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Nanopore Electrochemistry: A Nexus for Molecular Control of Electron Transfer Reactions

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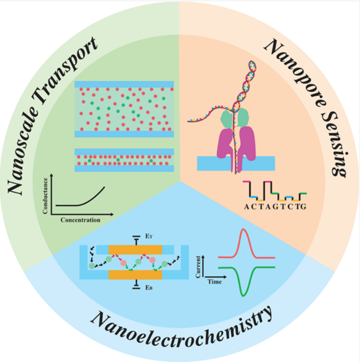
ABSTRACT: Pore-based structures occur widely in living organisms. Ion channels embedded in cell membranes, for example, provide pathways, where electron and proton transfer are coupled to the exchange of vital molecules. Learning from mother nature, a recent surge in activity has focused on artificial nanopore architectures to effect electrochemical transformations not accessible in larger structures. Here, we highlight these exciting advances. Starting with a brief overview of nanopore electrodes, including the early history and development of nanopore sensing based on nanopore-confined electrochemistry, we address the core concepts and special characteristics of nanopores in electron transfer. We describe nanopore-based electrochemical sensing and processing, discuss performance limits and challenges, and conclude with an outlook for next-generation nanopore electrode sensing platforms and the opportunities they present.



INTRODUCTION

Chemical measurement science benefited tremendously from the post-1960 emergence of sophisticated instruments coupled with automatic data analysis, which enabled researchers to separate, identify, and quantify targets at unprecedented levels.[1] Nowadays, the extensive overlap between fundamental research and practical applications[2,3] has brought within reach the ultimate goal of measurements which simultaneously exhibit ultrahigh sensitivity and selectivity in physical formats of reasonable cost and complexity. One powerful approach to realizing this ideal is to introduce mass-limited samples in ultrasmall confined volumes, sort the resulting mixtures, and quantify each entity one-by-one. Nanopores are biomimetic architectures that mimic the behavior of ion channels by confining targets to nanoscale volumes from which measurable signals can be generated *in situ*, thus recapitulating the key features of an ideal detection system.[4] In this Introduction, we trace the histories of nanopores and nanoelectrodes, the merging of the two streams in the early 2000s, and finally the genesis of nanopore electrode systems.

Pore-based analysis originated in 1953 with the resistive pulse detection scheme of Wallace Coulter,[5] which made possible the analysis of sub-μm particles and macromolecules in the Coulter Counter.[6,7] Soon after, the quest to characterize single biomolecules stimulated researchers to use nanopores commensurate in size with target analytes.[8,9] The human genome project then triggered the development of fast, cheap, and label-free DNA detection, and in 1996, Kasianowicz et al. detected single DNA strands using an α-hemolysin nanopore.[10] Follow-up work led to the emergence of solid-state nanopore sensors exhibiting performance characteristics comparable to those of biological nanopores.[11,12] Subsequently, Bayley and co-workers achieved single-nucleobase discrimination[13,14] leading Oxford Nanopore Technologies to launch a portable real-time DNA sequencing platform in 2014.

At the same time, enhanced understanding of nanofluidics opened the way to controlling molecular transport at unprecedented levels. Rice and Whitehead first described electrokinetic transport in nanoscale capillaries,[15] and the theory of potential driven flow was developed by Levine and co-workers.[16] During the 1980s and 1990s, fabrication of nanochannels became easier, less expensive, and more versatile.[17,18] In 1999, Ramsey and co-workers demonstrated the first in-plane nanoporous structure for sample preconcentration,[19] and later surface charge in nanocapillary array membranes was exploited to effect digital nanofluidic coupling between microfluidic chanels.[20] In 2004, Dekker and co-workers developed ion transport platforms that were governed by nanochannel surface charge.[21] Then, Yang and Majumdar developed ionic diodes, i.e., rectified nanofluidic ion currents.[22,23] Recently, Jiang and co-workers reported a biomimetic nanochannel sensing platform in which the response to ions and molecules is controlled by surface functionalization.[24]

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In contrast, nanoelectrodes (so-called ultramicroelectrodes) were developed in the 1980s, when electrochemists started

