to fructose-1,6-bisphosphate. Allosteric activation and inhibition of PFK1 by over ten metabolites and in response to hormone and growth factor signaling fine-tune glycolytic flux to meet energy requirements. Despite its critical role in glucose flux, the crystal structure of the mammalian biologically relevant PFK1 tetramer has not been resolved. Here, we report structures of the human platelet isoform of PFK1 (PFKP) in complex with ATP-Mg^{2+} and ADP at 3.1 and 3.4 Å, respectively. The structures reveal substantial conformational changes in the enzyme upon nucleotide hydrolysis. Further, the structures illuminate that mammalian PFK1 has a unique tetramer interface compared to the yeast Saccharomyces cerevisiae. Structure/function experiments demonstrate that hydrophobic interactions between protomers at the tetramer interface are essential for subunit assembly and enzymatic activity, while electrostatic interactions are required for optimal enzyme activity. With altered glycolytic flux being a hallmark of cancers, these new structures allow a molecular understanding of the functional consequences of 44 somatic PFKP mutations identified in human cancers. We characterize three of these mutations in vitro and show they have distinct effects on PFKP activity. Two mutations, R48C and N426S, attenuate allosteric inhibition by citrate and ATP, respectively, while one mutation, D564N, decreased the maximum velocity and substrate affinity. Expression of wild type and mutant PFKP in MTLn3 rat mammary adenocarcinoma cells altered lactic acid production, with cells expressing wild type and N426S excreting more lactate but cells expressing D564N excreting less when compared with GFP expressing controls. Taken together, the structures provide molecular insight into the allosteric regulation of mammalian PFK1 and provide a blueprint to guide therapeutic targeting of PFK1 activity to control dysregulated glucose metabolism in disease.

1029-Pos Board B6

Comparison of the Energetics of Histone Peptide Binding among Histone Readers

Suvobrata Chakravarty[1], Francisca Essel[2], Tao Lin[1].
[1]South Dakota State University, BROOKINGS, SD, USA, [2]Chemistry & Biochemistry, South Dakota State University, BROOKINGS, SD, USA.

To better understand the mechanism of histone peptide recognition, here we probed the energetic contributions to recognize the same histone H3 peptide by three different plant homeodomain (PHD) H3K4me0 readers: hKDM5B-PHD1 (first PHD finger of hKDM5B), hBAZ2A-PHD, and hAIRE-PHD1. The energetic contributions of residues differ significantly from one complex to the next. For example, H3K4A substitution completely aborts formation of the hAIRE-histone peptide complex, while it has a small destabilizing effect on binding of the other readers, even though H3K4 methylation disrupts all three complexes. Packing density suggests that methylation of more tightly packed Lys residues can disrupt binding, even if the energetic contribution is small. The binding behavior of hKDM5B-PHD1 and hBAZ2A-PHD is similar, and both possess a pair of Asp residues in the Zn knuckle for interaction with H3R2. These Asp residues make significant energetic contributions to the formation of the hKDM5B- and hBAZ2A-histone peptide complexes, suggesting that there are interactions in addition to those reported in the recent NMR structure, and the hKDM5B-PHD1 Zn knuckle is thus remodeled to account for the observed peptide interaction energetics. However, the presence of the Zn knuckle Asp in sequences of other PHD family members may not always be sufficient for histone peptide binding. This study highlights reader-histone peptide interactions in the context of energetic contributions, interfacial packing, and sequence-based reader subtype predictability.

1030-Pos Board B7

Simulating mTOR Hyperactivating Mutations to Understand Functionally Significant Structural Rearrangements

Steven Albanese[1], Jianing Xu[2], James Hsieh[2], John D. Chodera[3].
[1]Computational Biology, Gerstner Sloan Kettering, New York, NY, USA, [2]Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [3]Computational Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

The kinase mTOR is a common therapeutic target for a number of cancers and most prominently in clear cell Renal cell carcinoma where hyperactivating mTOR mutants can be found. In vitro data suggests that these mutants can be divided into different groups and that combining mutants from different groups has an additive effect on mTOR kinase activity. However, the mechanisms through which these mutants activate mTOR are not well characterized. To dissect these mechanisms, we sought to identify possible structural rearrangements in response to these point mutants using molecular dynamics simulations on Folding@home, a massively distributed computing network. Using these atomistic models, we use a variety of computable surrogates to examine how mutants impact substrate affinity. Understanding how these mutants are hyperactivating will provide insight into the development of future therapies.

