New and Notable

Computational Simulations of Peptide Binding to Proteins: How Scorpions Sting K^+ Channels

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Do not let the primary title "Modeling the structure of Agitoxin in complex with the *Shaker* K^+ channel" of the article by Eriksson and Roux lead you to think that this work is of interest only to a select cadre of channel zealots; the paper should be read by anyone interested in combining computational and mutagenesis approaches of analyzing molecular interactions between proteins. In the thermodynamic cyclic mutagenesis approach, the interaction energies between two residues in interacting proteins A and B are approximated by comparing effects on the binding energy of mutating the residue in A alone, the residue in B alone, and the simultaneous mutation of both residues. When first applied to the Barnase-Barstar interaction, for which the crystal structure had been determined, residues were always near each other when the approximate change in the interaction energy, $\Delta\Delta G_{bind}$, was relatively large. This approach was used in Rod MacKinnon's laboratory to analyze binding of Agitoxin2 (Agtx2) to *Shaker* K^+ channels. When his group determined the crystal structure of the bacterial K^+ channel, KcsA, they found its structure to be consistent with the major conclusions of these studies. Although the general orientation and location of the toxin could be crudely deduced from these data, fine details of its binding remained obscure.

Now Eriksson and Roux have developed a computational approach to quantitatively relate the binding of the toxin to the mutagenesis data. Their first stage uses distance constraints based on the strength of experimentally approximated residue-residue interactions to simulate many dockings of NMR-determined Agtx2 structures to a homology model of the *Shaker* channel. Side chains are flexible and backbone structures are somewhat constrained during initial molecular dynamic simulations; but all the final models are tested with molecular dynamics and explicit waters with no restraints. This conformational freedom may cope better with induced conformational changes than do most other docking algorithms. In the more unique second stage, changes in the binding energies of the toxin due to both single and dual mutations are calculated for each docked conformation. These are then compared with the experimentally determined values to evaluate which of many docked models is more likely to be correct. Surprisingly, they identified two equally plausible docking positions for the toxin.

How much confidence can we have that these simulations and energy calculations produce the correct answer? Their application of the methods to the Barstar-Barnase and a lysozyme-antibody complex produced impressive results. The simulated changes in free energies agreed well with experimentally determined binding energies, and the deviation of their best model of the Barnase-Barstar complex from the crystal structure was small. However, these modeling procedures rely upon assumptions that may not always be valid. The assumption that the homology model of the binding portion of the *Shaker* protein based on the KcsA structure is correct is supported by several observations: the homology model does not require insertions or deletions and the sequences of the P segments to which the toxins bind are 61% identical, only a few substitutions are required to make KcsA sensitive to blockade by AgTx2, and the locations and numbers of K^+ ions in the KcsA channel and simulations of ion permeation through KcsA agree quite well with conclusions drawn from experimental studies of other K^+ channels. The assumption that neither mutations nor binding substantially alter the backbone structure of AgTx2 is also consistent with numerous findings: the toxin is very thermodynamically stable with several of stabilizing disulfide bridges in its core, several homologous toxins have similar structures even though their sequences differ substantially, and numerous studies of mutated toxins indicate no substantial conformational changes. But, similar assumptions about the stability of the channel are more problematic. The K^+ binding region of the pore contains two highly flexible glycines (G444 and G446 in *Shaker*), and this portion of the protein has an unusual backbone structure that is stabilized by the presence of the K^+ ions. The K27 lysine side chain of the toxin has been shown experimentally to displace at least one of these K^+ ions. This could destabilize the protein. Effects of the dual mutation K27M/Y445F suggest interactions between these residues. In the KcsA crystal structure, the tyrosine analogous to Y445 is structurally and functionally important; its backbone carbonyl oxygen participates in forming one of the outer K^+ binding sites whereas its side chain hydroxyl group is buried within the protein where it H bonds to highly conserved threonine and tryptophan side chains of surrounding helices. Eriksson and Roux assumed that the amine group of the K27 side chain binds to the backbone

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carbonyl oxygen of Y445. They prudently excluded the K27M/Y445F data from their procedure of evaluating the models because their methods were unlikely to calculate adequately probable effects of the Y445F mutation on the backbone structure and effects of the competition with K^+ ions. Their model of AgTx2 docking may have been quite different if they had been unaware that the K27 amine group competes for K^+ binding and had included these data. Thus, the approach could produce erroneous results if not limited to mutations of surface residues that are unlikely to alter the backbone structure of either protein.

Despite some potential difficulties that are inherent in most docking algorithms, the methods presented here represent a substantial step forward in using computational methods to expand our limited knowledge of protein structures and interactions. This approach is reminiscent of techniques to solve NMR structures that place experimental constraints on molecular dynamic simulations. It has several potential applications, e.g., to design additional mutagenesis experiments to determine which of the two the conformation of the AgTx2-*Shaker* complex is better, to help develop homology models of other K^+ channels and their binding of other toxins, to design peptides with stronger binding affinities, and perhaps even to analyze binding of other organic molecules to proteins.