Prediction of H exchange from perfectly funneled structure based models

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ABSTRACT

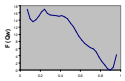
The principle of minimal frustration assumes that the overall landscape of proteins has evolved to be a rough funnel. When energetic frustration is low enough, topology alone becomes the key factor governing the folding. The structures of transition state ensembles, folding intermediates, and the mechanisms of dimerization, and domain swapping have been well predicted by models where frustration has been removed and topological information about the native state is the sole input. In this work we compare the protein motions simulated using perfectly funneled structure based models, and amide hydrogen exchange (HX) measurements under native conditions that report on the thermodynamics and kinetics of protein structures.

Prediction of ΔG opening reaction

Umbrella sampling (Qw bias) was performed to improve the sampling of very rare events as the open states under native conditions

$$Qw : \frac{2}{(N-1)(N-2)} \sum_{i<j-1} \exp\left(-\frac{(r_{ij}-r_{ij}^N)^2}{2\sigma_{ij}^2}\right)$$

WHAM analysis



Ubiquitin

Temperature was selected to match the simulated global stability and the real stability of the protein in the HX experiment

How can we structurally define the open and closed states?

Accessibility of the H-amide. Approximated by the number of contacts made by each residue

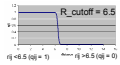
And / or

H-bond evaluation

We calculated the ΔG of the opening reaction by evaluating the probability of the number of native contacts (Qi) made by each residue during the simulations:

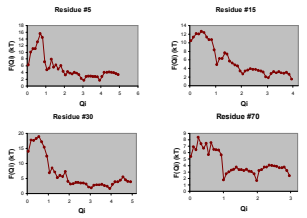
$Qi = \sum_j qij$ → # of native contacts of residue i

$qij = 0.5(1+\tanh(5(r\_cutoff - rij)))$ → qij is evaluated only for contacts that are made in the N state

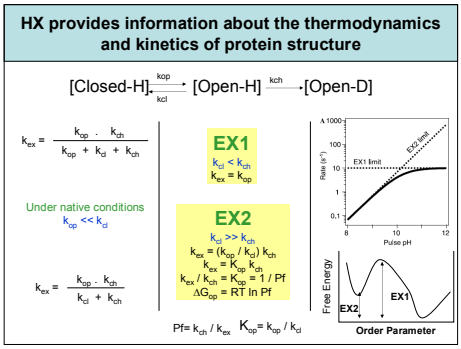


The open state was defined as the one with no native contact (totally unfolded)
The closed state was defined as the state of maximum probability (lowest free energy)

$$F(Qi) \sim -kB\,T \ln P(Qi)$$



HX provides information about the thermodynamics and kinetics of protein structure



AMGō with non additivity

Coarse grain model: only Cα, Cβ, and O

Attractive forces only between residues that make contact in the native structure.

Includes excluded volume effects.

Non additivity: forces depend on the structural context.
The weight of a pair interaction is modulated by the presence of other residues

$$H = H_{\text{backbone}} + H_{\text{na}}$$

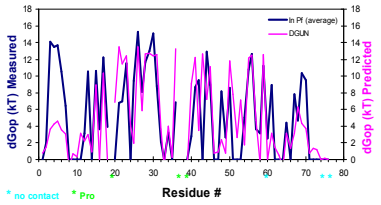
$$H_{\text{backbone}} = \lambda_{\phi\phi} V_{\phi\phi} + \lambda_{\chi} V_{\chi} + \lambda_{\text{ex}} V_{\text{ex}} + \lambda_{\text{harm}} V_{\text{harm}}$$

