PREFACE

The diversity of structures found for peptides and proteins in the biosphere is overwhelming with folds as simple as α-helical coiled coils to far more complex membrane conformations. In order to understand biological function at the most basic level, one needs a coherent description of the factors that lead to stable protein structures and, preferably, the ability to construct systems using the basic principles of chemistry and biophysics. It is this latter objective that drives much of the research in the field of protein design. The field benefited greatly from advances in chemistry, biophysics, and molecular biology that occurred in the 1980s. Automated peptide, protein, and nucleic acid synthesis combined with the development of cloning, mutagenesis, and expression of genes made preparation of new sequences routine. At the same time, expanded computer capability, combined with access to synchrotron radiation sources and high-field NMR and mass spectrometers, provided a previously unavailable level of determining sequence, structure, and, most recently, a computational basis for understanding protein structure and dynamics. These advances have led to a broad range of systems that define a significant element of modern synthetic biology.

Peptide, protein, and enzyme design may take on many different flavors. One branch of the field focuses on selective pressure to evolve new activities for proteins, enhancing existing catalysis or stabilizing already efficient systems. This field of molecular evolution has had remarkable impact for arriving rapidly at desired chemical transformations (McIntosh, Heel, Buller, Chio, & Arnold, 2015). A drawback, however, is that "directed evolution" (even if in a test tube) drives the modifications so that intermediate steps that provide insight on function are often missed. Nonetheless, combining computational efforts in conjunction with this directed evolution can now be used to identify key components of catalysis (Dodani et al., 2016). While directed evolution is an important component of the protein reengineering toolkit, there are other treatises that cover this area extensively, so it is not generally reflected in this compendium.

This does not mean that reengineering proteins are not contained herein as numerous methods are presented on this topic. In fact, this can be done by making mutations to existing proteins (Hu, Chan, Sawyer, Yu, & Wang, 2014; Nakajima et al., 2008), adding cofactors to systems that previously did not include such catalytic functionality (Dürrenberger & Ward, 2014; Miner et al., 2012), making protein fusions that provide photoaddressable

proteins that localize in membranes (Hallett, Zimmerman, Yumerefendi, Bear, & Kuhlman, 2016), altering cofactors to serve a different purpose, or attaching side chains (Hayashi, Sano, & Onoda, 2015) or metal binding sites to confer new protein–protein interactions (PPIs) (Speltz, Nathan, & Regan, 2015) or added stability (Salgado, Faraone-Mennella, & Tezcan, 2007; Schoene, Fierer, Bennett, & Howarth, 2014). These PPIs are critical for molecular recognition, but often are difficult to measure in their native environments so that new methodologies have been developed to quantify these interactions in vivo (Cherf & Cochran, 2015). Often, proteins are reengineered to enhance stability and so we examine this general strategy as well. A major limitation for the development of novel designed systems has been the limitation of amino acids that can be incorporated into proteins by bacterial expression. For this reason, advances for employing noncoded or even nonnatural amino acids are discussed (Coin, Perrin, Vale, & Wang, 2011; Hu et al., 2014). Additionally, libraries of antibodies or other proteins are often made as libraries so methods are needed to display these products (Frei, Kielian, & Lai, 2015).

While protein engineering relies on a previously evolved protein scaffold, de novo protein design approaches this topic from the most rudimentary levels by attempting to prepare existing or unknown protein folds using first principles of biophysics. DeGrado described the approach well (Bryson et al., 1995; DeGrado, Wasserman, & Lear, 1989) as a peptidic or protein system that is not directly related to any sequence found in nature yet folds into a predicted structure and/or carries out desired reactions. Because of its more straightforward rules for folding (Hartmann et al., 2016), α-helical coiled coils have been one of the major starting points for designed proteins with systems as simple as models for the two-stranded coiled coil GCN4 to elaborate multicomponent structures (Woolfson, Bartlett, Bruning, & Thomson, 2012). Computational methods, the most famous of these being Rosetta (Rohl, Strauss, Misura, & Baker, 2004), both for reengineering proteins and de novo design have appeared (Mitra, Shultis, & Zhang, 2013). While the present issue does not describe the computational aspects in detail, many workers have used these methodologies to develop functional designed scaffolds. There has been effort expended on using nonnatural amino acids to stabilize these systems (Buer, Meagher, Stuckey, & Marsh, 2012). A significant portion of the text is devoted to these de novo systems with additions of related cyclic peptides (Bock, Gavenonis, & Kritzer, 2013) and more recently prepared amide foldamers that mimic protein structure (Kudo, Maurizot, Kauffmann, Tanatani, & Huc, 2013).

