IN BRIEF

GENOMICS

Better base editors

Gehrke, J. M. et al. *Nat. Biotechnol.* https://doi.org/10.1038/nbt.4199 (2018).
Wang, X. et al. *Nat. Biotechnol.* https://doi.org/10.1038/nbt.4198 (2018).

With their ability to introduce single-base changes without cutting both strands of the DNA base, editors are the rising stars in the CRISPR portfolio. The past two years have seen advances in on-target activity, but targeting editors to a specific base while leaving those in the vicinity unchanged is still a challenge. For a C-to-T edit, a Cas9 nickase is fused to a cytidine deaminase, most commonly rat APOBEC1, and a uracil DNA glycosylase inhibitor that prevents repair of the edited base. To narrow the specificity of the base editor to a single C within the editing window, Gehrke et al. used human APOBEC3A, which requires a TCA/G motif for editing and hardly edits Cs in other sequence contexts. Independently, Wang et al. also found that human APOBEC3A has a narrower editing window and efficiently converts Cs to Ts in regions with high DNA methylation levels. *NR*

https://doi.org/10.1038/s41592-018-0154-4

NEUROSCIENCE

Modeling temperature during optogenetic illumination

Picot, A. et al. *Cell Rep.* **24**, 1243–1253 (2018).

Two-photon-based optogenetic manipulation of individual cells or ensembles of cells is a valuable approach for analyzing neuronal circuits. However, the necessary illumination schemes can heat up the targeted cells, and this has to be carefully evaluated. Picot et al. describe a model for predicting temperature increases with high spatial and temporal resolution. The researchers validated their model with phantom preparations and then applied it to illumination schemes that are in current experimental use, such as holographic illumination and spiral scanning. They found that illumination designed to optogenetically activate single cells or ensembles of up to 100 cells (using the opsin CoChR) did not lead to local temperature increases beyond 1 K, provided that the cells were not too close together and that the illumination frequency allowed for a sufficient temperature decay between pulses. The scripts used for the modeling are available and should be useful to others who want to evaluate their own experimental conditions. *NV*

https://doi.org/10.1038/s41592-018-0155-3

SENSORS AND PROBES

Lighting up proteins

Chen, Y. et al. *Angew. Chem. Int. Ed. Engl.* **57**, 12390–12394 (2018).

Numerous strategies exist for tagging proteins of interest for downstream applications such as fluorescence microscopy and spectroscopy. However, smaller, brighter, and more photostable probes are still needed. Chen et al. have developed a green fluorescent probe for protein labeling that is fluorogenic, meaning it turns on only when bound to its target. Their probe has two components: a peptide tag containing two cysteine residues that is used to tag the target protein, and a BODIPY-based dye conjugated to two maleimide groups. When free in solution, the dye is nonfluorescent. However, when both maleimide groups react with the cysteines on the peptide tag, the dye turns on. The researchers demonstrate that their labeling approach works in both bacterial lysates and living mammalian cells. *RS*

https://doi.org/10.1038/s41592-018-0156-2

CHEMICAL BIOLOGY

Evolving proteins with improved solubility

Wang, T. et al. *Nat. Chem. Biol.* **14**, 972–980 (2018).

Escherichia coli is the most widely used organism for recombinant protein expression, but the majority of nonbacterial proteins cannot be expressed in this bug in soluble form. Directed evolution is a promising approach for generating protein variants with a desired function that also have improved solubility, as demonstrated recently by Wang et al. Using their previously established phage display-based PACE (phage-assisted continuous evolution) system, the researchers additionally implemented the use of a split-intein minor coat protein III—which is required for the generation of infectious progeny phage—to enable two concurrent positive selections. This approach allows them to select for both function and improved solubility. They applied this soluble expression (SE)-PACE method to evolve antibody fragments with improved expression in just a few days, as well as to generate APOBEC1 cytidine deaminase variants with improved solubility and base-editing activity. *AD*

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