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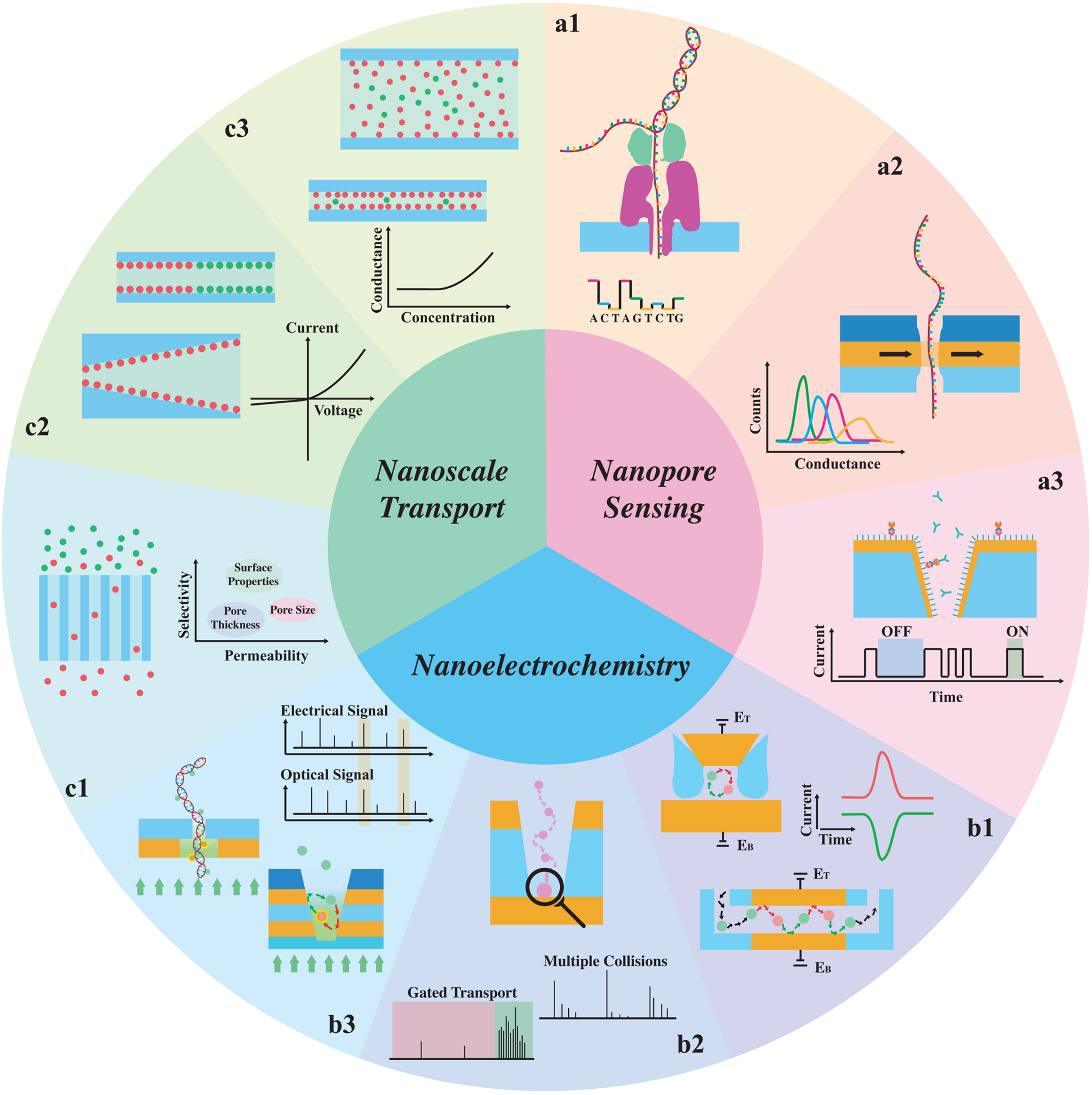

Figure 1. Schematic illustration showing recent progress in nanopore sensing (a), nanoelectrochemistry (b), and nanoscale transport (c), including nanopore-based DNA sequencing (a1); single nucleotide identification by nanopore tunneling current (a2); stochastic protein sensing by receptor modified nanopore (a3); single molecule detection by redox cycling (b1); multiple collisions or gated transport of nanoparticles in nanopores (b2); correlated optical and electrchemical analysis of single entities (b3); molecular sieving in nanopore arrays (c1); asymmetric nanopores and bipolar nanopore as ionic diodes (c2); and surface-charge governed ion transport in nanochannels compared to microchannels (c3).

