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Editorial

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Special Issue on Engineered DNA-Binding Proteins

Technologies for engineering new functions into proteins are advancing biological research, biotechnology, and medicine at an astounding rate. Building on fundamental research of natural protein structure and function, scientists are identifying new protein domains with previously undescribed properties and engineering new proteins with expanded functionalities. For example, recent discoveries of the underlying principles of protein−DNA interactions in various species are guiding the development of methods for engineering synthetic proteins that can be targeted to any DNA sequence. The first example of programmable DNA-binding proteins, synthetic zinc finger proteins (ZFPs), was first described two decades ago. Since then, the discovery of the DNA recognition code of Transcription Activator-Like Effectors (TALEs) and the repurposing of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system for genome engineering has increased the ease and speed with which new DNA-binding proteins with customized target site specificity can be created. ZFPs, TALEs, and CRISPR/Cas9 DNA-binding proteins can now serve as scaffolds for building enzymes that modify DNA sequence, transcriptional regulation, or epigenetic status at any site in the genome.

Gene circuits are a foundation of the synthetic biology field. The original gene circuits were built from naturally occurring transcription factors that respond to chemical or other environmental stimuli. In light of the movement toward increased complexity in synthetic biology, it is unsurprising that scientists have quickly adopted the ZFP, TALE, and CRISPR/Cas9 technologies to engineer new transcription factors and expand the versatility of the synthetic biology toolbox. However, to generate systems with dynamic regulation, it is still necessary to incorporate control functions into these proteins. Therefore, the ongoing efforts to incorporate protein domains that respond to extracellular stimuli, such as small molecules or light, will be critical to realizing the potential of engineered transcription factors for enabling more complex gene circuits. Alternatively, transcription factors may be engineered to respond to the intracellular conditions, such that transcription factor activity fluctuates dynamically with the system and also serves as a readout for the intracellular environment. This special issue of *ACS Synthetic Biology* highlights a sample of recent advances in each of these areas for engineering DNA-binding proteins, with a particular focus on developing transcription factor platforms for gene regulation.

As an introduction to this special issue, Moore, Chandrahas, and Bleris review the recent development of TALE engineering and its applications in synthetic biology. The review comprehensively summarizes the fundamental biochemistry of natural and engineered TALE proteins, available resources for engineered TALE proteins, and how the TALE technology has been applied thus far. Although focused on TALEs, this story is representative of other classes of engineered transcription factors, including ZFPs and CRISPR/Cas9, that have been repurposed from natural proteins. The authors also note that despite the remarkable level of progress, additional work is necessary to improve these nascent technologies and fully characterize their potential and their limitations. For example, studies are needed to develop effective means of delivering large TALE and Cas9 proteins, determine their immunogenicity, and further characterize DNA-binding specificity in the context of large complex genomes. Continuing to develop these tools for modulating gene expression is necessary to meet the needs of the next era of high-throughput science.

With the advent of user-friendly cloning platforms for building ZFPs, TALEs, and CRISPR/Cas9 proteins combined with the plummeting cost of DNA synthesis, genomic exploration has been simplified and become accessible to the general biomedical research community. For protein engineering experts, designing and constructing transcription factors with these platforms is a routine exercise. However, for scientists new to this field, there may be a steep learning curve. To address the need for reliable and automated grammar for transcription factor design, Purcell et al. have developed a computer-aided design software, GenoCad, to assist in this process. Construction of synthetic transcription factors relies on the modular nature of each of the components. Using experimental evidence as a guide, GenoCad determines the proper grammar for assembling the modular domains of ZFP-, TALE-, or CRISPR/Cas9-based transcription factors with diverse functionalities.

For many applications, including the construction of gene circuits, it is necessary to have precise control of gene expression. Two of the articles featured in this special issue have developed methods that enable inducible gene expression with engineered transcription factors. Mercer *et al.* developed a new class of inducible TALE transcription factors that respond to synthetic hormones. By fusing a customizable TALE transcription factor to the estrogen receptor homodimerization domain, progesterone homodimerizing domain, or retinoid X receptor-α/ecdysone receptor heterodimerization domain, they created hormone-responsive TALE transcription factors capable of regulating endogenous gene expression. This system can be useful for controlling both the timing and magnitude of gene regulation with the TALE transcription factor platform.

As an alternative to chemical control of protein activity, Engelke *et al.* developed a system for light-inducible control of gene expression. By genetically encoding a photocaged lysine into the nuclear localization signal (NLS) of a protein, they control whether the protein is located in the nucleus or cytoplasm by release of the photocage with UV light. For example, appending this photocaged NLS to the natural transcription factor FOXO3 enabled light-inducible translocation of FOXO3 to the nucleus and activation of FOXO3-driven transcription. The modularity of the system is demonstrated by appending the light-inducible NLS to the

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TEV protease. Because the system is modular, it is expected that the light-inducible NLS could also be used in the context of other engineered transcription factors, including ZFPs, TALEs, and CRISPR/Cas9, and this could be an exciting area of future research.

Synthetic proteins capable of sensing the intracellular environment are valuable for studying and regulating natural cellular processes. Suzuki et al. developed a calcium-dependent transcription factor (CaTF) that senses intracellular calcium levels and generates a transcriptional readout proportional to the intracellular levels of calcium. This was accomplished by engineering a calcium-sensitive protease recognition sequence linked to a nuclear export signal (NES) into the reverse tetracycline repressor (rTetR). Following increases in intracellular calcium, the NES is removed allowing the transcription factor to enter the nucleus and activate target gene expression. Because the NES and protease recognition sequence are modular, this system should also be portable to other engineered transcription factors, including those based on ZFPs, TALEs, and CRISPR/Cas9. This system is useful for monitoring intracellular events in real time at the single cell level. It also presents a way to interface a synthetic gene circuit with an endogenous signaling network. Such proteins are helping to provide answers to fundamental biological questions.

Taking inspiration from nature, the protein engineering field is collectively expanding the number of functionalities available in the synthetic biology toolbox. Such tools are enabling the precise study of fundamental aspects of cellular behavior, the engineering of cellular systems for biomanufacturing and bioremediation, and the development of a new class of gene therapies that manipulate the expression of endogenous genes. The applications of these gene regulation technologies include controlling cell decision making, reprogramming cell lineage commitment, monitoring cellular states, regulating synthetic gene circuits, and stimulating expression of therapeutic factors. In fact, engineered transcription factors have entered clinical trials for the treatment of human diseases and others are in preclinical development.

This special issue includes a review of a new transformative class of engineered transcription factors, software for generalizing the design of engineered transcription factors, and three new platforms for engineering peptide modules into transcription factors that enable dynamic control in response to extracellular or intracellular stimuli. Collectively, these tools have the potential to enable diverse new areas of synthetic biology. The challenge moving forward is to realize this potential by incorporating the tools into tangible benefits for science, technology, and medicine.

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