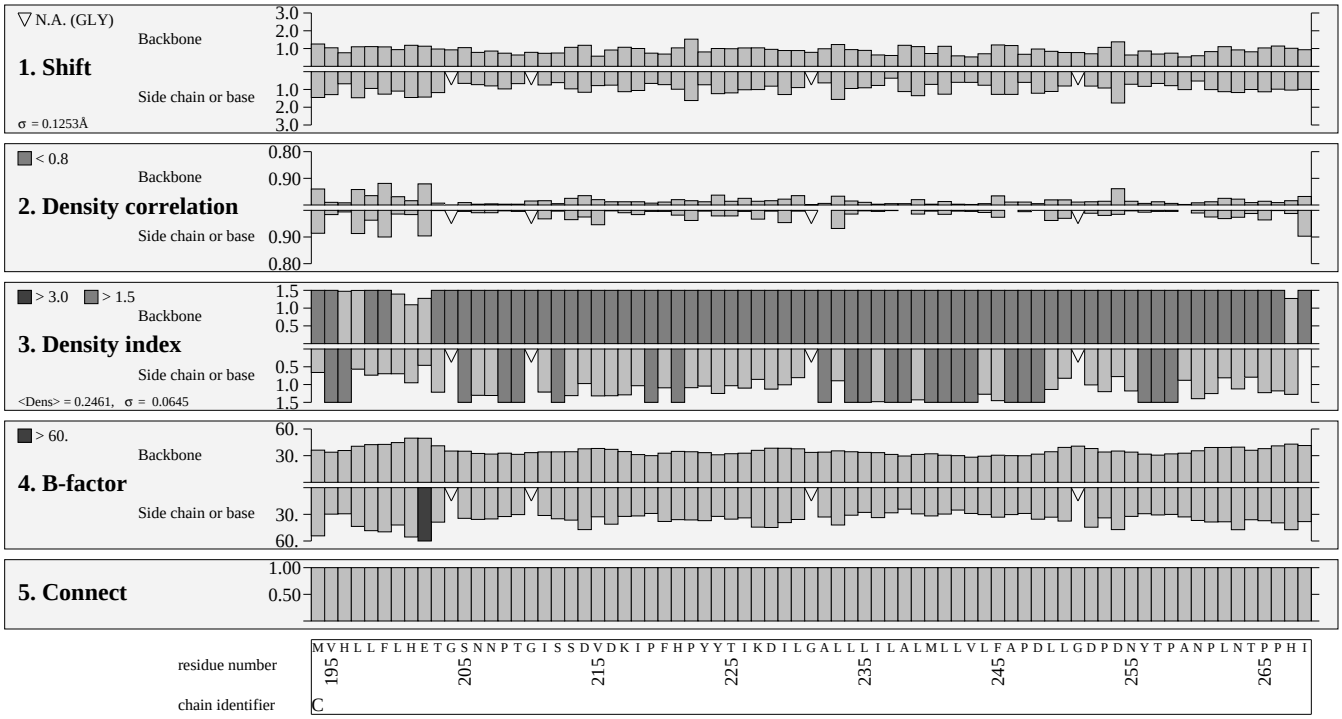


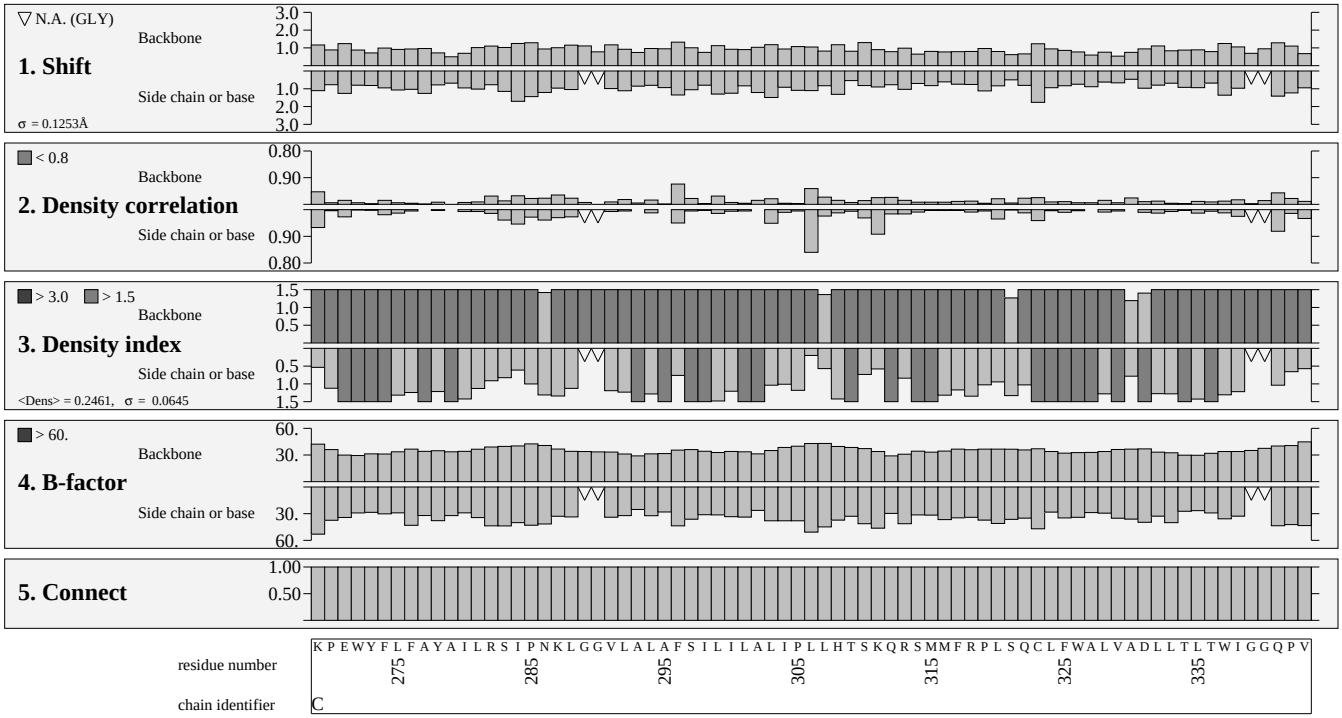
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (8)



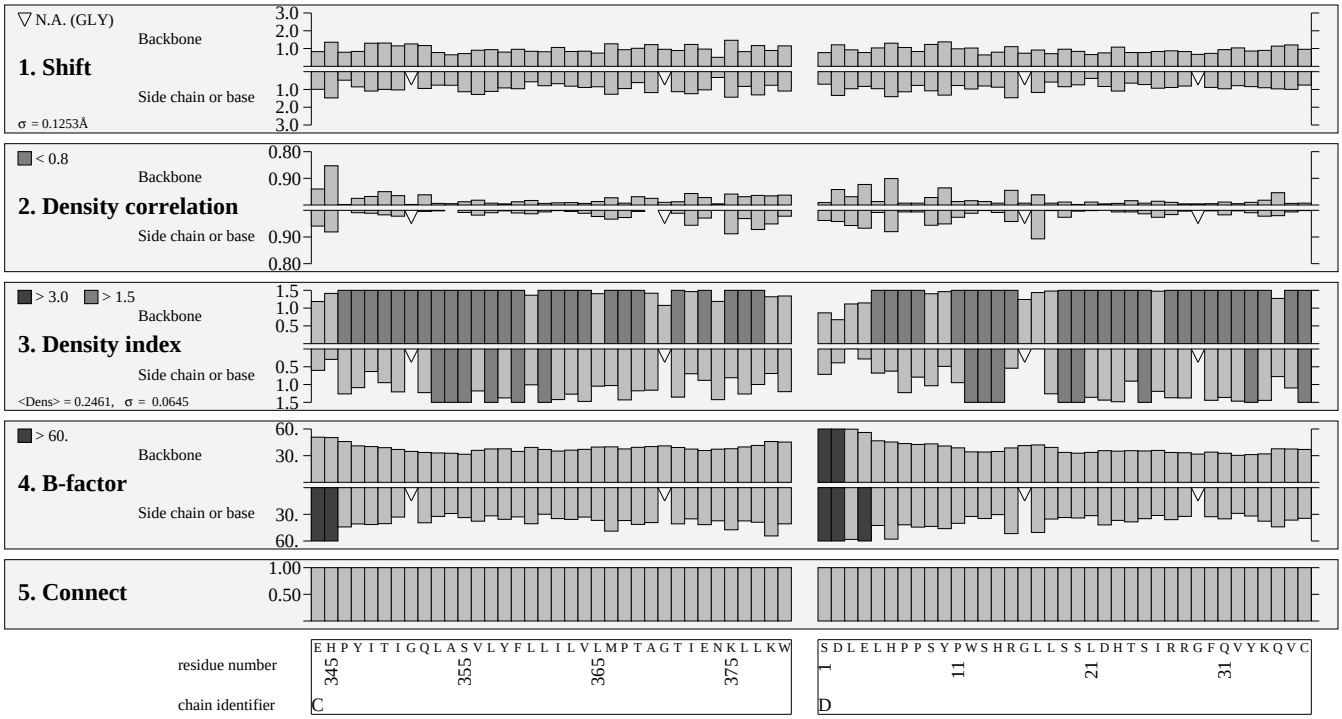


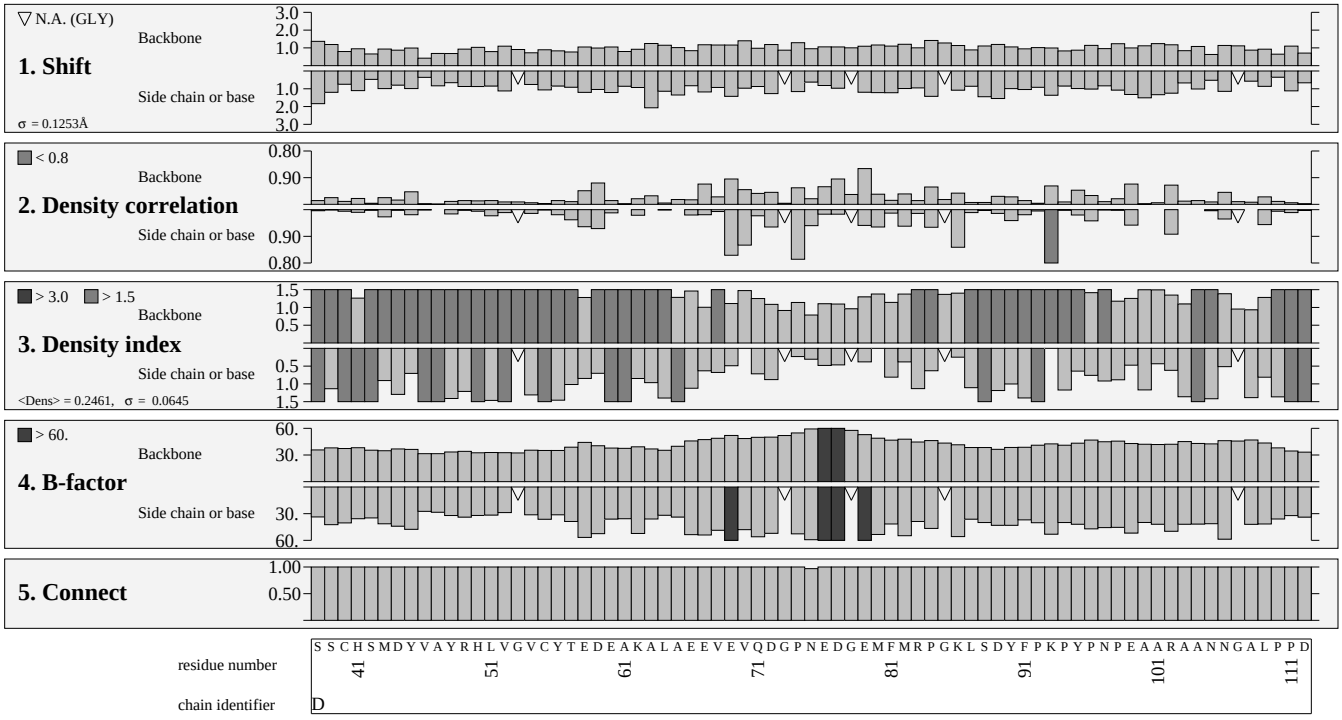
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (9)



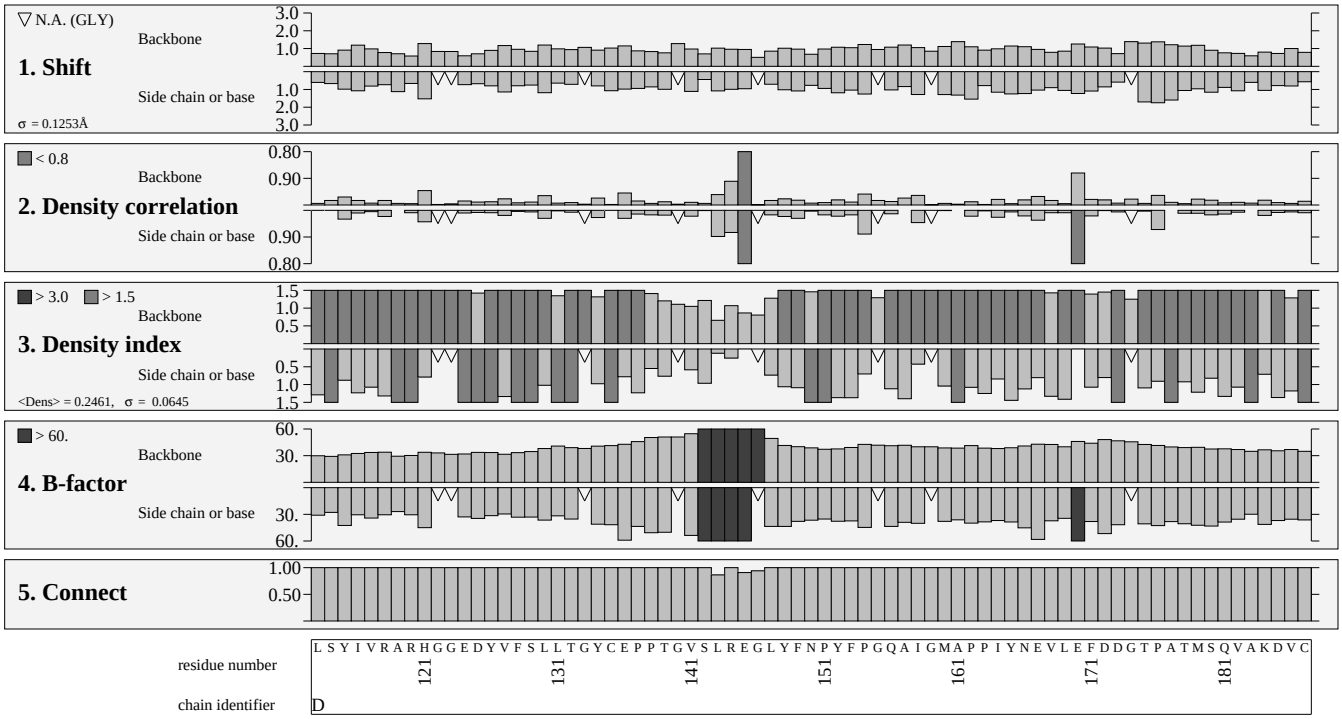


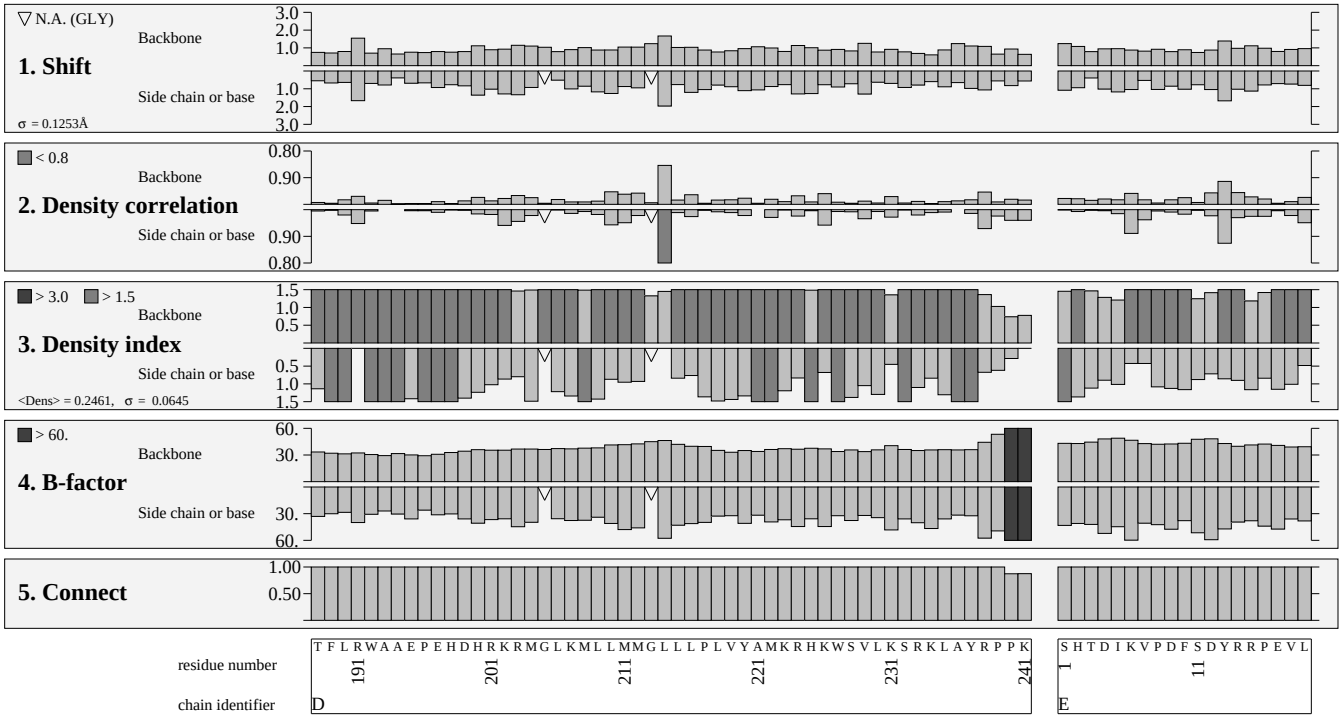
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (10)



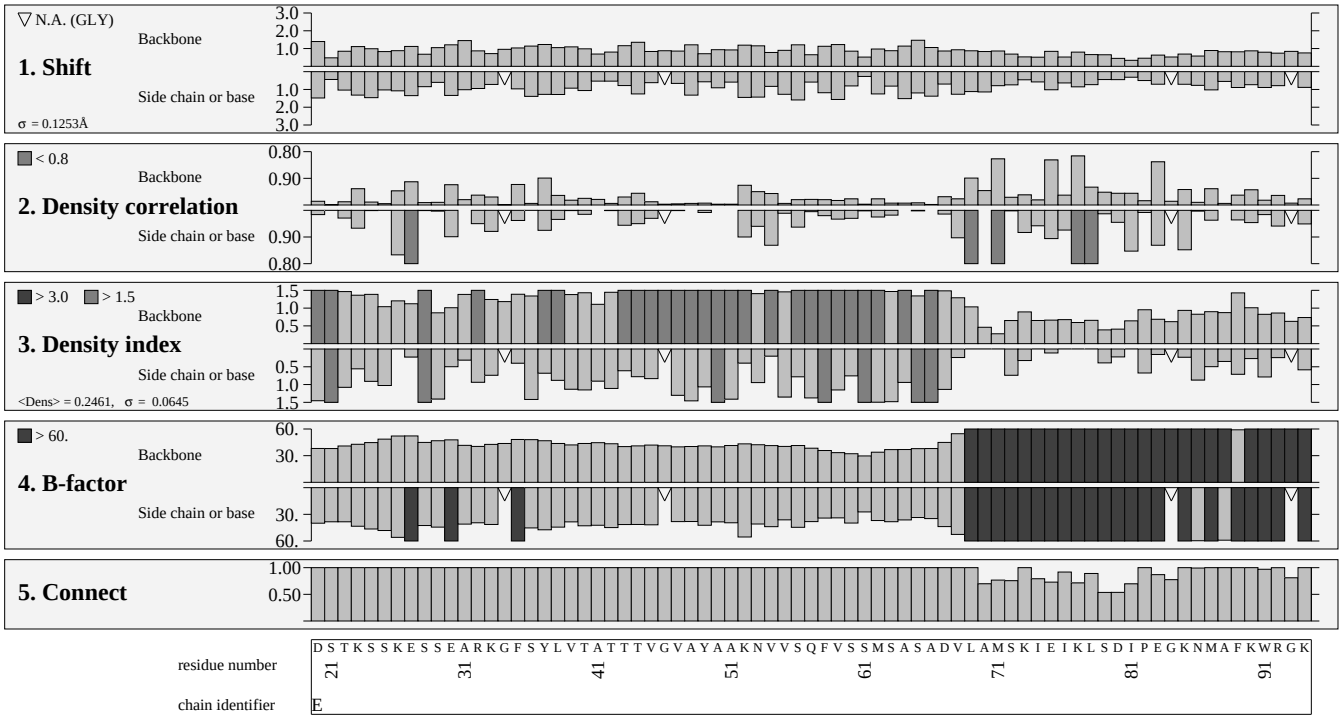


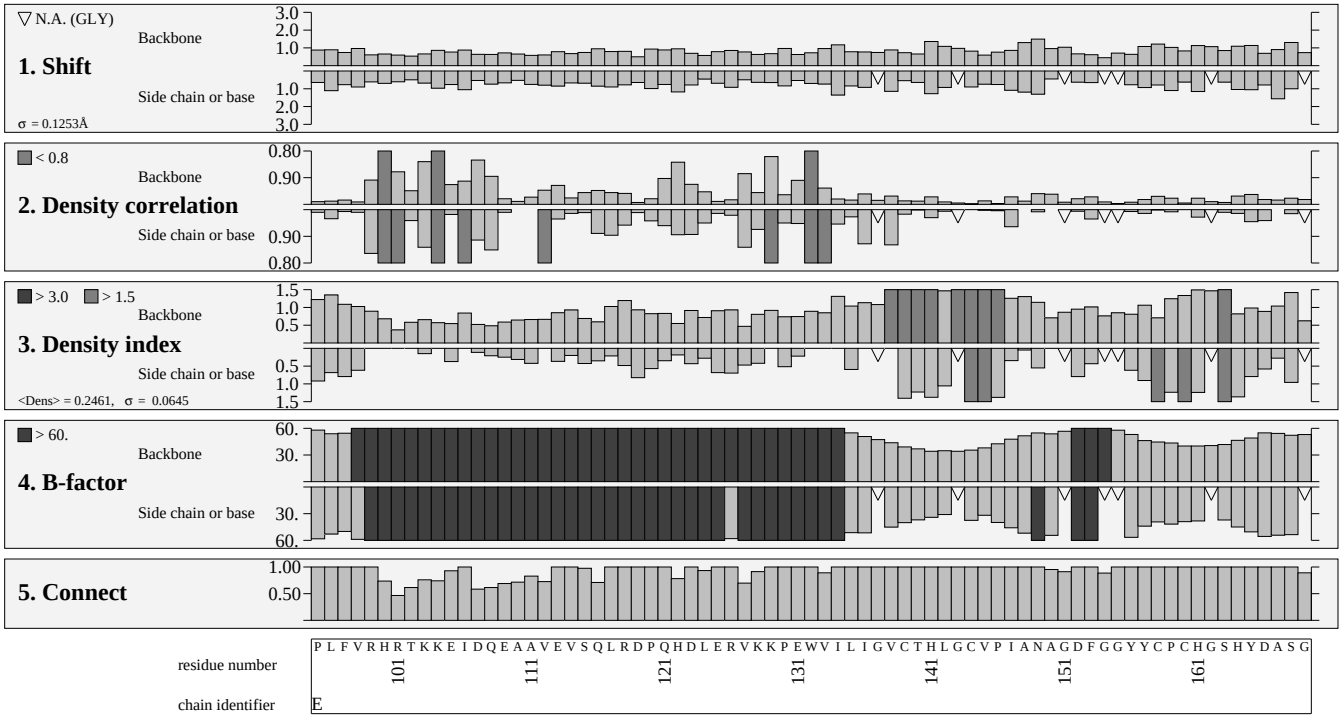
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (11)



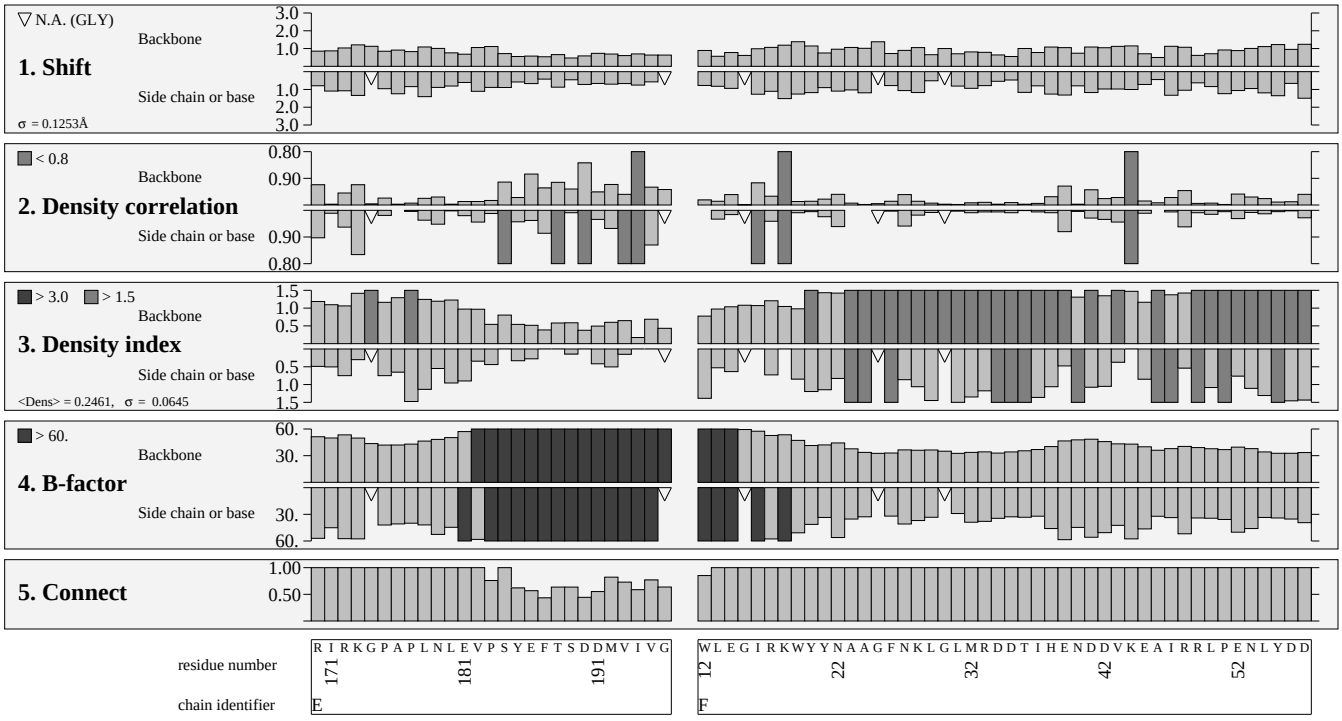


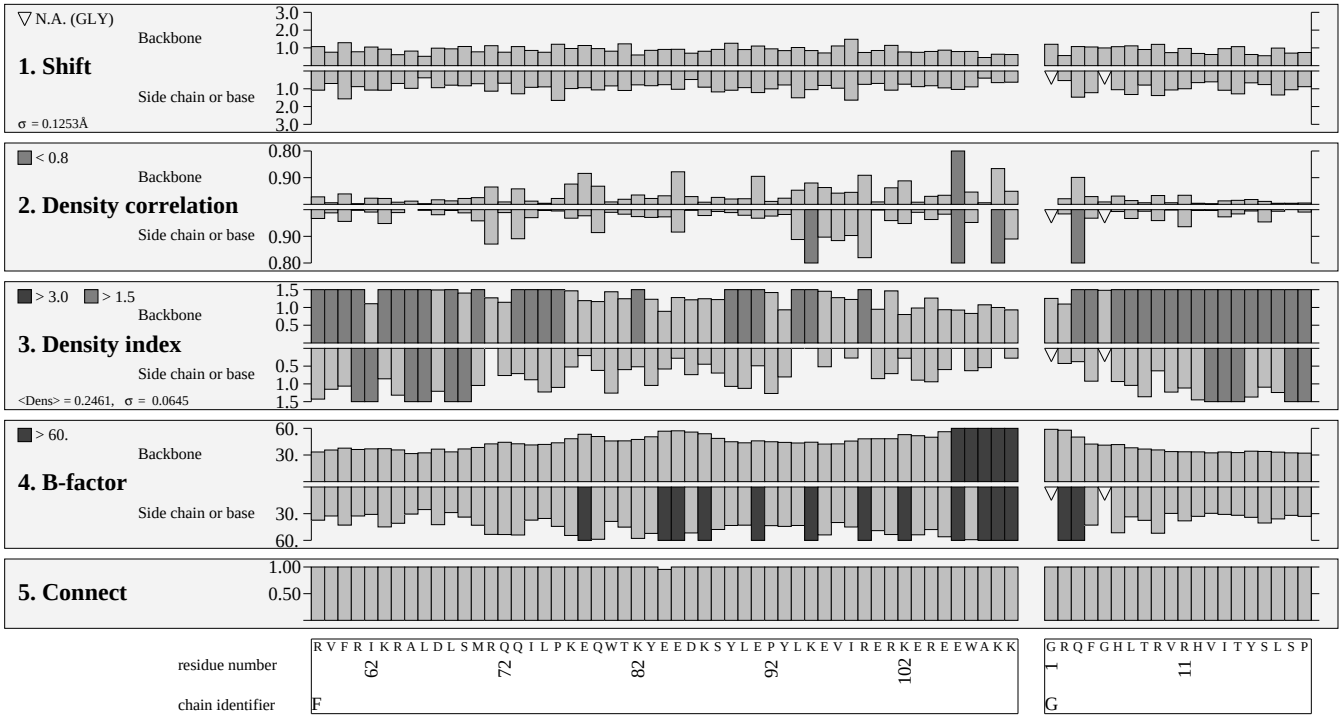
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (12)



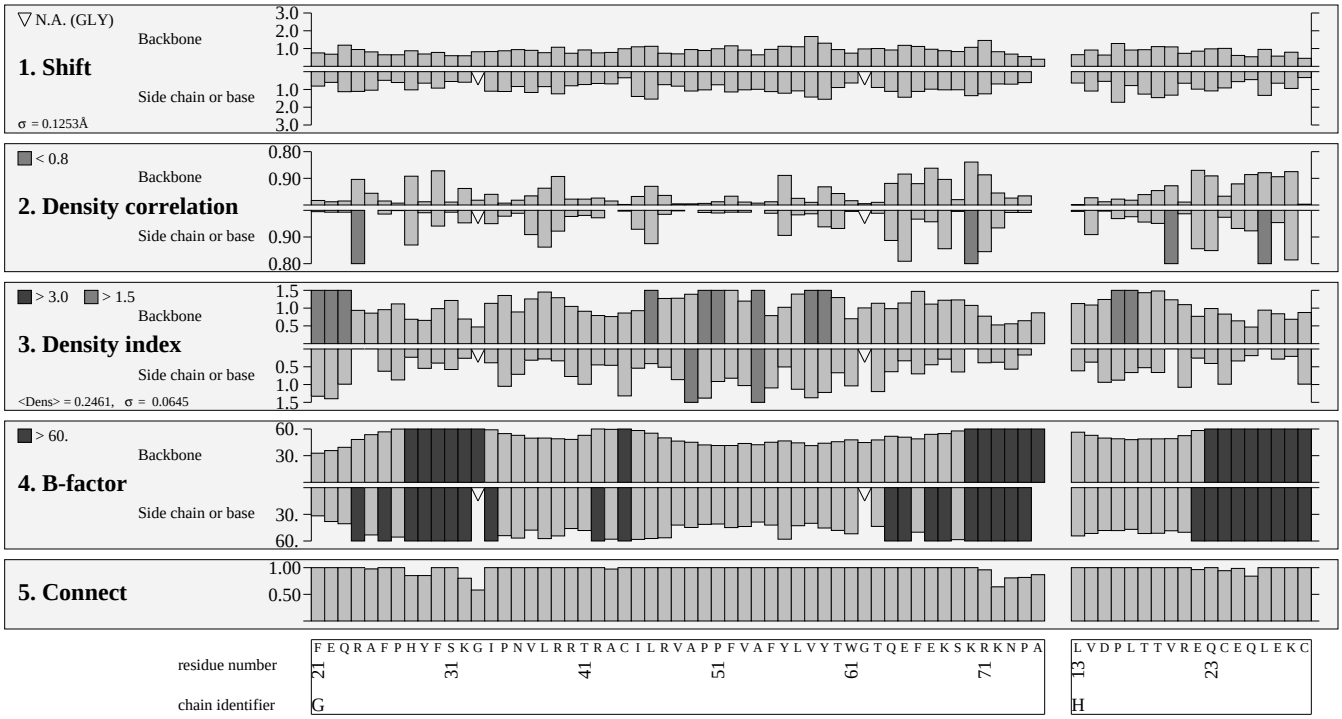


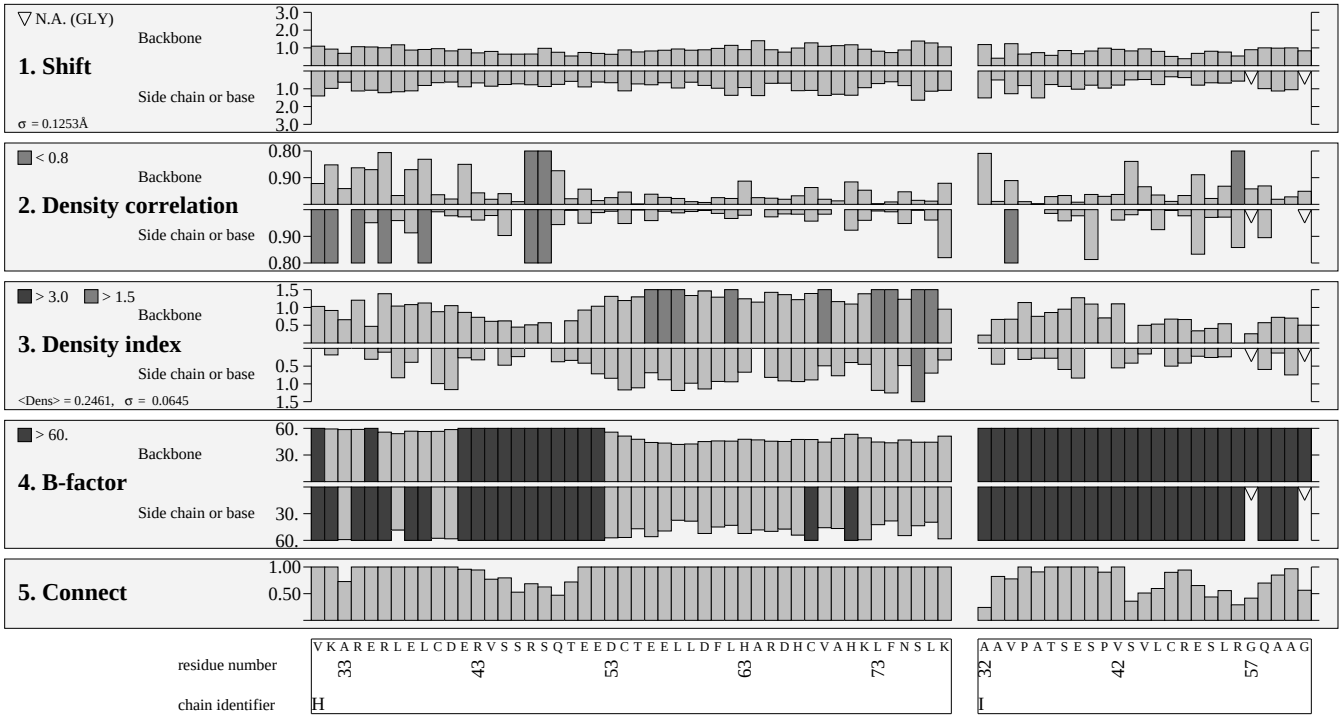
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (13)

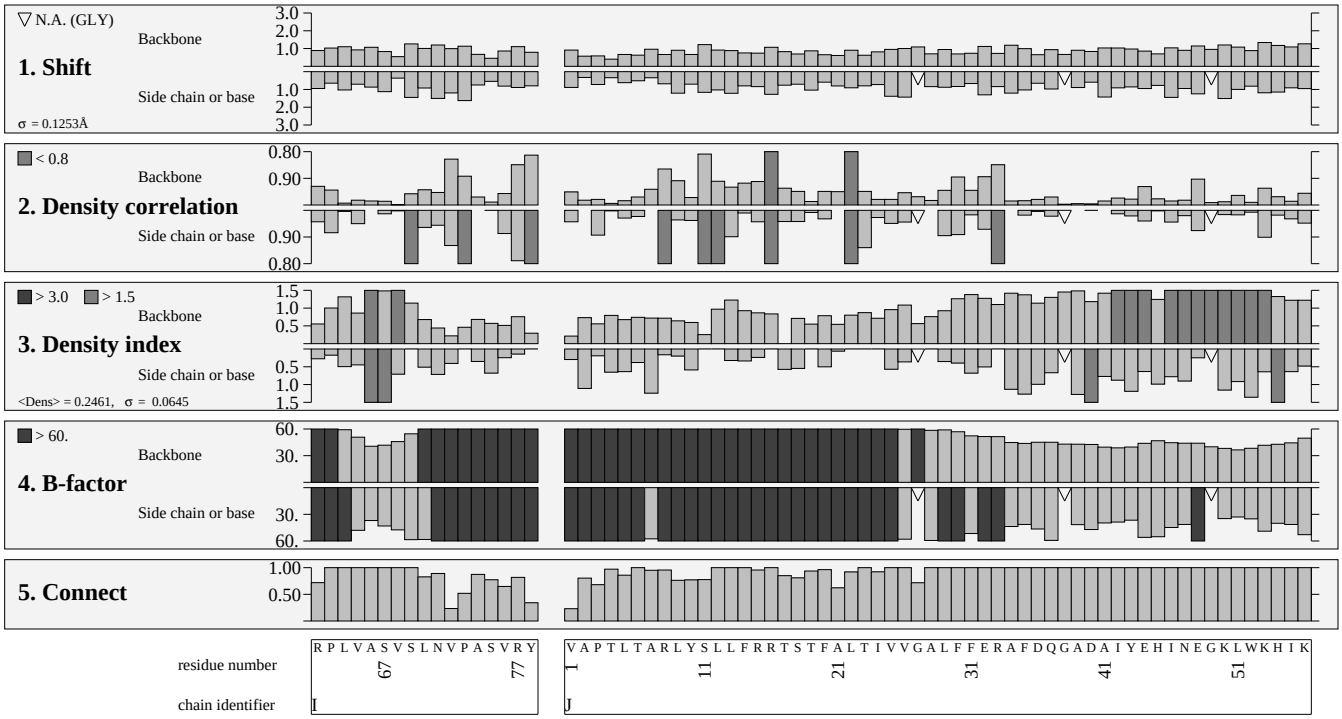


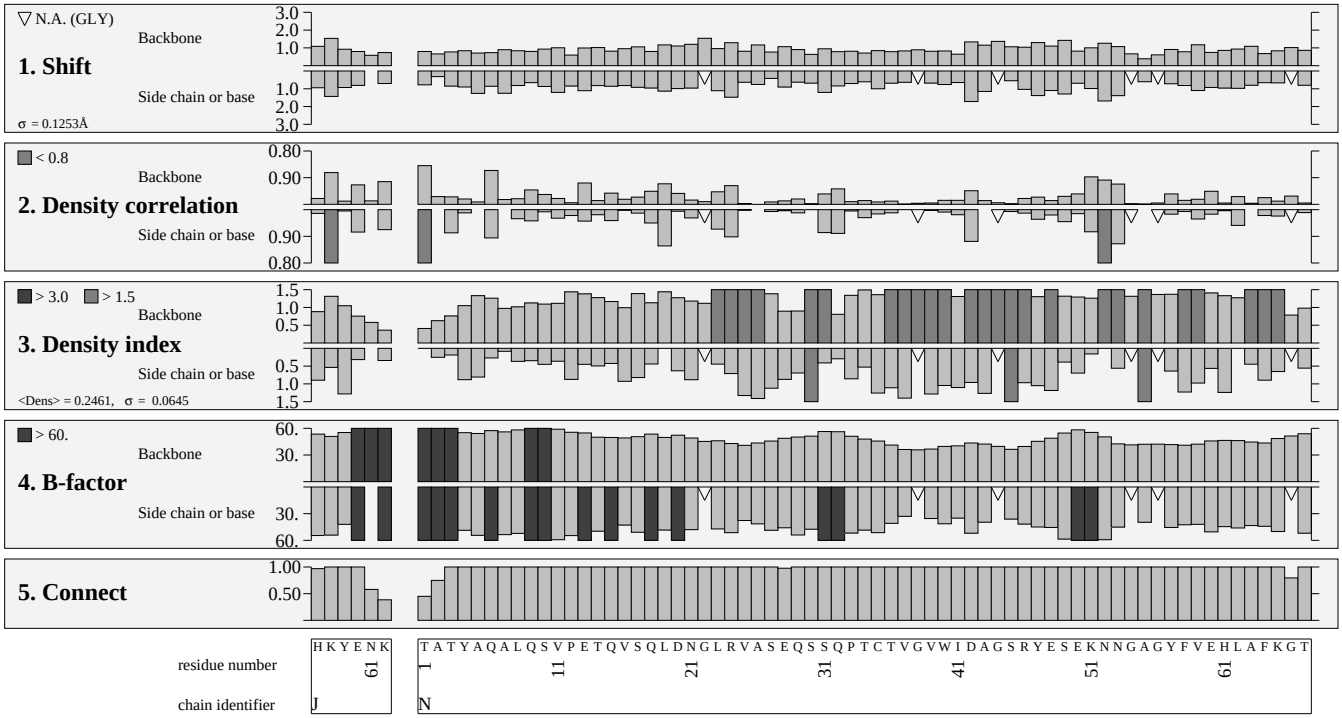


Structure Factor Check

1PP9

Local estimation (14)



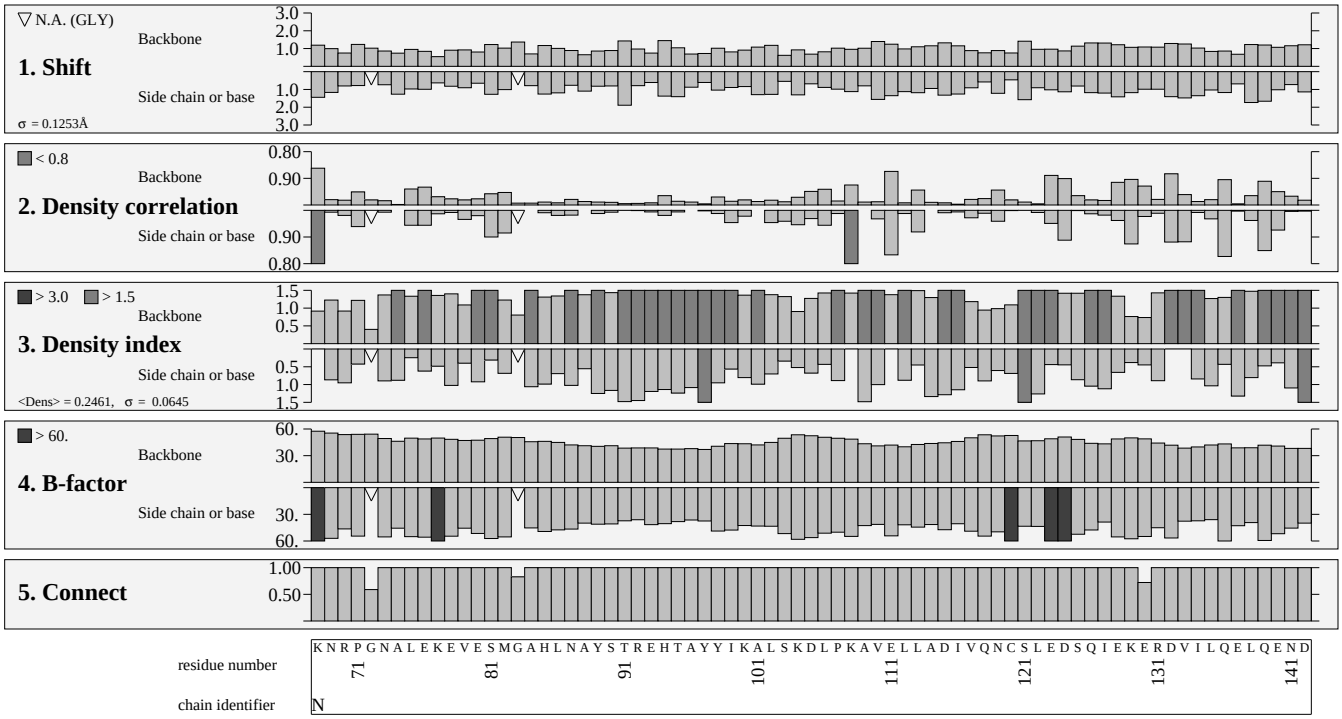


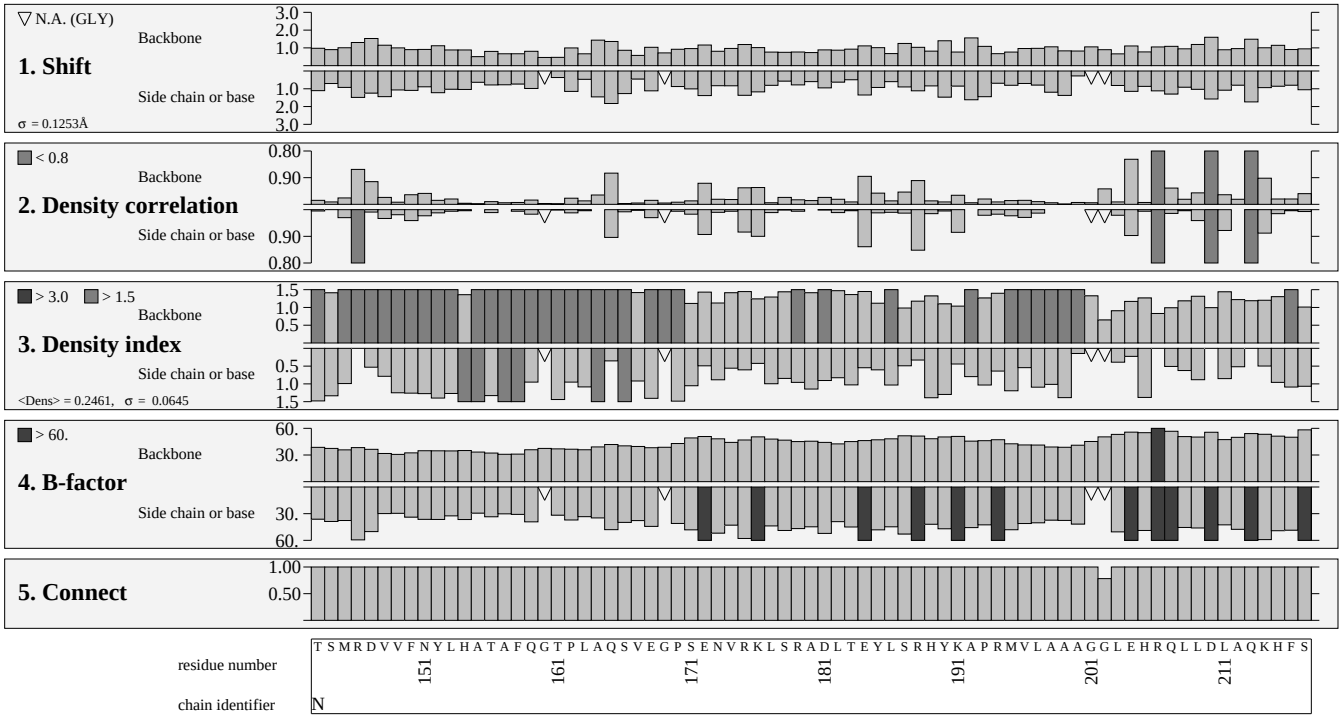
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (15)



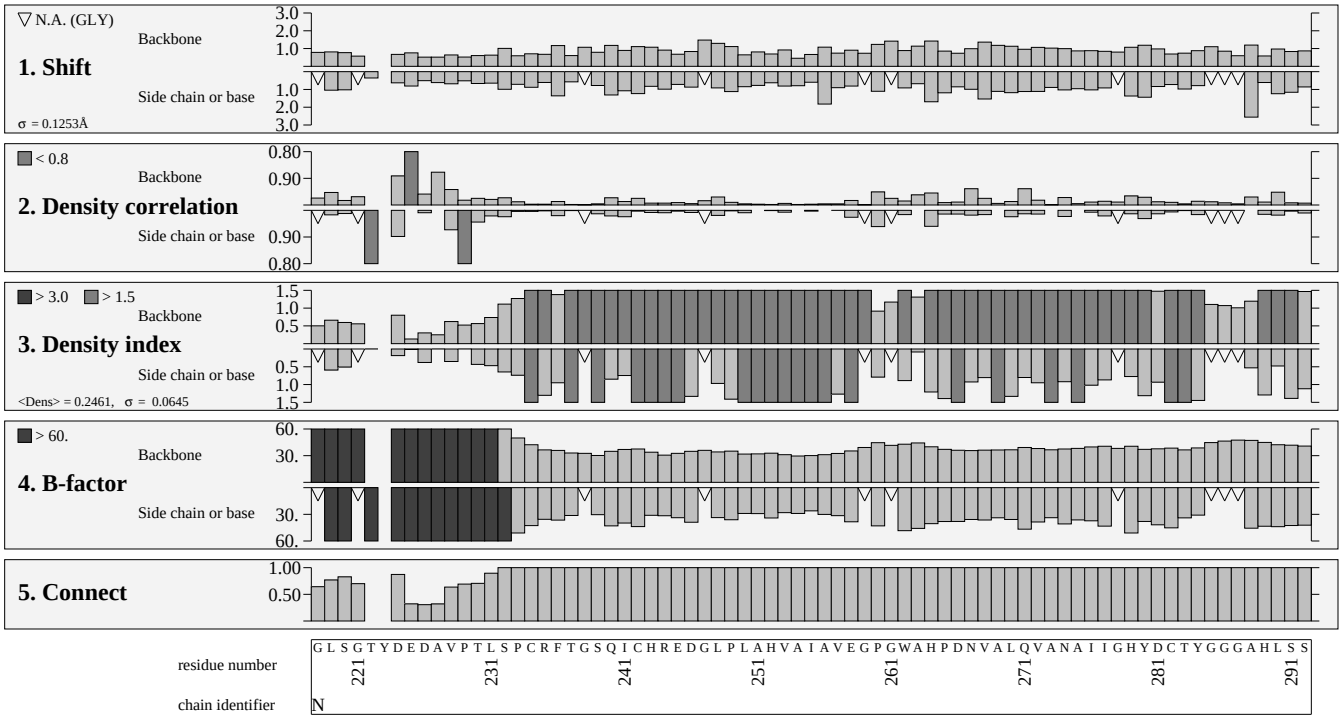


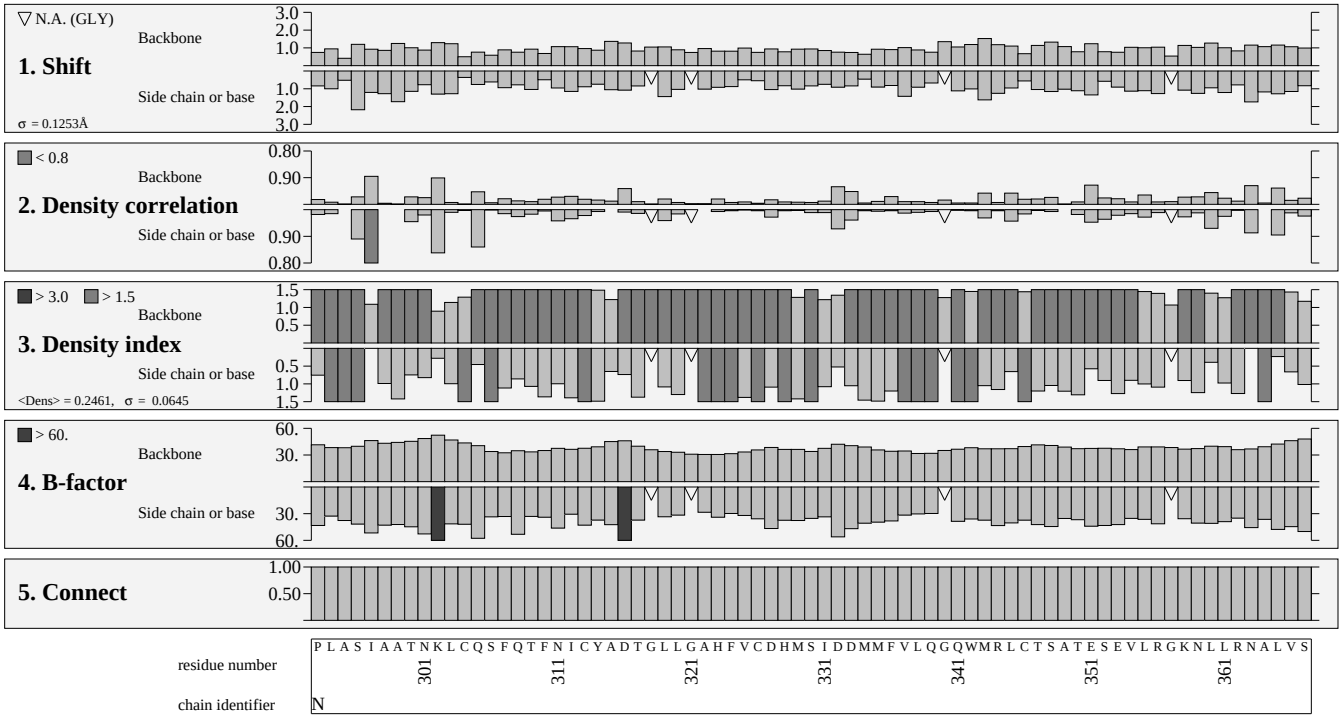
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (16)



