2159-Plat
Transient Disorder: Calcineurin as an Example
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How intrinsically disordered proteins and intrinsically disordered regions evade degradation by the cellular machinery evolved to recognize unfolded and misfolded chains remains a vexing question. One potential means by which this can occur is that the disorder is transient in nature. That is, the disordered state exists just long enough for it to be bound by a partner biomolecule and fold. Calcineurin (CaN) possesses a regulatory domain that appears to to be transiently disordered. Despite its transient nature, this disordered state is essential for activation of this enzyme. CaN is a highly-conserved, heterodimeric Ser/Thr phosphatase that plays vital roles in memory development and retention, cardiac growth, and immune system activation. Alterations in the regulation of CaN contributes to disorders such as Alzheimer's disease, Down syndrome, autoimmune disorders and cardiac hypertrophy. At low calcium levels the 95 residue regulatory domain in CaN appears to be folded. As levels rise, the CaN B chain binds calcium and undergoes a conformational change that releases the regulatory domain into a disordered state. The subsequent binding of CaM to CaN results in the regulatory domain folding. Folding of the regulatory domain in turn causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. The transient disordered state of the regulatory domain is an essential intermediate state in the process of activation.

Platform: Membrane Pumps, Transporters, and Exchangers II

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Transport Pathway in Cu^+ P-Type ATPases
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Cellular copper levels are carefully controlled to avoid toxic free copper while maintaining integrity of enzymatic copper centers. PIB-type ATPases transport Cu^+ ions across membranes with high specificity. Here, we use a range of molecular dynamics (MD) simulation techniques in combination with enzymatic assays and x-ray crystallography to determine the copper-transport pathway mediated by a Cu^+ transporting P-type ATPase (CopA). The simulation data provide insight into transport energetics and accompanying side-chain interactions that would be hard to observe experimentally. We also observed a unique mode of ion transport, where the dephosphorylation event was not obligatory coupled to the structural rearrangements that produce an occluded intermediate state. Hence, the generalized reaction scheme based on PII-type ATPases, such as SERCA, might not apply to all P-type ATPases.

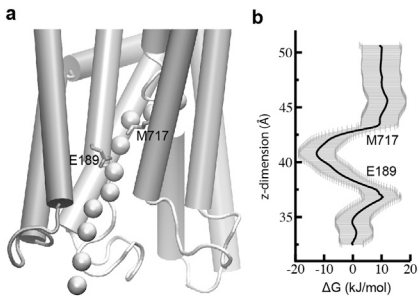

Figure 1: The simulated energetics associated with copper release.

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Somatic Mutations in the Na,K-ATPase Can Cause Hypertension
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Conn's syndrome, which accounts for at least 5% of hypertension cases, is caused by aldosterone-producing adenomas (APAs) of the adrenal gland that are highly prone to somatic mutations(1,2). From exome sequencing of ten APAs from the zona glomerulosa (ZG), we identified four with mutations in ATP1A1 encoding the α1-subunit of the Na^+/K^+-ATPase(3).
Expression of the most frequent mutation in ZG APAs, L104R ATP1A1, in Xenopus oocytes not only abrogated active ion transport, but allowed a significant inward current at physiological levels of Na^+ and K^+. Na^+ and H^+ can both leak, while K^+ at high concentrations acts as a non-conducting partial inhibitor. The three other ATP1A1 mutations found in APAs caused inward leaking currents under physiological conditions as well. Inward leaking is expected to depolarize ZG cells, causing opening of voltage-gated Ca^{2+} channels and increased aldosterone production in response to the rising cytoplasmic Ca^{2+} levels.
The L104 is completely conserved in Na^+,K^+-ATPases as well as in the closely related Ca^{2+}-ATPases SERCA and PMCA. The residue is situated one helical turn above a kink in the middle of transmembrane helix 1, and is only 4Å from a key ion binding residue. Recently, it was discovered in SERCA that a 12Å sliding of M1 relative to M4 creates an opening within the membrane towards the the ion-accepting sites in the E1 conformation(4).
Small ZG APAs with somatic mutations may thus prove to constitute a novel group of curable hypertension, and we can structurally and functionally explain why the mutations causes APAs.
1 Choi et al., Science, 2011.
2 Beuschlein et al., Nature Genetics, 2013.
3 Azizan, Poulsen et al., Nature Genetics, 2013.
4 Winther, Bublitz et al., Nature, 2013.

2162-Plat
Glutathionylation of the β1 Subunit Prevents the E1Na3 to E2P Forward Reaction in the Na^+, K^+ ATPase
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Exposure of voltage clamped cardiomyocytes to receptor-coupled oxidant signalling has been associated with a decrease in electrogenic Na^+, K^+ pump current activity vattributed to glutathionylation of the pump's β1 subunit. The mechanism by which glutathionylation, a reversible oxidative modification, affects turnover is not understood. We investigated the effect of β1 subunit glutathionylation on the E1Na3 → E2P forward reaction using the fluorescent probe anthryl-ouabain (AO) that binds to E2P species.
We induced glutathionylation in Na^+, K^+ ATPase enriched pig kidney membrane fragments by exposure to the chemical oxidant peroxynitrite. The Na^+, K^+ ATPase was stabilised in the E1Na3 poise by incubation in 100 mM NaCl and 20 mM Histidine. Glutathionylation of the β1 subunit was confirmed by immunoblotting techniques. MgATP was added to initiate the E1Na3 → E2P conversion and the E2P species was detected by the binding of AO. Addition of MgATP induced an increase in AO fluorescence indicative of a shift to E2P. The increase in fluorescence was blocked by the exposure to peroxynitrite. When glutathionylation was reversed by exposure to glutaredoxin 1, confirmed by the immublotting technique, the shift to E2P was restored as indicated by AO fluorescence.
In an independent series of experiments we utilised baseline glutathionylation of a fraction of the Na^+, K^+ ATPase that is detectable without exposure to oxidants. We followed the E1Na3 → E2P conversion with RH421 fluorescence. As expected, addition of MgATP markedly increased fluorescence. We added 2.5 mM dithiothreitol (DTT) to rapidly reverse glutathionylation. This further increased RH421 fluorescence indictating that DTT made additional Na^+, K^+ ATPase available to undergo the E1Na3 → E2P conversion.
We conclude that glutathionylation of the β1 subunit blocks the forward E1Na3 → E2P reaction.

2163-Plat
Discovery of Enzyme Modulators via High-Throughput Time-Resolved FRET in Living Cells
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We have used a "2-color" SERCA (sarco/endo-plasmic reticulum calcium ATPase) biosensor and a high-throughput fluorescence lifetime plate-reader (FLT-PR) to develop a high-precision live-cell assay designed to screen for small molecules that perturb SERCA structure. We used a construct derived from canine cardiac SERCA, in which red fluorescent protein (RFP) was fused to the N terminus and green fluorescent protein (GFP) to an interior loop. This 2-color SERCA was stably expressed in HEK-GnTI- cells, a strain that can be grown in monolayers or in suspension, as needed for automated transfer to multiwell plates. Fluorescence resonance energy transfer (FRET) was measured from GFP to RFP using the FLT-PR, which increases precision by a factor of 30 over a conventional intensity-based plate-reader, without