using them to detect trace analytes and perform transient electrochemical measurements.[25−27] During the 1990s Martin's group developed a robust synthesis of pore-based nanomaterials;[28] Bard and Fan designed an elegant purely electrochemical detection of a single molecule;[29] and Murray's group observed the charging of gold nanoparticles (quantized capacitors).[30] Inevitably, nanopores and nanoelectrodes were combined to yield single nanopore electrodes, by Zhang and White in 2004.[31] Later, single nanopore electrodes and nanopore electrode arrays with well-defined, reproducible pore geometry and size were fabricated lithographically.[32,33] Compared with early work, in which electrodes were embedded in track-etched or anodic aluminum oxide membranes, these well-defined nanopore electrodes enabled an additional level of control over transport and reactivity that was exploited to yield enhanced nanoscale electrochemical measurements. Subsequently, the term "nanoelectrochemistry" was coined to describe phenomena ranging from fabrication and characterization of nanoelectrodes to the applications of nanoelectrodes as ultrasensitive tools for electroanalysis.[34−38]

Here, we examine the overlapping regimes of nanopore sensing, nanoscale transport, and nanoelectrochemistry, shown in Figure 1. We start by introducing the core characteristics of the nanopore electrode, review progress over the past decade, and finally discuss the remaining limits and challenges and propose the outlook for next-generation nanopore electrodes and electrode arrays. Because solid-state nanopores, unlike biological nanopores, can take advantage of mature nanofabrication processes, flexible choice of materials, and easily altered functionality,[39,40] they will be focus of this outlook.

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DEFINING CHARACTERISTICS

Benefits of Nanoelectrodes. Nanoscale electrodes exhibit enhanced mass transport, enhanced faradaic currents, and negligible *iR* drop during electrochemical measurements.[34] As shown in Figure 2a, the benefits of nanopore electrodes can be classified as follows. (1) Nanopore electrodes provide small confinement volumes, which significantly enhance collision frequencies with the electrode surface.[41,42] A molecule with a diffusion coefficient of 10^{-5} cm^2/s will collide with the wall of a 1000 nm^3 nanopore millions of times more frequently than in a 1000 μm^3 micropore. (2) Mass transport driven by unscreened electric fields is efficient and tunable at the nanoscale.[43,44] For example, the field strength between two electrodes with a 10 nm gap is 10^6 V/cm at $\Delta E = 1$ V. In addition, the direction of mass transport can be easily switched.[45] Nanopores can be fabricated to be size-commensurate with the Debye length, producing strong coupling between the solution ion distribution and the nanopore surface charge, i.e., permselectivity.[21,46] (3) Nanopores can serve as nanoscale reactors. Individually addressable electrodes can be inserted into the nanopore and used to control electron transfer processes, thereby achieving vectorial coupling of reactions.[47] (4) Stochastic phenomena dominate when nanopore electrochemical measurements involve only one or a few molecules, making it possible to resolve the dynamics of single electron transfer events.[48]

Fabrication and Surface Modification. While the fabrication of nanopore electrodes is based on well-developed approaches for nanofabrication, there are some issues that are characteristic of nanopore electrodes compared to nanobands, nanodisks, etc. First, electrodes are commonly embedded into substrates, typically as a multilayer film, before pore formation. In addition, nanopore electrodes are typically combined with confined cavities or fluidic channels. Both of these considerations determine the shape, size, and type of nanopore electrodes that can be fabricated. As shown in Figure 2b, nanopore electrodes are fabricated by one of two principal approaches. Highly precise single nanopores can be prepared at the sub-10 nm level by state-of-the-art fabrication approaches, such as focused ion beam (FIB) milling[11,49] or e-beam lithography (EBL).[12,50] In contrast, massively parallel approaches, such as nanoimprint lithography,[51,52] nanosphere lithography,[53,54] self-assembly of block copolymers,[55,56] and nanoporous alumina templates,[57,58] can produce nanopore arrays over large areas. Once formed, nanopores may be surface modified as long as (1) the surface remains inert to redox reactions in the applied potential window and (2) small charging currents are maintained. In addition, it is desirable if the structures admit versatile, yet specific, methods to modify the electrode surface. So far, noble metals, e.g., Pt or Au, and carbon-based materials, e.g., graphite, carbon nanotubes, etc., are the most widely used electrode materials, Figure 2c, and these admit a rich portfolio of surface modification approaches,[4] imbuing the nanopore, for example, with stimulus-response (pH,[59] temperature,[60] light[61]) characteristics or molecular recognition capabilities.[62,63]

