(precursor of BNP which has additional 76 residues at the N-terminus). The dissociation constants and binding kinetics of anti-BNP mAb and the three forms of BNP derivatives were determined by FCS and fluorescence quenching. Comparisons of the thermodynamics results on the three forms of BNP provide insights on how the size and conformation of the antigen affect the antigen-antibody interactions.

1050-Pos Adiabatic Compressibility Variation During Amphiphile Binding To Bovine Serum Albumin

Najwa El Kadi[1], Nicolas Taulier[1], Wladimir Urbach[2], Marcel Waks[1]

[1] *CNRS, Paris, France*
[2] *ENS, Paris, France.*

Board B26

Serum albumin, the most abundant transport protein in the circulatory system, is made of three structurally homologous, predominantly alpha-helical domains. These domains denominated I, II and III, share the two primary protein binding sites existing for a wide variety of ligands/drugs. Since it has been crystallized, the protein has therefore attracted a renewed interest for the understanding of its drug transport and delivery mechanism. We present here the ultrasonic characterization of bovine serum albumin (BSA) during the binding of amphiphile molecules: a cationic surfactant cetylmethyl ammonium chloride (CTAC) and a fatty acid myristic acid (myr). We have investigated BSA by densimetric studies and adiabatic compressibility determinations and monitored the resulting conformational changes by circular dichroîsm. We have investigated first the BSA adiabatic compressibility as a function of CTAC concentration. The compressibility increases up to a CTAC/protein molar ratio of 20, decreasing then for higher ratio values. This result has been interpreted in terms of surfactant binding, water release, protein unfolding and/or aggregation. At the same time the alpha-helical content of BSA was decreasing starting at a ratio around 20. In contrast, volumetric measurements of myristic acid binding reveal a maximum at a myr/BSA molar ratio of 7, which corresponds precisely to the maximum number of binding sites as reported by recent NMR studies. At higher ratio values, a very slow decrease of the compressibility is observed, which might be interpreted by the formation of myr micelles. The binding does not induce observable changes in BSA secondary structure. These results are of value for the design and the developpement of new, tailor-made drug carriers, an important area for future research.

1051-Pos Mutations In PTEN's Phosphatase Domain Affect Binding To Phosphatidylinositol-4,5-bisphosphate and Phosphatidylserine

Roberta E. Redfern[1], Alonzo Ross[2], Arne Gericke[1]

[1] *Kent State University, Kent, OH, USA*
[2] *University of Massachusetts Medical School, Worcester, MA, USA.*

Board B27

PTEN is a phosphatase specific for the 3 position of the phosophoinositide ring that is deleted or mutated in many different disease states. PTEN association with membranes requires the interaction of its C2 domain with phosphatidylserine (PS) and most importantly, the interaction of its N-terminal end with phosphatidylinositol-4,5-bisphophate ($PI(4,5)P_2$). We have shown that the latter interaction is specific for $PI(4,5)P_2$ over all other phosphoinositides and this binding specificity is maintained for a short peptide representing PTEN's first 21 amino acids ($PTEN_{1–21}$). We hypothesize that some disease relevant mutations affect the extent and/or specificity of PTEN's binding to $PI(4,5)P_2$ or PS. We have shown that one particular cancer relevant mutation, K13E, leads to a loss of PTEN binding to $PI(4,5)P_2$, suggesting that the lysine in the 13 position is critical for binding. We have investigated several mutants that either vary the nature of the amino acid in the 13 position or alter the position of the lysine by switching it with neighboring amino acids. All mutations encountered abrogate binding of $PTEN_{1–21}$ to $PI(4,5)P_2$ and abolish specificity. Importantly, many of these mutations maintain the overall charge of the peptide, highlighting that the interaction between PTEN and phosphoinositides is specific and not governed only by non-specific electrostatic interactions. $PTEN_{H93R}$ is an autism relevant mutation in PTEN's phosphatase domain associated with a complete loss of *in vivo* activity. Our results indicate that this mutant protein binds more strongly to PS than the wild type protein and that the protein undergoes different conformational changes upon interactions with lipids than the wild type protein. We hypothesize that a new PS binding site is created by this mutation and that the strong binding prevents PTEN from "hopping", which limits PTEN's substrate access and reduces its activity.

Protein-Ligand Interactions - II

1052-Pos Carbohydrate recognition by anti-viral proteins

David F. Green

Stony Brook University, Stony Brook, NY, USA.

Board B28

The surfaces of many enveloped viruses are heavily glycosylated to avoid an immune response; the HIV envelope glycoprotein gp120 is a perfect example. This creates many challenges to the effective inhibition of viral-cell recogition, but also provides unique opportunities. Over the past several years, a number of prokaryotic proteins have been shown to inhibit HIV cell-entry by binding to gp120; these proteins specifically recognize the carbohydrate portion of the glycoprotein. Despite a number of structural and biochemical studies, a complete understanding of the origins of affinity and specificity for diverse carbohydrate targets has yet to be developed.

Using a range of computational approaches, we have developed detailed models of carbohydrate binding to the best-studied of these proteins, Cynanovirin-N. The known differences in binding affinities of a series of oligosaccharides are well-matched by calculations based on these models, and can be explained in simple structural

terms. The models additional provide insight into the mechanisms of multi-valent binding — a key property of these systems. A computational protein design protocol has further been able to suggest possible variants of Cyanovirin-N with enhanced affinity and specifity for a number of carbohydrate targets.

Work is on-going to extend these studies on Cyanovirin-N to the less-well characterized anti-viral proteins. In addition, the methodologies and insights developed through these studies may be applicable to a diverse set of problems in the molecular interactions of glycoproteins.

1053-Pos Drug Discovery for HIV-1 NCp7 and Structural Analysis Using NMR

Lingchun Yang, Deborah Kerwood, Philip N. Borer

Syracuse University, Syracuse, NY, USA.

Board B29

The Human Immunodeficiency Virus (HIV), responsible for the Acquired Immunodeficiency Syndrome (AIDS), is a member of the retroviral family. The nucleocapsid (NC) domain of the Gag precursor is critical for the recognition and packaging of the viral genome and appears to be important for viral particle formation. Structural analysis of NCp7 by NMR has already provided clues about the NC binding mechanism and suggested tools to defeat AIDS. When a drug candidate binds the protein, the electronic environment of the protein's binding site changes and results in perturbation of the chemical shifts of nuclei at that site. Two-dimensional ^{1}H-^{15}N heteronuclear single-quantum correlation (HSQC)-NMR spectroscopy can be used to screen for ligand binding by detecting the amide signals of the ^{15}N-labeled protein. Based on previous research in our group, 22 compounds (D1-D22) from the NCI Diversity set were found to interfere with NCp7 binding to the HIV-RNA packaging signal. The HSQC spectra of a series of ligands bound to NC have been obtained. The primary analyses show that D9 can bind with NCp7 and change the structure of NCp7 gradually with increasing ratio and finally denatures the protein when the molar ratio, D9:NCp7 is 8:1([NCp7]=0.1mM). D7, D8, and D11 also can denature NCp7 when the ratio is above 4:1. The comparison of HSQC spectra between free NCp7 and NCp7 bound with D17 or D19 proves that D17 or D19 first reacts with the second zinc finger, then the first zinc finger, and finally denatures the protein. These results suggest that these compounds may become the promising drugs which can interact with zinc fingers and change the structure of NCp7.

