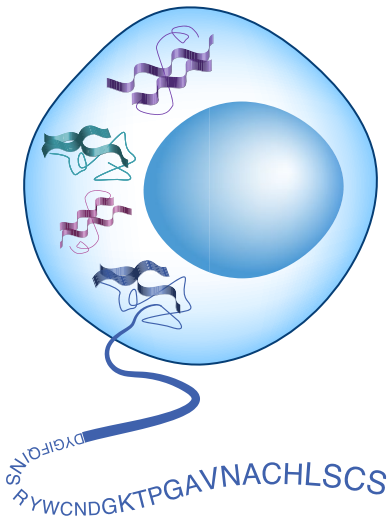
Single-cell proteomics

New technologies bring single-cell proteomics closer to reality.

Single-cell studies of the genome and transcriptome are all the rage as sequencing technologies are reaching maturity. No two cells are exactly alike; the only way to understand this biological heterogeneity, or to capture information from rare cells, is to profile them individually

Proteins represent the main functional machinery of cells, so how the expressed proteome differs from cell to cell is a question of high interest. However, capturing proteomic information from individual cells has proven to be a substantial technical challenge. Mass spectrometry—the workhorse technique of proteomics—enables near-complete proteomes to be detected and quantified, but such experiments are typically carried out with tens of thousands of cells or more. In a typical single mammalian cell, reliable analysis by mass spectrometry has been possible for only the most abundant proteins.

Newer approaches may soon bring change. Researchers in the mass spectrometry field are devising advanced sample preparation and separation approaches to reduce sample losses during processing, and therefore facilitate deeper, quantitative analysis of single-cell proteomes. Nanopore sensors, now an established technology for DNA and RNA sequencing on the single-cell level, are also being explored for protein sequencing—a bigger challenge, given the much greater complexity of amino acid chemistries in comparison to nucleic acids.



Proteomes of single cells may soon be routinely profiled. Credit: Marina Corral Spence/Springer Nature

Targeted, antibody-based approaches to protein profiling in single cells are also being increasingly applied on a broader scale. Mass cytometry, which relies on antibodies conjugated with rare-earth mass tags, now allows profiling of upward of 100 different protein targets in single cells in high throughput. In the Cell Atlas project, antibody-based immunofluorescence confocal microscopy was used to map the subcellular distributions of more than 12,000 human proteins across multiple cell lines (*Science* **356**, eaal3321; 2017).

Even an old method for protein sequencing—Edman degradation—is poised to make a comeback. By fluorescently labeling selected amino acids and imaging the decrease in fluorescence during consecutive rounds of Edman degradation, researchers recently showed that it is possible to obtain sparse peptide sequences that could be identified via matching to a reference protein database (*Nat. Biotechnol.* **36**, 1076; 2018).

We look forward to seeing intrepid methods developers turn these and other nascent ideas for single-cell proteomics into reality. ❐

Allison Doerr

https://doi.org/10.1038/s41592-018-0273-y

Building up bioluminescence

Improved luciferases and luciferins are poised to shine.

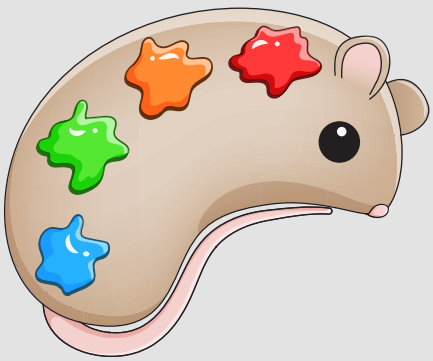
Tools like firefly and *Renilla* luciferase are commonly used in diverse assays. However, autocatalytic fluorescent proteins such as GFP still dominate in most imaging applications, especially those involving microscopy. Fluorescent proteins have the advantages of being independent of cofactor for fluorescence and having emission spectra that are narrow enough for straightforward multicolor imaging.

However, luciferases offer complementary advantages. Because these probes are not excited by light, bioluminescence imaging has minimal phototoxicity and succeeds at greater depths within tissue than imaging with fluorescent proteins. Bioluminescence is also uniquely suited to imaging in freely moving organisms. For these reasons, there has been a push in recent years to develop improved luciferases and luciferins.

One advance has come in the form of 'nanolanterns', which fuse luciferases with fluorescent proteins for bioluminescent resonance energy transfer to combine the power of luciferases with the spectral benefits and brightness of fluorescent proteins (*Nat. Commun.* **3**, 1262; 2012). Other groups have engineered improved luciferases that have higher substrate turnover, are brighter, or are color-shifted relative to their original versions. Alongside these developments, luciferins have been developed for optimal brightness, solubility, affinity, and color.

Perhaps the most useful of these combinations emit light in the near-infrared range, where tissues are most transparent (*Nat. Commun.* **7**, 11856, 2016; *Nat. Methods* **14**, 971–974, 2017). A stunning example of the power of these tools showed that an all-engineered, red-shifted luciferase–luciferin pair could be used to visualize individual tumor cells in the lungs of mice and to detect small numbers of neurons in freely behaving marmosets (*Science* **359**, 935–939; 2018).

Annother push to increase the versatility of luciferases depends on making them fully genetically encoded. This requires identification and expression of the enzymes involved in the synthesis of their luciferins—a challenging prospect because many aspects of these synthetic pathways are poorly understood. Fully genetically encoded bacterial luciferase systems have been developed (*Proc. Natl Acad. Sci. USA* **115**, 962–967; 2018), and headway made in this direction for other luciferases will greatly enable biological studies. Continued development of luciferases and their substrates will transform these tools into nearly ideal probes, especially for gentle imaging in living mammals. ❐



A diverse palette of bioluminescent tools will enable gentle imaging, especially in animals. Credit: Marina Corral Spence/Springer Nature

Rita Strack

https://doi.org/10.1038/s41592-018-0274-x