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The Many Facets of Protein Design: From Self-Assembled Materials to Vaccines

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Proteins are fascinating molecules: they efficiently perform an immense variety of functions ranging from catalysis to structural support and signal transduction. Even more fascinating is their structural diversity and complexity: they range from small, folded structures of less than thirty amino acids to enormous multiunit assemblies reaching millions of Da in size often required for complex biological functions. Not surprisingly, ever since the first hypothesis that proteins are made of simple amino acids by Emil Fischer and Franz Hofmeister, many attempts were made to build proteins *de novo*, i.e. from scratch, to understand the forces that define macromolecular function and to create polypeptides with new properties. Nonetheless, rationally creating even a very simple folded structure, a hallmark of proteins, proved to be a tough challenge. Almost a century after Fischer's and Hofmeister's hypothesis, DeGrado and coworkers demonstrated for the first time that very simple physico-chemical principles can be used to build helical bundles that have all the properties of typical proteins.[1–3] These prototypical proteins can be easily optimized and functionalized further.[4–6] Now, several decades later, advances in structural determination of protein structures allowed for development of powerful algorithms to predict and build proteins with novel structure and functions.[7,8] At the same time the principles of self-assembly, so powerfully used by nature itself, can be efficiently used to construct complex machinery that is right for the given task.

Astounding progress in computational methods, chemical biology and molecular biology techniques has enabled increasingly complex applications of protein design and engineering at the interface with biology. A collection of reviews in this issue of *Current Opinions in Structural Biology* summarizes recent advances in the design of complex self-assembling structures both *in vitro* and *in vivo*, applications of protein design for antibody and vaccine development, as well as new approaches to constructing new proteins using both natural and unnatural amino acid residues.

Given the success supramolecular assembly produced *in vitro*, it was only a matter of time before principles that guide protein assembly would be applied to creating new functionalities *in vivo*. Nguen and Ueno describe recent advances in creating new, intracellular structures that can serve as carriers for cargo delivery or even become artificial nanoreactors.

Panettieri and Ulijn review the recent progress in identifying the very basic thermodynamic and kinetic principles that drive supramolecular self-assembly in small peptides. Remarkable morphological and functional variability in assemblies formed by peptides as short as three

residues long allows for establishing important structure activity relationships that help guide design of complex materials with predetermined properties.

Formation of self-assembled structures has long been postulated to be one of the earliest mechanisms for macromolecular evolution. Liang *et al.* propose the concept of conformational evolution that occurs in the process of morphological and functional selection during the assembly process. Elucidation of the driving forces that drive oligomerization and structural diversification of amyloid-forming peptides will lead to better understanding of their role in disease.

The protein fold plays a key role in properly arranging multiple functional groups together with the substrate to promote chemical reactions. The same function can be fulfilled by supramolecular assemblies formed by small molecules, nucleic acids and polypeptides (and various combinations of all of the above). The review by Wang *et al.* focuses on progress in development of supramolecular enzyme-like catalysts.

Grayson and Anderson discuss the latest progress in using *de novo* designed helical bundles as scaffolds to create artificial enzymes. Rationally designed, well-folded, *in vivo* assembled catalysts facilitate redox transformations with efficiencies rivalling those of closely related natural enzymes opening the path for production of industrially relevant chemicals and rewiring cellular circuitry.

The use of non-canonical amino acids in protein design provides important means to introduce specific function in protein scaffold. Focusing on metal-chelating non-canonical amino acids, Almhjell and Mills highlight design efforts to generate proteins in which novel metal-binding sites impart catalytic activity, or are used to control the self-assembly into oligomers. Importantly, computational approaches such as Rosetta can be expanded to include non-canonical amino acids in the design process.

Ancestral proteins display important differences with extant ones, such as enhanced stability, functional promiscuity, and active site dynamics. These differences offer an alternative approach to protein engineering, as discussed by Sanchez-Ruiz and Ozkan. For example, the enhancement of thermal stability of Precambrian proteins compared to modern ones may provide better starting points for protein engineering projects.

