METHODS IN BRIEF

IMAGING

Small-angle X-ray scattering reaches new dimensions

Small-angle x-ray scattering (SAXS) is a powerful tool for imaging dense specimens at the nanoscale in two dimensions, but the generation of 3D reconstructions (also known as tomography) has been technically challenging with SAXS data. New work has overcome this hurdle to allow complete 3D imaging of dense biological samples. Schaff *et al*. used computed tomography with virtual tomography axes, which retained sample-orientation information that is normally lost and allowed for complete 3D mapping of collagen organization in tooth samples in real and reciprocal space. In separate work, Liebi *et al*. produced complete 3D reconstructions of collagen in bone samples by combining SAXS with tensor tomography. These methods pave the way for future 3D imaging of dense biological samples at the nanoscale.

Liebi, M. *et al*. *Nature* **527**, 349–352 (2015).

Schaff, F. *et al*. *Nature* **527**, 353–356 (2015).

GENE EXPRESSION

Synthetic data feed machine-learning predictors of splicing

Splicing is a complex and highly regulated process that generates enormous transcriptional diversity. Researchers have tried various approaches to learn the sequence-based determinants of splicing activity, with limited success. Rosenberg *et al*. identified universal splicing elements by training a machine-learning method with more than 2 million data points from a synthetic mini-gene assay. Each barcoded mini-gene included two closely spaced, fully degenerate 25-nucleotide sequences in either the donor or the acceptor region of an intron that split two exons. The authors transfected pooled plasmids into HEK293 cells and sequenced RNA to determine splice status, and they found that a model trained only on these synthetic data accurately predicted the effects of human genetic variants on isoform ratios. They also showed that splicing occurs preferentially at upstream donor sites and that exon inclusion is regulated cooperatively by *cis* elements.

Rosenberg, A.B. *et al*. *Cell* **163**, 698–711 (2015).

BIOCHEMISTRY

Comprehensive glycoprotein characterization

Protein glycosylation is a functionally important but very complex type of post-translational modification. It has thus remained an experimental challenge to comprehensively characterize glycan structures as well as their site-specific locations on proteins on a proteomic scale. Sun *et al*. have reported an approach that enables researchers to thoroughly characterize N-linked glycoproteins. Their method involves conjugation of digested peptides to a solid support; chemical modification of both peptide and glycan to facilitate mass spectrometry analysis, followed by the release of the glycans and peptides from the support; identification by mass spectrometry; and finally reconstruction of the intact N-glycopeptides from the mass spectrometry data. This approach provides information about the total N-glycan, peptide glycosite and glycoprotein content in complex samples and was used to observe the effects of an N-linked glycosylation inhibitor on ovarian cancer cells.

Sun, S. *et al*. *Nat. Biotechnol*. doi:10.1038/nbt.3403 (16 November 2015).

GENOMICS

Cooperative transcription-factor binding

The interplay between transcription factors (TFs) and DNA is still subject to intense investigation. Binding motifs for many TFs are known, as is the fact that TFs can bind as pairs to multiple motifs. What remains unclear is the prevalence of such pairings and whether DNA sequences determine binding. Jolma *et al*. systematically addressed this question with a strategy of selection and enrichment. They labeled TFs with different tags and enriched for DNA sequences bound by TF pairs in consecutive rounds of pulldowns. Most of the enriched TF pairs showed DNA-mediated cooperative binding and recognized composite motifs different from those of the individual TFs. The researchers estimate that ~25,000 TF pairs—a much larger number than previously anticipated—contribute to DNA regulatory interactions in cells.

Jolma, A. *et al*. *Nature* **527**, 384–388 (2015).