Data-mining the Fourth Dimension from Crystal Structures: Structure-Function-Entropy Relationships in Membrane Proteins

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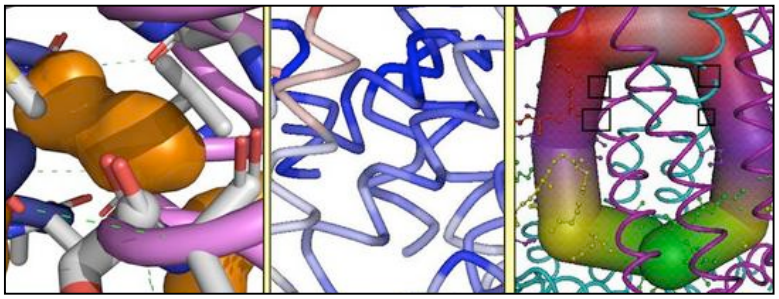

Crystal structures, the major source of protein structural information, are often regarded as static 3D snapshots of dynamic macromolecules. Thus, study of protein dynamics is often confined to computationally heavy simulations. Instead, here we data-mine membrane-protein structures directly. We show how local regions of distinct flexibility are fine-tuned to control specific functions. Protein features considered during the analysis include intra-protein cavities, B-factors normalized in a unique manner, and intrinsic degrees of freedom. These are correlated with evolutionary conservation, packing motifs, mutagenesis simulations, local deformations, functional pathways, and experimental validation.

First, we show how local flexibility acclimatizes photosynthetic energy conversion across a wide range of temperatures, from sub-tropical habitats to scalding hot springs[1]. A group of hitherto unrecognized intra-protein cavities and adjacent packing motifs jointly impart localized flexibility at the center of the photosynthetic complex. The motifs are evolutionarily conserved at the sequence and structural levels with consistent changes between mesophiles and extremophiles. We show experimentally that electron transfer rate accelerates with temperature (Arrhenius behavior) up till the physiological temperature (T_0). Above T_0, it levels-off, thus avoiding photo-damage due to reaction over-acceleration. A single mutation, conducted according to the *in silico* analysis, altered T_0. Moreover, *in silico* mutagenesis that abolished the cavity was applied *in vivo* and shown to eliminate the non-Arrhenius behavior and reduce the electron transfer activation entropy.

Second, to generalize our findings, we assess whether local flexibility, associated with unique hydrogen bonding patterns, confers specific functions. As recently reviewed[2], the frequent appearance of local distortions in transmembrane helices may enable precise positioning of functional groups or, in contrast, facilitate flexible functional movement. Data-mining a non-redundant dataset of crystal structures reveals that both roles of these local distortions are demonstrated. They are clearly divided according to the normalized temperature- (or B-) factor. In order to apply this measurement of crystal structure disorder to the local region of interest, normalization was confined to transmembrane backbone atoms. Further, in order to localize the helical deformations, intrahelical H-bonding parameters were utilized, rather than the conventional DSSP annotation. The approach provides guidelines for analysis of pivotal, evolutionary conserved loci.

Last, we assess whether the protein matrix disorder level *per se* can modulate function. We show that variation in local flexibility along electron transfer pathways explains the dilemma between two opposing requirements: optimizing rigid geometry required for donor-to-acceptor wave-function overlap and conferring flexibility to quickly dissipate heat thus avoiding a back-reaction. In photosynthetic complexes the first, fast and low-energy-gap steps take part along a rigid microenvironment thus ensuring chemical capture of the photo-excited sate. In contrast, the latter slow steps involving a high-energy-gap steps take part along a flexible microenvironment.

Cumulatively, data-mining the local variation in the intrinsic flexibility of crystal structures provides a tool to study dynamic mechanisms. The applied methods provide guidelines to analyze key functions, adding available, yet often neglected, data to our understanding of membrane proteins.

1. Kerner, O.*, Samish, I.*, Kaftan, D.*, Holland, N., Sai, P. S. M., Kless, H. & Scherz, A. (2006). Protein Flexibility Acclimatizes Photosynthetic Energy Conversion to the Ambient Temperature. *Nature* (In Press). *Equal contribution
2. Bowie, J. U. (2005). Solving the Membrane Folding Problem. *Nature* 438, 581-589