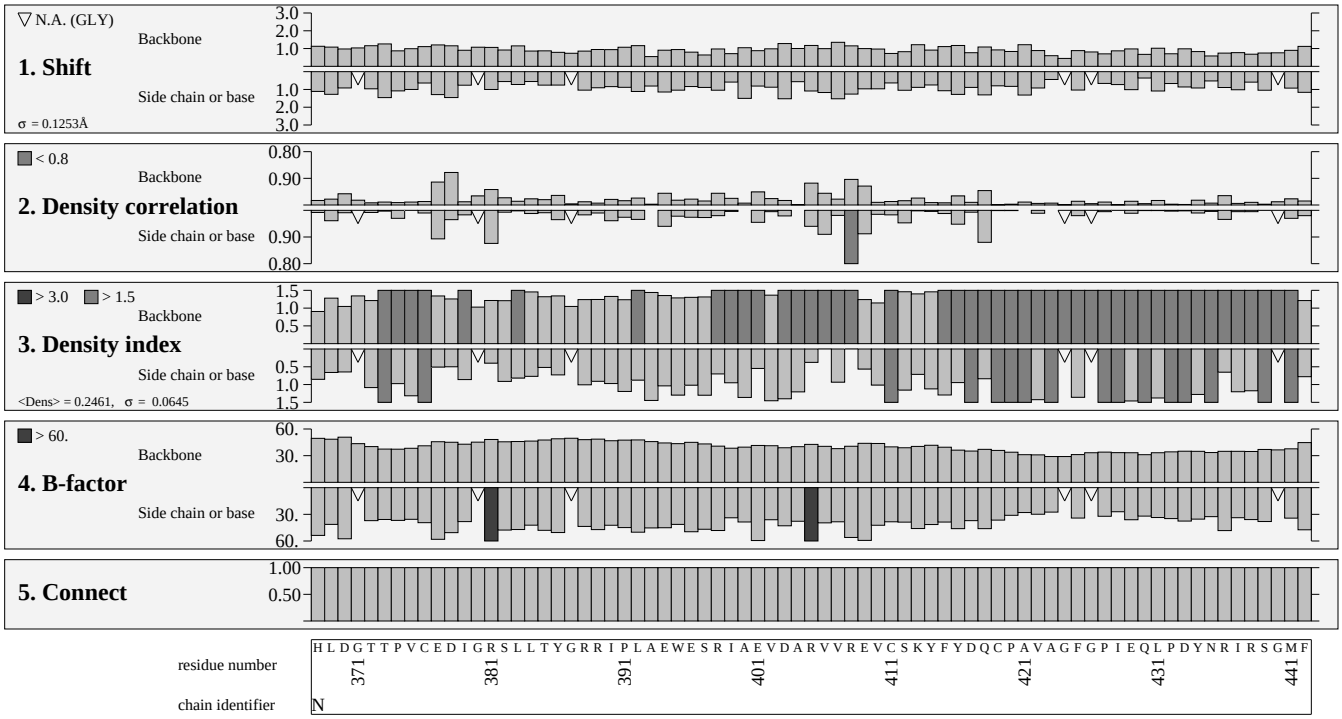


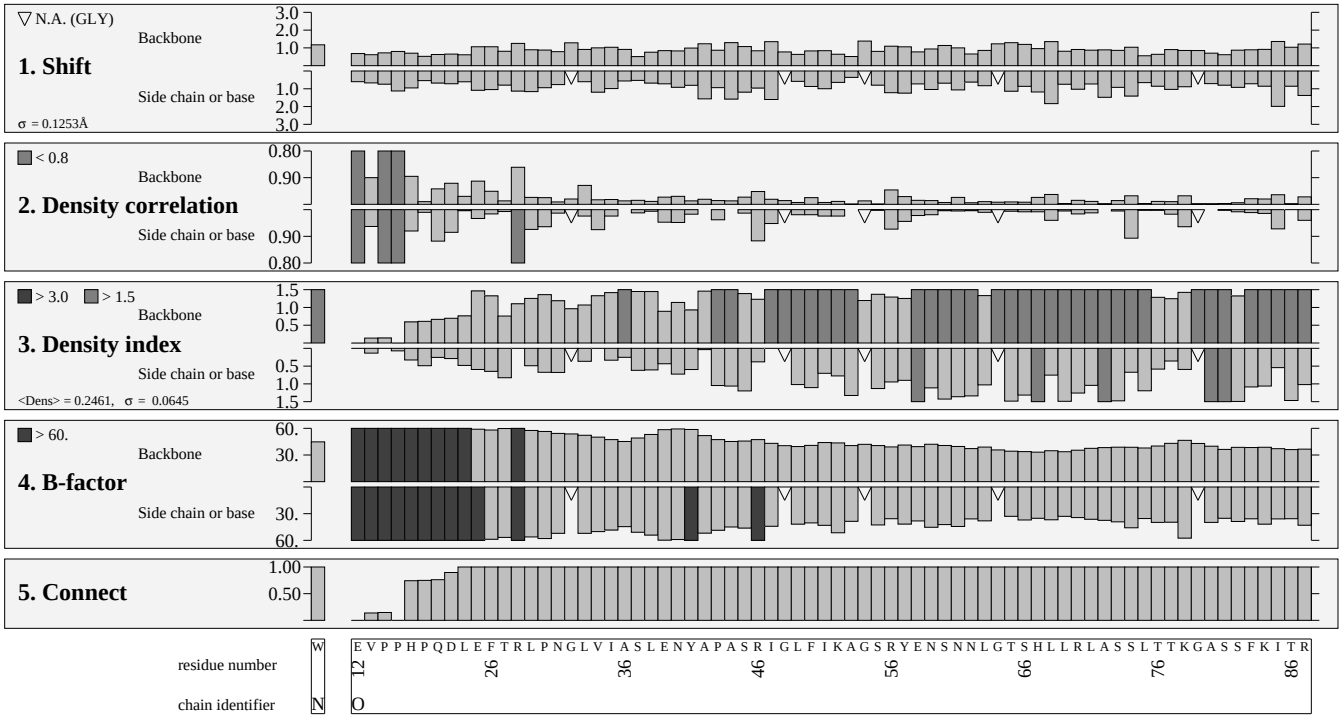
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (17)



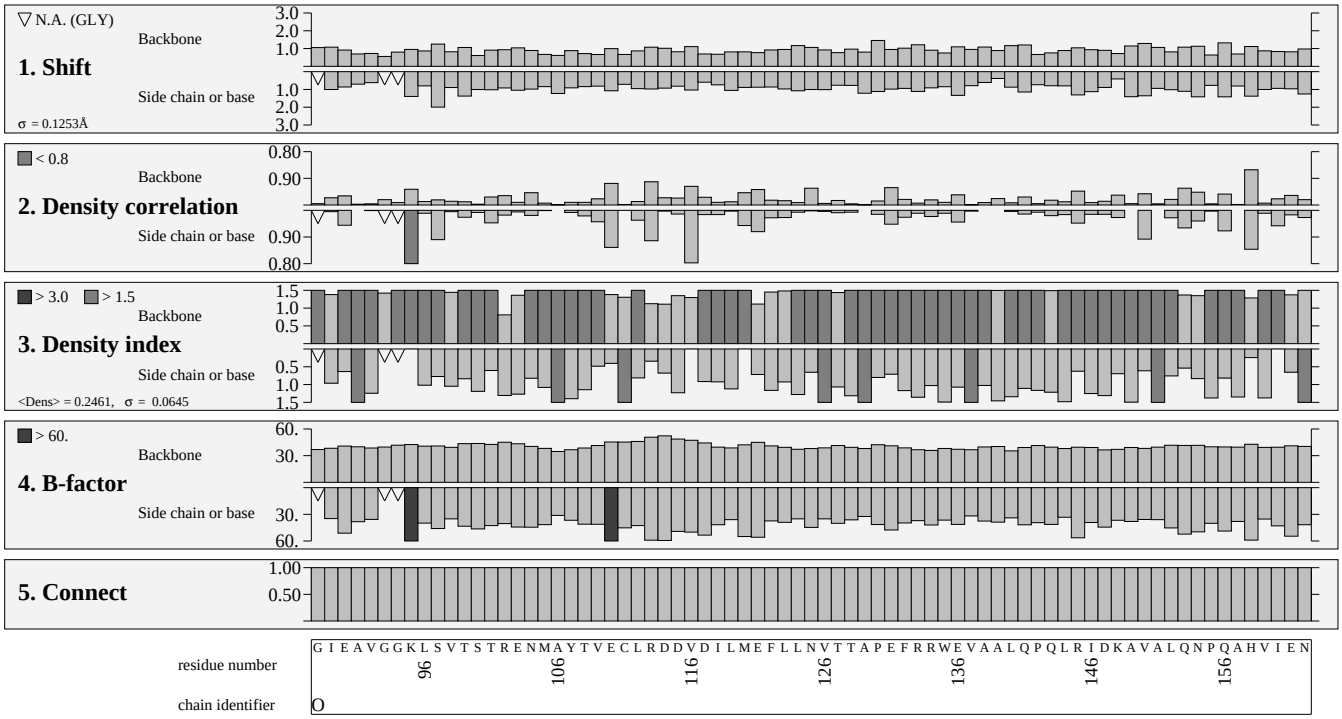


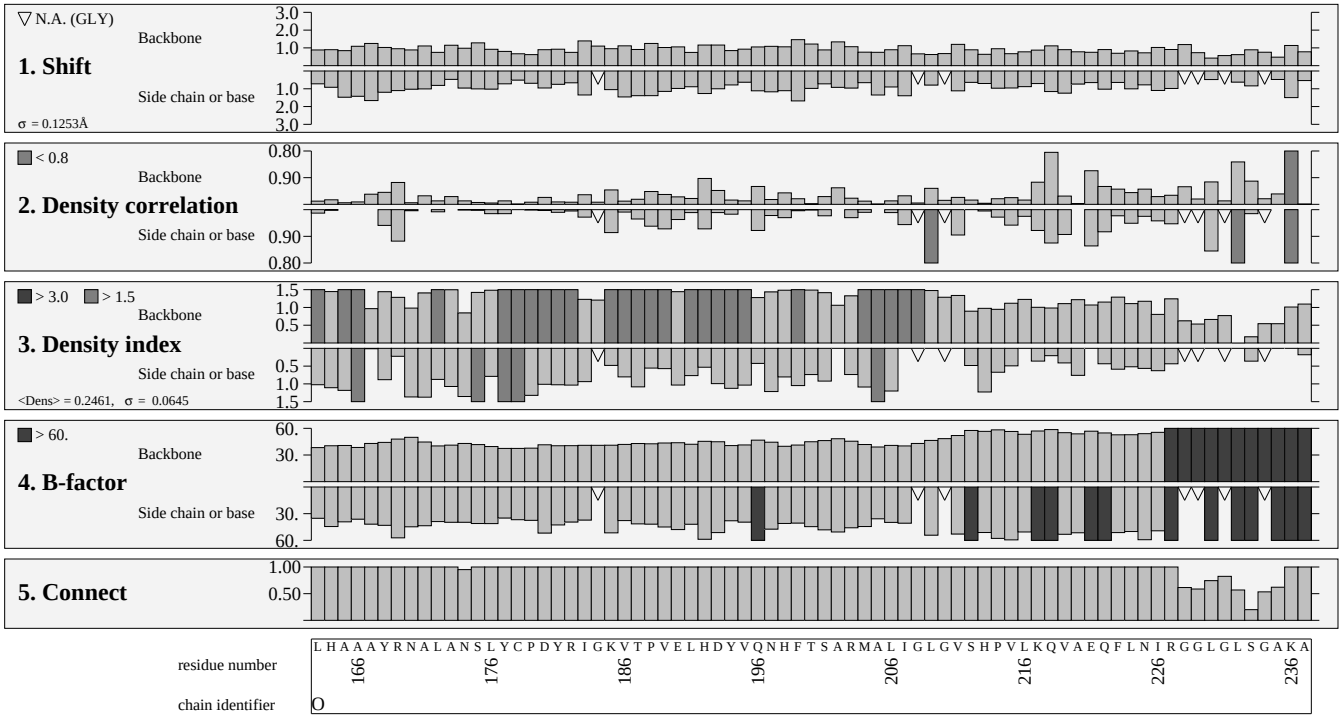
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (18)



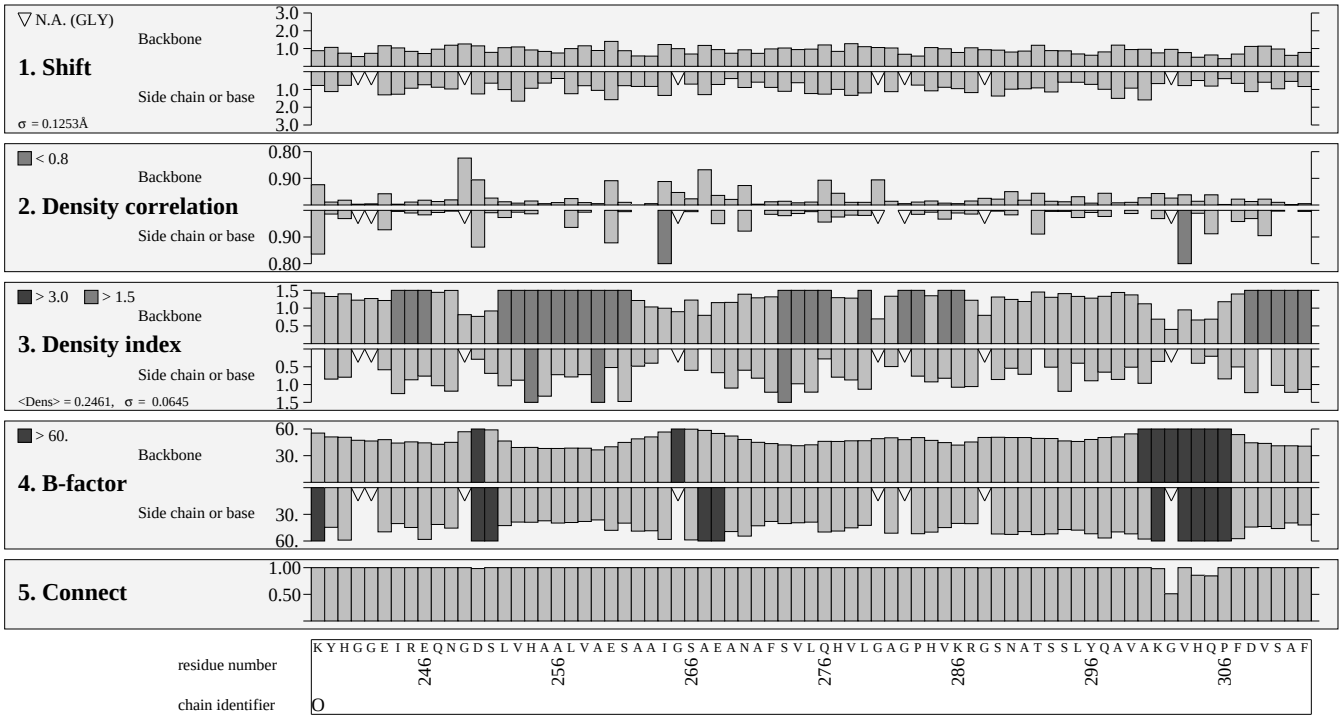


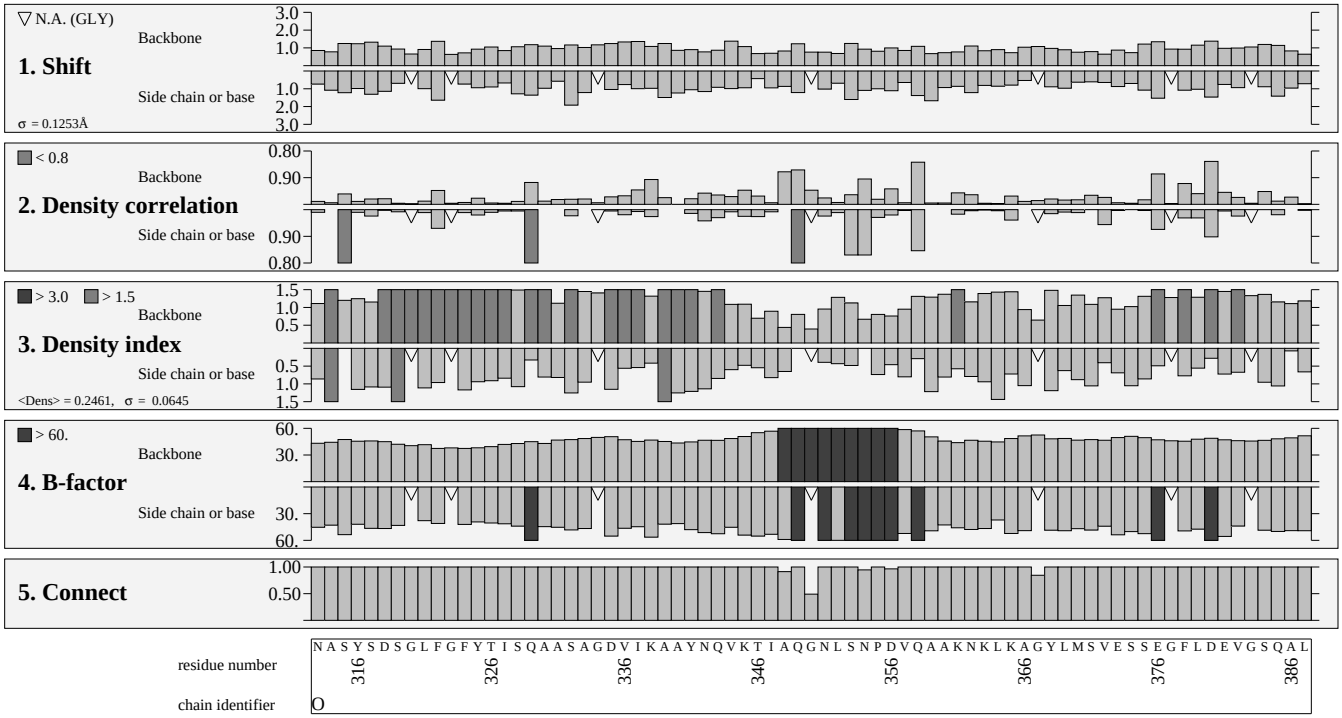
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (19)



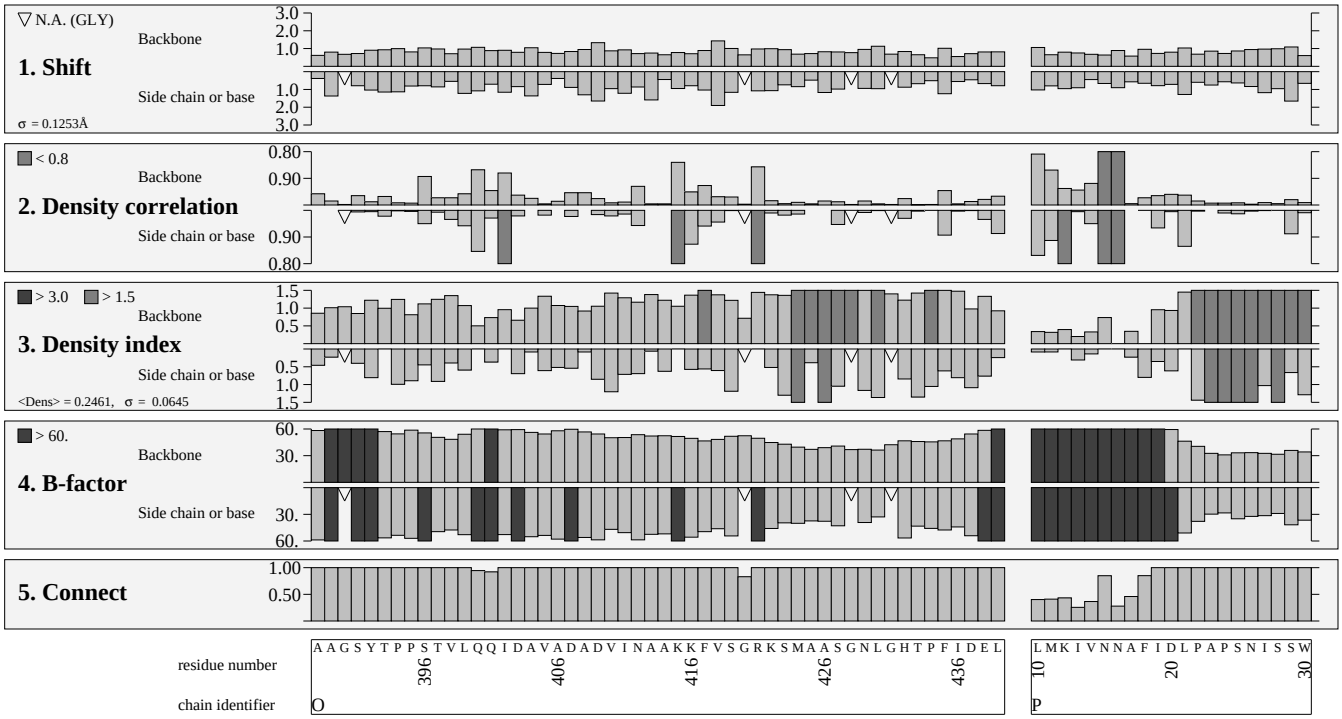


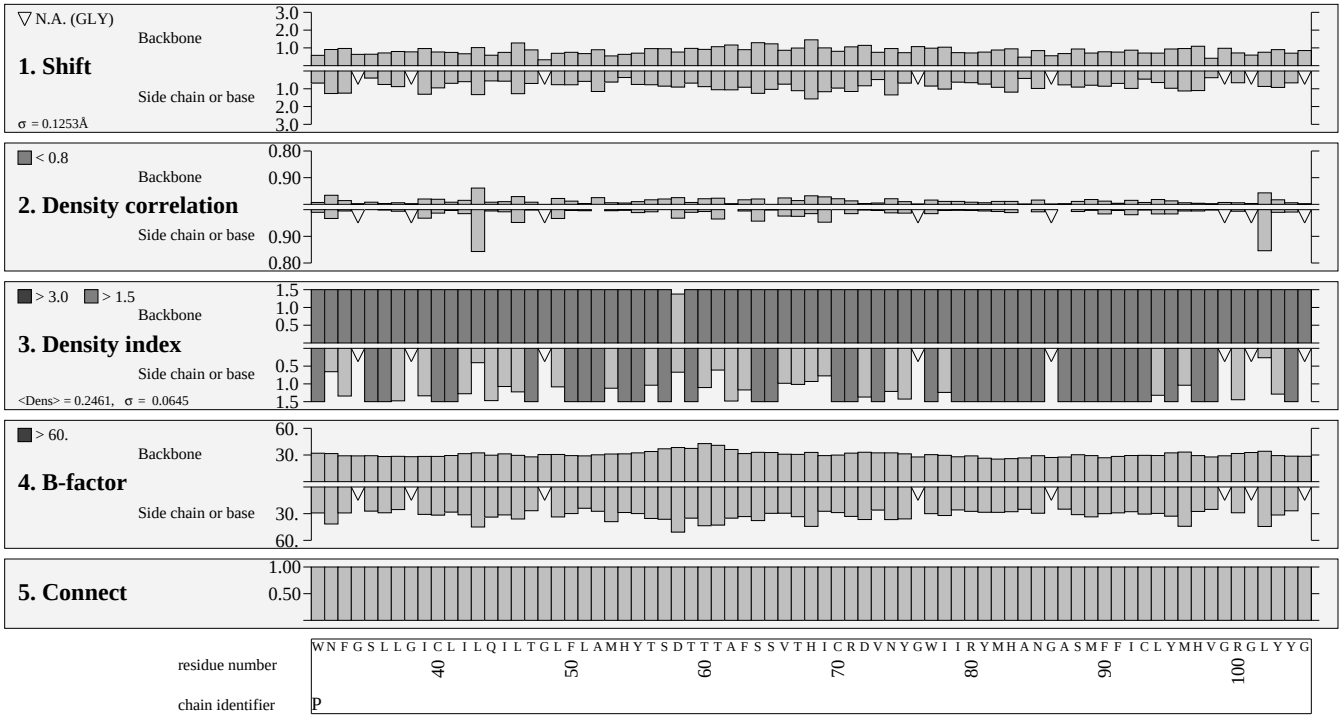
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (20)



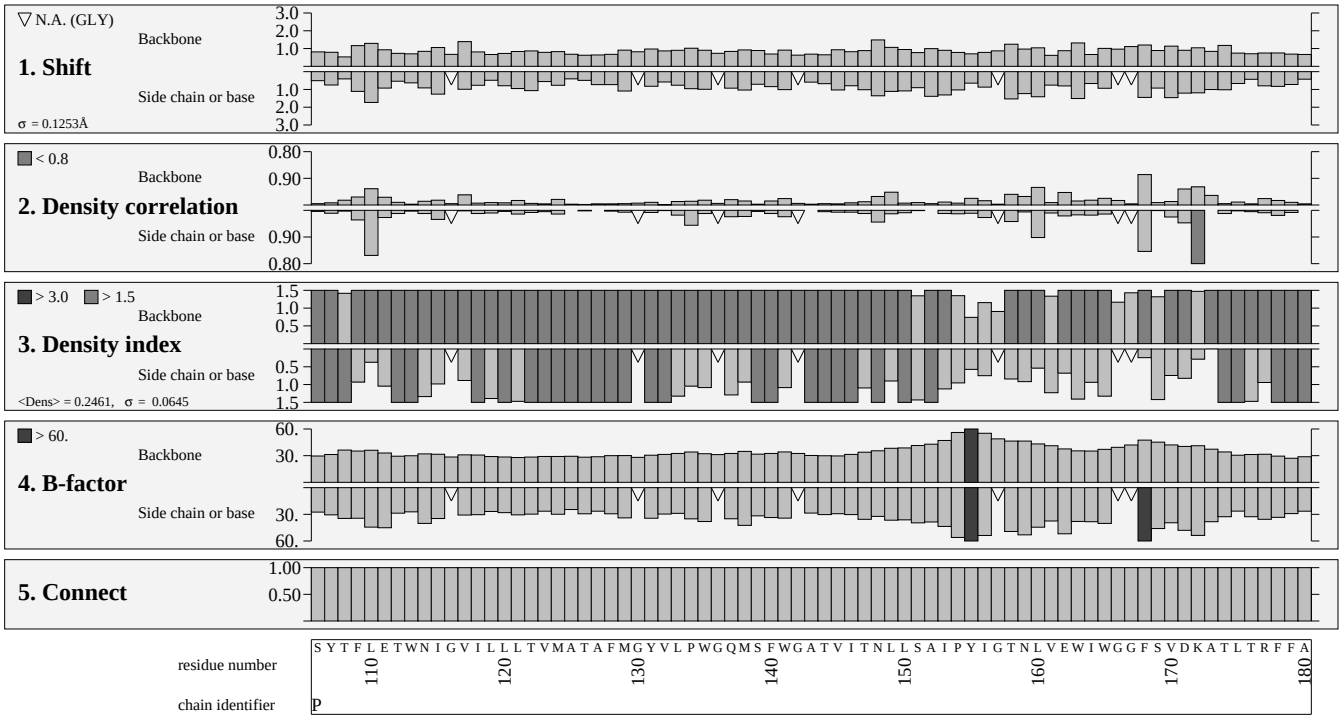


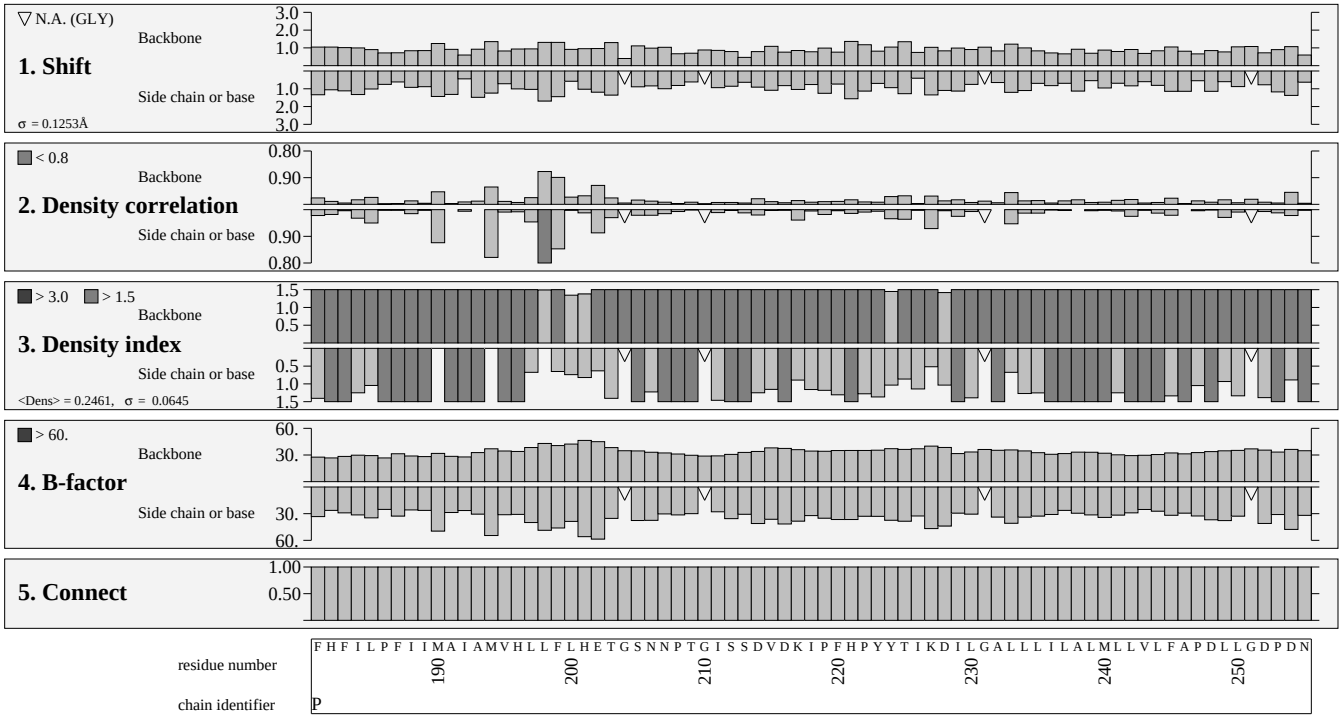
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (21)



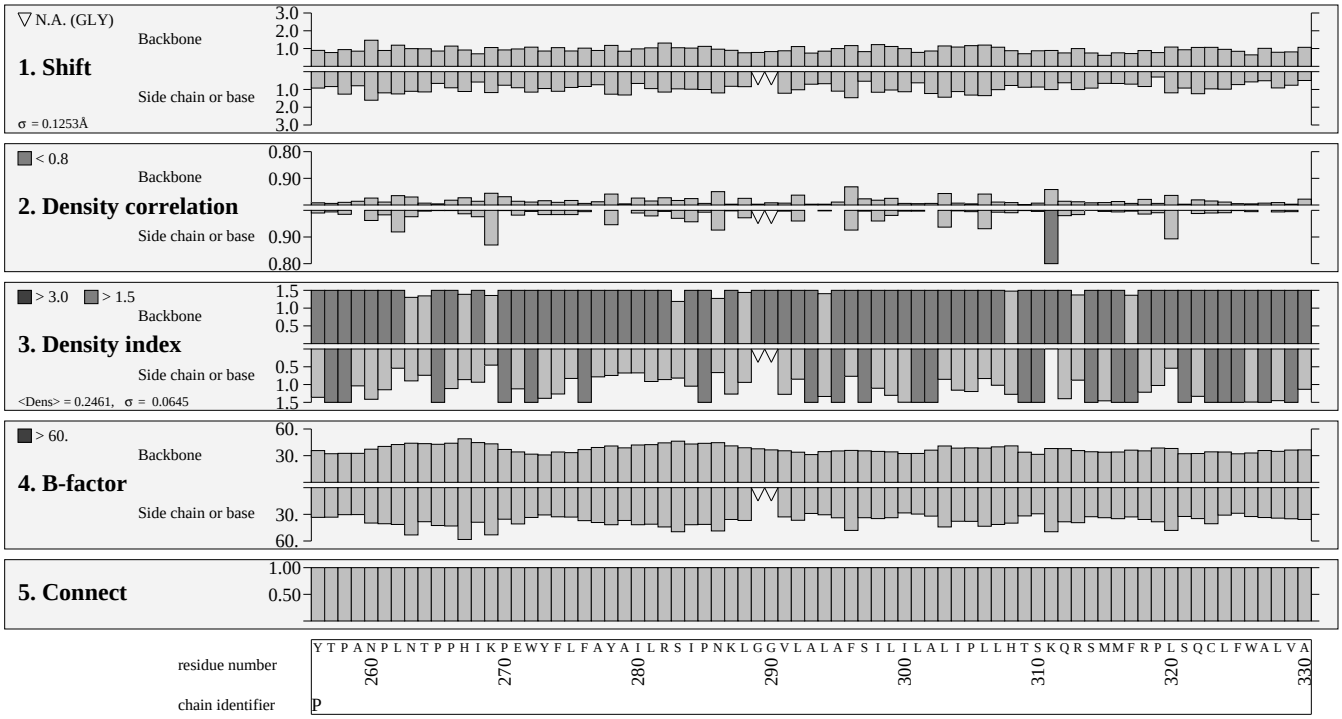


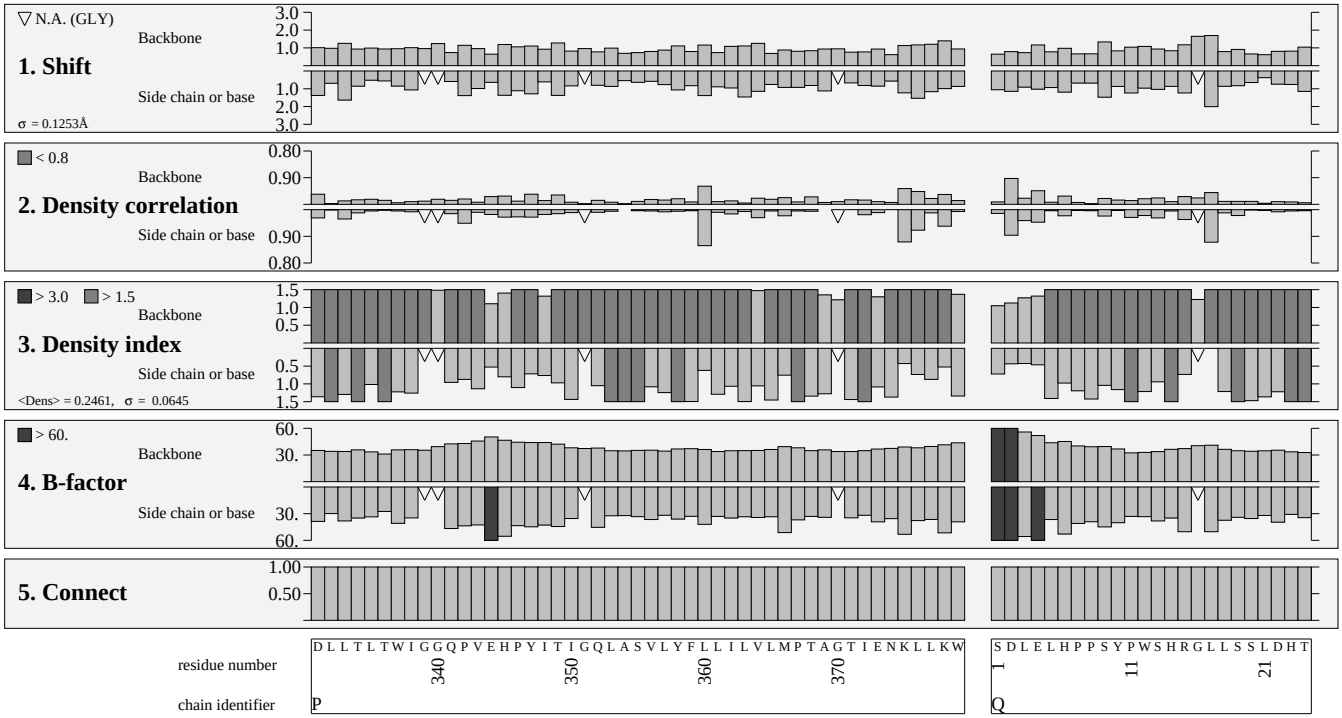
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (22)



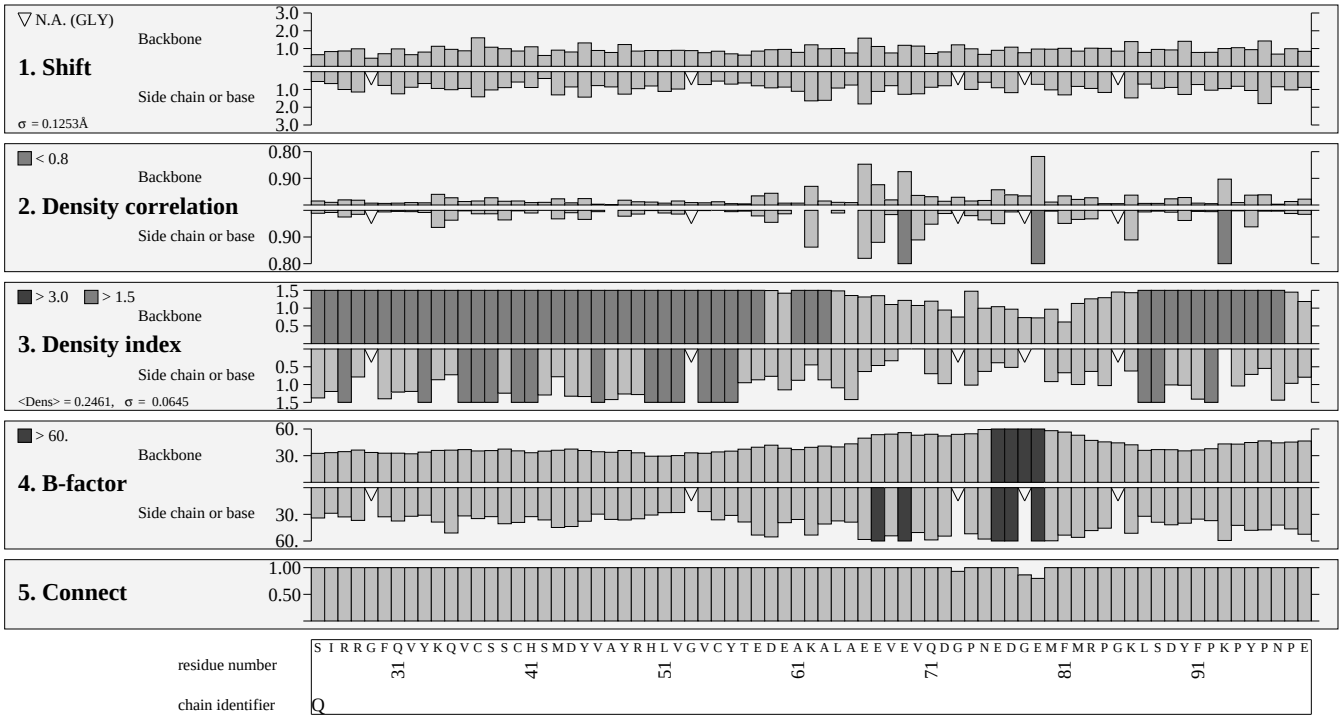


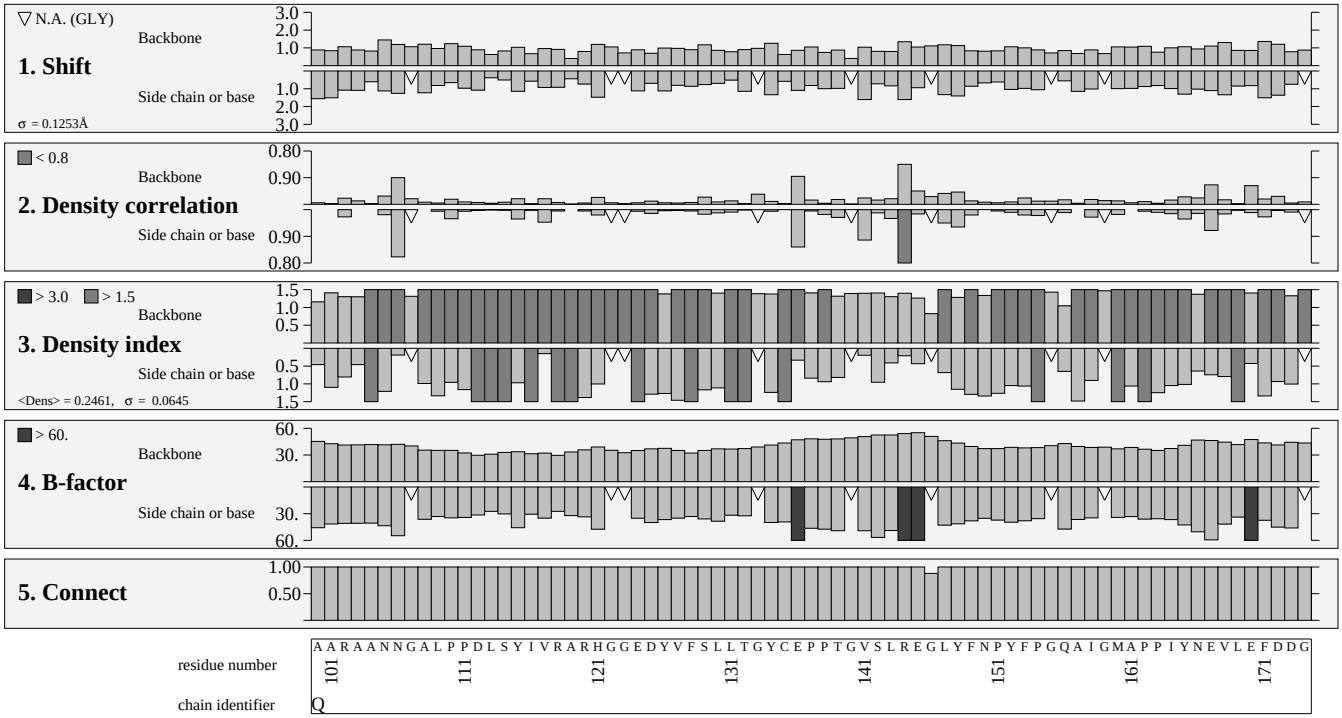
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (23)



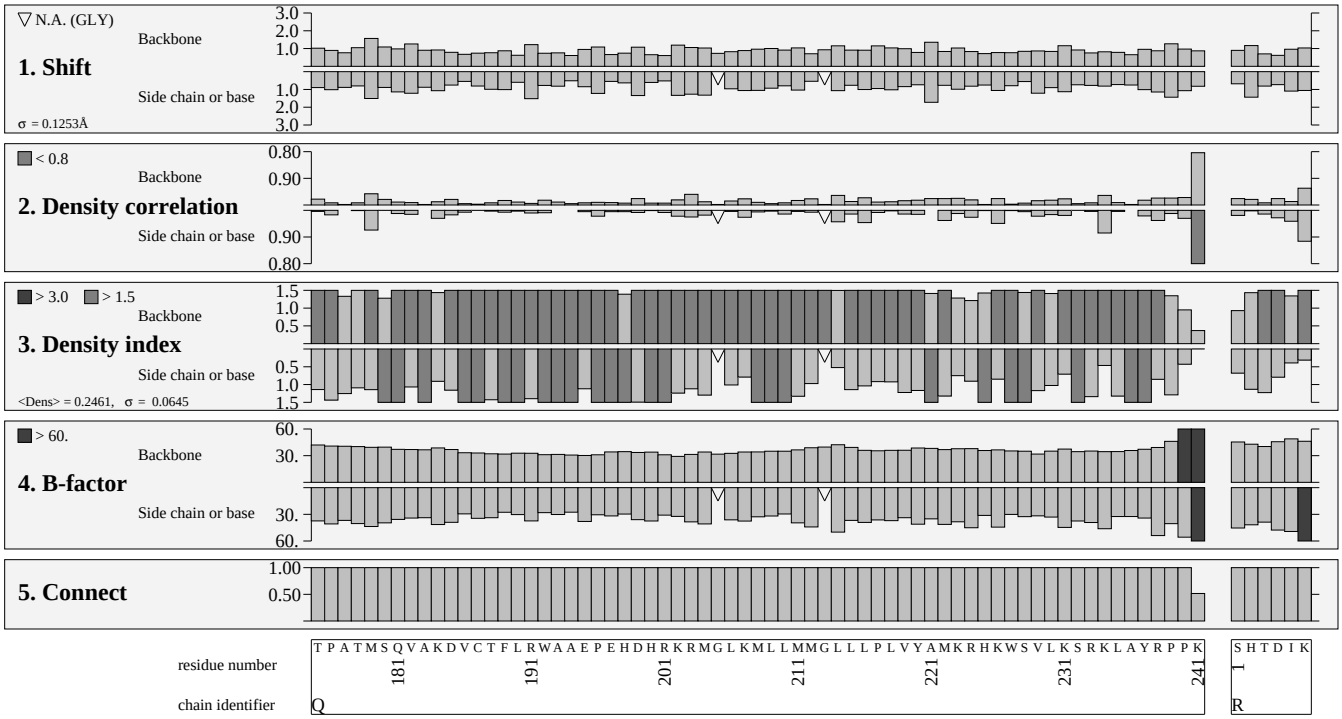


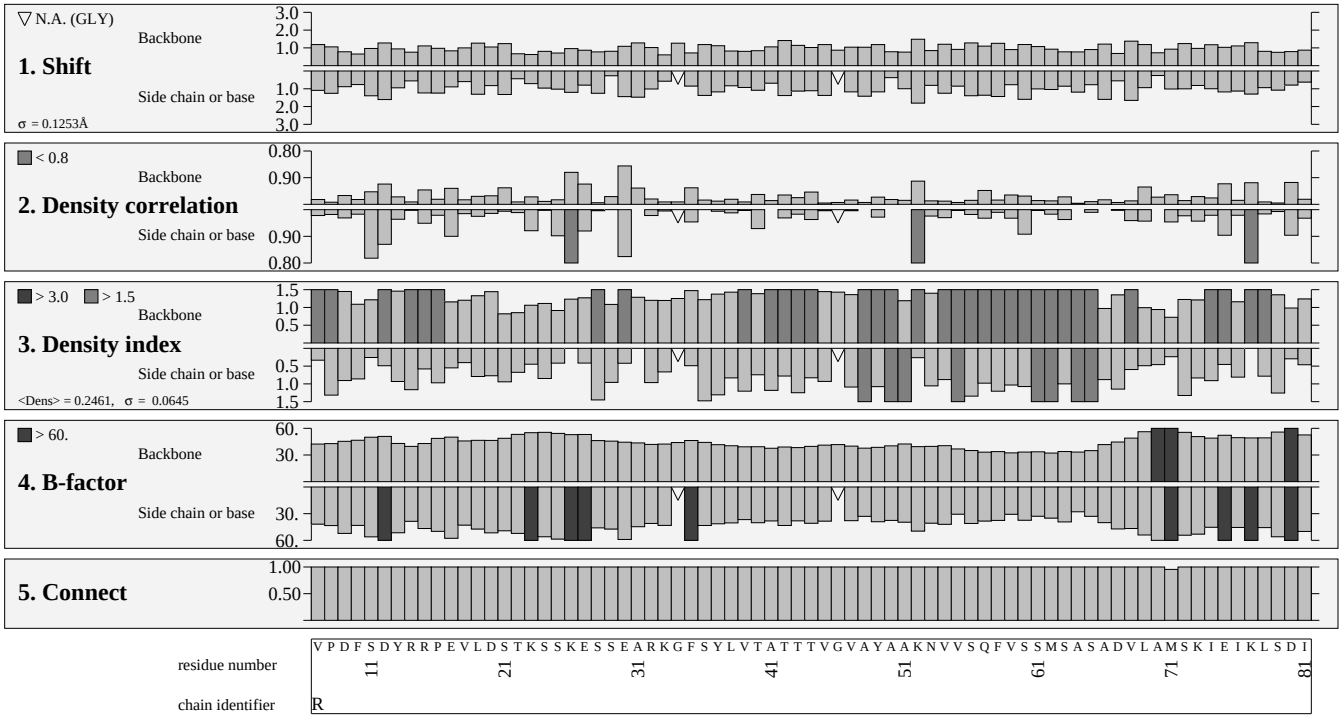
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (24)



