METHODS IN BRIEF

GENOMICS

Cas goes rogue

Conventional wisdom has it that Cas nucleases need guide RNAs to find and cleave their targets, but this may not always be true. Sundaresan *et al.* report that, given high enough manganese concentrations, certain Cas species cleave DNA randomly without any sequence specificity and without guide RNA. They tested Cas9 from *Streptococcus pyogenes* (SpCas9), the most widely used Cas protein for genome editing, and Cas9 and Cpf1 from *Francisella tularensis novicida* (Fno). Purified SpCas9 and FnoCpf1 degrade linear and circular single-strand DNA at 250 μm Mn^{2+}, and FnoCas9 nicks double-stranded DNA (dsDNA) at 500 μm Mn^{2+}—levels these proteins could encounter in a cell. Sundaresan *et al.* did not observe nonspecific activity of SpCas9 on dsDNA, so it is unlikely that this finding will impact the nuclease's usefulness, but it is something to keep in mind.

Sundaresan, R. *et al. Cell Rep*. **21**, 3728–3739 (2017).

IMAGING

Tilted light-sheet microscopy with 3D point-spread functions

In conventional light-sheet microscopy, a sample is illuminated by a thin layer of light perpendicular to the detection axis. This reduces the background illumination and improves single-molecule localization. However, these configurations come with certain drawbacks and are often complex and expensive. Gustavsson *et al.* present an easier and cheaper method to achieve super-resolution with a light-sheet microscope. They found that when they tilted the illumination plane, they could image cells all the way down to the coverslip. Only one objective is needed for this approach, which allows the use of a high-numerical-aperture objective. In combination with 3D point-spread functions, this system achieves localization precision to tens of nanometers without losing the advantages of light-sheet microscopy.

Gustavsson, A.-K. *et al. Nat. Commun*. **9**, 123 (2018).

CHEMICAL BIOLOGY

Light-based RNA structure probing

Hydroxyl radical probing remains the gold-standard approach for measuring RNA-nucleobase solvent accessibility, but it requires synchrotron radiation, and thus is challenging to apply in cells. Feng *et al.* now present light-activated structural examination of RNA, or LASER, a synchrotron-free alternative method for probing the solvent accessibility of purine nucleobases. The approach uses nicotinoyl azide, which, when hit with light, undergoes a rapid chemical reaction to form adducts with solvent-exposed guanosines and adenosines on a folded RNA. Such tagged sites are detected by reverse transcription of the modified RNA followed by denaturing gel electrophoresis. The method detects rapid changes in RNA structure after ligand binding and yields results very similar to those obtained by hydroxyl radical probing, but it works both outside and inside cells.

Feng, C. *et al. Nat. Chem. Biol.* https://dx.doi.org/10.1038/nchembio.2548 (2018).

IMMUNOLOGY

Antibody function revealed in high throughput

Next-generation sequencing has boosted the scale of immune profiling, by capturing the repertoire of T and B cell receptors that are enriched during adaptive immunity. In contrast, methods to profile antibody specificity typically require the laborious immortalization and *in vitro* expansion of individual B cells. Wang *et al.* are now accelerating the functional characterization of antibodies by using yeast engineered to display the human antigen-binding fragment (Fab) on its surface. To capture pairs of light and heavy chain antibody sequences from the same cell, they perform single B cell emulsion lysis followed by oligo-dT RNA capture, reverse transcription and overlap-extension PCR to create a single linked amplicon. The amplicon is then cloned into a yeast surface expression vector in a single step. The researchers used their method to identify neutralizing antibodies to HIV-1, Ebola virus glycoprotein and influenza hemagglutinin.

Wang, B. *et al. Nat. Biotechnol.* https://dx.doi.org/10.1038/nbt.4052 (2018).