Concentrating (on) Native Proteins to Control Cell Fate

Casim A. Sarkar

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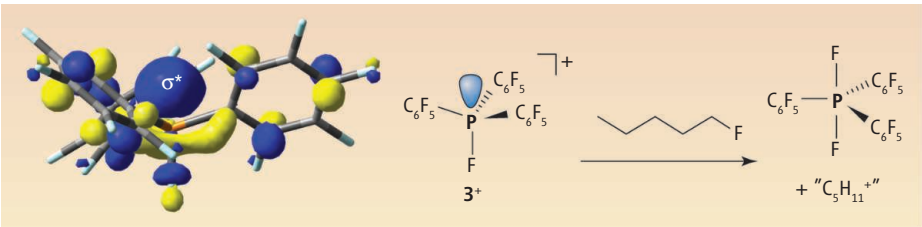

Lewis acidity at work. The fluorinated phosphonium cation 3^+ reported by Caputo *et al*. is a powerful Lewis acid that can cleave the C-F bonds of fluoroalkanes (**right**). This high Lewis acidity originates from the presence of a vacant coordination site defined by an empty σ* orbital whose main lobe is centered on the phosphorus atom (**left**).

which readily interact with 3^+ to form the difluorophosphorane $(C_6F_5)_3PF_2$. The thermodynamic drive for this reaction is so strong that 3^+ can abstract a fluoride anion from comparatively inert fluorocarbons (see the second figure), a class of molecules that, when volatile, contribute to the greenhouse effect. This fluoride abstraction reaction, which involves activation of a C-F bond, can be carried out in the presence of a hydride donor such as a trialkylsilane. Under these conditions, fluorocarbons are catalytically converted into alkanes through a process known as hydrodefluorination.

Cation 3^+ is not as effective as the silylium-based hydrodefluorination catalysts pioneered by Douvris and Ozerov (*12*), but it nonetheless has a number of unique features that could make this class of Lewis acids attractive as reagents and catalysts. One key point is that the phosphorus center of 3^+ has a full valence of eight electrons. As a result, salts of 3^+ are more stable and easier to handle than their silylium counterparts. The compound also contains ^{31}P and ^{19}F nuclei, making its chemistry easy to follow with nuclear magnetic resonance.

The results described by Caputo *et al*. go beyond the simple discovery of a new Lewis acid. They set the stage for broader developments in the chemistry of cationic Lewis acids containing phosphorus and other group 15 elements. For example, an antimony compound was recently used for the fluorescence sensing of fluoride in water at part-per-million concentrations (*13*). Such compounds may also find applications in asymmetric synthesis. The tetrahedral geometry of these cations should lead to increased steric interactions with incoming substrates—a phenomenon that could be exploited for asymmetric induction if chirality is present at the group 15 element.

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CELL SIGNALING

Concentrating (on) Native Proteins to Control Cell Fate

Synthetic genetic controllers modulate endogenous signaling networks to route cells toward desired behaviors.

Casim A. Sarkar

Synthetic biology has ambitious goals—to engineer and repurpose cells for applications ranging from medicine to energy (*1*, *2*). Such achievements require robust designs for programming cell behavior. Signaling pathways within cells integrate information from the environment to drive specific gene expression programs, so from an engineering perspective, these pathways represent prime interventional targets for harnessing control of cellular decision-making (*3*). The output of a signaling pathway ultimately depends on the dynamics of its constituents, which, in turn, are determined by their concentrations and interactions. Given that these properties are hard-wired into the genome of the organism, how might cell fate be artificially regulated without manipulating the host's DNA? On page 1358 of this issue, Galloway *et al*. (*4*) demonstrate that this can be accomplished by introducing synthetic genetic controllers into the host to modulate the activity of a native signaling pathway. Cell fate "rerouting" in yeast was accomplished by using these genetic controllers to conditionally increase the concentrations of key endogenous proteins in a mitogen-activated protein kinase (MAPK) pathway, thereby reshaping the signaling dynamics and cellular response without introducing new proteins into the network.