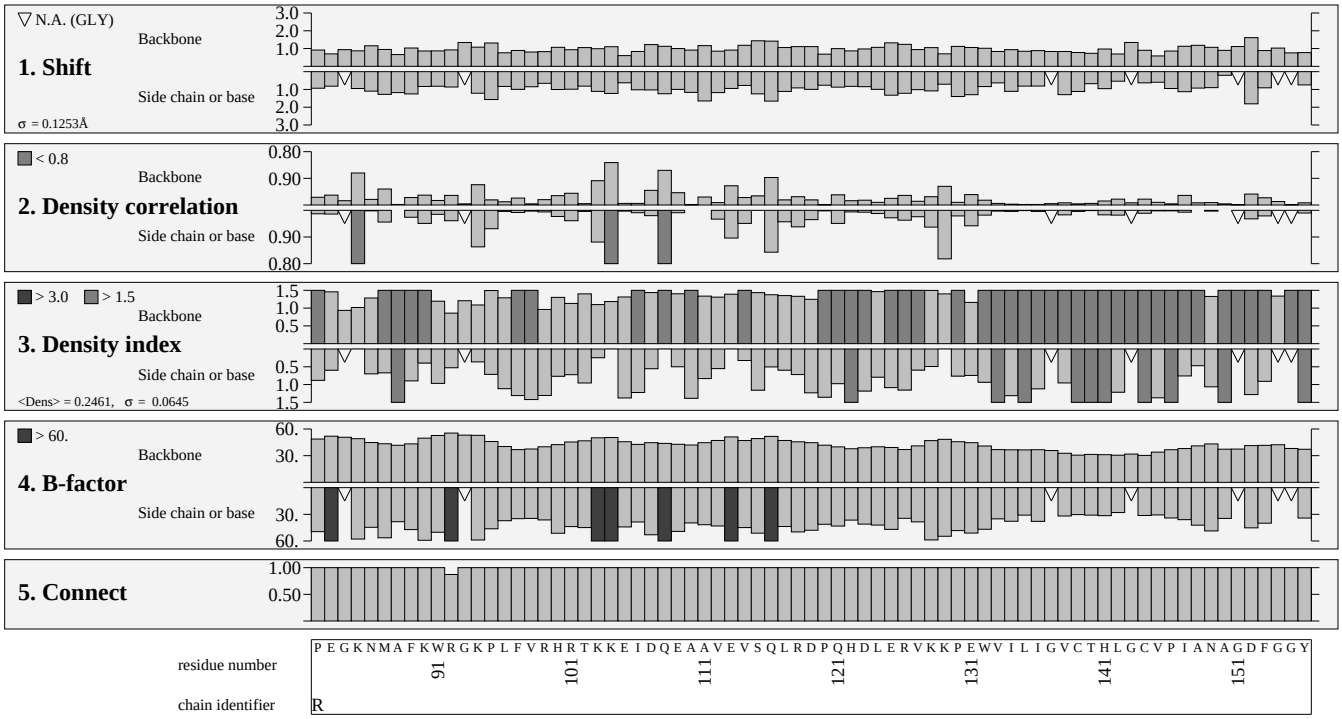


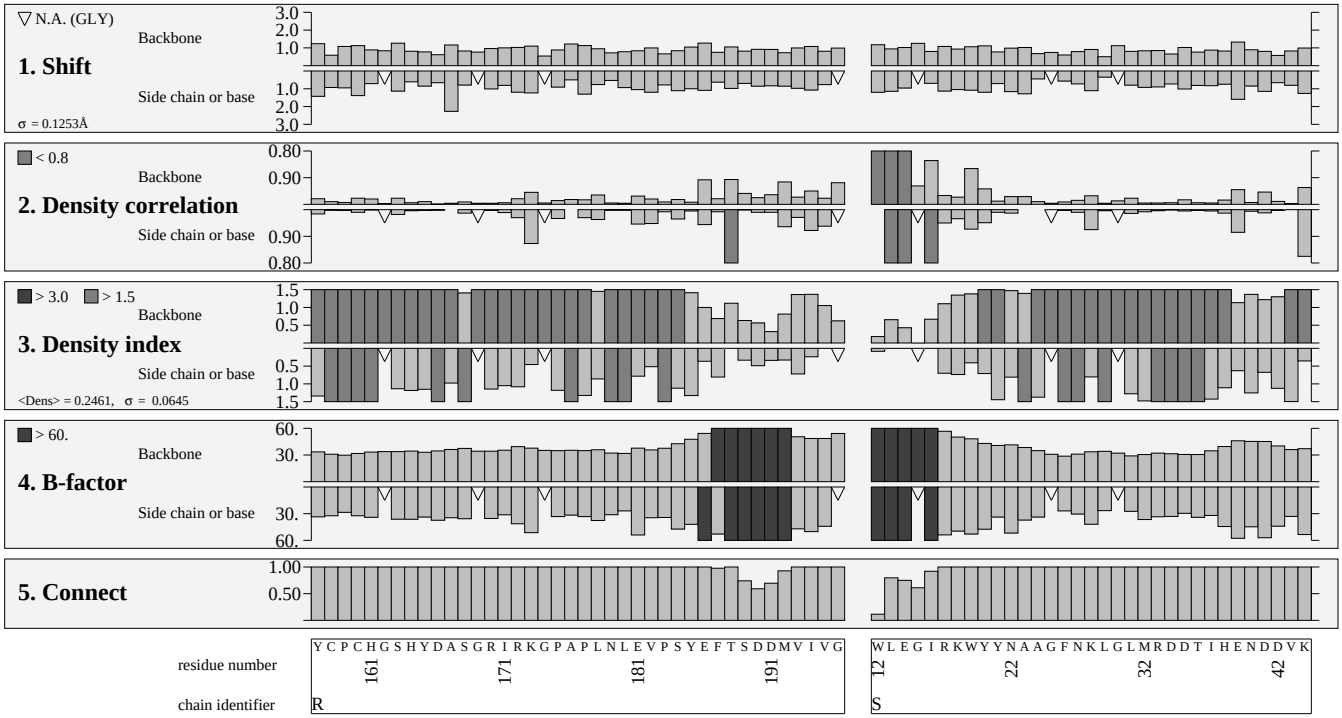
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (25)



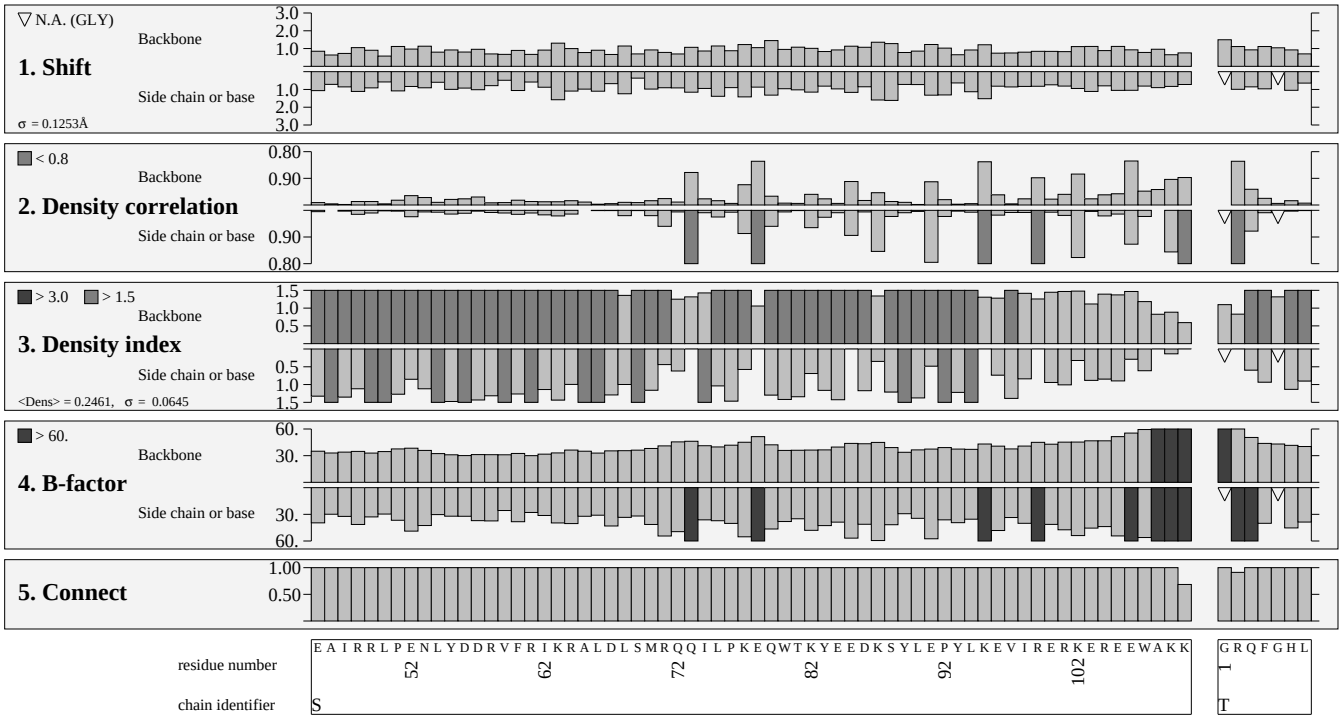


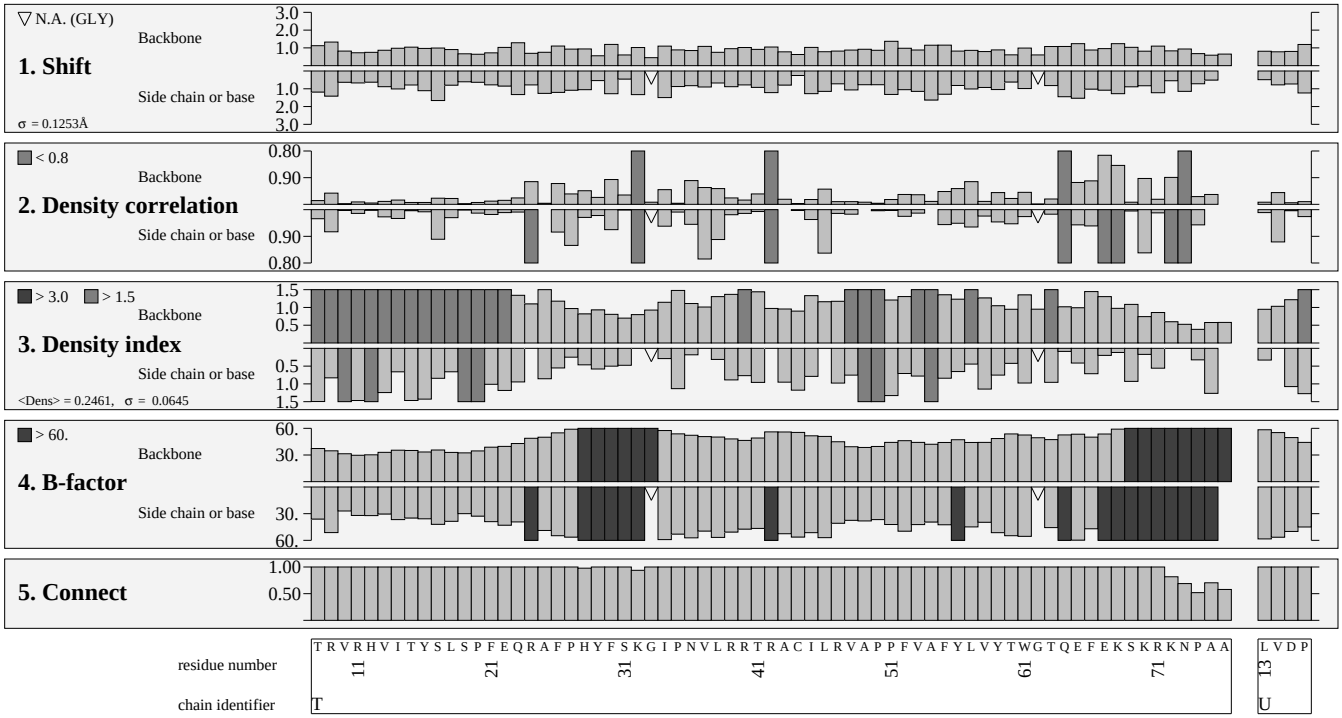
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (26)



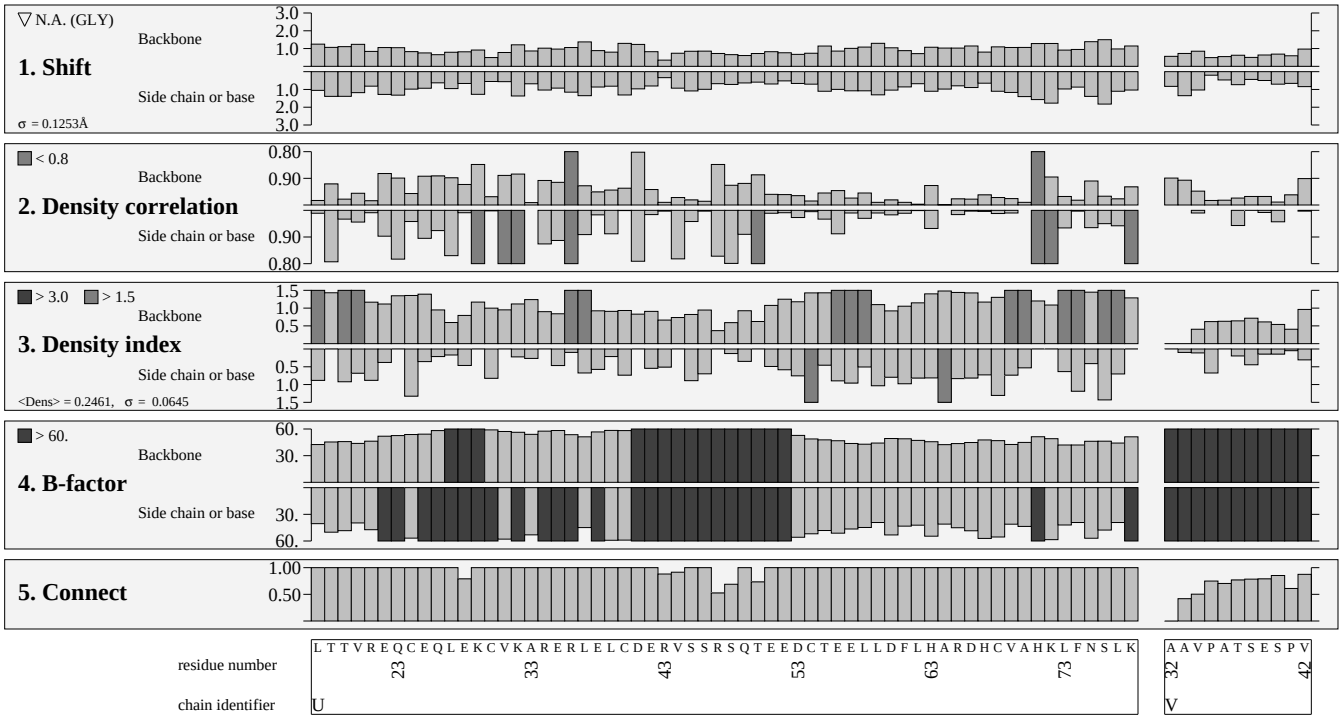


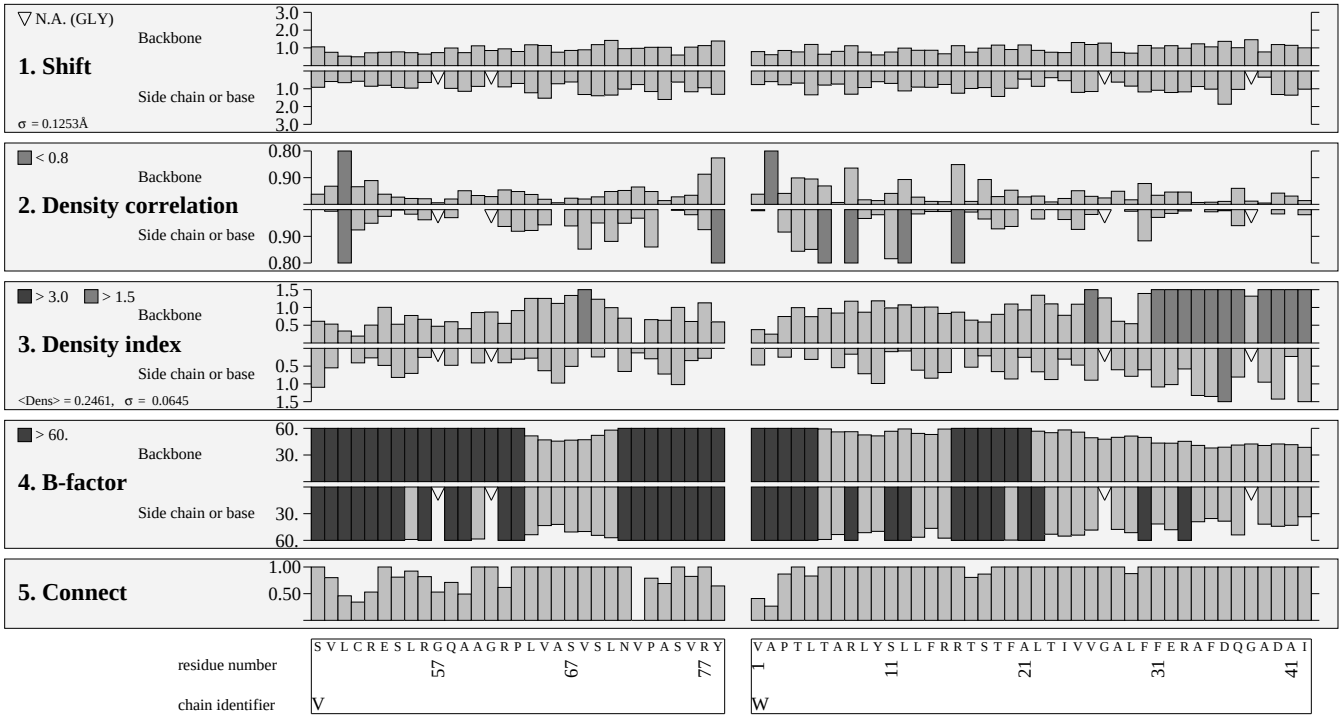
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (27)

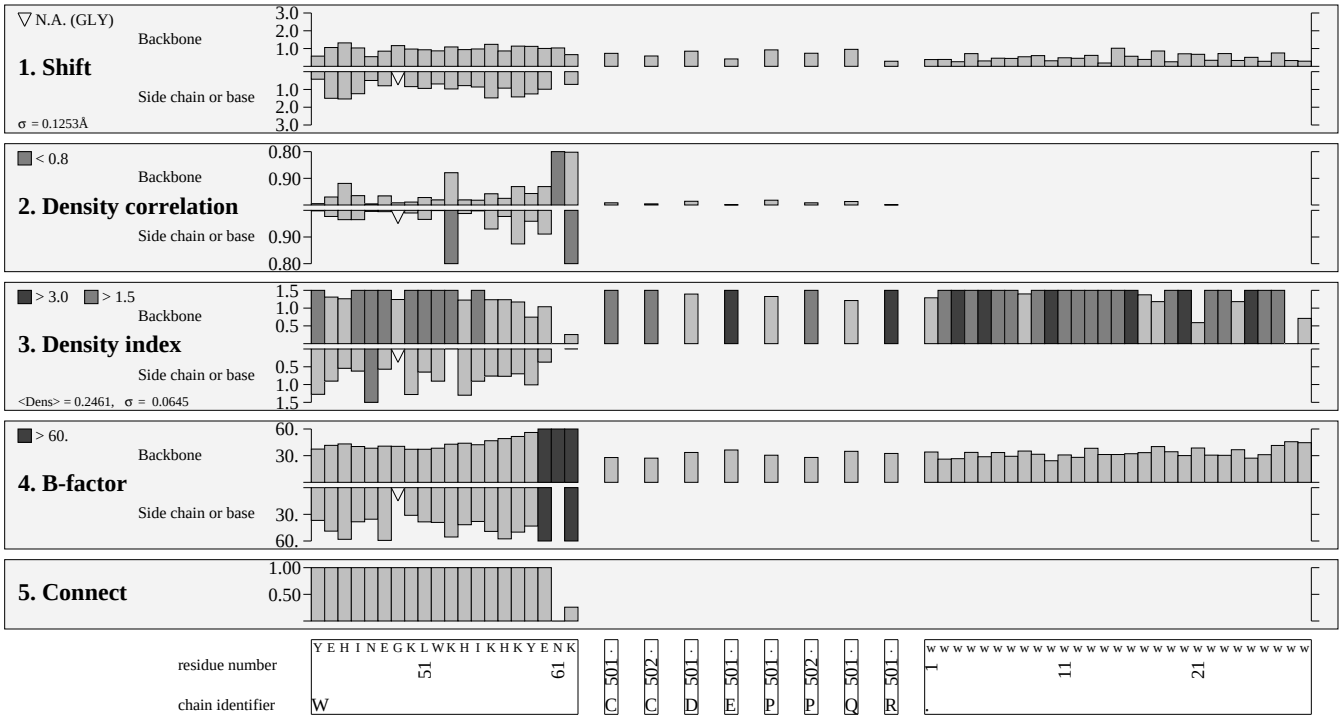


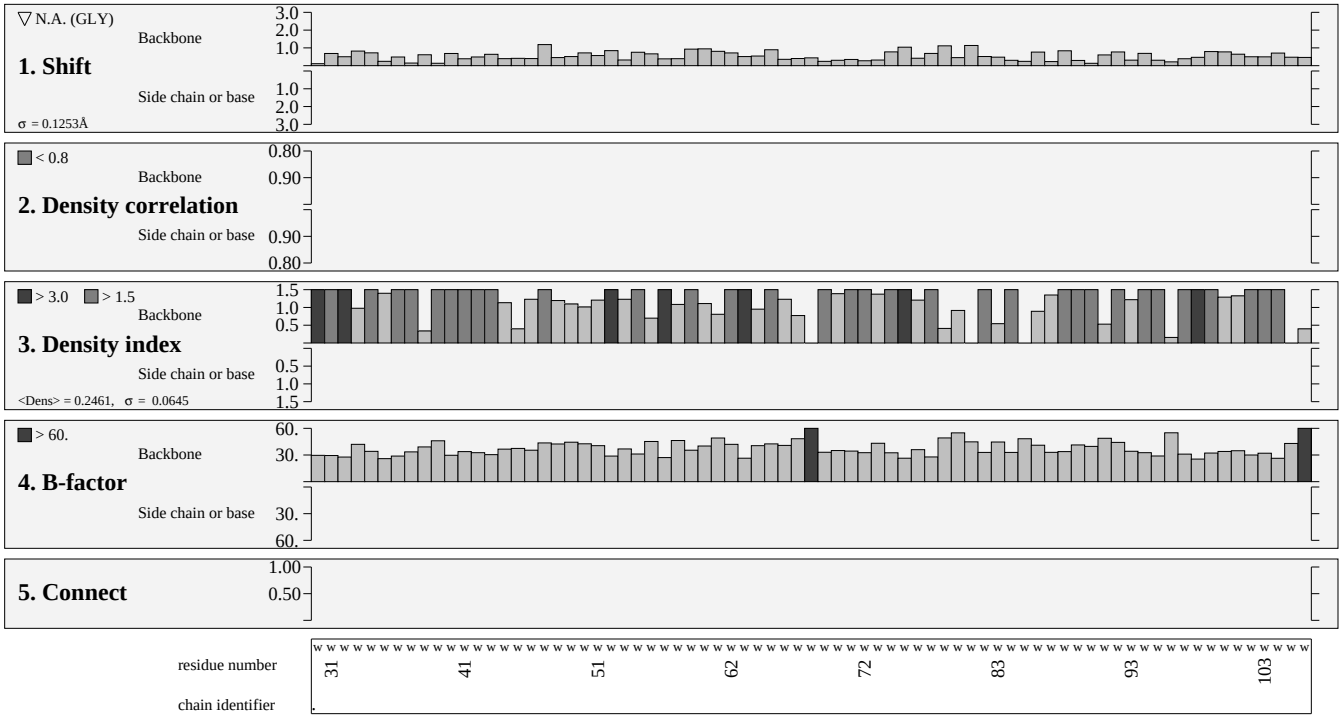


Structure Factor Check

1PP9

Local estimation (28)



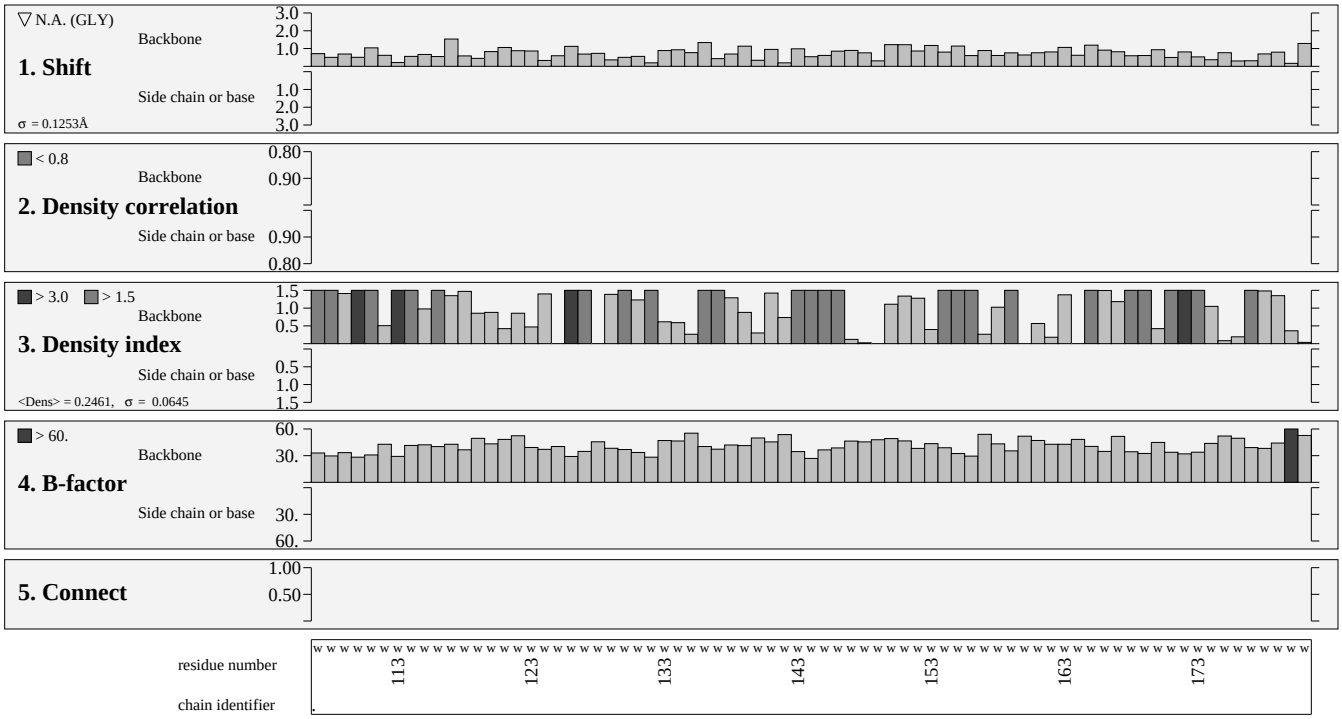


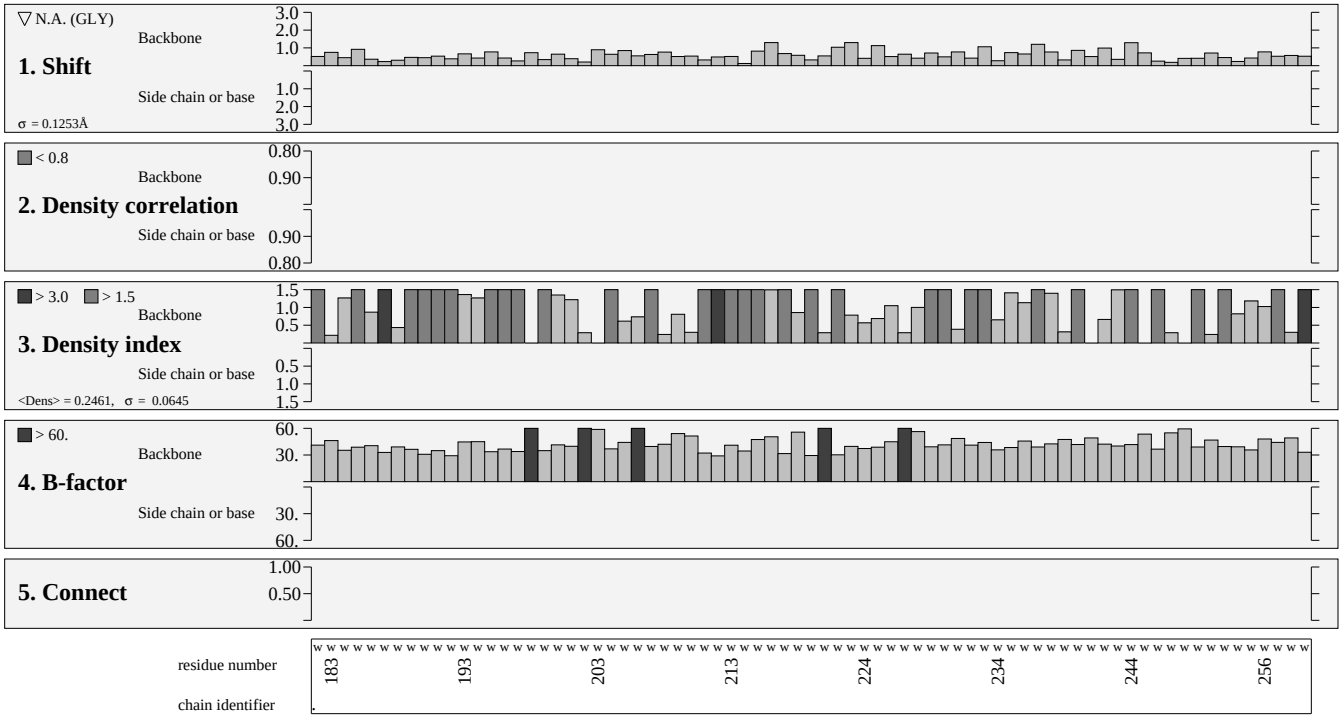
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (29)

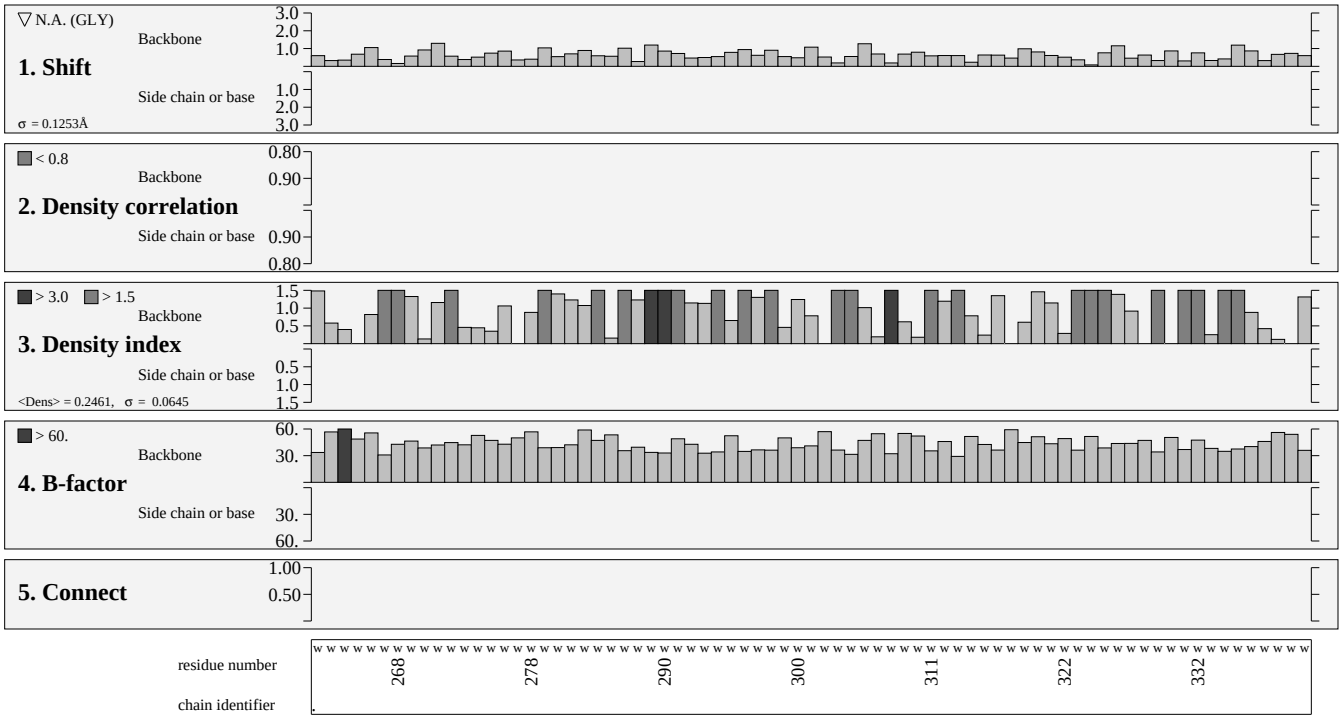


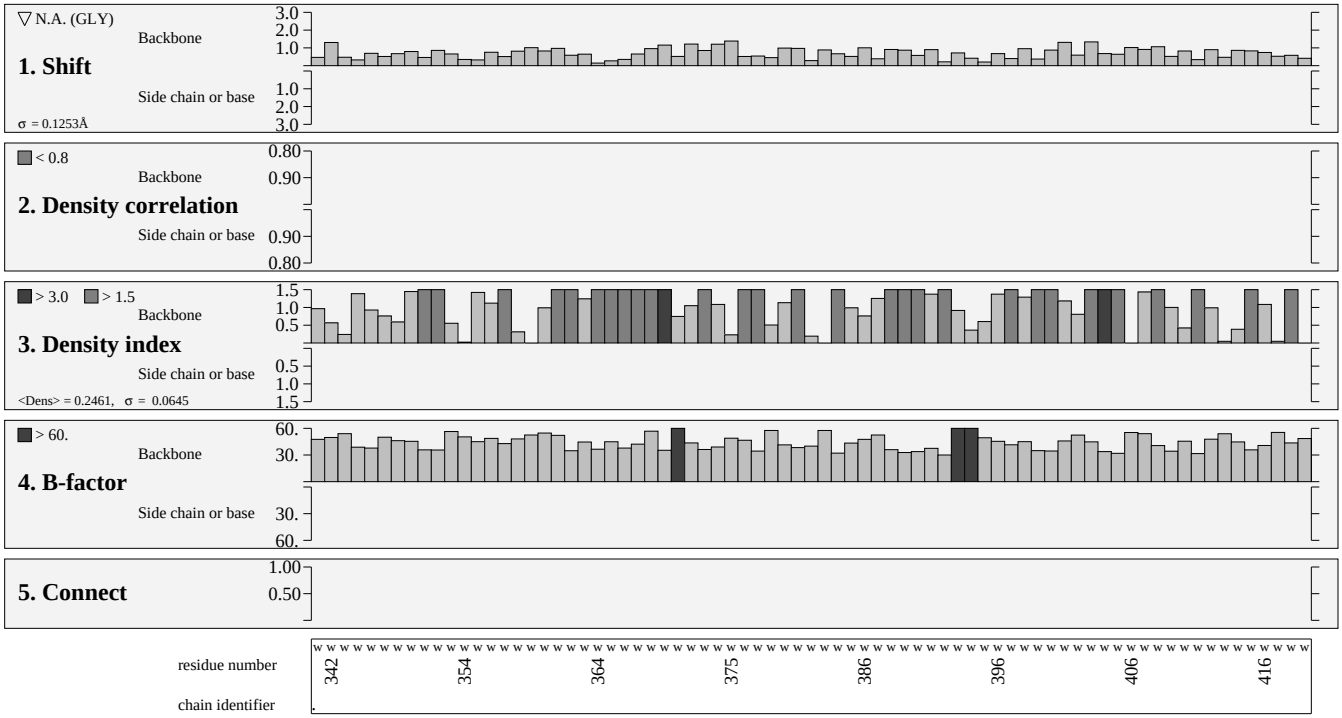


Structure Factor Check

1PP9

Local estimation (30)



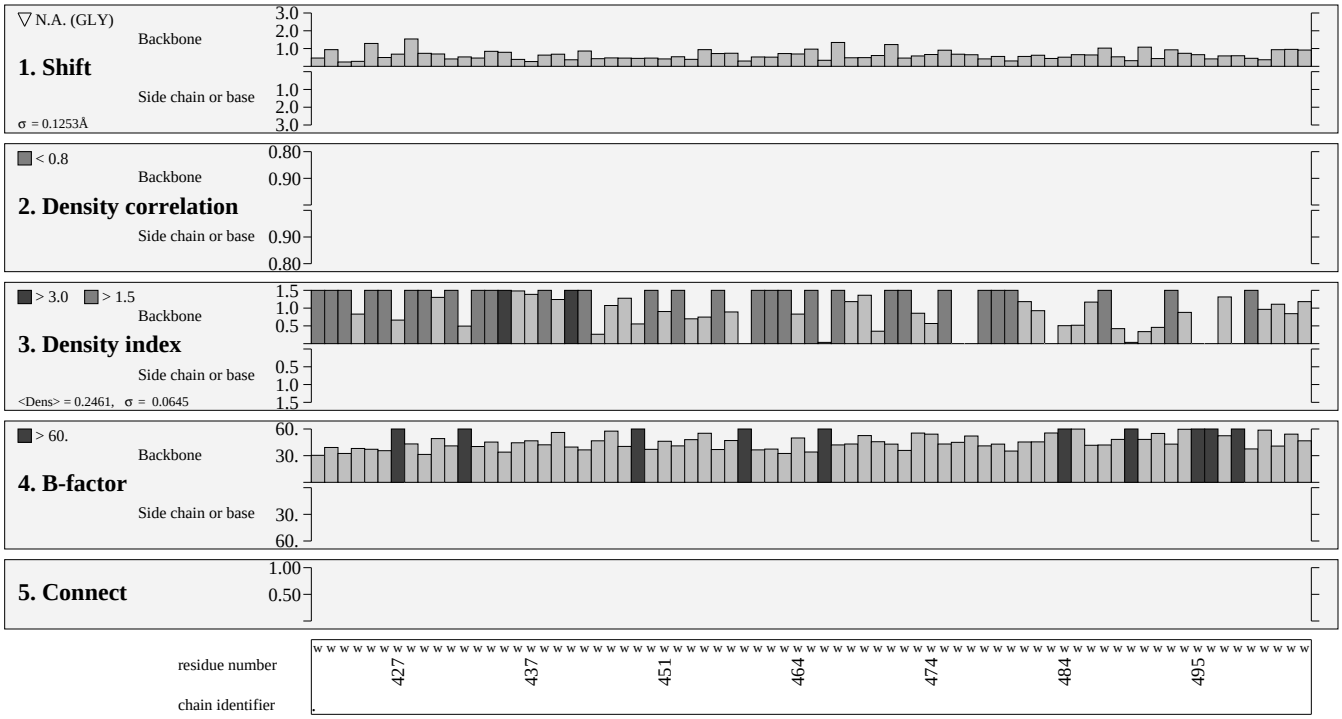


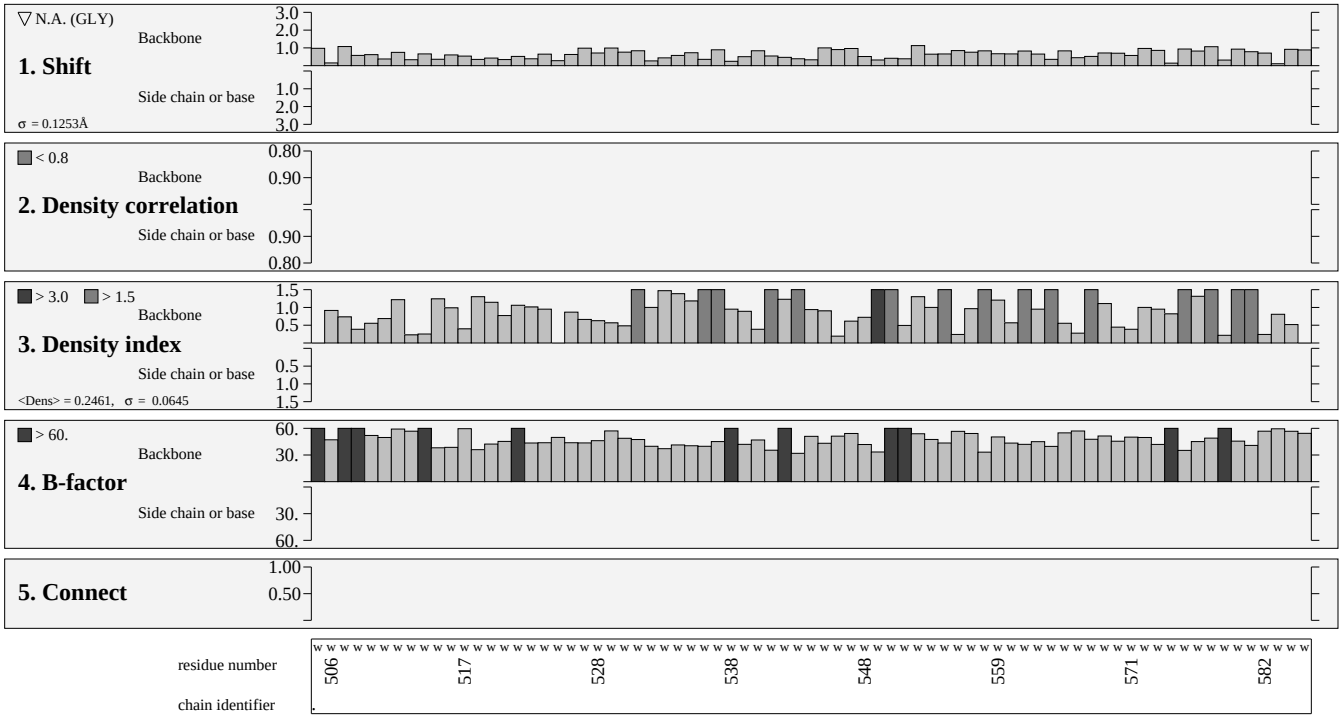
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (31)



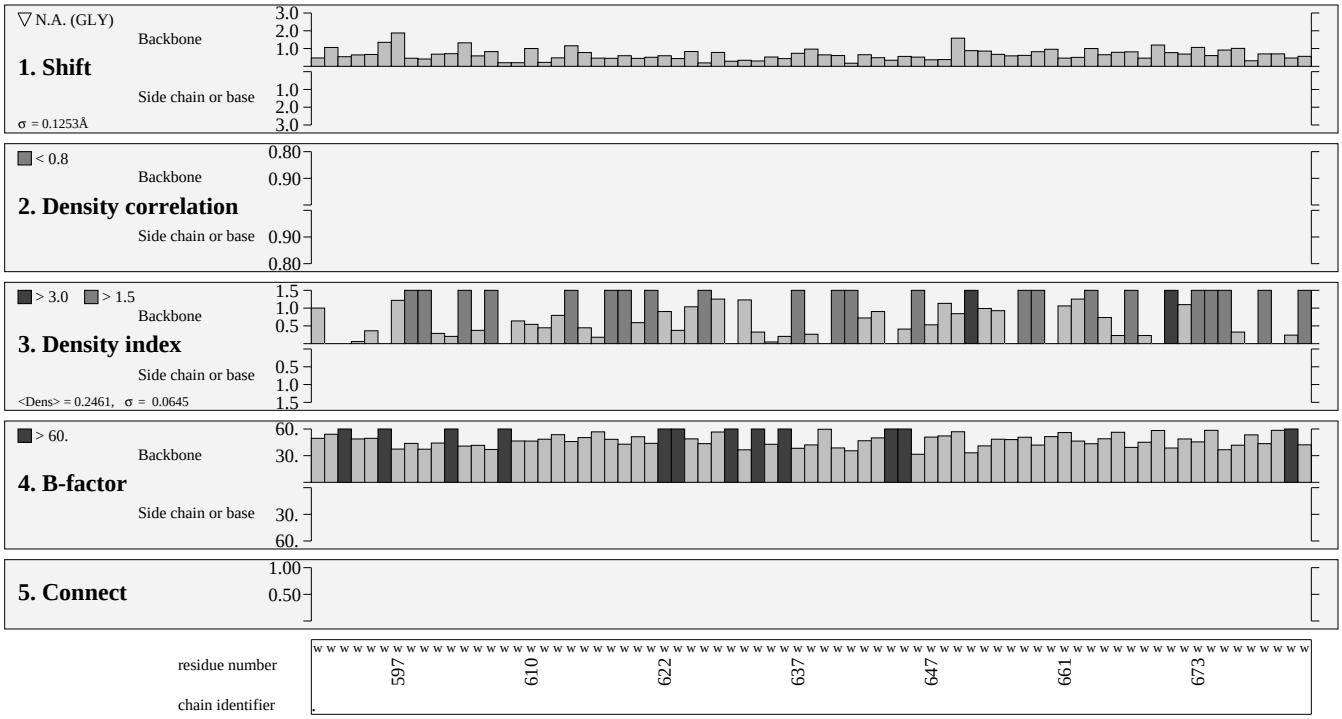


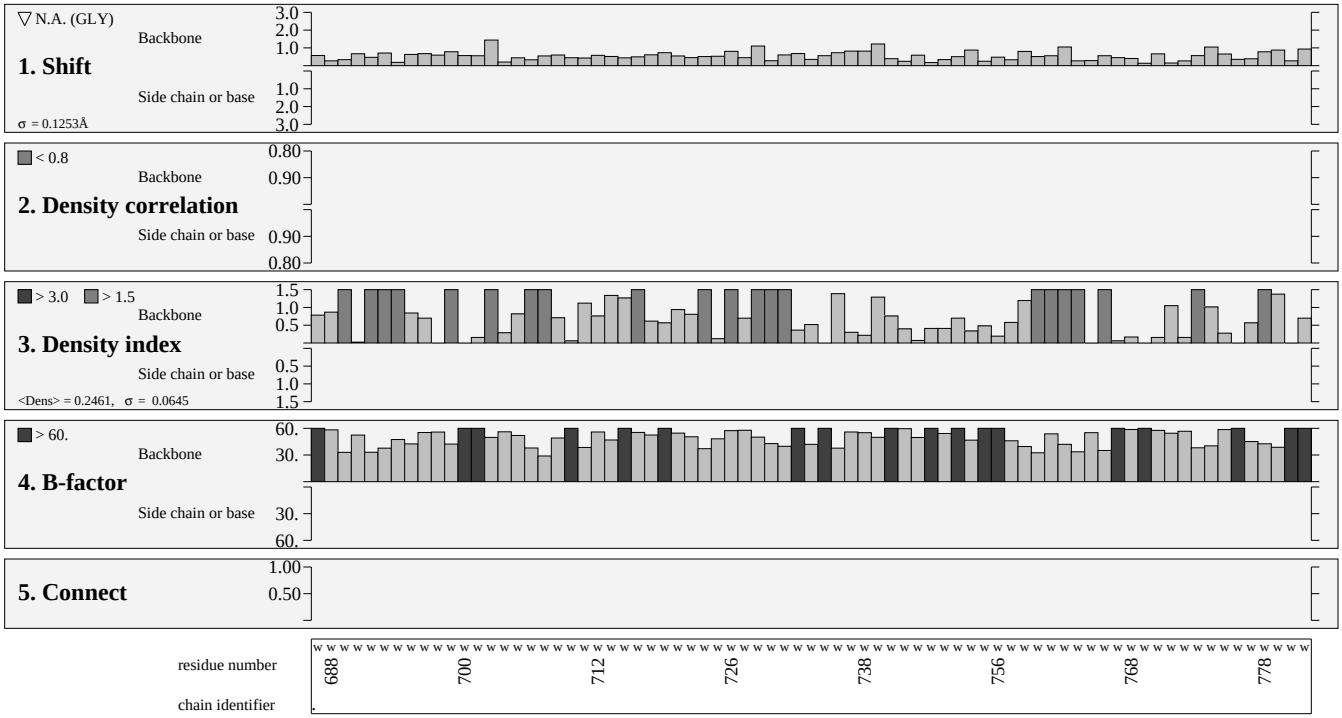
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (32)



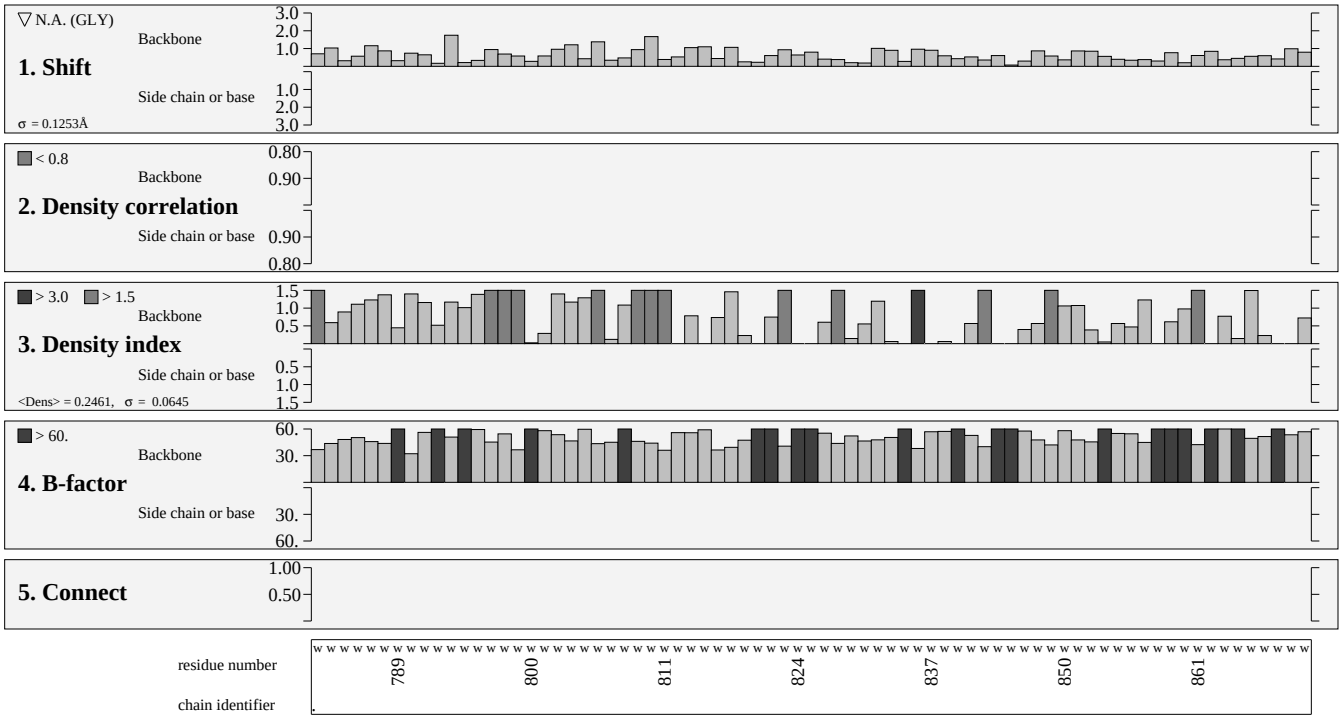


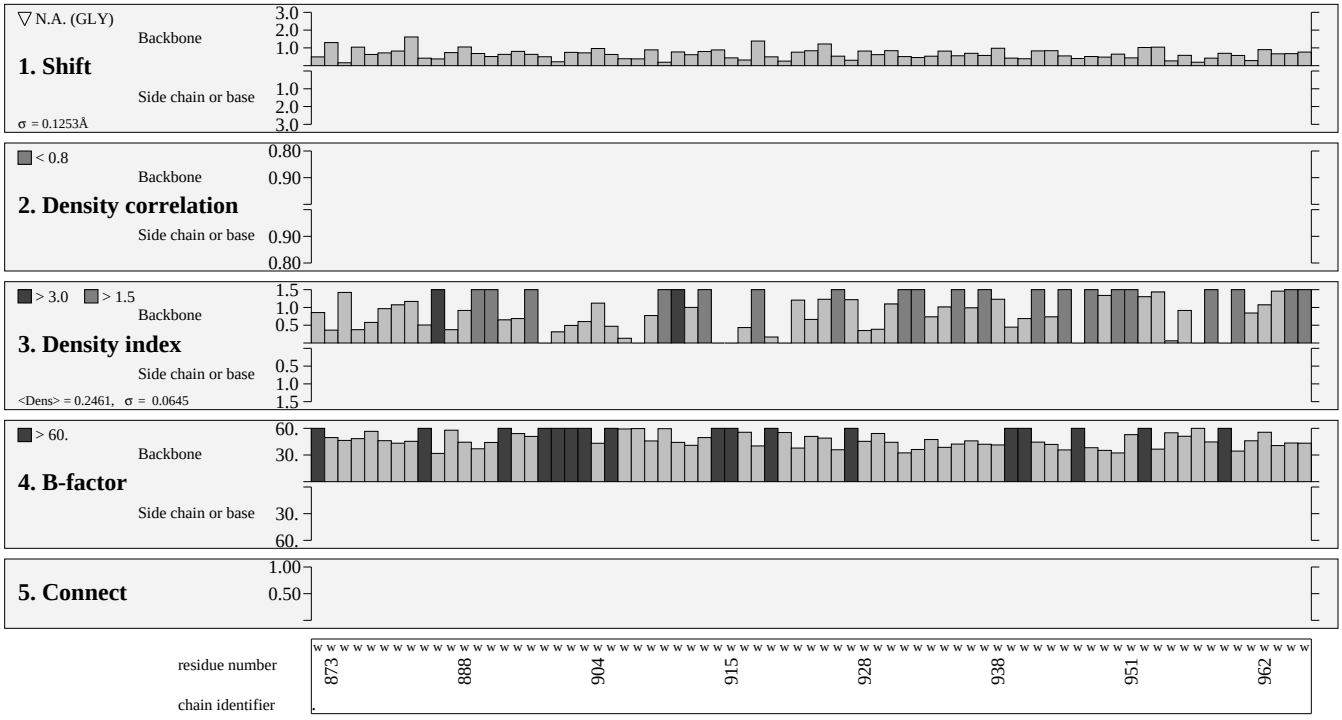
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (33)

