**In this issue**

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### XFELs go viral

Serial femtosecond X-ray crystallography using an X-ray free-electron laser (XFEL) is an exciting method for structure determination, but several aspects remain challenging. Sample delivery such that a large fraction of crystals are ‘hit’ by the laser is one such challenge. Meents and colleagues now describe a fixed-target sample delivery method that makes full use of the XFEL repetition rate and thus enables efficient data collection and rapid structure determination even on small quantities of sample. They apply this approach to solve the 3D structure of bovine enterovirus 2 to 2.3 Å; this is the first viral structure to be solved using an XFEL.

**Article p805**

### Expanding interactomes

The functionally critical role of interactions between proteins has motivated ongoing efforts to generate interactomes—maps of all protein–protein interactions in a sample. But even static interactomes are still incomplete. To solve this problem, the scale and speed of interaction mapping techniques need substantial improvement. Ecker and colleagues describe a scaled-up version of the workhorse yeast two-hybrid (Y2H) method. Their approach makes use of Cre recombinase to fuse the bait and prey plasmids of an interacting protein pair; the resulting reporter is read out by next-generation sequencing. Iterative all-by-all screening of Arabidopsis transcription factors identified thousands of previously unknown putative interactions, which illustrated the power of this massively scaled-up technique.

**Article p819**

### Subgene-level cancer drivers

The identification of genetic cancer drivers has been tackled at different levels, from gene regions to entire genes and pathways. Finding driver mutations at the subgene level is attractive, as it allows researchers to distinguish variants in the same gene that lead to different phenotypes. Godzik and colleagues present the classification and comparison of tools that detect cancer drivers at subgene resolution. The authors categorize the algorithms into four types depending on whether they work with linear sequences or take 3D protein information into account and whether they cluster mutations de novo or use externally defined regions to search for variants. Strengths and limitations of each category are presented, so researchers can make the most of the data to prioritize the many variants with hitherto unknown driver effect.

**Analysis p782**

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### Calibration for counting

Single-molecule localization microscopy has proven very useful for quantifying biology. For example, this technique can be used to count the number of subunits in protein complexes. However, such counting is complicated by problems that include incomplete protein labeling, complex fluorophore photophysics, and antibodies being labeled with variable numbers of fluorophores. Zanacchi, Lakadamyali and colleagues develop a calibration approach to correct for these issues and improve accuracy in quantifying protein copy number. Their approach makes use of a DNA origami chassis that can be precisely labeled with a protein of interest, in this case GFP, along with antibodies that bind to that protein. In doing so, the researchers can directly measure labeling density and efficiency to calibrate measurements of complexes made in cells. They demonstrate the method by counting GFP-tagged nuclear pore proteins.

**Brief Communication p789**

### Fast volumetric calcium imaging in the mouse brain

Calcium imaging approaches in the mouse are challenged by the scattering nature of brain tissue. While the widely used two-photon scanning microscopy circumvents this problem, this serial acquisition approach is inherently slow. On the other hand, light-field microscopy is a fast, parallel imaging technology, but its one-photon excitation is susceptible to scattering artifacts. Nöbauer and colleagues have developed a seeded iterative demixing (SID) strategy that extracts calcium traces from light-field images of the mouse brain. SID is computationally efficient and can run on a standard workstation. In the mouse brain, the combination of SID and light-field microscopy allows calcium imaging up to 380 μm deep, at a 30-Hz volumetric imaging rate.

**Article p811**