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ABSTRACTS ISSUE

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TABLE OF CONTENTS

Introduction to the 29th Annual Symposium of The Protein Society Abstracts Issue
Abstracts of the 29th Annual Symposium of The Protein Society. .............. 1–343
protein structure prediction, membrane protein-protein docking, and membrane protein design. The RosettaMembrane residue environment energy term is based on amino acid propensities in hydrophobic, interface, and water layers of the membrane and depends on the residue burial state – from being completely buried within a protein environment to being completely exposed either to the lipid or water environments. Residue buried state is determined from the number of residue neighbors within 6 and 10 Å spheres. The RosettaMembrane residue-residue interaction term is based on the propensities of amino acid pairs to be in close proximity to each other within hydrophobic, interface, and water layers. Results of our statistical analysis reveal fine details of favorable and unfavorable environments for all amino acids types in all membrane layers and residue burial states. We find that large hydrophobic amino acids are favorable facing the hydrophobic core of the lipid bilayer. Small amino acids are favorable facing the protein core within the hydrophobic layer of the membrane. Aromatic or positively charged amino acids and favorable facing the lipid head groups. Residue-residue interactions are often favored between polar and charged amino acids and also between some of small and large hydrophobic amino acids inside of the protein core within the hydrophobic layer of the membrane. These data will be useful for rational design of novel membrane protein structures and functions.

PL-031

**Coordinated gripping of substrate by subunits of a AAA+ proteolytic machine**

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Protein quality control’ or ‘Protein degradation’ Hexameric AAA+ protein-remodeling machines use conserved loops that line the axial pore to apply force to substrates during the mechanical processes of protein unfolding and translocation. An open question in the AAA+ field is whether pore loops from different subunits of the hexameric ring grip the substrate coordinately (all six subunits involved), independently (one subunit at a time involved), or partially coordinated (two or three subunits at a time). To answer this question, we studied covalently linked hexamers of the E. coli ClpX unfoldase bearing different numbers and configurations of wild-type and mutant pore loops and challenged these variants with protein substrates with a broad range of stabilities. We find that successful unfolding of increasingly resistant substrates requires the coordinated action of a greater number of wild-type pore loops. Our results support a mechanism in which a power stroke initiated in one subunit of the ClpX hexamer results in the simultaneous movement of all six pore loops, which coordinately grip and apply force to the substrate.

PL-032

**Structure and function of the Toc159 M-domain, and its role in targeting the preprotein receptor to the chloroplast outer envelope membrane**

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Chloroplast biogenesis and function rely on the import of thousands of nucleus-encoded preproteins from the cytosol. Preprotein import is supported by the Toc and Tic (Translocon at the outer and inner envelope membranes of chloroplasts) complexes, which work cooperatively to translocate preproteins across the double-membrane envelope to the chloroplast interior. Toc159 is one of the preprotein receptors of the Toc complex, is also encoded in the nucleus and post-translationally targeted to the chloroplast, and is comprised of 3 distinct domains: 1) the intrinsically disordered N-terminal Acidic (A-) domain; 2) the central GTPase (G-) domain; and 3) the C-terminal Membrane (M-) domain that anchors the protein to the chloroplast outer membrane (COM) through an unknown mechanism. The M-domain has no known homologues and does not contain a predicted trans-membrane domain, but does contain intrinsic chloroplast targeting information at the extreme C-terminus. The M-domain also contains
a predicted β-helix motif, which may be important for anchoring the protein to the COM. We are interested in characterizing the structure of the M-domain and determining the nature of its association with the COM, as part of our larger goal of understanding the role Toc159 plays in protein import into chloroplasts. We are also interested in defining the precise nature of the targeting information contained within the extreme C-terminus of Toc159, elucidating the targeting pathway that is used, and whether other COM proteins use this pathway. We will present our most recent data on the structure, function and targeting of the Toc159 M-domain.

PL-033

Structural investigation of NlpC/P60 protein acquired by Trichomonas vaginalis through a lateral gene transfer event

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Trichomonas vaginalis is an extracellular flagellated protozoan parasite that causes the most common non-viral sexually transmitted disease, with approximately 200 million cases worldwide annually. Nevertheless, the biochemical processes behind T. vaginalis infection and its interaction with the vaginal microbiota are still not well defined. In 2007 the draft genome sequence of Trichomonas vaginalis strain G3 was described, identifying 60,000 protein-coding genes. Of these, nine genes encode NlpC/P60-like members. This superfamily is widely represented in the different kingdoms of life and has diverse enzymatic functions, such as amidases, endopeptidases and acetyltransferases. Previous studies have shown that members of this superfamily hydrolyze specific peptide linkages in bacterial cell walls affecting germination, vegetative growth, sporulation and division or cell lysis/invasion. As a typical eukaryote, the protozoan parasite T. vaginalis does not have a cell wall itself. Previous studies suggest that the T. vaginalis NlpC/P60 genes were acquired via lateral gene transfer from bacteria and must have an important function, possibly controlling the vaginal microbiota and aiding parasite invasion and infection. To investigate the function of the NlpC/P60 family of proteins in T. vaginalis we have expressed, purified and crystallized a member TVAG_119910 and report its three-dimensional structure, determined at 1.5 Å resolution, by X-ray diffraction. The structure of the protein reveals a typical papain-like fold resembling peptidoglycan hydrolases from the NlpC/P60 family with a conserved cysteine and histidine; forming the catalytic residues. The protein contains two bacterial SH3 domains at the N-terminus. This domain acts as a general binding domain and is likely to aid the interaction of the NlpC/P60 domain with substrate components. Combined with biochemical and enzymatic characterization, the structure of this NlpC/P60 protein will help to elucidate the molecular origin of its hydrolase activity and to decipher their putative role in the parasite infection.

PL-034

Novel DNA polymerases from Red Sea brine-pools: new potential polymerases for PCR application

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The polymerase chain reaction (PCR) is a key tool in medical and biological research. The most common PCR reaction relies on the thermal cycling method that consists of repeated cycles of heating and cooling steps for DNA melting and extension by the DNA polymerase, respectively. The introduction of new DNA polymerases to the market is a major area of development that tremendously helped in improving the performance and quality of PCR. Nonetheless, PCR still requires optimization of salt and metal ion concentrations leaving a room in the market for introducing new DNA polymerases that are robuster in their salt and metal ion concentration dependence. In this study, we will present the characterization of a novel archaean DNA polymerase from the Red Sea brine-