Track: High Throughput Protein Science

ABS335 | Thermal and mechanical stability of highly-luminescent protein NanoLuc in presence and absence of chaperones

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Despite the extensive application of NanoLuc protein as a reporter enzyme, its mechanical behavior has never been investigated. This unexplored area intrigued our interest given NanoLuc's high thermal stability in comparison to the traditionally used Firefly Luciferase. Our Atomic Force Microscopy based Single Molecule Force Spectroscopy experiments on various polyprotein constructs of NanoLuc enabled us to explore various scenarios of this protein's unfolding and possibly misfolding. Our results strongly demonstrated that despite all the constructs had similar unfolding behavior, the refolding behavior differed. The percentage of successful refolding recordings of NanoLuc was greatly decreased when the protein was linked to itself. This was a contrary result from the construct in which the NanoLuc repeats were separated by other proteins or in the construct with a single NanoLuc protein. Additionally, Steered Molecular Dynamics Simulations of NanoLuc provided valuable insight into the unfolding pathways, in which the Cterminus end of the protein was the first to break apart from the rest of the protein. Lastly, thermal denaturation experiments of poly-NanoLuc proteins showed a sudden decrease in thermal stability at the denaturation temperature of 58C, while the monomeric NanoLuc remained mostly folded in same conditions. Addition of
the E. coli DnaK/DnaJ/GrpE chaperone system to the poly-NanoLuc proteins resulted to a 70% recovery of the initial bioluminescence. The spontaneous recovery in the absence of the chaperones showed no recovery, further supporting how poly-NanoLuc proteins are robust chaperone substrates.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS336 | Allosteric Regulation of Protein Kinase C beta Isoforms

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Protein Kinase C (PKC) isoforms are found in a wide range of cell types and direct diverse but specific functions in cells. Because of their ubiquity, PKCs are potential targets for treatment in a plethora of diseases, however the lack of structural and functional information on the isoforms limits the ability to develop effective pharmacologic agents. PKC beta (PKCB) isoforms PKCBI and PKCBII are of particular interest as a target for chemotherapeutic agents and structurally differ by only a Cterminal segment. In our work, we examine how the variable C-terminal segment alters the allosteric regulatory mechanism of PKCBI and PKCBII to provide isoform– specific activities. We assay the differential response of the two isoforms in response to different lipid compositions and inhibitors using in vitro kinase assays. Alternatively, we use limited proteolysis to probe variations in PKCB conformation in the presence of allosteric regulators and investigate how PKCBI and PKCBII mutants perturb function. Finally, we show that the allosteric regulatory mechanism of PKCBI can be altered by endoxifen, providing a “proof of concept” for isoform-specific allosteric regulators of PKCs. Overall, our data elucidates the underlying molecular basis by which the PKCB isoforms are regulated and can inform the development of highly specific treatments that exploit isoform dissimilarities.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS339 | SPADE: A Fast and Accurate Estimation of Entropy from the Molecular Surface Properties

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Estimating entropy is crucial for understanding and modifying biological systems, such as protein-ligand binding. Current computational methods to estimate entropy are resource-intensive. Here, we present SPADE1 (Surface Properties-based Accurate Descriptor of Entropy), a new method that estimates the gas-phase entropy of small molecules purely from their surface properties. The accuracy of SPADE in estimating gas-phase entropy for the 1263 small molecules in our curated database is within 12% of computationally expensive quantum mechanical or

molecular mechanical calculations. The small molecule gas-phase entropy can be computed in ≈ 0.01 CPU hours with an average run time of O(√Na), where Na is the number of atoms. We further show that the inclusion of the SPADE estimated entropy term for 243 ligands spanning ten protein targets improves the distance