NANOPORE ELECTRODE CAPABILITIES

The dramatic increase in research focused on nanopore electrodes and electrode arrays has resulted in a plethora of new and exciting capabilities for the chemical sciences—single entity electrochemistry, current and molecular rectification, scanning ion conductance mapping, concentration polarization, permselectivity, and correlated photonic and electrochemical measurements, to name a few. Rather than an exhaustive review, here we highlight a few of the forward looking nanopore electrode-enabled measurements that exhibit transformational new capabilities.

Single Molecule or Nanoparticle Electrochemistry. Single molecule electrochemistry is a holy grail, which is especially challenging due to the intrinsic Johnson noise at accessible gain−bandwidth conditions near 300 K.[64−66] Currently, there are two widely used strategies to detect single molecules electrochemically, both of which rely on current amplification by factors $>10^4$ to produce detectable currents in the fA range. Nanopore electrodes, especially those with two closely placed and independently addressable electrodes, are especially powerful in this context, as they support enhanced collision frequencies and thus large current amplifications. In the pioneering work of Fan and Bard, redox molecules were confined in the ultrasmall volume between a scanning electrochemical microscopy (SECM) tip and conductive substrate, thus facilitating fast and efficient redox cycling.[29] Subsequently, Sun and Mirkin used zeptoliter-volume recessed-disk electrodes to achieve quantized cyclic voltammograms of very few ($n < 3$) molecules, Figure 3a.[41] Recently, Unwin and co-workers reported a novel quadruple nanostructure electrode in which the current traces reflect the fluctuations from the oxidation and reduction of single molecules confined in the nanopore,[42] Figure 3b. Another current amplification strategy relies on catalysis. The high turnover rate of a catalytic site can convert non-redox-active substrates into redox-active products, producing detectable currents, enabling detection down to <100 molecules.[67,68] Importantly, the catalyst approach can extend the range of substrates to the detection of small numbers of nonredox active molecules, using designs in which the target molecule is sandwiched in a complex between component A bound to the electrode surface and component B, the catalyst that converts nonredox species into redox-active species.

Nanoparticles, vesicles, and droplets have also been addressed in single entity experiments, which are primarily focused on two aspects. First, the heterogeneity of electron transfer from individual nanoparticles or nanoclusters provides

