IN BRIEF

SENSORS AND PROBES

Afterglow probes made easy

Jiang, Y. et al. *Nat. Commun.* **10**, 2064 (2019).

Autofluorescence background can be a challenge in biomedical imaging, especially in imaging of tissues and animals. Alternatives to fluorescent probes such as luminescent and afterglow probes circumvent this issue by bypassing illumination light and emitting long after autofluorescence has occurred, respectively. Although potentially useful, afterglow probes are typically made from nanoparticles containing rare-earth metals or heavy metals, which can be toxic. Jiang et al. developed an approach to turn conventional fluorescent dyes into afterglow luminescent nanoparticles (ALNPs). ALNPs have three parts: a photosensitizer initiator that converts photoenergy into singlet oxygen, a singlet-oxygen-reactive molecule that serves as the afterglow substrate and forms an unstable chemiluminescent intermediate, and a fluorophore that serves as the afterglow relay unit to accept energy from the intermediate, gradually releasing it as photons. The researchers demonstrated that their ALNPs could be used for sensitive imaging in mice. *RS*

https://doi.org/10.1038/s41592-019-0483-y

GENOMICS

Expanded CIBERSORTx

Newman, A. M. et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-019-0114-2 (2019).

To know which cell types make up a tissue is to know more about its basic biology and function. But probing the heterogeneity of a tissue or tumor is challenging. While the advent of single-cell RNA-seq methods has enabled transcriptional profiling of thousands of cells, throughput is still limiting. Newman et al. introduce a machine learning method that extends the authors' previous tool CIBERSORT, developed for the estimation of cell-type abundance from bulk RNA-seq data, to CIBERSORTx, which adds the inference of cell-type-specific gene expression levels. The tool 'digitally purifies' the transcriptome of individual cell types from the bulk data without requiring the isolation of single cells. The authors apply CIBERSORTx to various blood and epithelial cancers; homing in on melanoma, they connect certain phenotypic states with driver mutations and their response to immune cells. *NR*

https://doi.org/10.1038/s41592-019-0486-8

CELL BIOLOGY

Zapalog: a reversible dimerizer

Gutnick, A. et al. *Nat. Cell Biol.* **21**, 768–777 (2019).

Chemical inducers of dimerization (CIDs) are useful tools for bringing specifically tagged proteins together or for sequestering such proteins at a particular place in the cell. However, commonly used CIDs such as rapalogs do not afford spatiotemporal control over their action, and they result in irreversible dimerization of target proteins. Gutnick et al. describe zapalog, a light-sensitive dimerizer that undergoes photolysis upon blue light illumination. Zapalog consists of two handles, one for binding to the DHFR domain and one for binding to the FKBP domain. Thus, proteins of interest need to be tagged with these domains. The researchers used their tool to study mitochondrial motility in axons by controlling the interaction between mitochondria and kinesin. They found that one population of mitochondria was stably anchored at presynaptic sites while another pool of mitochondria was motile. *NV*

https://doi.org/10.1038/s41592-019-0484-x

CHEMICAL BIOLOGY

CRISPR inhibitors

Maji, B. et al. *Cell* **177**, 1067–1079 (2019).

Precise and fast control of the CRISPR–Cas9 system is desirable for many applications. Maji et al. present the first small-molecule inhibitors of Cas9, cell-permeable and stable compounds of less than 500 Da. The researchers screened a library of 2,652 compounds in an in vitro assay based on changes in fluorescence polarization after binding of Cas9 to a fluorophore-labeled oligo containing 12 PAM sites. Two molecules showed a dose-dependent inhibitory effect that was confirmed in two cell-based assays. Cas9 was blocked in its ability to cleave the target, and dCas9 fused to transcriptional activators or base editors was similarly inhibited. The researchers then tested 641 structural analogs to the most potent inhibitor in a cell-based assay—they looked for an absence of cytotoxicity and dose-dependent inhibition, and found two analogs with increased potency. Exploring the mechanism of inhibition for one compound, they saw that it does not interfere with the Cas9–sgRNA interaction but blocks the binding of the complex to DNA. The authors propose that their high-throughput screening assay will enable the discovery of inhibitors for other Cas species. *NR*

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