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Perspective
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Perspective and Prospectus on Single-Entity Electrochemistry

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ABSTRACT: Single-entity electrochemistry (SEE) describes a recent trend in state-of-the-art electrochemistry applied to the study of individual "things." Conceptually, SEE covers fundamentals and applications of SEE, as well as methods and tools to make SEE measurements. SEE is especially appealing, as it unifies different branches of electrochemistry and comingles diverse approaches and techniques toward similar goals. In this Perspective, motivations and advantages of SEE are considered. A brief historical perspective and overview of recent ideas and directions in research in SEE are considered. In closing, future challenges, opportunities, and destinations related to SEE are discussed.

At the most basic level, single-entity electrochemistry (SEE) refers to the study of one "thing" at a time by electrochemical means. SEE is an overarching concept in measurement. The "thing" measured could be anything (a cell, a molecule, a reaction) that represents a unit of interest. A diversity of topics, which includes entities from fields such as cellular biology, catalysis, and organic chemistry, means SEE is inherently interdisciplinary, seeking to answer questions from all corners of science. Length scales for SEE measurements can include entities and processes 100s of micrometers to angstroms in size (Figure 1). A unifying theme of SEE is the measurement process, be it the instrumentation, the method or the data analysis used to extract information at the level of a single-entity. At its core, SEE is electroanalysis at the ultimate limits of our ability. The origins of SEE are sprinkled through the last decades of research. Starting as early as the 1950s, and becoming more prevalent with the advent of microelectrodes in the 1980s, SEE has sought to push the limits of electrochemical measurement to ever shrinking size scales.

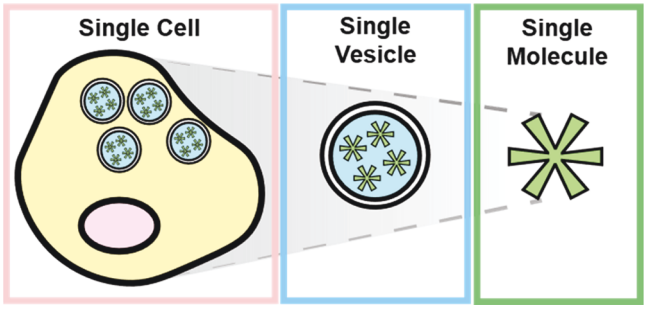

Figure 1. Single Entities. A single-entity is a "thing" that is measured one "thing" at a time. For example, one may desire to investigate neurotransmitter concentrations. Use of electrochemistry to measure the neurotransmitter concentrations associated with each of these "things", i.e., the amount of a neurotransmitter in a single cell, the amount of neurotransmitter in a single vesicle, or a single neurotransmitter itself would constitute "single-entity electrochemistry" or a "SEE" measurement.

A 2016 Faraday Discussion (organized by Unwin, Bartlett, Fermin, Gooding, Koper, and Vincent) coined the name "single-entity electrochemistry",[1] and prompted electrochemists to codify the concept of SEE as a special area of inquiry.[2−4] Thus, SEE has begun to take corporeal form as an organizing or a unifying theme in electrochemical measurement, and has been the subject of additional recent meetings, editorials[5−7] and reviews, and original research.

This perspective considers why we should make SEE measurements, pioneering experiments of the concept, recent triumphs, and a prospectus of the future for SEE measurements.

WHY SHOULD WE STRIVE TO MEASURE SINGLE ENTITIES VIA ELECTROCHEMISTRY?

There are two layers to this question. First, why should we measure single entities? Second, if we postulate such measurements are important, why use electrochemistry to do so?

