Human p52Shc Conformational Bias and Localization in c-SRC Activation

ARTICLE *in* BIOPHYSICAL JOURNAL · JANUARY 2014

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2013.11.2642

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2354-Pos Board B46

Architecture of Whole-Module and Bimodular Proteins from the 6-Deoxyerythronolide B Synthase

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The 6-deoxyerythronolide B synthase is a prototypical assembly line polyketide synthase (PKS). Models for the quaternary structures of a full PKS module and bimodule have been derived through small-angle X-ray scattering (SAXS) analysis of a systematic set of multidomain proteins whose fragments have previously been characterized at atomic resolution. The global architectures of these proteins suggest that the ACP can interact with the KS domain through two mutually exclusive channels during polyketide chain elongation and translocation.

2355-Pos Board B47

Self-Assembley of Dehaloperoxidase-Hemoglobin Probed by Backbone Dynamics using NMR Relaxation Experiments and Molecular Dynamics Simulation

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Dehaloperoxidase-hemoglobin (DHP) is a multi-functional protein isolated from the annelid marine worm Amphitrite ornata. It has been shown to function as an oxygen transporter, peroxidase, peroxygenase, oxidase and hydrogen sulfide oxidase with substrates that include a range of phenols and indoles. DHP primarily exists as a monomer in the solution (~90%), while is observed as a dimer in the X-ray crystal structure. The dimer-monomer equilibrium in solution has shown to be able to change the redox state of the protein which further regulates the multiple enzyme functions of DHP. The self-assembly behavior of DHP has been studied by NMR and MD simulations in terms of backbone dynamics. The R1, R2 and {1H} −15N NOE of the backbone amide N-H bonds have been measured using NMR relaxation experiments at multiple magnetic fields. The squared generalize order parameter S2 that describe the spatial restriction of the internal motions of amide N-H bond was extracted using the model-free analysis for each residue. S2 were also theoretically calculated from correlation functions based on MD simulations. The dynamic pattern of DHP monomer shows that the μs-ms slow motions experiencing by residues in the dimer interface are primarily responsible for association between each monomers. Moreover, the disulfide bond is formed between the only cysteine in DHP during the dimerization process which leads to the unique autoreduction phenomenon in DHP. While most heme proteins autooxidize, DHP will actually autoreduce if prepared in the ferric form because of its very high reduction potential (the highest known for a monomeric hemoglobin) and reduction by surface cysteines that form disulfide bonds during the dimerization process.

2356-Pos Board B48

Studying the Role of Protein Flexibility in Allosteric and Evolutionary Changes as Seen in PyrR Protein Family

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Oligomerisation is essential for the function of some proteins. The PyrR family of proteins are involved in pyrimidine operon attenuation. They are regulated by the presence of certain nucleotides such as guanosine monophosphate (GMP), which stabilises the tetrameric state. Notably, some members of this family can adopt a tetrameric oligomerisation state regardless of the presence of the GMP. Perica et al. (2012) previously found that this family, among several others, have differences in sequence outside the oligomeric interfaces and these are sufficient to explain the changes to their subunit geometry and oligomerisation states. Here, we compared the differences in structural flexibility linked to the changes in oligomeric state caused by a) specific mutations and, b) by the presence of bound GMP. We calculated the coarse-grained normal modes of dimeric units of the PyrR dimers and tetramers using an Elastic Network Model implemented in the Molecular Modelling ToolKit. We conducted several analyses including comparing the normal modes of these proteins with each other using the Bhattacharyya Coefficient similarity measure, correlations matrices, and the conformational overlap analysis, by which the contribution of these modes to the transition from one state to another can be quantified. Firstly, we found that while the dynamics were very similar between all the structures, there was a noticeable difference between the dimeric and tetrameric units. We also show that both sets of proteins transition from tetrameric to dimeric states similarly, indicating some overlap between the effects of allostery and evolution on oligomerisation in PyrR.

2357-Pos Board B49

Determining the 3D Topologies of Heteromeric Protein Assemblies by a Mass-Spectrometry Based Hybrid Approach

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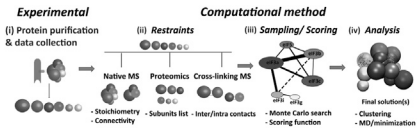
Describing, understanding, and modulating the function of the cell require elucidation of the networks and structure of its macromolecular assemblies. Here, we describe an integrative approach to determine the topologies of heteromeric assemblies using structural information derived from native mass spectrometry (MS), proteomics and chemical cross-linking MS. The method was developed and further assessed for robustness and accuracy using a benchmark of five hypothetical assemblies with simulated data and two assemblies with previously published MS data. With this benchmark in hand, we purify and characterize the yeast eukaryotic initiation factor 3 (eIF3) complex whose complete 3D topology has not been previously defined. Using a combination of MS-based data, we establish sub-stoichiometric binding of eIF5 and derive a high-likelihood structural model for the five subunit eIF3 complex. Our results further reveal two interaction modules within the eIF3 and are in accord with its role as a scaffold for other initiation factors. In conclusion, here we highlight the utility of a MS-based integrative approach for modelling complexes with unknown interactions and topology. The corresponding computational algorithm is implemented in the open source *Integrative Modeling Platform* (IMP).