(D7=NSC 48300, D8=NSC 305787, D9=NSC 107684, D11=NSC 65238, D17=NSC 305819, D19=NSC 13950)

1054-Pos Agonist/Antagonist Sensor in Opioid Receptors - Molecular Modeling Study

Michal Kolinski, Slawomir Filipek

International Institute of Molecular and Cell Biology, Warsaw, Poland.

Board B30

Opioid receptors belong to a large superfamily of G protein-coupled receptors (GPCRs). These receptors are essential for signaling across plasma membranes. Each GPCR responds to a single or multiple ligands by activating G proteins and thus giving rise to a highly amplified signaling cascade. GPCRs represent the largest and most versatile family of membrane receptors. Although so different in action all these receptors share the same topology - a bundle of seven transmembrane α-helices. Activated receptor undergoes conformational rearrangements leading to transmitting the signal to cell interior.

Opioid receptors (mu, delta and kappa) belong to family A (rhodopsin-like) of GPCRs. For the important role they play in the human body in controlling pain and stress, modulating immune responses and developing addiction the opioid receptors were subject of numerous investigations.

The first event in opioid receptor activation includes sensing of agonists and antagonists. To investigate this effect at the atomic level we chose a set of rigid ligands based on the structural motif of tyramine. Four antagonists were used and three agonists including morphine. Opioid receptors and their complexes were simulated in the membrane. Based on simulated annealing and molecular dynamics methods we propose a distinct mode (a sensor) of binding of antagonists and agonists in the active site of opioid receptors. The phenolic hydroxyl group of antagonists was bound to Y3.33 and that of agonists to H6.52. All analyzed agonists broke a hydrogen bond D3.32-Y7.43 and thus weakened the connection between transmembrane helices 3 and 7. Moreover, when antagonist was forced to bind H6.52 it also broke D3.32-Y7.43 in all opioid receptors.

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1055-Pos Coarse-Grained Modeling of Signal Transduction in Beta2 Adrenergic Receptor

Ilya A. Balabin, David N. Beratan

Duke University, Durham, NC, USA.

Board B31

Beta2 adrenergic receptor (B2AR) is a G-protein coupled receptor (GPCR) targeted by the catecholamines. Activating of B2AR causes smooth muscle relaxation resulting in dilation of bronchial passages, vasodilation in muscle and liver, relaxation of uterine muscle and release of insulin. Thanks to this function, B2AR is a prime pharmaceutical target is asthma therapy. However, B2AR agonists are also known to cause side effects such as increased heart rate and blood pressure, suggesting multiple signaling channels. Understanding the structural basis of signal transduction in B2AR will be immensely helpful in minimizing side effects of drugs that target the receptor. We perform a coarse-grained analysis of the B2AR structural motions induced by ligand binding. Effects of agonists and antagonists are explored, and strategies for designing new drugs to target B2AR are discussed.

1056-Pos Molecular Modeling of Syringomycin-E - membrane interactions

Edit Matyus, D. Peter Tieleman

University of Calgary, Calgary, AB, Canada.

Board B32

Syringomycin-E (SR-E) is a cyclic lipodepsipeptide produced by certain strains of the bacterium *Pseudomonas syringae pv. syringae*. It shows inhibitory effects against many fungal species, including human pathogens. Its primary biological target is the plasma membrane, where it forms channels comprised of at least six SR-E molecules. The asymmetric channel appears to directly incorporate 40 lipid molecules. The high-resolution structure of SR-E in solvents and the structure of the channels are currently not known. Studies of the SR-E channel structure may result in a better understanding of its biological activity.

We investigated in atomic detail the molecular features of SR-E in hydrophilic, hydrophobic and lipid bilayer environments by molecular dynamics simulation (MD). As a first step we built a model of SR-E and examined its structure in water and octane in 200 ns MD simulations including distance restraints derived from NMR NOE data. We determined structural preferences of SR-E in both solvents, in particular the importance of side-chain interactions in determining peptide stability. SR-E exists in two dominant structures whose relative probabilities depend on the polarity of the solvent, in agreement with published experimental data. These three-dimansional structures are used as a basis to investigate the interactions of SR-E with lipid membranes.

Activity of SR-E is significantly influenced by the lipid composition of the membrane, and they play a crucial role in channel gating. To study the influence of lipid charge and shape on peptide-lipid interactions and on pore formation we built DOPE and DOPS bilayers and incoporated six SR-E molecules in each lipid bilayer. These bilayer systems were simulated by long MD simulations. The results of trajectory analyses will be presented.

1057-Pos Structural and Biophysical Studies of the Interactions of Phosphatidylinostiol 3-kinase C2a with Clathrin

David P. Chimento[1], Thomas J. Murray[1], James Bedison[1], Yanqui Zhao[2], James H. Keen[2], Kathryn M. Fereguson[1]

[1] *University Of Pennsylvania, Philadelphia, PA, USA*
[2] *Thomas Jefferson University, Philadelphia, PA, USA.*

Board B33

We are interested in the potential regulatory role of the phosphatidylinostiol 3-kinase C2α (PI3K-C2α) in clathrin assembly and trafficking events. PI3K-C2α co-localizes with clathrin, and exogenous expression of PI3K-C2α affects clathrin-mediated endocytosis and sorting to the trans-Golgi network. Further, the association of clathrin with PI3K-C2α stimulates the kinase activity. Many clathrin interacting proteins contain a consensus sequence called a "clathrin-box" that mediates interaction with the terminal domain (TD) of the clathrin heavy chain. We are investigating the role of the clathrin-box and other possible binding regions from the N-terminal clathrin binding domain of PI3K-C2α for interactions with the clathrin terminal and ankle domains.

We have determined the X-ray crystal structure of a complex of the clathrin TD (amino acids 2-363; TD363) with amino acids 24-135 of PI3K-C2α (PI3K-C2α(24-135)). We observe only 6 amino acids of PI3K-C2α(24-135), located in a hydrophobic binding groove previously identified as the binding site for "clathrin-box" peptides. Surprisingly we find that this bound fragment is not the previously defined clathrin box (box1) of PI3K-C2α. Rather we identified a degenerate "clathrin-box" sequence (DLMVFP) that we term box2.

Surface plasmon resonance (SPR, Biacore) studies of PI3K-C2α (24-135) binding to TD363 show that this interaction is relatively weak (Kd ~ 100 μM) and dominated by clathrin box2. In further exploring the interaction of PI3K-C2α with clathrin we have observed higher affinity interactions (Kd ~ 1–5 μM) using either longer PI3K-C2α fragments, or clathrin fragments that comprise the terminal domain plus amino acids extending into the clathrin ankle domain. We anticipate that multiple contact points, each individually of rather low affinity, cooperate to form a stable complex between the PI3K-C2α and clathrin. Progress towards structural characterization of these multiple binding sites will be described.

1058-Pos Understanding the Mechanism of the Anti-angiogenic Activity of Suramin

Khalil Ibrahim, Karuppanan M. Kathir, Thallapuranam K. Kumar

University of Arkansas, Fayetteville, AR, USA.