1. To Separate Individual Responses from the Bulk. The most obvious reason is to determine what individual (single-entity) responses contribute to the bulk (ensemble) response typically observed. If we understand how a single-entity contributes to the ensemble response, an understanding of the underlying chemistry (or biology/physics/materials science) can be better determined. An example of this is the measurement of a catalyst. If we understood exactly how a single molecule turned over at a single catalyst, we could conceivably optimize the chemical transformation catalyzed. This example also underscores the difficulty in single-entity measurements. If we truly want to understand a single catalytic reaction as described, measuring a single particle, a single molecule, a single reaction would represent the ultimate in terms of characterization, and presents an incredible challenge that is often impossible with present measurement techniques. Further, making measurements at these scales in an innocent, nonperturbative manner can provide additional challenges. The example of a catalyst above also demonstrates the importance of statistics, populations and reproducibility in SEE measurements. Providing context for an individual SEE observation of a large number of comparable, repetitive measurements is important to extract meaningful information, and requires attention to rigor in analysis.

Dynamic SEE measurements are also important. For an ensemble measurement, convoluting time on top of a

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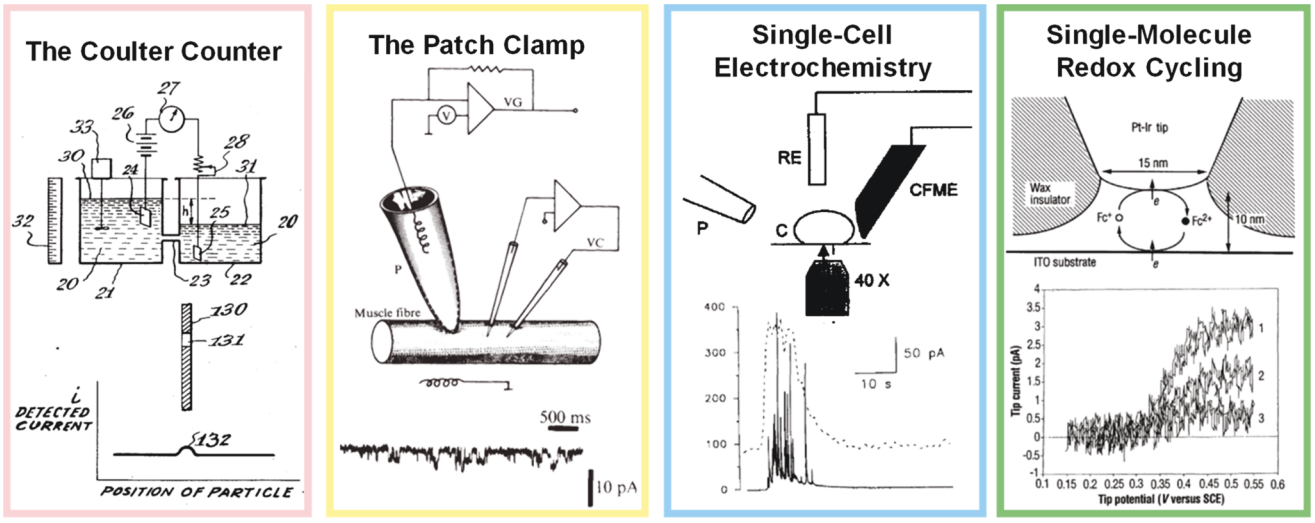

Figure 2. Pioneering experiments of single-entity electrochemistry. The Coulter Counter. An illustration from Coulter's patent for particle counting via resistive-pulse sensing. Two fluid reservoirs are connected by a narrow sensing channel. An ion current is generated by applying a potential difference across the sensing channel. Displacement of fluid in the small channel by analyte results in a transient change in current amplitude, to allow counting of single entities. **The Patch Clamp.** A glass pipette is sealed to the membrane of a cell to allow measurement of individual ion channels. An ion current trace for ion channel activity stimulated by addition of suberyldicholine is shown. **Single-Cell Electrochemistry.** A carbon microfiber electrode is placed near a cell of interest. Stimulation of the cell by a nearby ejection pipette invokes cellular release by the cell which is monitored by voltammetry or amperometry (shown bottom) at the carbon fiber. **Single-Molecule Redox Cycling.** An insulated nanoelectrode tip is moved close to a larger substrate electrode to create an ultrasmall electrochemical chamber. Trapping redox molecules in between the tip electrode and substrate electrode allows cycling and electrochemical amplification for the detection of single molecules, as exhibited by the potential dependent current response shown. Portions of figures adapted with permission from References 9 (United States Patent & Trademark Organization), 13 (Springer Nature), 17 (American Chemical Society), and 21 (American Associate for the Advancement of Science).

