SENSORS AND PROBES

Dyes for visualizing voltage

Ortiz, G. et al. *J. Am. Chem. Soc.* **141**, 6631–6638 (2019).

The ability to sense changes in membrane potential or voltage in electrically excitable cells such as neurons and cardiomyocytes is crucial for understanding their biological activities, and numerous dyes and genetically encoded sensors have been developed to monitor these changes. Ortiz et al. describe voltage-sensitive fluorescent indicators based on a carbofluorescein scaffold. To develop these probes, the researchers used chlorination and sulfonation of the carbofluorescein scaffold to lower the pK_a and prevent cyclization to a nonabsorbing form, respectively. From there, they incorporated the chlorinated sulfone carbofluoresceins into phenylenevinylene molecular wire scaffolds for voltage sensing. The indicators show high sensitivity and are red-shifted relative to fluorescein, which makes them useful for two-color applications with green fluorophores. The researchers demonstrated the dyes' performance on in vitro–cultured neurons and HEK cells, and they note that these dyes can be synthesized from readily available reagents. *RS*

https://doi.org/10.1038/s41592-019-0447-2

BIOPHYSICS

Predicting RNA–protein binding affinity

Kappel, K. et al. *Proc. Natl Acad. Sci. USA* **116**, 8336–8341 (2019).

Interactions between RNAs and proteins play a role in most major cellular processes. Because of their importance, scientists have studied them experimentally at close to a genome-wide scale in order to better understand the rules governing these interactions and their associated energetics. They have also been modeled computationally, but this is challenging because RNAs can exist in multiple unbound states and RNA–protein interactions are often associated with large conformational changes. To address this challenge, Kappel et al. developed the Rosetta-Vienna RNP-ΔΔG method to predict RNA–protein binding affinities. This structure-based framework marries secondary-structure-based calculations of unbound RNA free energies and a unified energy function for bound complexes. The researchers showed that the predicted binding energies have low errors for a diverse set of complexes, and further validated the approach by showing accurate predictions for PUM2 binding to hundreds of RNA sequences. *RS*

https://doi.org/10.1038/s41592-019-0445-4

NANOBIOTECHNOLOGY

More accurate nanopore sequencing

Noakes, M. T. et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-019-0096-0 (2019).

Many of the promises of nanopore sequencing are being realized—DNA and RNA and their modifications can be directly sequenced at low cost and with little sample preparation—but there is still room for improvement when it comes to accuracy. As the nucleic acid makes its way through the pore, it is drawn by the current applied to the membrane, but its movement is slowed to discrete steps by a motor protein. During these steps in translocation, the degree to which the nucleotides within the pore block conductance is recorded and then computationally deconvoluted to yield a base-resolved sequence. However, irregular steps by the motor enzyme are sources of substantial error. Noakes et al. now correct for these irregularities by applying variable voltage to the membrane that complements the discrete stepping and allows the continuous probing of conductance at each nucleotide. They were able to improve the per-base accuracy from 62% with constant voltage to 79% with variable voltage. *NR*

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NEUROSCIENCE

Neuron segmentation with deep learning

Soltanian-Zadeh, S. et al. *Proc. Natl Acad. Sci. USA* **116**, 8554–8563 (2019).

Calcium imaging with two-photon microscopy is widely used to monitor neuronal activity in the brain. As the generated datasets are large, automated analysis methods are typically employed. Soltanian-Zadeh et al. developed a deep-learning approach to improve the accuracy of neuronal segmentation, which is the first step in automated processing pipelines. The Spatiotemporal NeuroNet (STNeuroNet) consists of a 3D convolutional neural network that can capture the spatiotemporal dynamics of calcium-based activity. To validate their approach and compare its performance to that of alternative tools, the researchers made use of publicly available datasets that they manually corrected for misattributed neurons. The researchers found that their method outperformed popular tools while maintaining a reasonable processing speed. The tool is available at https://github.com/soltanianzadeh/STNeuroNet. *NV*

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