TOOLS IN BRIEF

STRUCTURAL BIOLOGY

Tags to disentangle Dicer

Primarily for its role in the silencing of genes via small RNAs, Dicer is an enzyme that fascinates biologists. Dicer proteins are large, complicated proteins that recognize double-stranded RNA (dsRNA) and chop it into precisely sized products. The structural and mechanistic details of how these activities are performed by different domains in this large protein remain largely unknown. Electron microscopy reconstructions of human Dicer have been reported, but Lau *et al.* set out to experimentally test working models of its domain architecture. They did so by introducing short amino-acid sequence tags targeted to specific functional domains in the protein. They purified the tagged proteins, visualized them by negative-stain electron microscopy and performed three-dimensional reconstructions. The strategy revealed the sites associated with different functional domains in the architecture of the large protein and allowed comparisons between different Dicer homologs, providing insight into the structural basis for small RNA production in eukaryotes.

Lau, P.W. *et al. Nat. Struct. Mol. Biol.* **19**, 436–440 (2012).

SENSORS AND PROBES

An ideal cyan fluorescent protein?

Cyan fluorescent protein serves as the donor fluorophore for the majority of genetically encoded sensors based on fluorescence resonance energy transfer, and scientists have continuously improved it over the years in an attempt to optimize its performance. Goedhart *et al.* examined the structures of some of these variants, trying to uncover the origins of these improvements. They noted one highly variable residue that did not appear to be optimized and subjected it to saturation mutagenesis. This resulted in a variant they named monomeric (m)Turquoise2 that exhibits faster maturation and a quantum yield of 93% as well as other beneficial characteristics. The protein's improved characteristics will be useful, but it is becoming clear that optimization of the popular fluorescent protein backbones is entering a period of diminishing returns.

Goedhart, J. *et al. Nat. Commun.* **3**, 751 (2012).

BIOPHYSICS

Nanocombinatorics for biology

There is much interest in understanding the role of substrate topology on the mechanical properties of cells and its possible biological consequences. Methods to study cell-substrate interactions are thus in demand. In recent work, Giam *et al.* describe the use of massively parallel polymer pen lithography to generate substrates with customized patterns. The pen arrays can be used either in a level or a tilted configuration, the latter enabling the rapid preparation of combinatorial libraries with topological features that range in scale from microscale to nanoscale. The researchers used these tilted arrays to generate gridded fibronectin patterns of varying size and spacing. They observed differences in the amounts of osteogenic markers in mesenchymal stem cells plated on nanoscale- versus microscale-patterned fibronectin.

Giam, L.R. *et al. Proc. Natl. Acad. Sci. USA* **109**, 4377–4382 (2012).

SENSORS AND PROBES

RNA sensors for small molecules

Small-molecule sensors for live imaging studies are relatively few. To add to the toolbox, Paige *et al.* propose a method that couples small-molecule binding RNA aptamers—short sequences that can be selected *in vitro* for desirable binding properties—to a conditional fluorophore. The group previously engineered Spinach, an aptamer that causes a modified fluorophore from GFP to fluoresce upon binding. Destabilizing one stem of a stem-loop in Spinach caused it to stop functioning, but attaching a small molecule–binding aptamer sequence to the stem resulted in a reporter that was structured when bound by the small molecule and caused the fluorophore to fluoresce. The group generated Spinach-based sensors for adenosine 5′-diphosphate (ADP), S-adenosylmethionine (SAM) and other molecules in *Escherichia coli* in this way.

Paige, J.S. *et al. Science* **335**, 1194 (2012).