population, especially if the population undergoes dramatic change during an experiment, provides challenges in interpreting data. However, if we can follow a single entity temporally, a deeper understanding can be obtained. A recent review from White and co-workers underscores the advantages of nanoscale electrochemical measurements in dynamic electrochemical studies and expounds on the most significant challenges of making fast, low current measurements.[8]

2. To Enable New Concepts in Electrochemical Measurement. The chance to measure single entities can open the path to completely new types of measurements. An example of this is if we consider the transition from a filtration membrane to DNA sequencing by protein channels. At first glance this may seem unrelated, but what if—instead of separating an ensemble of molecules through a filtration membrane—one sought to pass a single entity through a single pore? What other experiments could be realized? The Coulter counter (described below) used this approach to realize single-entity measurement with micron scale single pores to characterize single cells. The concept was extended further in recent years to realize a method to sequence individual nucleic acids as they travel through a single protein channel. Taking the concept of a membrane that filters at large scales to the scale of a single-entity, thus realizes completely new modes of interrogation.

Because SEE measurements often operate at very small size scales, size dependent phenomenon that emerge can also lead to new methods of measurement. Small electrodes lead to different properties for electrochemical measurements, particularly when diffusion and capacitance are considered, which can change the scope of measurement, for example, the time scales, that can be accessed. Further, when merged with other fields, for example plasmonics in SEE nanoparticle measurements, nanoscale phenomenon wholly absent at larger scales can be exploited.

3. Because We Can. SEE measurements often represent an ultimate challenge. Pursuit to test the limits of what can be measured is often compelling justification, as continual surprises are revealed as we increase the sensitivity, range, time scale and statistics of measurement. This sentiment must not be lost in the modern world of translational science, where clear applications are often required. Fundamental, basic research and scientific inquiry driven by curiosity remain vital to scientific growth. Basic research is key to opening new, original lines of inquiry and real paradigm shifts in science. Seminal discoveries with immense contributions to society (e.g., penicillin, magnetic resonance spectroscopy, conducting polymers) underscore such contributions. As SEE opens the door to new views at exceedingly complex, small and fast scales, a better understanding of the natural world around us will surely emerge with impact we have yet to envision.

WHY USE ELECTROCHEMISTRY FOR SINGLE-ENTITY MEASUREMENTS?

Nonelectrochemical methods can also be used to make single-entity measurements, most obviously spectroscopy, but electrochemistry is attractive for a number of reasons. First, the process of moving an electron or ion from one entity to another is a key step in a multitude of important chemical processes. To probe the movement of charge for a single entity is fundamentally an electrochemical process and is at the heart of SEE. Second, electrochemistry has the inherent sensitivity required to make single-entity measurements. Advances in electronics for electrochemistry and electrophysiology have

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provided basic tools that can be employed for SEE. Through clever electrochemical methods, for instance redox cycling (vide infra), SEE measurements can be amplified to the levels of single molecules. And third, the semiconductor industry, a huge technology driver, has an insatiable need to go to faster, smaller, more reliable materials and devices. The tools of semiconductor manufacturing go hand in hand with tools for SEE and provide new routes to SEE experiments. Photolithography, focused-ion beams, reactive-ion etching, chemical vapor deposition, and many other fabrication tools have been key to materials and methods of SEE.

Pioneering Experiments of Single-Entity Electrochemistry. The invention of scanned probe microscopies, the nanotechnology boom, and the need to make quantitative biochemical measurements at the level of single cells or even single molecules has created an environment in which SEE measurements flourished. There were many early advances, but four key experiments, the Coulter counter, the patch-clamp, single-cell electrochemistry and single-molecule redox cycling (Figure 2) have proven especially inspirational in providint a glimpse into what could be achieved, and especially in the case of the Coulter counter and patch clamp, proving that such approaches had real-world application. These four experiments are considered below.