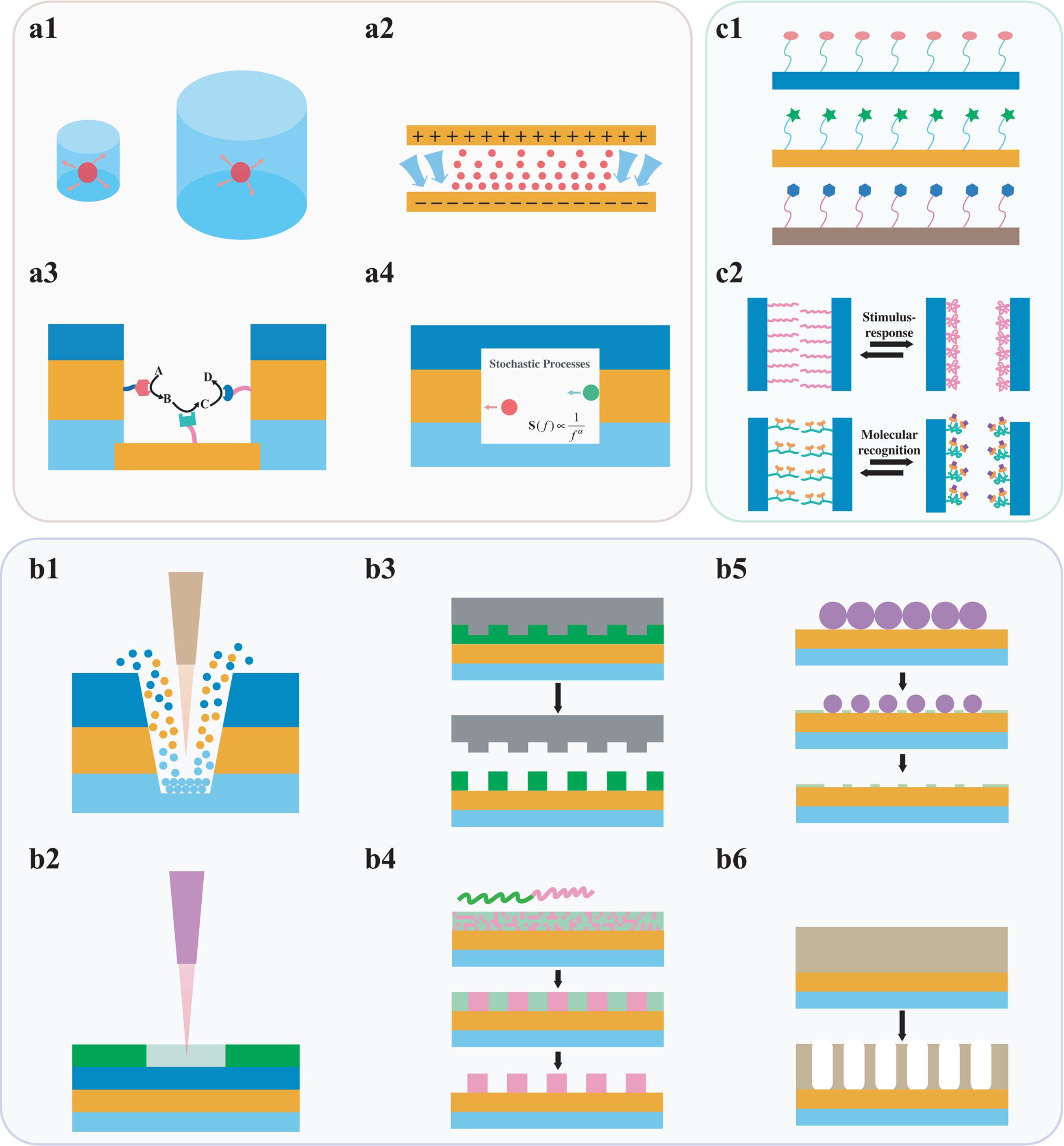

Figure 2. Schematic illustration of the defining nanopore electrode characteristics (a), fabrication methods (b), and surface modification (c) of nanopore-based sensors. Characteristic behaviors include the confinement effect (a1); strong electric fields (a2); vectorially coupled reactions (a3); and stochastic processes (a4). Pertinent fabrication methods include ion beam milling (b1); electron beam lithography (b2); nanoimprint lithography (b3); direct self-assembly of block copolymers (b4); nanosphere lithography (b5); and anodic aluminum oxide nanoporous templates (b6). Chemical modification strategies include orthogonal chemical processes for surface modification (c1) and nanopores with external stimuli, e.g., pH, temperature, ion strength, light, electric or magnetic field, and bioaffinity agents, e.g., protein, DNA, RNA, and metabolites (c2).

useful kinetic information that can be used, for example, to develop more powerful electrocatalysts.[69,70] Second, material released from vesicles or droplets provides a natural *ex vivo* mimic of extracellular release processes, e.g., neurotransmitters released from neurons.[71,72] Early work addressing single nanoparticle collisions was conducted on ultramicroeletrodes by Bard et al.[73] and others.[74−76] Since these early reports, it has become possible to sequester a few nanoparticles, or even one.[77,78] Recently, Long et al. reported an innovative nanopore bipolar electrode to control the dynamic self-assembly of gold nanoparticles,[79] Figure 3c. Similarly, White and co-workers proposed a super-resolution imaging method to map the trajectories of fluorescent nanoparticles around the tip of a nanopipette,[80] Figure 3d. These are just a few examples

