NANOPORES

No small matter

The confined geometry of nanopores enables a wealth of chemistry and analysis to be conducted at the single-molecule scale. Yi-Lun Ying, Aleksandar P. Ivanov and Vincent Tabard-Cossa report on recent developments discussed at the 2020 Nanopore Electrochemistry Meeting.

One of the most significant challenges in modern analytical sciences is the ever-growing need to characterize single-molecules and molecular assemblies with high spatial and temporal resolution. Nanopores are nanoscale-sized channels that address this challenge by providing a confined space for detecting single entities such as small molecules, nucleic acid polymers, proteins, viruses and nanoparticles using an electrical signal[1–3]. When a voltage is applied across the nanopore, its confined geometry forms a 3D sensing interface that is probed continuously by an ionic current. Molecules that are temporarily confined or transported through the nanopore can be identified by their current modulation. Various methods could be used to controllably construct nanopore structures in a range of soft and solid-state materials. These may include the self-assembly of proteins in lipid bilayers or block copolymer membranes, the drilling or dielectric breakdown of solid-state membranes, or even by simple pulling of glass capillaries down to nanoscale dimensions[3,4].

By confining single molecules, nanopore sensors could access information on the chemical and physical properties of molecular entities, which is remarkably wealthier than only looking at ensemble-average measurements. The approach has already proven its prowess for long-read nucleic acid sequencing and is making its first strides towards the formidable goal of achieving protein sequencing. Beyond sequencing, it offers an elegant solution to a diverse array of contemporary challenges, for example, by providing novel insights into outstanding ionic and molecular transport problems, controllable chemical synthesis, precision diagnostics and advances in green energy conversion.

The 2020 Nanopore Electrochemistry Meeting (9–14th October) provided an opportunity for scientists worldwide to share recent advances and exchange ideas on the growing number of potential applications that continuously drive the rapid development of nanopore analysis (Fig. 1). The virtual meeting was hosted and organized by Yi-Tao Long and Hong-Yuan Chen from Nanjing University, China and Mathias Winterhalter from Jacobs University, Germany. Over 800 participants from across the globe came together to attend over 60 presentations and discuss more than 1000 questions online. These discussions covered an extensive range of nanopore-related topics spanning across physical and life sciences, medicine and engineering. The speakers shared new insights on biological suprabiomolecular assemblies, bionics, functional (bio)materials, chemical reactions and unconventional transport in nano-confined environments. There was significant focus on emerging nanopore methods for controllable chemical synthesis, protein analysis, future precision diagnostics using molecular barcodes and carriers, biosensing and molecular information storage using DNA nanostructures, single-cell analysis, and for providing new insights into the dynamics of biological processes.

One exciting trend was the growing versatility of biological nanopores. The hallmark of the protein nanopore is the atomically defined interior, which offers a designable bioreaction interface and excellent control of the local environment. Hagan Bayley's team (University of Oxford,