The Coulter Counter. In 1949 Wallace H. Coulter filed a patent titled "A method for counting particles suspended in a fluid", which described what eventually came to be known as the Coulter Counter (Figure 2).[9] The device measures ion current which flows between two electrolyte filled chambers that are connected by a small central channel. In Coulter's original description, a pressure difference was generated by the difference in fluid height between the two chambers. The pressure difference generated a fluid flow from one chamber to the other through the central channel. When a single particle of appropriate size was carried through the central channel, the momentary change in the volume of electrolyte in the channel resulted in a change in the current measured, which allowed for individual particles to be counted, providing a very early example of a SEE measurement. Early on the concept was extended to characterizing the size and shape of biological cells and eventually viruses and is in fact used today in clinical and laboratory settings on a regular basis. The Coulter Counter is conceptually simple and can generate statistically relevant data by measuring the passage of many particles. Perhaps most impressively, Coulter's device lives today in practice as a commercial product, with applications in biomedical and research settings for characterizing and counting individual cells.[10] Decades later, the Coulter Counter concept would be extended further, especially to biological macromolecules, as described in more detail below.

Patch-Clamp Electrophysiology. Although technically electrophysiology, the patch-clamp[11,12] approach to studies of single ion channels has been hugely influential in electroanalytical chemistry. This award-winning technique, first described by Neher and Sakmann in 1976[13] showed at a relatively early time the opportunity to measure single proteins, in the form of ion channels. In the patch clamp a glass pipette is sealed to a section of a cell membrane to allow electrical measurement of ion currents through individual cells or ion channels. Because of the importance of ion channels, the biological implications of science learned from this approach have been immense. A multitude of variations in configuration of the original patch clamp experiment have been realized, with voltage and current clamp protocols, as well as different patch configurations at use today. Efforts beyond single pipette patches toward automated patch clamp methods have been somewhat effective in developing routes to high throughput screening of ion channel and receptor activity.[14,15] Importance for this work was magnified outside of the electrophysiology community, where electrochemists quickly realized the utility of high bandwidth, low noise current amplifiers and automated machines for pulling pipettes in electrochemical studies.

Single-Cell Electrochemistry. More recently (1982), Wightman and co-workers described single-cell electrochemical measurement of neurotransmitters at single chromaffin cells[16] that sparked significant subsequent efforts in SEE. Key to these experiments was the development of microscale electrodes, which could be positioned very close to a cell of interest.[17] The cell was then stimulated by release of nicotine, which resulted in release of catecholamines from the chromaffin cell, that were electrochemically detected at the microelectrode. Catecholamines measured in this manner were commonly recorded by both fast-scan cyclic voltammetry (for species identification) and amperometry (for quantitative temporal release). This seminal report underscored the need for instrumental advances to realize SEE measurements. Here, carbon fiber microelectrodes were needed to position the electrode at distances close enough to catch catecholamines released as they diffused away from the cell. The low capacitance of microelectrodes also allowed fast measurements necessary to quantify release. Additionally, advances in electronics and hardware were required to realize fast data acquisition with fast-scan cyclic voltammetry. This work opened the door to a significant number of cell types, releasates and biological measurements related to neurotransmitter release from single cells, and eventually even single vesicles.[18,19]

Single-Molecule Redox Cycling. The development of scanned probe microscopies was a key step in the nanotechnology revolution. Bard and co-workers developed the most popular electrochemical scanned probe, the scanning electrochemical microscope (SECM),[20] which has been a pivotal tool for micro and nanoscale electrochemistry. In 1995, Fan and Bard described a SEE experiment based on SECM that created generator-collector electrode experiments carried out at the scale of a single redox molecule.[21] In these experiments, a nanometer scale sharpened metal tip was insulated in wax and brought to a conducting surface (an electrode). Under appropriate conditions, a small volume of solution with a single redox molecule (a ferrocene derivative) could be trapped in a pocket formed by the surface, electrode and wax insulation. With the electrode tip poised at an oxidizing potential, the single ferrocene species was oxidized to the ferrocenium species. The ferrocenium species then diffused to the surface electrode and was reduced to the original state, providing an oxidation−reduction cycle (redox cycling). For conditions used in these experiments, a single molecule could undergo 10^7 roundtrips between the two electrodes in one second, to give 1 pA of current flow, and allowed detection of single electrochemically active molecules for the first time. This experiment challenged technical limits and reinforced a key tool for SEE measurements, namely chemical amplification of redox reactions, in this case realized by diffusional trapping. Such amplification has proven key in a number of more modern SEE techniques. These types of experiments have also pushed the limits of instrumentation and experimental design.

