

CHEMICAL BIOLOGY

Kinase clamping

By introducing two point mutations in the ATP-binding pockets of kinases, researchers can specifically and irreversibly inhibit these enzymes and determine how much kinase activity is needed for downstream signaling.

Some experiments are straightforward, others much less so. Kevan Shokat has good examples for both. He says: "It is easy to relate the dose of a drug to a phenotype..., but it is always difficult to relate inhibitor occupancy to phenotype." Putting a drug, for example, a kinase inhibitor, on cells and measuring the effect gives researchers important insights into the potency of the drug, but it is impossible to infer how much of the inhibitor physically binds to the ATP pocket of the kinase and what effect partial inhibition has on downstream signaling. Shokat and his team at the University of California in San Francisco realized that to explain the signaling capacity of a kinase at a defined inhibitor occupancy, new tools were needed.

He outlines their strategy: "We wanted a chemical genetics system in which we could

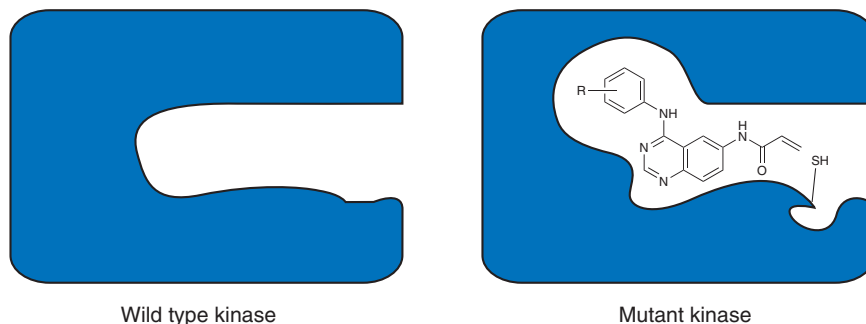


Figure 1 | Chemical genetics strategy to irreversibly inhibit a kinase. A point mutation in the ATP-binding pocket creates the space for the inhibitor to enter; a cysteine introduced by a second mutation facilitates irreversible binding of the inhibitor.

clamp the kinase activity at 10% or 20% inhibition, and see how the pathway responds to this inhibitor occupancy and how it is coupled to the downstream elements."

Shokat's team built upon their previous innovation in chemical genetics where they had introduced a space-creating gatekeeper mutation into the ATP-binding pocket of a kinase, thereby making it receptive for

bulky ATP analogs that cannot bind to the wild-type kinase. Now they added a cysteine residue at the entrance of the ATP-binding pocket: the gatekeeper mutation allows a bulky inhibitor to enter, and the cysteine facilitates the formation of an irreversible link between kinase and inhibitor (Fig. 1).

To correlate kinase activity with downstream signaling, they first incubated cells

MUTAGENESIS

DESIGNER RECEPTORS FOR EVERY BODY

Using a directed evolution approach, researchers demonstrate a way of creating 'designer' receptors that are specifically activated by a ligand with no other biological activity in the cell.

G protein-coupled receptors (GPCRs) are an important class of membrane proteins that orchestrate a wide variety of cellular responses by binding environmental cues and relaying this information into the cell.

With an interest in investigating what individual neuron populations do in the brain, Bryan Roth and his colleagues at Case Western Reserve University School of Medicine and University of North Carolina Medical School set out to expand the toolbox for working with GPCRs. "The idea was that we could stick in an artificial receptor and turn those neurons off with a compound that does nothing [else in the cell], and that would tell us what the function of those neurons is in intact animals," says Roth.

His team set out to create a receptor, activated only by a pharmacologically inert compound, that they could express in any tissue or organ in the body to turn the activity of that tissue on or off. The ligand they chose for this purpose

was clozapine-*N*-oxide (CNO). A related compound binds rat M_3 receptors, so the researchers hypothesized that directed evolution of this receptor could be used to change its specificity. What they found experimentally, however, "is that you may want to start with an intermediate ligand which is equidistant between the native ligand and the ligand that you will eventually evolve the receptor toward, because you might not get it in one step; in our case, it did take repeated cycles of random evolution," says Roth.

Using mutagenic PCR, they produced libraries of modified M_3 receptors and selected them in yeast for activation by an intermediate ligand. After several rounds of mutagenesis, the team obtained receptors that were activated by this ligand and then repeated the process to evolve the chosen mutants for CNO responsiveness.

As the goal was to use these receptors in mammalian cells, the researchers introduced the identified mutations into a human M_3 receptor, and after screening this library of mutants in HEK T cells, they identified a double-mutant M_3 receptor that was activated exclusively by CNO. As proof of principle, Roth and colleagues found that immortalized human pulmonary artery

NEWS IN BRIEF

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 M₃ 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 potentially activated by an inert ligand. *Proc. Natl. Acad. Sci. USA* **104**, 5163–5168 (2007).

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).