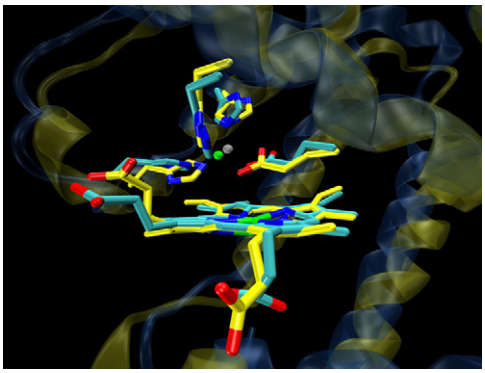
Synthetic Biology Select

Cell

In this issue, *Leading Edge* highlights synthetic biology, a field that is deepening our understanding of the design principles of life and increasing our mastery of biological systems for practical applications in biotechnology and medicine. Reports from the recent literature, discussed in this Synthetic Biology Select, include the rational design of a functional enzyme, an approach for creating more stable proteins, and systems-level analyses that reveal unexpected sophistication in the regulatory networks of microorganisms.



Overlay of the crystal structure of the rationally designed protein (cyan) with the structure predicted by computational modeling (yellow). Image courtesy of Y. Lu.

A How to Guide for Building an Enzyme

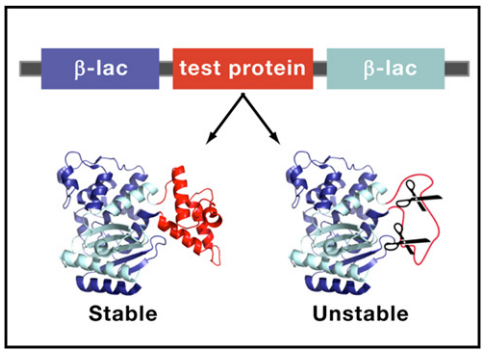
In a forceful demonstration of rational protein design, Yeung et al. (2009) report the transformation of sperm whale myoglobin into a functional nitric oxide reductase. Adding to the difficulty of this feat, crystal structures for native nitric oxide reductases are currently lacking. Hence, the proposed mechanism of catalysis had to be inferred from homology to subunits of heme copper oxidases (such as cytochrome *c* oxidase). The authors' efforts ultimately entailed the introduction of three histidines and one glutamine to create a non-heme binding site for iron, an essential cofactor for reductase activity. This synthetic site is then shown to confer iron binding, and remarkably, the engineered protein acquires the ability to reduce nitric oxide (NO) to nitrous oxide (N_2O). When the designed protein is crystallized, the structure of the rationally designed iron binding site closely matches the structure predicted by computational modeling. These findings show how synthetic enzymes can provide insight into the mechanism of action of enzymes and illustrate the power of in silico modeling for the design of synthetic proteins.

N. Yeung et al. (2009). Nature. Published online November 25, 2009. 10.1038/nature08620.

Selecting for Stability

Most amino acid substitutions decrease a protein's stability. Given this tendency, what tools do protein engineers have at their disposal when enhanced stability is their goal? Recent work by Foit et al. (2009) provides a clever means to select for improved stability through an in vivo screen in the bacterium *Escherichia coli*. To do this, they insert a test protein between the N- and C-terminal halves of TEM1-β-lactamase, an antibiotic resistance protein. These tripartite fusion proteins are then expressed in *E. coli* and grown in the presence of the antibiotic penicillin V, which TEM1-β-lactamase counteracts. Using this system, the authors show for a collection of test proteins that antibiotic resistance correlates with their stability. Taking this a step further into the realm of protein engineering, the authors then test a library of mutants for the protein Im7 (immunity protein 7) and succeed in identifying mutations that improve Im7's stability. However, these amino acid substitutions come with an interesting side effect, in that many disrupt the interaction of Im7 with the toxin colicin, its native interacting partner. Thus, this approach offers a straightforward means to engineer a protein with a desired stability and also reveals evidence of evolutionary tradeoffs between function and stability. For in-depth discussion of the tradeoffs involved in designing a different type of biological system—signal transduction pathways—see the Review in this issue by Christina Kiel, Eva Yus, and Luis Serrano (page 33).