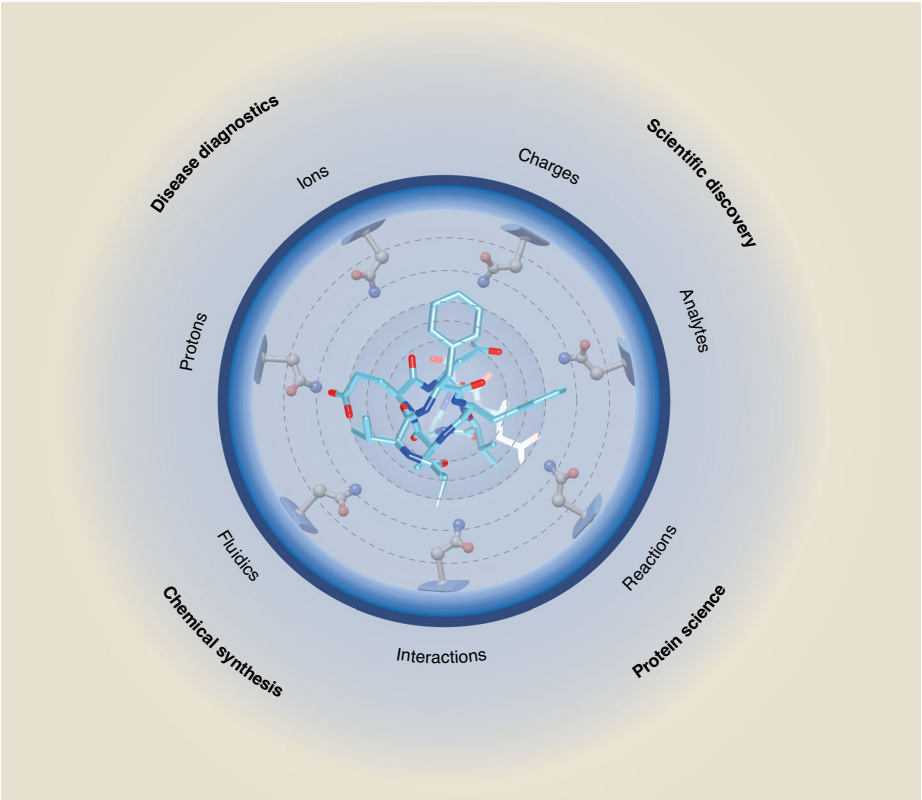

Fig. 1 | The 2020 Nanopore Electrochemistry Meeting discussed a wide range of topics. A new emerging domain in measurement sciences, nanopore electrochemistry, uses a nanopore to characterize single entities with spatial resolution down to sub-nanometre scale and temporal resolution reaching sub-microseconds. The electrochemical confinement of protons, ions, charges, analytes, reactions, interactions and fluidics inside nanopore channels offers a powerful toolkit for the analytical sciences and provides new insights into outstanding scientific questions in protein science, controllable chemical synthesis, and precision diagnostics.

UK) shared their latest results using an α-hemolysin nanopore as a "nanoreactor" for single-molecule covalent chemistry. When an analyte molecule covalently attaches to the reactive site on the pore interior wall, the chemical reaction can be inferred by a change in ionic current. The intermediates in a reaction can be registered and their lifetimes recorded, yielding the rate constant for all steps. In an organic chemistry context, Scott L. Cockroft (University of Edinburgh, UK) showed that nanopores could resolve rapid click reaction equilibria. Henry S. White (University of Utah, USA) discussed acid–base chemistry in base-flipping dynamics. Learning from the quantum-confined superfluid (QSF) effect in biological ion channels, Lei Jiang's group (Technical Institute of Physics and Chemistry, China) aimed to arrange single molecules in artificial nanopores to improve the efficiency of chemical reactions. Yunfei Chen (Southeast University, China) discussed dehydrated ion interactions in a highly confined nanopore solution. Chemistry under nanopore confinement thus permits many notable applications, including in situ site-specific modification of biopolymers, selective transmembrane signalling in synthetic cells and enantioselective catalysis. Nanopore confinement also enables the control of electrochemical phenomena such as permselective ion transport (when an ion is preferentially transported), gated transport and redox cycling. To this end, Paul W. Bohn (University of Notre Dame, USA) showed hierarchically organized nanopore electrode arrays to control the transport and reactivity of redox molecules.