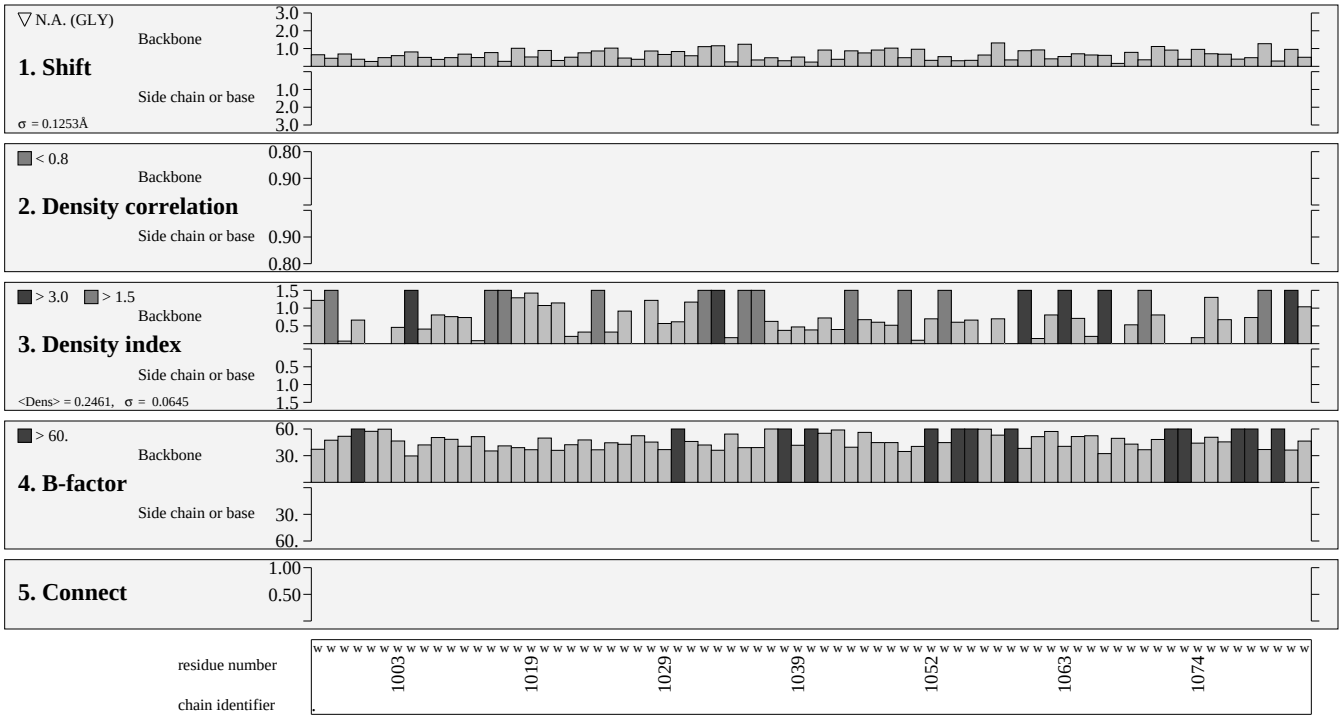


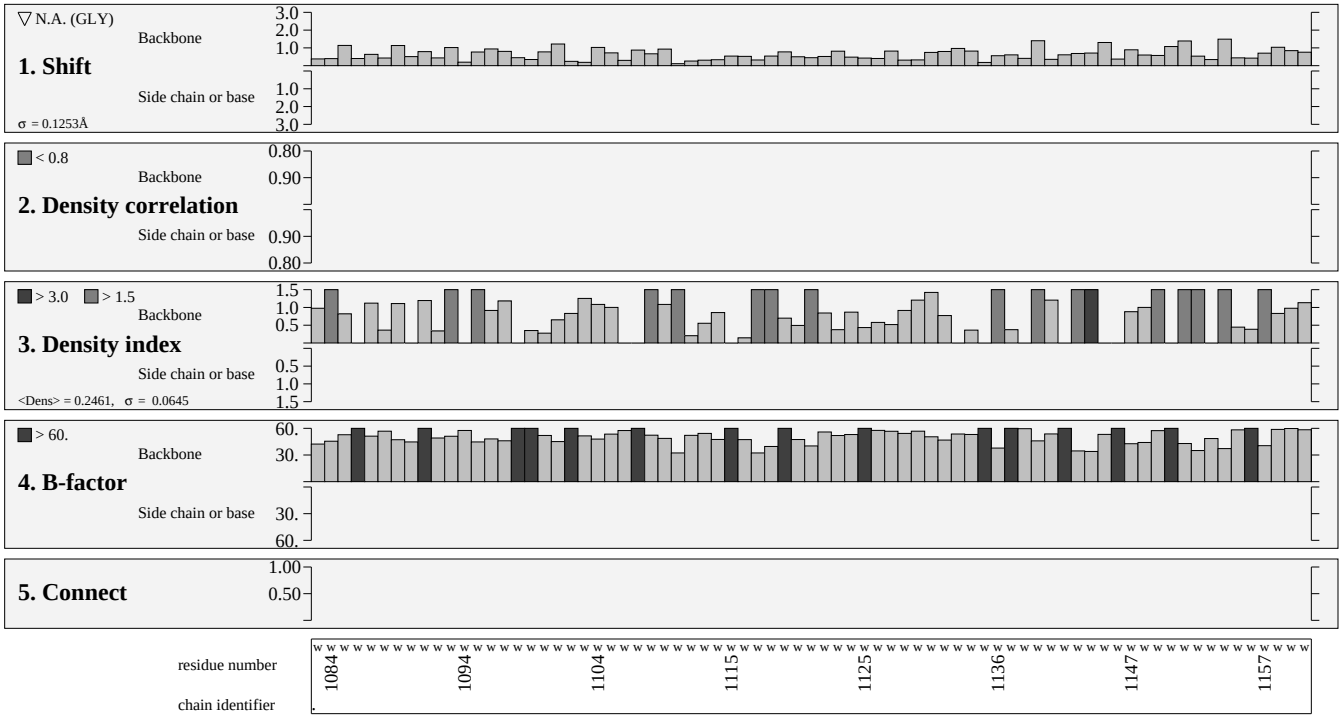


Structure Factor Check

1PP9

Local estimation (34)



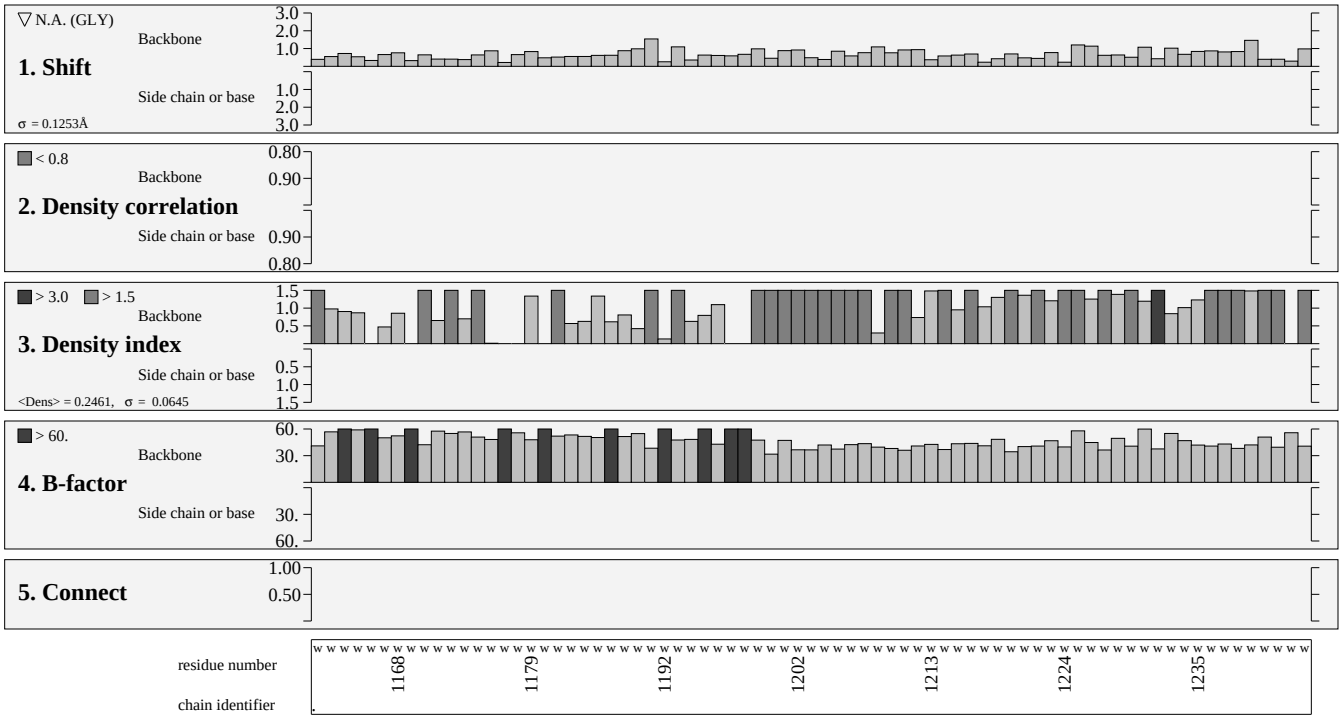


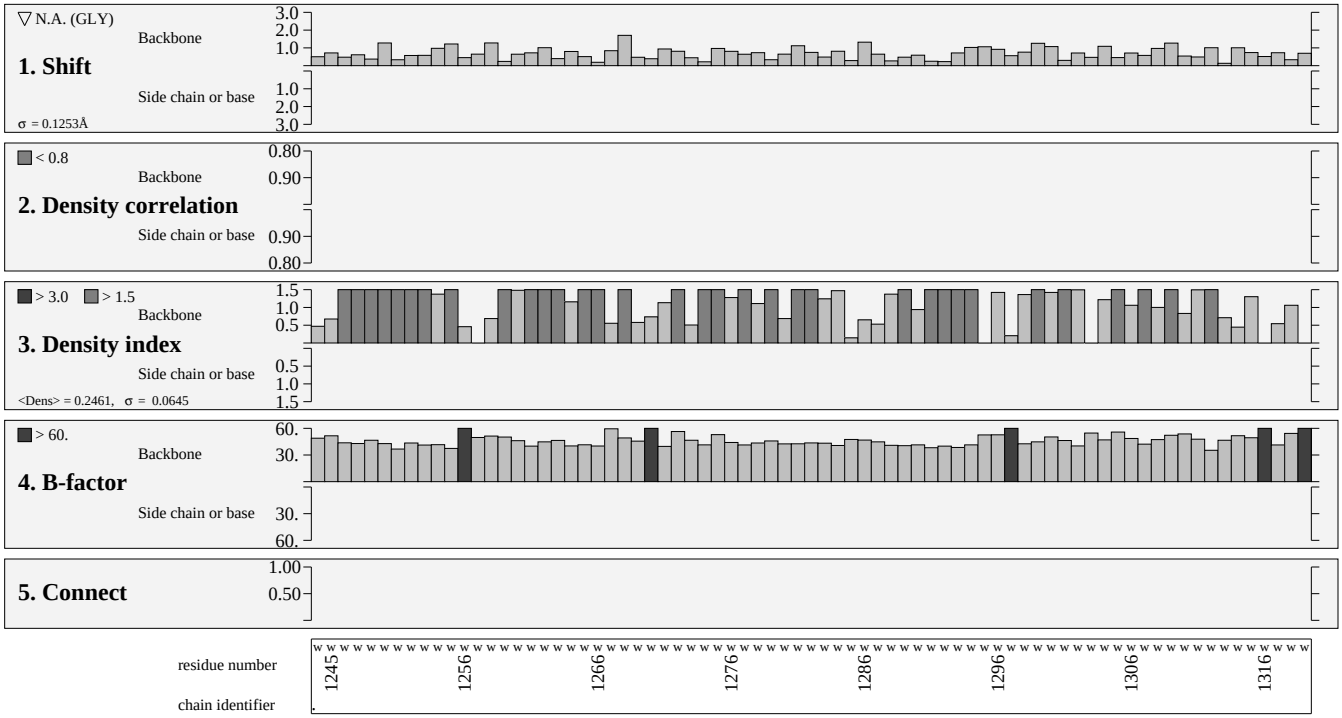
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (35)



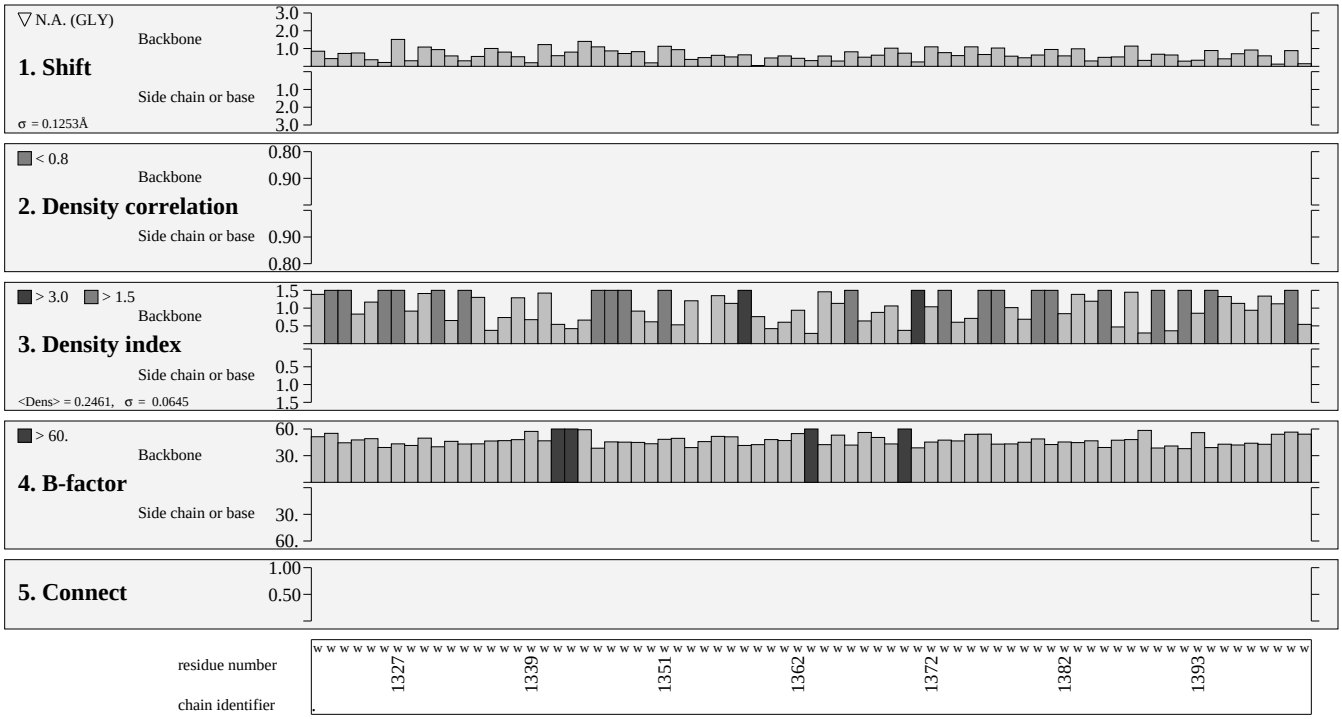


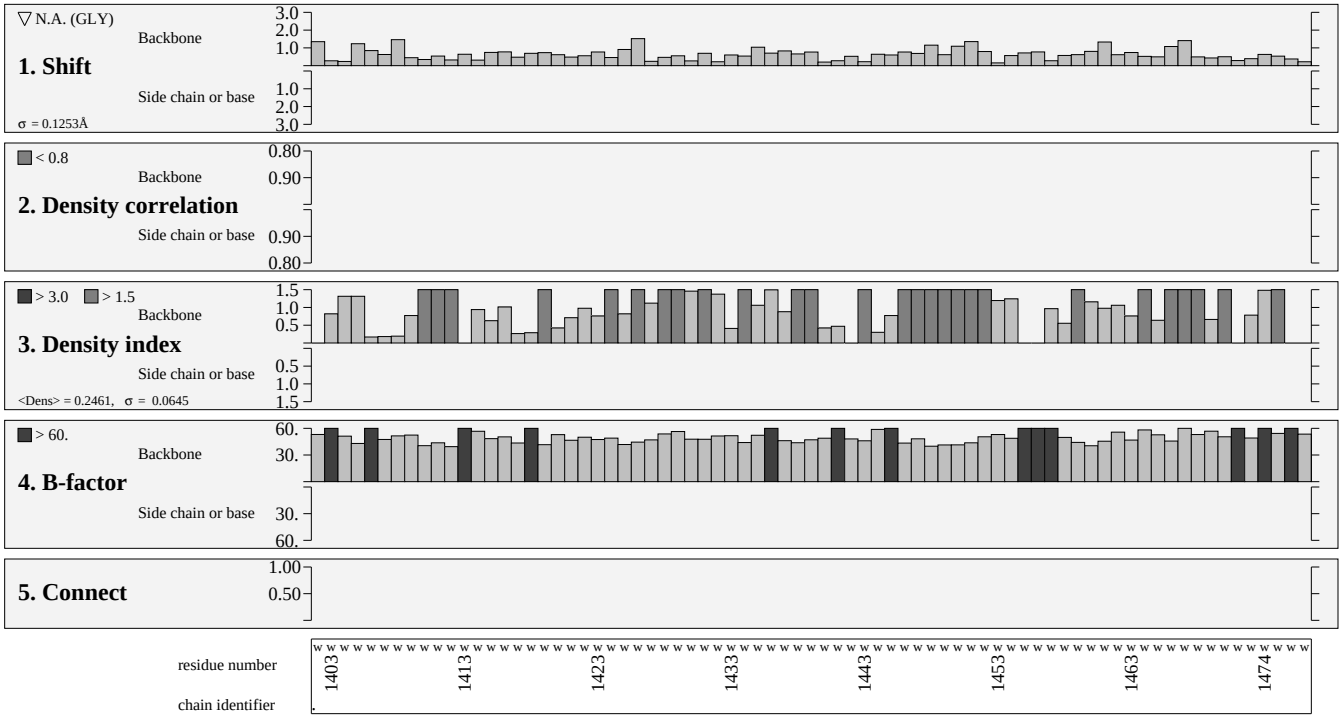
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (36)



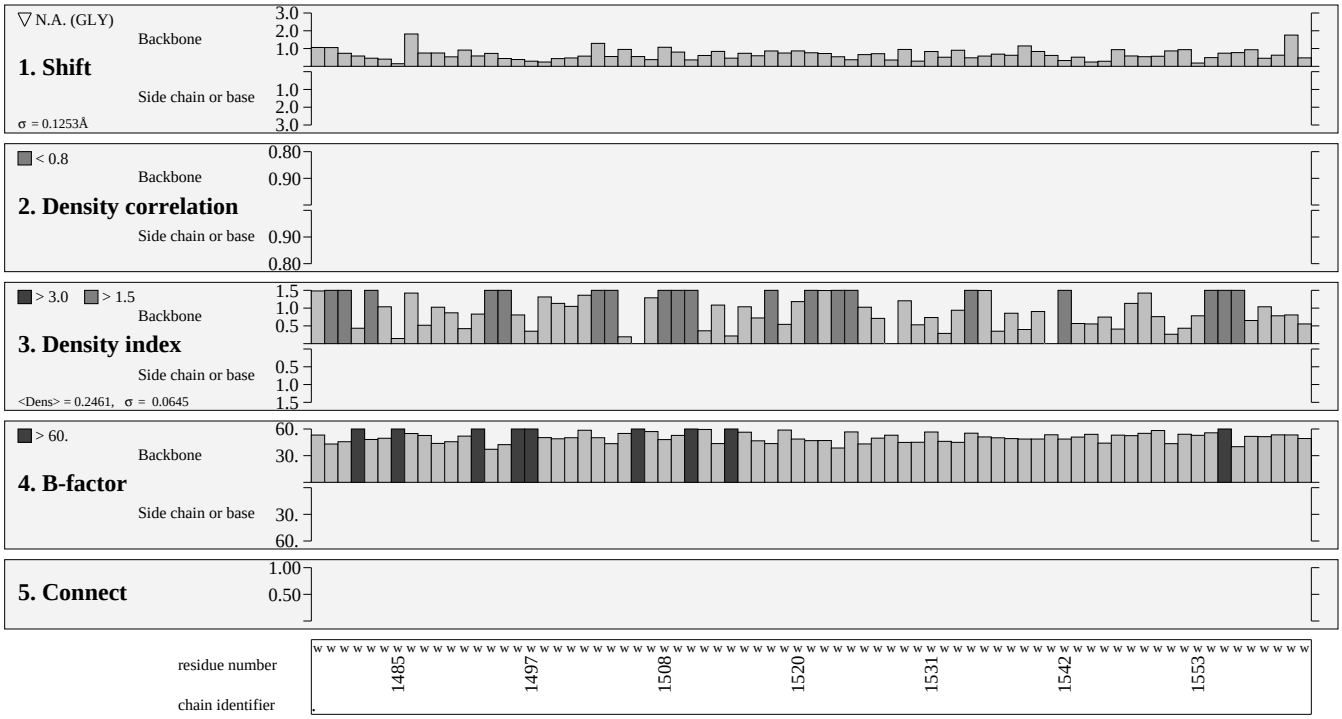


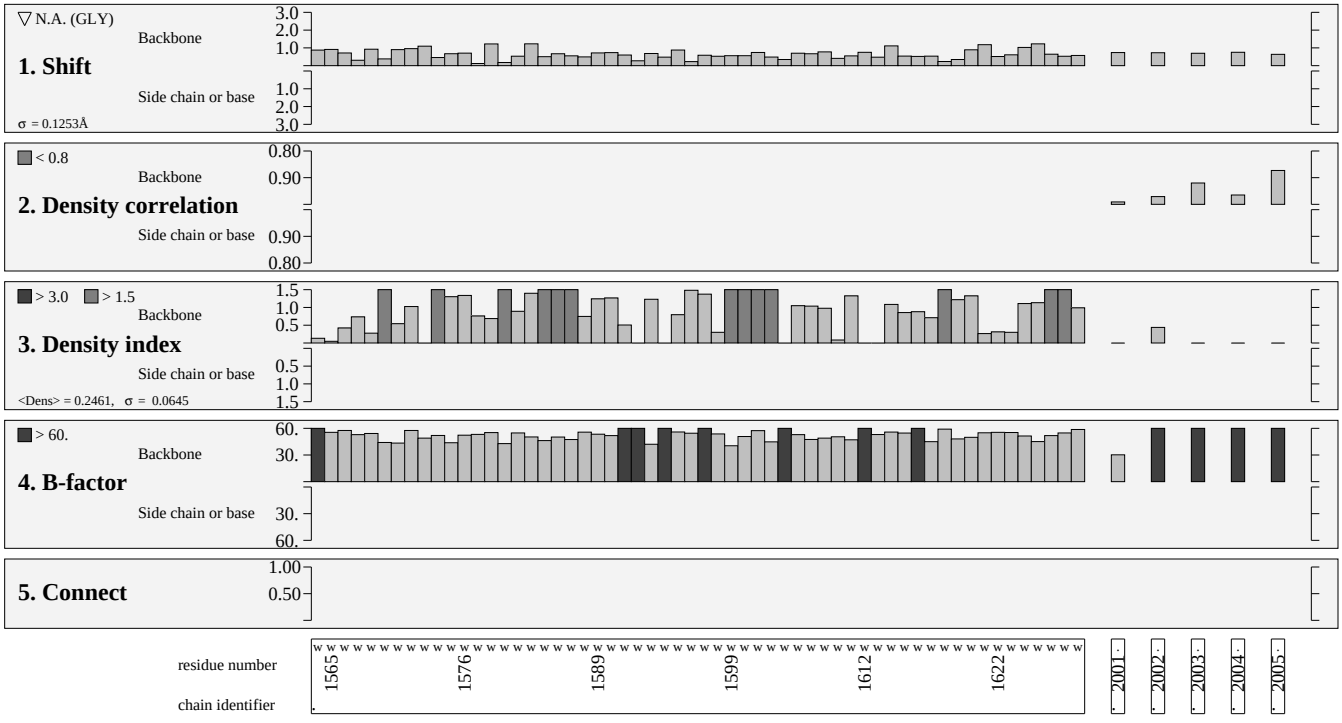
SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (37)



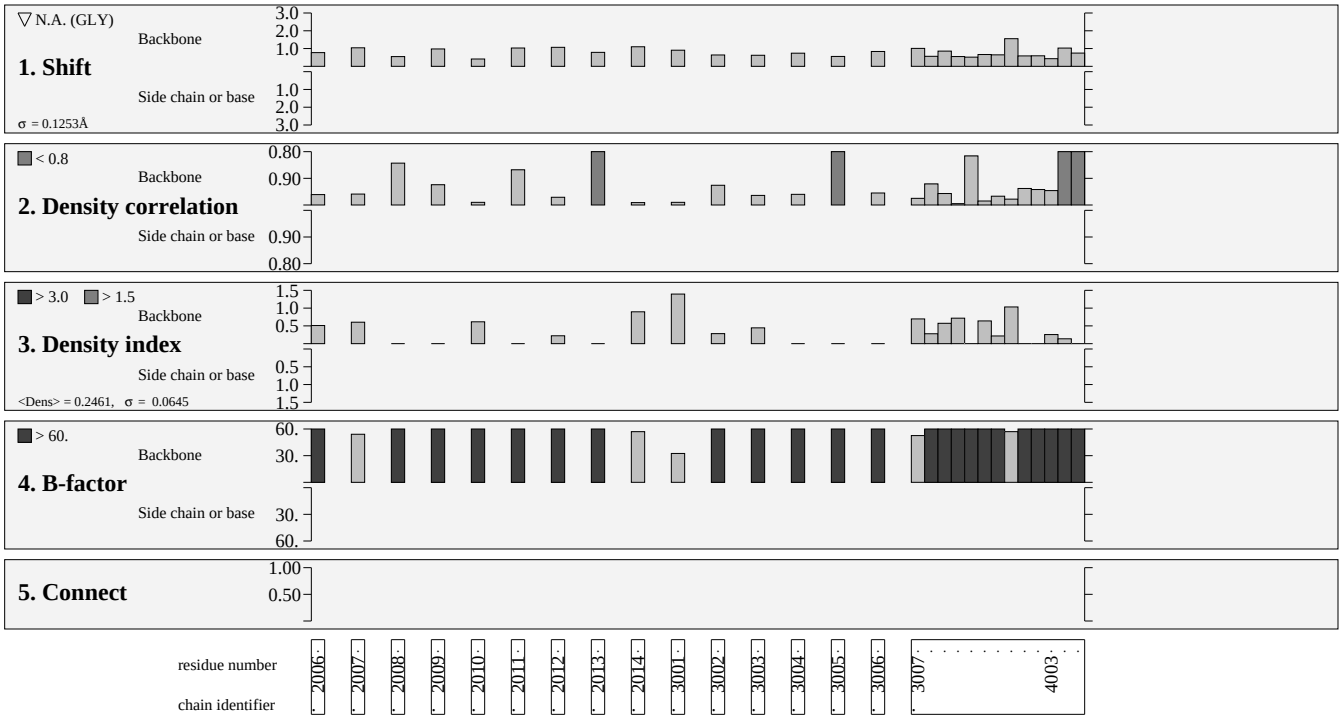


SFCHECK 6.0.5

Structure Factor Check

1PP9

Local estimation (38)



Structure Factor Check
1PPJ

Title: BOVINE CYTOCHROME BC1 COMPLEX WITH STIGMATELLIN AND ANTIMYCIN
Date: 16−JUN−03
PDB code: 1PPJ

Crystal

Cell parameters:
a: 128.53 Å b: 168.75 Å c: 231.53 Å
α: 90.00° β: 90.00° γ: 90.00°

Space group: P 21 21 21

Number of NCS−operators: 1
NC−symmetry only for information

Model

33549 atoms (1370 water molecules)

Number of chains:	51
Volume not occupied by model:	45.0 %
<B> (for atomic model):	49.8 Å ²
σ(B):	16.04 Å ²
Matthews coefficient:	2.79
Corresponding solvent % :	55.52

Refinement

Program:	CNS 1.1
Nominal resolution range:	93.5 − 2.10 Å
Reported R−factor:	0.224
Number of reflections used:	285060
Reported Rfree:	0.26
Sigma cut−off:	N.A.

Structure Factors

Input

Nominal resolution range:		93.5 − 2.10 Å
Reflections in file:		285058
Unique reflections	above 0:	285058
	above 1σ:	278499
	above 3σ:	213616

SFCHECK

Nominal resolution range:	93.5 − 2.10 Å
(max. from input data, min. from author)	
Used reflections:	285058
Completeness:	97.8 %
R_stand(F) = <σ(F)>/<F> :	0.079
Anisotropic distribution of Structure Factors	
ratio of eigen values:	1.0000 0.7563 0.6853
B_overall (by Patterson):	38.5Å ²
Optical resolution:	1.75 Å
Expected opt. resol. for complete data set:	1.75 Å
Estimated minimal error:	0.029 Å

Model vs. Structure Factors

R−factor for all reflections:	0.250
Correlation factor:	0.931
R−factor:	0.253
for F > 2.0 σ	
nom. resolution range:	93.53 − 2.10Å
reflections used:	278697
Rfree:	0.274
Nfree:	13880
R−factor without free−refl.:	0.252
Non free−reflections:	264817
<u> (error in coords by Luzzati plot):	0.323 Å
Estimated maximal error:	0.104 Å
DPI:	0.189 Å

Scaling

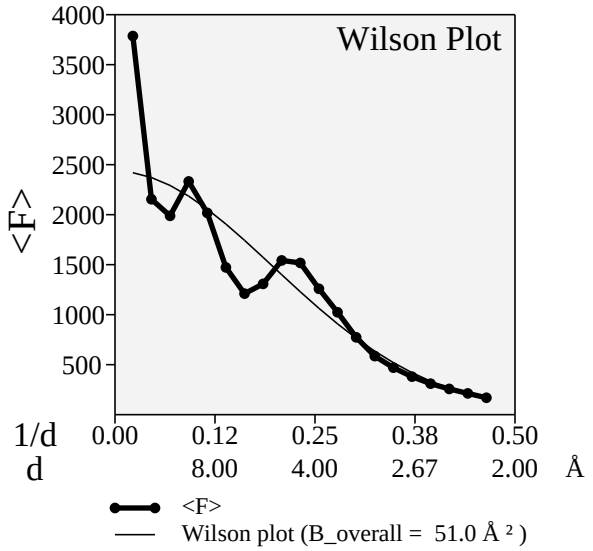
Scale:	1.010
Bdiff:	−4.23

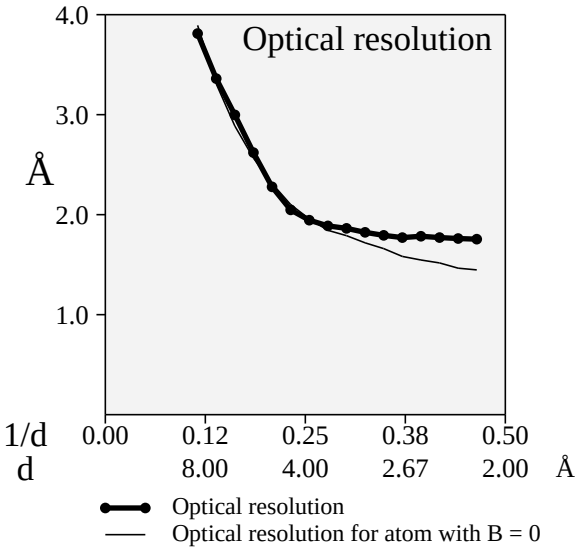
Anisothermal Scaling (Beta):
4.0089 0.5542 −0.7787 0.0000 0.0000 0.0000

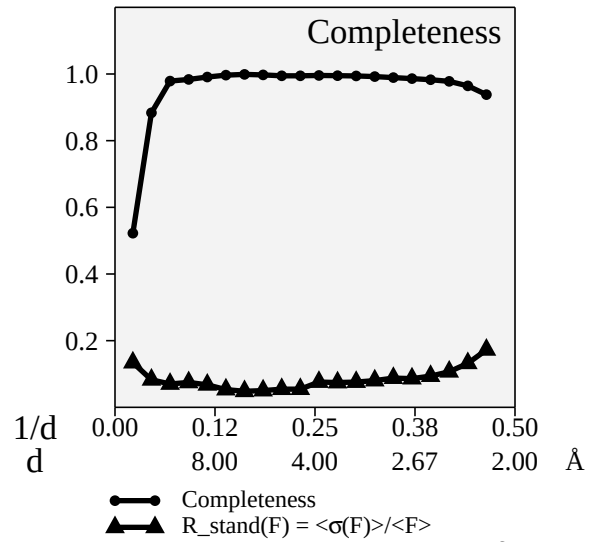
Solvent correction − Ks,Bs: 0.841 250.032

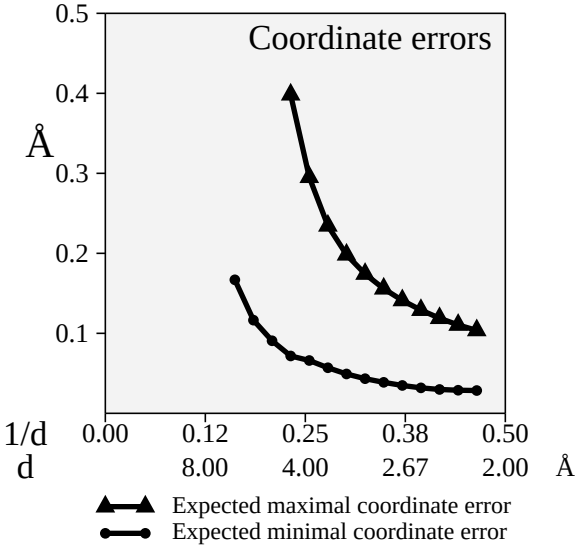
Structure Factor Check

1PPJ

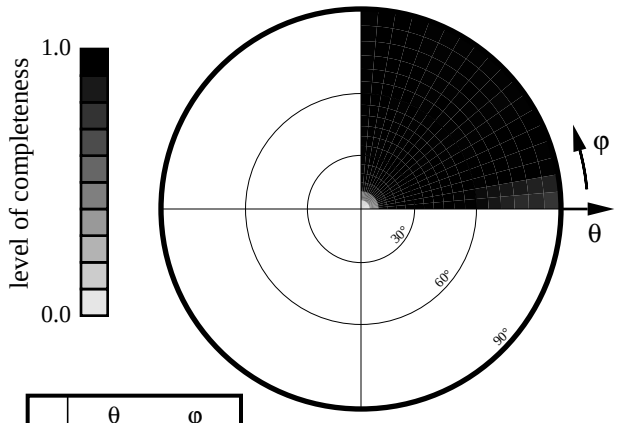






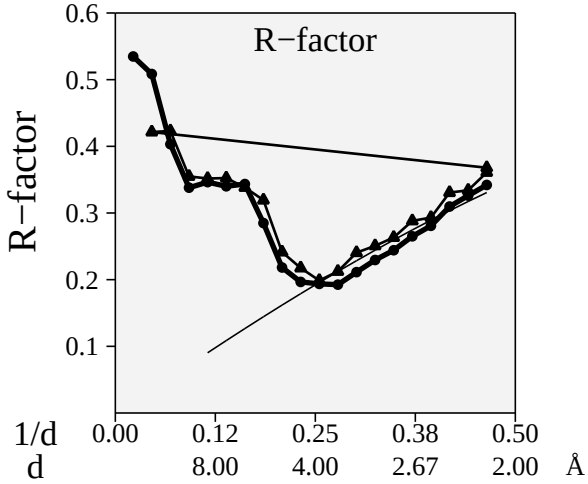


Stereographic projection of
the averaged radial completeness



	θ	φ
h	90.00	0.00
k	90.00	90.00
l	0.00	0.00

polar coordinates of the
crystallographical axes

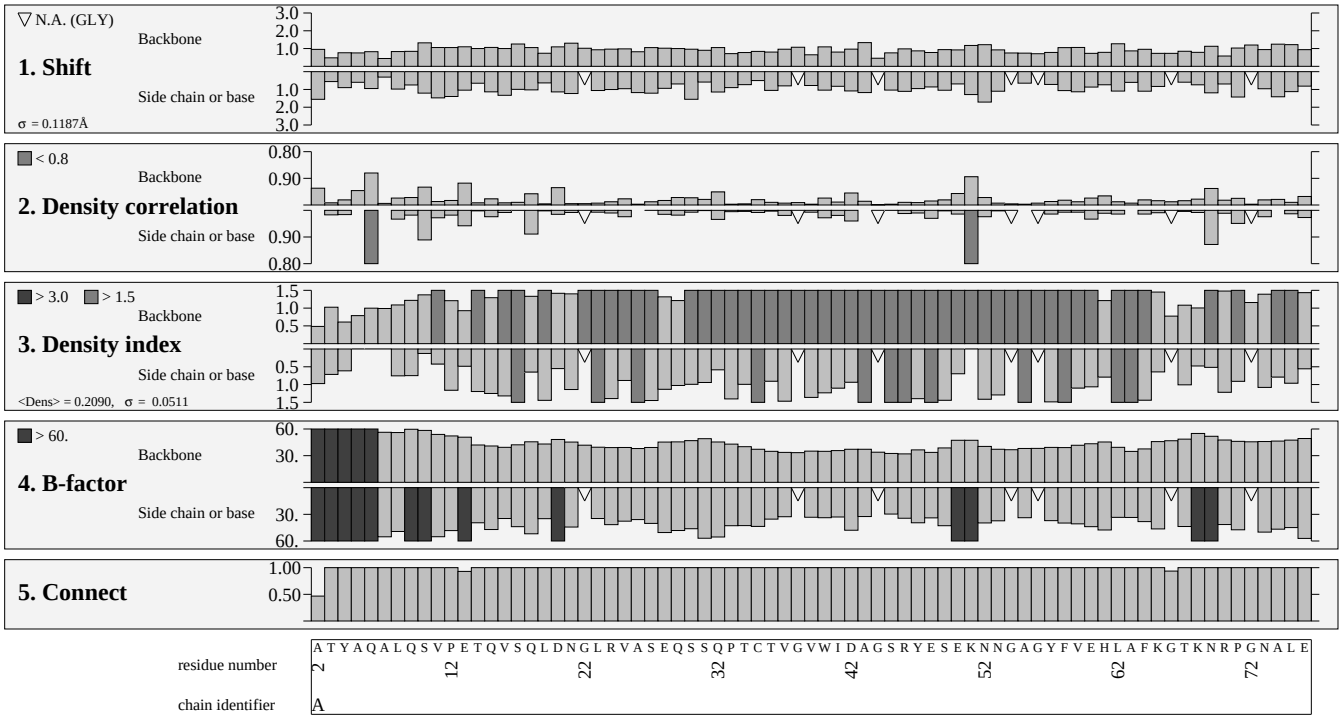


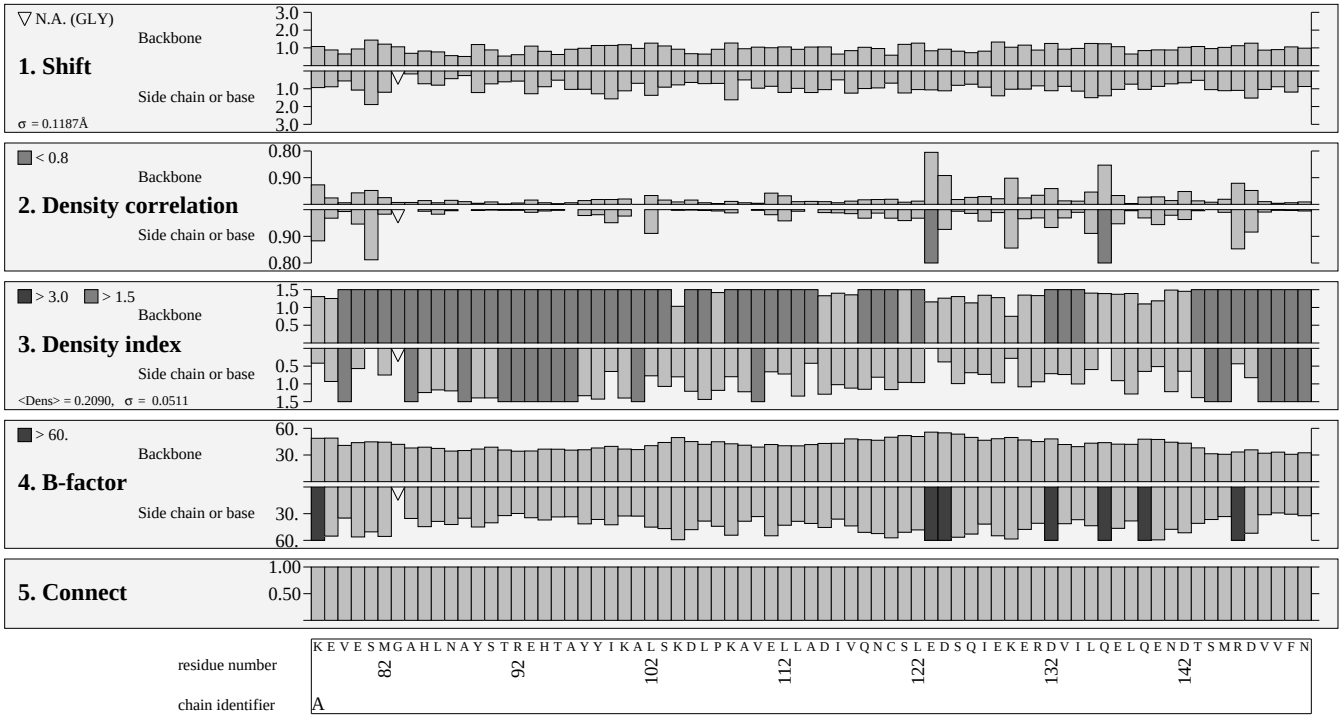
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation



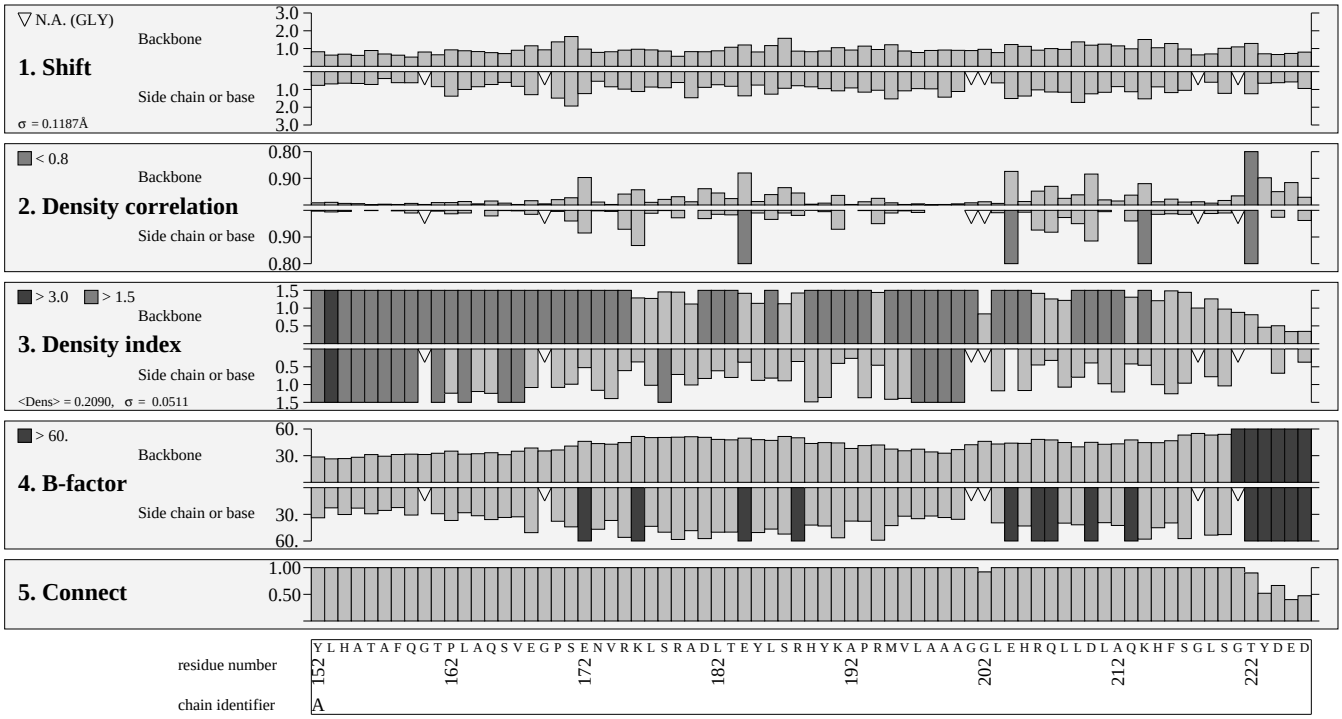


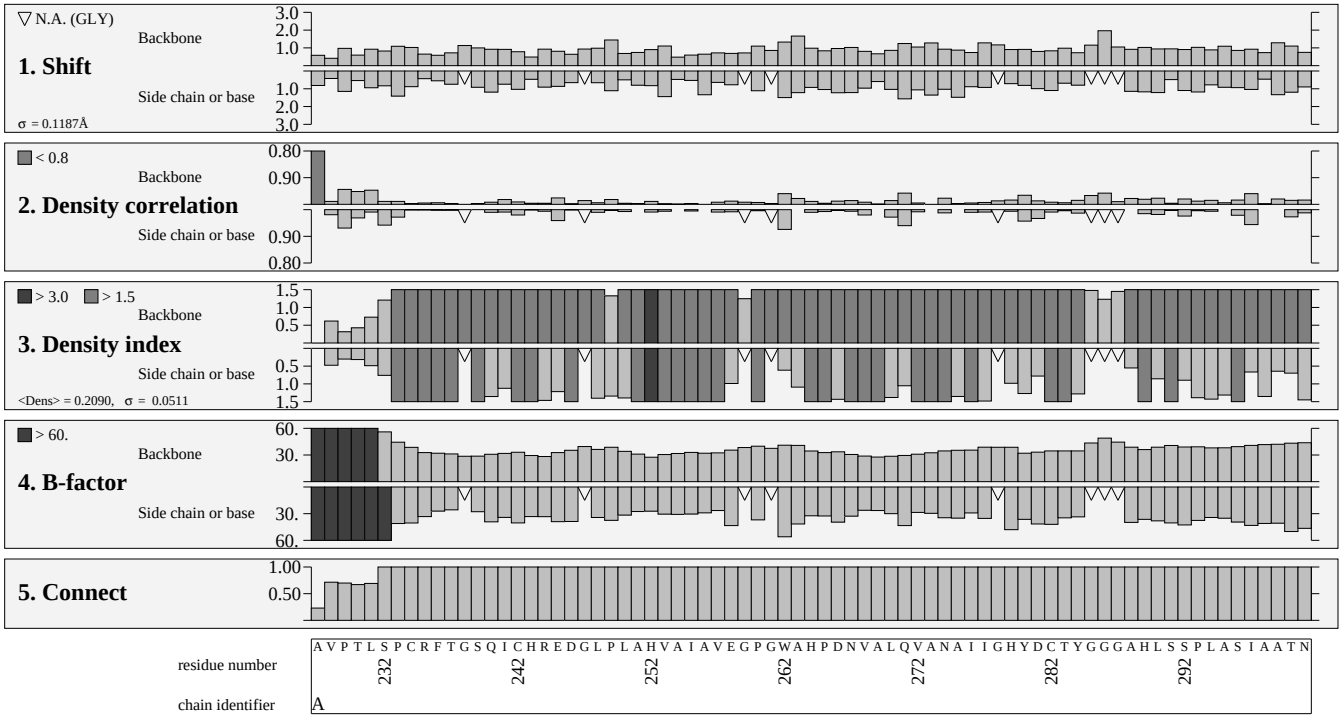
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (2)



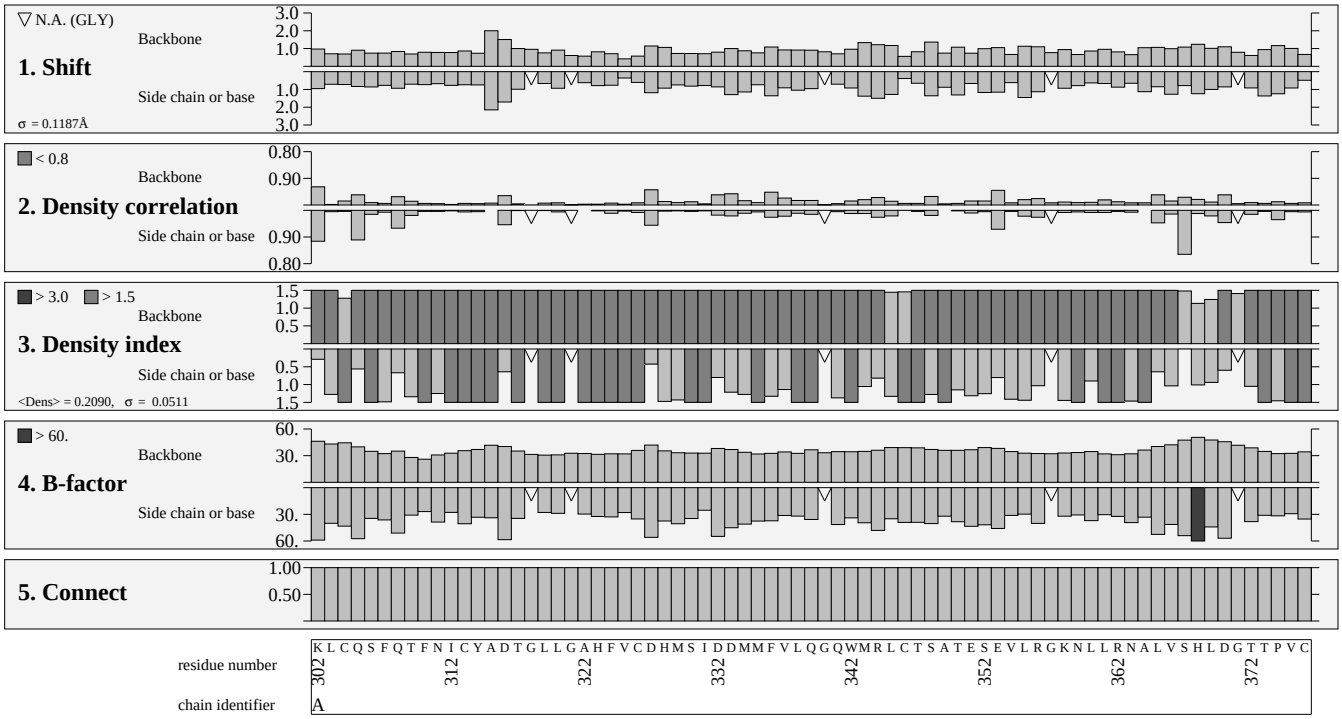


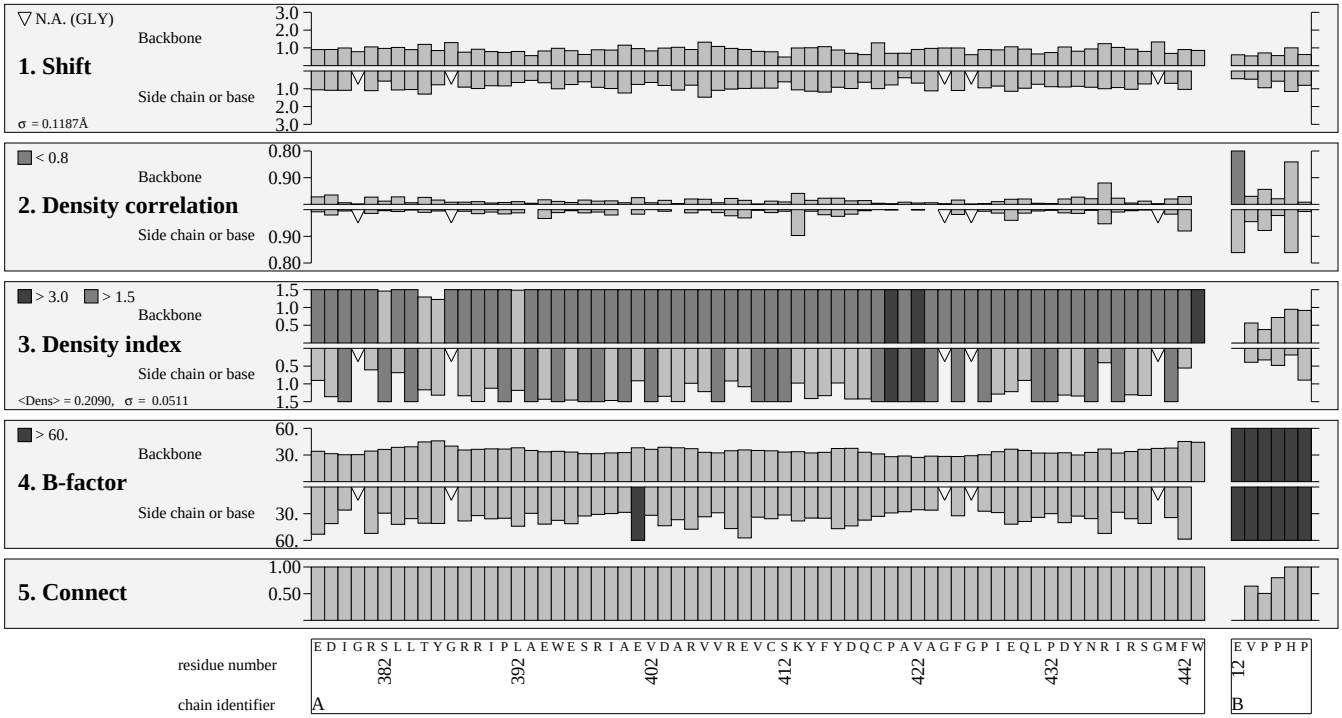
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (3)



