Published in final edited form as:

Methods Enzymol. 2016 ; 580: xvii–xxii. doi:10.1016/S0076-6879(16)30242-7.

Preface: Peptide, Protein and Enzyme Design

Vincent L. Pecoraro

Department of Chemistry and Program in Biophysics, University of Michigan, Ann Arbor, Michigan 48109-1055, USA

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 1980's. 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 et al 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 re-engineering toolkit, there are other treatises that cover this area extensively, so it is not generally reflected in this compendium.

This does not mean that re-engineering proteins is not contained herein as numerous methods are presented on this topic. In fact, this can be done by making mutations to existing proteins (Hu et al 2014; Nakajima, et al 2008), adding cofactors to systems that previously did not include such catalytic functionality (Dürrenberger et al 2014; Miner et al 2014), making protein fusions that provide photoaddressable proteins that localize in membranes (Hallett et al 2016), altering cofactors to serve a different purpose or attaching side chains (Hayashi et al 2015) or metal binding sites to confer new protein-protein interactions (PPIs) (Speltz et al 2015) or added stability (Salgado et al 2007; Schoene et al 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 and Cochran 2015). Often, proteins are re-engineered 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 non-coded or even non-natural amino acids are discussed (Coin et al 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 and Lei 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 (DeGrado et al 1989; Bryson et al 1995) 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 (Woelfson et al 2012). Computational methods, the most famous of these being Rosetta (Rohl et al 2004), both for re-engineering proteins and *de novo* design have appeared (Mitra et al 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 non-natural amino acids to stabilize these systems (Buer et al 2012). A significant portion of the text is devoted to these *de novo* systems with additions of related cyclic peptides (Bock et al 2013) and more recently prepared amide foldamers that mimic protein structure (Kudo et al 2013).

It is believed that roughly a third to one half of native proteins bind metals either for their function, their structure or to transport these ions to or within cells (Waldron and Robinson 2009; Thomson and Gray 1998). Thus, it is appropriate that in this volume roughly the same proportion of articles address methods to design metalloproteins (Yu et al 2013). In studies such as these one must not only consider the factors that determine a specific secondary structure or protein-protein interaction, one must also address the thermodynamics, kinetics and dynamics of metal ion interactions with the peptides and proteins (Farrer and Pecoraro 2003; Ghosh et al 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 (Zhang et al 2011.; Plegaria et al 2015b; Roy et al, 2014), structural (Lombardi et al 2000; Plegaria et al 2015a; Fragoso et al 2015; Mocny and Pecoraro 2015) or catalytic function (Cangelosi et al 2014; Durrenberger and Ward 2014; Miner et al 2014; Nakajima, et al 2008; Reig et al

2012; Tegoni et al 2012; Zastrow et al 2012). However, this field is not restricted to biomimics as new catalysis is explored by building unique cofactors (Dürrenberger and Ward 2014; Hayashi et al 2015; Popp and 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.

References

- Bedford NM, Ramezani-Dakhel H, Slocik JM, Briggs BD, Ren Y, Frenkel AI, Petkov V, Heinz H, Naik RR, Knecht MR. Elucidation of Peptide-Directed Palladium Surface Structure for Biologically Tunable Nanocatalysts. *ACS Nano*. 2015; 9:5082–5092. [PubMed: 25905675]
- Berwick MR, Lewis DJ, Jones AW, Parslow RA, Dafforn TR, Cooper HJ, Wilkie J, Pikramenou Z, Britton MM, Peacock AFA. De Novo Design of Ln(III) Coiled Coils for Imaging Applications. *Journal of the American Chemical Society*. 2014; 136:1166–1169. [PubMed: 24405157]
- Bock JE, Gavenonis J, Kritzer JA. Getting in shape: controlling peptide bioactivity and bioavailability using conformational constraints. *ACS Chemical Biology*. 2013; 8(3):488–99. [PubMed: 23170954]
- Bryson JW, Betz SF, Lu HS, Suich DJ, Zhou HX, O'Neil KT, DeGrado WF. Protein Design: A Hierarchic Approach. *Science*. 1995; 270:935–941. [PubMed: 7481798]
- Buer BC, Meagher JL, Stuckey JA, Marsh ENG. Structural basis for the enhanced stability of highly fluorinated proteins. *Proc Nat Acad Sci USA*. 2012; 109(13):4810–4815. [PubMed: 22411812]
- Cangelosi V, Deb A, Penner-Hahn JE, Pecoraro VL. A *de novo* designed metalloenzyme for the hydration of CO₂. *Angewandte Chemie*. 2014; 53:7900–7903. [PubMed: 24943466]
- Cherf GM, Cochran JR. Applications of Yeast Surface Display for Protein Engineering. *Methods in Molecular Biology* (Clifton, N.J.). 2015; 1319:155–175.
- Coin I, Perrin MH, Vale WW, Wang L. Photo-Cross-Linkers Incorporated into G-Protein-Coupled Receptors in Mammalian Cells: A Ligand Comparison. *Angew Chem Int Ed Engl*. 2011; 50:8077–8081. [PubMed: 21751313]
- DeGrado WF, Wasserman ZR, Lear JD. Protein design, a minimalist approach. *Science*. 1989; 243:622–628. [PubMed: 2464850]
- Dodani SC, Kiss G, Cahn JKB, Su Y, Pande VS, Arnold FH. Discovery of a regioselectivity switch in nitrating P450s guided by molecular dynamics simulations and Markov models. *Nature Chemistry*. 2016; 8:419–425.
- Dürrenberger M, Ward TR. Recent Achievements in the Design and Engineering of Artificial Metalloenzymes. *Current Opinions in Chemical Biology*. 2014; 19:99–106.
- Farrer BT, Pecoraro VL. Kinetics of Hg(II) Encapsulation by a Weakly Associated Three-Stranded Coiled Coil. *Proceedings of the National Academy of Sciences USA*. 2003; 100:3760–3765.

