METHODS IN BRIEF

SEQUENCING

Single-molecule protein interaction detection

Gu *et al.* bring the power of single-molecule sequencing technology to profile protein interactions with a new approach called single-molecule-interaction sequencing, or SMI-seq. The approach is based on tagging proteins with DNA barcodes that can be read out by sequencing. First, proteins are barcoded with DNA tags by ribosome display or enzymatic conjugation; next, the barcoded proteins are randomly immobilized in a gel matrix; then, the DNA barcodes are amplified into *in situ* polymerase colonies ('polonies'). Barcoded proteins in a complex mixture can thus be identified and quantified by DNA sequencing, and protein-protein interactions can be detected by sequencing colocalized polonies. Gu *et al.* demonstrated the utility of this powerful platform for profiling the binding repertoire of an antibody and also for screening for small molecules that mediate G protein–coupled receptor activation.

Gu, L. *et al. Nature* doi:10.1038/nature13761 (21 September 2014).

IMAGING

Super-resolution imaging in live animals

Technical issues make it difficult to track single molecules in a living organism despite the development of super-resolution imaging techniques that improve light microscope resolution to a few nanometers. Zhan *et al.* now show that complementation-activated light microscopy (CALM) can be used to follow individual voltage-dependent calcium channels in the muscle cells of living adult roundworms. CALM relies on fusing a protein of interest to a nonfluorescing GFP that lacks a single β-strand, which can be added back at low levels for complementation and sparse molecular labeling; it does not need photobleaching or low protein levels. The authors used dystrophin-mutant worms as models for human Duchenne muscular dystrophy and discovered that dystrophin modulates calcium-channel positional organization in muscle cell membranes and neuromuscular synapses by transducing changes in muscle tension.

Zhan, H. *et al. Nat. Commun.* **5**, 4974 (2014).

PROTEOMICS

Phosphohistidine proteomics

Mass spectrometry–based proteomic methods for detecting phosphorylation of serine, threonine and tyrosine are becoming well established, but this important regulatory post-translational modification (PTM) is also known to occur on other amino acid residues such as histidine. Oslund *et al.* describe a method for global proteomic profiling of phosphohistidine proteins, which they applied to study differences in *Escherichia coli* cells grown on glycerol versus on mannitol. First, to enrich for peptides containing this PTM, they utilized a pan-phosphohistidine antibody. Next, they found that collision-induced dissociation fragmentation of phosphohistidine-containing peptides in the mass spectrometer produced characteristic neutral-loss species. This observation allowed them to develop a software tool to identify phosphohistidine-containing peptides in mass spectrometry data by their characteristic fragmentation pattern.

Oslund, R.C. *et al. J. Am. Chem. Soc.* **136**, 12899–12911 (2014).

MOLECULAR BIOLOGY

Large-scale transcription factor binding prediction

Of the approximately 170,000 eukaryotic transcription factors (TFs) believed to exist, only about 1% have characterized binding sequences. Weirauch *et al.* address the knowledge gap by using two protein-binding microarrays to determine the binding motifs of 1,032 cloned TFs, which come from over 130 species and represent 54 of 80 known DNA binding-domain classes. On the basis of sequence similarity, the researchers inferred motifs for another 58,000 TFs, with 89% accuracy according to cross-validation. Motifs are enriched in promoters, chromatin-immunoprecipitation sequence fragments and *Arabidopsis thaliana* expression quantitative trait loci. Weirauch *et al.* also develop an algorithm that generates ranked lists of human TFs whose binding can be altered by disease-associated genetic variants. The binding motif information is available in the catalog of inferred sequence preferences of DNA-binding proteins (CIS-BP) (http://cisbp.ccbr.utoronto.ca/).

Weirauch, M.T. *et al. Cell* **158**, 1431–1443 (2014).