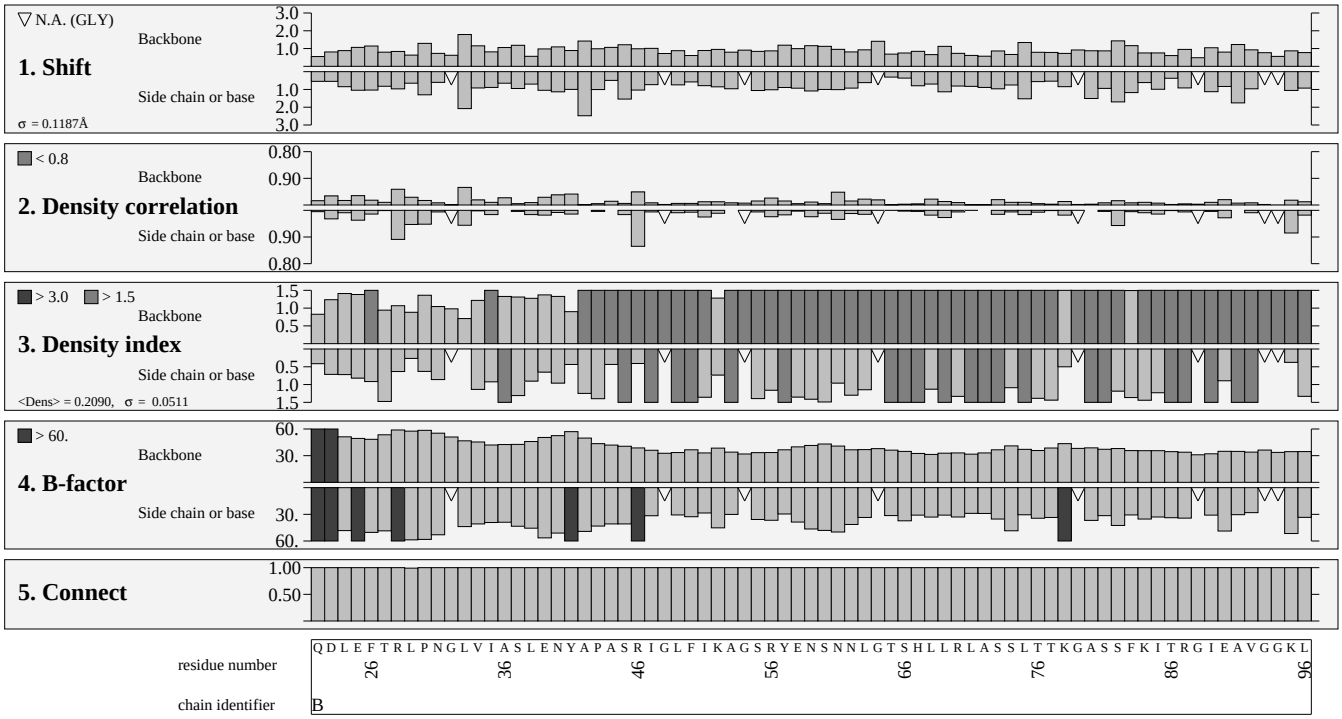


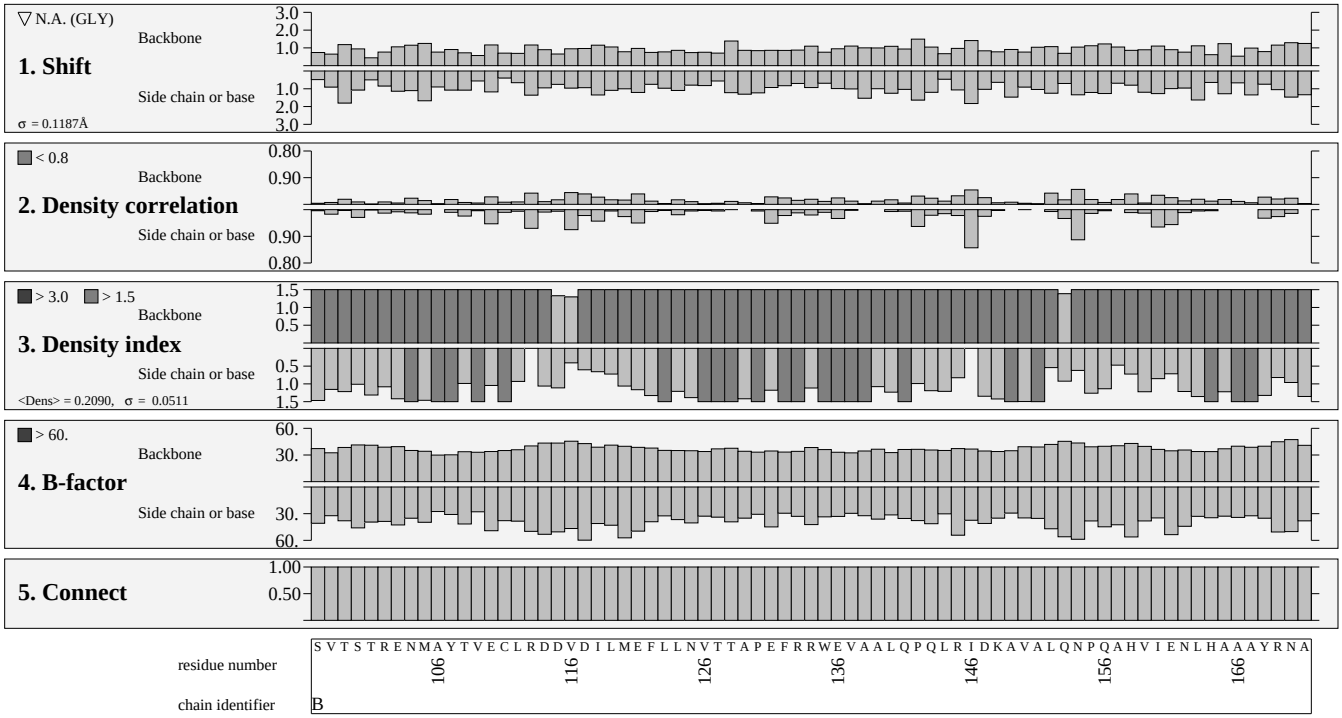
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (4)



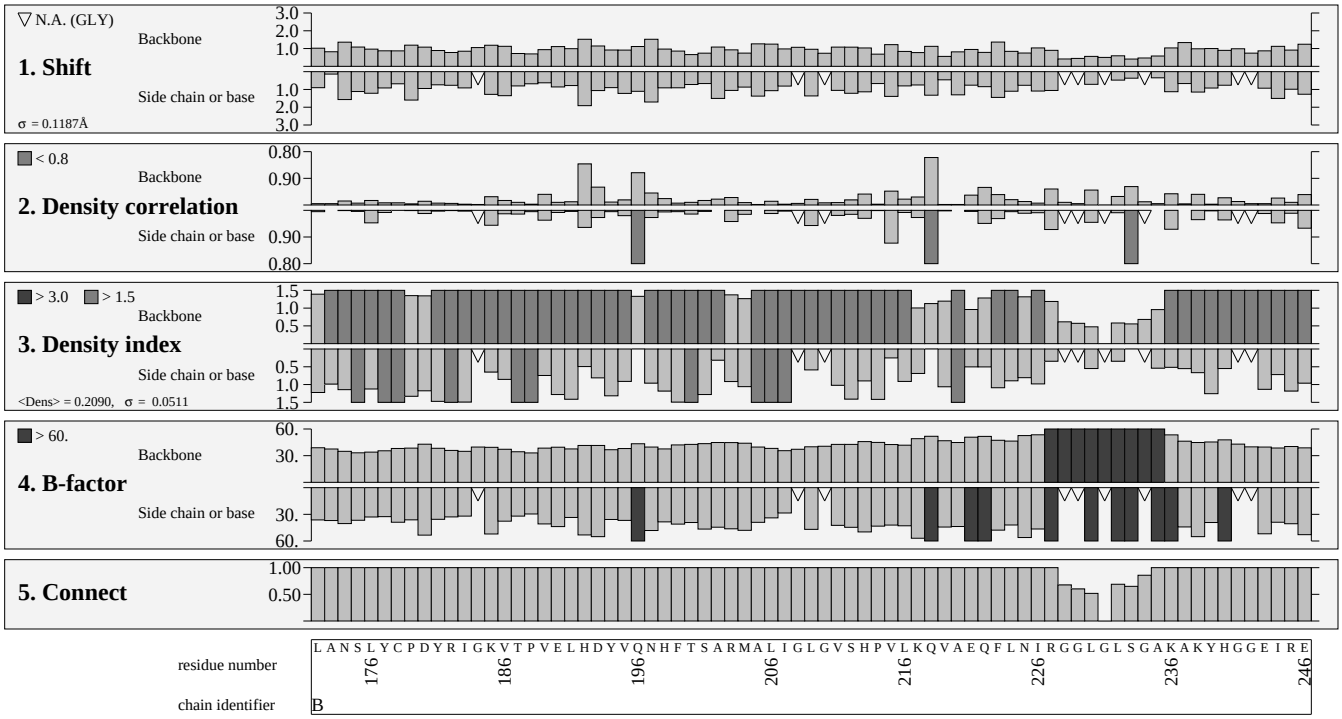


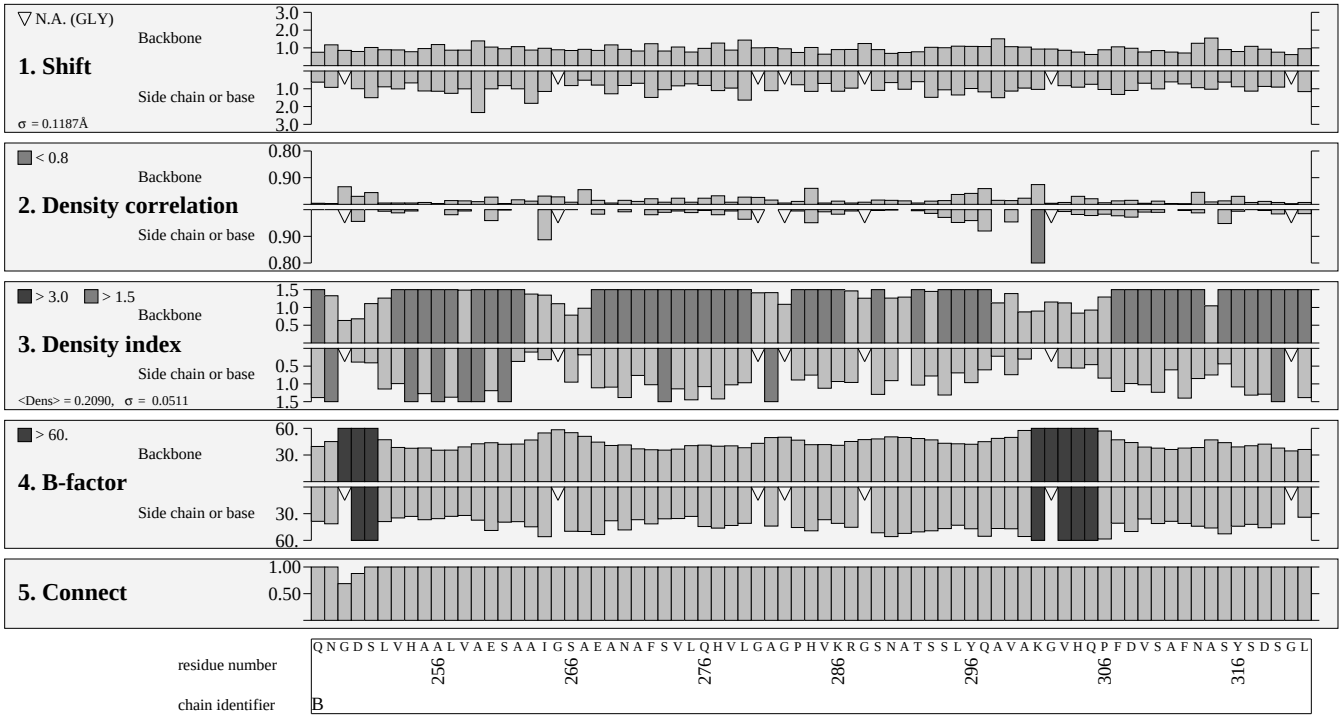
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (5)



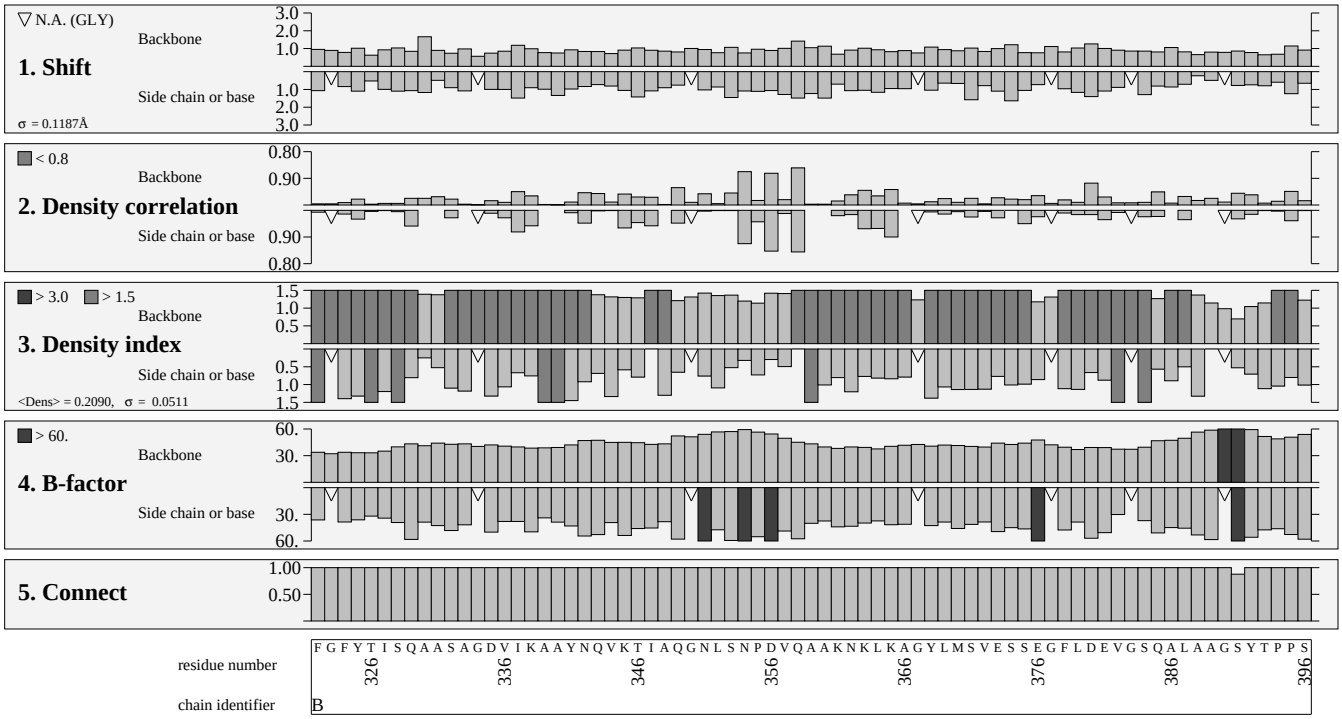


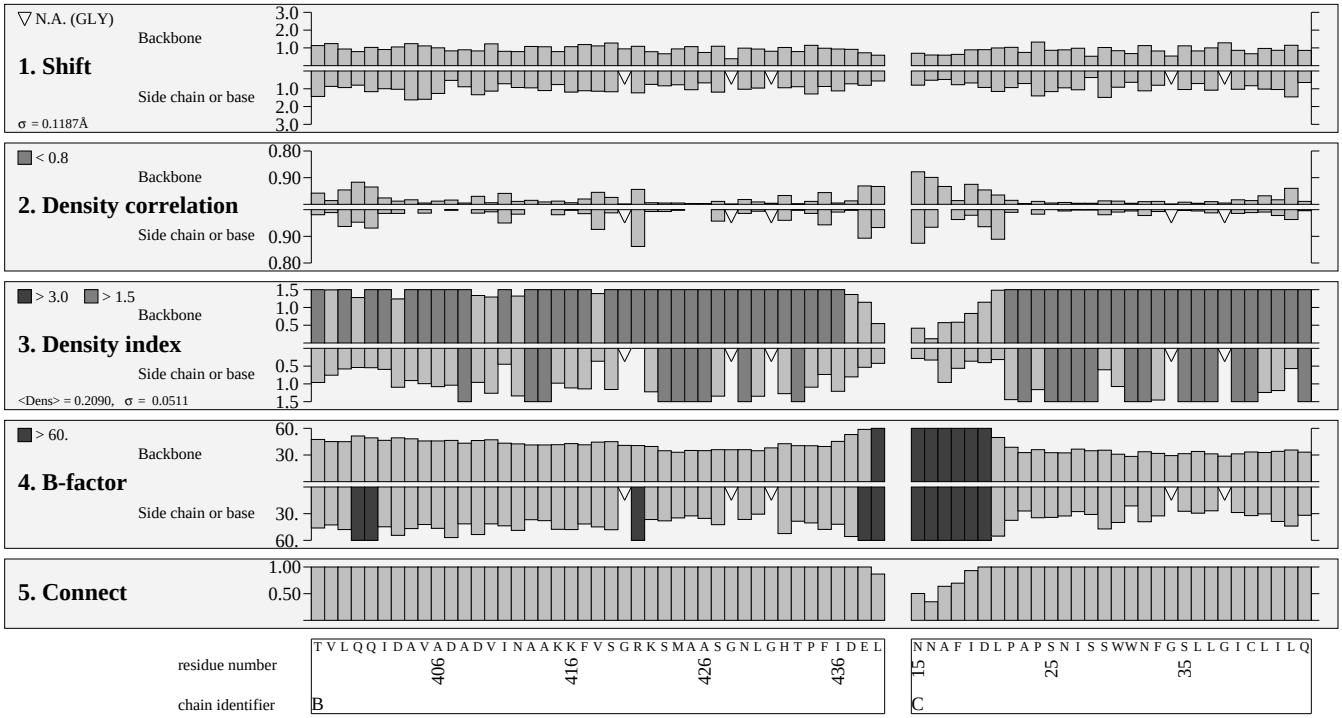
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (6)



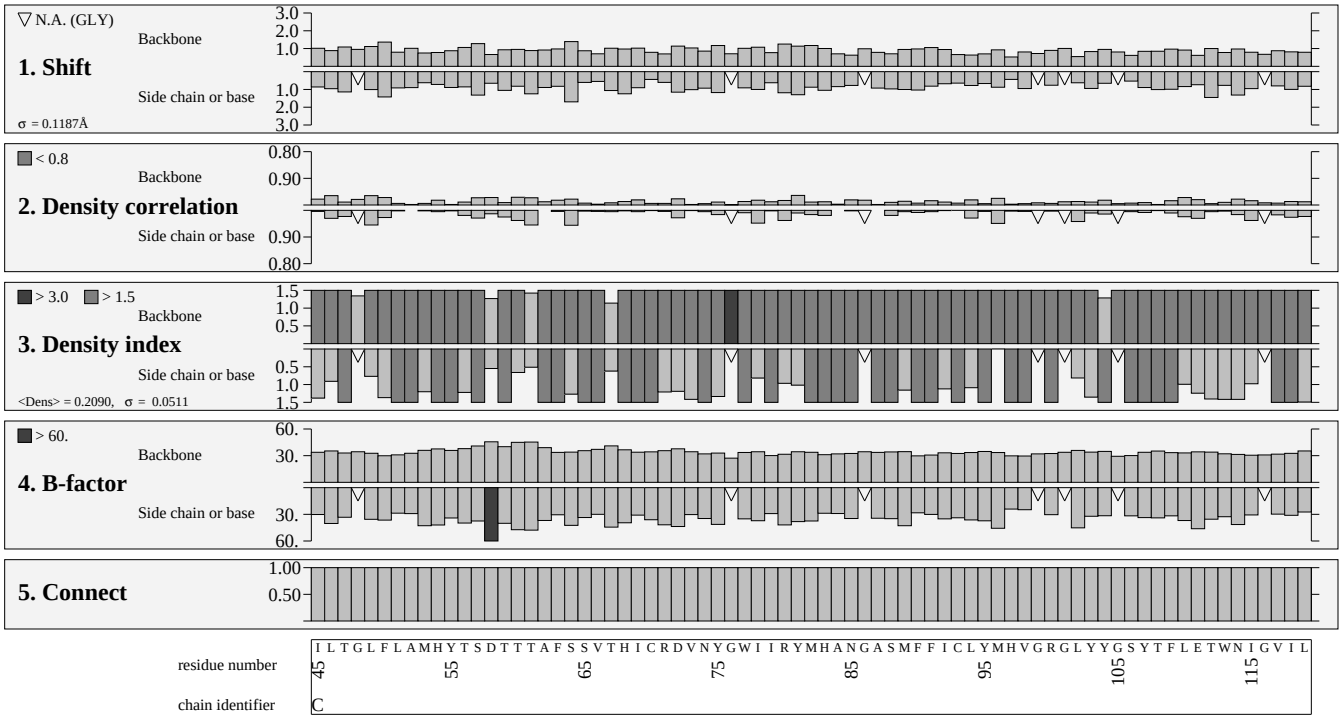


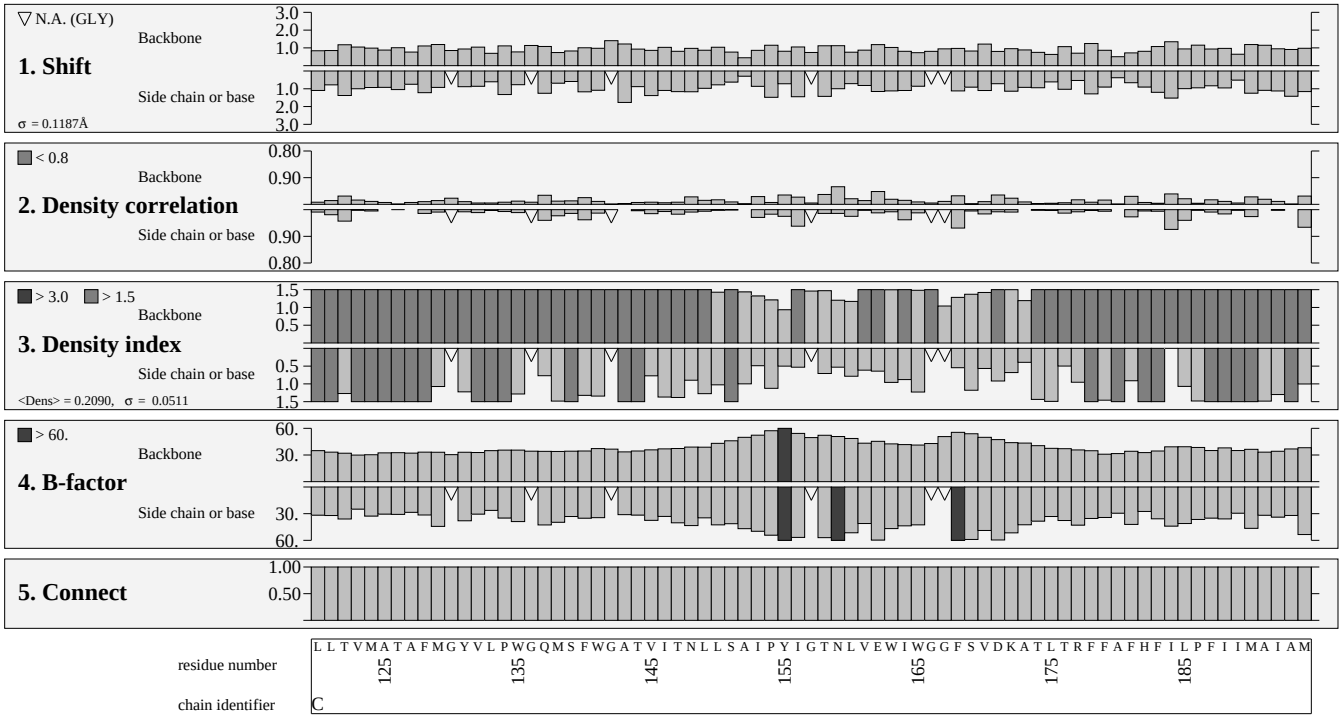
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (7)



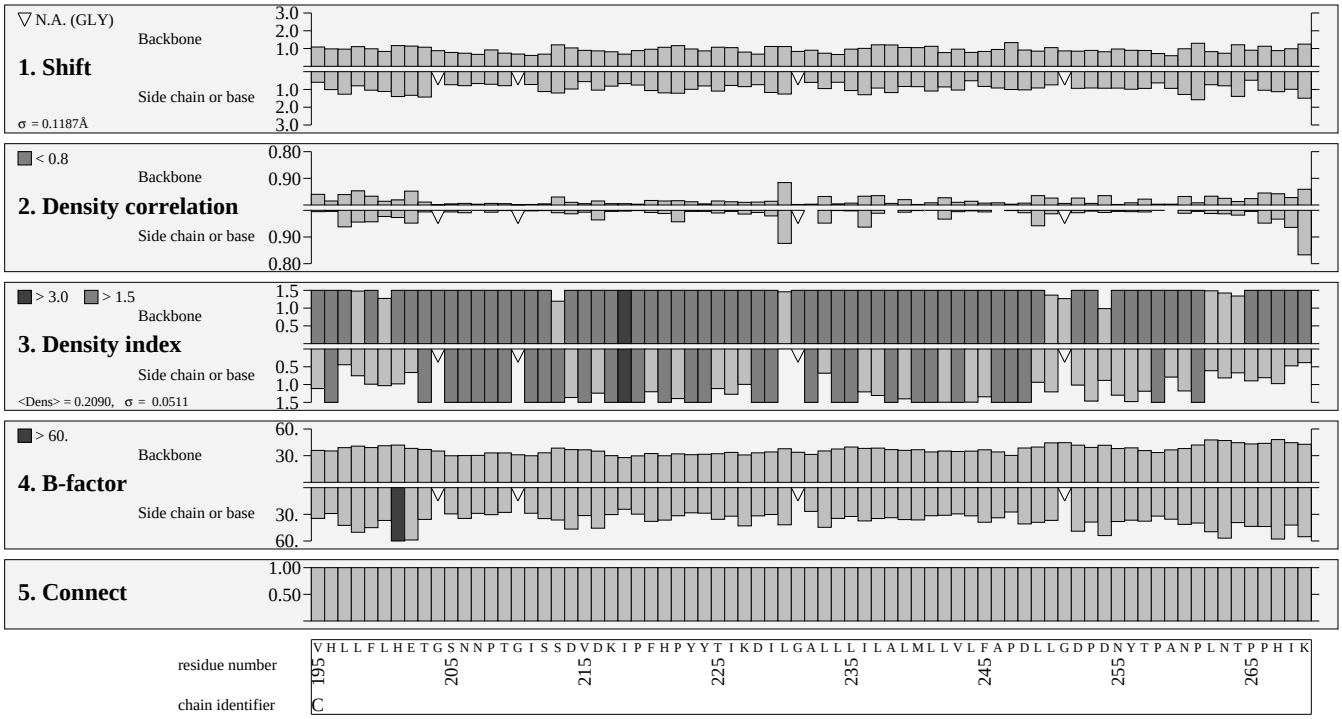


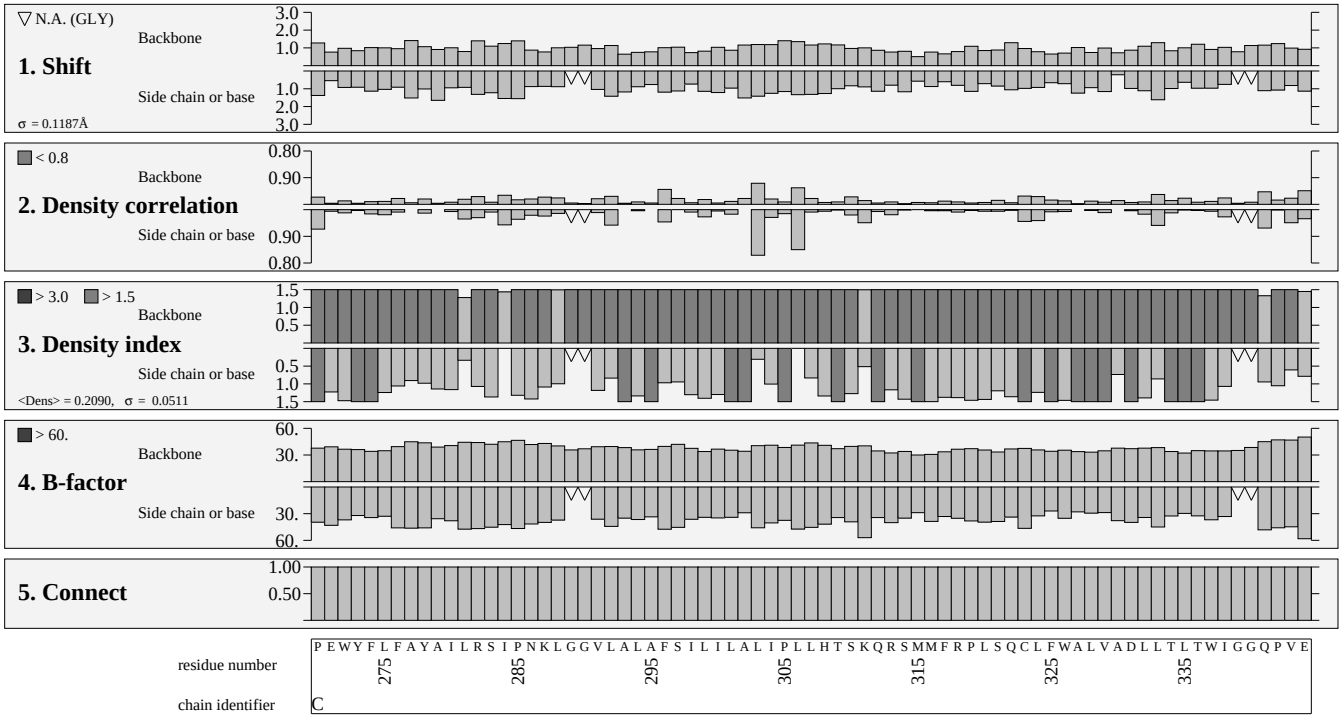
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (8)



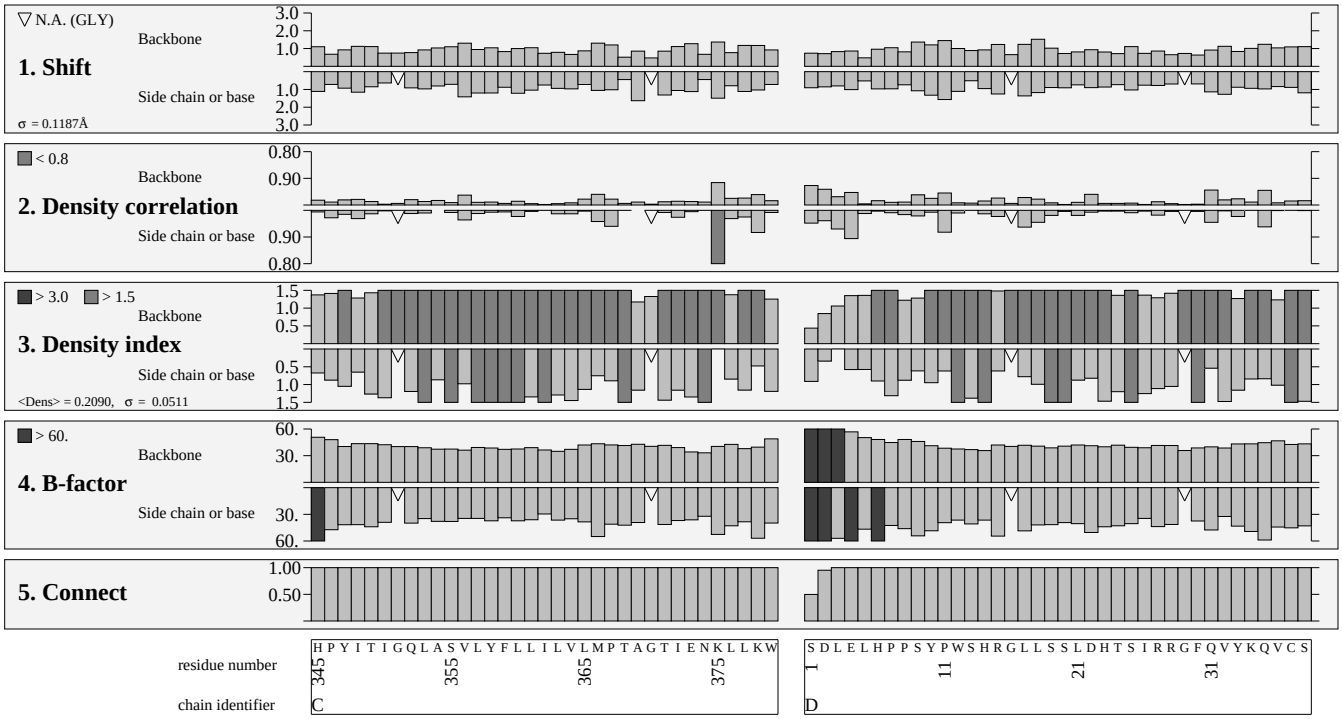


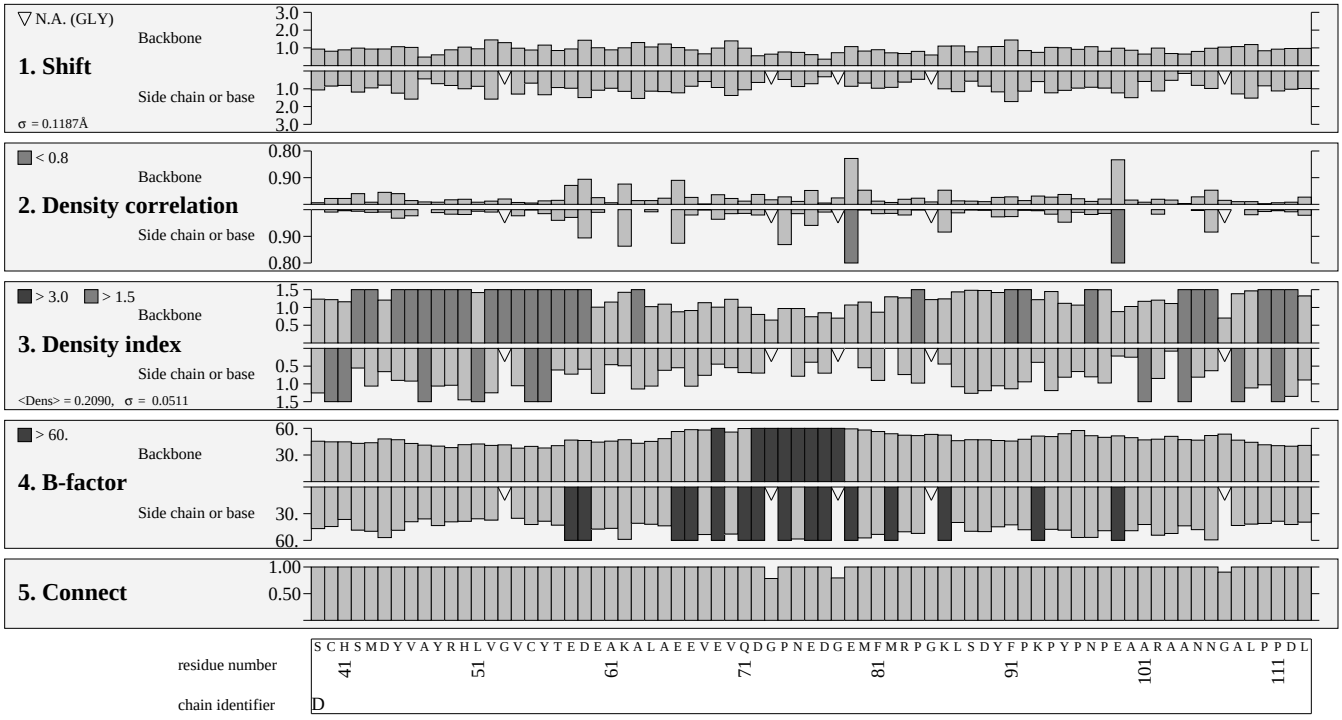
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (9)



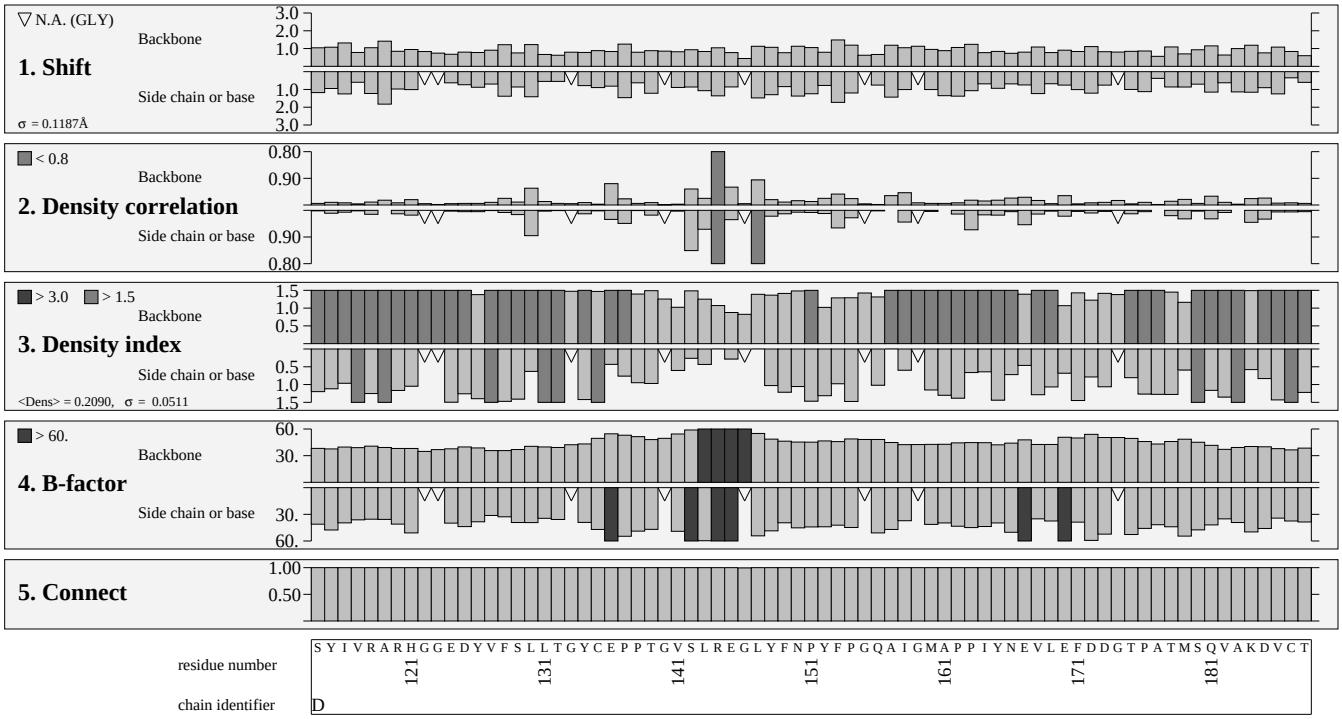


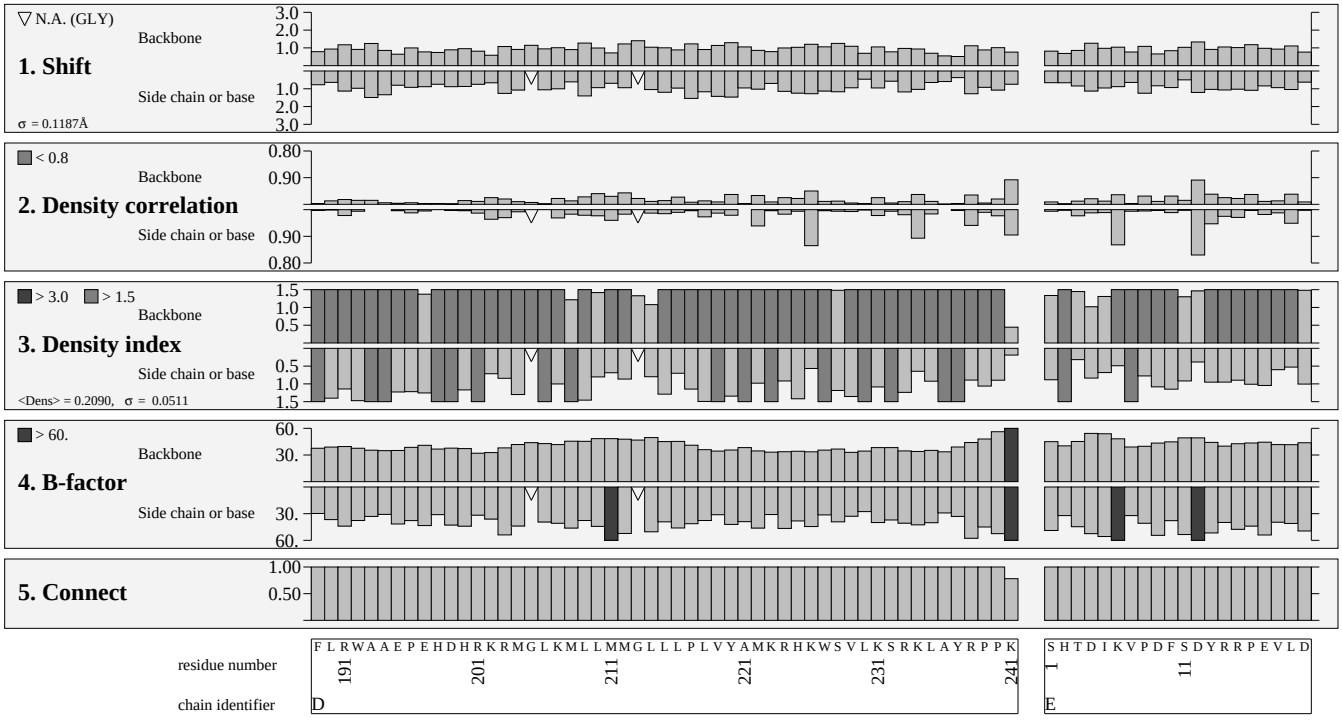
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (10)



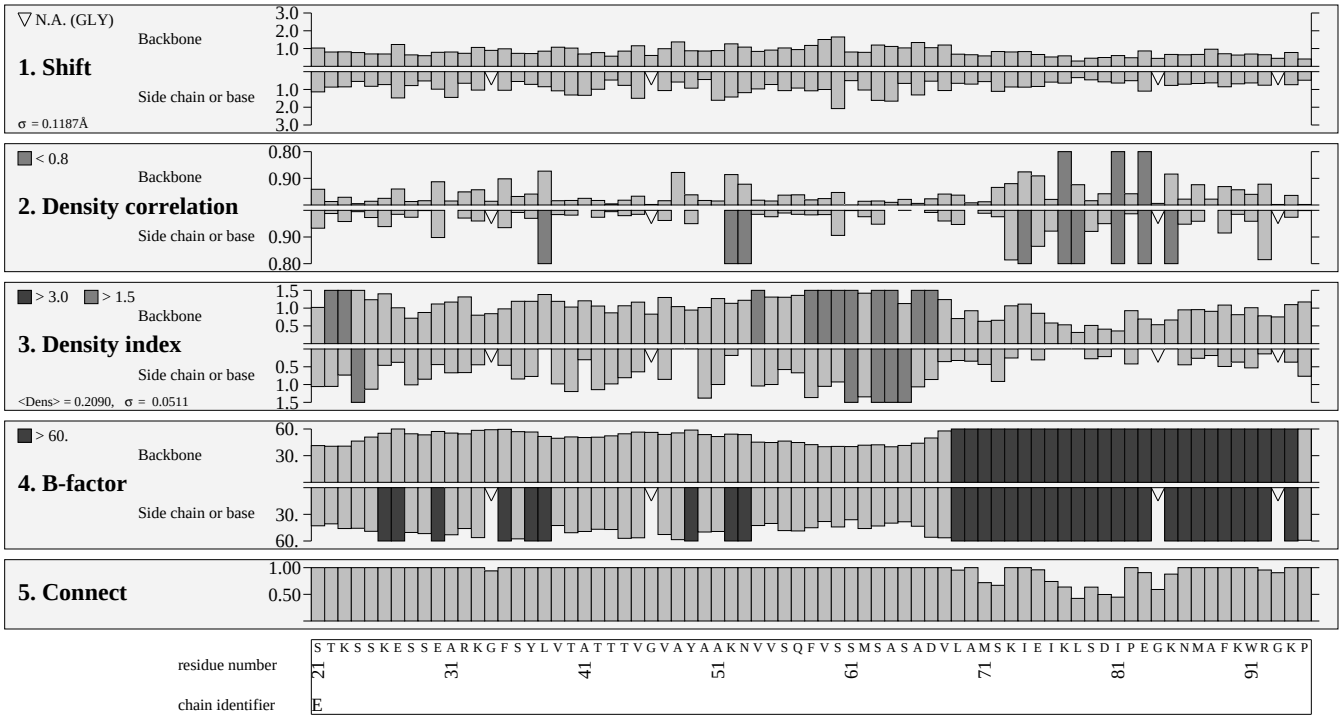


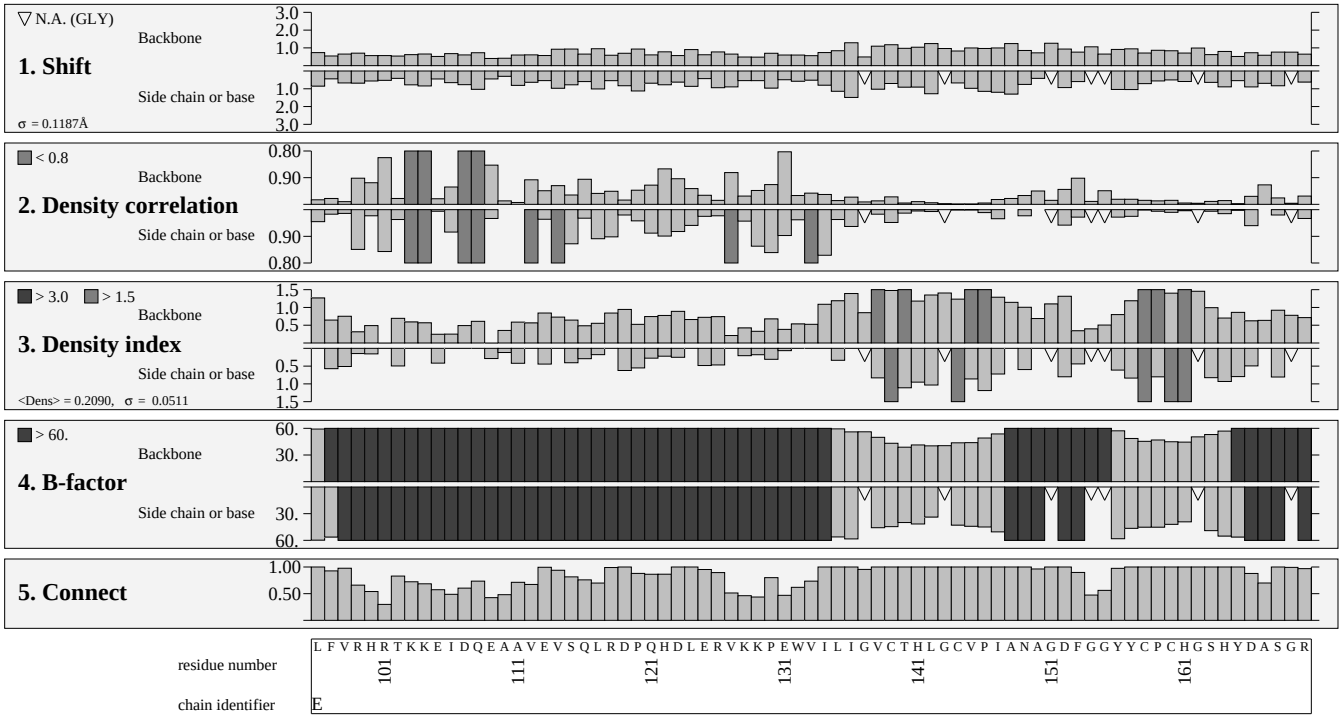
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (11)



