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Protein Dynamics from X-Ray Crystallography Data: Modeling Structural Heterogeneity with Inverse Kinematics

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Short Abstract: We present a method to accurately model heterogeneity in experimental protein main-chain fragments. A computer program using our method was developed to automatically identify and model multiple conformations of a fragment that fit the experimental X-ray data, together with their likelihoods. Additionally, our software can be used to calculate energetically plausible pathways between the conformations, thus providing insight in the local dynamics of the main chain.

Long Abstract:
We present a method to accurately model heterogeneity in experimental protein main-chain fragments. A computer program using our method was developed to automatically identify and model multiple conformations of a fragment that fit the experimental X-ray data, together with their likelihoods. Additionally, our software can be used to calculate energetically plausible pathways between the conformations, thus providing insight in the local dynamics of the main chain.

X-ray crystallography requires well-ordered protein crystals to produce diffraction images. Accurately determining the atomic coordinates of mobile fragments in a protein structure remains a challenge with this technique. For such fragments a simple temperature factor model, i.e. an averaged conformation together with a harmonic parameter to account for atomic vibrations, is often not adequate to explain the experimental data. For example, the electron density may indicate multimodal disorder, where the protein main chain adopts two or more distinct conformations for a number of contiguous residues. Recognizing features in such areas of overlapping electron density is difficult, even for a trained crystallographer. There may also be regions where poor crystal packing leads to dynamic disorder of fragments in the macromolecule. In these regions the electron density is difficult or even impossible to interpret. At the same time, a protein's ability to bind to other molecules is typically facilitated by these mobile regions, and deformable fragments thus play a vital role in understanding the docking process and the protein's function. Such fragments of the protein main chain are better represented by an ensemble than a single, averaged conformation.

We have combined a fast inverse kinematics algorithm with real space, torsion angle refinement in a two stage approach to fit fragments to the electron density between two anchor points. The first stage samples a large number of closing conformations, guided by the electron density. These candidate conformations are ranked according to density fit, and

then clustered. Top-ranking conformations from distinct clusters are simultaneously subjected to torsion angle, subchain refinement in the second stage. Optimization steps are projected onto the null space of the subchain, thus preserving rigid geometry and closure. Using conformational distance as a target function, this null space optimization yields pathways between conformations.

The algorithm was tested on synthetic data as well as experimental electron density. Using synthetic atomic coordinates and calculated electron density, the software correctly identified and modeled two distinct conformers of length 8, separated by 4.4A, to within 0.9A and 0.6A of the true conformers at a resolution of 1.5A. Using real data, the software found two distinct conformers of length 8, separated by 2.6A, to within 0.9A and 0.5A of the conformers deposited in the PDB (1VME) at a resolution of 1.8A.

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