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Methods, techniques, assays and protocols

Applied biomedical research needs more of them to be more broadly useful, reproducible and robust.

The scientific process is a structured method, yet not one that is defined in detail. Scientific knowledge and scientific applications advance through rational guesses, observation, the formulation of hypotheses or theories, experimentation and computation, as well as recurrent testing, data analyses, validation and replication (not necessarily in this order).

Acquiring and refining scientific knowledge, and devising science-based solutions to problems, thus requires rationality, objectivity, empiricism, scepticism, peer review and many other scientific practices and values (with accountability and transparency becoming increasingly crucial). Robust methods are also required as well as painstakingly detailed protocols — that is, step-by-step instructions for carrying out a specific method or technique. Regardless of whether a method is referred to as an 'approach', 'a set of techniques', a 'workflow' or an 'assay' (or analogous wording, depending on the customs of the research area and the method's purpose; for instance, an assay usually refers to a test for the detection or quantification of molecules or substances or their activities), a method tends to be broader in scope than a protocol, and sufficiently flexible or modifiable to fit the actual research hypothesis, problem or set-up.

In applied biomedical research, methods and protocols are indispensable for unravelling the workings of biomedically relevant biological systems (molecular, cellular, and at the organ and whole-organism levels) and of mechanisms of disease, and for diagnosing conditions and devising treatments. Biomedical methods and protocols (including laboratory and clinical-trial protocols as well as standard operating procedures) can also serve as common communication and collaboration tools across disciplines, and the most widely used methods have contributed considerably to the most-cited research of all time (R. Van Noorden et al. *Nature* **514**, 550–553; 2014).

The degree of utility is an essential editorial consideration in how manuscripts that report methods are assessed at *Nature Biomedical Engineering*. We pursue the publication of methods (but not protocols, which editors at *Nature Protocols* often commission for recent papers reporting methods) that enable the acquisition of biomedical data or knowledge that were otherwise difficult to capture, that facilitate the efficient analysis of big biomedically relevant datasets, that address a clear biomedical, translational or clinical need, that would seem to have a broader appeal (because they would be applicable to multiple research areas, for instance), that advantageously surpass existing procedures (for example, they are easier, cheaper or more efficient to run or implement), or that enhance the utility of already broadly used methods. (Some of these considerations are also relevant to papers published in *Nature Methods*, yet the journal focuses on serving researchers actively involved in laboratory practice.)

In this issue of *Nature Biomedical Engineering*, we highlight eight methods that exemplify these utility considerations.

In one Article, Philipp Holliger and colleagues describe a method for the rapid discovery of antibodies with binding affinities in the low-nanomolar to mid-picomolar range, as they show for the antigens human interleukin-7 and human epidermal growth factor receptor 2. The method leverages array-based assays, next-generation sequencing and high-throughput screening of antibody libraries to probe of the order of 10^8 antibody–antigen interactions, in 3 days. The generated datasets can also be used to train machine-learning models that accelerate the antibody-discovery process. The method has clear broad utility in helping accelerate antibody discovery and the exploration of genotype–phenotype relationships.

Another high-throughput method for the discovery of biomolecules included in this issue serves a clear clinical need. The method allows for the large-scale mass spectrometric quantification of glycopeptides in blood plasma samples as potential disease biomarkers, as Markus Ralser, Christoph Messner and colleagues show by using it to quantify about 1,000 glycopeptide features in the plasma glycoproteomes from patients with COVID-19.

The discovery and development of molecular drugs benefit from knowledge of interactions between the drugs and drug transporters. In another Article in this issue, Giovanni Traverso and co-authors report a method for acquiring interaction profiles between orally administered drugs and intestinal drug transporters. The method requires the modulation of the expression of drug transporters in intact porcine tissue explants via the ultrasound-enhanced delivery of small interfering RNAs. Moreover, the authors used the drug–transporter relationships that they obtained to train a random forest model for the classification of the interaction profiles. Because drug transporters determine the rates of absorption and elimination of therapeutics, by taking into account their interactions with the intestinal transportome, this type of method combining ex vivo tissue and machine learning may help to accelerate the development and formulation of oral drugs.

Technologies for single-cell sequencing allow for the classification of cells into subgroups according to their characteristics and functionality. Yet, the functional profiling of single cells has been a methodological bottleneck, particularly for highly heterogenous immune cells. Lih Feng Cheow and co-authors report an assay for the profiling of the cytotoxicity of killer cells in relation to their cellular phenotype and cytokine secretion at single-cell resolution. It relies on the detection of an initially intracellular fluorescent protein that has been 'painted' by a nearby lysed cell on the surface of the lysing killer cell. The assay can be integrated with flow cytometry and single-cell RNA sequencing, and could also be used to analyse molecular pathways associated with cell cytotoxicity and to seek correlates of immune responses.