Board B34

Angiogenesis is a cellular process that involves the sprouting of new blood vessels from pre-existing ones. Fibroblast growth factors (FGFs) play a crucial role in the regulation for angiogenesis and tumor metastases. Therefore, intensive research efforts are on to develop drugs that can specifically inhibit FGF-induced angiogenesis. FGFs exhibit their cell proliferation activity by binding to the extracellular D2 domain of their cell surface receptor. Suramin ({carbonyl bis[imino-3,1-phenylenecarbonylimino(4-methyl-3,1 phenylene)carbonylimino]}-bis(1,3,5-naphthalenetrisulfonic acid) hexasodium salt) has been previously shown to inhibit FGF-induced tumors. In this context, in the present study, we investigate the interaction of suramin with the extracellular D2 domain of the FGF receptor (FGFR). Results of the isothermal titration calorimetry (ITC) experiments suggest that suramin binds to the D2 domain of FGFR with a reasonably high affinity ($K_d \sim 10^{-6}$ M). ITC experiments, carried out a various salt concentrations, showed that suramin-D2 domain interaction is mostly stabilized by ionic interactions. Size exclusion chromatography profiles of the D2 domain, obtained in the presence and absence of suramin, show that the drug binds to the receptor domain in a 1:1 stoichiometry. Equilibrium unfolding experiments monitored by far UV circular dichroism reveal that the D2 domain is significantly stabilized by suramin.

^{1}H-^{15}N chemical shift perturbation data show that the suramin binding sites are mostly contributed by residues located at the N- and C-terminal ends of the D2 domain. Interestingly, some of the residues that bind to suramin are located at the FGF-D2 domain interface. Therefore, it appears that suramin inhibits the cell proliferation activity of FGF by preventing its interaction with FGFR. The results of this study are expected to pave way for a rational design of drugs against FGF-induced tumors.

1059-Pos Dimerization Of The Fgfr Is Prerequisite For Fgf Signaling

Ryan Thurman, Rajalingam Dakshinamurthy, Fei Guo, Joshua Sakon, Suresh Kumar Krishnaswamy Thallampuranam

University of Arkansas, Fayetteville, AR, USA.

Board B35

Fibroblast growth factors (FGFs) are ~ 16 kDa heparin binding proteins that regulate key cellular processes such as angiogenesis, differentiation, morphogenesis, wound healing and tumor growth. FGFRs consist of three extracellular ligand binding domains (D1, D2, D3), a single transmembrane helix, and cytoplasmic tyrosine kinase domain. Cell surface-bound HSPGs (heparan sulfate proteoglycans) supported dimerization or polymerization of the FGFRs are thought to be required to activate the signaling pathway. The D2 domain is suggested to bind with both HSPGs and FGFs to form a ternary complex. Recent X-ray crystallography data has showed D2 domain formed a crystallographic 2-fold symmetry dimer with the interface residues including Y155 and which is different from other crystallographic studies of FGFRs. In this study, D2-Y155 has been mutated and characterized using various biophysical techniques including multidimensional NMR spectroscopy. Results from thermal denaturation studies using fluorescence and circular dichroism indicates that the D2-Y155A mutation reduced the melting temperature of the protein by 10°C to14 °C to result in a Tm of 38 °C. Urea and guanidine hydrochloride induced denaturation also confirm a significant destabilization of protein caused by the Y155A mutation. Isothermal titration calorimetry (ITC) experiments of D2-Y155A with sucrose octasulfate (SOS) indicates a reduced binding affinity (Kd ~ 4.78 μM) when compared to the D2 (Kd ~2.59 μM) wild type.

1060-Pos Understanding the Molecular Mechanism Underlying the Auto Inhibition of the Fibroblast Growth Factor Signaling

Lindsay Rutherford, D. Rajalingam, T. K. S. Kumar*

Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR.

Board B36

Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation. The FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR, this binding induces dimerization of FGFR, which is an essential step in FGF signaling. Fibroblast growth factor receptor (FGFR) extracellular domain consists of three Ig domains D1, D2, and D3. Between the Domains D1 and D2 is a short span of acidic residues called the "acid box". The D1-D2 linker is thought to play a role in regulation of FGF interaction with FGFR. Many of the FGF binding sites can be found on the extracellular D2 domain of the receptor. It is believed that "acid box" can regulate FGF binding to FGFR. The "acid box" can mimic heparin like compounds and bind at the heparin binding sites located on the surface of the D2 region of FGFR. In the present study, we synthesized a twenty-eight amino acid box region peptide and studied its interaction with D2 domain of FGFR using various biophysical techniques including multidimensional NMR spectroscopy. Our results clearly show that the acid box peptide binds to the ligand binding domain of the fibroblast growth factor receptor.

1061-Pos Molecular cloning, Overexpression and Biophysical Characterization of D3 domain of Fibroblast Growth Factor Receptor

TRINH D. TRAN, Rajalingam Dakshinamurthy, Thallapuranam Krishnaswamy Sureshkumar

UNIVERSITY OF ARKANSAS, Fayetteville, AR, USA.

Board B37

Fibroblast growth factor receptor (FGFR) is comprised of a transmembrane domain, a cytoplasmic domain, which is responsible for the tyrosine kinase activity, and an extracellular ligand-binding domain. The ligand-binding domain consists of three immunoglobulin-like domains (D1, D2, and D3). The FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR. Ligand binding induces dimerization of FGFR, which proves to be an essential step in FGF signaling. The D3 domain determines the specificity of the ligand binding (FGF binding), while the D2 domain includes the primary site for ligand and heparin binding. In this study for the first time, we report the cloning, expression, biophysical, and biological characterization of the D3 domain of FGFR2. D3 domain has been expressed in *Escherichia coli* in high yields. The protein was purified to homogeneity using affinity chromatography. Results of the steady-state fluorescence experiment show that the recombinant D3 domain is in a folded conformation. Far-UV circular dichroism (CD) data suggest that D3 domain is an all beta-sheet protein. The findings of the present study not only pave way for an in-depth structural investigation of the molecular mechanism(s) underlying the interaction of FGF with D3 domain but also provide avenues for developing agonists and antagonists for the treatment of FGF-induced pathogenesis.

1062-Pos Understanding the Role of Divalent Cations in FGF Signaling

Karuppanan M. Kathir, Sivaraja Vaithiyalingam, Suresh Kumar K. Thallapuranam

University of Arkansas, Fayetteville, AR, USA.

Board B38

Fibroblast Growth Factors are important molecules that regulate key biological processes such as, limb regeneration, wound healing and tumor growth. FGFs require heparin or heparin sulfate to activate their transmembrane receptor (FGFR). FGFR is composed of three immunoglobulin (Ig)-like domains (D1, D2 and D3). The ligand (FGF) - induced dimerization of FGFR leads to the juxtaposition of the cytoplasmic tyrosine kinase domain and its subsequent trans autophosphorylation. However, very little is known on the exact events involved in the regulation of FGF signaling process. Previous studies had shown that divalent cations bind to the D2 domain of FGFR. The bound cations have been shown to regulate the mitogenic activity of FGF. In addition, copper is known to be involved in non-classical FGF release. In this study, we investigate the role of divalent cations (Cu^{2+}, Zn^{2+}) in modulating the structural events leading to FGF signaling. Isothermal titration calorimetry (ITC) experiments reveal that the divalent cations bind to the D2 domain with high affinity. Results of the Far UV circular dichroism and steady state fluorescence experiments reveal that the metal ions trigger a significant conformational change in the D2 domain. The divalent cation binding sites on the D2 domain have been mapped using ^{1}H-^{15}N chemical shift perturbation. The divalent metal ions appear to increase binding affinity of sucrose octa sulfate (SOS), a structural analogue of heparin, to the D2 domain. Results of ITC and ^{1}H-^{15}N chemical shift perturbation experiments show that affinity of the D2 domain to bind to FGF decreases significantly in the presence of the divalent cations. The results of our study for the first time elucidate the role of divalent cations in the regulation of the FGF signaling process.