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In particular, signal/noise ratio and instrument bandwidth have constantly been challenged by SEE approaches. Presently, significant challenges in addressing these limits remain.

These four early experiments exemplify conceptual leaps and inspiration to what could be achieved with SEE experiments. Pivotal to each of the experiments were both a realization of an insightful design in measurement and advances in hardware and tools for the measurement. This spirit has persisted and remains key to SEE advances pursued today.

MODERN DIRECTIONS IN SINGLE-ENTITY ELECTROCHEMISTRY

More recently, within the context of previous experiments, SEE has flourished. The most prominent areas of SEE are highlighted below, each presented as a vignette at a high-level overview. Since exhaustive reviews of each area have previously been prepared, the vignettes are not comprehensive in scope of discussion, but illustrative of the directions presently being studied. (More detail on unique areas is present in recent comprehensive literature reviews referenced in each vignette.) The areas described are not necessarily independent, with many of the areas cross-fertilizing each other. For instance, nanoscale electrodes are used in many areas (e.g., single-cell studies, nanoscale electrochemical imaging). The cross-fertilization further underscores the interdependence of instrument and technique development on fundamental and applied studies of SEE.

Nanoscale Electrodes. Small capacitances, thin diffusion layers and ability to provide a spatial probe at ever shrinking scales, make small electrodes a critical component of many SEE experiments. In addition to facilitating other areas of SEE, nanoscale electrodes themselves comprise an active area of SEE research, allowing new fundamental studies of small domain, fast time scale electrochemistry. Only recently has reproducible, routine fabrication of true nanoscale electrodes become more commonplace (Figure 3).[22−24] Nanoscale electrodes have proliferated recently for two reasons, first, methods to fabricate and characterize electrodes have improved, most notably through routine use of focused ion beams for custom fabrication of known electrode geometries. Second, glass pipettes, which are much easier to fabricate in the absence of a central metal filament, have become more accepted by electrochemists as nanoscale electrodes, typically as probes of conductance or ion transfer.[25] Further, filling nanoscale pipettes with carbon via pyrolysis of carbonaceous gases has popularized nanoscale carbon electrodes. Pulled pipettes with multiple barrels also offer the chance to incorporate multielectrode probes for more complex studies. Hybrid nanoelectrode designs that take advantage of decades of research in patch-clamping, ion transfer at the immiscible interface between electrolyte solutions, and chemically modified electrodes have also proven useful, often in SEE areas of research described in more detail below. Recent reviews of the state of the art in nanoelectrode research provide rich resources of the most up-to-date advances.[22,26−29]

Single-Cell Electrochemistry. SEE measurements of individual cells have made significant progress, even moving to subcellular SEE measurements. A large body of research has continued in the vein of amperometric measurements of neurotransmitter release pioneered by Wightman (vide supra) and has progressed to characterization of single vesicles. Nanoscale electrodes have even been positioned in the synapse of cultured cells to observe exocytotic release.[30] As electrodes have shrunk, insertion of electrodes into cells has become more commonplace and has been used for sample collection at single cells and subsequent genetic analysis.[31,32] Chemically modified nanoelectrodes have also been inserted into cells to interrogate subcellular electrochemical processes (Figure 4).[33] Future research on single cells holds promise to measure metabolic pathways, although significant work remains to determine the biological consequences of a physically invasive cellular probe as opposed to an extracellular electrode. Many hybrid electrode designs, which also incorporate nanopores or ion transfer electrodes, in addition to faradaic electrodes, find application in single-cell SEE measurements. Recent comprehensive reviews of the state of research related to single-cell electrochemistry are excellent sources for more detail.[19,27,34−36]

