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EDITORS LETTER

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Protein Research at Its Best

Also known for its sports teams, its role in the American Revolution, its baked beans, and its exceptional universities, Boston, Massachusetts was the venue for the 25th Anniversary Protein Society symposium. Held at the Marriott Hotel in Copley Place, this conference featured a wide variety of seminars and poster presentations at the frontiers of protein science research. Although it is impossible to review all of the outstanding work presented at this meeting, this Editor's Letter will summarizes a few of the talks I had the pleasure of attending.

The incorporation of unnatural amino acids into peptides permits the engineering of proteins with new functionalities. Jason Chin, from the MRC Laboratory of Molecular Biology, delivered a captivating talk that described the development of what he called a "parallel genetic code". His group is able to incorporate unnatural small molecules into proteins through the use of engineered translational machinery such as orthogonal tRNA/tRNA synthetase pairs and engineered ribosomes. In a particularly noteworthy study, his group incorporated multiple unnatural amino acids using orthogonal tRNA/tRNA synthetase pairs and synthetically evolved ribosomes that recognized quadruplet codons and the amber stop codon.[1]

Protein quality control is critical to robust cellular functioning. Damaged or misfolded proteins must therefore be degraded to prevent deleterious aggregation in the cell. Robert Sauer, from the Massachusetts Institute of Technology, described some of his latest work in elucidating the mechanism of ClpXP, important proteins involved in the unfolding and degradation of damaged proteins. His talk focused specifically on describing the mechanism by which ClpX functions. Through a series of biochemical and biophysical characterization, Sauer determined that ClpX, an ATP-dependent asymmetric, hexameric "motor", binds a target protein which is followed by ATP-binding/hydrolysis producing a "power stroke" of 15° pulses that first unfold and subsequently drives substrate proteins into a lower compartment where ClpX degrades them.[2]

The precise functioning of transport proteins in a cell is essential and hence so is understanding the underlying mechanism by which they function. A big step forward would be to determine the structures of these proteins. In an attempt to systematically solve the high resolution structures of mammalian membrane proteins, Douglas Rees, from the California Institute of Technology, presented a "funnel" approach to solve the structures of several of these transport proteins. This approach is part of the National Institute of Health's "Protein Structure Initiative" that brings together leading investigators to use new ways to solve biomedical problems. In an attempt to solve the structures of some of the 521 annotated human transport proteins, this systematic approach involves the use of protein expression in *Pichia pastoris*, which can generate 200−300 g of biomass per liter in 2−4 days. Rees also tests various detergents (such as C8E4, β-OG, DDM, *etc.*) to determine which can most effectively isolate these proteins from the membrane while maintaining their solubility. The stability of these proteins is assessed by following its intrinsic tryptophan fluorescence. Rees concluded his talk by summarizing ways to improve the quality of protein crystals and data collection techniques for those that diffract poorly.

The use of fusions of green fluorescent proteins is an established method for the visualization of proteins in the cell. However, a major drawback is the large size of this protein tag. In her talk, Alice Ting, from the Massachusetts Institute of Technology, spoke about a new methodology, PRIME (PRobe Incorporation Mediated by Enzymes), which involves engineering "fluorophore ligases" such as lipoic acid ligase (LplA) from *Escherichia coli* to attach fluorophore 7-hydroxycoumarin to proteins that are fused to a 13-amino acid LplA recognition sequence.[3] With its relatively small size and highly specific labeling of target proteins within 10 min, this methodology is likely to find broad application as an imaging tool.

Gerhard Wagner, from Harvard Medical School, has been a pioneer in studying protein dynamics and in the development of nuclear magnetic resonance (NMR) as a tool for solving protein structures. In his talk, Wagner provided a wonderful overview of his seminal observations starting with what he described as the "stone age" of NMR when performing assignments to a 58-residue protein, basic pancreatic trypsin inhibitor (BPTI), up to the development of triple resonance experiments, which forms the basis for protein structure studies today. Acknowledging huge technological gains since the nascence of the field, Wagner proceeded to provide the audience with some of the more recent work on determining the solution structure of the human voltage dependent anion channel (VDAC-1) using membrane mimicking "nanodiscs".[4] Additionally, he outlined some of his latest work in determining the structures of therapeutically relevant nonribosomal peptide synthetases.

Transthyretin amyloidosis is a disease caused by protein aggregation and accumulation in body tissues. The dissociation of tetrameric transthyretin (TTR), a thyroxine carrier in the cerebrospinal fluid and holo-retinol in the blood, has been recognized as the underlying rate-limiting step for amyloidogenesis. Stabilization of the TTR tetramer native state over the dissociative transition state by small-molecule ligands is recognized as a potential approach to preventing TTR amyloid fibril formation.[5] Jeffrey Kelly, from the Scripps Research Institute, described work in his lab on finding a drug that has recently been approved by the European Medicines Agency for treatment of transthyretin amyloidosis. His lab in conjunction with Pfizer, Inc. identified the compound Vyndaqel (also called "tafamidis") that when administered orally as a single dose to patients at 20 mg/day slowed peripheral neurological impairment and disease progression over an 18 month clinical trial period.

New antibiotics that target essential bacterial enzymes are highly desirable. MraY, a key translocase that plays a role in bacterial cell wall biosynthesis, is one such target. Christopher Walsh, from Harvard Medical School, offered a seminar on the biosynthetic assembly of pacidamycins, uridyl peptide antibiotics produced by *Streptomyces ceoruleorubidus*, which target MraY. Through some outstanding biochemical work, his group identified the enzymes and elucidated their complex mechanisms

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required for the biosynthesis of the tetrapeptidyl nucleoside pacidamycin scaffold from amino acid and nucleoside units.[6]

Epigenomes are typically defined by chromatin structure. It is now well-known that histone modifications play a major role in determining chromatin structure and its role in processes such as transcription and replication. Dynamic control of the position and the chemical property of these modifications appear to play a role in gene regulation. Tom Muir from Princeton University gave a great talk on the use of protein ligation methodologies to assist in deciphering the "histone code". One such approach was the use of an expressed protein ligation (EPL), a protein semisynthetic method that could be used to generate acetylated, phosphorylated, methylated, and ubiquitylated histones, and the application of these modified histones in understanding chromatin biology.

Alzheimer's disease (AD) is the most common form of neurodegenerative disorders affecting as many as 5 million Americans. According to Christopher Dobson, from the University of Cambridge, the economic burden of patient care for those suffering from AD is likely to rise from $172 billion in 2010 to $1 trillion in 2050. AD occurs through aberrant behavior of proteins in the form of misfolded proteins as amyloid β (Aβ) causing amyloid plaques and Tau protein tangles in the brain. In order to study the underlying mechanism for protein aggregation, his group rationally mutagenized 17 variants of Aβ42 peptide based on computational predictions to form a correlation between the tendencies of these peptides to aggregate and their corresponding effects on neuronal dysfunction.[7]

The 25th Anniversary Protein Society symposium covered a host of topics for protein research aficionados with great talks from researchers at the front-line in finding answers to some of the most pertinent questions in protein science.

Jitesh Soares
Managing Editor, ACS Chemical Biology

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