# Posters: Enzyme Function, Cofactors, and Post-translational Modifications

**2599-Pos**

**Activity and Substrate Specificity of Factor XIII Resulting from Different Activation Pathways**

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Factor XIII (FXIII) is a transglutaminase that catalyzes the formation of a calcium-dependent isopeptide bond between an acyl donor and a primary amine substrate. Plasma FXIII $A_2B_2$ is proteolytically activated by the concerted action of thrombin and low mM $Ca^{2+}$ (FXIIIA*) whereas cellular FXIII $A_2$ is activated non-proteolytically by high mM $Ca^{2+}$ concentration (FXIIIA°). Previous studies demonstrated that these activation pathways result in FXIIIA species that differ conformationally and functionally. The activities and substrate specificities of FXIIIA* and FXIIIA° were further examined using monodansyl cadaverine (MDC) incorporation and UV-spectrophotometric assays. In the MDC assay, reactive glutamines within fibrinogen αC (233-425) (Q237, Q328, Q366) were crosslinked by FXIII (A* versus A°) to MDC (amine substrate), and results were evaluated by SDS-PAGE. The assay revealed that with αC WT, Q328P (Seoul II), and Q237only, FXIIIA* had more transglutaminase activity than FXIIIA°. In contrast, αC E396A and αC 389stop (233-388) exhibited no substantial differences in activities between FXIIIA* and A°. This observation may be due to loss of αC E396 and surrounding residues from the putative FXIII anchoring site αC (389-403). From the UV-spectrophotometric assay, kinetic parameters for FXIII A* and FXIIIA° were determined using glutamine donor peptides (K9 (1-10), $\alpha_2$ AP (1-15) and $\alpha_2$ AP (1-15, Q4S)) crosslinked with the amine DMPDA. Km values for FXIIIA* were modestly lower than FXIIIA° for K9 (1-10) and $\alpha_2$ AP (1-15, Q4S) and higher for $\alpha_2$ AP (1-15). The smaller kinetic differences observed with peptides may be due to amino sequence or the need for larger FXIII substrates that better mimic physiological targets. Overall, FXIIIA activity can be a function of its activation pathway, in addition to substrate size and sequence.

**2600-Pos**

**Solution Structure Studies of Ess1 Interactions with the RNAPII CTD Suggest a Dual Binding Mechanism that Differs from that of Human Pin1**

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Peptidyl-prolyl cis/trans isomerases (PPIases) catalyze the isomerization of the peptide bond that precedes specific proline residues. An essential PPIase in S. cerevisiae, ScEss1, is conserved; its human ortholog is known as Pin1. ScEss1 targets the carboxy-terminal domain (CTD) of the large subunit in RNA polymerase II (RNAPII) and in turn, regulates DNA transcription as well as RNA processing. ScEss1 isomerizes peptide bonds within the many heptad (YSPTSPS) repeats in the CTD. To understand the structure and function of ScEss1 in solution, we first obtained chemical shift assignments of backbone and side chain 1H, 13C, and 15N nuclei. Secondary chemical shifts and NMR relaxation data confirm that a well-structured, α-helical linker domain connects the protein's WW and PPIase domains, in contrast to the more flexible linker found in human Pin1. Therefore, the WW and catalytic PPIase domains in ScEss1 tumble together as a unit unlike in Pin1, where they tumble independently. The structured linker in ScEss1 may have functional significance for how the enzyme interacts with its substrates. To test this, we performed NMR titration experiments with phosphorylated CTD peptides containing one to five heptad repeats. Our binding studies of ScEss1 to CTD peptides found that a patch on ScEss1 PPIase domain is only involved in binding when the CTD peptide is longer than 4 repeats. Our data suggest that binding affinity for the CTD is increased when the CTD peptide is sufficiently long to engage both WW and PPIase domains simultaneously. We therefore conclude that ScEss1-CTD binding is length-selective and likely due to the rigid structure of Ess1. These structural and binding data suggest that substrate selectivity for ScEss1 is different than its human ortholog, Pin1.

