a perspective of utilizing emerging techniques through integrative approaches [2-4]. Subsequently, we review specific biological processes associated with CgA and derived peptides in neuroendocrine, immune, and digestive systems. Finally, we discuss biomedical applications of CgA as a biomarker, suggesting future directions toward translational and precision medicine.

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180-Pos

SPR and HDXMS Analysis of Interactions between Complement Component 3 and Thrombomodulin

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We have used surface plasmon resonance (SPR) and hydrogen/deuterium exchange mass spectrometry (HDXMS) to study the interaction between thrombomodulin and complement component 3 (C3) Complement component 3 (C3) is at the junction of three different complement activation pathways (classical, lectin, and alternative). Activation to C3b is a key step in the innate immune response that allows for the formation of important multi-protein complexes that ultimately participate in pathogen clearance. When misregulated, complement can lead to inflammatory disease and autoimmune disorders. Several regulatory proteins for C3b are known, but the molecular details of interactions between these proteins have not yet been elucidated. Thrombomodulin (TM), and specifically its N-terminal lectin-like domain (TMD1), has been identified as a possible regulator of complement through interactions with C3 or C3b, and the known regulator CFH may also be required. Using SPR, we see that C3 or C3b can bind to a surface coated with TMD1, and full kinetic studies are underway. Using HDXMS, we see that TMD1 interacts with C3b, and there is a lesser interaction with C3. TMD1 tends to make C3b more accessible to deuterium exchange, while C3 tends to be less accessible to deuterium exchange in the presence of TMD1. This difference suggests a possible role for TMD1 in regulating a key step in the complement pathway. We will next investigate the role of CFH in these interactions.

181-Pos

Amyloid Beta Oligomerization Probed by Single-Molecule FRET

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Amyloid beta protein (Aβ) is one of the key proteins implicated in Alzheimer's disease (AD) pathology. The characteristic amyloid plaques consisting of amyloid fibrils are present in the brain of nearly all AD patients, but growing evidence suggests soluble oligomers may be more toxic. However, it is difficult to track and probe oligomers that may transiently appear during the aggregation and are presumably a heterogenous mixture. We took the power of single-molecule Förster resonance energy transfer (FRET) and investigated soluble oligomers of Aβ. We performed the experiment after mixing 42-residue Aβ labeled with Alexa 488 (donor) and Aβ labeled with Alexa 594 (acceptor) with different mixing ratios. Fibril formation of dye-labeled Aβ was confirmed by EM. We monitored the aggregation using a plate reader and sampled the solution at different phases of aggregation for single-molecule free-diffusion experiment. In this experiment, a freely-diffusing molecule in solution emit a burst of fluorescence photons during its brief residence in the focal volume. The burst duration and brightness analysis reveal the presence of very small population of oligomers with the size much larger than decamer. To further investigate the oligomer characteristics, we recorded time-lapsed movies of the surface deposition of oligomers and fibrils of dye-labeled Aβ. We found that the fluorescence lifetime of oligomers and longer fibrils are different, suggesting the conformation of the monomer unit is different between the small oligomers and large fibrils that lead to different quenching environment of fluorophores. In addition, the growing speed is much faster for fibrils than oligomers. Our study shows that the oligomer population is very low and most of them may not grow into long fibrils, which are consistent with the observation of the long lag time of the aggregation process.

182-Pos

The Structure and Mechanism of a Unique Rieske-Type Mono-Oxygenase Enzyme from the Human Gut Microbiota Implicated in Cardiovascular Disease

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Our work focuses on the biochemical and structural characterisation of a unique Rieske oxygenase enzyme called CntA recently discovered in Acinetobacter baumannii from the human gut microbe community. In complex with a reductase enzyme CntB, this enzyme system plays a role in metabolising dietary carnitine into trimethylamine (TMA). TMA is subsequently metabolised by human liver enzymes to trimethylamine-oxide (TMAO) which is implicated in cardiovascular conditions like atherosclerosis. Until recently, it was thought only aromatic-like substrates could be metabolised by such Rieske-type enzymes and warrants investigation of its structure and mechanism. CntA has canonical features similar to other Rieske enzyme systems with a 2[Fe-2S] cluster and a mononuclear iron site whilst CntB has a 2[Fe-2S] cluster. Focussing on CntA, a characteristic feature of other such oxygenase enzymes is a bridging aspartate between the Rieske centre and the mononuclear iron thought to be part of the electron transport pathway. Instead, CntA possess a glutamate bridging residue which is critical for activity as mutating into an aspartate abolishes activity. Additionally, 2 cysteine residues adjacent to the bridging aspartate, not seen in other classes of Rieske enzymes provide further intrigue to this enzyme with their roles not yet known. Furthermore, a comprehensive substrate preference for CntA is yet to be ascertained to better understand this intriguing enzyme system. We present the crystal structure for CntA co-crystallised with L-carnitine revealing a unique ligand binding mode. We investigate the electron transfer pathway with EPR measurements in trying to ascertain a mechanism and possible role structurally and/or mechanistically for the cysteine residues. In addition, we have profiled a range of substrates building a pharmacophore model for activity and have also discovered novel inhibitors against this enzyme system.

183-Pos

Structural Insights into an ATP-Dependent Ribokinase from *Arabidopsis Thaliana*

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Recent studies in *Arabidopsis thaliana* have established a metabolic link between purine catabolism and the degradation of the corresponding nucleosides. In particular, during nucleoside hydrolysis, ribose product is released, and phosphorylated by an ATP-dependent ribokinase (RBSK) into ribose-5′-phosphate for its entry into a central metabolism recycling the sugar carbons from nucleosides. Here, we report the crystal structure of RBSK from *Arabidopsis thaliana* (AtRBSK) in three different ligation states: an unliganded state, a ternary complex with ribose and ATP, and a binary complex with ATP in the presence of Mg^{2+}. Only in the presence of Mg^{2+}, ATP in the binary complex adopts a catalytically competent conformation, providing a mode of action for Mg^{2+} in AtRBSK activity. The structural data combined with activity analyses of mutants allowed assignment of functional roles for the active site residues. Overall, this study provides the first structural characterization of plant RBSK, and experimentally validates a previous proposal model concerning the general reaction mechanism of RBSK.

184-Pos

MDMX Acidic Domain Requires the WF Motif for the Initiation of the Secondary Interaction with the P53DBD

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MDMX is an oncoprotein that inhibits p53 by reversibly binding the p53TAD and the p53 DNA binding domain (p53DBD). The canonical binding between the p53TAD and the p53 binding domain of MDMX drive the initial interaction that is required for stable inhibition of p53. Secondary interactions between the p53DBD and two additional domains of MDMX, the acidic domain (AD) and