L. Foit et al. (2009). Mol. Cell ***35****, 861–871.*



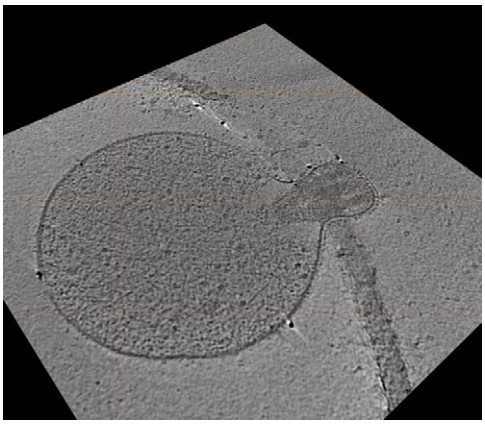
Tripartite fusion proteins in which a test protein is inserted into the antibiotic resistance gene *TEM1-β-lactamase* (*β-lac*) can be used to select for amino acid substitutions that improve protein stability. Figure courtesy of J. Bardwell.

How Yeast Know When It's Time to Grow

It makes intuitive sense that the availability of energy, such as glucose, would be a primary determinant of a cell's growth rate. However, according to a recent examination by Youk and van Oudenaarden (2009), the growth landscape for yeast is substantially more complex than anticipated. In yeast, glucose uptake is modulated by glucose sensors, which promote the expression of six primary hexose transporters (HXT). Youk and van Oudenaarden decouple the

sensing of extracellular glucose from its transport by deleting the endogenous HXT genes. They then replace the genes one at a time and put them under the control of a promoter that is induced by doxycycline but not glucose. Surprisingly, this decoupling reveals that an increase in the concentration of glucose does not invariably stimulate the rate of yeast growth. In some cases, increasing the glucose concentration actually decreases the growth rate. Moreover, the HXT replacement strains all behave differently with respect to the effect of glucose concentration on growth rate. Yet, this is not to say that growth rate is unpredictable. By plotting all of the data together, the authors show that growth rate is determined by two independent variables, the rate of glucose uptake and the extracellular concentration of glucose, and that these variables can be fit into a relatively simple equation that defines a growth landscape. This analysis reveals the functional importance of the interaction between the modules that control glucose import and sensing. By uncovering the underlying design principles controlling growth rate in yeast, this study may inform ongoing efforts to engineer biological systems that respond dynamically to changing environmental conditions. The theme of engineering dynamic control into heterologous pathways is explored in an Essay in this issue by William Holtz and Jay Keasling (page 19).

*H. Youk and A. van Oudenaarden (2009). Nature **462**, 875–879.*



A cryo-electron tomogram of *Mycoplasma pneumoniae*. Image courtesy of A.-C. Gavin.

Dissecting and Reconstructing *Mycoplasma*

Three recent studies (Kühner et al., 2009; Yus et al., 2009; Güell et al., 2009) suggest that the gene, protein, and metabolic networks of *Mycoplasma pneumoniae*, a bacterium with one of the smallest known genomes, display regulatory control previously thought to be limited to eukaryotes. In the first paper, Kühner et al. use mass spectrometry as part of a genome-wide screen to define the protein complexes of *M. pneumoniae*. This effort provides evidence that the organism has ~200 protein complexes that form from among the organism's 689 open reading frames. Building on existing structural data, the authors then map some of the most prevalent proteins and complexes onto cellular electron tomograms to produce a dramatic representation of cellular organization. Yus et al. undertake the reconstruction of the metabolic network of *M. pneumoniae* and use it to design a minimal culture medium containing only 19 ingredients to support growth. Their study further shows that *M. pneumoniae* has a greater capacity to adjust gene expression in response to specific metabolic perturbations than might be anticipated given that it is adapted to a specialized environment and has very few transcription factors compared to more complex bacteria. The transcriptional dynamics of *M. pneumoniae* are examined in-depth by Güell et al. Their study reveals the existence of 341 operons, many of which display production of alternate transcripts under different growth conditions. In addition, antisense transcripts are found in unanticipated large numbers. The sophisticated way in which gene expression is regulated in *M. pneumoniae* indicates the presence of underappreciated mechanisms that control gene expression, which when more completely understood might be used in the engineering of microbial gene networks. (The use of transcriptional feedback mechanisms in the design of cellular memory networks is discussed in this issue in an Essay on page 13 by Devin Burrill and Pamela Silver.) Cumulatively, these ambitious studies add new layers of insight into biological design and will undoubtedly provide food for thought for those undertaking the construction of synthetic biological systems or contemplating the challenges of creating synthetic organisms.

*S. Kühner et al. (2009). Science **326**, 1235–1240.*
*E. Yus et al. (2009). Science **326**, 1263–1268.*
*M. Güell et al. (2009). Science **326**, 1268–1271.*

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