It is believed that roughly a third to one-half of native proteins bind metals for their function, their structure, or to transport these ions to or within cells (Thomson & Gray, 1998; Waldron & Robinson, 2009). Thus, it is appropriate that in this volume roughly the same proportion of articles address methods to design metalloproteins (Yu, Penner-Hahn, & Pecoraro, 2013; Yu et al., 2014). In studies such as these one must not only consider the factors that determine a specific secondary structure or PPI, but must also address the thermodynamics, kinetics, and dynamics of metal ion interactions with the peptides and proteins (Farrer & Pecoraro, 2003; Ghosh & Pecoraro, 2004). Thus, one must determine how to place metal ligands in desired constructs in a way that will satisfy the bonding preferences of the metal (ligand type and number and coordination geometry) without destabilizing the existing scaffold. Further complicating these studies is the need to encapsulate metals in more than one oxidation state, a requirement that is essential for electron transfer proteins and redox active catalysts. In essence such multiple redox systems present the problem of simultaneously building two metalloproteins representing each metal oxidation level that must then be merged into one. In some instances, workers have expended effort to develop de novo designed scaffolds as simple coiled coils, sometimes called maquettes (Zhang et al., 2011), and others have taken a redesign strategy. Both approaches are represented in this collection. In terms of systems investigated, many design studies attempt to mimic or reproduce electron transfer (Plegaria, Herrero, Quaranta, & Pecoraro, 2015; Roy et al., 2014; Zhang et al., 2011) and structural (Fragoso et al., 2015; Lombardi et al., 2000; Mocny & Pecoraro, 2015; Plegaria, Duca, et al., 2015) or catalytic function (Cangelosi, Deb, Penner-Hahn, & Pecoraro, 2014; Dürrenberger & Ward, 2014; Miner et al., 2014; Nakajima et al., 2008; Reig et al., 2012; Tegoni, Yu, Berseloni, Penner-Hahn, & Pecoraro, 2012; Zastrow, Peacock, Stuckey, & Pecoraro, 2012). However, this field is not restricted to biomimics as new catalysis is explored by building unique cofactors (Dürrenberger & Ward, 2014; Hayashi et al., 2015; Popp & Ball, 2010). Another area of great interest is the ability of a protein to recognize another protein or an inanimate surface. New methods for linking proteins together using metal-directed protein self-assembly (Salgado et al., 2007) or exploring protein interactions with inorganic surfaces (Bedford et al., 2015) are burgeoning fields. There are even new strategies for obtaining clinically relevant MRI or luminescent imaging agents using designed proteins containing rare-earth ions (Berwick et al., 2014). In all these areas, one will

find chapters herein that provide detailed insight on the methods necessary to achieve these types of exciting and heretofore unknown metalloproteins.

As one can see from this Preface, the field of protein design is vast; therefore, it is impossible to present the broad range of methods in a single, limited volume. Fortunately, other volumes of *Methods in Enzymology* and related series provide additional information that the reader may find helpful. It is my hope that the sampling of different techniques represented here will assist individuals presently looking for alternative strategies to solve problems associated with their protein design projects. In particular, I will consider this project a success if new researchers are inspired and assisted by these chapters to develop their own programs in this field.

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