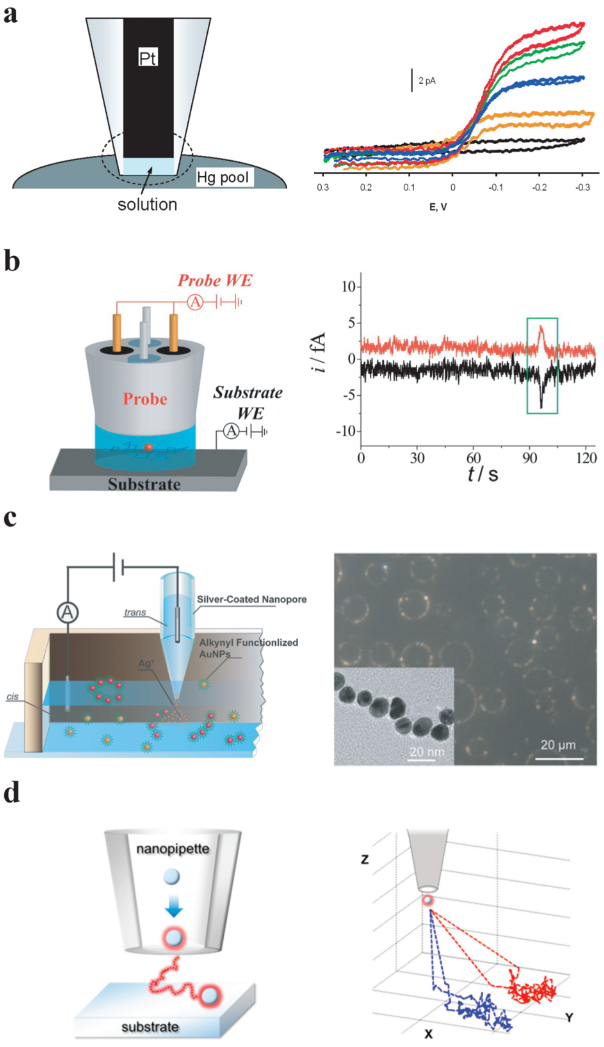

Figure 3. (a) Schematic illustration of a nanopore electrode immersed into Hg. The limiting current values of cyclic voltammograms correspond to zero (black), one (orange), two (blue), three (green), and four (red) molecules. (b) Schematic illustration of four electrode configuration, where the molecules are confined within the nanogap electrochemical cell. Current−time plots are from carbon working electrode (red line) and substrate working electrode (black line), where symmetric peaks indicate a single molecule event based on highly efficient redox cycling. (c) Schematic illustration of the self-assembly of gold nanoparticles (AuNPs) at the tip of a nanopore electrode, where the dark-field (20 μm scale) and TEM (20 nm scale) images present several and a cornel of microcyclic AuNP structures, respectively. (d) Schematic illustration of the manipulation of the fluorescent nanoparticle by nanopipettes, where the trajectories of each nanoparticle are captured in real time by the electron multiplied CCD detector for three-dimensional super-resolution imaging. Panel a reproduced with permission from ref 41. Copyright 2008 American Chemical Chemistry. Panel b reproduced with permission from ref 42. Copyright 2015 American Chemical Society. Panel c reproduced with permission from ref 79. Copyright 2017 Wiley-VCH. Panel d reproduced with permission from ref 80. Copyright 2017 American Chemical Society.

illustrating the broad interest in single entity electrochemistry; readers may refer to recent comprehensive reviews for additional details.[81−84]

High Density Nanopore Sensing Array. The experiments above highlight the push to single entity level detection in a single nanopore electrode. A natural extension is multiple nanopore electrodes on one device, i.e., nanopore electrode arrays (NEAs), either to enhance signal without losing the unique features of nanoscale electrode or to operate as multiplex sensors.[85,86] In order to avoid the problem of overlapping diffusion profiles in high density nanopore arrays,[34] Bohn and co-workers fabricated high density NEAs with two closely placed intrapore electrodes, Figure 4a, so that reversible redox couples undergo coupled reduction and oxidation reactions at oppositely biased top and bottom electrodes. The collection efficiency of redox species for both electrodes is close to 100%,[87,88] which results in both greatly enhanced redox cycling and selectivity,[89−91] Figure 4b. Furthermore, electrochemical events can be efficiently converted to optical, e.g., fluorescence, readout by coupling the redox cycling signal to a distal reporter cell with a bipolar electrode.[92] Recently, a high porosity permselective membrane was integrated with an NEA to mediate molecular transport, enhancing the selectivity to analytes of different charge.[93] The permselective membrane serves as an ideal ion gate, controlling the access of charged analytes to the nanopore. Rectified redox cycling currents have also been observed raising the possibility of ionic diode functionality.

> The dramatic increase in research focused on nanopore electrodes and electrode arrays has resulted in a plethora of new and exciting capabilities for the chemical sciences—single entity electrochemistry, current and molecular rectification, scanning ion conductance mapping, concentration polarization, permselectivity, and correlated photonic and electrochemical measurements, to name a few.