The secretions of immune cells can affect them and their neighbouring cells, yet identifying genetic regulators of the secretions involves the sorting of a large number of cells according to their secretion patterns. Shana Kelley, Edward Sargent and co-authors describe in an Article also included in this issue a high-throughput method leveraging microfluidics for the analysis of the secretion levels of large populations of immune cells. The method allowed the authors to discover highly co-expressed kinase-coding genes that regulate the secretion of interferon γ

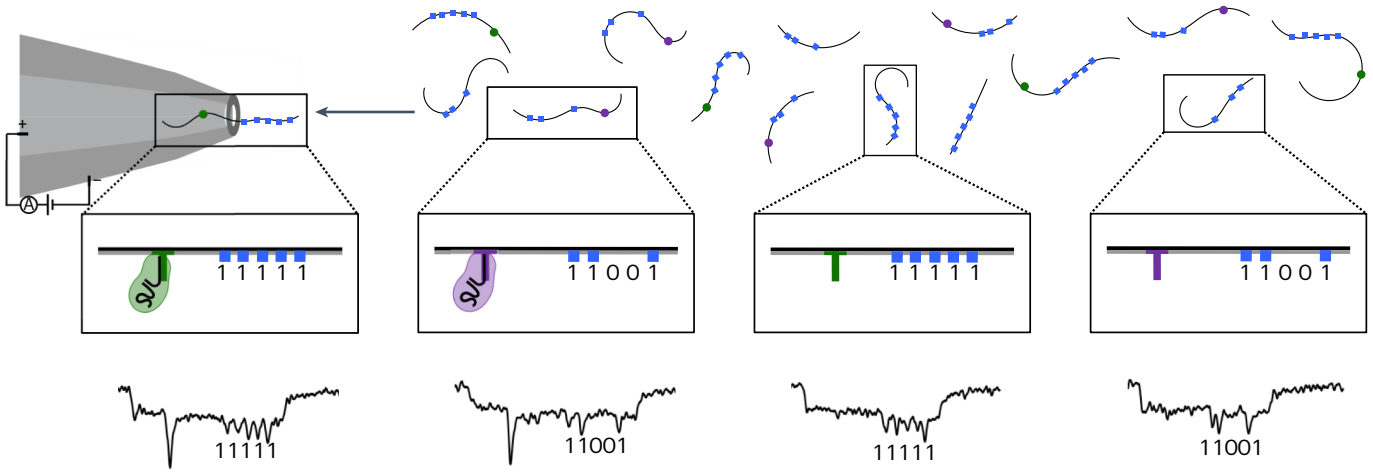

Fig. 1 | A method for sensing the DNA-mismatch tolerance of catalytically inactive Cas9. The schematic shows a solid-state nanopore (grey), two different DNA nanostructures (11111 and 11001) with two DNA overhangs (green and purple) and either with bound Cas9 (left) or without bound Cas9 (right) mixed together in solution, and the ionic-current traces (bottom) resulting from the translocation of the nanostructures through the nanopore. Figure adapted from the Article by Keyser and colleagues, under a Creative Commons license CC BY 4.0 .

by helper T lymphocytes, and may facilitate the discovery of therapeutic targets for autoimmune diseases.

Another microfluidic-based high-throughput screening method, described by Alan Wong and colleagues, enables new possibilities: the discovery of genetic and cellular drivers of the formation of syncytia (multinucleated cells resulting from cell–cell fusions) induced by the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The method takes advantage of droplet microfluidics and strategies for size-exclusion selection to screen (via large-scale mutagenesis and the genome-wide generation of gene knockouts via CRISPR) libraries of spike-variant-expressing 'sender' cells fusing with 'receiver' cells expressing the receptor angiotensin-converting enzyme 2 (ACE2). This method enables the exploration of any common and unique determinants of the virus-induced formation of syncytia.

This issue also includes an Article describing an extension to the utility of a widely used method. Seok-Hyun Yun, Sheldon Kwok and collaborators show that flow cytometry can be used to track and repeatedly measure the same cells using more markers and fewer colours, as the researchers show for three back-to-back cycles with more than ten markers per cycle. Such multi-pass high-dimensional flow cytometry takes advantage of cellular barcoding via microparticles emitting near-infrared laser light.

In another example of advantageous functionality, Ulrich Keyser and co-authors used DNA barcoding and solid-state nanopores to probe, with higher specificity and speed than had been possible, binding events between catalytically inactive Cas9 (the most used ribonucleoprotein in genome editing) and any pre-defined short sequence of double-stranded DNA. The method requires barcoded linear DNA with Cas9-binding double-stranded DNA overhangs that are sensed via changes in ionic current as the DNA translocates through solid-state nanopores (Fig. 1). Assessing the DNA-mismatch tolerance of catalytically inactive nucleases could inform diagnostic applications relying on the detection of single base-pair changes.

More important than the specific editorial rationale for why we published the methods included in this issue is the reasonable evidence of reproducibility and robustness that they provide. Indeed, validation of the findings with additional datasets or samples, the benchmarking of a new technique against established methods, the verification of the results against alternative methods, and replicability efforts by different experimenters (when possible, under blinded conditions) are a core part of the scientific method.

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