3339-Pos Board B67

Heme-Transfer Mechanism of Structurally Similar Isd NEAT Domains of Staphylococcus Aureus Exhibiting Different Affinities for Heme

Yoshitaka Moriwaki[1], Tohru Terada[1], Jose M.M. Caaveiro[1], Yousuke Takaoka[2], Itaru Hamachi[2], Kouhei Tsumoto[1], Kentaro Shimizu[1].
[1]The University of Tokyo, Tokyo, Japan, [2]Kyoto University, Kyoto, Japan.

Near Transporter (NEAT) domains of the iron-regulated surface determinant (Isd) proteins play an important role in importing heme from host animals to Staphylococcus aureus. IsdH, IsdA, and IsdC are anchored to the cell wall and display one or more copies of NEAT domain. The order of transfer of heme between the NEAT domains is as follows: IsdH-NEAT3 → IsdA-NEAT → IsdC-NEAT, though the three-dimensional structures are quite similar. In this study, we measured the difference of the affinities for heme between these NEAT domains and found that the affinity gradually increased in the downstream receptors. To gain insight into the atomistic mechanism for the difference, we performed in silico MD simulation and in vitro site-directed mutagenesis. The simulations showed that a cluster of negatively charged residues in the first loop between strand β1b and the 310 helix (loop 1 region) adversely influences the interaction with the propionate group of heme. Meanwhile, the higher affinity of IsdC was partly attributed to an internal salt-bridge formed between Glu88/Arg100 on binding to heme. On the other hand, we also observed that Phe130 of IsdC makes the β7-β8 hairpin region less flexible in the ligand-free form, which leads to reducing the entropy loss on binding to heme. Based on these calculations, we confirmed that substitution of these key residues decreased its affinity for heme. Importantly, these affinity-decreased IsdC mutants did transfer heme back to IsdA. Moreover, substitution of the loop 1 region in IsdH-NEAT3 for IsdC-type residues reversibly increased its affinity for heme and did not transfer to IsdA. Thus, NEAT domains have evolved the characteristic residues on the common structural scaffold so that they exhibit different affinities for heme and promote the efficient transfer of heme.

3340-Pos Board B68

Exploring How Phosphorylation Influences C-I Interaction and Calcium Sensitivity of Troponin by Molecular Dynamics Simulations

Yuanhua Cheng[1,2], Steffen Lindert[2], Peter Kekenes-Huskey[2], Vijay S. Rao[1], Paul R. Rosevear[3], J. Andrew McCammon[2], Andrew McCulloch[2], Michael Regnier[1].
[1]University of Washington, Seattle, WA, USA, [2]University of California, San Diego, San Diego, CA, USA, [3]University of Cincinnati, Cincinnati, OH, USA.

During β-adrenergic stimulation, cTnI is phosphorylated by PKA at sites S23/S24, located at the N-terminus of cTnI. This phosphorylation has been shown to decrease K_{Ca}, decrease pCa_{50}, and weaken C-I interaction. However, as the N-terminus of cTnI (residues 1-40) has not been resolved, the structural basis of how PKA phosphorylation influences cTn structure and calcium binding still remains elusive. Here, for the first time, we built up the cTn complex structure (including residues cTnC 1-161, cTnI 1-172, and cTnT 236-285) based on Rosevear's NMR structure. To mimic phosphorylation status, we constructed a bis-phosphomimics model by mutating S23/S24 of cTnI to aspartic acid. Then, 150 ns duplicated molecular dynamics (MD) simulations were performed on both WT and TnI-S23DS24D models. Through residue-residue contact analysis, we found that introducing these two phosphomimic mutations to sites S23/S24 significantly alters the native C-I interaction, except in the switch/inhibitory regions of cTnI. This phosphorylation also leads to the formation of intra-subunit interaction between N-terminus and inhibitory peptide of cTnI. Then, we studied how this phosphorylation influences the calcium handling in site II by calculating the distance between calcium and its coordinating residues. We saw that Ca^{2+} could coordinate with four residues (D65, D67, D73, E76), and S69 is the most flexible residue, which is in agreement with our previous observation. We also analyzed the interhelical angle and distance between A/B and B/C helices and quantified the exposure of hydrophobic surface in cTnC related to cTnI switch peptide binding. Simulations of an entire cTn complex model and its bis-phosphomimics status can elucidate the dynamic interplay among cTnC, cTnI and cTnT subunits, as well as the cellular progress during β-adrenergic stimulation on a molecular level. HL65497, HL11197 (MR), 8P41GM103426 (AM & AM).