**2601-Pos**

**Biophysical Investigation of the Function of Enzyme 3DL1**

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Protein function is often tied to structure, so structural comparisons for a protein or enzyme of unknown function may help to elucidate its function. An enzyme of unknown function (PDB ID 3DL1) derived from Klebsiella pneumonia has been studied using various methods to predict and test enzyme function. Crystal structures of 3DL1 with and without zinc bound show that zinc binds at a site with sequence and structural similarity to known metallopeptidases. These crystal structures also show that zinc binding affects the secondary structure of the protein. Computational methods for sequence and structure comparisons were made of 3DL1 to other protein sequences and structures. These show that 3DL1 may be a hydrolase with possible peptidase activity. 3DL1 was expressed in E. coli cells and purified via metal-affinity chromatography. Size exclusion chromatography was used to further purify 3DL1. To study the secondary structure of 3DL1 in the presence and absence of zinc, circular dichroism (CD) spectroscopy was used. The CD data allowed for determination of the stoichiometry of binding of the zinc ion(s) to the protein. It was expected that one zinc ion would bind per protein molecule. Moreover, enzymatic assays were performed using various relevant substrates to monitor the activity of 3DL1 in the presence and absence of zinc. These assays were performed with small molecule substrates and were monitored by UV/visible spectroscopy. Because 3DL1 has some structural similarity to gelatinase A, the activity of 3DL1 with a larger protein substrate was also tested in a gelatin-based assay. The relevance of this project comes from the necessity to identify potential enzymatic action of proteins of unknown function. Through this, some proteins could become useful for treatment of human disease or lead to a greater understanding of their cause.

**2602-Pos**

**Ligand Binding Studies of a Trimethoprim-Resistant Dihydrofolate Reductase by Fluorine NMR**

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Antibiotic resistance is a worldwide problem. The excessive use and misuse of antibiotics have resulted in the development of drug resistance. One example involves the antibiotic trimethoprim (TMP). TMP selectively inhibits the bacterial enzyme dihydrofolate reductase (DHFR) over its human counterpart. Inhibition of DHFR results in inhibition of DNA synthesis and ultimately in cell death. Unfortunately, extensive use and dissemination of TMP over the last forty years has resulted in the development of drug resistance in the form of highly transmissible plasmids. R67 dihydrofolate reductase is a plasmid-encoded enzyme that confer resistance to TMP. Our lab has studied how R67 DHFR works *in vitro* using diluted conditions. However, most proteins function inside cells under crowded and heterogeneous environment. As $^{19}F$ labeled proteins in complex mixtures can be characterized by NMR, we report our initial expression and characterization of R67 DHFR. To obtain such information, we have labeled R67 DHFR with fluorine at different positions in the indole ring and showed that fluorine incorporation into R67 DHFR does not affect the structure and function of the protein. We have characterized the $^{19}F$ NMR spectra of apo R67 DHFR and optimized fluorine incorporation. Furthermore, fluorinated R67 DHFR shows different signals for binding of cofactor, $NADP^+$, and substrate, dihydrofolate (DHF). We have obtained similar dissociation constants for the cofactor and substrate of R67 DHFR by both NMR and ITC. In our next step, we will measure dissociation constants in more complex solutions. We envision using fluorinated protein to quantify the effect of osmolytes and crowders on the binding affinity of substrate and cofactor towards R67 DHFR.

**2603-Pos**

**The Dissociation Mechanism of the Processive Cellulase *Tr*Cel7A**

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Cellulase enzymes catalyze the hydrolysis of recalcitrant cellulose into soluble sugars, and are thus a key biocatalyst in the growinglignocellulosic bioeconomy. Cel7A from *Trichoderma reesei* (*Tr*Cel7A) is a widely used