- Fragoso A, Carvalho T, Rousselot-Pailley P, Correia dos Santos MM, Delgado R, Iranzo O. Effect of the Peptidic Scaffold in Copper(II) Coordination and the Redox Properties of Short Histidine-Containing Peptides. *Chemistry – A European Journal*. 2015; 21:13100–13111.
- Frei JC, Kielian M, Lai JR. Comprehensive mapping of functional epitopes on dengue virus glycoprotein E DIII for binding to broadly neutralizing antibodies 4E11 and 4E5A by phage display. *Virology*. 2015; 485:371–382. [PubMed: 26339794]
- Ghosh D, Pecoraro VL. Studying Metalloprotein Folding Using *De Novo* Peptide Design. *Inorganic Chemistry*. 2004; 43(25):7902–7915. [PubMed: 15578824]
- Hallett RA, Zimmerman SP, Yumerefendi H, Bear JE, Kuhlman B. Correlating in Vitro and in Vivo Activities of Light-Inducible Dimers: A Cellular Optogenetics Guide. *ACS Synth Biol*. 2016; 5(1): 53–64. [PubMed: 26474029]
- Hartmann MD, Mendler CT, Bassler J, Karamichali I, Ridderbusch O, Lupas AN, Hernandez Alvarez B. α/β Coiled Coils. *eLife*. 2016; 5:e11861. [PubMed: 26771248]
- Hayashi T, Sano Y, Onoda A. Generation of new artificial metalloproteins by cofactor modification of native hemoproteins. *Israel Journal of Chemistry*. 2015; 55:76–84.
- Hu C, Chan SI, Sawyer EB, Yu Y, Wang J. Metalloprotein design using genetic code expansion. *Chemical Society Reviews*. 2014; 43:6498–6510. [PubMed: 24699759]
- Kudo M, Maurizot V, Kauffmann B, Tanatani A, Huc I. Folding of a linear array of α -amino acids within a helical aromatic oligoamide frame. *Journal of the American Chemical Society*. 2013; 135:9628–9631. [PubMed: 23763658]
- Lombardi A, Summa CM, Geremia S, Randaccio L, Pavone V, DeGrado WF. Retrostructural analysis of metalloproteins: Application to the design of a minimal model for diiron proteins. *Proceedings of the National Academy of Sciences USA*. 2000; 97:6298–6305.
- McIntosh JA, Heel T, Buller AR, Chio L, Arnold FH. Structural Adaptability Facilitates Histidine Heme Ligation in a Cytochrome P450. *Journal of the American Chemical Society*. 2015; 137:13861–13865. [PubMed: 26299431]
- Miner KD, Mukherjee A, Gao YG, Null EL, Petrik ID, Zhao X, Yeung N, Robinson H, Lu Y. A Designed Functional Metalloenzyme that Reduces O₂ to H₂O with Over One Thousand Turnovers. *Angewandte Chemie Int Ed*. 2012; 51:5589–5592.
- Mitra P, Shultis D, Zhang Y. EvoDesign: de novo protein design based on structural and evolutionary profiles. *Nucleic Acids Research*. 2013; 41:W273–W280. [PubMed: 23671331]
- Mocny CS, Pecoraro VL. *De Novo* Protein Design as a Methodology for Synthetic Bioinorganic Chemistry. *Accounts of Chemical Research*. 2015; 48:2388–2396. [PubMed: 26237119]
- Nakajima H, Ichikawa Y, Satake Y, Takatani N, Manna SK, Rajbongshi J, Mazumdar S, Watanabe Y. Engineering of *Thermus thermophilus* cytochrome C552 : Thermally tolerant artificial peroxidase. *ChemBioChem*. 2008; 9:2954–2957. [PubMed: 19006152]
- Plegaria JS, Duca M, Tard C, Deb A, Penner-Hahn JE, Pecoraro VL. *De Novo* Design of Copper Metallopeptides Inspired by Native Cupredoxins. *Inorg Chem*. 2015a; 54:9470–9482. [PubMed: 26381361]
- Plegaria JS, Herrero C, Quaranta A, Pecoraro VL. Electron Transfer Activity of a de Novo Designed Copper Center in a Three-Helix Bundle Fold. *Biochimica Biophysica Acta-Bioenergetics*. 2015b; 522–530.
- Popp BV, Ball ZT. Structure-Selective modification of aromatic side chains with dirhodium metallopeptide catalysts. *Journal of the American Chemical Society*. 2010; 132:6660–6662. [PubMed: 20420453]
- Reig AJ, Pires MM, Snyder RA, Wu Y, Jo H, Kulp DW, Butch SE, Calhoun JR, Szyperski T, Solomon EI, DeGrado WF. Alteration of the oxygen-dependent reactivity of de novo De Fe proteins. *Nature Chemistry*. 2012; 4:900–906.
- Rohl, Ca; Strauss, CEM.; Misura, KMS.; Baker, D. Protein structure prediction using Rosetta. *Methods in Enzymology*. 2004; 383:66–93. [PubMed: 15063647]
- Roy A, Sommer DJ, Schmidt R, Brown C, Gust D, Astashkine A, Ghirlanda G. A De Novo Designed 2[4Fe-4S] Ferredoxin Mimic Mediates Electron Transfer. *Journal of the American Chemical Society*. 2014; 136:17343–9. [PubMed: 25437708]

