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252-Pos Board B52
Rational Design of Human Fibroblast Growth Factor with Enhanced Stability
Ali Haydar, Becky Kerr, T.K.S. Kumar.
Fibroblast growth factors (FGFs) constitute a large family of heparin-binding proteins which control the proliferation, differentiation, and migration of a variety of cell types. For its transduction of cellular signals, FGF must bind to the extracellular domains of fibroblast growth factor receptor (FGFR), a transmembrane tyrosine kinase receptor. The resulting FGF-FGFR complex leads to receptor dimerization which stimulates a phosphorylation cascade within the cell resulting in a variety of cellular outcomes.
FGF also binds to a second class of receptors for which it has a lower affinity, heparan sulfate proteoglycans (HSPGs). Heparanase treatment of intact cells has shown that FGF fails to bind to FGFR resulting in non-proliferation of the cells, indicating the importance of HSPG-binding. Closely related to heparan sulfate is heparin, a structurally-similar proteoglycan which can bind to FGF isofunctionally.
The role of heparin in FGF-FGFR interactions has been discussed greatly. Heparin is believed to play a role in FGF stability, protecting it from thermal denaturation and proteolytic degradation. It has also been proposed that HSPG will increase the binding affinity of FGF for FGFR and will stabilize the dimerization of FGFR. Some studies have shown, however, that heparin binding may not be essential to FGF-FGFR binding. The goal of this study was to define the role of heparin in human FGF-1 (hFGF-1) stability and cell proliferation activities. A His-tagged quadruple mutant with mutations made in the defined heparin-binding region of hFGF-1 at the N128K, Y139K, G140R, and Q141K positions (Quad-His) was created prior to the beginning of this study, and the mutant was compared against wild type hFGF-1 through a variety of biophysical techniques. Experiments were conducted with both heparin and sucrose octasulfate (SOS), an analog commonly used as a structural and functional mimic for heparin.

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Sequence Dependence of Coiled-Coil Formation and Stability Studied Using a Coarse-Grained Atomistic Model
Sara Sadeghi, Eldon Emberly.
The recurrent coiled-coil domain in proteins consists of multiple helices that intertwine to form a superhelix. Superhelical formation is driven primarily by the packing of hydrophobic residues that pattern each helix making up the coil. An examination of the sequences forming naturally occurring coiled-coils reveals a high degree of variation, yet all the sequences ultimately fold into left-handed superhelical structures. We have developed a coarse grained atomistic folding model for coiled-coil formation that only requires a hydrophobic-polar pattern for the input sequence[1] and are able to generate coiled-coil structures that have a low root mean square distance(RMSD) to known structures. We have considered different parameters in our energy function and tried to optimize them to get lowest RMSD. Besides correctly predicting structure from sequence, we have applied the model to study both the thermodynamic and mechanical stability to sequence mutations for the selected coiled coils. The model provides insights into key sequence characteristics that aid both stability and the kinetics of coil formation.
[1] S.Sadeghi & E.Emberly ; Phys. Rev. E.80, 061909 (2009).

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Structural Basis of WHAMM to Regulate Golgi Membrane Transportation via Interacting with Both Microtubules and Membranes
Qingtao Shen, Kenneth G. Campellone, Charles V. Sindelar, Matthew D. Welch, Hongwei Wang.
WHAMM protein is a newly identified mammalian nucleation-promoting protein, and it is comprised of an N-terminal domain that mediates Golgi membrane association, a coiled-coil domain (CC) that binds to microtubule, and a WCA domain that stimulates Arp2/3-mediated actin polymerization. WHAMM protein plays key roles in both maintaining Golgi structure and facilitating anterograde membrane transport. The molecular mechanism for WHAMM to fulfill its functions remains unknown. We have used cryo-electron microscopy (cryo-EM) to investigate the structural basis of WHAMM's interaction with microtubules and membranes.
Our in vitro pelleting assay shows that both full-length WHAMM protein and the CC domain have high binding affinity to Taxol-stabilized microtubules at around 360 nM. This is verified by Cryo-EM observation that both full-length WHAMM protein and CC assemble around microtubules within a few minutes of incubation. Image analysis of the micrographs indicates that the proteins assemble with stoichiometric binding to the alpha-beta tubulin heterodimers in the microtubule lattice. We performed three-dimensional reconstruction of the WHAMM and CC assemblies around microtubules and revealed that WHAMM binds to the outer surface of microtubules via direct interaction between its CC domain with tubulin dimer. The assembly of WHAMM onto microtubule exposes the N-terminal domain ready to bind membranes.
We have further used fluorescence microscopy to demonstrate that vesicles made of lipids from liver extract can be recruited to microtubules in the presence of WHAMM in an in vitro assay. Cryo-EM of such a specimen verified the cross-bridge between the lipid vesicles and microtubules by WHAMM and showed that the interaction causes the vesicles to change their shapes along the microtubules.

