A cross-linking–mass spectrometry bonanza

The combination of chemical cross-linking and mass spectrometry is being used increasingly to study protein-complex architecture as well as protein-interaction networks. These measurements reveal distances between amino acids and indicate physical protein–protein interactions. Three papers report methodological advances in cross-linking combined with mass spectrometry. Chait and colleagues describe an optimal protocol for isolating cross-linked protein complexes at endogenous levels using GFP-tagged transgenic cell lines, which allowed them to model the architectures of multisubunit protein complexes in their near-native state. Aebersold and colleagues report a computational approach for the analysis of quantitative cross-linking–mass spectrometry data that allowed them to study the structural differences between discrete states of protein complexes. Finally, Heck and colleagues report an integrated workflow and software tool for identifying intra- and interprotein cross-links on a proteomic scale.

Brief Communication p1135, Articles p1185 and p1179

Light-sheet microscopy with four orthogonal views

Light-sheet microscopy has become a valuable tool for imaging relatively large-volume specimens with minimal light exposure. However, light-sheet microscopy still suffers from limits in terms of axial resolution, sample size and the ability to image nontransparent samples. These issues have been at least partially addressed by several recent methods, including methods that combine multiple views of a sample to improve isotropic resolution. Keller and colleagues built on these approaches to develop the IsoView microscope, which uses simultaneous light-sheet illumination and fluorescence detection along four orthogonal directions. IsoView microscopy can be used to image relatively large, highly scattering samples such as whole Drosophila larvae with high temporal resolution as well as increased spatial resolution and decreased resolution anisotropy relative to conventional light-sheet methods.

Article p1171

Enhancing the range and precision of Cas9

Two elements are required to cleave the genomic target site of the Cas9 nuclease: the presence of a three-nucleotide protospacer-adjacent motif (PAM) on the target to interact with a domain in Cas9, and a complementary guide RNA to form an R loop and prepare the target DNA for cleavage by Cas9. This requirement for a particular PAM limits the range of Cas9. Wolfe and colleagues now add a third licensing step to allow precise targeting of Cas9 to any genomic locus. They attenuated the affinity of Cas9 for its PAM sequence, allowing binding to more motifs, and fused Cas9 to a zinc-finger domain that can be designed to bind any sequence downstream of the PAM. They show precise cleavage of target sites with far fewer off-target effects than observed with wild-type Cas9.

Article p1150

Imaging biomechanical properties

The hydromechanical properties of cells can vary with their environment, their age and their pathological state. Scarcelli and colleagues have developed a noncontact method to assess these properties on the basis of Brillouin light scattering. In this type of scattering, the interaction of light with the cellular material results in a frequency shift. In contrast to invasive methods such as atomic force microscopy and bead displacement by optical tweezers, Brillouin microscopy can be used to measure cellular viscoelasticity without mechanical probing. The researchers validated their method on cells subjected to different osmotic conditions and then applied their method to cells grown on or in 2D and 3D matrices.

Brief Communication p1132

Finding RNA-protein networks

RNAs and their binding proteins form an intricate regulatory network that controls many cellular functions. Current protocols for identifying RNAs that interact with a protein of interest involve immunoprecipitation or cross-linking. Wickens and colleagues introduce an in vivo RNA-tagging approach in yeast in which the potential RNA-binding protein is fused to a polymerase that will add uridines to any RNA the protein binds. The length of the U-tag is an indication of the binding affinity of the protein and can thus be used to distinguish regulatory proteins from those that merely sample an RNA. RNA Tagging can be used to identify the transcriptome-wide targets of proteins, as the authors show for a well-characterized ribonucleoprotein and a protein of the secretory pathway without any known RNA-binding motif.

Article p1163