# METHODS IN BRIEF

GENOMICS

## A functional assay for promoters

Gene expression is regulated by the carefully orchestrated interplay between enhancers, which bind transcription factors, and promoters, which convert the enhancers' signals into transcription. But what constitutes a promoter sequence that strongly responds to an enhancer, and how large can the dynamic range of this response be? Alexander Stark *et al.* systematically tested genomic sequences in *Drosophila melanogaster* for their ability to act as minimal promoters. Their self-transcribing active core promoter sequencing (STAP-seq) revealed up to 1,000-fold difference in gene activity with different minimal promoters coupled to the same enhancer. These data allowed the researchers to predict the strength of a core promoter from its sequence and will allow insight into the mechanism of transcriptional regulation. They will also enable the design of highly responsive transcriptional regulatory elements for high transgene expression.

Arnold, C.D. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3739 (2016).

IMAGING

## Count on FRET for stoichiometry

Determining protein stoichiometry in complexes is a long-standing challenge in biochemistry, and numerous *in vitro* approaches are useful for making such measurements. However, measuring complex stoichiometry in living cells represents a much more challenging problem. Ben-Johny *et al.* address this challenge using a FRET-based approach that exploits the fact that donor- and acceptor-centric metrics of FRET efficiency are differentially affected by the ratio of donor to acceptor molecules in a complex. Comparing the two FRET efficiencies allows the relative stoichiometry of two proteins to be determined. The researchers validated the method on concatemers of fluorescent proteins with known composition and used the method to study a long-standing question regarding calmodulin binding to ion channels under high and low $Ca^{2+}$ levels.

Ben-Johny, M. *et al. Nat. Commun.* **7**, 13709 (2016).

BIOPHYSICS

## Probing protein mechanics with an electric field

Proteins can be thought of as tiny machines that carry out mechanical functions in the cell. Such mechanics, however, can be very challenging to study. Hekstra *et al.* describe an approach to following conformational changes in proteins over time in response to applied strong electric field pulses, the idea being that large external electric fields exert forces that stimulate the movement of atoms throughout the protein. The method, electric field–stimulated X-ray crystallography (EF-X), uses timed, fast synchrotron X-ray pulses to capture a series of diffraction snapshots of a protein crystal of interest upon perturbation by electric field pulses. In applying the approach to a human PDZ domain as a model system, the authors observed conformational motions on the submicrosecond timescale that were similar to those observed upon ligand binding.

Hekstra, D.R. *et al. Nature* **540**, 400–405 (2016).

NEUROSCIENCE

## Optimized optical recordings of voltage sensors

Voltage sensors have been steadily improving, but they are still difficult to record with current optical technologies. Marshall *et al.* provide an optimized approach for optical recording of membrane voltage dynamics in freely moving mice. To remove motion artifacts, the researchers coexpressed and recorded a red fluorescent protein together with a green fluorescent voltage sensor. They sinusoidally modulated excitation of the two fluorescent proteins in order to deal with optical crosstalk between the fluorophores. Furthermore, they optimized a multimode optical fiber for their experimental setup, and used it for two-color illumination and recording. And finally, the researchers developed a custom unmixing algorithm to separate the fluorescent channels. They used this approach to analyze the contribution of two classes of dopamine-responsive neurons to local field potentials in the mouse striatum.

Marshall, J.D. *et al. Cell* **167**, 1650–1662 (2016).