$$H_{\text{na}} = -\tfrac{1}{2}\sum_i |E_i|^l$$

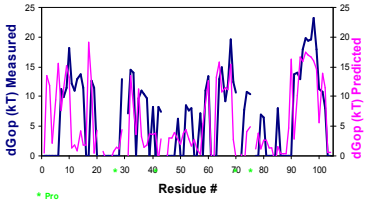
$$E_i = \sum_j \epsilon_{ij}(r_{ij}) = -\sum_j \left|\frac{\epsilon}{a}\right|^{1/p} \theta(r_c - r_{ij}^N)\gamma_{ij} \times \exp\left(-\frac{(r_{ij}-r_{ij}^N)^2}{2\sigma_{ij}^2}\right)$$

$$\sigma_{ij} = |i-j|^{0.15}\ \text{Å} \qquad a = \frac{1}{8N}\sum_i \left|\sum_j \gamma_{ij}\theta(r_c - r_{ij}^N)\right|^p \qquad \gamma_{ij} = \begin{cases} 0.125 & |i-j|<5 \\ 0.5 & \text{otherwise} \end{cases}$$

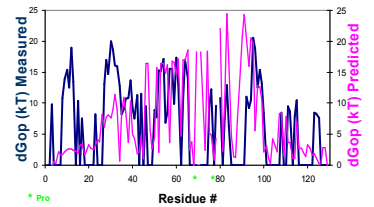
Ubiquitin



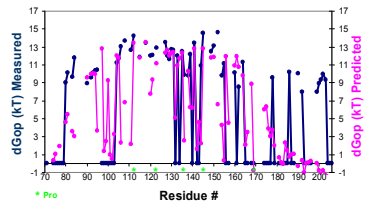
Cyt-C (1akk)

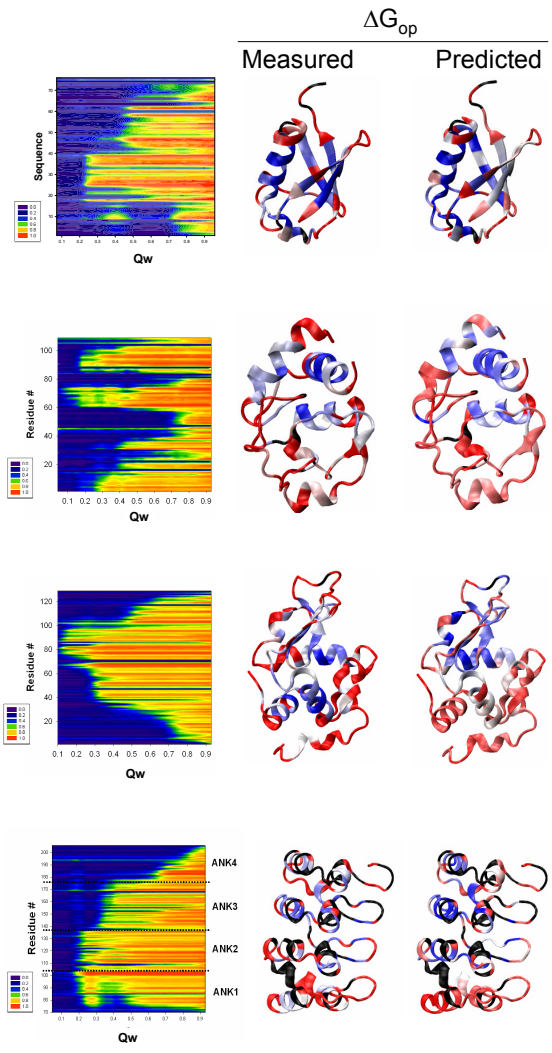


HEWL



IkBα (ANK 1-4)





CONCLUSIONS

The method presented in this work demonstrated to be capable of predicting the general features of the HX pattern under native conditions of ubiquitin and cytochrome-c, and to a lower extent the one of HEWL and IkBα. The agreement is remarkably given the simplicity of the model used, and allows further improvements varying the amount of non additivity used, weighting differentially the energies of the interacting residues, and most importantly making and evaluation of the H-bonding properties of the H-amide of each residue. Applications related to the interpretation of the energetics of proteins and parameterizations of force fields based on HX experiments are envisioned.