Chemistry & Biology, Vol. 11, 1475–1482, November, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.11.005

Previews

Engineering Switches, Genetically

Ostermeier, Guntas, and Mitchel describe a new approach to design enzymes that are allosterically regulated by an unrelated ligand [1]. The resulting protein, constructed by nonhomologous recombination and genetic screens, displays switch-like behavior.

Naturally occurring proteins currently represent the best examples of soft (molecularly compliant) nanoscale devices. These devices can sometimes behave as switches, modulating their principle catalytic or binding activity based upon the action of a second binding partner. The advent of large-scale sequencing efforts has dramatically increased the number of known proteins, providing a potentially vast parts store that can be scavenged to create fundamentally new activities. While there is great interest among a new breed of molecular hot rodders to raid this databank, the challenge ahead lies in putting parts together effectively, because coupling the activity of two proteins is somewhat more difficult than putting a new carburetor on your car.

In this issue of *Chemistry & Biology*, Guntas et al., [1] provide an elegant example of how circular permutation, nonhomologous recombination, and genetic screens can be combined to purposefully create a novel type of protein-based switch. Conceptually, the idea is straightforward, take an enzyme (here β-lactamase) and make its activity dependent upon the binding of an unrelated ligand such as maltose. To do this, the authors chose to create a fusion protein that had both β-lactamase and maltose binding protein (MBP) domains in the same chain. Maltose binding protein seemed a particularly attractive regulatory domain because it is known to undergo a significant conformational change upon binding the ligand [2].

The design challenge they faced was determining how to put these two proteins together in a way that couples their activities. This is a problem that has been approached by other labs in making sensors or altering modular signal transduction pathways. For example, various FRET-based sensors have been designed by tethering the fluorescent dye pair CFP and YFP using a domain that undergoes a conformational change upon action by a cellular protein [3–5]. Similarly, Lim and coworkers have shown that novel scaffold-based signaling cascades can be engineered by assembly of fusion proteins from a series of recognition domains that undergo conformational changes upon seeing some signal [6]. In these cases, tethering the pieces together is sufficient to create the intended switch.

Ostermeier and coworkers faced a more challenging problem because their fusion protein switch needed to be engineered such that ligand-dependent conformational changes (pulling, pushing, moving about) in MBP altered the activity of the enzymatic domain. In their previous work, the Ostermeier group created a series of new proteins by nonhomologous recombination of the β-lactamase chain into MBP [7]. In principle, this library contains a series of new proteins that have β-lactamase inserted into MBP at every position in the MBP chain. However, because the nonhomologous recombination protocol relies on DNAase treatment, MBP sequences near the insert site typically contain either deletions or insertions. This rough "cut and paste" library makes it necessary to perform genetic screens that identify fusion proteins that bear both activities. In their first attempt, this approach resulted in two β-lactamases that vary k_{cat}/K_m by 1.7- to 1.8-fold in the presence of an MBP ligand.

To do better, the authors had to realize that there were probably better sites within β-lactamase than the N and C terminus on which to pull and tug and thereby change its activity. This is where circular permutation comes into play. Circular permutation is the process of creating a series of linear molecules that would result if the chain were cyclized end to end and then reopened somewhere else in the chain. This idea can be applied generally to biomolecules be they DNA [8], RNA [9], or proteins [10]. In many proteins (such as β-lactamase), designing a series of circular permutations is straightforward because the N and C terminus are close in space and can be joined by a short linker peptide.

The Ostermeier group created a library of β-lactamase circular permutations by joining the N and C terminus with a flexible peptide linker and then opening the chain randomly at internal positions (see Figure 1 in the accompanying paper [1]). They then inserted this library into MBP via nonhomologous recombination as before and screened the resulting variants. Statistically, they determined that the resulting library started with more than 27,000 MBP-β-lactamase fusion proteins. After screening, they performed detailed kinetic analysis of the best variant (termed RG13), demonstrating that this protein has ~25-fold swing in activity based on the presence or absence of maltose.

What lessons can we take from this study? First, that allosterically regulated enzymes (switches) can be created via recombination and genetic means. In fundamentally soft materials like proteins, context and conformation matter and are usually coupled together in a complicated way with the primary sequence [11]. Engineering through genetics provides a powerful operational approach that enables solving a design problem without explicitly understanding all the underlying variables.

This operational approach may seem less satisfying than computational design methods set out to accomplish the same phenomenon [12, 13]. However, genetic and computational design approaches are more similar than they are different. In silico, many different designs are tested and sieved by combining energetic calculations and search-limiting algorithms in an effort to identify the most "fit." In this view, computational protein designers may be seen as electronic geneticists.

The newly designed molecule reported here represents an important proof-of-principle and is potentially useful both for its intended purpose (i.e., as a switch) and also to provide a methodology for linking the function of

two unrelated proteins. We now have a huge parts store in our backyard waiting for creative scientists to assemble the pieces and realize new devices that never existed in nature. We need design protocols that are appropriate for the soft, nanoscale systems that are folded proteins. As we begin to design these new systems, it is becoming increasingly clear that genetic approaches to engineering represent the future of soft, nanoscale protein design.