1031-Pos Board B8

Promiscuity and Polyreactivity of Antibodies and their Binding Modes during B-Cell Differentiation

Franca Fraternali[1], Julie Laffy[2], Deborah Dunn-Walters[3].
[1]King's College, London, United Kingdom, [2]Randall Division of Cell and Molecular Biophysics, King's College, London, United Kingdom, [3]Division of Infection, Immunity and Inflammatory Disease, King's College, London, United Kingdom.

Many diversification mechanisms occurr during central tolerance leading to a wide array of antibodies (Abs) with a range of binding specificities, some of which lead to polyreactivity. The repertoire of preB cells (before central tolerance), immature B cells (during central tolerance) and naïve B cells in the peripheral blood (after central tolerance) has been interrogated by high throughput sequencing of heavy and light chain Ig genes. It has been shown that older people display a less diverse repertoire of antibody-producing cells, and that this loss of diversity is associated with poor health. Within a population of responding cells there are favoured characteristics of a the Ab gene CDRH3 loop that is crucial in binding the foreign antigen. We hypothesise that favoured physico-chemical CDRH3 characteristics make a structure capable of binding to multiple foreign antigens (polyspecific).

The ability to predict this promiscuous behaviour with respect to binding specificity would greatly improve the efficiency of the therapeutic antibody discovery process. From these data we want to extract the molecular determinants playing a role in polyreactivity by exploiting structural and dynamical information. We use statistical analyses to classify CDRH3 physico-chemical properties and Molecular Dynamics (MD) simulations at the atomistic and/or coarse grained levels to characterise flexibility requirements for binding multiplicity.

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1032-Pos Board B9

Implication of Natural Polymorphism in Hinge Region of HIV-1 Protease on Protein Conformations, Local Structures and Backbone Dynamics

Zhanglong Liu[1], Xi Huang[1], Lingna Hu[1], Linh Pham[1], Katye Poole[2], Yan Tang[2], Brian P. Mahon[2], Wenxing Tang[2], Kunhua Li[1], Nathan E. Goldfarb[2], Ben M. Dunn[2], Robert McKenna[2], Gail E. Fanucci[1].
[1]Department of Chemistry, University of Florida, Gainesville, FL, USA, [2]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, USA.

HIV-1 protease (PR) serves as an important drug target because its inhibition generates immature non-infectious virions. However, drug-pressure selected mutations, along with natural polymorphisms, can lead to drug resistance to HIV-1 protease inhibitors (PIs), which change the drug binding configuration and equilibrium protease conformational sampling. As was suggested in our previous study, (1) a new protease conformation, curled-open, was identified in constructs carrying natural polymorphisms including E35D, and consequently, these constructs displayed higher protein backbone dynamics compared with wild-type subtype B construct characterized by nuclear magnetic resonance (NMR) spectroscopy. In the present work, we discuss the impact of the single E35D natural polymorphism on generating a minor protease conformation (curled-open), and consequently, influencing the protein structure and dynamics. The combined results from Double electron-electron resonance (DEER), NMR spectroscopy and X-ray crystallography structures provide a possible structural mechanism for altered conformational dynamics.

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1033-Pos Board B10

Catching Excited States in the Act: Functional Unfolding in E. Coli Adenylate Kinase

Jeremy A. Anderson[1], Ananya Majumdar[1], Vincent J. Hilser[2].
[1]Biophysics, Johns Hopkins University, Baltimore, MD, USA, [2]Biology, Johns Hopkins University, Baltimore, MD, USA.

Excited states of proteins are important for many functions, however they are difficult to study due to their low population and transient nature. Using an entropy-enhancing, valine to glycine mutation scheme we are able to stabilize a locally unfolded (LU) excited state in the LID domain of E. coli adenylate