535

Development of an all-atom force field including hydration using protein decoys: optimization of torsional and solvation parameters

Yelena Arnautova, Harold Scheraga

Department of Chemistry and Chemical Biology, Cornell University, Baker Laboratory, Ithaca, NY, 14853

We have developed a new method for all-atom force field parameterization, with inclusion of solvation. The method is based on optimization of free energy gaps between sets of native-like and non-native conformations generated for each protein. The method was applied to optimization of the all-atom force field including the ECEPP-05 intramolecular energy and the solvation energy described by the solvent-accessible surface area model of Ooi, T.; Oobatake, M., Nemethy, G.; Scheraga, H.A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, 84, 3086. The parameters of the backbone torsional energy terms and of the solvent model were optimized to stabilize the native-like structures of six medium size (~20-70 amino acids) proteins with different folds against competing low-energy decoys. The ability of the resulting force field to distinguish native-like from misfolded conformations was validated for a set of nine nonhomologous proteins. The set included proteins with α, β, and α/β folds containing ~30-70 amino acids. For each protein, decoys with 2-4 Å backbone root-mean-square deviation and correct experimental topology emerged as those with the lowest energy.

536

Network pattern of residue packing in helical membrane proteins and its application in membrane protein structure prediction

Zhijun Li[1], Vagmita Pabuwal2

1Bioinformatics, University of the Sciences in Philadelphia, 600 South 43rd Street, Philadelphia, PA, 19104, 2Chemistry & Biochemistry, University of the Sciences in Philadelphia, 600 South 43rd Street, Philadelphia, PA, 19026

De novo protein structure prediction and homology modeling techniques have been extensively used in studies of helical membrane proteins as well as structure-based drug design efforts. Developing an accurate scoring function for structure discrimination and validation remains a challenge. Network approaches based on the overall network pattern of residue packing have been proven to be useful in soluble protein structure discrimination. It is thus of interest to apply similar approaches to the studies of residue packing in membrane proteins. In this work, we first carried out such analysis on a set of diverse and non-redundant native helical membrane protein structures using the network tool previously developed for the analysis of soluble proteins. To explore the potential application of the findings, we applied the same approach to two test sets of total 101 computationally constructed G-protein coupled receptor (GPCR) models, constructed using either *de novo* or homology modeling techniques. Models in these test sets have sequence identity with the bovin rhodospin varying from approximately 20% to 95%. Results of this large-scale analysis indicate that such an approach is very effective for discriminating less native membrane protein folds from native ones and the findings by studying native membrane proteins are good indicators of a native fold. These findings should be of help for the investigation of the fundamental problem of membrane protein structure prediction. This work is supported by the Research Starter Grant in Informatics from the PhRMA Foundation.

537

How effective for fold recognition are relative orientations between contacting residues in proteins?

Sanzo Miyazawa[1], Robert Jernigan2

[1]Graduate School of Engineering, Gunma University, 1-5-1 Tenjin, Kiryu, Gunma, 376-8515, Japan, [2]Laurence H. Baker Center for Bioinformatics and Biological Statistics, Iowa State University, 112 Office and Lab. Bldg., Ames, IA, 50011-3020

We estimate the statistical distribution of relative orientations between contacting residues from a database of protein structures and evaluate the potential of mean force for relative orientations between contacting residues. Polar angles and Euler angles are used to specify two degrees of directional freedom and three degrees of rotational freedom for the orientation of one residue relative to another in contacting residues, respectively. A local coordinate system affixed to each residue based only on main chain atoms is defined for fold recognition. To evaluate the fully-anisotropic distributions of relative orientations as a function of polar and Euler angles, we use a method in which the observed distribution is represented as a sum of δ functions each of which represents the observed orientation of a contacting residue, and is evaluated as a series expansion of spherical harmonics functions. The sample size limits the frequencies of modes whose expansion coefficients can be reliably estimated. High frequency modes are statistically less reliable than low frequency modes. Each expansion coefficient is separately corrected for the sample size according to suggestions from a Bayesian statistical analysis. As a result, many expansion terms can be utilized to evaluate orientational distributions. Also, unlike other orientational potentials, the uniform distribution is used for a reference distribution in evaluating a potential of mean force for each type of contacting residue pair from its orientational distribution, so that residue-residue orientations can be fully evaluated. It is shown by using decoy sets that the discrimination power of the orientational potential in fold recognition increases by taking account of the Euler angle

dependencies and becomes comparable to that of a simple contact potential, and that the total energy potential taken as a simple sum of contact, orientation, and (ϕ, ψ) potentials performs well to identify the native folds.

