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Structural Relationships Between Retaining and Inverting GT-B Glycosyltransferases as Revealed by Structure Analysis of Sucrose Synthase-1 from *Arabidopsis Thaliana*
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Sucrose synthase-1 from *Arabidopsis thaliana* (AtSus1) is a member of the retaining GT-4 glycosyltransferase subfamily (i.e. the product sucrose has the same alpha configuration as the sugar donor UDP-glucose) within the larger family of metal-independent GT-B glycosyltransferases. A typical GT-B fold comprises of two structurally similar Rossmann folds (GT-B_N and GT-B_C) connected by two hinge regions. We have determined the structure of AtSus1 in catalytically competent closed state with UDP-glucose or fructose and UDP bound at the active site. Active site analysis provides future evidence for S_Ni-like reaction mechanism and functions of conserved residues His438, Glu675, Lys585 and Arg580. Structural Comparison of AtSus1 with inverting and retaining GTs of known structures revealed two interesting features of GT-B glycosyltrasferase. First, three loop regions have distinct lengths and differing positions between the inverting and retaining GTs and play an important role in determining the direction of attack on the anomeric carbon of the sugar moiety by the sugar acceptor. Second, a simple rigid body motion of GT-B_N domain relative to GT-B_C domain may be sufficient for the movement of the loops to open or close the attacking route. This domain motion suggests the two hinge regions connecting the two individual Rossmann folds in a particular orientation will stabilize either retaining or inverting reaction outcome over the other. Further understanding of the hinge region will provide new insights for glycosyltransferase engineering and may open up a broader substrate spectrum for glycosyltransfer reactions.
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Mapping Protein Structure Changes with Cysteine Labeling Kinetics by Mass Spectrometry
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Protein unfolding, disassembly, and aggregation underlie many diseases, but detailed study of these processes in intact cells has been limited. Cysteine Shotgun labeling utilizes cell-permeable fluorescent dyes to label exposed cysteine residues. We applied it to study protein structure changes in response to mechanical stress on red blood cell ghosts in live mice and in whole-cell lysates in native versus urea-denaturing conditions. Labeling rate constants are calculated for any given Cys site by normalizing the protein labeling kinetics to the rapid labeling under denaturing conditions. Proteins can be identified and further analyzed by mass spectrometry to pinpoint specific, susceptible domains involved. A number of proteins contain cys with a wide variety of rate constants. This study focuses on human and mouse spectrin, Filamin A and B, Talin, and pyruvate kinase. These various proteins contain many cysteine-rich domains and have been amenable to studying by this in-cell technique. Results are confirmed by studies of purified protein.

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Structure and Function of the Peripheral Membrane Protein P2 from Human Nervous System Myelin
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The myelin sheath is a multilayered, tightly packed membrane wrapped around axons, enabling the rapid saltatory transmission of nerve impulses. Myelin carries a set of specific proteins, most of which are either integral or peripheral membrane proteins. In the peripheral nervous system, one of the myelin-specific proteins is P2, a member of the fatty acid binding protein (FABP) family that binds monomeric lipids inside its barrel structure. P2 is a peripheral membrane protein, and in addition to binding membrane surfaces, it is able to stack lipid bilayers. We performed a detailed structure-function analysis of recombinant human peripheral nerve P2. The liganded crystal structure of the wild-type human P2 protein was determined at 0.93-Å resolution, allowing a very detailed analysis of fatty acid binding. In addition, 14 structure-based mutants were produced and characterized with respect to crystal and solution structure as well as membrane interactions and lipid binding. While the surface charge distribution of P2 presents two positively charged faces as putative membrane binding sites, the mutations with largest effects on P2 function did not affect the basic surface residues. Instead, it is likely they affect the dynamics and structure of the helical lid domain, which is expected to open and close upon lipid binding and to partially insert into the membrane. Furthermore, a hinge domain mutant could be crystallized in the absence of bound lipid. We also show that P2 gets oriented when binding to membrane surfaces. Overall, our current data allow a very detailed understanding of the structure-function relationships in myelin P2, a unique FABP, which is able to promote membrane multilayer formation.

3091-Plat
Membrane Targeting of the N-Terminal Ubiqutin-Like Domain of Kindlin-2 is Crucial for its Regulation of Integrin Activation
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Kindlin is a three-member subfamily of FERM (four-point-one, ezrin, radixin, moesin) containing proteins that dramatically enhance talin-mediated integrin alphaIIbbeta3 activation, supporting their role as co-activators of integrins. Among them, kindling-2 is widely expressed and highly concentrated at integrin-rich cell-ECM adhesions. By binding to integrin beta cytoplasmic tail via its C-terminal FERM-like domain, kindlin-2 promotes integrin activation. Intriguingly, this activation process depends on the N-terminus of kindlin-2 (K2-N) which precedes the FERM domain. The molecular function of K2-N is unclear. Here we present the solution structure of K2-N, which displays a ubiquitin fold similar to that observed in kindlin-1. Using chemical shift mapping and mutagenesis, we found that K2-N contains a conserved positively charged surface that binds to membrane enriched with highly negatively-charged lipids. We show that while wild-type kindlin-2 is capable of promoting integrin activation, such ability was significantly reduced for its membrane-binding defective mutant. These data suggest a membrane-binding function of the ubiquitin-like domain of kindlin-2, which is likely common for all kindlins to promote their localization to the plasma membrane and control integrin activation.

3092-Plat
On the Structural Space of Protein-Protein Interfaces
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All major cellular processes in living cells are dependent on protein-protein interactions. At the heart of protein-protein interactions are protein-protein interfaces where the direct physical interactions occur. One may view the collection of all possible protein interface structures as a structural space. Understanding the nature of this space not only advances our fundamental knowledge about proteins but has profound implications for protein-protein interaction prediction and design. Can one explain the observed space of structures just by the principles of physics or does evolution need to be invoked as well? Here, we focus on interface structures of dimers, i.e., a complex formed by two protein monomers. By developing and applying an efficient structural alignment method, iAlign, we study the structural similarity of >1000 representative protein-protein interfaces. We present results of comparing experimental (native) interface structures formed by proteins whose *monomers adopt different structures* and show that most native interfaces have a close structural neighbor with similar backbone C geometry and interfacial contact pattern. To understand the possible origin of this interface similarity, we build artificial complexes from a library of randomly generated, compact homopolymeric structures and compare the structure of their interfaces to native interfaces. We show that most of native complexes can find an artificial counterpart, and vice versa. Moreover, from protein-like sequences further designed to be thermodynamically compatible with the artificial structures, native-like artificial protein complexes with strong favorable interactions emerge, albeit at a small probability.

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Dynamic Protein-Protein Complexes: How Alternative Interactions Create Ensembles and How Solution NMR and MD Simulations can Characterize them
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It is now recognized that protein-protein interactions in solution are dynamic, especially if the binding affinities are only moderately strong. Dynamics,