overexpressing the mutated kinase with a submaximal dose of inhibitor, then stimulated the kinase and chased with a saturating dose of the inhibitor linked to a fluorophore. The amount of fluorescent kinase shows how much of the enzyme was available for activation after addition of the first submaximal dose of inhibitor. The measurement of downstream substrates of this kinase indicates the activity that a given inhibitor occupancy allows.

A few wrinkles in the technique remain to be ironed out: the cysteine residue is essential for irreversible inhibitor binding, but it is not always obvious from sequence alignments where the cysteine should be placed. Shokat is also concerned that studies in populations of cells may make a clean separation of pathway activities difficult, and he is therefore pursuing a single-cell model. "That way," he explains, "we can do single-cell clamping of a kinase and measure the exact downstream phosphoactivation of that single cell."

Additionally, his team is working on a system in which they can independently inhibit two kinases to answer questions about how kinases in different pathways interact.

Looking beyond the interest of his own laboratory, Shokat sees a good chance that this chemical genetics approach will be applicable for other classes of enzymes. A space-creating gatekeeper mutation and a cysteine in the ATP-binding pocket might make other classes of enzymes susceptible to a high-affinity inhibitor—an attractive strategy, especially for enzymes, such as ATPases, for which no high-affinity inhibitor exists now.

If Shokat's approach finds followers, kinases will not remain the only enzymes that are being clamped.

Nicole Rusk

RESEARCH PAPERS

Blair, J.A. *et al.* Structure-guided development of affinity probes for tyrosine kinase using chemical genetics. *Nat. Chem. Biol.* **3**, 229–238 (2007).

smooth muscle cells lacking the M_3 receptor but expressing this designer receptor responded to CNO and effected downstream signaling. Also, in hippocampal neurons expressing a different designer receptor as well as an endogenous receptor, CNO specifically controlled the engineered receptor, inducing neuronal silencing—while the native receptor was unaffected.

Considering the wide range of natural ligands that bind various GPCRs, receptors could be engineered to be activated by essentially any ligand using this strategy. Other scientists are already using the receptors generated in this study as tools—with mice. But Roth sees other applications for these designer receptors: "Where we'd like to see it go further is not only using these as tools for addressing questions of interest to the basic scientist, but also to use these as therapeutic tools for tweaking the activity of engineered tissue in humans. And I think ultimately this is where this is going to go."

Irene Kaganman

RESEARCH PAPERS

Armbruster, B.N. *et al.* Evolving the lock to fit the key to create a family of G protein–coupled receptors potently activated by an inert ligand. *Proc. Natl. Acad. Sci. USA* **104**, 5163–5168 (2007).

NEWS IN BRIEF

IMAGING AND VISUALIZATION

Fluorescent reporter proteins without oxygen

Fluorescent proteins such as GFP are extremely useful, genetically encodable probes for biological imaging. All proteins derived from the GFP family, however, require molecular oxygen as a cofactor for chromophore fluorescence and therefore cannot be used in anaerobic systems. Drepper *et al.* now describe the engineering of blue-light flavin mononucleotide–based fluorescent proteins that do not require oxygen for formation of the chromophore. These proteins should find application for imaging in anaerobic microbes or hypoxic solid tumors.

Drepper, T. *et al. Nat. Biotechnol.* **25**, 443–445 (2007).

GENE TRANSFER

Guiding Sleeping Beauty

Transposons are mobile genetic elements that integrate into the genome at random. Although the efficiency of their integration makes them suitable as gene delivery vectors, their unspecific insertion can be problematic when it disrupts genes or regulatory elements. Yant *et al.* now show that by fusing the Sleeping Beauty transposon to a DNA-binding domain, they can direct the integration to specific sequences in human cells.

Yant, S.R. *et al. Nucleic Acids Res.*; published online 7 March 2007.

MICROARRAYS

Making protein arrays with AFM

Atomic force microscopy (AFM) is a useful technology for manipulating molecules on surfaces. Though it showed potential for the creation of protein arrays, this application was limited to very stable proteins, as it required relatively harsh conditions unfriendly to delicate proteins. Tinazli *et al.* now demonstrate the fabrication of 'rewritable' protein nanoarrays under physiological conditions using AFM-based nanolithography.

Tinazli, A. *et al. Nat. Nanotechnol.* **2**, 220–225 (2007).

BIOPHYSICS

Tethering with bacteriophage

Khalil *et al.* describe the application of the M13 filamentous bacteriophage as a tether in single-molecule assays. Through genetic manipulation, reactive groups can be displayed on one end of the bacteriophage for tethering to a bead, while proteins of interest can be displayed on the phage surface for biophysical investigation. The authors describe a robust method for generating the tethers and fully characterize their biophysical properties via optical trapping.

Khalil, A.S. *et al. Proc. Natl. Acad. Sci. USA* **104**, 4892–4897 (2007).

IMAGING AND VISUALIZATION

Cell-penetrating quantum dots

Quantum dots are very popular probes for imaging owing to their broad adsorption range, narrow emission spectra and excellent photostability. Getting them into living cells is tricky, however, and even once in, they have a tendency to stay trapped in endosomes. Duan and Nie report the use of hyperbranched copolymer coatings to empower quantum dots with cell-penetrating and endosome-disrupting capabilities, which should facilitate their application for imaging in live cells.

Duan, H. & Nie, S. *J. Am. Chem. Soc.* **129**, 3333–3338 (2007).