2358-Pos Board B50

Native Ion Mobility-Mass Spectrometry: from Flexible Proteins to Ion Channels

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After a brief introduction into the field of native mass spectrometry and ion mobility analysis of protein complexes, we discuss how high-mass modified instrumentation can give powerful insights into the stoichiometry, subunit composition, size and shape of biomolecular particles.

We will show recent data on HFQ complexes which act as an RNA chaperone and are involved in the regulation of gene expression through facilitating post-transcriptional interactions between non-coding sRNAs and mRNA. The example of SMC proteins, which are crucial for Structural Maintenance of Chromosomes, illustrates the ability of ion mobility approaches to link information on complex assembly with the topology of the functional unit in heterogeneous systems.

Recently, we have also been studying ion channels in detergent micelles and have been able to show the gradual opening of the Mechanosensitive Channel of Large Conductance in response to the binding of a charged drug molecule inside the channel which mimics the pressure on the bulk membrane. We show that we can characterize snapshots of the gradual opening of the channel, by using collision cross sections obtained from ion mobility measurements in combination with molecular modelling.

2359-Pos Board B51

Human p52Shc Conformational Bias and Localization in c-SRC Activation

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c-SRC is a non-receptor protein tyrosine kinase that serves as an upstream regulatory molecule for a wide array of signaling cascades and proteins such as C-Jun N-terminal Kinase (JNK). Chronic JNK activation is known to alter cellular function and lead to human disease including peripheral insulin resistance in type II diabetes. Thus, modulating c-SRC activation via therapeutic intervention is a potential means for treating human disease. c-SRC is activated by saturated fatty acids and cytoplasmic regulatory proteins. One such protein, p52Shc,

associates with c-SRC in membrane fractions leading to JNK activation via phosphorylation of both c-SRC and p52Shc. The mechanism by which this occurs isn't known. The primary objective of this study is to characterize the p52Shc - c-SRC association as a means to determine how c-SRC is activated.
In order to study the binding between c-SRC and p52shc, we expressed both proteins in Escherichia coli and purified them for biochemical and biophysical characterization. We demonstrate that full-length human p52Shc partitions between both membrane and soluble fractions with distinct biophysical, conformational and associative properties. The soluble and membrane associated p52Shc forms have different buffer preferences. Furthermore, the membrane associated form shows two circular dichroism minima at 222 nm and 208 nm while the soluble form shows only one distinct minimum at 210 nm. This is surprising considering the thermal unfolding is the same between both forms. This data would suggest that conformational differences between the two p52Shc forms are important in the binding and activation of c-SRC. Binding studies with both forms and c-SRC are currently underway.

2360-Pos Board B52
Non-Canonical Modular Domain Interactions Dictate PKCα Function
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The use of modular protein domains has emerged as a prominent feature of increasing phylogenetic complexity. Linking modular domains within a single protein allows complex regulation while conserving the sequence and structure of the individual domains. For instance, spatio-temporal control of signaling proteins is often achieved by stringing together a conserved catalytic domain with one or more regulatory modules. These modules can play multiple roles including masking the catalytic site to inhibit basal activity (auto-inhibition), releasing auto-inhibition through conformational changes triggered by second messenger stimuli, and facilitating translocation to subcellular compartments through binding secondary messengers or scaffolding proteins. Each additional module in a signaling protein provides a combinatorial enhancement to its regulation and cellular function. The protein-context independent structure and cellular function of individual modules have been extensively researched using biophysical approaches such as x-ray crystallography and NMR. Most modular domains have an evolutionarily conserved canonical function. However, coordination of interactions between these domains remains largely unexplored primarily due to the reliance on reductionist structural and biochemical approaches. As a corollary, our current structural understanding of modular signaling proteins does not adequately address the versatility of their cellular function. Using the uniquely persistent ER/K α-helix derived from the lever arm of myosin VI combined with genetically encoded fluorophores we have previously developed a methodology termed SPASM to both observe and modulate intra- molecular interactions between domains in multi-domain proteins. Using human protein kinase C α (PKCα) as a model multi-domain signaling protein, we have uncovered intra- and inter- molecular interactions involving each of its modular domains. These interactions contribute to context-dependent spatio-temporal regulation of PKC function in cells. Our findings highlight the importance of intra-molecular interactions in biologically critical multi-domain proteins.