1063-Pos Interplay Between the Intracellular Domain of Amyloid Precursor Protein and Wnt Signaling

Fangfang Zhou[1], Xiufang Zhang[1], Nanming Zhao[1], Huaxi Xu[2], Yeguang Chen[1], Yandao Gong[1]

[1] *Tsinghua University, Beijing, China*
[2] *The Burnham Institute, La Jolla, CA, USA.*

Board B39

Amyloid precursor protein (APP), a key protein in pathogenesis of Alzheimer's disease (AD), is a type I transmembrane protein which can be cleaved by β- and γ-secretase to release the amyloidogenic β-amyloid peptides (Aβ) and the APP intracellular domain (AICD). While Aβ has been widely believed to initiate pathogenic cascades culminating AD, the physiological functions of AICD remain elusive. Here, we demonstrate that the AICD, independent of Fe65, is stabilized by canonical Wnt signaling mediated by β-catenin and then the accumulated AICD inversely inhibits Wnt signaling by enhancing the kinase activity of GSK3β through interacting with GSK3β and enforcing the association of GSK3β and Axin. Furthermore, based on the inhibition of Wnt signaling, AICD induces the promoter activity and protein expression of CDK inhibitors: p15, p16 and p21 with or without NGF stimulation, inhibiting the cell proliferation and facilitating the neurite outgrowth in neural PC12 and N2a cells.

1064-Pos The Activation and Repression of YlaC, ECF Sigma Factor, by Formation of Disulfide Bond in YlaD, Anti-sigma Factor, and Adding Mn^{2+} Ions at Condition on Interaction of YlaC and YlaD in *B. subtilis*

Han-Bong Ryu, InJi Shin, Hyung-Soon Yim, Sa-Ouk Kang

Seoul National University, Seoul, Republic of Korea.

Board B40

YlaC, one of seven ECF sigma factors of *B. subtilis*, has the function of gene expression for response to oxidative stress agent such as hydrogen peroxide or diamide. YlaD, anti-sigma factor of YlaC, is membrane protein having HX_3Cys-X-X-Cys motif acting as oxidative stress sensing domain and ZAS family (zinc-binding anti-sigma factor) in *Streptomyces coelicolor*. We now report that purified YlaC interacts with purified YlaD *in vitro* by oxidized and reduced state of YlaD or adding Mn^{2+} ions at condition on interaction of YlaC and YlaD. Reduced YlaD obtained by DTT treatment at YlaD binds to YlaC. Reversely, YlaC is released from interaction between YlaC and YlaD when oxidizing agent such as hydrogen peroxide or diamide added. Also, adding Mn^{2+} ions at condition on interaction of YlaC and YlaD obtains the same results as oxidizing reagents treatment at YlaD. These interactions of YlaC and YlaD *in vitro* studies indicate that redox state of YlaD is critical for YlaD binding to YlaC and Mn^{2+} ions release YlaC from YlaD binding to YlaC. Also, i*n vitro* transcription studies compensate for results of interaction of YlaC and YlaD *in vitro* studies. Two promoters, P_{yla} and Spx-dependent, have existence in the *ylaABCD* operon. It is known that Spx-dependent promoter responds to oxidative stress. P_{yla} promoter is not known. YlaC induces transcription from P_{yla} and Spx-dependent promoter. Hydrogen peroxide and diamide induce the transcription of *ylaC* transcripts but *ylaA* transcripts did not induced. These results suggest that YlaC may induce the transcription from P_{yla} and Spx-dependent promoter by different mechanism of releasing YlaC from YlaD binding to YlaC

1065-Pos The Allosteric Effect of a Single Point Mutation Can be Summed Up in One Individual Subunit of B. stearothermophilus Phosphofructokinase

Stephanie Perez, Gregory D. Reinhart

Texas A&M Univ, College Station, TX, USA.

Board B41

Gly184 is a key residue in conferring the allosteric response in *Bacillus stearothermophilus* PFK (BsPFK). As compared to wild-type, the substitution G184C has been shown to have a seven-fold decrease for PEP inhibition in BsPFK at 25°C. To further investigate how this alteration disrupts the complex inhibition pathway, the mutation was introduced into one subunit of hybrid tetramers that isolate the individual heterotropic interactions for BsPFK: 23Å, 30Å, 33Å, 45Å. The effect of G184C in each individual interaction was determined and compared to wild-type. In BsPFK, the four heterotropic interactions isolated in these hybrids account for the heterotropic inhibition observed for both wild-type and G184C. In the 32Å interaction, there was a two-fold decrease in PEP inhibition and a less dramatic decrease was seen in the 22Å interaction. There was almost no change in PEP inhibition observed in the remaining 30Å and 45Å heterotropic interactions. The allosteric effects of single perturbations in BsPFK have previously been thought to have a more global affect in the tetramer, however, this is the first residue shown to contain its effects in the single mutated subunit.

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1066-Pos Short Electronegative Sequences Mediate Binding of Obscurin to sAnk1

Benjamin R. Busby, Maegen A. Borzok, Aikaterini Konstrogianni-Konstantopoulos, Robert J. Bloch

University of Maryland, Baltimore, Baltimore, MD, USA.

Board B42

Obscurin is a 6,620 amino acid protein that surrounds the contractile apparatus at M-bands and Z-disks. It has been proposed to link the contractile apparatus to network sarcoplasmic reticulum (nSR) by binding via its C-terminal domain to a small form of ankyrin (sAnk1), an integral protein of the nSR. Two regions in the C-terminus have been shown to bind sAnk1, but their binding characteristics have not been compared quantitatively. We generated GST fusion constructs, and prepared biotinylated, synthetic peptides of the 30 residue minimal binding sequences of each, and assayed their binding to sAnk1 fusion constructs by surface plasmon resonance. Histagged $sAnk1_{29–155}$ constriucts bound to the $Obsc_{6316–6345}$ peptide with 90 nM affinity, whereas they bound to the $Obsc_{6231–6260}$ peptide with a much higher K_D (lower affinity). When the minimal binding region of sAnk1, $sAnk1_{60–130}$ was assayed, the $Obsc_{6231–6260}$ peptide binding became even weaker, but there was no significant change in binding of $Obsc_{6316–6345}$. We obtained similar results using fusion protein constructs of the obscurin sequences, rather than oligopeptides. We also assayed the ability of the synthetic peptides in solution to inhibit binding. Consistent with our direct binding assays, the $Obsc_{6316–6345}$ peptide was several-fold more potent an inhibitor than $Obsc_{6231–6260}$, when tested against fusion proteins containing either sequence. Finally, we studied the ability of both constructs to bind to eight mutants of sAnk1. We found that mutants of sAnk1 modulated binding of sAnk1 to both constructs similarly. Our results suggest that $Obsc_{6316–6345}$ predominates in binding to sAnk1, but that the same residues on sAnk1 that bind to this sequence with high affinity also bind to $Obsc_{6231–6260}$, albeit with lower affinity. We find it remarkable that a 6620-residue protein requires ≤ 30 amino acids to bind with nM affinity to a membrane ligand.