538

Molecular and structural characterization of FK506 binding protein 35 from Plasmodium falciparum

Ho Sup Yoon, Cong Bao Kang, Hong Ye

School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore, 637551, Singapore

Despite multilateral research efforts, malaria is still one of major human health threats. Studies indicate that the parasite rapidly develops resistance to anti-malarial drugs. In recent years, efforts have been made on the development of vaccines against the parasite. Unfortunately, a high degree of antigenic variation is hindering our endeavor toward the development of malarial vaccines. In view of this, developing drugs against authentic parasite targets would provide an alternative option in combating malaria.

The immunosuppressive drug FK506 binds its cellular targets FKBP family proteins and modulates important cellular processes including transcription. Interestingly, recent emerging data indicate that FK506 shows anti-malaria activities. The mechanism of the drug action against the parasite would involve molecular interaction with FK506 binding proteins. Newly identified FK506 binding protein 35 from *Plasmodium falciparum* (PfFKBP35), which is assumed to be only FK506 binding protein family member in the parasite, emerges as a novel molecular target for the development of anti-malaria drugs against *Plasmodium falciparum*. Currently, molecular basis of the growth inhibition of the parasite by the treatment of the drug remains unclear. To understand the underlying molecular mechanism of FK506 on PfFKBP35, in this study we first examined molecular and biochemical characteristics of PfFKBP35 and then determined three-dimensional structure of its FK506 binding domain by solution NMR spectroscopy. PfFKBP35 showed a basal activity in inhibiting the phosphatase activity of calcineurin in the absence of FK506, but the presence of FK506 greatly enhanced its calcineurin-inhibitory activity. Our NMR data indicated that the FKBD binds FK506 with a high affinity. The overall structure resembles those of the canonical FKBP family proteins. Differences were observed in the regions of β3/4 and β5/6 loops, which are important for ligands binding and peptidylproryl *cis-trans* isomerase (PPIase) activities.

Protein Structure/Function Studies (539 - 589)

539

Role of Thrombin Residues in Recognition and Hydrolysis of the Factor XIII Activation Peptide Sequence

Muriel Maurer, Giulia Isetti, R. Cory Lucas

Chemistry, University of Louisville, 2320 South Brook Street, Louisville, KY, 40292

The serine protease thrombin helps to activate Factor XIII (FXIII) in the last stages of blood coagulation. To better understand the roles of individual thrombin residues in recognition and hydrolysis of the FXIII activation peptide, mutations within thrombin's aryl and apolar binding site are being explored. Kinetic studies have been carried out with thrombin mutants (W215A, E217A, W215A/E217A, I174A, L99A, and W60dA) targeting the substrates FXIII (28-41) V34 AP and FXIII (28-41) V34L AP. As recorded previously with plasma FXIII, the cardioprotective FXIII V34L AP sequence is a better substrate for wild-type thrombin than V34AP. The thrombin residue W215A provides an important platform for binding and directing FXIII APs for proper hydrolysis. Loss of this platform leads to decreases in kinetics, particularly to the kcat of FXIII V34L AP. E217 also plays a supporting role, but the E217A mutation is not as detrimental as W215A. W215A/E217A is unfavorable for both activation peptides. The substrate peptides can readily bind, but they cannot be oriented for effective hydrolysis. This thrombin double mutant imparts a synergistic effect in the presence of FXIII V34 AP but the effect is mostly additive with V34L AP. Kinetic studies with I174A indicate that this thrombin residue is more crucial for interactions with the larger V34L AP segment. The L99A mutation greatly hinders binding and hydrolysis of both APs. The V34L, however, is able to partially compensate for the loss perhaps by increasing contact within the aryl and apolar sites. The W60dA thrombin shows improvements in substrate binding relative to L99A but hydrolysis remains comparable. The V34L AP continues to better handle this mutation. Understanding how specific FXIII and thrombin residues participate in binding and control hydrolysis may lead to the design of coagulation enzymes whose degree of activation and optimal target site can be controlled. (NIH R01HL068440)