Typical biological nanopores also provide some of the most advanced detectors for proteomic analysis at the single-molecule level. Juan Pelta (University of Paris-Saclay, France) described steps toward nanopore sequencing of proteins using a wild-type aerolysin nanopore, a powerful sensor for size discrimination of peptides. Together with Jan C. Behrends (University of Freiburg, Germany), they revealed that the aerolysin nanopore could identify single amino acid differences within a polyarginine carrier. By tuning the two sensing regions inside a mutant aerolysin nanopore, Yi-Tao Long's group (Nanjing University, China) demonstrated enhanced sensitivity of nanopores for identifying peptide post-translation modifications and DNA lesions. Giovanni Maglia (University of Groningen, the Netherlands) introduced a sequencing concept based on assembling a nanopore protein sequencer with a proteasome that unfolds the protein structures. Biological nanopores are naturally suitable to study protein–protein interactions (PPI) and protein conformational states. Liviu Movileanu (Syracuse University, USA) showed a sensor platform based on a tether, a peptide adaptor and a protein receptor that could identify low- and high-affinity PPI in a complex mixture. Proteins are folded into 3D shapes of different sizes and solid-state nanopores have diameters that can be tuned easily to study a wide range of protein structures and biomolecular complexes. Moreover, solid-state nanopores allow current recordings in harsh conditions such as high voltage (force), high temperature, extreme pH and strong denaturants. Cees Dekker (TU Delft, the Netherlands) presented "NEOtrap", a technique for long-term trapping (minutes to hours) of individual proteins inside a solid-state nanopore. A DNA-origami nanosphere is docked to a lipid-coated nanopore, forming a confined region and a highly charged environment where proteins can be trapped via electro-osmotic flow. Michael Mayer (Université de Fribourg, Switzerland) shared his vision of using polymer and lipid-coated solid-state nanopores for high-resolution characterization of single protein amyloid particles. Sébastien Balme (Université de Montpellier, France) described how conical track-etched nanopores, with their high aspect ratio, are well suited to characterize protein protofibril growth or degradation.

In terms of emerging healthcare applications, it was exciting to see several innovative applications employing solid-state nanopores. Amit Meller (Technion, Israel) shared the latest results from his lab's work towards using portable nanopore devices for rapid analysis of clinical samples. The technology allows for quantification of RNA expression based on the synthesis and single-molecule counting of gene-specific cDNAs without the need for amplification. Joshua Edel's and Aleksandar Ivanov's groups (Imperial College London, UK) demonstrated multiplexed microRNA detection directly in native clinical samples without the need for sample processing using electro-optical nanopore readouts of molecular carriers grafted with molecular beacons. Justin Gooding (University of New South Wales, Australia) adopted a different approach by using magnetic nanoparticle carriers to separate analytes selectively and performed nanopore detection of bioanalytes in whole blood. Vincent Tabard-Cossa (University of Ottawa, Canada) reported on a digital scheme capable of quantifying the concentration of a protein biomarker from serum using DNA nanostructures as proxies for the presence ("1") or absence ("0") of the target captured via a magnetic bead-based sandwich immunoassay.

The majority of these new solid-state nanopore technologies largely rely on molecular probes or carriers based on DNA nanostructures functionalized with recognition sites that enable selective detection of specific analytes. The carrier usually enables more-efficient transport and detection of analytes with a heterogeneous charge that may otherwise be challenging to sense. There are, however, other applications. Ulrich Keyser's group (University of Cambridge, UK) demonstrated how DNA nanotechnology could be used for information storage and data processing using nanopores. They showed how the density of information could be expanded by increasing the number and the structure of barcodes grafted to a long linear DNA carrier, including 4-way, 6-way and 12-way DNA junctions, that would correspond to logical bits with depths beyond 0 and 1.

While the COVID-19 pandemic forced many of us to distance physically, this virtual meeting's format can serve as a model for bringing the scientific community closer. A forum for early-career scientists and a training workshop for newcomers took place within the meeting to encourage the next generation of researchers in the nanopore field. The 2020 Nanopore Electrochemistry Meeting was just the start of many more international interactions within this diverse community: following the conference's success, the Nanopore Weekly Meetings were launched in late October and take place online every Monday. These meetings provide an open platform for researchers worldwide to share new results and exchange ideas on a variety of nanopore-related topics. ❐

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Competing interests

The authors declare no competing interests.