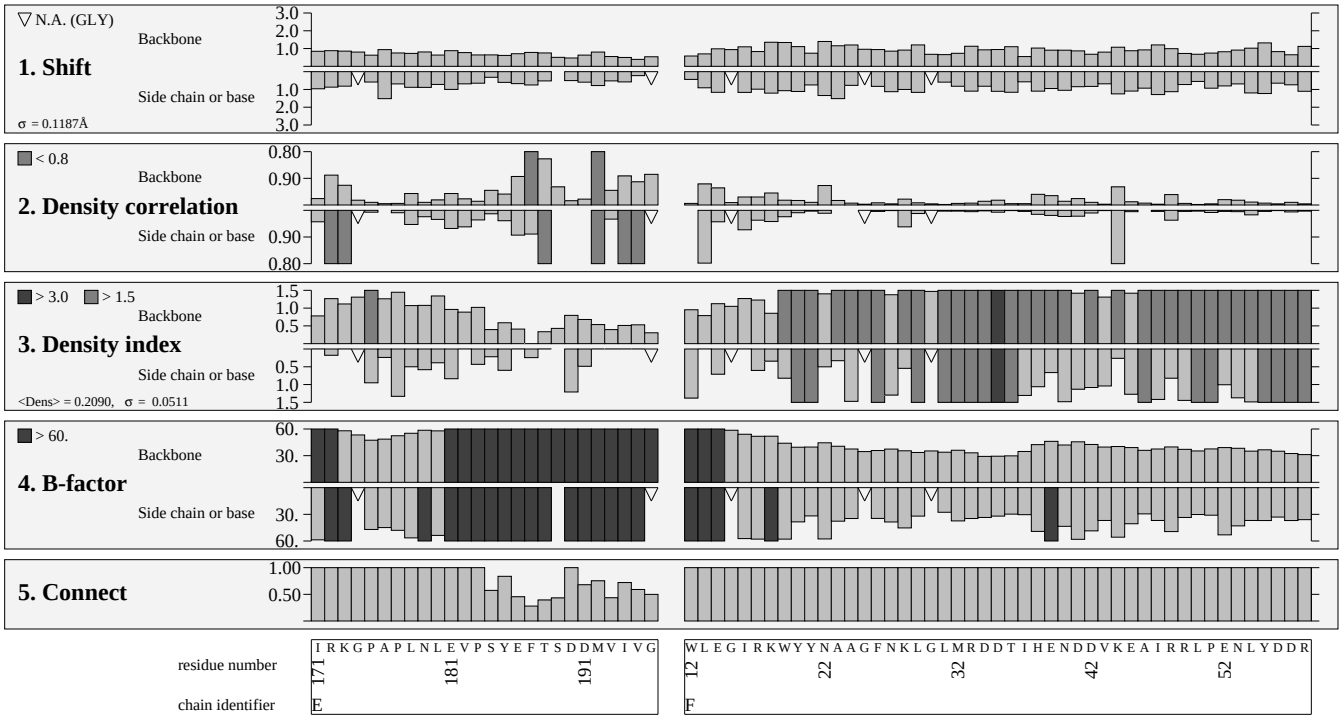


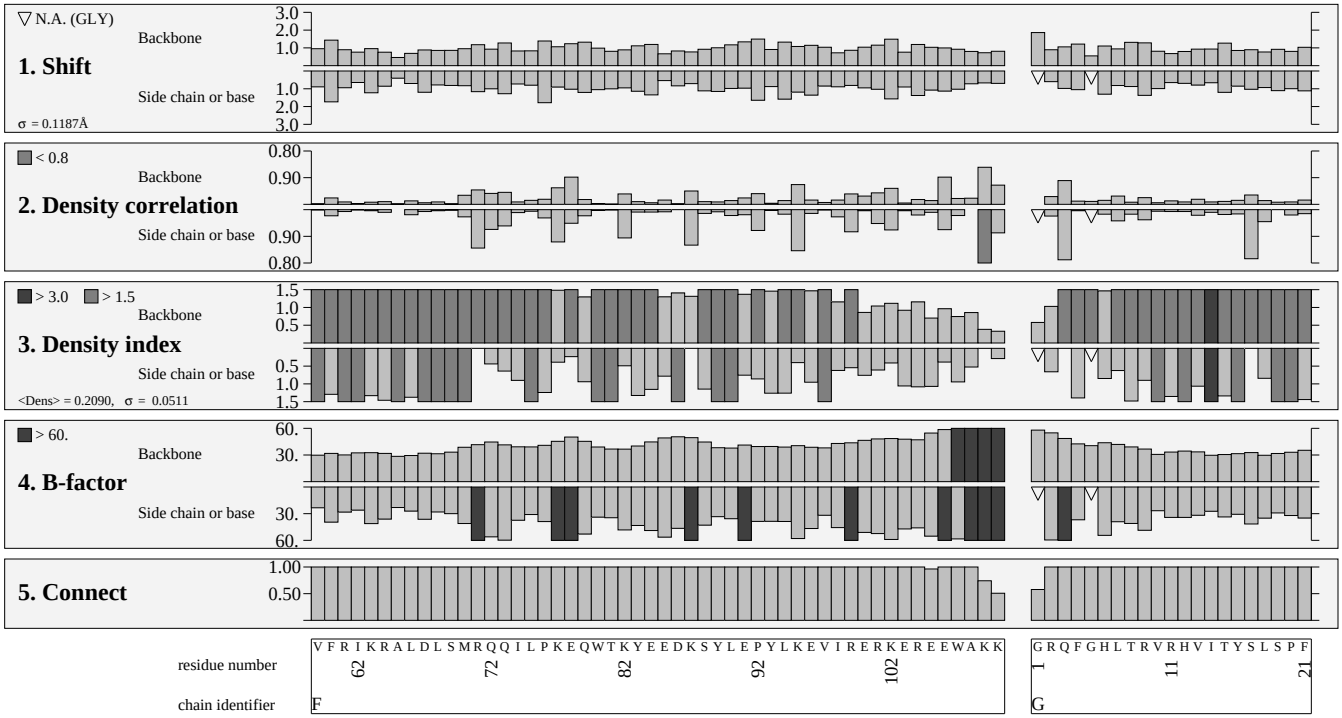
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (12)



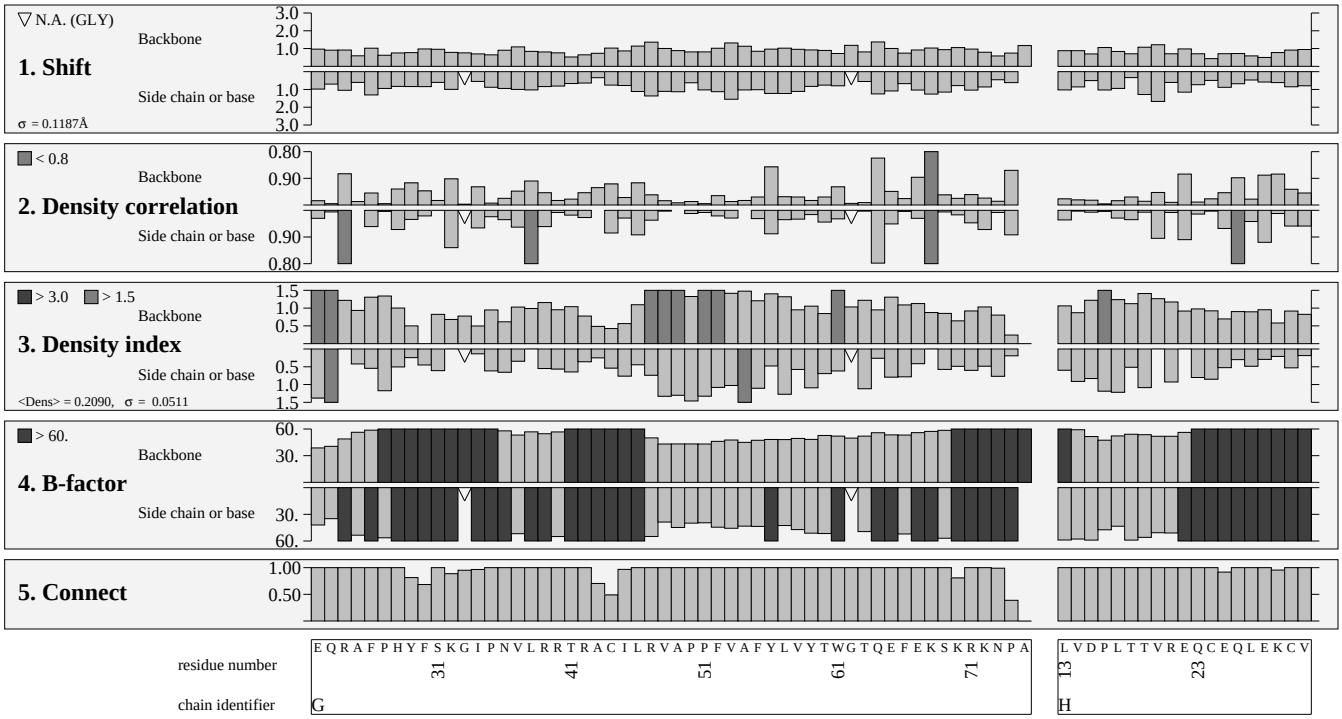


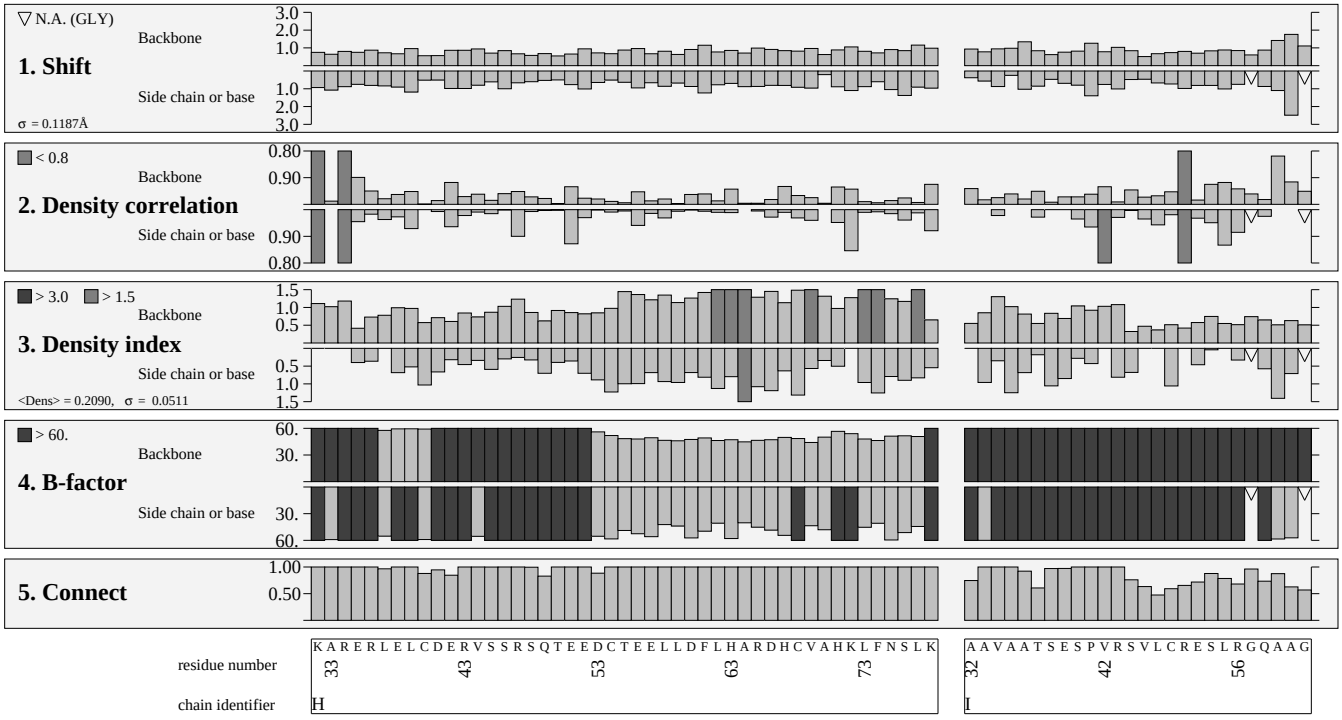
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (13)



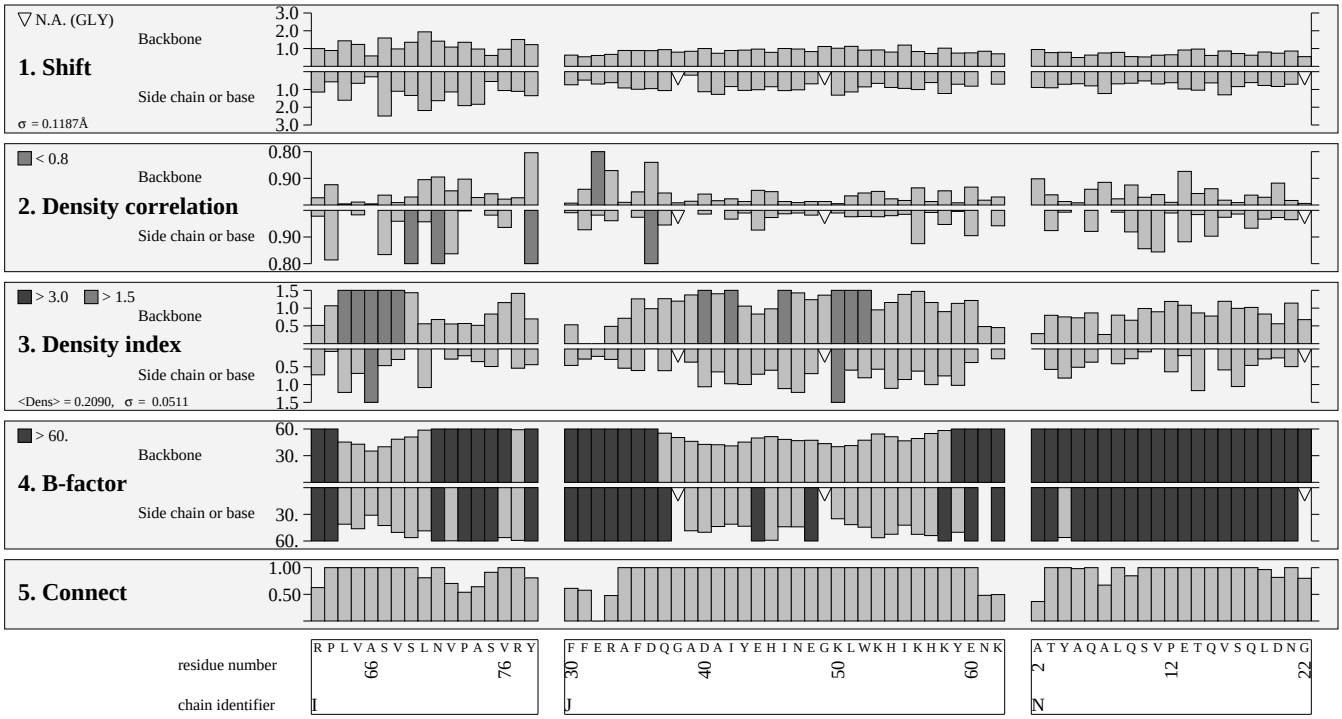


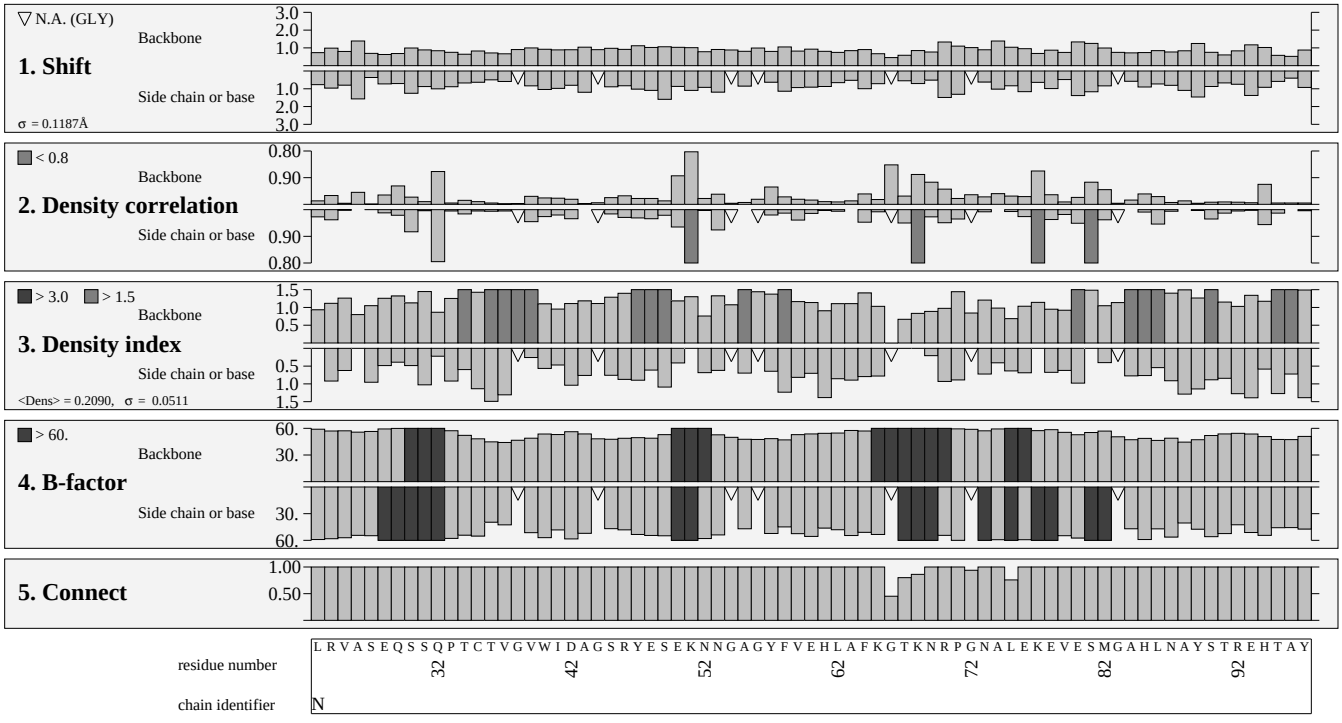
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (14)



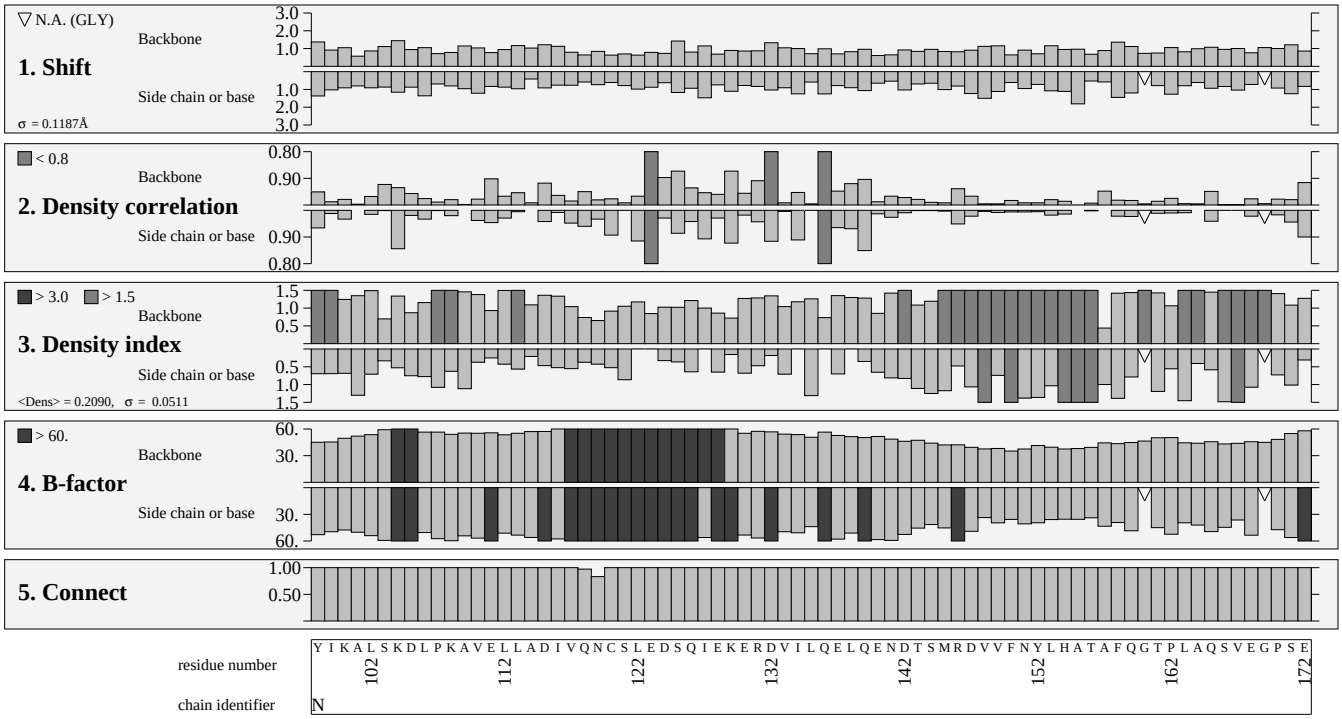


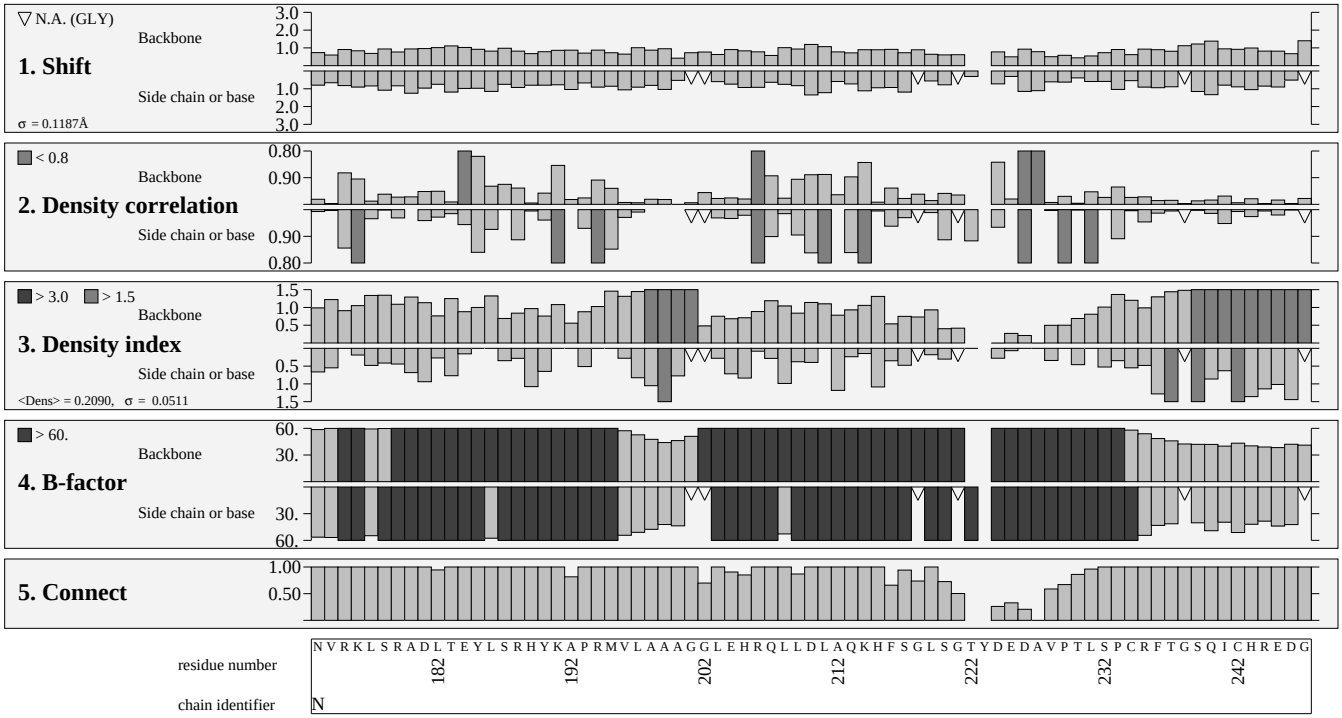
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (15)



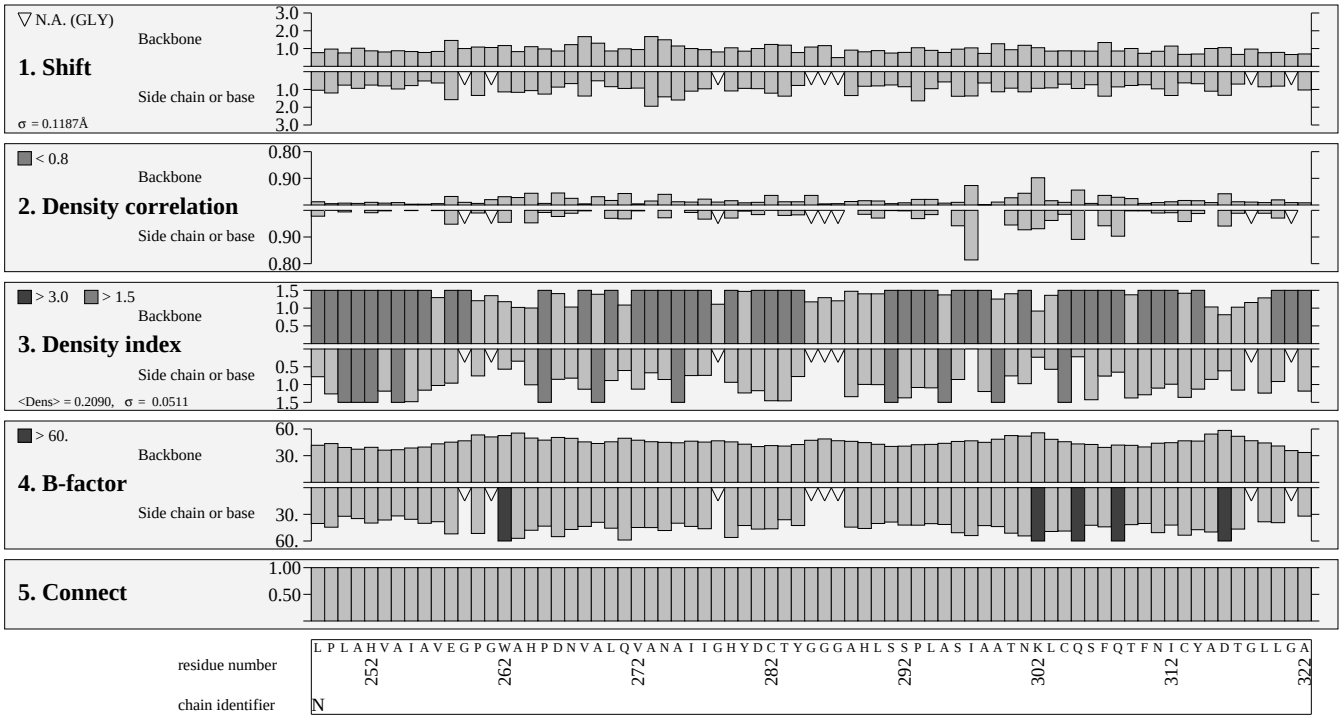


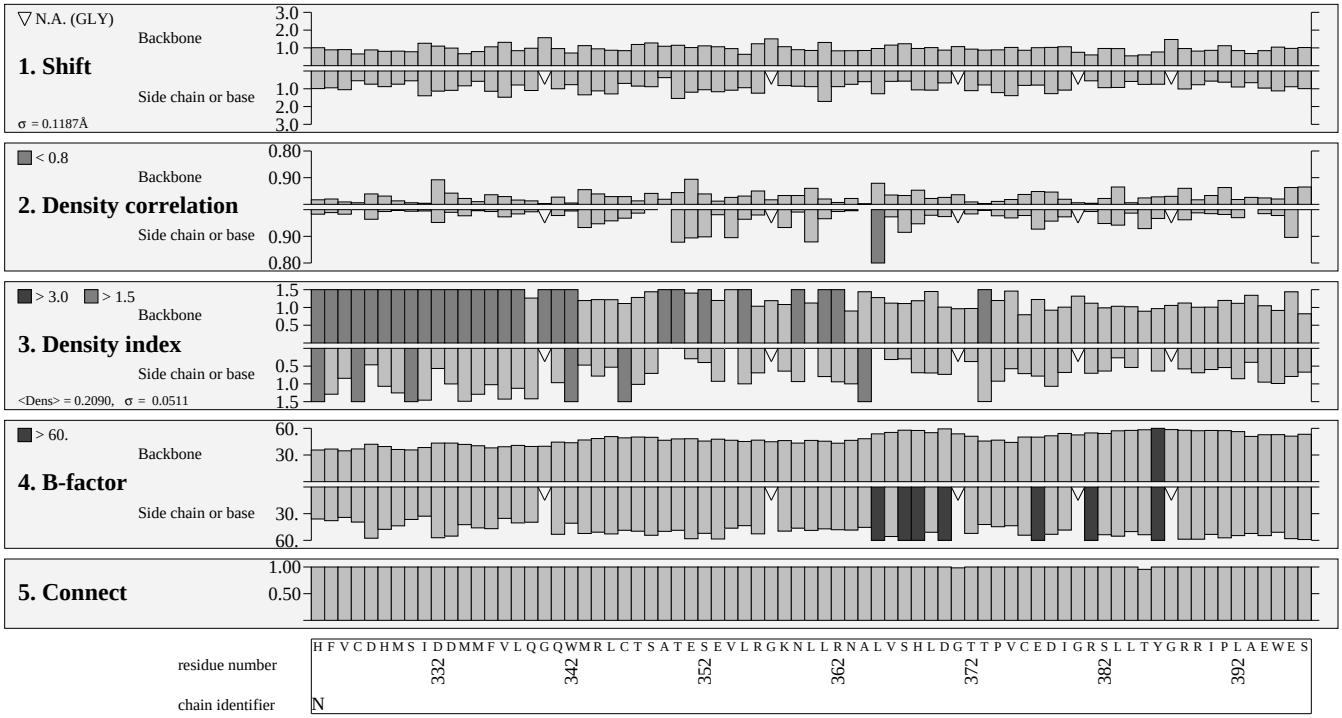
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (16)



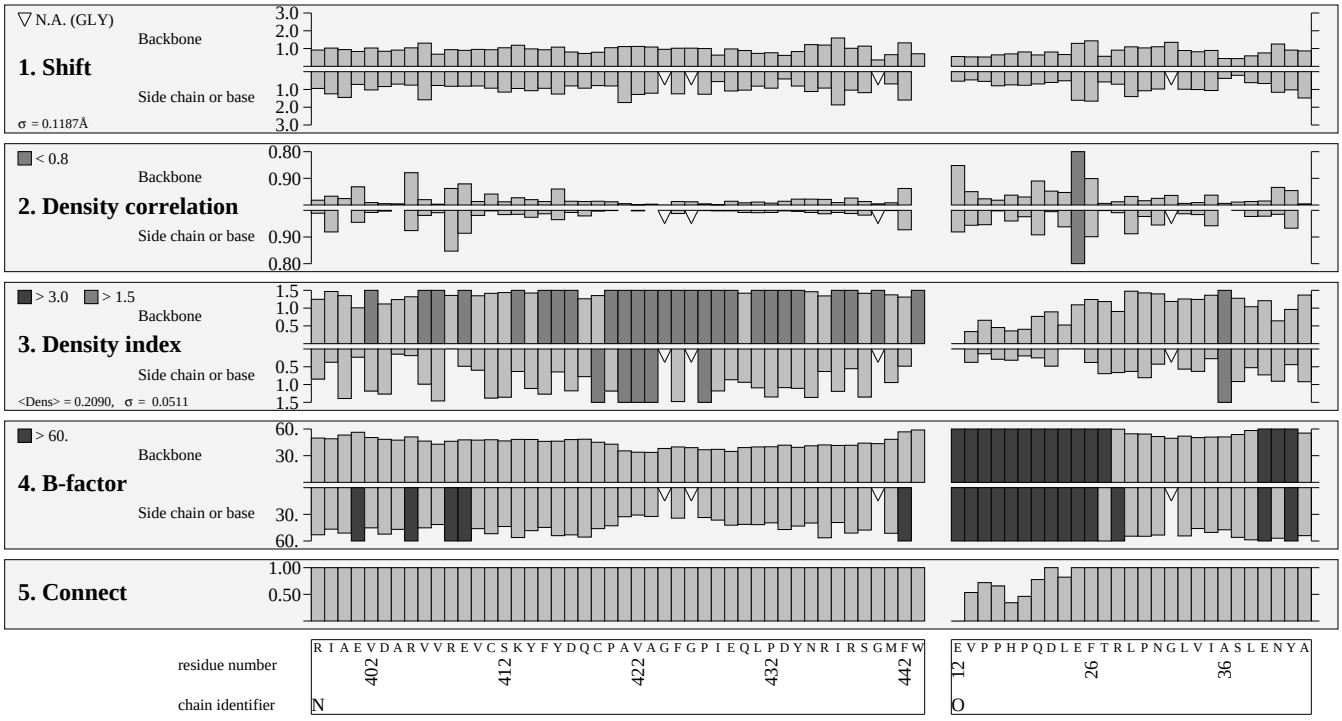


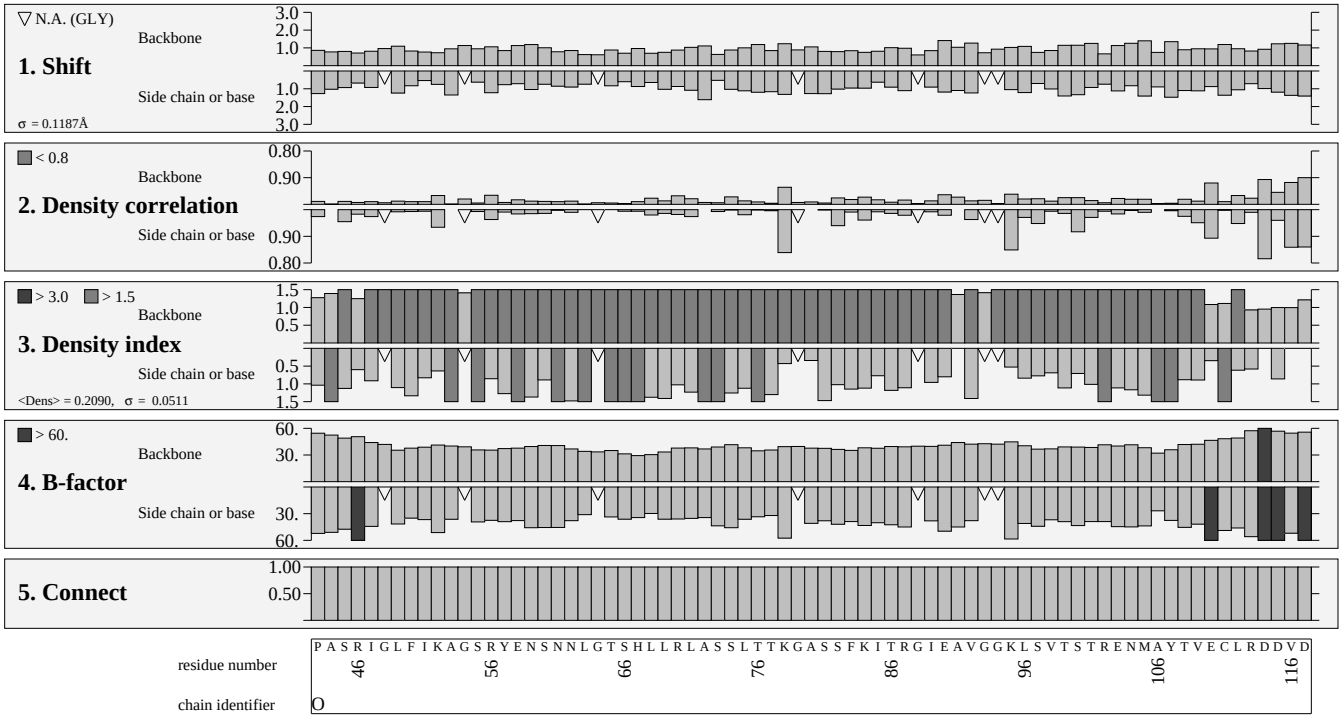
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (17)



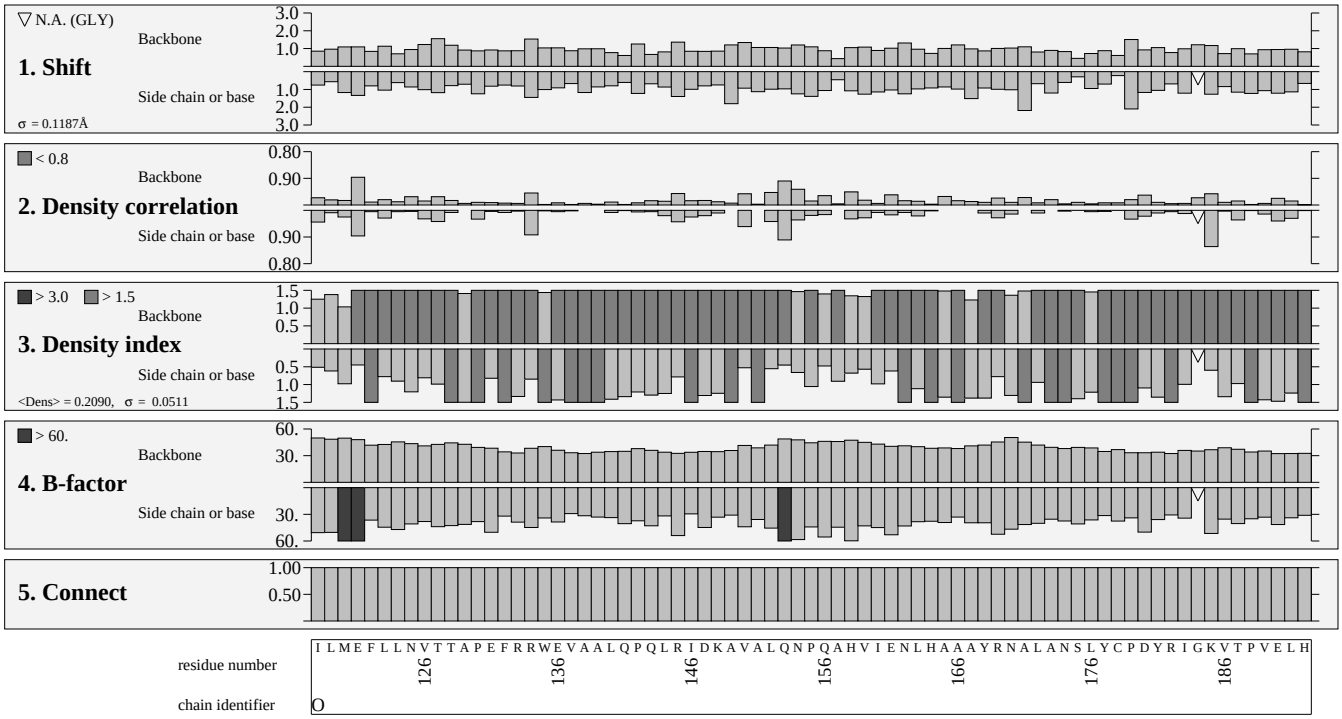


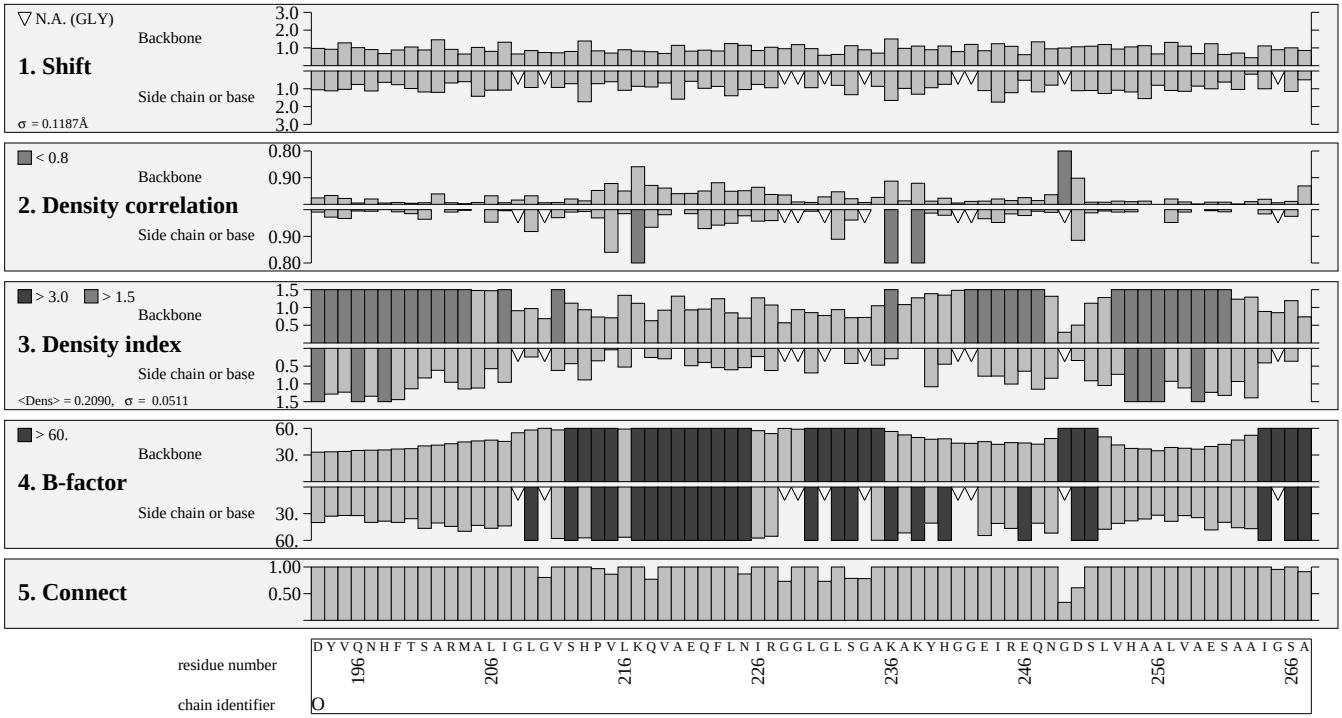
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (18)

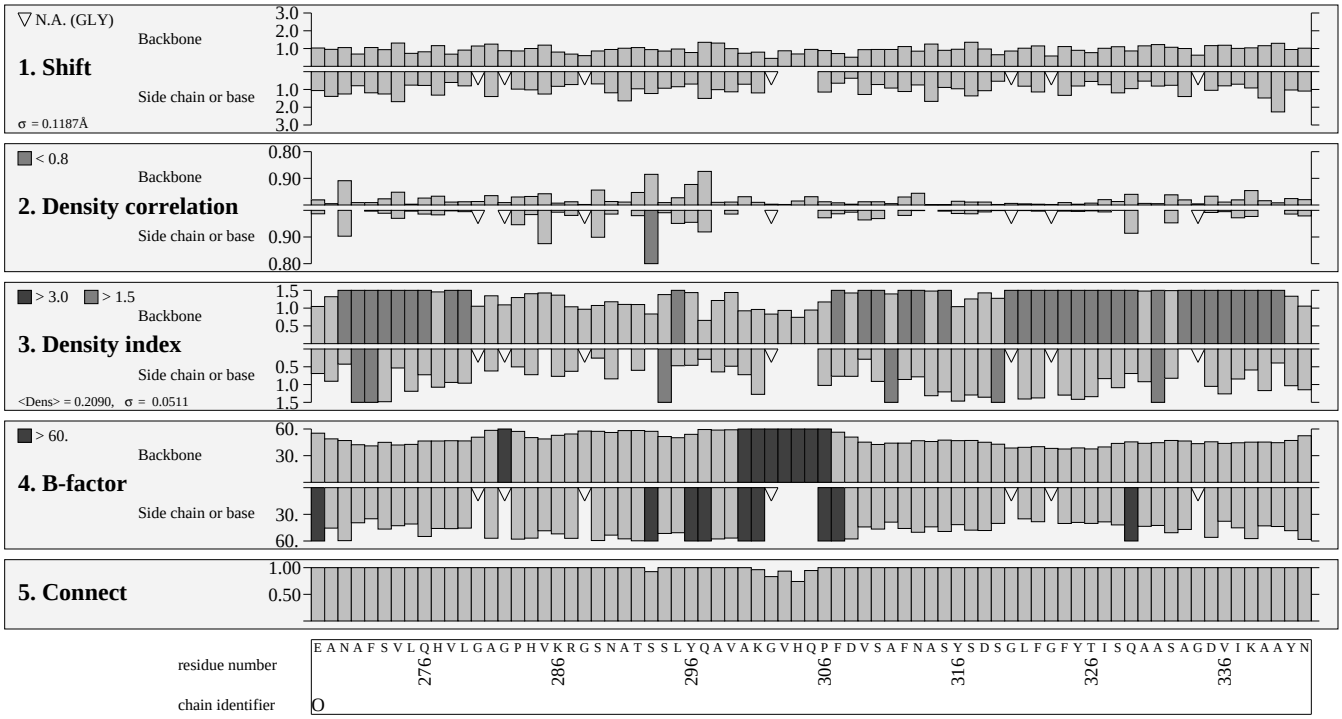


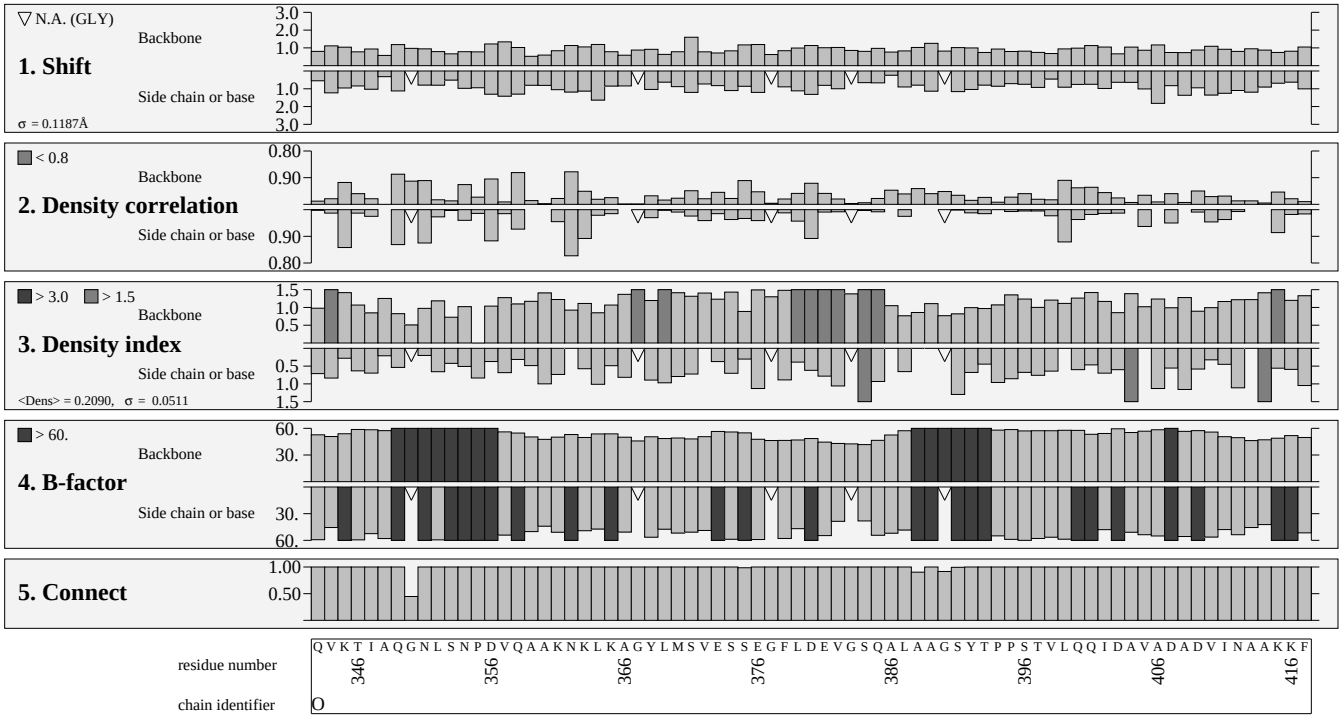


Structure Factor Check

1PPJ

Local estimation (19)



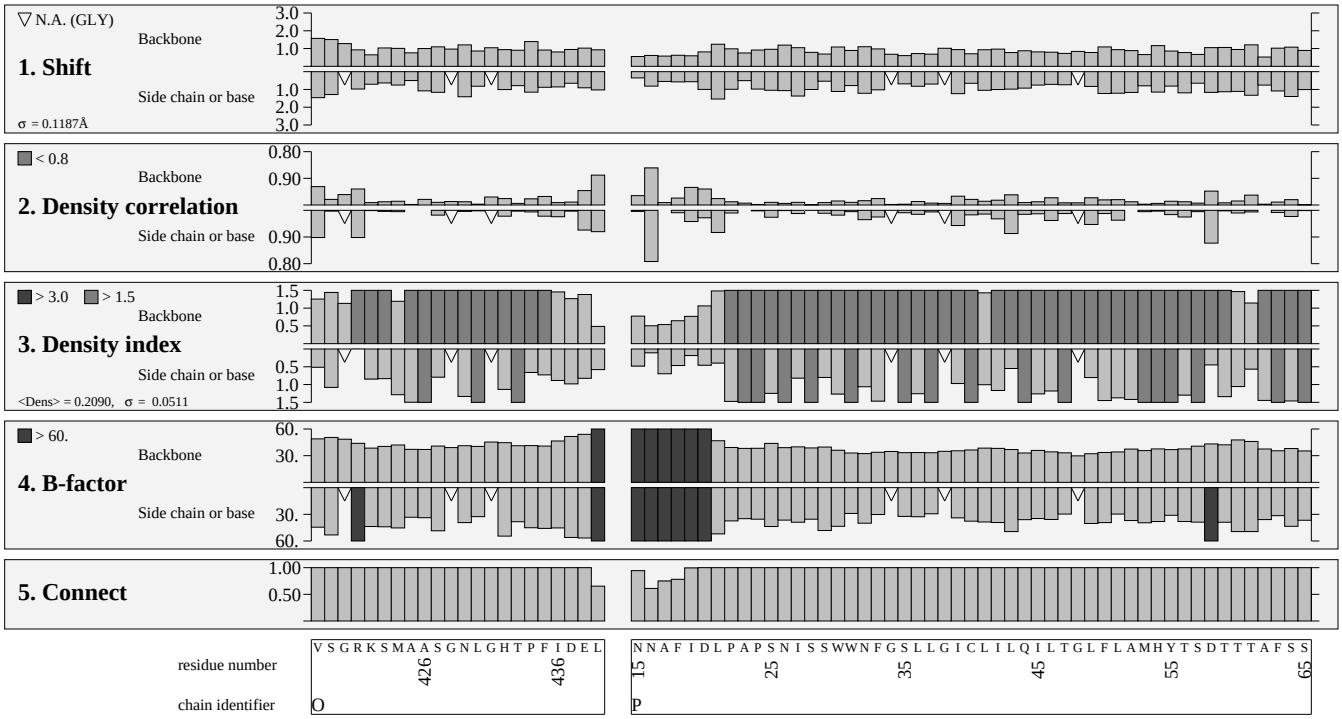


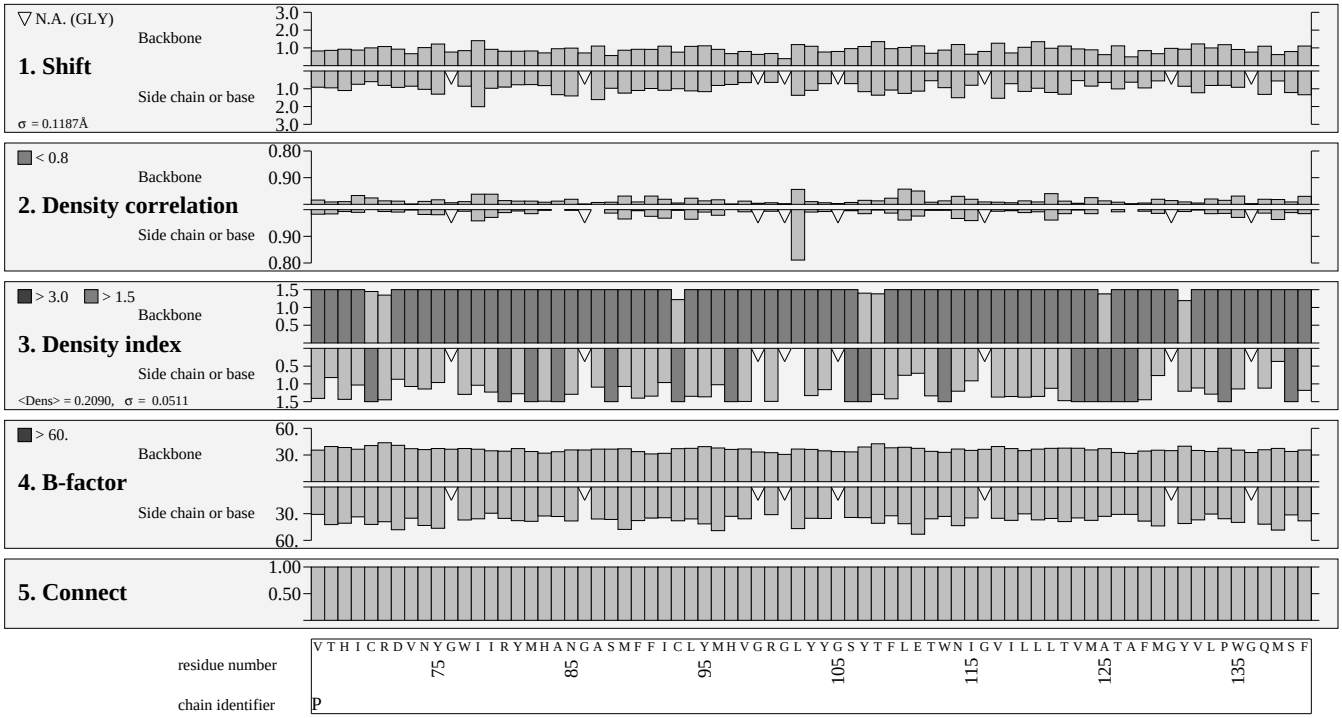
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (20)