1067-Pos Identifying peptides that bind to the C-terminus of erythroid beta spectrin using a phage display

Chloe Antoniou[1], Yuanli Song[1], Sang Thai[2], Adnan Memic[2], Brian K. Kay[2], L. W.-M. Fung[1]

[1] *Department of Chemistry and*

[2] *Department of Biological Sciences, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL, 60607, USA.*

Board B43

Spectrin is a major cytoskeletal protein in a wide-range of cells that consists of alpha and beta subunits that form functional tetramers. Recently, spectrin isoforms have been shown to interact with many proteins in neuronal cells both as an alpha-beta complex and as individual subunits. Proteins that associate with beta spectrin in neurons include, amongst others, ankyrin, various types of sodium and calcium channel, neurofascin, protein kinase C, other cytoskeletal proteins etc. We have focused on the tetramerization region of spectrin in the cytoskeleton. A recombinant protein of the C-terminal region of beta spectrin was used as a target protein to screen for interacting peptides. A library of 10-amino acid peptides displayed on phages was used, and we have identified six peptides that bound to our target protein. The peptides were then synthesized and characterized by circular dichroism. Their association with spectrin isoforms were studied by isothermal titration calorimetry methods to further explore the interactions. The effects of these peptides on alpha and beta spectrin association will be discussed.

1068-Pos Molecular Simulation of the Scorpion BmP02 with the Small-conductance Calcium-activated Potassium Channels

Mehriar Amininasab[1], Maryam Rouhani[2]

[1] *Department of cell and molecular biology, Faculty of science, University of Tehran, Tehran, Iran (Islamic Republic of)*

[2] *Department of biophysics, Faculty of science, University of Tarbiat-moddaress, Tehran, Iran (Islamic Republic of).*

Board B44

The interaction of scorpion toxin BmP02 from *Buthus martensi karsch* with a small conductance calcium activated potassium channel rsk2 was simulated in the following steps. In the first step the overall orientation of the toxin with respect to rsk2 was determined according to the default Brownian dynamics algorithm implemented in the MACRODOX simulation package. Among all with the minimum potential energies of interactions, two modes of binding were

identified, selected and utilized as the starting complexes for the second step of simulations. In this step a 5 ns molecular dynamics simulations was performed in NPT conditions on each selected model using the GROMACS simulation package, version 3.3.1.

In general, the BD simulation and subsequent molecular dynamics refinement reveals the critical residues for the recognition and interaction between BmP02 and rsk2 complexes.

1069-Pos Calmodulin and Calcineurin: Mechanisms of Molecular Recognition

Susan E. O'Donnell, Madeline A. Shea

University of Iowa, Iowa City, IA, USA.

Board B45

Cardiac hypertrophy results from stress on cardiomyocytes, which contributes to heart failure. Calcineurin (CaN) is a dimeric Ser/Thr phosphatase, which when constitutively active, induces hypertrophic responses. The activity of CaN is controlled by intracellular calcium and requires calmodulin (CaM), a major Ca^{2+} sensor in eukaryotes. The individual roles of the two homologous domain of CaM in the activation CaN are not known.

Thermodynamic properties governing the interactions of CaN and CaM were studied. Fluorescence anisotropy was used to determine the binding affinity of CaM for a peptide (CaNp), representing the CaM-binding domain of CaN subunit A. Dissociation constants (K_d) were determined for the N-domain ($CaM_{1–80}$), C-domain ($CaM_{76–148}$) and full-length form of CaM ($CaM_{1–148}$) in the presence and absence of saturating calcium (10 mM). Calcium-saturated $CaM_{1–148}$ had a significantly higher affinity for CaNp than either of its two domains, with an estimated K_d of 1 pM. Domain-specific mutants of CaM were studied to determine how mutations *between* and *within* Ca^{2+}-binding sites alter the interactions between CaM and CaNp.

Fluorescence-monitored equilibrium calcium titrations of $CaM_{1–148}$, $CaM_{1–80}$, and $CaM_{76–148}$ showed how CaNp affected the Ca^{2+}-binding properties of CaM. The Ca^{2+} affinity of the N- and C-domain sites of $CaM_{1–148}$ increased significantly with the greater effect on the N-domain, evidently through interdomain cooperativity. Studies of mutants revealed that CaNp significantly improved the Ca^{2+}-binding properties of both domains of $CaM_{1–148}$ mutants, while there was little effect on corresponding domain fragments. Thus, CaNp restored the Ca^{2+}-binding properties of $CaM_{1–148}$ mutants, even when residues located in the Ca^{2+}-binding sites were compromised.

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1070-Pos Potentiation and Inhibition of the nACHR alpha 7 subtype by choline and a spirofuropyridine alpha 7 agonist

David Gurley, Jeff Smith, Tim Piser

Astrazeneca, Wilmington, DE, USA.

Board B46

The α7 neuronal nicotinic receptor (α7 nAChR) is a homopentameric ligand-gated ion channel expressed in mammalian central nervous system regions associated with cognitive processing. α7-selective agonists have demonstrated activity in rodent models of cognition. A hallmark of α7 receptor activation is rapid activation and desensitization, occuring on the order of milliseconds. Also, pre-application of agonists appear to inhibit subsequent ACH-mediated current by desensitization. Potency for desensitization of the receptor is much higher then potency to activate the receptor.

Choline is a full agonist on the α7-homomeric subtype (Papke et al., 1996). Additionally, on the α4β4 nAChR subtype, choline was shown to potentiate ACH-mediated currents (Zwart and Vijverberg, 2000). We further explored the activity of choline on the rat a7 receptor expressed in xenopus oocytes. Brief incubations with choline, at biologically relevant concentrations, much lower than those necessary to directly gate the α7 receptor, potentiated current mediated by low concentrations of ACH (30microM). Also, performing dose-response experiments with ACh revealed that co-application with choline resulted in a pronounced leftward shift in the ACH concentration-effect curve.

AZ1 is an α7-selective agonist with EC50 for activation in the high nanoM range when measured by net charge transfer and low microM range when measured by peak on rat α7. However, positive behavioural effects with this compound occur at plasma concentrations as low as ∼1.5nM, well below threshold for eliciting observable channel activation. We found that, like choline, AZ1 potentiates ACh-mediated current from α7 at concentrations well below those needed for direct activation of the receptor. These results provide a novel mechanism we call, positive orthosteric modulation, to explain behavioral effects observed with doses of AZ1 that are unlikely to elicit channel opening in vivo.

1071-Pos A Model for the Interaction of a Long chain alpha-neurotoxin with Muscle type nAChR

adak Nasiripourdori, Bijan Ranjbar, Hossein Naderi-manesh

Tarbiat modares university, tehran, Iran (Islamic Republic of).