MAPK pathways are attractive targets for cell engineering because they are ubiquitously expressed in eukaryotic organisms and are vital cogs in the decision-making machinery, regulating cellular processes such as differentiation, proliferation, motility, and death (*5*). The concentrations of the constituent proteins in the MAPK cascade can vary substantially across organisms (*6*), which in turn can alter the signal-processing characteristics of the network (*7*).

Galloway *et al*. focused on a yeast mating behavior that is governed by a pheromone called α-factor. This stimulus activates a MAPK pathway that stops cell division and promotes a mating response. To identify concentration-dependent control points in this pathway, the authors increased the expression of individual signaling proteins in the network. In the absence of pheromone, they identified Ste4 as a protein whose overexpression results in pathway activation. In the pres-

Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA. E-mail: csarkar@umn.edu

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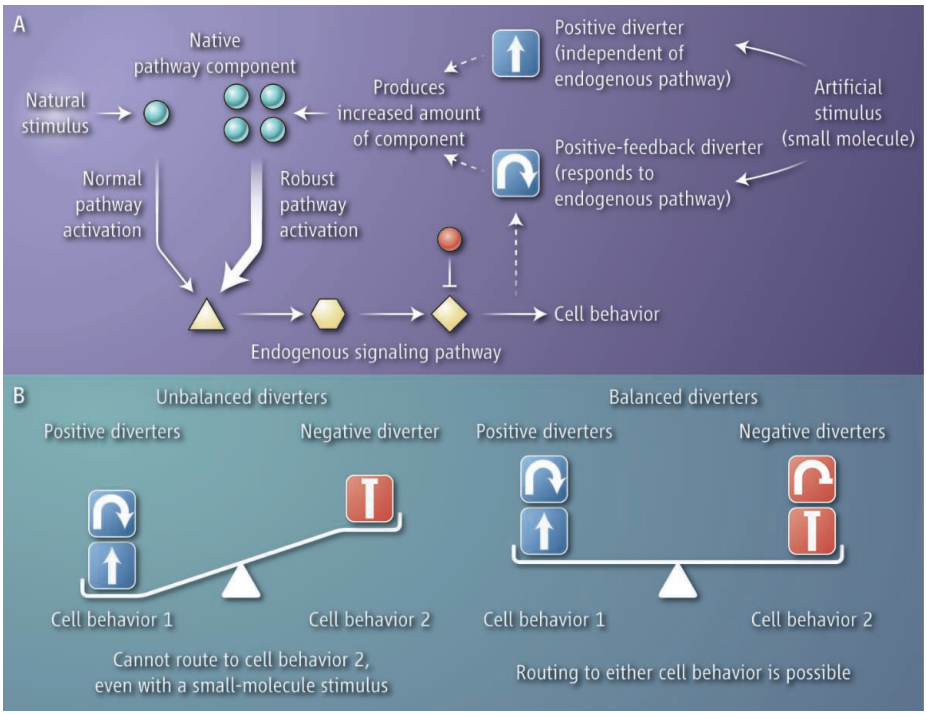

Controlling cell behavior with molecular network diverters. (**A**) A cell signaling pathway that is normally activated by a natural stimulus can be artificially controlled by increasing the expression of a key native pathway component. This can be accomplished by introducing "diverters" (synthetic genetic elements) into the cell that respond to an artificial stimulus. Positive diverters can increase pathway activity in the absence of a natural stimulus by increasing the concentration of a pathway activator (blue circle); negative diverters (not depicted) can decrease pathway activity in the presence of a natural stimulus by increasing the concentration of a pathway repressor (red circle). (**B**) A signaling network may be modulated by several diverters to enable conditional routing of a cell to multiple different fates, but this requires an appropriate balance of positive diverters (blue) and negative diverters (red) so that each small-molecule inducer has enough sway to redirect the cell toward the corresponding fate.

ence of α-factor, the authors identified Msg5 as a pathway constituent whose overexpression can override this pheromone and attenuate pathway activity.