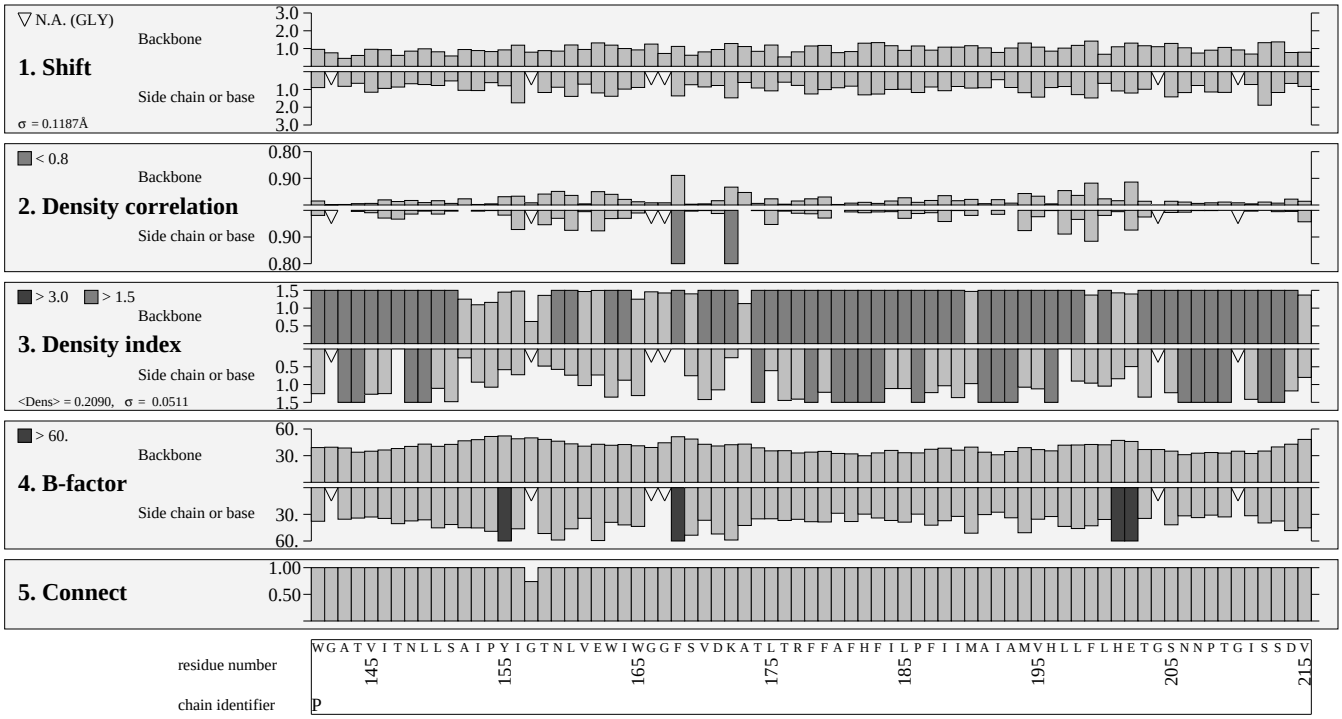


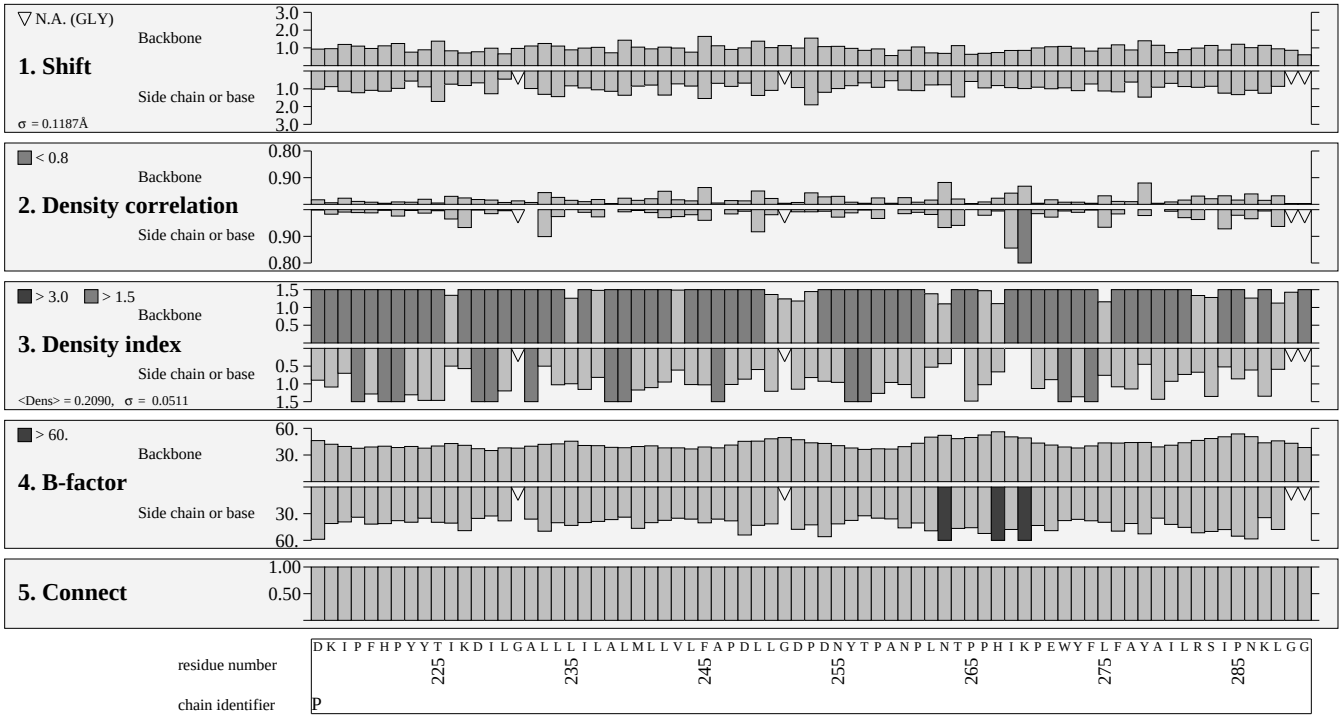
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (21)



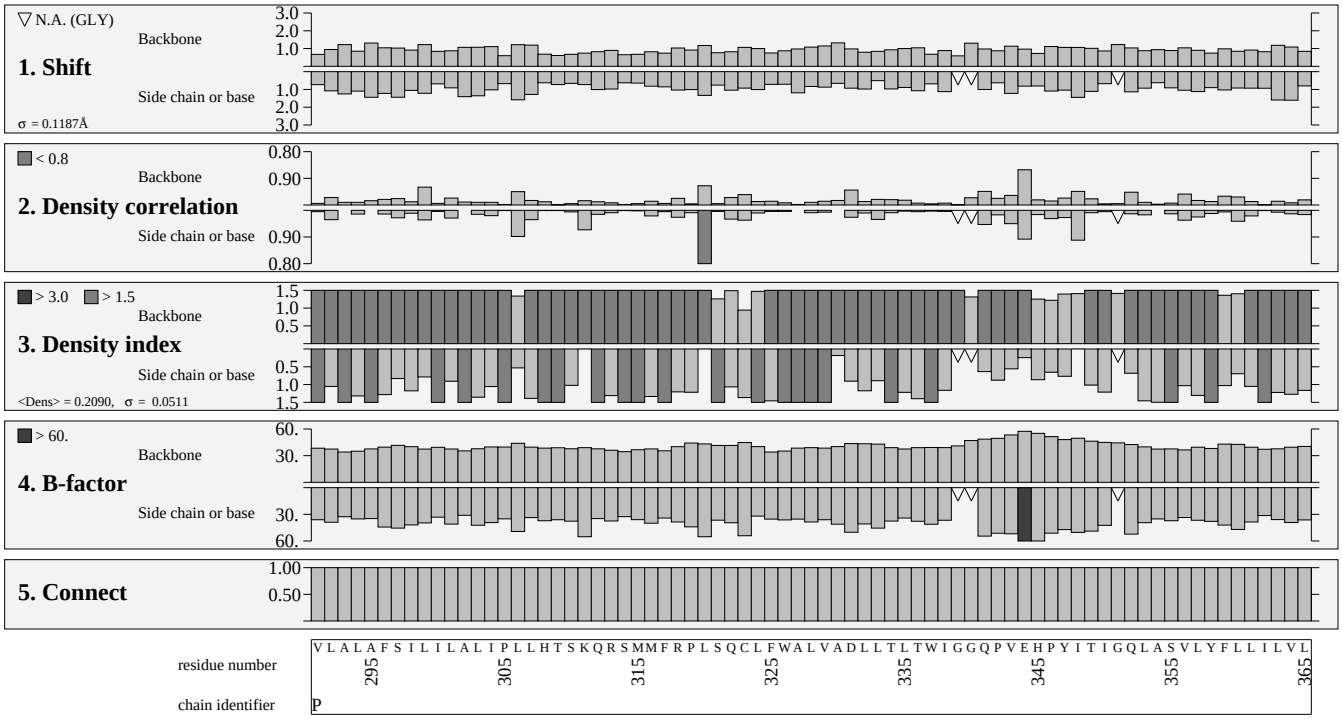


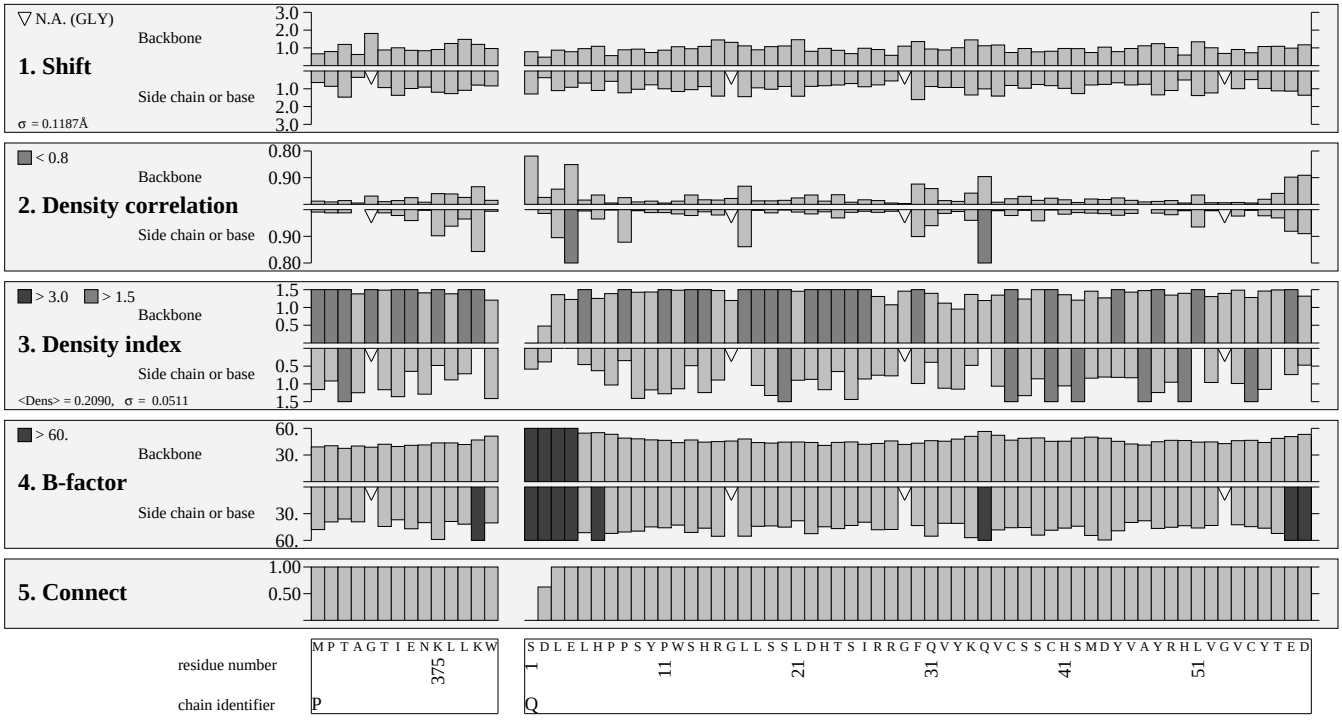
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (22)



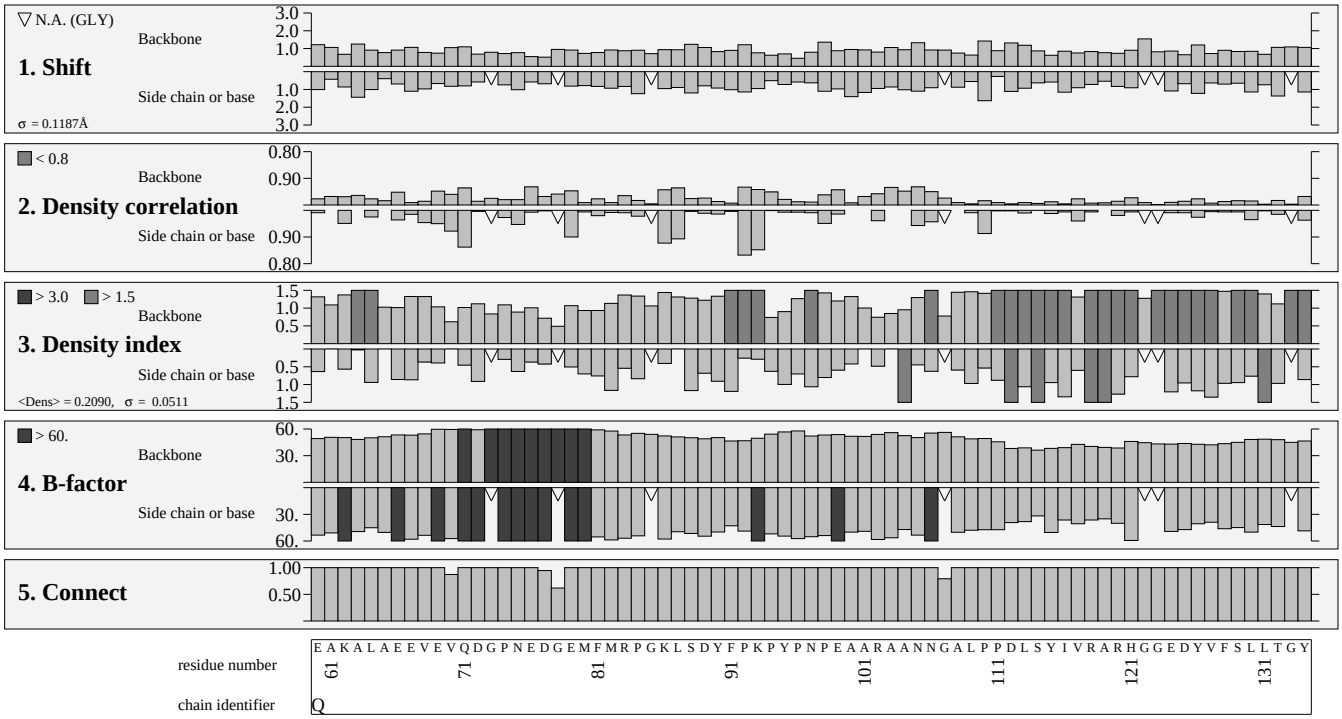


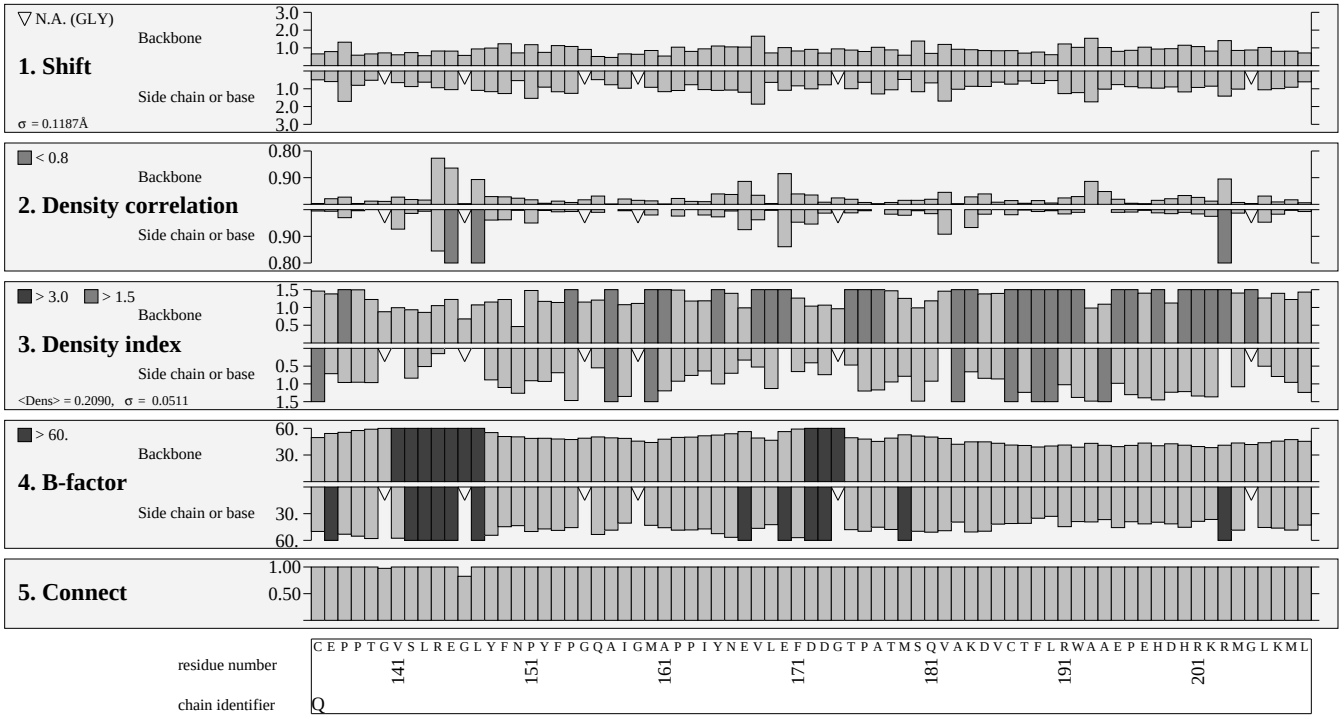
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (23)



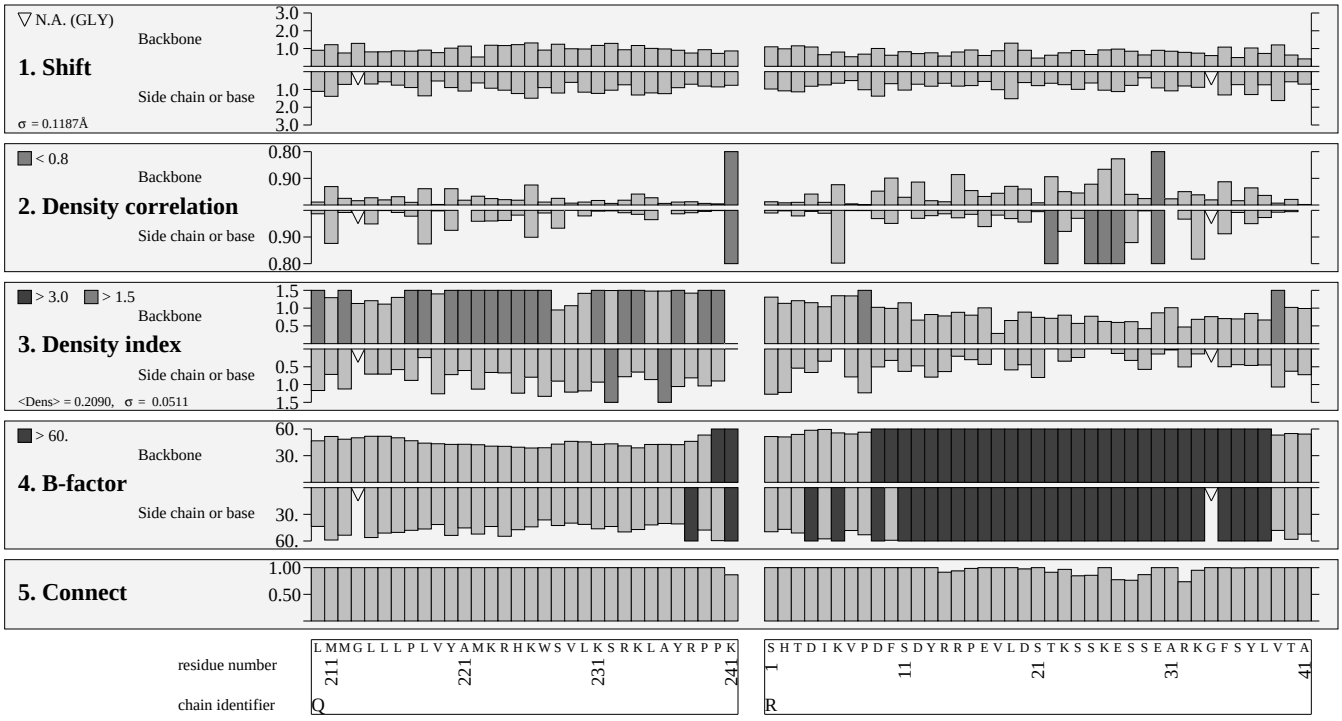


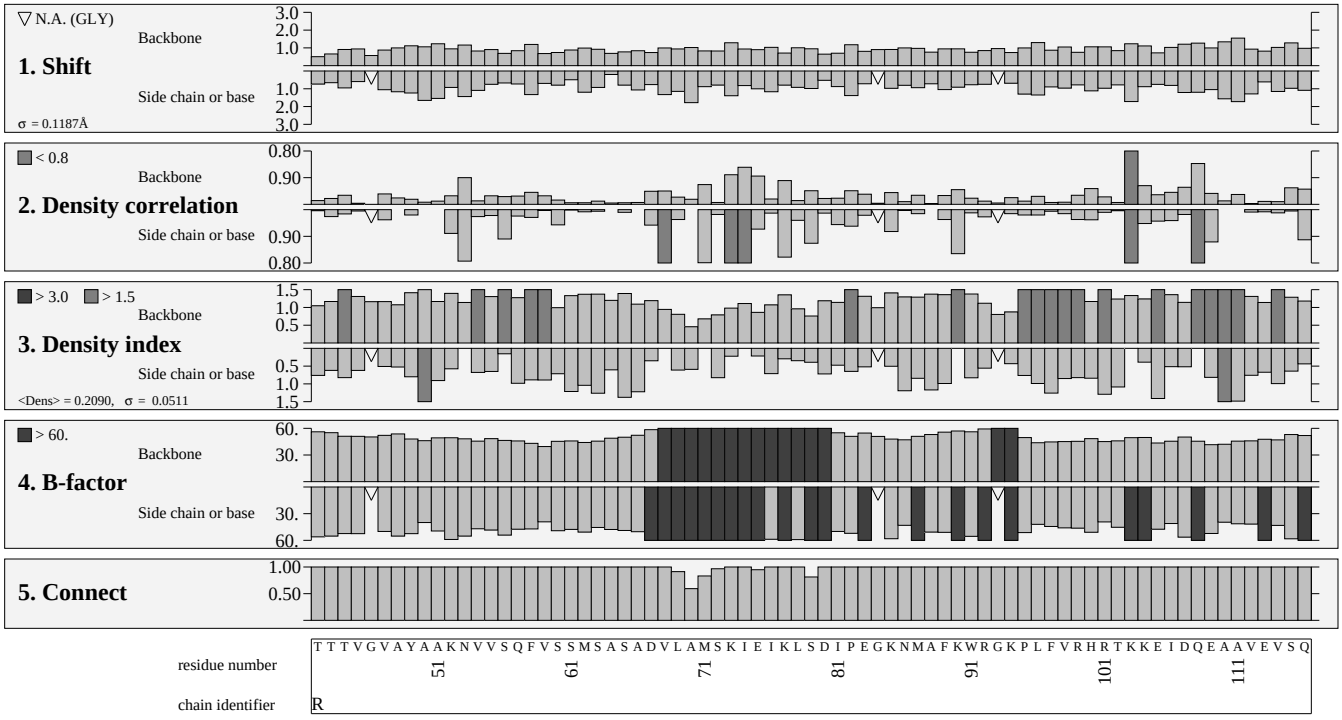
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (24)



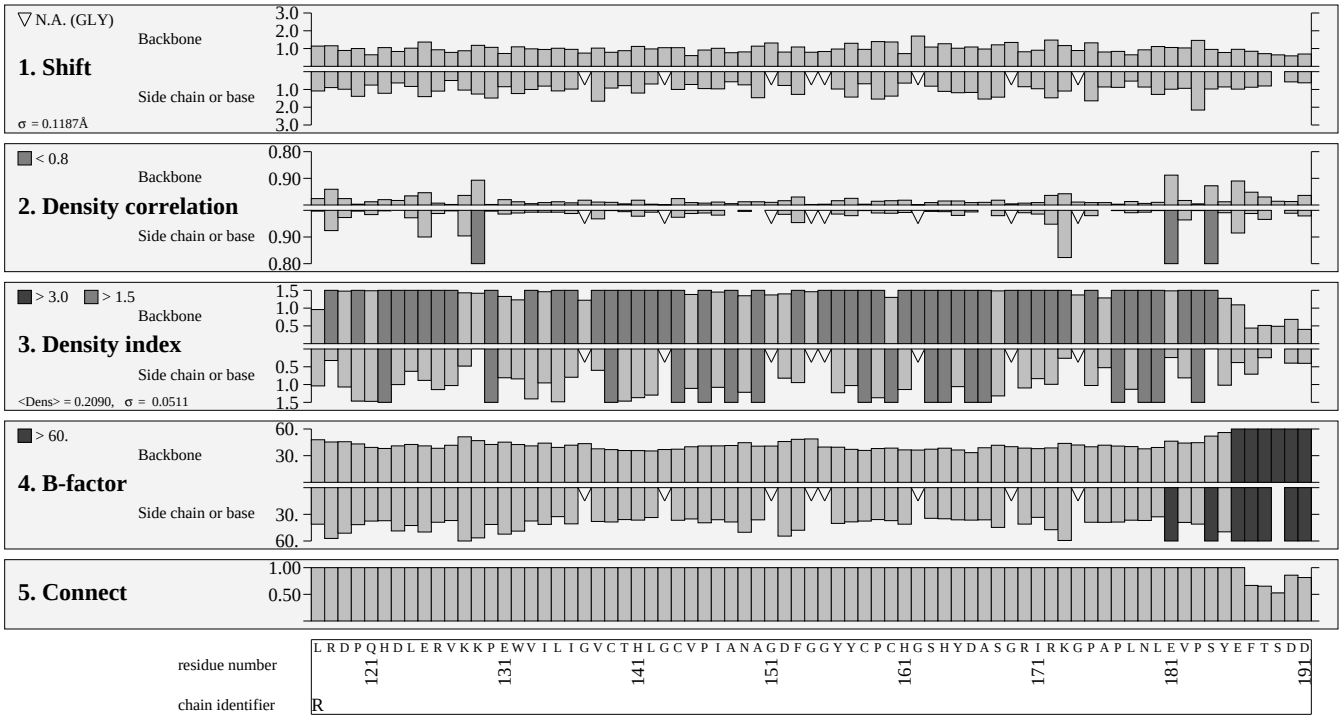


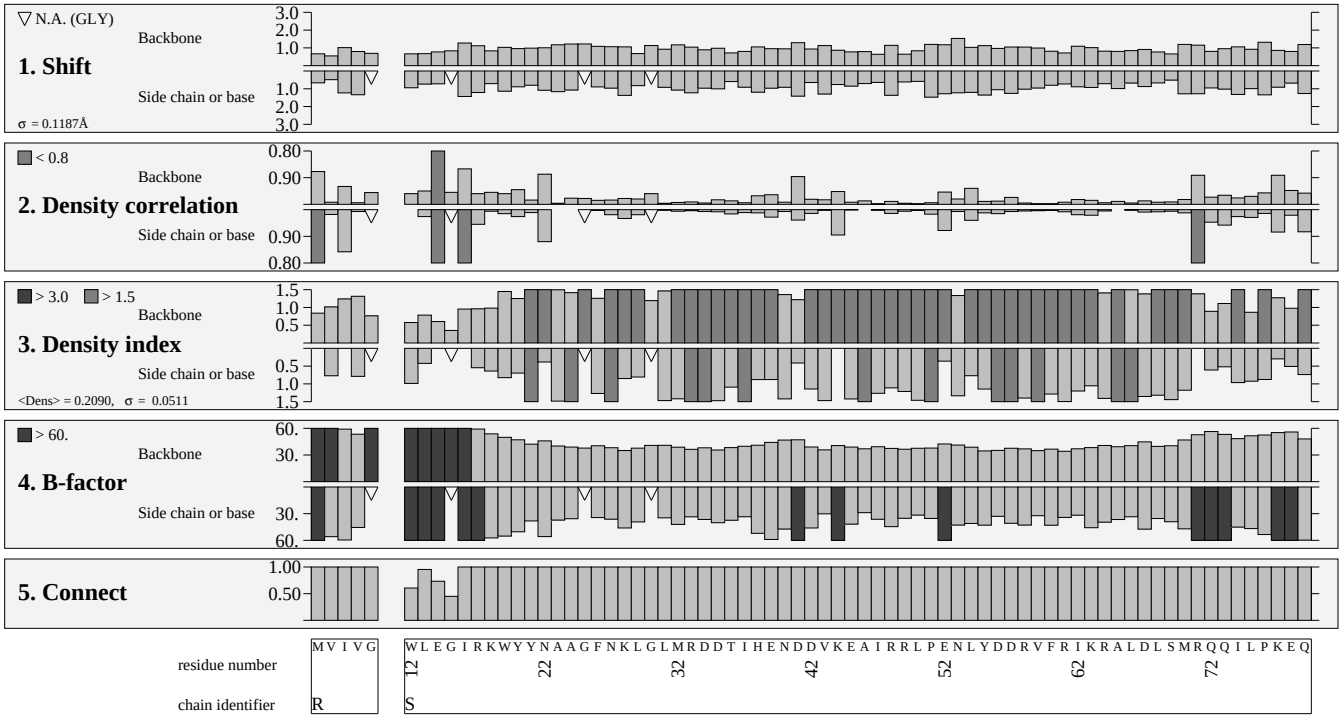
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (25)



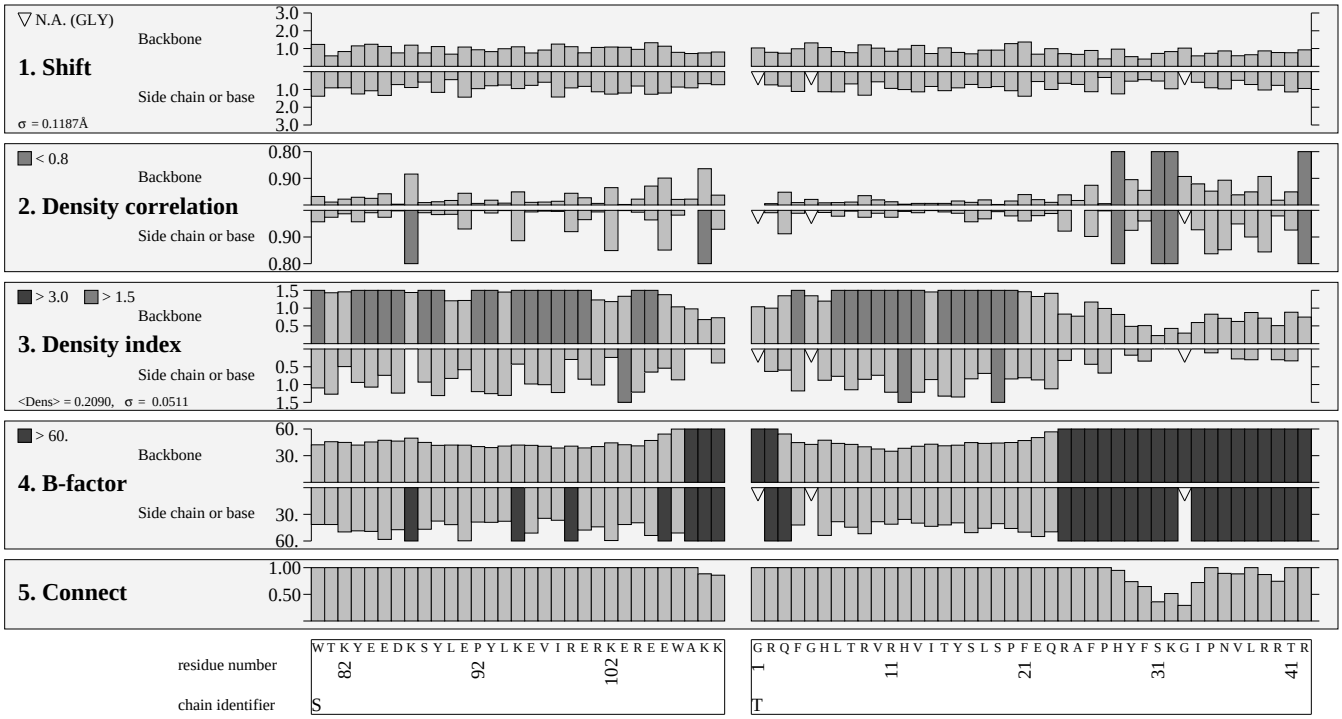


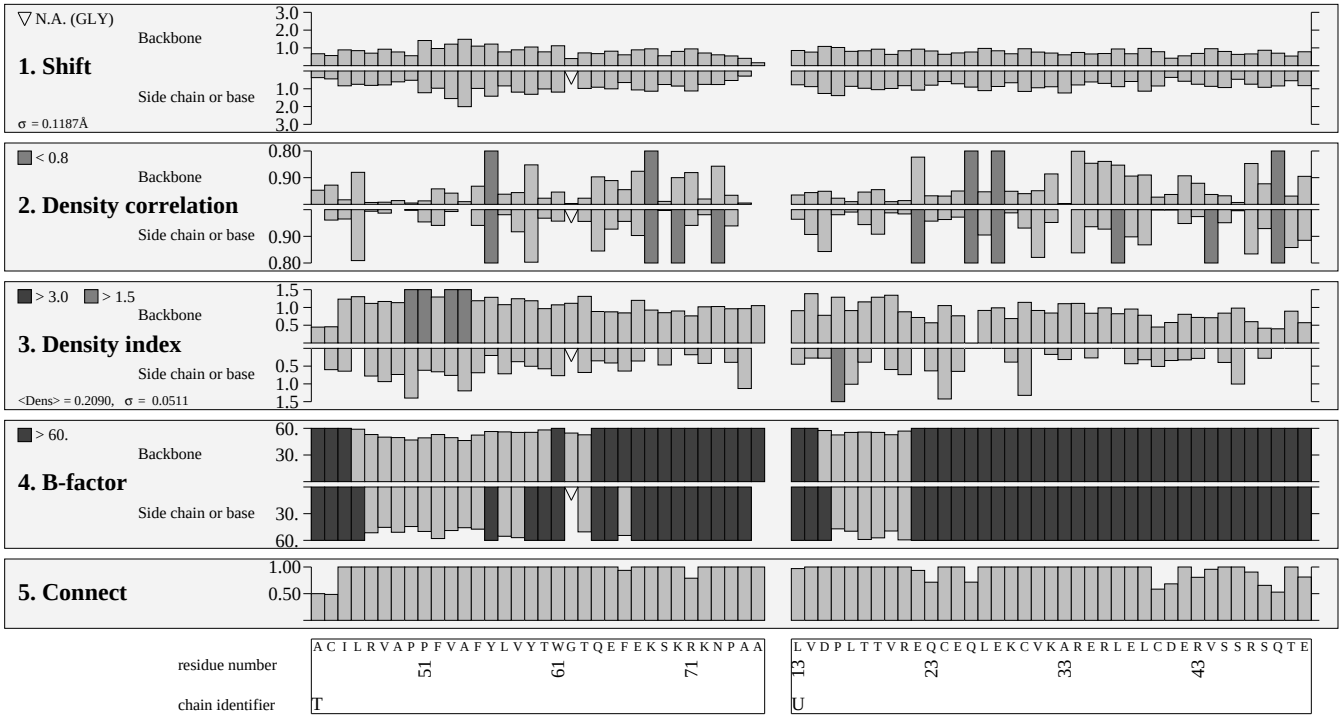
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (26)

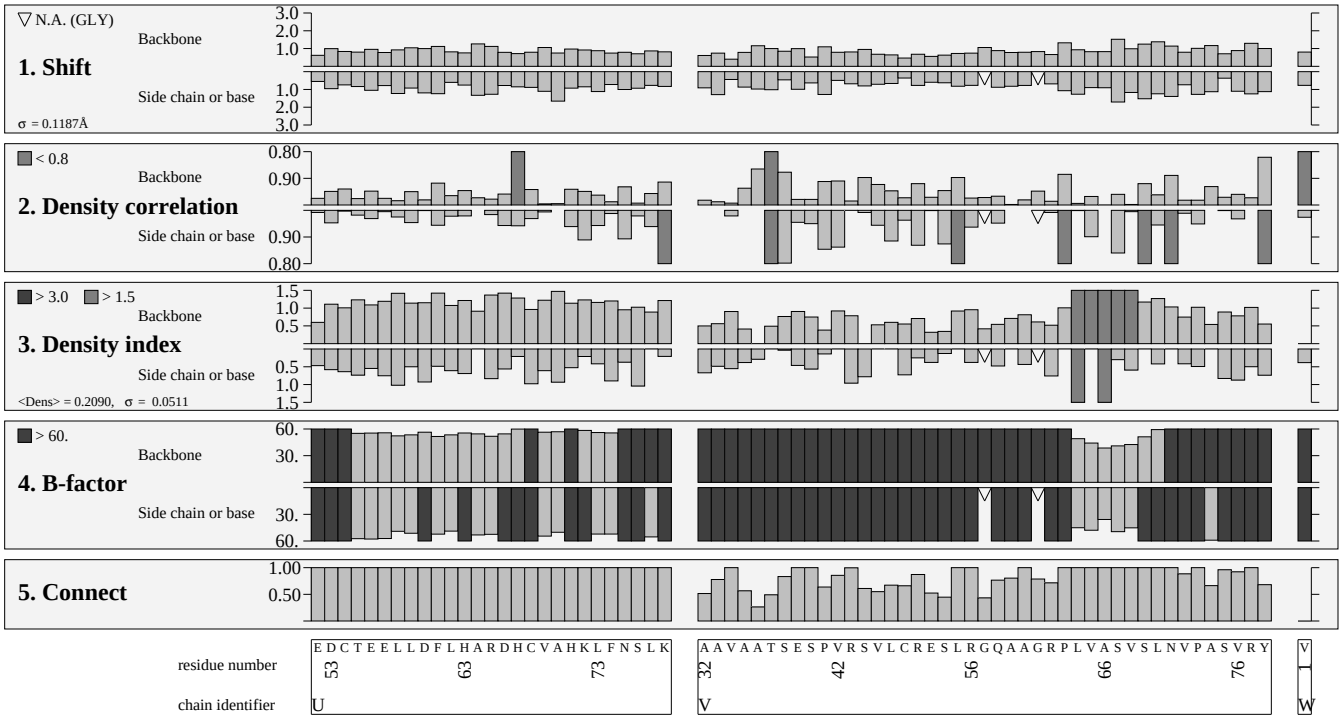


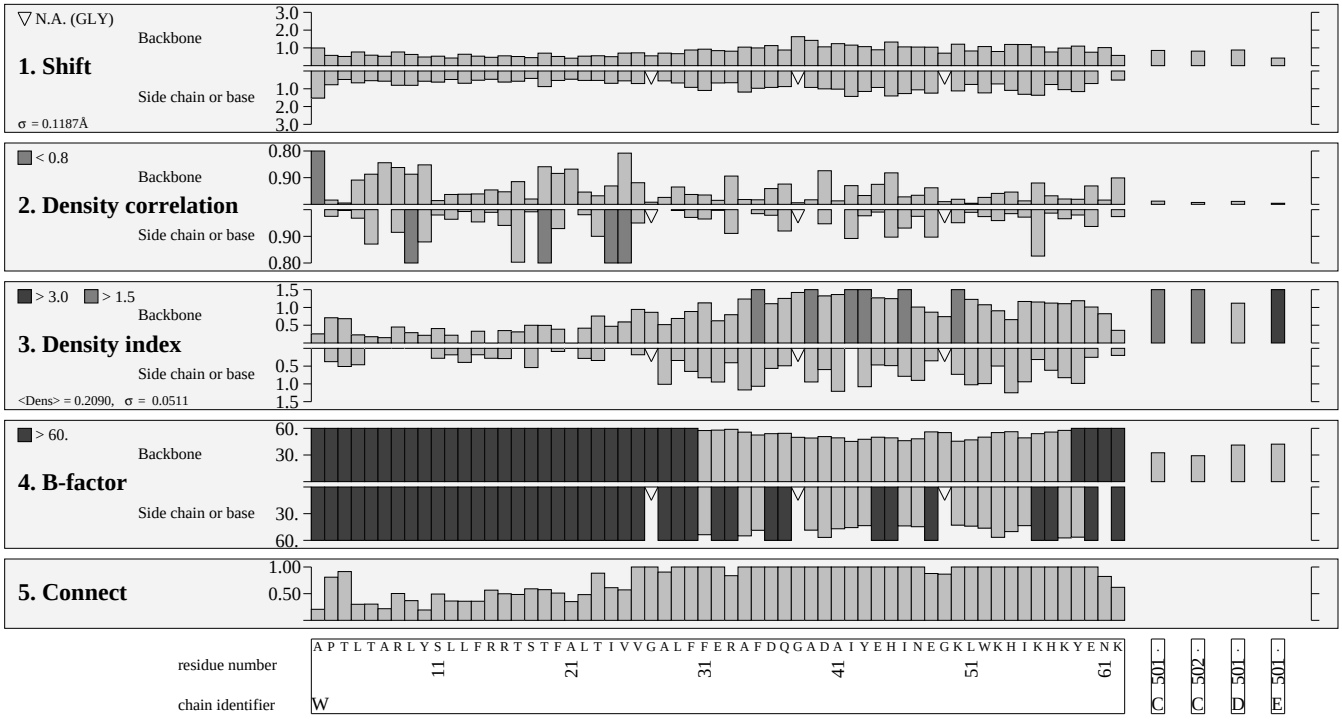


Structure Factor Check

1PPJ

Local estimation (27)



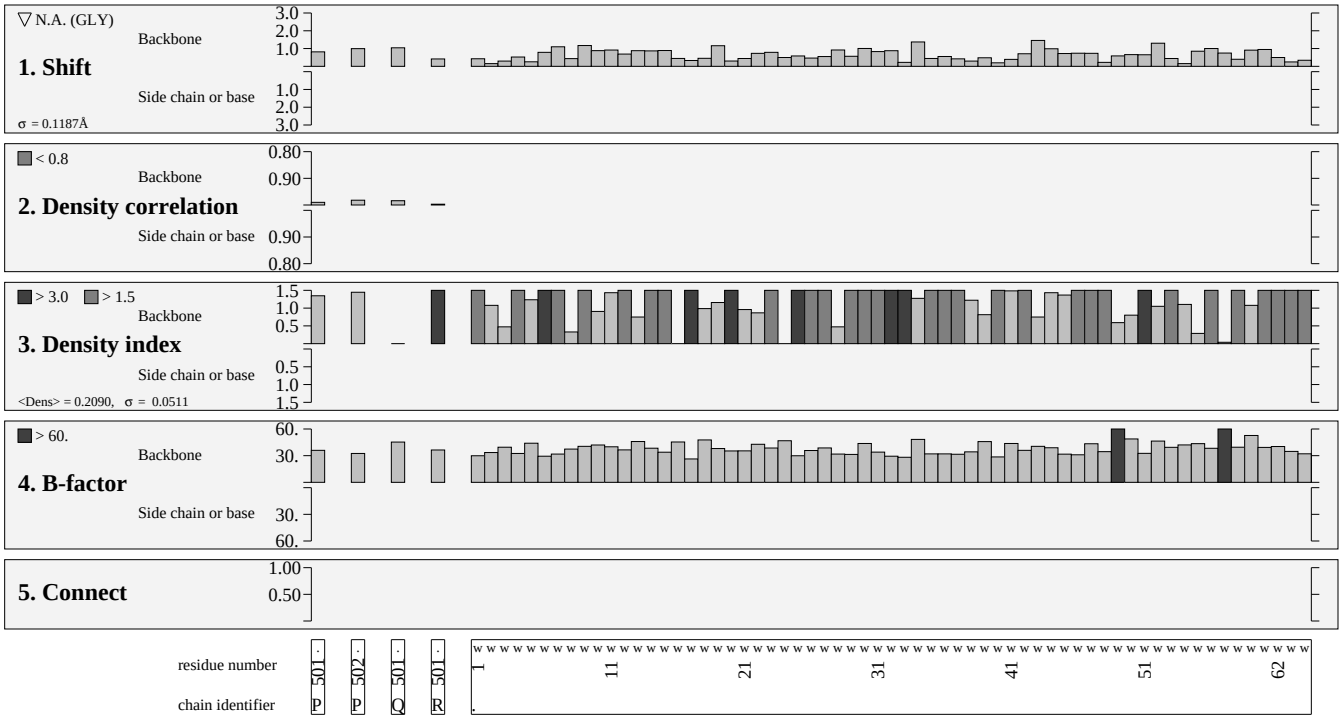


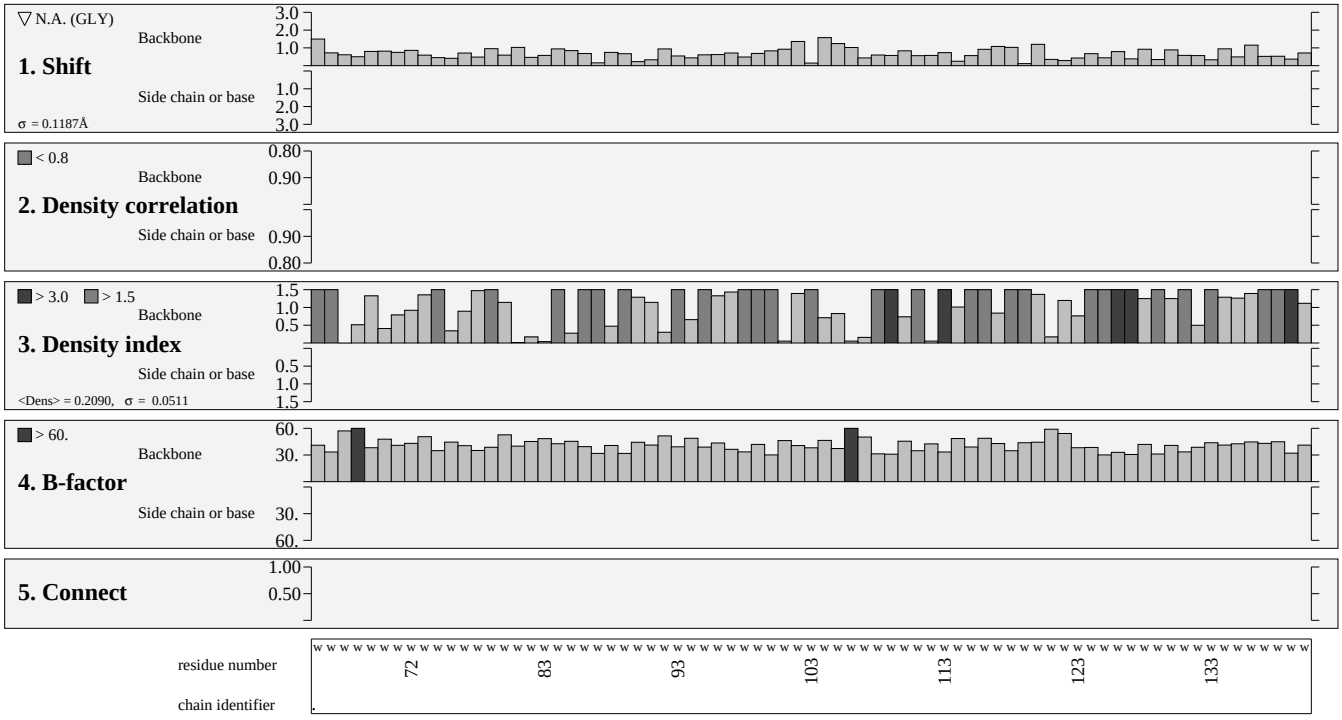
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (28)