Board B47

Molecular dynamics simulations of a model of ligand binding domain of Torpedo nicotinic acetylcholine receptor in complex with a long-chain alpha-neurotoxin from Naja Naja oxiana is conducted. The model is based on muscle type nAChR(2BG9) resolved by electron microscopy which appears to exist under the basal or resting state.Two simulations are run,one is receptor with toxin as a ligand and the other is receptor free of ligand, in order to give insight into the details of the interactions and conformational changes upon binding of a long-chain neurotoxin to the nAChR.Our docking results confirm the claim that *T*.nAChR (2BG9) is in basal or resting state, which favors binding to the alpha neurotoxins. Details of interaction shows that the long chain alpha neurotoxin interacts in so many ways similiar to that of other well-characterized alpha neurotoxins, such as alpha-bungaro toxin and alpha-cobratoxin. The main structural changes seen upon binding of neurotoxin compared to the apo form of nAChR,is that ligand-bonded nAChR is more compact and less dynamic in certain domains compared to the apo form.Most changes and dynamic motions are seen in loops and the lowes part of the LBD.the most distinctive loop motion appears to be that of the C-loop, the main constituent of binding site.In the

binding site, motions are much restricted and residues are more ordered in the presence of the ligand bound. In addition, some critical interactions in the binding site are studied, e.g. the triad of K145/D200/Y190.the salt bridge formed between K145/D200 remains intact and is even stabilized during the 5 nsec MD simulation, while it is obviously more labile in apo form.This is in agreement with the claim that alpha neurotoxins stabilize the resting state.

1072-Pos Interactions of the Anti-Psychotic Drug Trifluoperazine with Calmodulin and its Effects on Calcium Binding Affinity

Michael D. Feldkamp, Madeline A. Shea

Univeristy of Iowa, Iowa City, IA, USA.

Board B48

The anti-psychotic drug trifluoperazine (TFP) is an antagonist of calmodulin (CaM), binding in its hydrophobic clefts. Three crystal structures of TFP bound to calcium-CaM show 1, 2 or 4 TFP per CaM, but the RMSD of their backbones is < 1 Ã.... Their conformations are similar to CaM bound to CaMKII peptide, which increases the calcium affinity of CaM. However, the energetics of TFP binding to CaM, its effects on calcium binding, and possible interactions with apo (calcium-depleted) CaM are controversial.

Equilibrium calcium titrations monitored by changes in steady-state fluorescence of intrinsic Phe and Tyr residues were used to evaluate the effect of TFP on the calcium affinity of full length CaM (CaM1-148), N-domain (CaM1-80) and C-domain (CaM76-148) over a range of TFP:CaM ratios. Despite similarities between structures of CaM-TFP and CaM-peptide complexes, low levels of TFP (1:1, 2:1 ratios) decreased the calcium affinity of CaM. TFP had the greatest effect on calcium binding to sites III and IV, in the C-domain of CaM1-148, but affected both domains. At an 8:1 ratio of TFP:CaM, the effect reversed and the calcium affinity of CaM increased.

Stoichiometric titrations of TFP binding to CaM monitored by 1H-15N-HSQC NMR also identified the C-domain of CaM1-148 as most perturbed by TFP, whether apo or calcium-saturated. The maximal stoichiometry of TFP binding to apo CaM1-148 was 3:1; for calcium-CaM, it was 4:1. Addition of calcium to apo CaM1-148-TFP caused significant perturbation to the 1H-15N-HSQC spectrum, indicating the structure of apo CaM1-148 bound to TFP changed significantly upon calcium binding. This demonstrated that the TFP-CaM interfaces respond to calcium ligation, and that there is a hierarchy of binding TFP to its multiple sites.

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1073-Pos Thermodynamics of Nickel Ion Binding to Human Growth Hormone

Ali Akbar Saboury, Safora Amiri

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran (Islamic Republic of).

Board B49

Human growth hormone (hGH) is a single domain globular protein containing 191 amino acids and approximately molecular mass of 22 kDa. hGH plays an important role in somatic growth through its effects on the metabolism of proteins, carbohydrates and lipids. Following our reports recently on the binding properties and structural changes of hGH due to its interaction with a number of metal ions, a binding study of nickel ions by hormone has been done at 27°C in NaCl solution (50 mM) using an isothermal titration calorimetry. There is a set of three identical and noninteracting binding sites for nickel ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 40 μM and −16.5 kJ/mol, respectively. Thermodynamic parameters of nickel ion binding are compared to the other metal ions. The molar entropy of binding is 29.3 J K^{-1} mol^{-1} for Ni^{2+}, less than of Cu^{2+} and more than of Ca^{2+}, Mg^{2+}, Fe^{3+} and Co^{2+}, means that the disorder of the protein structure due to the binding of nickel ions is more than to the other ion metals studied, except Cu^{2+}. From spectroscopic data, it is expected that nickel ions can prevent from the aggregation of the hormone.

1074-Pos Role of Annexin in the Non-Classical Secretion Signal Peptide-less Proteins

Anna E. Daily, Rajalingam Dakshinamurthy, Suresh Kumar Krishnaswamy Thallampuranam

University of Arkansas, Fayetteville, AR, USA.

Board B50

Members of the S100 family of proteins are involved in a variety of calcium-signaling events and various human diseases such as cystic fibrosis, cardiomyophathy, and several types of cancer. S100A13, a member of the S100 family, exhibits ubiquitous expression in a broad range of tissues. S100A13 is known to be involved in the non-classical export of signal peptide-less proteins such as fibroblast growth factor (FGF-1) and interleukin-1Î± across the cell membrane. To achieve non-classical transport, S100A13, in complex with a signal peptide-less protein, binds to annexin II which exhibits an inducible flip-flop mechanism across the cell bilayer and by which helps the multiprotein release complex to traverse the membrane bilayer. In the present study the interaction of S100A13 and annexin II is characterized using various biophysical techniques including multidimensional NMR spectroscopy. We identified and synthesized using solid-phase peptide synthesis techniques a 15-amino acid N-terminal region of the annexin II, which bind to several members of S100 family. Fluorescence titration experiments on apo-S100 and calcium bound form with annexin II indicate that the annexin II peptide binds strongly to holo-S100A13 compared with apo-S100A13. Results of the intrinsic tryptophan fluorescence, isothermal calorimetry (ITC), Circular Dichroism (CD), and multidimensional NMR experiments will be discussed in detail.

1075-Pos Conformational Regulation of Soluble Guanylate Cyclase

Jasmin Kristianto, Yu-Hsi Chang, Raymond M. Esquerra, Nancy C. Gerber

san francisco state university, San francisco, CA, USA.

Board B51

Soluble Guanylate Cyclase (sGC), a member of the nucleotide cyclase family, is the principal physiological receptor of Nitric Oxide (NO) with primary function to catalyze the formation of cGMP from GTP. Upon NO binding, the enzyme's activity increases by almost 400-fold, and the produced cGMP acts as a second messenger, regulating the physiological function of several important enzymes that promote vasodilation, smooth muscle relaxation, and platelet aggregation. Carbon monoxide has also been implicated as a signaling molecule acting through sGC, although it appears to require the presence of an allosteric effector to achieve full activation. Although the structure of sGC has not been elucidated, it is known to be a heme containing heterodimer with alpha and beta subunits. Our experimental objective is to understand the conformational changes of the enzyme upon activation by NO and CO and how the allosteric regulation occurs. Using both fluorescence spectroscopy and Magnetic Circular Dichroism (MCD), we probed the different conformational complexes occurring in sGC upon activation by NO, CO and the effector YC-1. MANT-GTP, a fluorescence analog of GTP, was also utilized to examine the enzyme's active site. Despite the similar levels of activity, the NO and CO/YC-1 bound enzymes are structurally distinct. MCD measurements showed the sGC heme forming a 6-coordinate complex upon binding with CO/YC-1; a structure indistinguishable from carbonmonoxy Myoglobin, while adopting a 5-coordinate complex in the presence of NO. The distinction was further confirmed via fluorescence spectroscopy of the four endogenous Tryptophans. Using fluorescence, we have also determined that YC-1 binds at a distance of 60 Å from the heme, within close proximity to the GTP binding site. Collectively, the data has allowed us to propose a mechanism on the allosteric regulation employed to fully activate sGC via the CO and NO gasses.