To understand the effect of protein overexpression on signaling dynamics, it is also important to consider how the concentration is increased. One way to boost expression of a gene is to use a constitutive or pathway-independent genetic element called a promoter. With such a promoter, the concentration of the gene's corresponding protein can be increased without changing the topology of the signaling network. In synthetic MAPK cascades with the same network topology, varying the concentrations of pathway members can enable predictable tuning of the output response (*7*). Alternatively, gene expression can be enhanced using a pathway-responsive promoter. In this case, not only is the protein concentration increased, but the network structure is also changed by introducing a feedback loop. Endogenous MAPK pathways have been rewired using feedback-driven artificial protein interactions to rationally alter signaling dynamics (*8*).

To modulate protein concentrations, Galloway *et al*. constructed a series of molecular network "diverters." Each diverter consisted of three genetic elements: a pathway-independent or pathway-responsive promoter to control gene expression; a concentration-dependent pathway regulator (i.e., the gene encoding either the pathway activator Ste4 or the pathway repressor Msg5); and one or more transducers that enable small-molecule control of protein synthesis (*9*). These diverters were assembled in plasmids that were introduced into the yeast but remained separate from the host's native genetic material. The modular design of the diverters facilitates piecewise engineering of the component parts and their overall function enables conditional increase in expression of a key protein of interest, with or without network rewiring (see the figure).

Although Ste4 overexpression increases pathway activity, a diverter that drives constitutive expression of this protein with a small-molecule inducer only elicited weak routing to the mating fate (in the absence of the natural pheromone stimulus). However, by changing to a diverter in which expression of Ste4 was under control of a pathway-responsive promoter, a positive-feedback loop was created that robustly amplified the effects of Ste4 on the pathway. By contrast, for routing to the nonmating fate in the presence of both pheromone and a different small-molecule inducer, feedback-driven expression of Msg5 could not elicit the desired outcome, whereas constitutive expression of this protein could do so. Msg5 is a negative regulator of the MAPK pathway, so unlike Ste4, its effect is attenuated when its expression is driven by a pathway-responsive promoter.

What if a cell was engineered to harbor both of these diverters—that is, one for pathway-responsive expression of Ste4 and one for constitutive expression of Msg5? Could regulation of multiple cell behaviors be achieved? Galloway *et al*. tried this, but "background cross talk" got in the way—basal activity from the uninduced Msg5 diverter antagonized the ability of the Ste4 diverter to route the cell to the mating fate. Through computational modeling, the authors identified additional genetic elements that could enhance the function of the two original diverters while buffering against undesirable cross talk. Importantly, when combining diverters that act on the same signaling network to route cells toward different behavioral outcomes, their strengths must be appropriately balanced so that the small-molecule inducers can elicit the desired effects. For example, in the study by Galloway *et al*., a system with both constitutive and positive-feedback diverters for Ste4, but only the constitutive diverter for Msg5, was unbalanced. The mating phenotype could be induced with the appropriate small-molecule stimulus (in the absence of the natural pheromone stimulus), but the nonmating fate could not be induced in the presence of pheromone and the other small molecule. The final system design consisted of two genetic controllers for the expression of Ste4 and two for the expression of Msg5 (one constitutive and one pathway-responsive for each); this balanced structure enabled inducible cell fate routing in multiple directions.

To complement the promising strategy developed by Galloway *et al*., methods could be developed to further streamline its implementation. Identification of key control nodes in a signaling pathway of interest may be guided by computational modeling and sensitivity analyses (*10*). This can be useful when working with a network containing many proteins or a network whose activity may be largely insensitive to overexpression of individual proteins but sensitive to simultaneous perturbation of two or more proteins. Exper-

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imentally, directed evolution is a powerful optimization tool (*11*) that may be useful in refining promoter and transducer sequences to achieve the desired balance among diverters.

Galloway *et al*. demonstrate that natural cell signaling pathways can be coaxed to elicit desired cell fate outcomes using molecular network diverters. The approach does not require new protein components to achieve this control, but instead relies on conditionally augmenting the concentrations of existing signaling proteins in the cell. Furthermore, this cellular reprogramming strategy can be implemented without manipulating the host's genome; rather, the native genetic material is supplemented with plasmids harboring the diverters. For synthetic biologists, these features should make the approach attractive for implementation in a variety of organisms, including higher eukaryotes.

