A refined approach to proteins at low resolution

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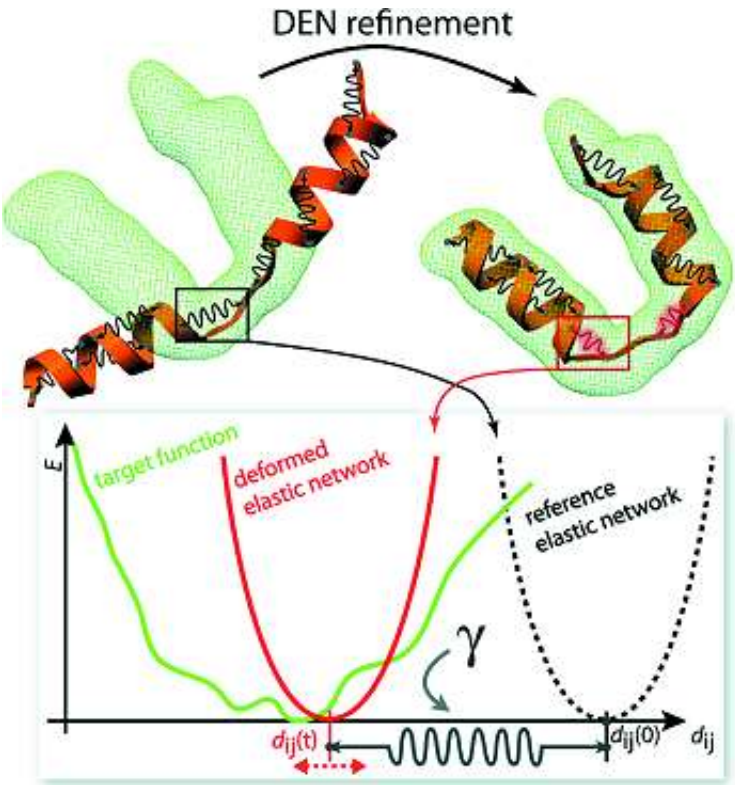

A starting model (orange, left) is refined against experimental data. Credit: Schröder et al

Membrane proteins and large protein complexes are notoriously difficult to study with X-ray crystallography, not least because they are often very difficult, if not impossible, to crystallize, but also because their very nature means they are highly flexible. The result is that when a structure

can be obtained it is often of low resolution, ambiguous and reveals a mosaic-like spread of protein domains that sometimes create more puzzles than they solve.

Now, Gunnar Schröder of the Institute of Complex Systems at the Forschungszentrum Jülich and the University of Düsseldorf, Germany and colleagues at Stanford University School of Medicine, USA have reviewed their earlier refinement technique known as Deformable Elastic Network (DEN) and found ways to optimize it successfully for the investigation of several particularly problematic protein structures including soluble proteins and membrane proteins up to a resolution limit ranging from 3 to 7Å.

The team explains that advances in X-ray technology and light sources have in recent years led to structures for previously intractable proteins such as the ribosome, transcription complexes and even viruses. The details then lie in a successful refinement that can provide valuable information about the structure in question despite lower resolution than would normally be desirable. "The interpretation of low-resolution diffraction data is generally difficult," the team says, "owing to the unfavorable ratio of parameters (variable degrees of freedom, such as flexible torsion angles or Cartesian atomic coordinates) to observables (observed diffraction intensities)." Ambiguities and errors of interpretation abound.

The DEN approach begins with a model, a prediction, of the target structure containing as much information as is known ahead of the insertion of the diffraction data, and determines which features of the model ought to be adjusted to fit the diffraction data emerging from the X-ray experiments. In other words, a null hypothesis is applied; those parts of the model not predicted to alter the diffraction data are retained as is. Distances between randomly chosen pairs of atoms within the structure are tested and tweaked accordingly within a distance restraint,

customarily referred to as the elastic network potential. This approach guides the structure towards the lowest energy landscape; it nudges it along towards an optimal structure wherever that may lie in the landscape, rather than forcing it in a particular direction.

The team has undertaken a proof of principle on their DEN approach with five representative protein cases with a particular focus on one example: the ß2 -adrenergic receptor, a membrane-bound protein consisting of seven transmembrane helices which belongs to the class of G-protein coupled receptors. The structure of the activated form of this protein was refined from a 3.5Å resolution data set in complex with an agonist and a nanobody that had been used to facilitate the crystallization process. They also had success with photosystem I, a membrane-protein complex involved in plant photosynthesis, which consists of 2334 amino acids in 12 polypeptide chains; an error-rich data set for AAA-ATPase p97, a hexameric protein complex in which each of the protomers contains an N-terminal domain and two nucleotide-binding domains; and the human myxovirus resistance protein (MxA), an enzyme that helps defend against viruses.

"We envision several extensions of DEN refinement that could potentially improve its performance and applicability," the team reports. They add that using more than one reference model from multiple known structures might allow the DEN restraints to be defined more precisely still with appropriate weight being given to each input model. "This would increase the overall amount of information used to guide the refinement," the team explains.

More information: Schröder, Levitt & Brunger. (2014), *Acta Cryst.* D70, 2241-2255; DOI: 10.1107/S1399004714016496

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