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Understanding Functional Tuning, Robustness, and Evolvability in Proteins By High-Throughput Biophysics
Wouter D. Hoff.
Proteins consist of only 20 different amino acids with very modest chemical reactivity, but perform a breathtaking range of functions. A central question in biophysics is to understand how proteins achieve such functional versatility. A key recent advance in understanding protein structure-function relationships is the robustness of proteins against point mutations, which implies that only a small subset of residues determines functional properties. We tested this prediction using photoactive yellow protein (PYP), a 125-residue prototype of the PAS domain superfamily of signaling proteins. PAS domains are defined by a small number of conserved residues of unknown function. Our high-throughput biophysical measurements on a complete ala scan of purified PYP mutants characterizing active site properties, functional kinetics, stability, and production level reveal that 124 mutants retain the characteristic photocycle of PYP, but that the majority of substitutions significantly alter functional properties (Philip et al., PNAS 2010, early edition 10.1073/1006660107). Only 35% of substitutions that strongly affect function are located at the active site. Unexpectedly, most PAS-conserved residues are required for maintaining protein production. The photocycle kinetics are significantly altered by substitutions at 58 positions and span a 3,000-fold range, allowing us to identify conserved interactions governing allosteric switching in PYP. The data also shed light on two classic examples of functional tuning of active site groups, shifts in pK_a value and shifts in absorbance maximum for spectral tuning, and result in a novel model for spectral tuning based on changes in the shape of the energy surfaces involved (Philip et al., 2010, PNAS 107: 5821-5826). The results show that PYP combines robustness with a high degree of evolvability, imply production level as an important factor in protein evolution, and provide new insights into the functional tuning of active site residues.

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Local Conformational Fluctuations of the Backbone Affect the pK_a Values of Ionizable Groups in Proteins
Brian M. Doctrow, Kelli L. Baran, Jamie L. Schlessman, Bertrand García-Moreno.
The pK_a values of all 20 Asp and Glu residues in staphylococcal nuclease (SNase) have been measured previously using NMR spectroscopy. Some of these pK_a values are shifted relative to the normal values for Asp and Glu in water, but most are not. This does not agree with structure-based pK_a calculations, according to which most of the pK_a values are depressed because of strong Coulomb interactions with basic residues. This discrepancy persists even in calculations that treat the protein interior with the dielectric constant of water, suggesting that the crystal structure does not fully represent the ensemble average in solution. This idea is supported by calculations showing that the acid unfolding properties of SNase are reproduced better when the protein is treated as an ensemble of structures rather than as a static structure. We have tested the hypothesis that subtle, local conformational fluctuations contribute significantly to the pK_a values of ionizable groups. This was examined experimentally by measuring pK_a values of Asp, Glu, and His residues with NMR spectroscopy in variants of SNase with Gly substitutions at strategic locations, designed to enhance the flexibility of the backbone. Such substitutions should affect pK_a values without affecting the protein structure. Consistent with our hypothesis, several Gly substitutions were found to shift the pK_a values of ionizable groups without affecting the structure noticeably. Efforts are underway to determine what the effects of Gly substitutions on structure and fluctuations are. Our results suggest that the high apparent polarizability of proteins might be due to local or subglobal unfolding. We will present a computational model designed to capture the effects of local unfolding on pK_a values in proteins.