Correlated Electrochemical and Optical Detection. Direct electronic detection of quantized events characterized by the passage of a few electrons is limited by the Johnson noise floor. In contrast, shifting to the more tractable problem of photon detection would allow the sensitivity issue at low analyte numbers to be addressed.[94−97] To achieve this, the bottom ring of dual-ring NEAs can be used both as a working electrode and as the optical cladding layer of a zero mode waveguide (ZMW).[98,99] The resulting electrochemical ZMWs (E-ZMWs) are ideal systems to investigate the singe molecule spectroelectrochemistry[100] and have been used to probe single molecule dynamics of immobilized[101] and freely diffused enzymes[102,103] of the fluorigenic flavoenzyme monomeric sarcosine oxidase, by modulating the fluorescence ON and OFF with applied potential, Figure 4c. The electrochemical and fluorescence signals are correlated, revealing single molecule fluctuations across the nanopore array. This method holds great promise for the study of vectorially coupled enzyme reactions at single molecule sensitivity.

■ CHALLENGES AND LIMITS

As successful as nanopore electrochemical structures have been, there exist both practical and fundamental limits to perform-

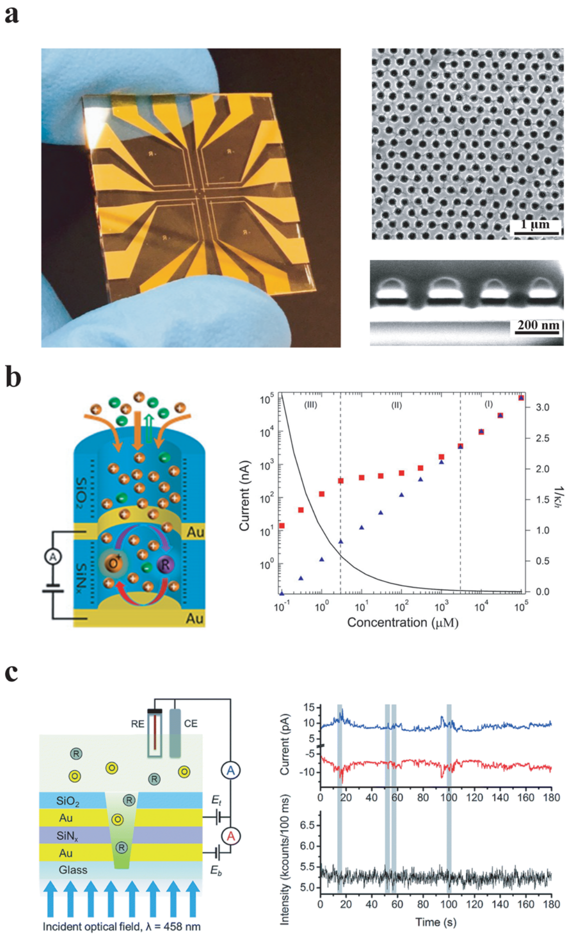

Figure 4. (a) Photo of 8 pairs of nanopore electrode arrays (NEAs) on an electrochemical chip, where SEM images indicate the plan view (top) and side view (bottom) of NEAs, respectively. The gray, white, and black layers in cross sectional SEM image are SiO_2, gold, and SiN_x, respectively. (b) Schematic illustration of ion migration and accumulation in NEAs, as demonstrated by large current amplification at low ionic strength. (c) Schematic illustration of dual-ring NEAs serving as E-ZMWs, where the voltage-sensitive dye molecules are only excited inside the nanopore. The electrochemical and fluorescence signals are correlated, revealing single molecule population fluctuations across the nanopore array. Panel a reproduced with permission from ref 91. Copyright 2017 Royal Society of Chemistry. Panel b reproduced with permission from ref 90 (Copyright 2016 American Chemical Society) and ref 89 (Copyright 2014 American Chemical Society). Panel c reproduced with permission from ref 103. Copyright 2017 Royal Society of Chemistry.

ance. The resulting challenges and limits constitute a list of attractive candidates for new breakthroughs.