Dissecting the function of complex natural proteins has long been a goal of protein design, particularly for ones for which structural characterization is challenging. G-protein coupled receptors, which are the target of approximately a third of the drugs currently available, stand out for the impact that a more accurate characterization could have for the development of novel therapies. Despite remarkable progress in the field, resulting in landmark structures of several members of the family, the low level of expression, limited stability outside the native membrane environment, and inherent conformational dynamics still pose challenges to the full characterization of this class of proteins. Barth and colleagues discuss applications of protein engineering methods to facilitate structural studies by stabilizing the active or inactive state of the receptors, and to alter the receptor's function by either changing ligand specificity, or modulating signaling strength, or rewiring signaling by changing its interactions with effector proteins.

Fischman and Ofran discuss computational methods to aid the discovery of antibodies with specific biological functions. Rather than focusing on generating high affinity antibody-antigen complexes, these methods highlight the importance of selected few residues, forming the epitope, in generating biologically relevant antibodies. The result is the design of focused, epitope-specific libraries that produce antibodies with increased specificity and/or functionality, for example by blocking interactions with the natural binder for a given epitope.

Antibodies against post-translational modifications (PTMs) are increasingly in high demand to help dissect the biological role of PTMs in physiological and diseased states. Identifying and quantifying these modifications is very challenging, because a high level of affinity and selectivity over the non-modified protein is needed. Koide and colleagues discuss novel methods to develop next-generation antibodies, starting from lead antibodies obtained through traditional immunization, as well as through directed evolution and structure based design and applying several steps of affinity maturations. Specific structural features are identified in next-generation anti-PTM antibodies, resulting in novel binding mechanisms and expanded recognition surfaces. A deep, structure-based understanding these mechanisms in turns results in improved starting points to generate novel anti-PTM antibodies.

Finally, Correia and colleagues address novel approaches to the design of immunogens that can elicit focused and neutralizing antibodies, primarily for diseases recalcitrant to traditional vaccine development. The review surveys methods to abolish non-neutralizing epitopes, to stabilize dynamic epitopes, to display desired epitopes on scaffolding proteins, and to raise broadly neutralizing antibodies by targeting the germline precursor. These methods are based on structural analysis of the desired antigen-antibody interactions, and employ state of the art protein design strategies. This novel strategy holds promise for the development of precision vaccines.

Biography

Ivan V. Korendovych is an Associate Professor of Chemistry at Syracuse University. He received B.S. and M.S. degrees in inorganic chemistry from the National Taras Shevchenko University of Kyiv and a Ph.D. degree from Tufts University working on mechanistic aspects of oxygen activation and anion recognition. After postdoctoral studies at the University of Pennsylvania School of Medicine, Dr. Korendovych joined the faculty at Syracuse University in 2011. The main foci of the Korendovych Lab include protein design and engineering, development of probes for cancer imaging, and medicinal chemistry. Prof. Korendovych received many awards including an Alexander von Humboldt Research Fellowship, an ACS Division of Inorganic Chemistry Young Investigator Award and ORAU Ralph E. Powe Junior Faculty Award.

Giovanna Ghirlanda is a Professor of Chemistry at the Arizona State University. Professor Ghirlanda received a B.Sc./M.Sc. degree in Medicinal Chemistry and a Ph.D. in Organic Chemistry from the University of Padova, Italy, with a thesis on the redox catalytic properties of supramolecular metallocomplexes. Prior to joining ASU, she pursued

postdoctoral work at the University of Pennsylvania specializing on *de novo* design of peptides and proteins. Her current research integrates protein design, chemical biology, and bioinorganic chemistry to produce novel proteins with desired functions on the design of functional proteins. A major thrust is in the area of sustainable fuel production, designing protein-based hybrid metalloenzymes that catalyze hydrogen production and carbon dioxide reduction in mild conditions, and on protein-glycan recognition.

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