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Editorial overview: From powerful tools to useful products: protein engineering after 35 years of directed evolution

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Tijana Z Grove studied physical chemistry at the University of Belgrade, Serbia and earned her Ph.D. in Chemistry from Iowa State University. She was an NIH postdoctoral fellow at Yale University with Lynne Regan, where she used protein engineering for assembly of stimuli-responsive biomaterials and scaffolds. While faculty in the Virginia Tech Chemistry Department, she established a nationally and internationally recognized research program at the intersection of chemistry, material science, and biotechnology. Currently, she is principal and founder of Zarkovic Grove Consulting, LLC specializing in protein engineering and nanoscience.

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Thomas J Magliery earned his A.B. at Kenyon College, and his Ph.D. with Peter G. Schultz at the University of California, Berkeley, where he used directed evolution to help lay the foundations for *in vivo* unnatural amino acid mutagenesis. He was an NIH postdoctoral fellow at Yale University with Lynne Regan, and is currently an associate professor of chemistry and biochemistry at The Ohio State University. His lab uses high-throughput and statistical approaches to understand the determinants of protein stability and other physical properties, and also engineers enzymes, antibodies, and signaling molecules for applications as

The year 2020 has been unusual on many accounts. We started planning this section in relative placidity of 2019, on the coattails of the 2018 Nobel prize in chemistry awarded to Frances H. Arnold, George P. Smith, and Sir Gregory P. Winter for their pioneering contributions to protein engineering through directed evolution of enzymes and, with phage display, of peptides and antibodies. Perhaps mostly, the award calls to mind the remarkable power of selective processes in the face of enormous sequence space, and in the face of an incomplete understanding of what properties sequence changes will elicit. While our foundational knowledge of the forces that stabilize proteins and the chemical mechanisms that underlie catalysis have expanded remarkably, even in combination with the most powerful computational tools of the day, engineering of structures, let alone functions, on demand, is still a challenge. The marginal stability of natural proteins and the unpredictable epistatic effects of mutations often at a distance are frequently confounding. Even remarkably accurate placement of active site residues does not guarantee high enzymatic activity.

Another lesson of the 2018 prize is the importance of tools, such as phage display to optimize binding, or microtiter plate assays to optimize function. Those tools today have been expanded through ingenuity, and also complemented with powerful computational approaches and with statistical approaches applied to natural evolutionary data and more recently to next-generation sequencing data from directed evolution itself. Although each of these tools has shortcomings in their current implementation, the combined results have been extraordinary. Engineered enzymes are now used to produce biofuels and pharmaceuticals. Engineered and even designed antibodies are becoming staples of therapies against autoimmune diseases and cancer, and may play a key interventional role in the COVID-19 epidemic ahead of other modalities. The scale of engineering has increased apace, from the design of small, soluble proteins and enzymes, to novel folds, to novel assemblies and even macroscale materials.

Therefore, with the Nobel committee's recognition of some of the great tools of protein engineering in mind, we chose to organize this section around engineering and design tools with an emphasis on what can be done right now, and what will soon be possible. Our goal was explicitly to show some of the frontiers and applications, even a bit away at times from the common definition of 'protein engineering and design.' The collection of manuscripts in this issue presents a broad cross section of computational, high-throughput, and statistical approaches to get at

diagnostic and therapeutic agents. He is interested in the commercialization of academic intellectual property, and he recently obtained an M.B.A. at OSU's Fisher College of Business.

functional aspects of protein design and engineering, with a special eye on the applications.

Sarel Fleishman *et al.* review the current possibilities afforded at the crossroads of statistical and atomistic design. Some of the greatest challenges to design have been revealed through lab evolution, such as the limited tolerance of scaffolds for mutations due to marginal stability, the complexity of chemical mechanisms, and the surprising importance of mutations far from the active site. These factors confound computational approaches and also limit the types of libraries that are suitable for directed evolution. But statistical analysis of phylogeny, such as through ancestral reconstruction, has afforded useful focusing of both approaches. The results at the intersection of statistical genomics, computer simulation, and experimental evolution are compelling.

Enzyme engineering has seen many remarkable successes, but nearly all rational and computational approaches need the power of selection to arrive at catalysts that even approach nature's power. The set of tools to generate new functions from existing enzymes continues to expand, and Wolf-Dieter Fessner *et al.* describe the expansion of microtiter methods into microfluidic sorting methods that can dramatically alter the throughput of experiments. The authors focus on the use of cleverly designed UV-VIS, fluorescence, and mass spectrometric readouts to elicit a broad range of useful transformations, but they also describe methods to cull libraries for stability using nanoDSF as a high-throughput screen. The combination of these factors gets us ever closer to on-demand catalysts of industrial utility.

One of the remarkable features elicited by evolution is regulation, but nearly 60 years after Monod–Wyman–Changeaux we still lack a sufficient understanding to tune allosteric regulation at will, let alone design it from whole cloth. Corey Wilson *et al.* review several studies that show the power of tinkering and selection to understand and rebuild some of the key features in the model LacI family. They also describe the tools and challenges involved in designing allosteric control into new scaffolds, with a special attention to the utility and limitations of statistical coupling methods. At the level of molecular machines, structure is function, and some of the greatest challenges arise from making mutations that affect higher order regulatory functions without perturbing basal functions, or even stability.

Somewhere between the re-design of small proteins and design of macromolecular assemblies lies the engineering and design of new folds and topologies. The number of known folds and topologies is now essentially static, but there is good evidence that the universe of possible stable folds is much larger. Roman Jerala *et al.* review key concepts in fold design as well as some of the challenges related to the secondary structural building blocks afforded by polypeptides. The authors compare the design challenges today for proteins to those that have been tackled for several decades in DNA nanotechnology as inspiration for new applications.

While nature uses proteins widely as materials, our engineering and design of macro-level properties necessary for new material applications has lagged molecular-level properties considerably. Design at the intermolecular level has proven to have its own challenges, but the engineering of assemblies underlies many potential useful applications of proteins as materials. Sagar Khare *et al.* review the bottom-up design of such higher-order assemblies, with a special emphasis on the topologies that have been explored to date, such as filaments, two and three dimensional assemblies, and fractal

superstructures. The authors drill down into the design of stimulus responsiveness and the opportunity afforded by DNA hybrid materials. One area with exciting results already is in the design of vaccine materials, but the potential utility in areas like nanoreactors and drug delivery is on the horizon.

Aitziber Cortajerena *et al.* take a broad view of the design successes and challenges in bionanomaterials. The authors especially look at the intersection of proteins and protein assemblies with non-protein materials, such as nanotubes and metal-organic frameworks, and they consider as an example how these can be used together for novel catalytic applications. Many of the challenges remain in the synthesis of these materials and in the fundamental protein design of the constituent units.

Jin Kim Montclare *et al.* take a deeper dive into a specific challenge in protein materials and review the design and engineering of proteins and peptides as hydrogels. The review focuses on the use of simulations to imbue 'smart' properties such as environmental responsiveness, but it also gives some perspective on the key problems such hydrogels may one day solve in areas like adhesion and drug delivery. Again, many of the challenges are underpinned by the shortcomings of fundamental molecular design approaches.

Our collection was created between the 2018 chemistry Nobel and the 2020 coronavirus pandemic, which may in retrospect look like an inflection point in protein engineering and design. The work of the last nearly 40 years starting with directed evolution and site-directed mutagenesis has given us a powerful and expanding toolbox, but what those tools have wrought have only just begun to make deeper impacts in healthcare, industrial processes, and smart materials to change the world around us with remarkable precision.