High Bandwidth Limits and Current Fluctuation. One of the stiffest technical challenges is to measure ultrasmall currents (<1 pA) at bandwidths (>1 MHz) corresponding to nanopore residence times at reasonable cost.[104] Even though 100 kHz is sufficient for stochastic experiments of most small target molecules, biomacromolecular targets such as DNA or proteins require higher frequencies to distinguish internal composition.[105] Achieving these performance goals will require careful device design that simultaneously maximizes sensitivity while minimizing parasitic capacitance.[106]

Nanoelectrode Design and Fabrication. Despite substantial improvements in nanofabrication, there is still an urgent need to develop methods to fabricate nanopore electrode structures with sub-10 nm feature sizes. Direct-write techniques, such as FIB and EBL, are limited to lab-scale structures. In addition, FIB milling implants conductive impurities, leading to current leakage problems, especially at high frequency.[107,108] EBL is limited by e-beam scattering during exposure, the development of e-beam resists after exposure, and subsequent pattern transfer to the underlayer.[109,110] Nanotemplate-based parallel processing approaches, e.g., nanoimprint lithography, nanosphere lithography, and block copolymer nanotemplates, are promising, but they need further development to provide high precision nanopatterning at production size scales.[111−114]

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Specificity and Multiplex Sensing. Another significant factor affecting performance of nanopore electrode systems is the specificity between target and interferences. One straightforward approach is to exploit biomolecular recognition at the surface of a nanoelectrode.[115,116] However, surface modification inside the ultrasmall confined volume of a nanopore is still tedious and inefficient, and nanopore electrode sensing constructs must ensure efficient electron transfer, even after surface modification—a particularly challenging problem for biorecognition motifs, such as enzymes.[117,118] If these problems can be solved, then multiple sensing units may be realized within a single nanopore to effect vectorially coupled reactions, or alternatively to differentially modify different regions of nanopore arrays for high throughput multiplex sensing.

■ NEXT-GENERATION NANOPORE ELECTRODES

Nanopore-based and nanopore electrode based sensors have benefited from the growth and maturation of nanotechnology. In linear succession, Wallace Coulter's 1950s idea of counting particles in a fluid was followed by Richard Feynman's oft-quoted 1959 essay "There is Plenty of Room at the Bottom",[119] which had a tremendous catalyzing impact on the scientific community. The technological developments flowing from these two seminal events were the intellectual ancestors to the human genome project, and after two decades the $1,000 genome has ushered in the era of personalized genomics and precision medicine.[120] What will come next? Nanopore electrode systems are certainly poised to be integrated into contemporary point-of-care devices—not only reading DNA but identifying a range of proteomic and metabolomic biomarkers related to human health and wellness.[121−123]

Incorporating new passive and active electrode materials is one area for potential elaboration of nanopore electrode characteristics. Over the past decade, solid-state nanopores and two-dimensional nanopore arrays have advanced to exhibit excellent performance, in some cases competing with biological nanopores. However, the insertion of electronic components into nanopore systems has the potential to extend the contemporary capabilities to efficiently control molecular transport, directly monitor electron transfer processes, and rapidly record electrical signals.[124−126] Gold and carbon are dominant electrode materials in nanopore electrode systems, and there are a number of interesting examples using carbon nanotubes or, more recently, graphene as electrode materials in nanopores.[127−129] However, there are now a myriad of newly characterized two-dimensional materials, e.g., molybdenum disulfide (MoS_2)[130] and hexagonal boron nitride,[131] promising candidates that exemplify new opportunities for nanopore sensing.

Lastly, the emergence of novel transport-reaction models suitable for application at the nanoscale and the development of powerful simulations together provide experimentalists with a direct way to predict the performance of new nanopore electrode sensors before testing as well as a way to assess performance afterward.[132,133] The modeling of graphene-based nanopore sensors is just one example.[134−136] Calculations can guide optimization of the number of graphene layers, pore diameter, and graft density of surface functional groups before fabricating graphene nanopores in the lab.

> Nanopore electrode systems are certainly poised to be integrated into contemporary point-of-care devices—not only reading DNA but identifying a range of proteomic and metabolomic biomarkers related to human health and wellness.

The topics highlighted here necessarily represent only a small fraction of the innovative work at the nexus where nanopore electrodes connect electron transfer and molecular control. Overall, there is a great deal of synergy in the opportunities before the nanopore community, and the exciting new directions that nanopore electrochemistry is poised to take should lead to a bright future and even more transformative surprises.

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Notes

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