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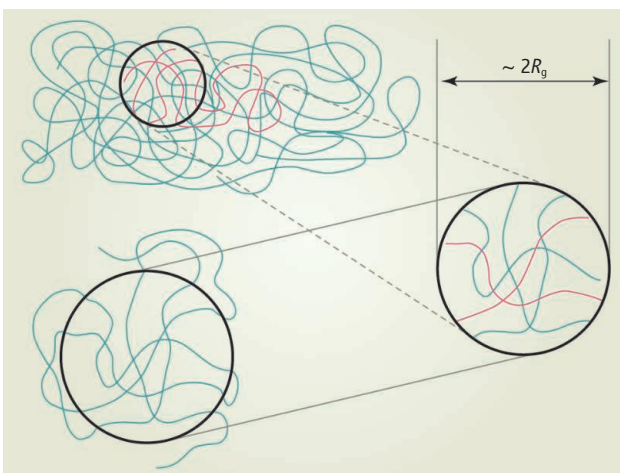
MATERIALS SCIENCE

Polymers Find Plenty of Wiggle Room at the Bottom

The glassy dynamics of an isolated polymer chain is found to be identical to that of a bulk material.

Thomas P. Russell

In his 1959 lecture "There's Plenty of Room at the Bottom," Richard Feynman discussed the prospects of developing future nanoscale technologies (*1*). There, he stated that the homogeneous properties of amorphous plastics and glasses would render them the materials of choice for such applications. Today, polymeric materials—particularly glassy, noncrystalline polymers—do play a key role in the manufacturing sector, but these tend to be bulk materials. Realizing Feynman's vision of nanomachines based on polymers would require an understanding of the properties of polymeric materials as they are shrunk to comprise a few or even single polymer chains. But the characterization of such nanoscopic amounts of material, and how they may differ (or not) from the bulk, remains a challenging and controversial area. On page 1371 of this issue, Tress *et al*. (*2*) have developed a technique using a nanoelectrode and broadband dielectric spectroscopy (BDS) to characterize tiny volumes of polymers and isolated polymer chains. Their results lead to the remarkable conclusion that the glassy dynamics of an isolated polymer chain is identical to that seen in the bulk.



A close up view. The sphere shows a volume taken from bulk polymer defined by the dimension of a single chain. The ends of the segments can then be connected to get a single chain. Tress *et al*. (*2*) find that these behave in the same way.

Nanoimprint lithography, step-and-flash lithography, templating for bit-patterned media or low–dielectric constant materials, organic photovoltaics, and batteries are just a few examples where polymers can be molded into nanoscopic objects, or where the polymer self-assembles into a morphology with nanoscopic features, or where a morphology is kinetically trapped at nanoscopic length scales. As the size scale of the features decreases, we must remain cognizant that they are comparable to or even less than the radius of gyration of the polymer chain, R_g—a characteristic dimension that describes the volume pervaded by an unperturbed polymer chain in the bulk. Consequently, with decreasing size, there must be some compression or stretching of the polymer chain to accommodate the confining geometry. This deformation comes at the cost of an elastic retractive force, a very strong entropic force that we are all familiar with from stretched rubber bands. As objects get smaller, the surface-to-volume ratio increases and interfaces play an ever more important role. Because polymers are incompressible, we must understand how the change in density of the polymer at an interface is accommodated by the long-chain molecules, particularly those with a center of mass that is located within one radius of gyration (or less) from the interface. How does the presence of a surface or interface influence the configuration of the polymer chain and the dynamics of the polymer either in segments or as a full chain? Equally important is the fact that synthetic polymers, regardless of the method of synthesis, have a finite molecular weight distribution, which translates into a distribution of molecular sizes and therefore a distribution in the response of different chains to confinement.

The ability to assess the properties of nanoscopic elements (structural, electrical, magnetic, optical, etc.) has given rise to many exceptional developments in tools to probe isolated nanoscopic elements, as well as the creative use of existing tools to measure collections of nanoscopic elements. Synchrotron sources and free electron lasers provide suffi-

Polymer Science and Engineering Department, University of Massachusetts, Amherst, MA 01003, USA, and Advanced Institute of Materials Research, Tohoku University, 2-1-1 Katahira, Aoba, Sendai 980-8577, Japan. E-mail: russell@mail.pse.umass.edu