1076-Pos Structural and Biophysical Basis of Poxvirus Interaction with Host Cell-Surface Chondroitin Sulfate

Joseph Schoeniger[1], Masood Hadi[1], Brent Segelke[2], Diana Roe[1], Yooli Light[1], Marites Ayson[1], Adam Zemla[3]

[1] *Sandia Labs, Livermore, CA, USA*
[2] *Lawrence Livermore National Labs, Livermore, CA, USA*
[3] *Lawrence Livermore National Lab, Livermore, CA, USA.*

Board B52

Binding to cell-surface carbohydrates is a critical step in the interaction of orthpoxviruses with host cells. Inhibiting the activity of the chondroitin sulfate (CS) binding protein D8L present on the surface of Vaccinia intracellular mature virus (IMV) greatly attenuates cell-binding and infectivity. D8L and a structurally dissimilar heparan sulfate binding protein H3L are two of a small number of IMV membrane proteins known to stimulate production of neutralizing antibodies. Most genes, including those encoding CS-binding proteins are, highly homologous across the orthopoxviridae. Since gross-sequence differences cannot be used to generate discriminating antibodies, understanding the structural and biophysical basis of the interaction of these proteins with their cell-surface receptor ligand and other small molecules can assist efforts to improve affinity reagents to detect the biothreat agent smallpox and discriminate it from other pox viruses. Such understanding may also form the basis for developing improved vaccines or new antipox drugs. Based on homology models, we identified a positively charged CS-binding groove on the protein surface, and predicted residues involved in CS binding. We used CS-binding assays and calorimetry to characterize CS-binding affinity and stability of purified recombinant wild-type and site-specific mutants of CS-binding protein expressed in E. coli. We experimentally identified key amino acids involved in CS binding, confirming many of our predictions, and other residues important for binding and protein stability. We recently determined an X-ray structure of CS-binding protein, minus its transmembrane anchor, at a resolution of 1.6 Angstroms. This is one of only two structures of a poxvirus IMV membrane protein. We will discuss implications of the structure for the molecular mechanism of CS-binding, potential of the protein as a drug target, and possibility using this information to develop affinity reagents for discriminating between orthopoxviruses.

1077-Pos Molecular Interactions In The ccd Addiction System

Natalie De Jonge, Lieven Buts, Abel Garcia-Pino, Lode Wyns, Remy Loris

VUB, Brussels, Belgium.

Board B53

Plasmid addiction systems consist of a plasmid-encoded toxin: antidote pair that serves to stabilize low-copy number plasmids in bacterial populations. The ccd addiction module on the E. coli plasmid F was the first TA module described and the first for which the mode of action of its toxin was elucidated. It encodes a toxin, CcdB, that targets DNA gyrase, and an antitoxin, CcdA, that can antagonize CcdB by forming a non-covalent complex. We analyzed the molecular interactions within the ccd system by means of a series of biophysical techniques and crystallography. The crystal structure of CcdB in complex with the C-terminal domain of CcdA shows an entity with a 2:1 CcdB:CcdA stoichiometry in which the CcdA domain is wrapped around the CcdB dimer in a largely alpha-helical conformation. Binding studies between CcdB and CcdA-derived peptides are indicative of a system with negative cooperativity in which the first ligand binds with high (picomolar) affinity and the second ligand with a substationally reduced (micromolar) affinity. This negative cooperativity is compatible with our crystal structure as binding of a second C-terminal part would require a conformational change in at least one of the proteins to avoid steric overlap. As the CcdA and gyrase-binding site of CcdB do not overlap, the rejuvenation mechanism of CcdA probably occurs though an allosteric mechanism rather than through competitive inhibition.

1078-Pos Biophysical Characterization of Human Interleukin 1α

Rajalingam Dakshinamurthy, Suresh Kumar Krishnaswamy Thallapuranam

University of arkansas, Fayetteville, AR, USA.

Board B54

Interleukin-1α is a 19 kDa protein that plays a crucial role in the regulation of a number of key cellular processes. IL-1α is a cytokine and a potent mediator of body's response to inflammation, microbial invasion, tissue injury, and immunological response. Recent studies also suggest that IL-1α also has a role in wound healing, Alzheimer's disease and tumor growth. Interestingly, unlike many other proteins, IL-1α lacks the N-terminal signal peptide and has been shown to be secreted through a non-classical route, which is independent of the conventional ER-Golgi secretory pathway. IL-1α is secreted into the extracellular compartment as a binary complex with S100A13, calcium binding protein. The non-classical secretion of IL-1α is proposed as ideal model to understand the ER-Golgi independent secretion of a large number of proteins that lack the N-terminal signal sequence. In this study for the first time, we report the cloning, expression, biophysical, and biological characterization of the human interleukin-1α. Human IL-1α has been expressed in *Escherichia coli* in high yields and purified to homogeneity using affinity chromatography. Results of the steady-state fluorescence, far-UV circular dichroism and 2D NMR experiments show that the recombinant IL-1α is in a folded conformation. Recombinant IL-1α was observed to exhibit strong cytostatic effect on human umbilical vascular endothelial cells. ITC experiments show that the recombinant IL-1α binds strongly to S100A13, a calcium binding protein that chaperones the *in vivo* release of IL-1α into the extracellular compartment. Using ^{1}H-^{15}N chemical shift perturbation data, the IL-1α binding sites on the S100A13 have been successfully mapped. The findings of the present study not only pave way for an in-depth structural investigation of the molecular mechanism(s) underlying the non-classical release of IL-1α but also provide avenues for the rational design of potent inhibitors against IL-1α mediated pathogenesis.

1079-Pos Anesthetic Interaction Sites in Closed- and Open-channels of nAChR (α4)2(β2)3

LU Tian Liu, Yan Xu, Pei Tang*

University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

Board B55

Nicotinic acetylcholine receptors (nAChR) are putative anesthetic targets. Because of its abundance in brain and high sensitivity to anesthetics, the nAChR (α4)2(β2)3 was chosen for searching for anesthetic binding sites and understanding how anesthetics modulate the receptor functions. Halothane binding in the (α4)2(β2)3 in closed- and open-channel conformations were examined using AutoDock under various docking conditions. For both channel conformations, the halothane binding energy was in the range of $-3.2 \sim -3.9$ Kcal/mol. Over 60% of halothane binding occurred in the interfacial region between the extracellular and transmembrane (TM) domains, including the Cys loops, β1–2 loops, and TM2-3 loops. The majority of remaining halothane-binding sites were found in the extracelluar domain, mainly in the MIR region and some in the A loops. Less than 2% of halothane binding was found in the TM domain of the closed conformation, but no binding site was found in the TM domain of the open state. A noticeable difference between the two conformations was that halothane bound to inter-subunit regions (α4/β2 and β2/β2) only in the closed state. The binding sites could be perturbed by even subtle protein structure changes, as evidenced in the fact that one of β2 subunits had no halothane binding in both closed- and open channel models. A close inspection of halothane binding sites indicated an amphiphilic nature of the sites consisting of both hydrophobic and polar hydrophilic (both neutral and charged) residues. Many of these results are in good agreement with previous findings from photoaffinity labeling experiments. Substantial halothane binding in the interfacial region of the (α4)2(β2)3 implicates the potential roles of anesthetics in destructing signal transduction from agonist binding in the extracellular domain to channel gating.

