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Self-consistent Assignment of Asparagine and Glutamine Side-Chain Amide Rotamers

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Short Abstract: We present a method based on potentials of mean force to validate and correct the amide side-chain orientation of asparagine and glutamine in protein structures which is often indistinguishable in electron densities obtained from X-ray experiments. Our method shows excellent agreement with expert annotated data. Availability: http://flipper.services.came.sbg.ac.at

Long Abstract:

We address the rotamer assignment problem of asparagine (Asn) and glutamine (Gln) side-chain amides encountered in protein structures solved by X-ray crystallography. The amide groups in the side-chains of Asn and Gln are specific examples of functional groups which act simultaneously as hydrogen bond donors and acceptors. These amide groups frequently participate in hydrogen bond networks, ligand binding, protein protein interactions, and catalysis. The electron density near the nitrogen and oxygen atoms of Asn and Gln amide groups is compatible with two rotamers which are related by a two-fold symmetry axis. Therefore, electron density maps obtained from X-ray diffraction experiments of protein crystals yield the positions of the oxygen and nitrogen atoms with high precision but not their identity resulting in the perpetual assignment of wrong rotamers in the order of 20 to 25%. However, incorrect rotamers in general result in unfavorable interactions and should be clearly detectable by proper energy calculations.

Here we present an automated method to assign the correct rotamer with high accuracy. We derive a mean force heavy atom potential [1] from the complete crystal environment of a set of 833 highly resolved PDB [2,3] protein chains with resolutions better than 1.6A and R-factors smaller than 0.25. Any two protein chains from this set have less than 20% sequence identity. Using the potentials, for each Asn/Gln residue we compute the interaction energy E1 of the original conformation as found in the PDB and the corresponding energy E2 of the alternative rotamer. The energy difference E1-E2 indicates the correct rotamer. In case of a positive energy difference (ie. E1>E2) the alternative conformation is taken. The potential functions are compiled from a database with incorrect rotamers and therefore wrong information is integrated into the potentials. We thus repeatedly correct the input structures and recompile potentials from the resulting corrected data set. Despite the high error rate this refinement procedure converges quickly to a stable set of self-consistent potentials. Potential functions before and after refinement are similar in shape and the final potentials exhibit pronounced valleys and peaks. The final potentials are then used for assigning the correct rotamer of Asn/Gln residues in an arbitrary PDB file.

We compare our assignments with an expert annoted set of 100 protein structures [4] and find sensitivity and selectivity values higher than 90%. Although the amide rotamer

assignment problem is specific to X-ray crystallography we find a similar error rate in protein structures solved by NMR experiments. When allowing the amide plane to rotate freely around its C-C bond we demonstrate that the lowest potential energy is found within 25 degrees of the correct conformation in 75% of all cases. We also discuss the upper limit of the rotamer error rate for which the refinement procedure still converges. A web service is available at http://flipper.services.came.sbg.ac.at.

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