

to determine the optical constants in a depth dependent manner. By modeling the reflectivity spectra, in combination with angular resolved reflectivity, we show that it is possible to extract accurate optical constants in cases intractable with current techniques. Due to the large number of parameters inherent in such free-form modeling, we use the maximum entropy method to refine the underlying model in fitting the measured reflectivity data.

Keywords: spectrometry, XAS, reflectivity

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Evolution and dynamics of protein complexes

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There is an abundance of data on protein interactions and protein complexes, both from conventional small-scale experiments collected over the decades, including three-dimensional structures, and more recently by large-scale functional genomics experiments. We can draw on this information to ask whether (i) non-functional protein interactions constrain protein sequences and (ii) whether protein structures harbor information about conformational changes upon binding to each other.

In order to answer the first question, we project evolutionary and systems information onto 397, 196, and 701 proteins of known structure from *E. coli*, *S. cerevisiae* and *H. sapiens* respectively. We find that the propensity of proteins to interact in a non-specific manner with other proteins is inversely correlated with their abundance in *E. coli* and *S. cerevisiae*. This tendency is most pronounced at surface residues, suggesting that high abundance proteins have evolved to have a less sticky surface. In *E. coli* and *S. cerevisiae*, we also find that the evolutionary conservation of an amino acid is positively correlated with the stickiness of the surface environment around it. Thus, residues in sticky surface patches are evolutionarily more constrained, possibly because they are more likely to trigger non-functional interactions if they mutate. Although significant, the impact of protein stickiness is comparatively small in shaping the physico-chemical properties and evolution of *H. sapiens* proteins. This suggests that promiscuous protein-protein interactions are freer to accumulate in species with a small effective population size; a phenomenon akin to junk DNA accumulation.

While non-functional interactions shape protein sequence and structure, functional protein interactions require not just sequence but also structural complementarity, which often involves conformational changes. We have analyzed the relationships between the structures of proteins and the conformational changes that they undergo upon binding. We find that the relative solvent accessible surface area of both free and bound subunits can be used to predict the magnitude of binding-induced conformational changes. We demonstrate that the relative solvent accessible surface area of monomeric proteins is useful as a simple proxy for intrinsic flexibility and for predicting conformational changes upon binding. In addition to the predictive power of this correlation, it reveals a strong connection between the flexibility of unbound proteins and their binding-induced conformational changes, consistent with the conformational selection model of molecular recognition.

Keywords: bioinformatics, protein complexes, protein interactions

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Structural insight into the regulation of AMP-activated protein kinase

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The energy sensor AMP-activated protein kinase (AMPK) plays a central role in regulating cellular metabolism and energy homeostasis and is thus a major drug target for obesity, type 2 diabetes and related metabolic disorders. AMPK is a heterotrimeric enzyme composed of catalytic α -subunit and regulatory β - and γ -subunits and characterized by its ability to undergo allosteric activation upon AMP binding to the γ -subunit. However, the molecular basis for this allosteric regulation remains unclear.

The catalytic α -subunit contains an N-terminal Ser/Thr kinase domain (KD) and an autoinhibitory domain (AID). We determined the crystal structures of an unphosphorylated fragment of the AMPK α -subunit (KD-AID) from *S. pombe* and of a phosphorylated kinase domain from *S. cerevisiae* (Snf1-pKD). Structural analyses indicate that AID binds, from the 'backside', to the hinge region of its kinase domain and constrains the mobility of helix α C, hence resulting in an autoinhibited KD-AID with much lower kinase activity than that of the kinase domain alone. Further *in vitro* kinetic studies demonstrate that disruption of the KD-AID interface reverses the autoinhibition and these AMPK heterotrimeric mutants no longer respond to the change in AMP concentration.

Structural studies on AMPK core provide information on the heterotrimer formation, but the competitive binding of ATP or AMP results in little, if any, conformational changes. In contrast to previous results, our co-crystallized core structures of mammalian AMPK, in complex with either two ATP or three AMP, exhibit different nucleotide/protein stoichiometries and significant conformational differences on the γ -subunit. We set up a minimal kinetic model for AMPK regulation with two functional nucleotide binding sites and demonstrated that the predominant regulatory site on the γ -subunit binds AMP 125-fold more tightly than ATP. Together, our structural and biochemical data have shown the primary mechanism of AMPK autoinhibition and provide a relatively comprehensive view for its allosteric regulation by AMP/ATP exchange.

Keywords: AMP-activated protein kinase, autoinhibition, allosteric activation

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A pseudokinase mediates cell wall integrity in *Mycobacterium Tuberculosis*

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