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1080-Pos Collagen Binding Domain - As a novel drug delivery of Parathyroid Hormone (PTH)

Sagaya T Leena Philominathan[1], Osamu Matsushita[2], Robert Gensure[3], Joshua Sakon[1]

[1] *University of Arkansas, Fayetteville, AR, USA*
[2] *Kitasato University, Sagamihara, Japan*
[3] *Ochsner Children's Hospital, New Orleans, LA, USA.*

Board B56

Monthly injection of parathyroid hormone (PTH) fused to CBD can increase bone mineral density (BMD) by 15% in mice within 2 months. PTH marketed not only needs to be injected daily but also will require a year to achieve the BMD increase. Pharmacokinetics of PTH-CBD is under investigation. To better understand the role of CBD in the pharmacokinetics and to help us to further optimize in PTH-CBD, through biophysical studies were conducted. For example, CBD with tighter binding to collagen may increase BMD even faster. Collagen binding domain (CBD), a C-terminal segment of bacterial collagenase (ColG) is responsible recognizing and binding to collagen triple helix. CBD binds tighter to collagen in the presence of Ca^{2+} (5.72×10^{-5}M). X-ray crystal structure is solved for CBD at 1.35Å resolution. Extensive mutagenesis of the conserved surface residues in CBD, in conjunction with collagen-binding studies identified key residues involved in CBD:collagen binding interface. Three possible collagen orientations on CBD were proposed based on the study. Since attempts to co-crystallize CBD and collagen did not yield crystals, NMR methods were used to determine the precise orientation of collagen on CBD. Titration results identified the involvement of additional residues on CBD in collagen binding and ruled out one of the earlier binding models we

proposed. Interestingly the binding surface identified is concave that could force collagen to be bent.

1081-Pos Mechanism Of Calmodulin Association With Connexin32 Derived Peptides

Ryan Dodd, Daniel Stolady, Zawahir Chowdhury, Gerald Connolly, Camillo Peracchia, Katalin Torok

SGUL, London, United Kingdom.

Board B57

Calmodulin (CaM) has been shown to modulate the chemical gating of gap junction channels[1]. Two calmodulin binding regions have been previously described in connexin32 (Cx32), one in the N-terminal and another in the C-terminal tail[1,2]. Using Ca^{2+} binding-deficient CaM mutants, CaM12 and CaM34, we have investigated the interaction of the N and C-lobe CaM Ca^{2+} binding sites with Cx32 derived peptides by stopped-flow kinetics at pH 7.0 at physiological ionic strength and 2 mM Mg^{2+}. Interaction with Cx32 1–19 was found to engage all 4 Ca^{2+} binding sites. The CaM C-lobe showed higher affinity (Ca^{2+} dissociation rate constant (k_{off}) $3.6 \pm 0.03\ s^{-1}$) than the N-lobe (k_{off} $56 \pm 1.8\ s^{-1}$). In contrast, Cx32 208–226 at 10 μM only affected the C-lobe CaM Ca^{2+} binding sites (k_{off} $6.1 \pm 0.1\ s^{-1}$). At 20 μM, however, 3 Ca^{2+} sites were involved: the Ca^{2+} binding sites of the C-lobe (k_{off1} $11.6 \pm 0.4\ s^{-1}$ and k_{off2} $1.67 \pm 0.05\ s^{-1}$) and an N-lobe site (k_{off} $11.6 \pm 0.4\ s^{-1}$). Dissociation constant K_d values were 1 and 3.4 μM CaM for Cx32 1–19 and 208–226, respectively. CaM conformation as measured by FRET[3] in the complexes was semi-compact which together with the Ca^{2+} kinetic data suggests separate functions for the C- and N-terminal lobes for CaM. Our data show that the C-terminal Cx32 208–226 peptide binds one CaM lobe only and suggest trans-domain CaM binding as a possible mechanism for chemical gating of gap junctions.

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Protein Physical Chemistry

1082-Pos Entropy Definition of Non-Equilibrium Biological Macromolecules

David V. Svintradze

Tbilisi State University, Tbilisi, Georgia.

Board B58

Novel entropy definition for non-equilibrium biological macromolecules will be proposed in this paper and shown that non-equilibrium systems like biological macromolecules can form stable states. Also summary temperature of non-equilibrium systems will be defined and clarified that in such systems each sub-system has different temperature. Entropy of the system couldn't be *0* at $0\ ^0K$, i.e generally thermodynamics third low couldn't be right for non-equilibrium systems. Let take single biological macromolecule situated in a virtual closed box as a closed system and define entropy of such system. Let consider that one of the principal determinants of biomacromolecular structures' are intermolecular hydrogen bonds and discuss some sub-system where number of hydrogen bonds are n, assuming that in hydrogen bonds $A_iH—B_i$ ($i=1,2...n$) proton has two possible energetic levels E_0 and E (or two possible quantum states), first corresponds to non-formation of hydrogen bonds and second corresponds to involving of proton in hydrogen bonds with energy E. So there could be some kind of swinging of proton between two energetic negative atoms A_i and B_i by hole effect, where time of this swinging could be appropriate time for protons during the effect. The relaxation time of such hydrogen bonds will be endlessness thus proton will never stand on one energetic level in hydrogen bonds. If it is considered that such formalism of hydrogen bonds is correct then it could be said that:

1. it is non-equilibrium state;
2. degree $\hat{o}$ of non-equilibrium state of the sub-system is proportional to number of hydrogen bonds involved in this sub-system; and
3. entropy is a function of two parameters E and $\hat{o}$, $S=S(E,\hat{o})$.

Thus finally we will suggest conclusions that different parts of biological macromolecules could have different temperature and entropy could not be *0* at $0\ ^0K$.

1083-Pos A Geometric Construction To Aid In Qualitative Analysis Of Ternary Mixture Phase Separation Conditions

George M. Thurston, David S. Ross

Rochester Institute of Technology, Rochester, NY, USA.

Board B59

Understanding the molecular origins of the phase boundaries of eye lens protein solutions can help us understand loss of transparency of the eye lens in cataract disease, the leading cause of blindness. It has recently been found that eye lens alpha and gamma crystallin mixtures need finely tuned interactions to avoid opacity, in that thermodynamic stability of their highly concentrated mixtures depends non-monotonically on alpha/gamma interaction strength[1]. Experiment and theory have long shown that ternary mixture phase separation typically depends non-monotonically on molecular interaction strengths. Here we present a geometric construction to aid qualitative analysis of phase separation conditions, in the space of the three independent components of the Hessian of the intensive Gibbs free energy. In the simplest mean-field models, a generic non-monotonic dependence of stability on interaction strengths is associated with conic sections formed by the intersection of planes with the elliptical cone of spinodal conditions. A similar construction can help in analyzing more complicated, realistic free energy models.