TOOLS IN BRIEF

GENETICS

Optogenetic mutagenesis in worms

Traditional mutagenesis relies on toxic chemicals or radiation; Noma and Jin now report a mutagenesis method that is completely safe for the experimenter. In their approach, the mini singlet-oxygen generator (miniSOG) is targeted to nuclei in the *Caenorhabditis elegans* germline. Upon illumination with blue light, miniSOG produces reactive oxygen species that lead to a variety of germline mutations. Although the mutation rate is four- to fivefold lower than with standard chemical mutagenesis under the conditions used, the spectrum of mutations is broader. miniSOG induces base pair changes not typically observed in chemical mutagenesis, as well as deletions ranging from 1 to about 1,500 base pairs in length. In addition, the researchers show that miniSOG-mediated chromosome breaks can facilitate the integration of transgenes into the genome.

Noma, K. & Jin, Y. *Nat. Commun.* **6**, 8868 (2015).

MOLECULAR BIOLOGY

Nanobody tools

Conventional antibodies can add tens of nanometers to the size of a structure that they label. Single-chain camelid antibodies, or 'nanobodies', are exciting because of their potential as much smaller protein labels, and they should enable more quantitative measurements of macromolecular structures. Pleiner *et al*. report a series of nanobodies that target components of the nuclear pore complex (NPC). They demonstrate that these tools can be prepared via cytoplasmic expression (as opposed to secretion into the bacterial periplasm) and conjugated to fluorophores at added surface cysteines using maleimide chemistry, apparently without disrupting their function. They used the fluorophore-labeled tools for localization-based super-resolution imaging of NPCs and also demonstrate purification of endogenous complexes via nanobodies fused to an affinity tag.

Pleiner *et al*. *eLife* doi:10.7554/eLife.11349 (3 December 2015).

SENSORS AND PROBES

A DIVERSE strategy for discovering peptide tags

Peptides that covalently bind small-molecule targets have uses in numerous biological applications, including protein tagging. However, methods to discover or improve such peptides are limited by technical issues such as the ability to screen very large libraries. To overcome this obstacle, Kawakami *et al*. developed a selection strategy called directed *in vitro* evolution of reactive peptide tags via sequential enrichment (DIVERSE). In DIVERSE, a large (10^{14}) DNA library is transcribed and translated in one pot. During translation, puromycin linkers are introduced that link the mRNA to the peptide; the mRNA is then reverse transcribed, yielding peptides that are covalently linked to cDNA. Using this strategy, the team found peptides that covalently bound a target small molecule; by conjugating the small molecule to a fluorophore, they were able to image tagged proteins in live cells.

Kawakami, T. *et al*. *Chem. Biol.* **22**, 1671–1679 (2015).

CHEMICAL BIOLOGY

Efficient unnatural amino acid incorporation

The ability to incorporate unnatural amino acids with unique chemistries into proteins by reprogramming the cell's translational machinery has had an impact on multiple areas of biological research. However, the technique has suffered from problems of inefficiency, especially when it comes to incorporating unnatural amino acids at multiple sites in a protein. Amiram *et al*. describe a greatly improved approach that allowed them to incorporate the unnatural amino acids *p*-acetyl-L-phenylalanine and *p*-azido-L-phenylalanine (which contain useful chemical handles for labeling) at multiple sites in a protein by evolving new aminoacyl-tRNA synthetases with up to 25-fold–improved activities. They also report aminoacyl-tRNA synthetases with readily tunable specificities for 14 additional unnatural amino acids. The researchers demonstrated the ability to synthesize elastin-like polypeptide fusion proteins containing an unnatural amino acid at up to 30 sites, at high yield and with high fidelity.

Amiram, M. *et al*. *Nat. Biotechnol.* **33**, 1272–1279 (2015).