METHODS IN BRIEF

GENOMICS

Bias in ChIP-seq data

Even well-established methods, such as chromatin immunoprecipitation followed by deep sequencing (ChIP-seq), are not immune to inherent bias, as Teytelman *et al.* discovered when using ChIP-seq to look at where in the yeast genome a protein complex that represses transcription binds. To their surprise, the researchers found components of the complex associated with a lot of highly expressed genes, a finding not in line with the known function of this complex. Following up with analyses of other proteins, Teytelman *et al.* discovered that highly expressed loci were always enriched in ChIP peaks, regardless of which protein was pulled down. They caution the scientific community that any ChIP-seq result should be checked against the expression of the locus and that unexpected findings need to be validated independently.

Teytelman, L. *et al. Proc. Natl. Acad. Sci. USA* **110**, 18602–18607 (2013).

STRUCTURAL BIOLOGY

A solution to the phasing problem

X-ray free-electron lasers (XFELs) provide very intense femtosecond X-ray pulses, allowing useable diffraction data to be collected from a stream of many thousands of tiny microcrystals before they are destroyed by radiation damage. This emerging approach, known as serial femtosecond crystallography, is proving to be very useful for determining structures of proteins that cannot be coaxed to form the large crystals necessary for conventional X-ray diffraction. To date, all protein structures solved using serial femtosecond crystallography have relied on a data analysis method known as molecular replacement, in which known related structures are used to obtain crystallographic phases for the new protein data. Barends *et al.* show that it is possible to borrow the method of heavy-atom doping from conventional crystallography. Heavy atoms intensify X-ray scattering and allow phases to be determined. This allowed them to solve the structure of lysozyme at 2.1 Å resolution without any prior structural knowledge.

Barends, T.R.M. *et al. Nature* doi:10.1038/nature12773 (24 November 2013).

SEQUENCING

Transcriptome of a single nucleus

High-throughput RNA sequencing can be used to measure gene expression in single cells, but some cells are difficult to isolate because they are delicate or embedded in complex tissues. As an alternative, Grindberg *et al.* show that it is possible to access the transcriptome of single cells by sequencing RNA from their nuclei. Nuclei are generally easier to isolate than whole cells and do not require heating, which is associated with enzymatic dissociation of whole cells, thus reducing the chance of disturbing the transcriptional state of the cell. The researchers applied a common single-cell RNA sequencing protocol to nuclei from single mouse hippocampal neurons and demonstrated that the sequencing results were very similar to those for whole neuronal progenitor cells, despite the fact that the nucleus can contain 10–100-fold less mRNA.

Grindberg, R.V. *et al. Proc. Natl. Acad. Sci. USA* **110**, 19802–19807 (2013).

MOLECULAR ENGINEERING

Engineering detergent-stable membrane proteins

Engineering detergent-stable variants of integral membrane proteins would enable many studies, including structural studies, of this important class of proteins. Scott and Plückthun describe a bacterial encapsulation technique that permits selection for detergent-stable membrane proteins from a large mutant library. When encapsulated bacteria expressing the protein of interest are solubilized, the resulting nanocontainer retains (that is, prevents the exit or loss of) the solubilized protein variant and its encoding gene but allows entry of a fluorescently labeled ligand. Detergent-stable variants of the examined protein can then be isolated by iteratively identifying ligand-binding clones. The researchers applied the approach to evolve detergent-stable variants of two G protein–coupled receptors.

Scott, D.J. & Plückthun, A. *J. Mol. Biol.* **425**, 662–677 (2013).