Track: Structure and Dynamics Perspectives on Enzyme Function

ABS022 | Filamentation/Polymerization as a Novel Wide-Spread and Evolutionarily Conserved Enzyme Regulatory Mechanism

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We use a model system, the sequence dependent endonuclease SgrAI, to investigate important mechanistic and biological questions regarding enzyme filamentation and enzyme regulation. SgrAI forms structured assemblies of heterogeneous stoichiometries under conditions where its DNA cleavage activity is 200-1000 fold accelerated, and surprisingly, its DNA sequence specificity is altered (Biochem. 49, 8818). We have shown that these assemblies are helical filaments composed of SgrAI (Biochem. 52, 4373, Structure 21, 1848), and have proposed hypotheses for 1) how filamentation activates the DNA cleavage activity of SgrAI, as well as 2) how filamentation modulates the enzyme's DNA sequence specificity using the sequence specific energy of DNA distortions (Structure 27, 1). We have also carried out a complete kinetic investigation to create a full computational model of the entire DNA cleavage pathway including filamentation and all forward and reverse rate constants for each step (JBC 293, 14585 & 14599). This model has allowed us to predict the behavior of SgrAI within a cell, at biologically relevant concentrations, and showed that the filamentation mechanism, and in particular the slow filament assembly step, allows SgrAI to target invading DNA while minimizing damage to its host genome (J. Virol. 93, e01647). We now present new Cryo-EM structural data supporting the hypothesis that filamentation stabilizes a conformation of SgrAI where a second divalent cation binds in the active site, thereby enhancing the rate of DNA cleavage (JBC, in press). We also propose a model for control of SgrAI filamentation and DNA sequence specificity involving a disorder-to-order transition in SgrAI. Finally, new kinetic modeling also show how the secondary recognition sequences alter the thermodynamic states of SgrAI to affect filamentation and DNA cleavage preferences.

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

ABS023 | Biochemical and Biophysical Characterization of Small Molecule Inhibition of Gankyrin as a Therapeutic Strategy for Cancers

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Gankyrin is a seven-ankyrin repeat containing oncoprotein associated with the 26S proteasome assembly that regulates numerous oncogenic and inflammatory pathways through facilitating protein-protein interactions. As a chaperone protein, gankyrin binds to the S6 ATPase subunit of the 19S regulatory cap of the 26S proteasome that enhances both mouse double minute 2 homolog (MDM2) and cyclin-dependent kinase 4 (CDK4) mediated proteolysis of tumor suppressor proteins (TSP) p53 and retinoblastoma protein (Rb), respectively. Previous studies have shown that gankyrin overexpression increases the degradation of tumor suppressor proteins resulting in uncontrolled cell proliferation and the onset of various cancers. The first small molecule identified for gankyrin inhibition, cjoc42, has been rudimentarily characterized to disrupt the interaction with S6 ATPase as a therapeutic approach for both liver and breast cancer. Here we show using a structure-based drug design approach that novel small molecule cjoc42 derivatives show marked improvement in inhibition of tumorigenesis with concurrent increases in TSP expression levels. Further biophysical evaluation revealed that these cjoc42 derivations mode of action induce global gankyrin unfolding. These findings also provide insight into clarifying essential residues within gankyrin that are required for small molecule interaction. Taken together, this work aims to establish optimal targeting of tandem repeat proteins to increase selectivity, mitigate off-target activity, and conceptualize a biochemical approach coupled with drug discovery.

Track: Protein Science Addressing Health Disparities

ABS024 | Mass spectrometry based protein assays for diagnosis and prognosis of COVID-19 infection

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