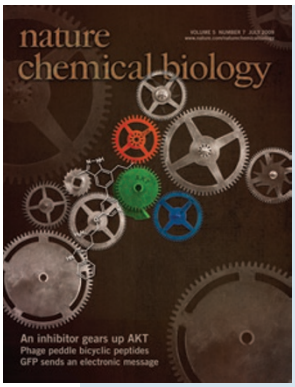


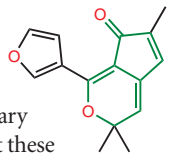
An inhibitor gears up Akt

In the absence of growth factors, the pro-proliferative kinase Akt is in the inactive, unphosphorylated state. Upon growth factor stimulation, Akt is activated by phosphorylation of Thr308 and Ser473. Unexpectedly, the ATP-competitive Akt inhibitor A-443654 has also been found to induce hyperphosphorylation of these Akt regulatory sites. This activation could be caused by inhibition of off-target kinases, by alterations in pathway-mediated feedback following Akt inhibition or as a direct result of inhibitor binding. To decipher the mechanism, Okuzumi *et al.* mutated the gatekeeper residue to generate an analog-sensitive (*as*) allele of Akt and synthesized an analog of A-443654 that inhibited *as*Akt but not wild-type Akt. Using these chemical genetic tools, the authors found that inhibitor binding directly triggered Akt hyperphosphorylation. This unexpected effect of small-molecule occupancy of the ATP binding site provides new insights into the biological regulation of kinase activity and has implications for the therapeutic use of Akt inhibitors. [**Articles, p. 484; News & Views, 448**] *JK*

Linking lipids

Lipids are gaining increasing importance as their varied biological roles become clearer, but challenges in obtaining sufficient quantities of pure lipids have limited these investigations. Previous research demonstrated that an engineered glycosidase, *endo*-glycoceramidase II (EGC), could attach different sugars to D-erythrosphingosine to yield various glycosphingolipids, but an enzyme capable of producing high yields of these lipids with modified lipid tails was not available. Hancock *et al.* now use a high-throughput screen to search for EGC variants that can accommodate diversity in the lipid cosubstrate. Several mutants were identified against each of two substrates, sphingosine and phytosphingosine, with the same D314Y mutation observed in each case. This mutation, which primarily served to increase k_{cat}, was sufficiently activating to generate 5 mg of purified product from the phytosphingosine substrate. The rational combination of productive mutations highlighted other EGC improvements and may point toward the further rational design of enzyme function. [Articles, p. 508] *CG*

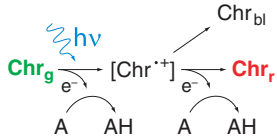
Stacking the screening deck



High-throughput screening should be like looking for a needle in a haystack: there are more than 10^{60} distinct molecules with 30 or fewer heavy atoms, while a relatively large high-throughput screening library might only include about 10^6 small molecules. Against these odds, Hert *et al.* looked for explanations for why high-throughput screening succeeds. To address this question, the authors quantified the similarity of purchasable chemicals, which constitute the vast majority of screening libraries, to metabolites and natural products, which by definition have biological activity. The authors then compared this to the level of similarity observed between a proxy for all of chemical space and the same set of bioactive molecules and found that screening libraries are heavily biased towards biogenic molecules. To further capitalize on this trend, Hert *et al.* identified molecules that are currently absent from screening libraries that might be added to enhance the likelihood of identifying hits against difficult targets. [**Articles, p. 479**] *JK*

Written by Catherine Goodman & Joanne Kotz

GFP reddens with redox

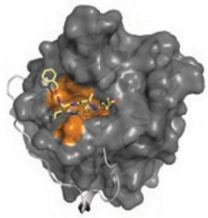


Green fluorescent protein (GFP) is an integral tool in chemical biology research, but the native function of this glowing protein has not been resolved. It was previously known that GFP could photoconvert into a red fluorescent state under anaerobic conditions, but these conditions are not thought to be representative of the protein's *in vivo* environment. Bogdanov *et al.* now demonstrate that GFP can also photoconvert into a red state under aerobic conditions in the presence of electron acceptors. This process was effective with both chemical and biological electron acceptors and can proceed in a one- or two-step manner. *In vitro* experiments with other fluorescent proteins suggest that the reaction requires a tyrosine-based chromophore. Finally, successful 'redding' in mitochondria, cells and a button polyp suggests that this reaction is biologically relevant, though it remains to be seen when and how cells take advantage of this function. [Brief Communications, p. 459] *CG*

Mind your mushrooms

Multiple cases of acute mushroom poisoning have been recorded, but the toxic agent has eluded identification. Matsuura *et al.* have now solved this mystery by determining both the true poisonous mushrooms and the small molecule responsible. By comparing the oral administration of water extracts of mushrooms collected in different sites, the authors determined that only one group—local to Kyoto—is the 'true' toxic *Russula subnigricans* species. Examination of toxic extracts led to the characterization of cycloprop-2-ene, a 4-carbon molecule known in synthetic chemistry, as the bioactive molecule. The introduction of methyl groups at one or both alkene carbons diminished or abrogated toxicity, as did an ene polymerization that occurred at high concentrations (that is, during purification). This report will enable additional testing to establish the mode of action of this tiny compound. [**Brief Communications, p. 465**] *CG*

Targeting MARTX toxin



Following secretion from some pathogenic bacteria, the MARTX toxin forms a pore in the host cell membrane. The central region of the toxin is then inserted into the host cytoplasm, where the eukaryotic small molecule inositol hexakisphosphate 6 ($InsP_6$) activates a cysteine protease domain, which autocatalytically processes the toxin to yield effector domains that alter the host cell cytoskeleton. Shen *et al.* have now developed the first small-molecule inhibitors of the cysteine protease domain of the *Vibrio cholerae* MARTX toxin. Using these chemical tools, along with structural and biochemical approaches, the authors defined the substrate specificity of the protease domain and identified multiple functionally relevant cleavage sites within the toxin. The authors further demonstrated that inhibiting the protease blocks toxin activity. These results provide the first insights into MARTX processing and suggest the protease domain as a potential drug target. [Articles, p. 469] *JK*