dipoles, etc.) dominate chemical activity, hence solution properties. Protein charge can be determined accurately using a combination of electrophoretic and hydrodynamic measurements. It is essential to measure charge since calculated values (e.g., from isoelectric point determinations) may be in serious error. Monoclonal IgGs (mAbs) provide an important example where charge must be measured. Charge determinations for 11 different mAbs in 100 mM KCl at pH 6.0 show that calculated values overestimate the charge by ~17, with the discrepancy increasing to ~50 at pH 5.0. The mechanisms underlying charge suppression are unclear. There is nothing obvious in IgG structure (e.g., buried side chains, H-bonding, clustered charged side chains) that would account for the suppressed charge. It seems likely that weak ion binding (either site or territorial) by IgGs may occur since changing the solvent ionic strength and ion composition influence charge suppression beyond their Debye-Hückel effects. The unusual charge properties of IgGs may have both in vivo and in vitro significance. In vivo, charge suppression may provide a ''buffer'' that allows high plasma concentrations of IgGs with different amino acid compositions. Charge also may be important in Fc receptor binding of IgGs. Analysis of isolated Fc and Fab fragments reveals that the Fc fragment charge is less than +1 at pH 6.0, where the calculated charge is +9. In vitro, IgG charge correlates with increased solubility and reduced solution viscosity, properties that are important in drug formulation.

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1085-Plat

A Near Atomic Resolution Model of the Microvillus and the Organization of the Brush Border

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Microvilli are ~1-μm long finger-like projections present on the apical surfaces of certain, specialized absorptive epithelial cells. A highly symmetric hexagonal array of thousands of these structures form the brush border, which in addition to providing a significant increase in surface area also serves a barrier function against invading pathogens. Here, for the first time, we present an atomic model of the protein cytoskeleton responsible for this dramatic cellular morphology. This model incorporates spectroscopic, crystallographic, and microscopic data reported by several groups over the last 30 years into a single cohesive macromolecular complex composed of actin, fimbrin, villin, brush border myosin (Myo1A), calmodulin, and brush border spectrin. The biological, biochemical, and biophysical implications stemming from this model will be discussed.

1086-Plat

Structural Study And Modeling Of The Influenza Viral Ns1 Protein

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The influenza NS1 protein is an intriguing molecule that performs a large number of functions and interacts with different types of molecules including proteins and nucleic acids. The N-terminal domain and the C-terminal domain structures of the molecule have been solved and both domain structures exist in a dimeric arrangement. Based on docking studies and using a loop-modeling algorithm developed in our laboratory, we have developed structural models for the NS1 dimeric complex. These models are compared to each other and to the Bornholdt & Prasad model. The bindings of both the double-stranded nucleic acid molecule and the single-stranded poly(A) mRNA to the NS1 protein are investigated. The relative small interface surface areas for the dimeric complex are consistent with the conservation of the domain structure during the dimerization process. Structurally, considering the NS1 protein as a two-domains signaling molecule is discussed. While there is a lack of sequence homology, there exist similarities between the domain structures of the NS1 protein and other signaling molecules.

Platform T: Ion Motive ATPases

1087-Plat

Cryoelectron Microscopy of an ATP-dependent Cu pump from Archaeoglobus fulgidis

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CopA is an ATP-dependent Cu pump that belongs to the P1 subfamily of P-type ATPases. This subfamily shares core catalytic domains with other members of the family, such as Ca-ATPase and Na,K-ATPase, for which the X-ray crystal structures have been determined. Members of the P1 family are characterized by extended N-termini, which contain tandem repeats of metal binding domains (MBD). Compared to other P-type ATPases, P1 pumps have two extra transmembrane helices near the N-terminus and truncation of four C-terminal transmembrane helices. CopA from Archaeoglobus fulgidis is an unusual P1 pump because, in addition to the N-terminal MBD, it also has a C-terminal MBD. Although there is some uncertainty about their precise function, MBDs have been proposed to participate in regulation, in targeting, and in transfer of Cu to the transport sites. To study the structural disposition of the MBDs, we have expressed constructs of CopA with truncation of the N-terminus and the C-terminus either individually or together. We have used cryoelectron microscopy and helical reconstruction to determine structures of these constructs. Comparison of the double truncation with the C-terminal truncation revealed the location of the N-terminal domain. We constructed an atomic model by fitting X-ray crystal structures of relevant fragments into our map, which suggests a regulatory role for the N-terminal domain. By imaging somewhat wider tubular crystals with better order, we have been able to determine the structure of the C-terminal truncation at higher resolution. This new structure reveals the architecture of the transmembrane domain and allows us to place the extra two transmembrane helices with greater precision. Additionally, we are working on a structure of the N-terminal truncation, which should reveal the location of the C-terminal domain and help us determine its role in Cu transport.

1088-Plat

Molecular Insights Into The Modulation Of Sodium Binding Affinity And Voltage Sensitivity Of The Sodium-Potassium Pump From Molecular Dynamics Simulations, Electrophysiology And Structure

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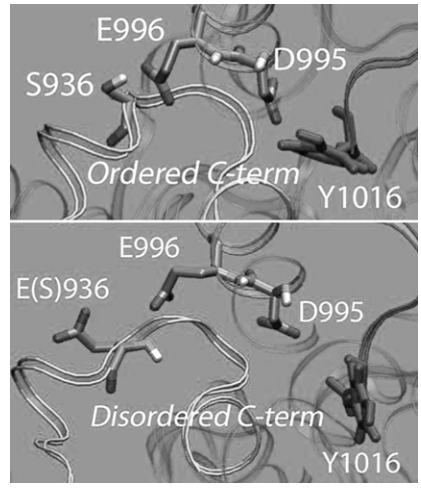
The Na^+-K^+ ATPase couples ATP hydrolysis to the export of three Na^+ ions and the import of two K^+ ions into the cell. It has been proposed that the C-terminus controls the pump's Na^+-binding affinity, but the molecular details of the pump's voltage sensitivity and regulation remain unknown. Combining data from Molecular Dynamics simulations, Electrophysiology and Crystallography, we propose a novel molecular mechanism of voltage sensitivity and regulation of the pump by the C-terminus.

When transmembrane electrical potentials are applied in simulations, a controversial PKA phosphorylation site: Ser936 becomes more accessible to cytoplasmic kinases than in the crystal structure. Phosphorylation of Ser936 has a disordering effect on the C-terminus, which is linked to Ser936 by a hydrogen-bonding bridge involving Asp995 and Glu996. Electrophysiological studies in *Xenopus* oocytes confirm the predictions and show that Ser936Glu, Asp995His and other related mutants distinctly alter the Na^+-binding affinity and voltage sensitivity. The study addresses a long-debated possible regulatory role of residue Ser936, and shows how the regulatory C-terminus is linked to Ser936 and Asp995, mutations in which cause Familial Hemiplegic Migraine.



1089-Plat

Cross-Linkable, Gain-of-Function Phospholamban (PLB) Mutant Reveals the Molecular Mechanism of SERCA2a Inhibition

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The PLB monomer inhibits the Ca2+ pump of cardiac sarcoplasmic reticulum (SERCA2a) by decreasing the apparent Ca2+ affinity of the enzyme. Here we