3341-Pos Board B69

A Complete Configurational Ensemble Approach to Expand Lsd1/Corest Druggability

James C. Robertson[1], Nate C. Hurley[1], Julie M. Kneller[1], Nadeem A. Vellore[1], Andrea Mattevi[2], Riccardo Baron[1].
[1]University of Utah, Salt Lake City, UT, USA, [2]University of Pavia, Pavia, Italy.

Lysine specific demethylase-1 (LSD1/KDM1A) in complex with the corepressor protein CoREST is a promising target for epigenetic drugs yet no therapeutics targeting LSD1/CoREST are currently available. Extended molecular dynamics (MD) simulations have indicated that LSD1/CoREST nanoscale clamp dynamics are regulated by substrate binding and highlighted key hinge points of this large-scale motion as well as the relevance of local residue dynamics. Prompted by the urgent need for new molecular probes and inhibitors to understand LSD1/CoREST interactions with small-molecules, peptides, protein partners, and chromatin, we undertake here a complete configurational ensemble approach to expand LSD1/CoREST druggability. The independent algorithms FTMap and SiteMap and a newly developed Druggable Site Visualizer (DSV) software tool were used to predict and inspect favorable binding sites on an ensemble of structures generated by MD simulation. We found that three hinge points revealed by MD simulations are new potential targets for the discovery of molecular probes to block association of LSD1/CoREST with chromatin or protein partners. A fourth region was also predicted from simulated configurational ensembles and was experimentally validated to have strong binding propensity for a small peptide. This prediction would be prevented when using only the X-ray structures available (including the X-ray structure bound to the same peptide), which underscores the relevance of protein conformational dynamics in protein interactions. A fifth region was also highlighted corresponding to a small pocket on the AOD domain. This study sets the basis for future virtual screening campaigns targeting the five novel regions reported herein and for the design of LSD1/CoREST mutants to probe LSD1/CoREST binding with chromatin and various protein partners. The newly developed computational methods are being further validated on various protein receptors and have shown promising preliminary results.

3342-Pos Board B70

The Acidic Residues of the IκBα PEST Sequence are Responsible for "Stripping" NFκB from DNA

Holly E. Dembinski, Elizabeth A. Komives.
Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA, USA.

Nuclear factor kappa B (NFκB) transcription factors are responsible for the regulation of more than 150 target genes, their expression is induced by many classes of stimuli, and NFκBs play essential roles in the healthy regulation of cellular development and proliferation in inflammatory and immune responses. Diseases such as cancer, heart disease, Alzheimer's disease, and AIDS can be attributed to the aberrant regulation of NFκB. The transcriptional activity of NFκB is controlled by its inhibitors, the IκBs. IκBα, in particular, dynamically responds to extracellular stimuli releasing a burst of NFκB that enters the nucleus and activates hundreds of target genes. The transcriptional activation is short-lived, and our lab has been investigating the mechanism of post-induction repression. We previously showed that IκBα actively dissociates or "strips" NFκB from DNA. Analysis of the crystal structures of NFκB (RelA/p50) with DNA and with IκBα shows that the IκBα PEST sequence, which is rich in glutamate and aspartate residues, forms similar electrostatic contacts to NFκB as the DNA. Given this, we hypothesized that the IκBα PEST sequence electrostatically repels DNA from NFκB during the stripping process. Here we present fascinating results that show that the individual and collective, conservative mutation of these acidic residues to their amide counterparts does not affect the binding affinities of these mutants to NFκB; however, the mutant in which all five acidic residues are neutralized is incapable of stripping NFκB from DNA and instead forms a stable IκBα-NFκB-DNA ternary complex.

3343-Pos Board B71

Structural Basis for Ca^{2+} Selectivity of a Voltage-Gated Calcium Channel

Lin Tang[1], Tamer M. Gamal El-Din[1], Jian Payandeh[2], Gilbert Q. Martinez[1], Teresa M. Heard[1], Todd Scheuer[1], Ning Zheng[1], William A. Catterall[1].
[1]University of Washington, seattle, WA, USA, [2]Genentech Inc., South San Francisco, CA, USA.

Voltage-gated calcium (Ca_V) channels catalyze rapid, highly selective influx of Ca^{2+} into cells despite a 70-fold higher extracellular concentration of Na^+. How Ca_V channels solve this fundamental biophysical problem remains unclear. Here we report physiological and crystallographic analyses of a calcium selectivity filter constructed in the tetrameric bacterial Na_V channel Na_VAb. Our results reveal Ca^{2+} interactions with two high-affinity Ca^{2+} binding sites followed by a third lower affinity site that Ca^{2+} would occupy as it moves inward through the pore. At the entry to the selectivity filter, Site 1 is coordinated by a quartet of the acidic residue, D178, which plays a critical role in determining Ca^{2+} selectivity. In the center of the selectivity filter, Site 2 is constructed of the carboxyl side chains of D177 and the backbone carbonyls