2361-Pos Board B53
The Histidine Button Dictates the Conformation of the pH-Sensitive Region of Troponin I
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Along with calcium binding, association of the switch region of troponin I (TnI) to the regulatory N-domain of troponin C (NTnC) is a key step in the regulation of contraction in cardiac and skeletal muscle. The TnI-NTnC interaction has been structurally characterized and the orientation of the switch region of TnI relative to NTnC is known to be very similar for cardiac and skeletal muscle; however, the region of TnI immediately following in the sequence differs substantially and is involved in the differential pH sensitivity of myocytes and cardiomyocyte observed during ischaemia. A single amino acid substitution (A162H, the histidine button) in the acidosis-sensitive cardiac TnI turns the cardiac muscle into the acidosis-resistant phenotype of skeletal muscle. The cause of this improvement is attributed to electrostatic interactions promoted by the histidine button in TnI with NTnC, but little is known about the structural characteristics of this system. In this study, we used NMR spectroscopy to determine the conformation of the pH-sensitive region of cardiac TnI A162H and skeletal TnI when bound to cardiac NTnC at pH 6. The results show that the pH-sensitive regions of both isoforms are in very similar conformations and resemble the conformation of the skeletal TnI when bound to skeletal NTnC. This indicates that the histidine, and not the NTnC isoform or other TnI residues, is the main determinant of the conformation of the pH sensitive region of TnI. The promotion of a conformational change in cardiac TnI in the presence of A162H at low pH then leads to the stabilization of TnI and of the TnI-NTnC interface to continue the contraction mechanism in the face of acidosis.

2362-Pos Board B54
Purification and Structural Analysis of the Anti-Viral Protein BST-2
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BST-2 is a human extracellular transmembrane protein that inhibits the release of viruses such as HIV-1 and Ebola from the cell surface. Viruses can evade this inhibition through antagonistic viral protein interactions with BST-2. The BST-2 is a homo-dimer that forms a coiled-coil connected by three disulfide bonds. Recent cellular studies suggest that the extracellular domain of BST-2 is flexible or structurally dynamic. However, x-ray crystallography suggests the coiled-coil structure is rigid. The goal of this study is to understand the relation between the full-length BST-2 structure and function, and the mechanism of viral protein binding. Through limited proteolysis, protein fluorescence, and small-angle x-ray scattering analysis we show that there is a flexible region and a rigid region in the extracellular portion of BST-2. The flexibility of the full-length protein is still unknown. We have purified both the membrane-bound protein of BST-2 and the viral antagonist protein, Vpu for biochemical and structural characterization. We are optimizing conditions for crystallizing the full-length BST-2, Vpu and the BST-2/Vpu complex. This will help us understand how BST-2 functions and the antagonistic interactions with viral proteins.

2363-Pos Board B55
Probing G Protein-Coupled Receptor Dimerisation by FRET and DEER
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G protein-coupled receptors (GPCRs) are the largest class of eukaryotic membrane proteins. They trigger intracellular signalling cascades by activation of heterotrimeric G proteins. They are of great pharmaceutical interest, with approximately 40% of marketed drugs targeting GPCRs. It has been shown that GPCRs can form oligomers in phospholipid bilayers in vivo and in vitro, affecting both ligand binding and G protein coupling [1].
Neurotensin receptor 1 (NTS1) is one of few GPCRs that can be produced in E. coli in an active state, and has been implicated in conditions such as schizophrenia and Parkinson's and postulated as a biomarker for various cancers [2]. NTS1 has been shown to dimerise in lipid bilayers [3], and though a crystal structure of NTS1 in detergent was recently published [4], there is still no structural data on the receptor and its dimer in a membrane environment.
We use a range of biophysical techniques to characterize the structure and function of NTS1 in model membrane systems, including ensemble and single molecule Förster resonance energy transfer (FRET), and double electron-electron resonance (DEER, also known as PELDOR). Fluorescence or nitroxide spin probes are attached to engineered cysteines on the transmembrane helices. By measuring intradimer distances between the probes on each monomer, we are studying dimerisation behaviour of NTS1 to produce a model of its dimeric structure in a more native lipid environment.
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2364-Pos Board B56
Tug-Of-War Between Thermodynamic Stability and Actin-Binding Function of Tandem Calponin-Homology Domains
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Tandem calponin-homology (CH) domains constitute a major class of actin-binding domains that include dystrophin and utrophin, the two key proteins involved in muscular dystrophy. Despite their importance, how their structure