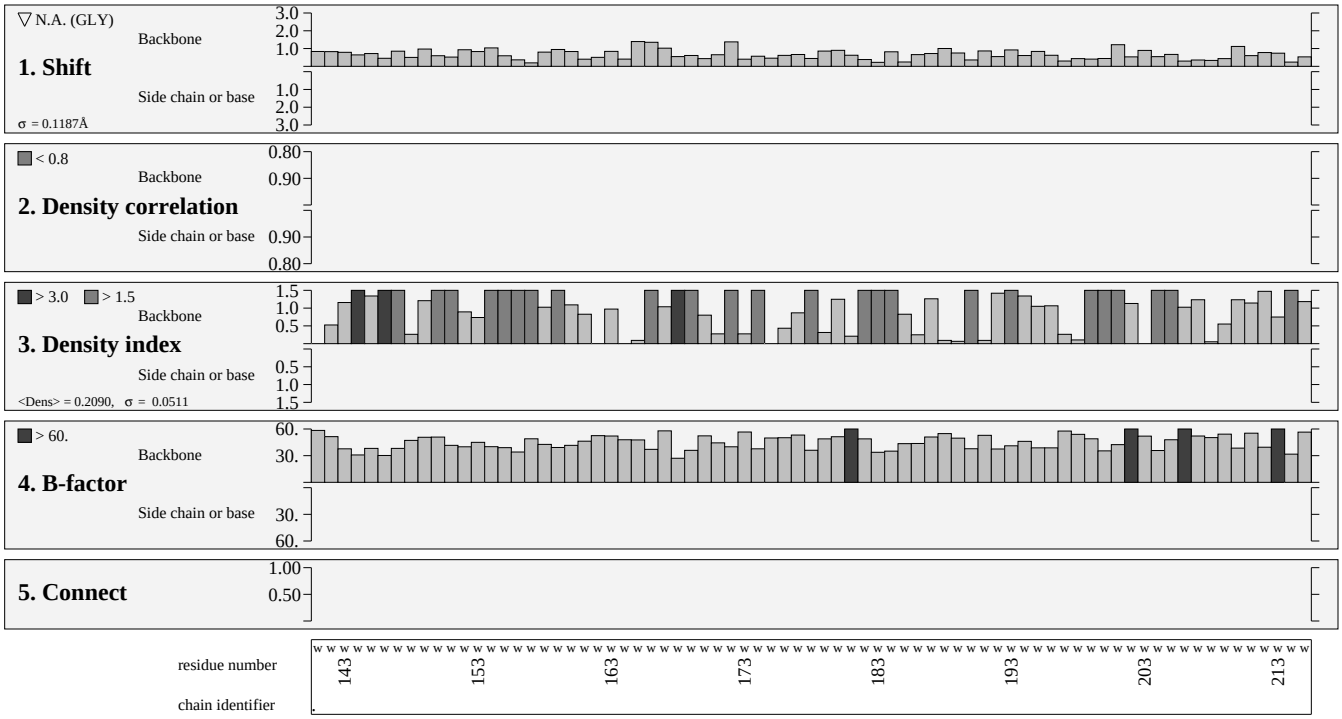


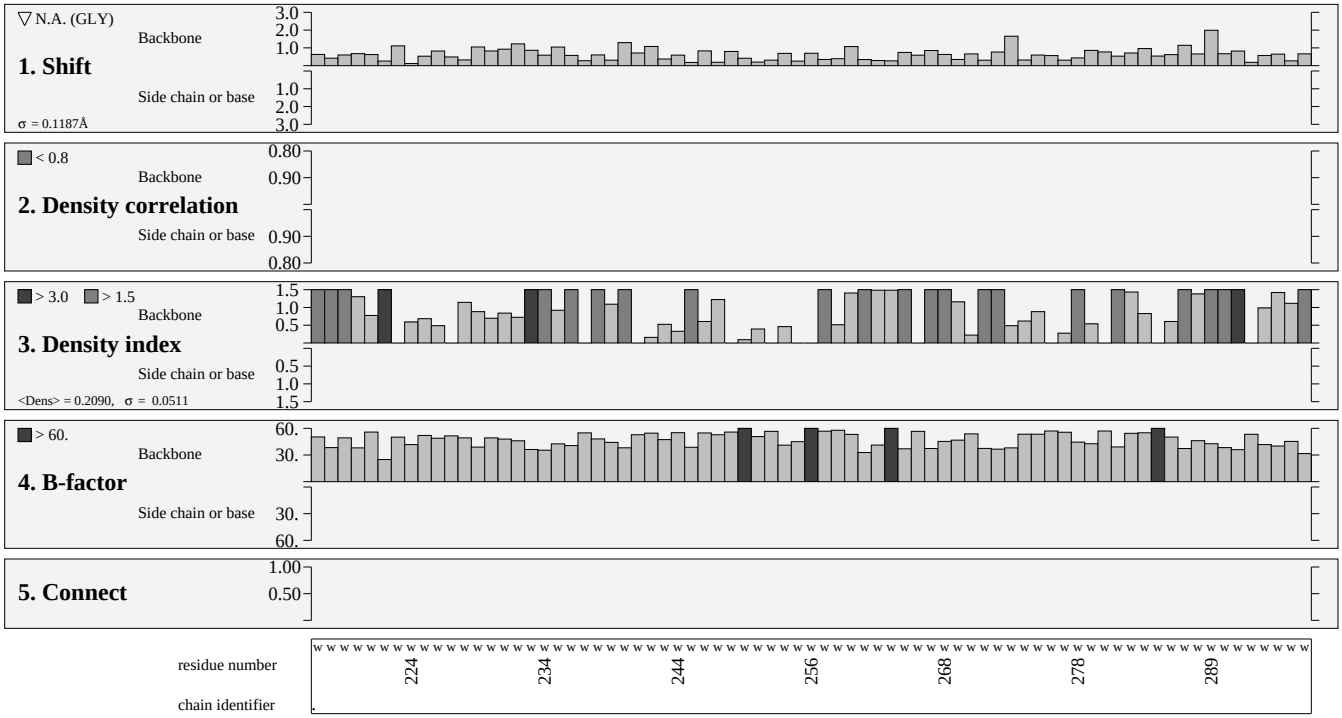
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (29)



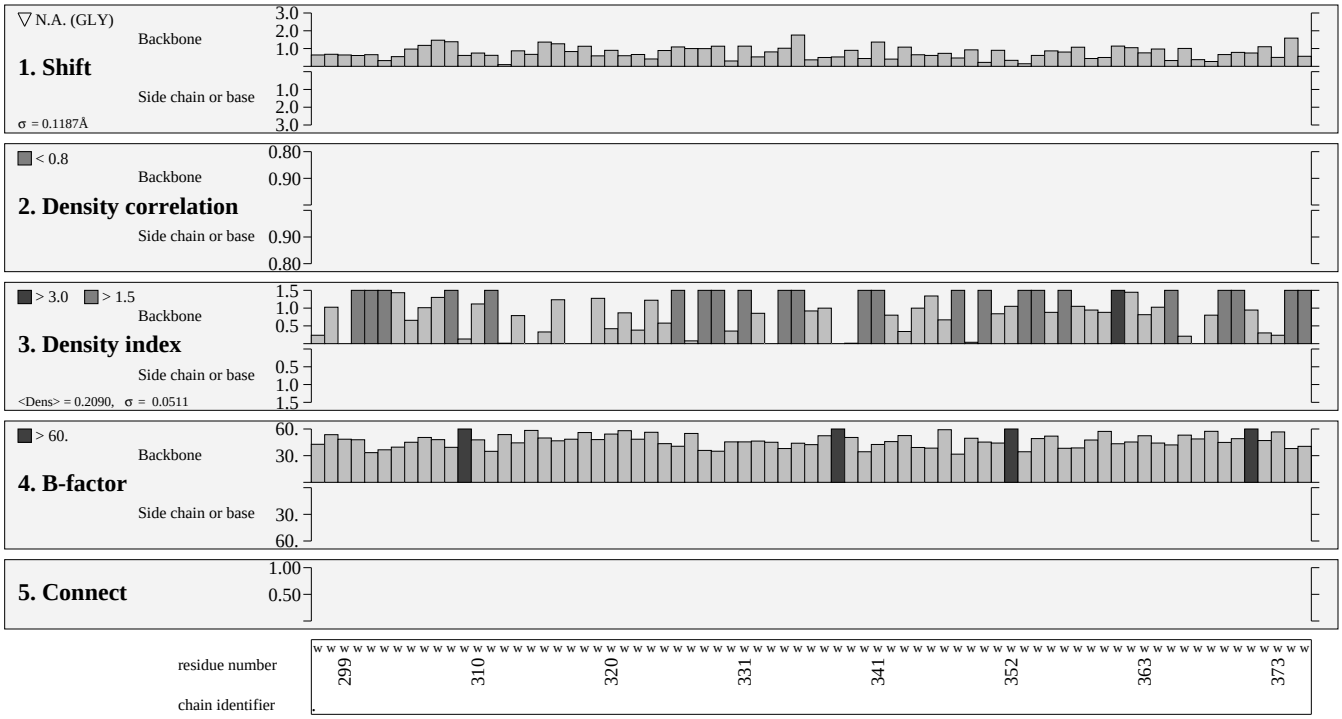


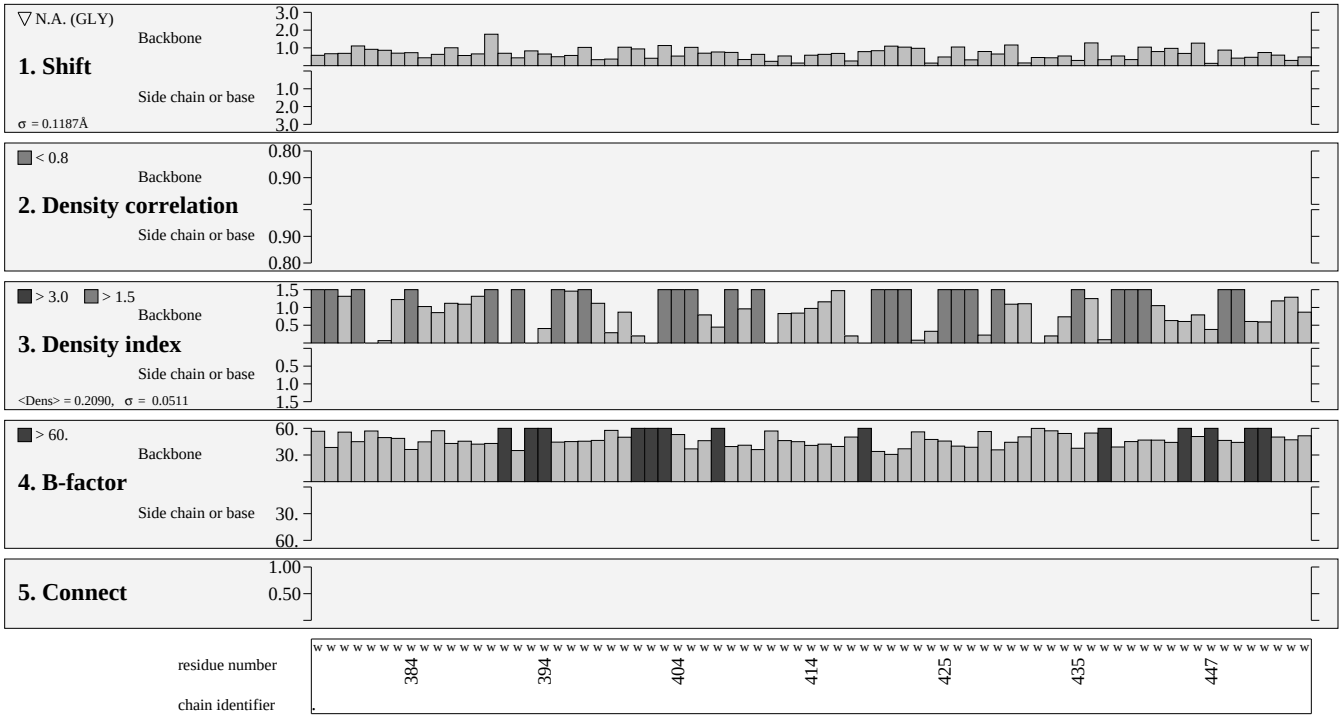
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (30)



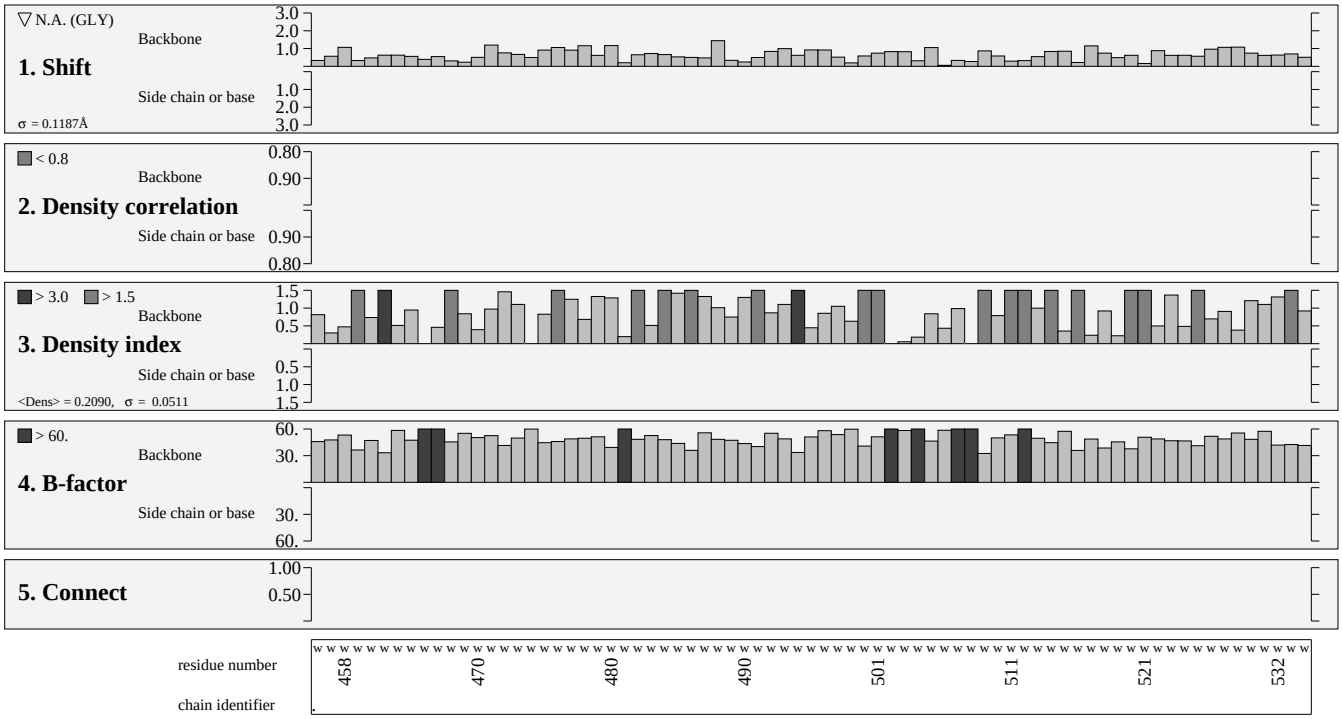


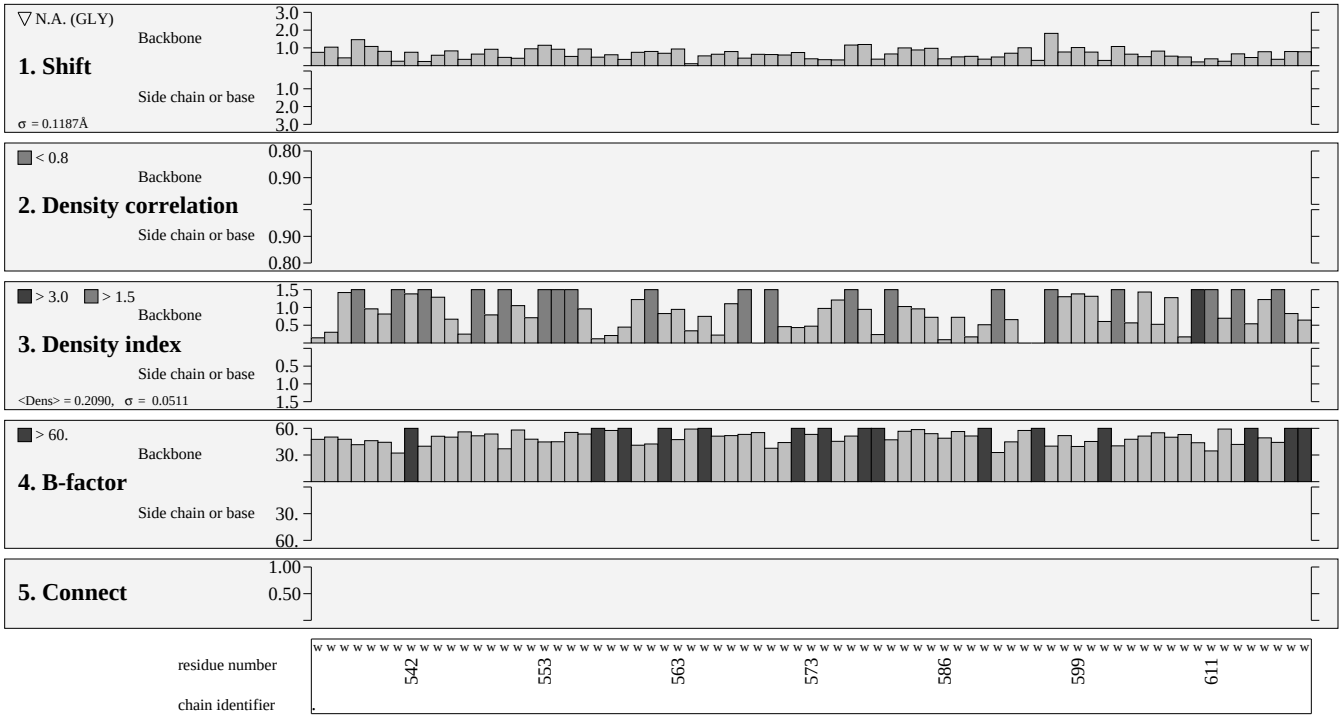
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (31)



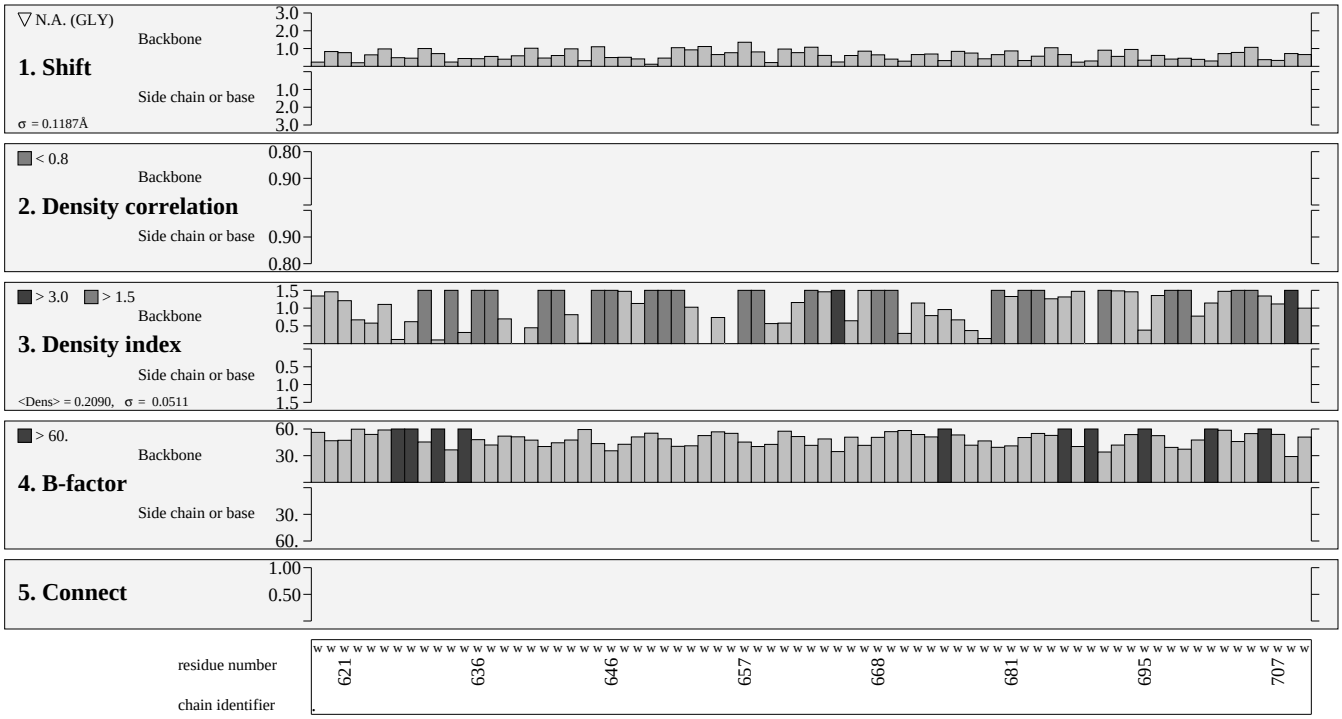


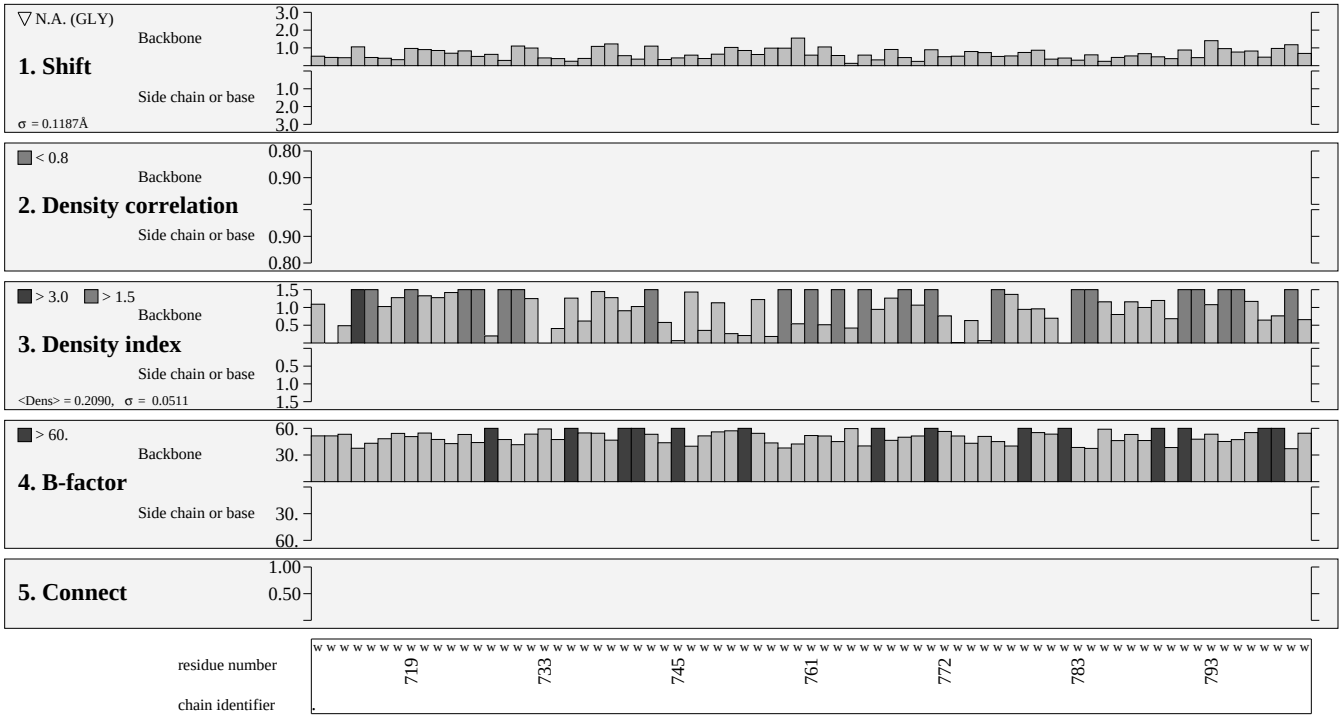
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (32)



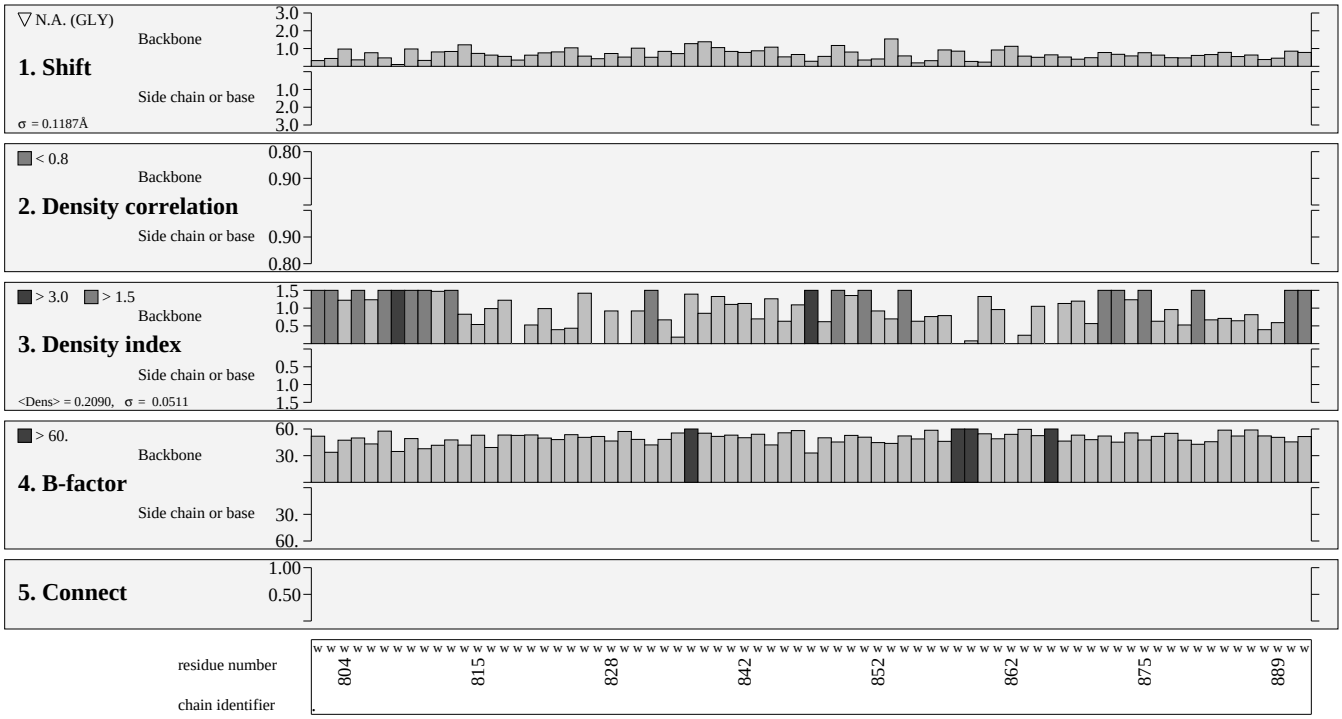


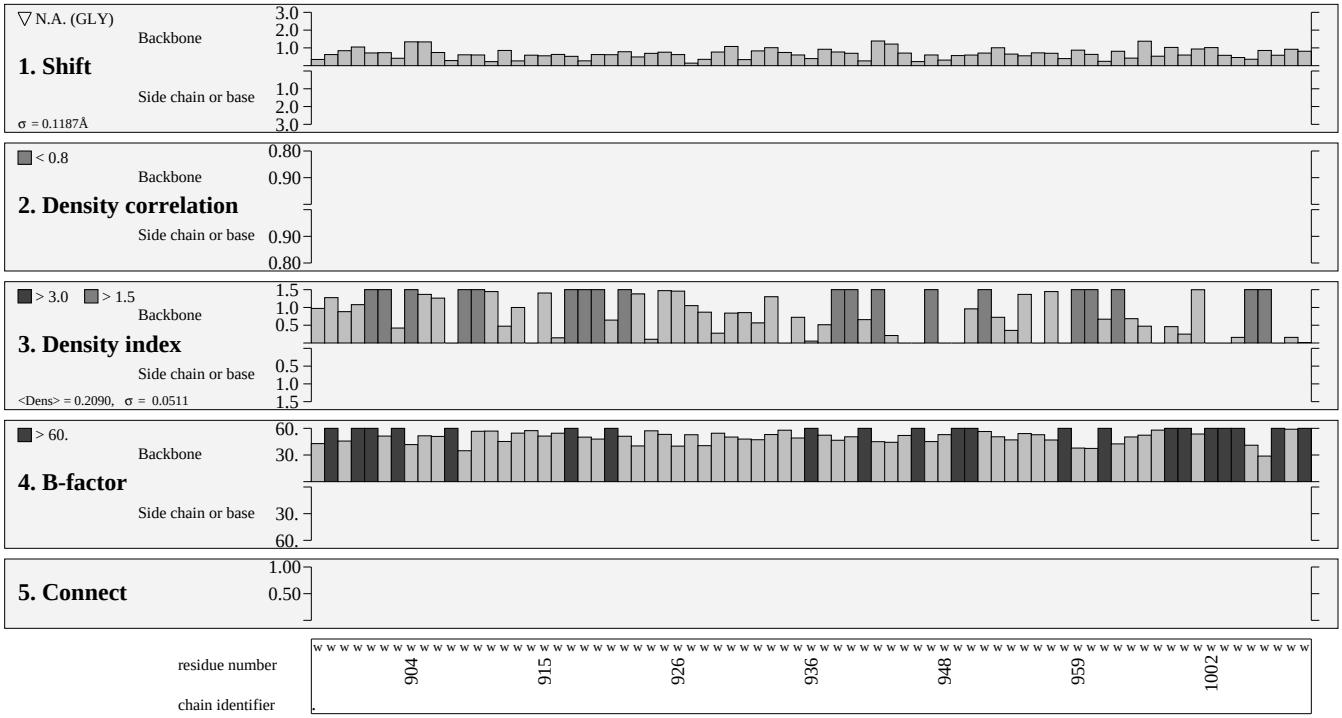
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (33)



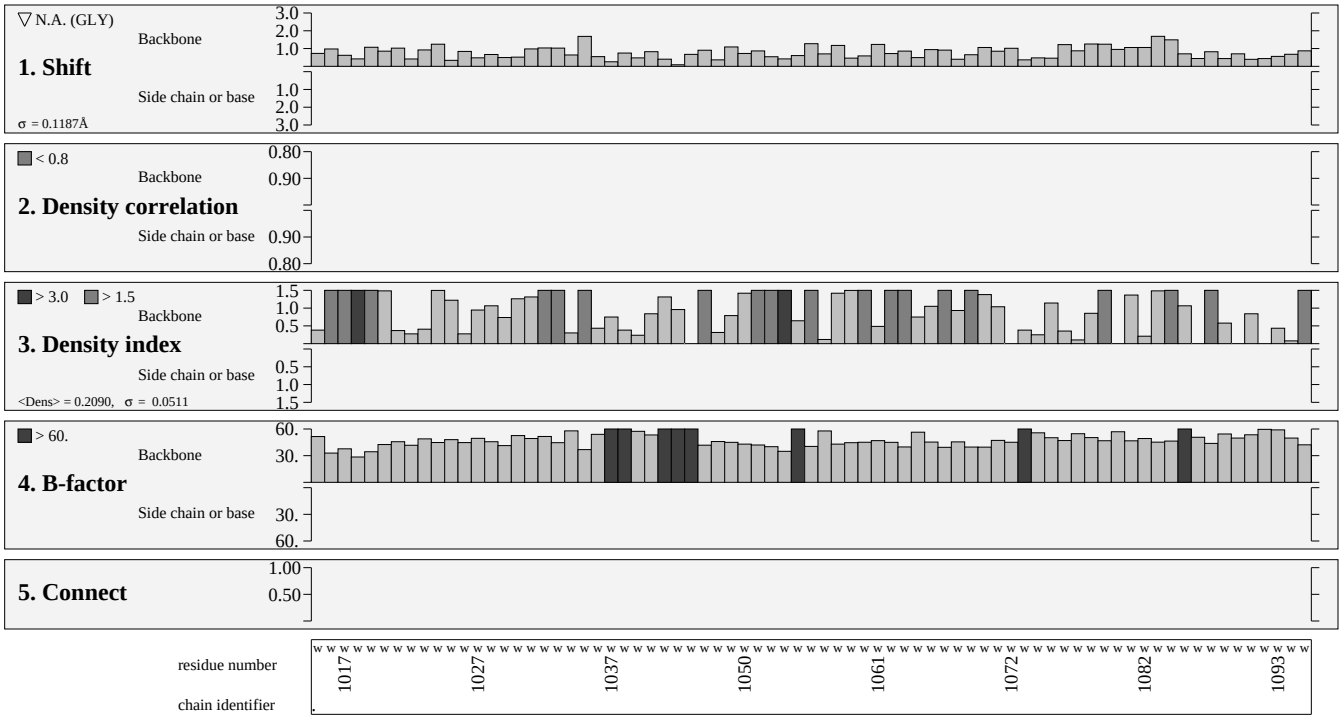


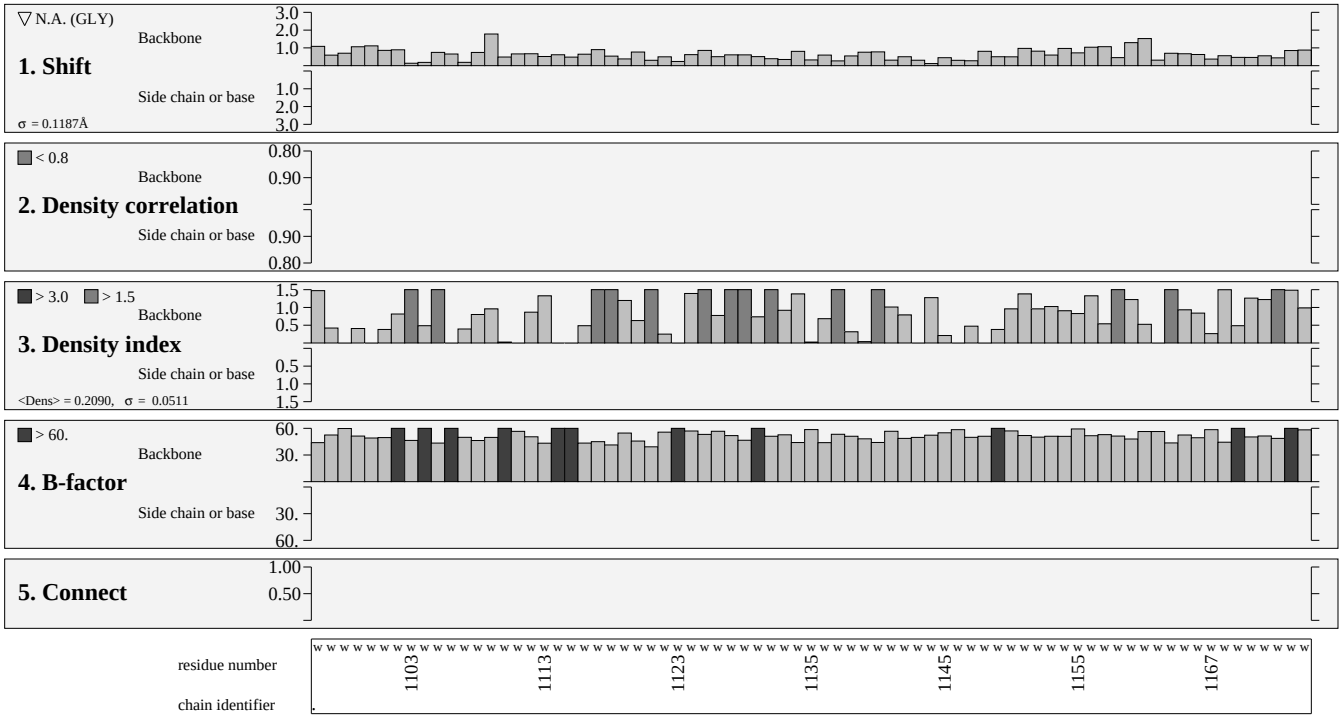
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (34)



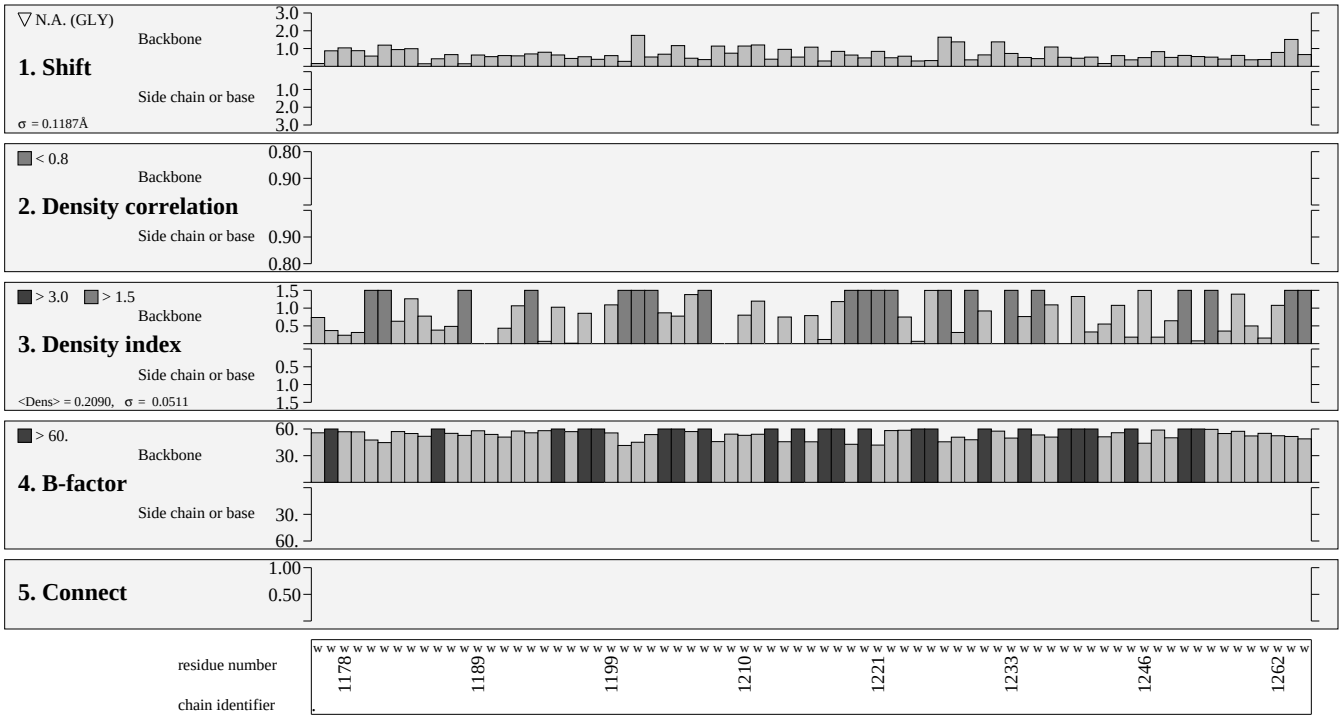


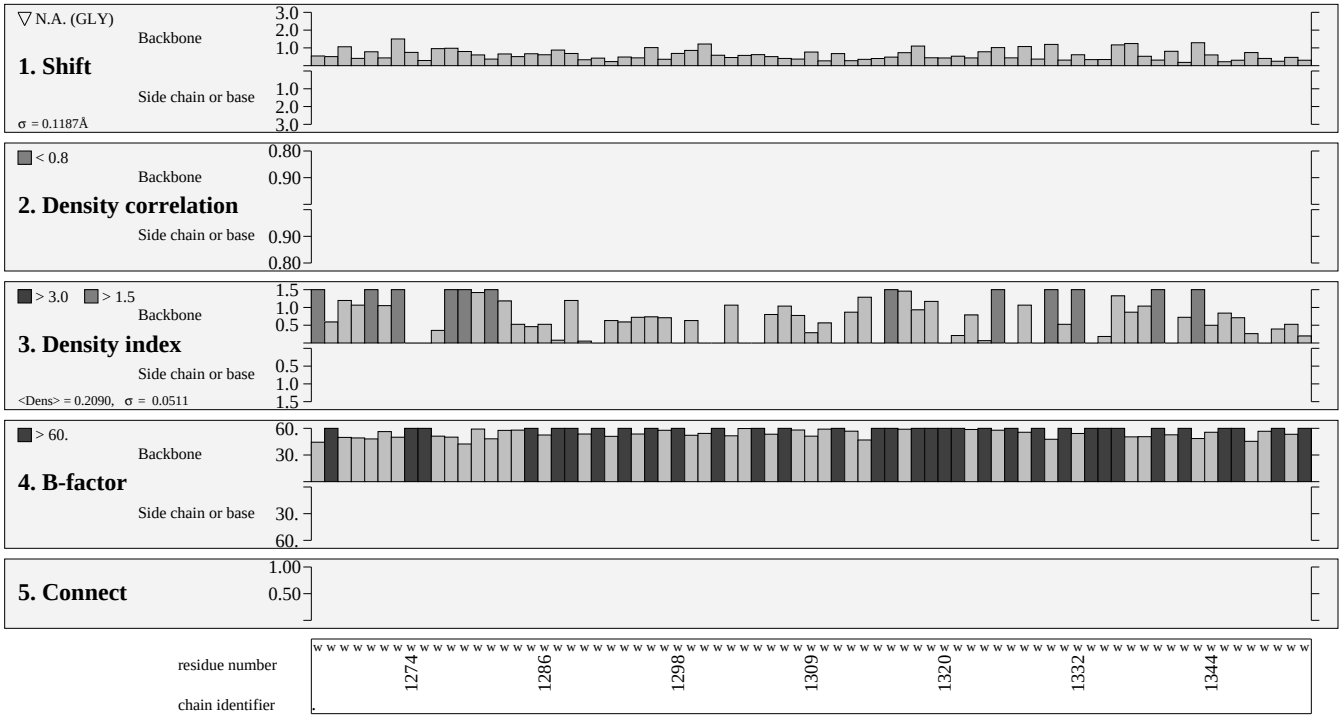
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (35)



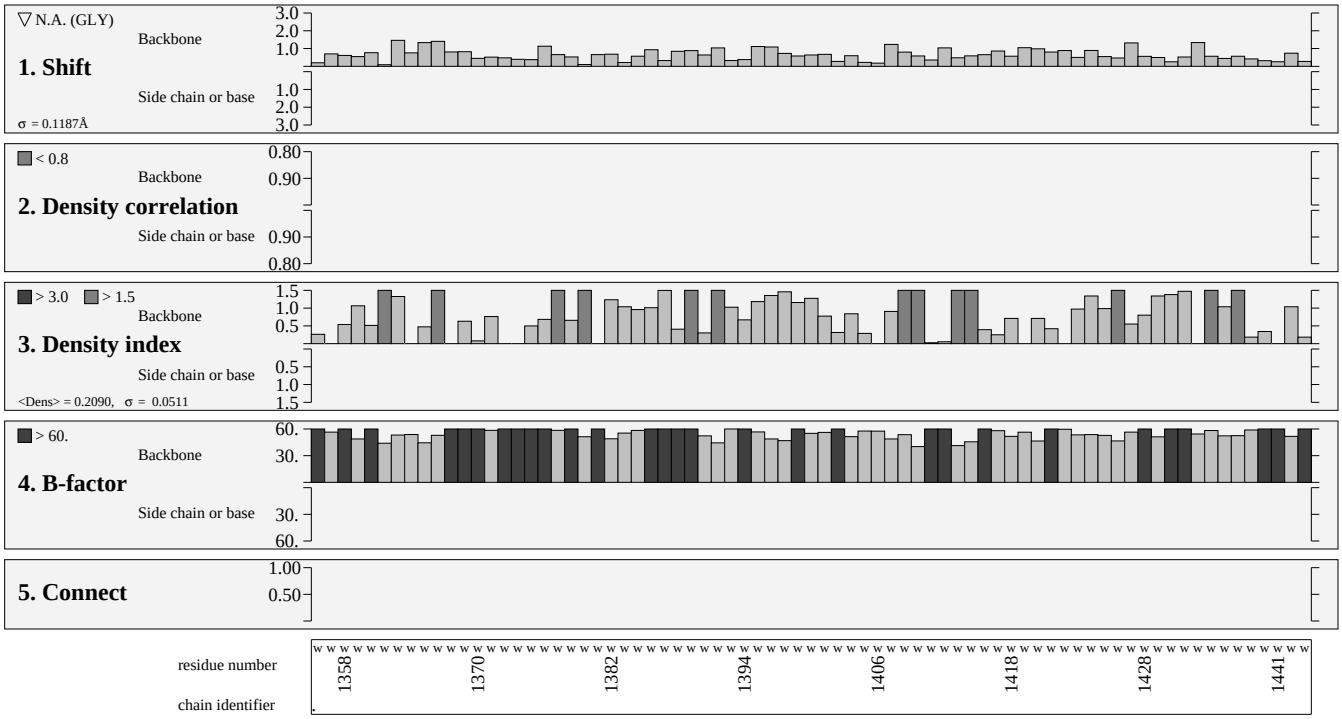


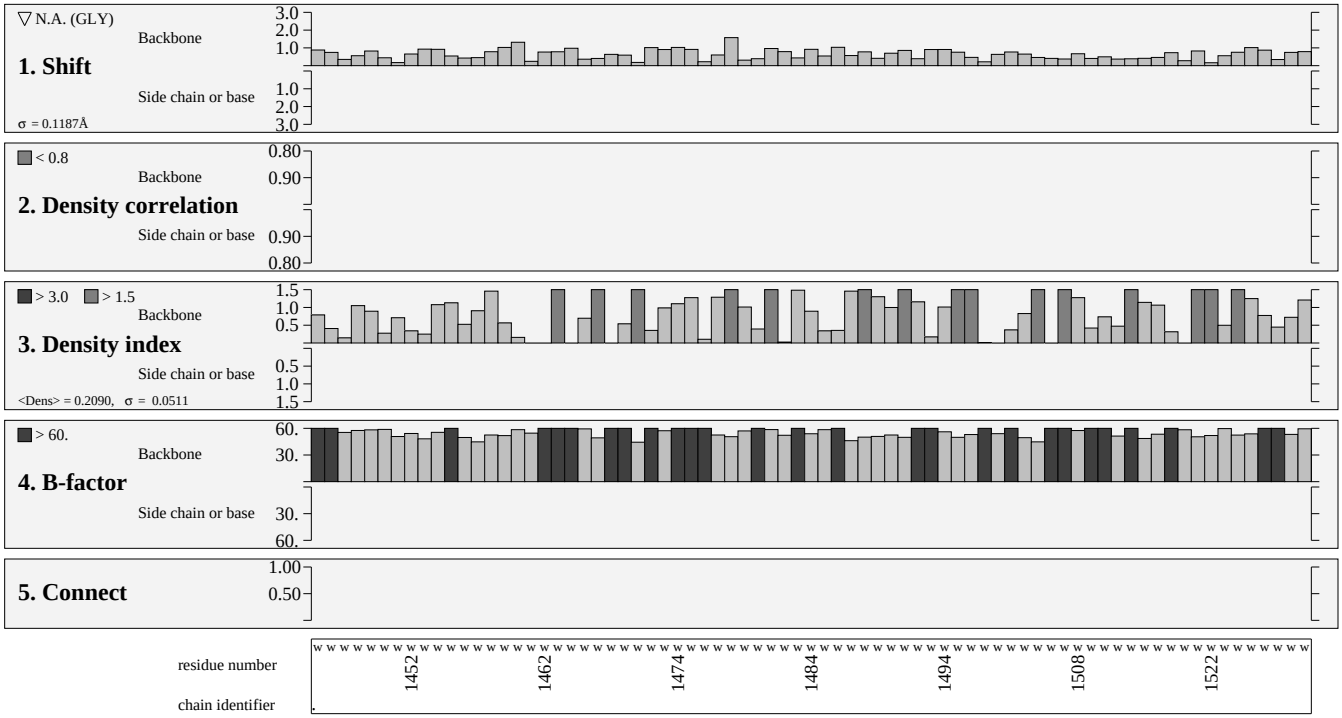
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (36)



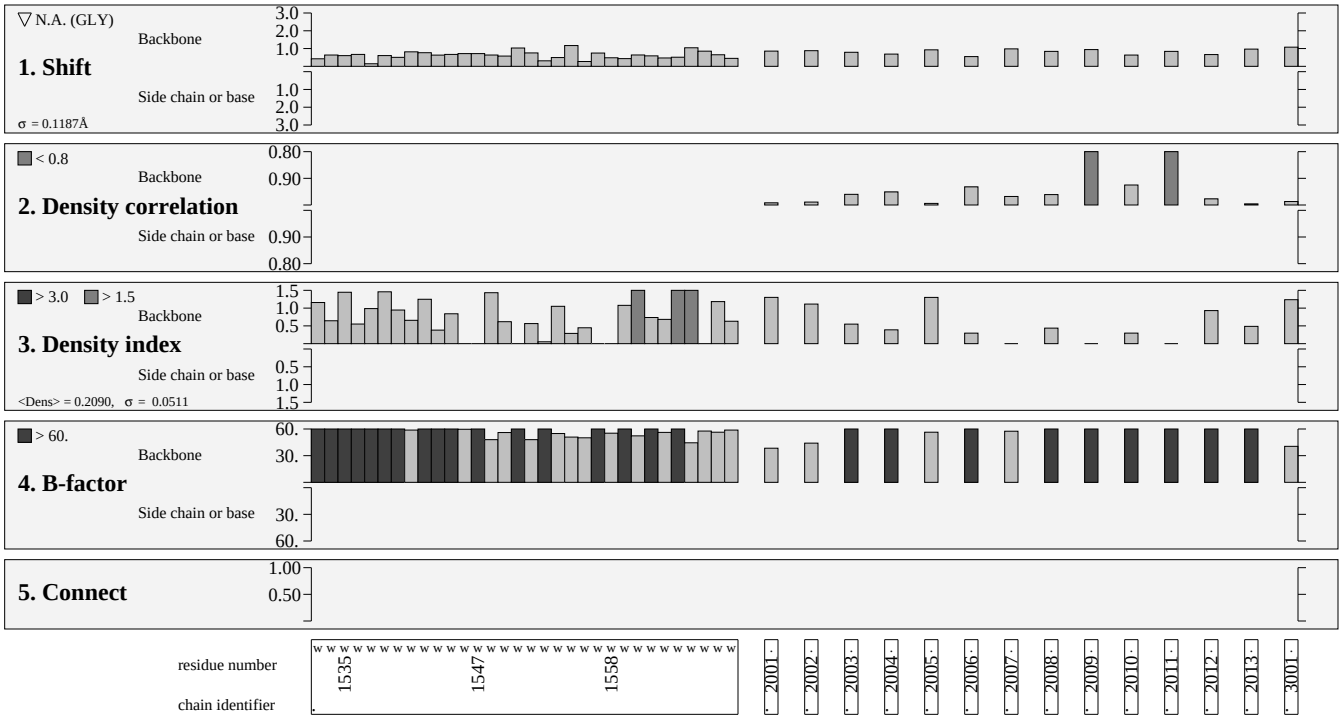


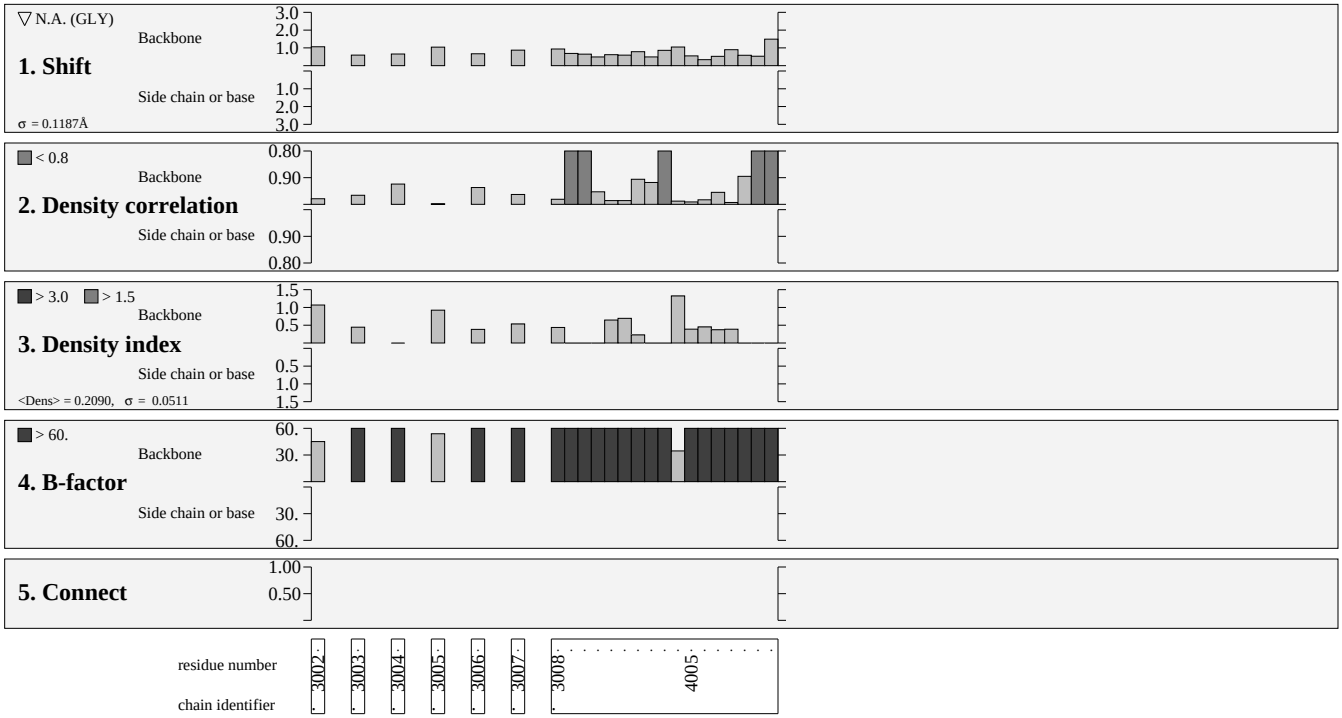
SFCHECK 6.0.5

Structure Factor Check

1PPJ

Local estimation (37)





SFCHECK 6.0.5