- Salgado EN, Faraone-Mennella J, Tezcan FA. Controlling protein-protein interactions through metal coordination: Assembly of a 16-helix bundle protein. *Journal of the American Chemical Society*. 2007; 129:13374–13375. [PubMed: 17929927]
- Schoene C, Fierer JO, Bennett SP, Howarth M. SpyTag/SpyCatcher Cyclization Confers Resilience to Boiling on a Mesophilic Enzyme. *Angewandte Chemie*. 2014; 53:6101–6104. [PubMed: 24817566]
- Speltz EB, Nathan A, Regan L. Design of Protein-Peptide Interaction Modules for Assembling Supramolecular Structures in Vivo and in Vitro. *ACS Chemical Biology*. 2015; 10(9):2108–2115. [PubMed: 26131725]
- Tegoni M, Yu F, Berseloni M, Penner-Hahn JE, Pecoraro VL. Designing functional type 2 copper centers within alpha-helical coiled coils that have nitrite reductase activity. *Proc Nat Acad Sci (USA)*. 2012; 109:21234–21239. [PubMed: 23236170]
- Thomson AJ, Gray HB. Bio-inorganic chemistry. *Current Opinion in Chemical Biology*. 1998; 2:155–158. [PubMed: 9667942]
- Waldron KJ, Robinson NJ. How do bacterial cells ensure that metalloproteins get the correct metal? *Nature Reviews Microbiology*. 2009; 7(1):25–35. [PubMed: 19079350]
- Woolfson, DN.; Bartlett, GJ.; Bruning, M.; Thomson, AR. *Current Opinion in Structural Biology*. Elsevier; New York: 2012. New currency for old rope: from coiled-coil assemblies to α -helical barrels; p. 432-441.
- Yu F, Cangelosi V, Zastrow ML, Tegoni M, Plegaria J, Tebo A, Mocny C, Ruckthorn L, Qayyum H, Pecoraro VL. Protein design: towards functional metalloenzymes. *Chemical Reviews*. 2014; 114:3495–3578. [PubMed: 24661096]
- Yu F, Penner-Hahn JE, Pecoraro VL. *de Novo* Designed Metallopeptides with Type 2 Copper Centers: Modulation of Reduction Potentials and Nitrite Reductase Activities. *Journal of the American Chemical Society*. 2013; 135:18096–18107. [PubMed: 24182361]
- Zastrow ML, Peacock AFA, Stuckey JA, Pecoraro VL. Hydrolytic Catalysis and Structural Stabilization in a Designed Metalloprotein. *Nature: Chemistry*. 2012; 4:118–123.
- Zhang L, Anderson JLR, Ahmed I, Norman JA, Negron C, Mutter AC, Dutton PL, Koder RL. Manipulating Cofactor Binding Thermodynamics in an Artificial Oxygen Transport Protein. *Biochemistry*. 2011; 50:10254–10261. [PubMed: 22004125]