Visualization of unfavorable interactions in protein folds

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ABSTRACT

Summary: Three dimensional structures of proteins contain errors which often originate from limitations of the experimental techniques employed. Such errors frequently result in unfavorable atomic interactions. Here we present a new web service, called Interaction Viewer, for the visualization and correction of such errors. We show how the Interaction Viewer is used in combination with the NQ-Flipper service to spot strained asparagine and glutamine rotamers and we emphasize the convenience of this service in correcting such errors.

Availability: The web service is integrated with the NQ-Flipper service and accessible at http://flipper.services.came.sbg.ac.at

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Protein structures contain a variety of errors. Such errors often originate from experimental limitations like poor and diffuse electron densities derived from X-ray analysis, or insufficient and ambiguous distance constraints obtained from nuclear magnetic resonance (NMR) experiments. A specific example is the asparagine (Asn) and glutamine (Gln) rotamer problem in X-ray analysis. The electron density of these residues is frequently compatible with two distinct rotamer states. In such cases experimental information is insufficient to determine the identities of oxygen and nitrogen atoms in the amide groups of these side-chains. However, in many cases these states are clearly distinguished by favorable or unfavorable interactions with the surrounding atoms and it has been repeatedly demonstrated that such errors can be identified quite reliably (McDonald and Thornton, 1995; Hooft et al., 1996; Davis et al., 2007; Weichenberger and Sippl, 2006a,b, 2007).

It is highly desirable that errors in protein structures are removed on a regular basis which requires that current protocols in experimental structure determination are supplemented by additional error correcting cycles. The primary output of available error recognition programs consists of scores which indicate unfavorable interactions, incorrect atom positions, or other problems (Sippl, 1993; Weichenberger and Sippl, 2006b; Wiederstein and Sippl, 2007). The interpretation of such scores is usually straightforward but requires some understanding of the principles and inner workings of the software. Moreover, the sceptical user may not trust numeric scores so that it is necessary to visualize flagged errors and the associated atomic conformations in three dimensions. In general this is a rather cumbersome exercise, which is hampered by the overall complexity of protein structures. To facilitate error recognition, to alleviate acceptance of tagged errors, and to foster error correction it is therefore, necessary to provide automated tools for the visualization of offending interactions in atomic detail. In such closeup views erroneous atomic conformations are often revealed in a most drastic way so that the nature of errors becomes obvious.

We have implemented a new software application, called Interaction Viewer, for the immediate visualization of strained atomic conformations in protein structures which meets these requirements. We have now integrated the Interaction Viewer with the NQ-Flipper service (Weichenberger and Sippl, 2006a) so that strained interactions of Asn and Gln residues with surrounding atoms are quickly visualized in three dimensions by selecting the respective radio button from the NQ-Flipper score list. The chief goal of this communication is to provide a primer for the use of the Interaction Viewer (Figure 1), to emphasize once more the high frequency of incorrect Asn/Gln rotamers found in protein structures, and to stress the simplicity in correcting such errors. Moreover, we encourage the structural biology community to use this and related services to correct Asn/Gln rotamers found in protein structures, and to stress the acceptability of tagged errors, and to foster error correction it is therefore, necessary to provide automated tools for the visualization of offending interactions in atomic detail. In such closeup views erroneous atomic conformations are often revealed in a most drastic way so that the nature of errors becomes obvious.

The error rate in the assignment of Asn or Gln side-chain rotamers is rather impressive. On average the structures deposited in PDB have more than 20% incorrectly assigned Asn and Gln rotamers. This number has to be compared to random assignments where the expected error rate is 50%. These errors in rotamer assignment are responsible for more than half a million incorrect atom positions in the PDB repository. In particular, even high quality structures determined to a resolution of 1.5 Å or better generally contain rotamers that violate basic physico-chemical principles. Another unexpected result is that the error rate found in structures determined by NMR exceeds the error rate of X-ray structures (Weichenberger and Sippl, 2006b). This is surprising since the structures determined by NMR are based on the interpretation of interatomic distance constraints rather than electron densities.

Over many years the rate of incorrect rotamers found in the weekly releases of new structures in PDB has remained at this high level. Given that there are now several on-line tools available to detect and to correct such errors it is indeed surprising that these tools are virtually neglected by the X-ray and NMR communities. But we note one exception. In a recent survey of structures deposited by the various structural genomics initiatives we observed the very
same error rate of 20% incorrect rotamers for all consortia except for the Joint Center for Structural Genomics whose comparatively small average error rate of 10% is outstanding.

The individual side-chain amides of Gln and Asn have the capacity to form four hydrogen bonds. A flip to the incorrect rotamer frequently results in most unfavorable electrostatic interactions. Although the importance of hydrogen bonds is undisputed, their role in protein folding is a controversial issue due to the many energetic and entropic factors that are thought to contribute to their stability (Baldwin, 2007). Hydrogen bonds and other interactions in proteins are conveniently studied by potentials of mean force (Sippl, 1990, 1995, 2006). They provide quantitative information on the variation in energy as a function of atom pair distances. The potentials are used by the NQ-Flipper application. In Figure 1 we provide an example of a recently released high resolution structure containing more than 40% unfavorable rotamers. These rotamers are detected by the NQ-Flipper service. Their subsequent visualization reveals the nature of the unfavorable interactions in atomic detail. Such violations are offending in the sense that they violate the most basic physico-chemical principles. Incorrect rotamers frequently have inverted hydrogen bonds where several pairs of like charges are in close proximity. Such configurations are properly called anti-hydrogen bonds. Anti-hydrogen bonds are impossible to achieve in reality since the associated high energies lead to the immediate disintegration of the respective protein structure.

REFERENCES