Richard W. Roberts
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125

Selected Reading

1. Guntas, G., Mitchell, S.F., and Ostermeier, M. (2004). Chem. Biol., this issue, 1483–1487.
2. Sharff, A.J., Rodseth, L.E., Spurlino, J.C., and Quiocho, F.A. (1992). Biochemistry *31*, 10657–10663.
3. Miyawaki, A., Llopis, J., Heim, R., McCaffery, J.M., Adams, J.A., Ikura, M., and Tsien, R.Y. (1997). Nature *388*, 882–887.
4. Ting, A.Y., Kain, K.H., Klemke, R.L., and Tsien, R.Y. (2001). Proc. Natl. Acad. Sci. USA *98*, 15003–15008.
5. Zhang, J., Ma, Y., Taylor, S.S., and Tsien, R.Y. (2001). Proc. Natl. Acad. Sci. USA *98*, 14997–15002.
6. Dueber, J.E., Yeh, B.J., Chak, K., and Lim, W.A. (2003). Science *301*, 1904–1908.
7. Guntas, G., and Ostermeier, M. (2004). J. Mol. Biol. *336*, 263–273.
8. Wu, H.-M., and Crothers, D.M. (1984). Nature *308*, 509–513.
9. Pan, T., Gutell, R.R., and Uhlenbeck, O.C. (1991). Science *254*, 1361–1364.
10. Baird, G.S., Zacharias, D.A., and Tsien, R.Y. (1999). Proc. Natl. Acad. Sci. USA *96*, 11241–11246.
11. Xia, T., Frankel, A., Takahashi, T.T., Ren, J., and Roberts, R.W. (2003). Nat. Struct. Biol. *10*, 812–819.
12. Dahiyat, B.I., and Mayo, S.L. (1997). Science *278*, 82–87.
13. Dwyer, M.A., Looger, L.L., and Hellinga, H.W. (2004). Science *304*, 1967–1971.

Chemistry & Biology, Vol. 11, November, 2004, DOI 10.1016/j.chembiol.2004.11.003

Targeting α-Synuclein in Parkinson's Disease

α-Synuclein aggregation into fibrils is associated with the pathogenesis of Parkinson's disease (PD). Li et al. provide strong evidence that rifampicin interacts with α-synuclein and inhibits its fibrillization [1]. Rifampicin could be a promising candidate for therapeutic application for PD.

Sometimes an observation may produce a hypothetical link between two apparently unrelated events. For example, the observation by McGeer et al. and Namba et al. that anti-leprosy-treated elderly patients have less dementia and senile plaques in their brains than nontreated patients has created a link between the antileprosy drug rifampicin and neurodegenerative diseases [2, 3]. How this hypothesis has been pursued and what might be the potential consequences for PD and other cell-degenerative diseases will be discussed here.

In vivo protein aggregation into fibrillar deposits is strongly associated with cell degeneration and the pathogenesis of a number of progressive cell-degenerative diseases. These include fatal neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), the transmissable spongiform encephalopathies (TSEs or prion diseases), the pancreatic β cell degenerative disease type II diabetes (T2D), and several other localized or systemic amyloidoses [4]. In all these conditions, a disease-specific polypeptide or protein aggregates into fibrillar deposits. Recent evidence suggests that common molecular events may underly the pathogenesis of the different "protein aggregation" diseases.

Parkinson's disease is the most common human neurodegenerative movement disorder and affects ~1% of the elderly population. Although symptomatic treatment strategies are available, PD has remained a noncurable disease [5]. Primary clinical symptoms of PD are bradykinesia, resting tremor, muscular rigidity, and difficulty with balance. PD is neuropathologically characterized by a marked and progressive degeneration of dopaminergic neurons and by the presence of fibrillar cytoplasmatic inclusions (Lewy bodies [LBs]) and dystrophic neurites (Lewy neurites [LNs]) in the *substantia nigra* region of the brain [6]. Although the loss of dopamine neurons is certainly related to the major clinical symptoms of PD, the causes and the pathogenesis of this multifactorial disease as well as that of related "synucleinopathies" are still largely unknown.

The major components of both LBs and LNs are fibrillar aggregates of α-synuclein [6, 7]. α-Synuclein is a widely expressed, neuronal presynaptic protein that appears to play a role in membrane-associated processes and synaptic plasticity and has been linked to learning and development processes [6]. While the mechanism(s) of formation of LBs and LNs and their association with PD is (are) yet not understood, several lines of evidence suggest that α-synuclein fibrillization is associated with PD [6, 8]. Similarily to other protein aggregation diseases, both neurotoxic and neuroprotective roles have been attributed to the endproducts of α-synuclein aggregation, the fibrillar α-synuclein deposits [6, 8]. α-Synuclein fibril formation in vitro proceeds via the conversion of the 140 amino acid residue protein, that appears to be "natively unfolded," into ordered, β sheet-rich oligomers also termed "protofibrils" [8]. α-Synuclein protofibrils or alternatively folded/assembled oligomers