P450s, remain unknown primarily due to challenges in handling these membrane-spanning proteins. Here we studied for the first time with single molecule resolution the function of POR(2). To ensure that we recorded the inherent behavior of POR we reconstructed the full length enzyme in “native like” membrane patches - Nanodiscs. Nanodiscs reconstitution increased POR stability ~2 fold as compared to POR solubilized in detergents highlighting the need for membrane environment to reliably study transmembrane protein behavior. Importantly it allowed the immobilization of individual POR enzyme on microscope slides(3-5) and their single molecule readout using the preflourescent analogue resazurin. Measurements and statistical analysis of individual catalytic turnover cycles shows POR to sample at least two major functional states. Each of these states could have different specificities for each P450 downstream partners providing cues for their selective activation(6) but to date remained masked in bulk kinetics.


1128-Pos Board B79
Interaction of Ribonuclease III with the Regulatory Macromdomain Protein YmdB Analyzed by Docking Calculations and SPR Experiments
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Ribonuclease III (RNase III) is a conserved bacterial endonuclease that cleaves double-stranded dsDNA structures and is essential in diverse DNA maturation and decay pathways [1,2]. RNase III is subject to multiple levels of regulation, allowing fine-tuning of its catalytic activity depending on the cellular physiological state. The regulatory macromdomain protein YmdB interacts with RNase III, and an increase in YmdB levels correlates with a decrease in RNase III activity in vivo [3]. However, the molecular details of the YmdB- RNase III interaction are not yet known. Here, docking calculations and computationally-driven mutagenesis were combined with surface plasmon resonance (SPR) experiments to identify energetically important determinants of the YmdB- RNase III interaction. The computational results reveal two alternative YmdB binding sites in RNase III: one located in the N-terminal nucleic domain (RIIID) (also indicated by co-immunoprecipitation and chemical cross-linking [3]), and a novel site in the C-terminal dsRNA-binding domain (dsRBD). The binding site in the RIIID is composed of a cluster of negatively charged residues that interact with a conserved arginine in YmdB, and the importance of this interaction is confirmed by SPR analysis of the YmdB Arg to Ala mutation. These results suggest a mechanism of RNase III regulation in which YmdB can bind separate sites in a concentration-dependent manner, leading to inhibition of catalytic activity.


1129-Pos Board B80
Targeting the Human DEAD-Box RNA Helicase, DDX3, as a Novel Strategy to Inhibit Aggressive Breast Cancer Metastasis
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DDX3 is a human DEAD-box RNA helicase that is expressed at a high level in aggressive breast cancer cells. The increased expression of the DDX3 protein downregulates E-cadherin expression, which results in an increase in the aggressive breast cancer cells’ metastatic properties. Aggressive breast cancer cells are insensitive to many of the present drugs, and new therapeutic agents that halt aggressive breast cancer cells’ metastasis are much needed. DDX3 is an ideal drug target to halt aggressive breast cancer metastasis because DDX3 is not essential for healthy cells’ metabolism, yet it is an important regulator of aggressive breast cancer cell motility; hence, inhibiting the DDX3 function is not expected to lead to side effects in a therapeutic context. The separated catalytic core of the DDX3 protein possesses no ATPase or helicase activity, and DDX3 N- and C- auxiliary domains are required for DDX3 function. We are investigating the mechanism employed by DDX3 auxiliary domain to modulate DDX3 function with the ultimate goal of using this understanding to find small molecule inhibitors of the DDX3 protein. The small molecule inhibitors will serve as lead compound for drugs that stop aggressive breast cancer metastasis. Our experimental results show that the C-terminal of DDX3 is involved in tethering of the DDX3 protein to RNA, and DDX3 dimer formation. Experiments are in progress to identify the C-terminal amino acid residues involved in RNA tethering, dimer formation and the role of the dimerization for the DDX3 physiological function. Moreover, by screening a library of 2000 natural products we were able to find three specific inhibitors of DDX3 ATPase activity. We are currently investigating these inhibitors exact mode of action.

1130-Pos Board B81
Kinetics and Thermodynamics of the ATPase Cycle of the DEAD-Box Protein Dhp5
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DEAD-box proteins are ubiquitous ATPase motor proteins involved in all aspects of RNA metabolism including folding, splicing, and trans-splicing and ribonuclease remodeling. Although all characterized DEAD-box proteins share a similar ATPase cycle, differences in individual ATP utilization rate and equilibrium constants confer unique motor properties for carrying out physiological functions, analogous to cytoskeletal motors (e.g. myosin, kinesin, dynein). RNA helicase functional diversity is therefore contained in their ATPase cycles, and variability introduced through interactions with regulatory partners. Dhp5 is a yeast DEAD-box protein that, in conjunction with several regulatory proteins and small molecules, plays an essential role in mRNA export from the nucleus to cytosol. We present a kinetic and equilibrium analysis of the Dhp5 ATPase as context for studying the role of the regulatory partners.

1131-Pos Board B82
Substrate Binding Effects of Carbohydrate Binding Modules on the Catalytic Activity of a Multifunctional Cellulase
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CelE from Clostridium thermocellum is a glycoside hydrolase family 5 enzyme that has broad specificity for hydrolysis of plant biomass polysaccharides, particularly cellulose, xylan, and mannan. A fusion of CelE with the well-studied carbohydrate binding module CBM3a was created, CelE_CBCM3a, and construct alone was able to give high-yield hydrolysis of both cellulose and hemicellulose in pretreated plant biomass. In this study, ninety different CBMs from the C. thermocellum genome were fused to GFP, and the specific binding interactions of the robotic cell-free translated GFP_CBCM constructs were determined with a panel of soluble and insoluble substrates. Most CBMs tested were able to bind at least one substrate, and several GFP_CBCM displayed unanticipated binding capabilities. We also asked whether these CBMs could yield different catalytic capabilities when fused to the multifunctional enzyme, CelE. Different CelE_CBCM fusions were also expressed using robotic cell-free translation, and our results show that some CBMs enhanced cellulose hydrolysis through specific interaction between the CBM domain and polysaccharide. In addition, different CelE_CBCM fusions gave lichenan-, xylan-, mannan-, and biomass-specific improvements in the catalytic activities. The methods described here provide a useful system that can be used to test the function of other CBM domains and to assist in the design and testing of new engineered enzyme formats.

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