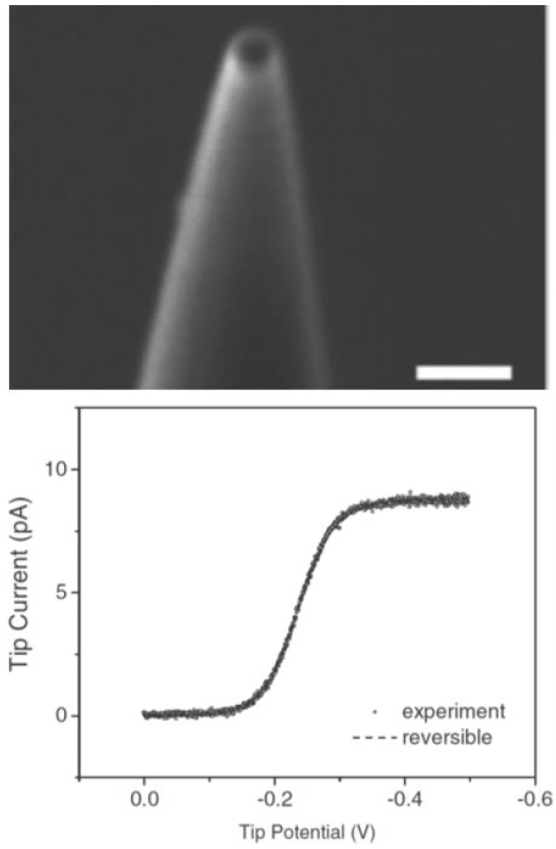

Figure 3. Nanoscale electrode fabrication and characterization. (top) Electron micrograph of a focused ion beam milled carbon nanoelectrode. Scale bar indicates 200 nm. (bottom) Cyclic voltammogram (CV) of 1 mM $Ru(NH_3)_6Cl_3$ recorded with a 29 nm radius carbon nanoelectrode. Dashed line (overlays with experiment) indicates simulated CV results. Figures adapted from ref 22 with permission from the Electrochemical Society.

Nanoscale Electrochemical Imaging. True nanoscale electrochemical imaging has recently become more accessible, and as such, has opened a new experimental space for SEE measurement. These strides come from advances in probe fabrication and instrumentation, most notably feedback methods that provide robust chemical imaging at small scales. New applications of SEE to measurement of nanoparticles, subcellular imaging, nanopore imaging, and many others have all been realized recently with nanoscale electrochemical imaging. In a recent report, measurements at discrete nanoparticles and even subentity measurements within

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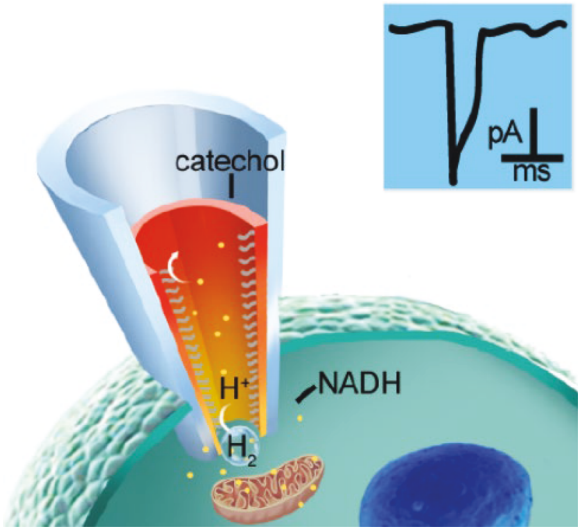

Figure 4. Cartoon of an intracellular nanoscale electrode for monitoring intracellular electrochemistry. A gold bipolar electrode is coated with catechol molecules that promote NADH/NAD^+, which generates an electrochemically measurable signal in the form of H_2 bubbles produced. Adapted from ref 33 with permission from the American Chemical Society.

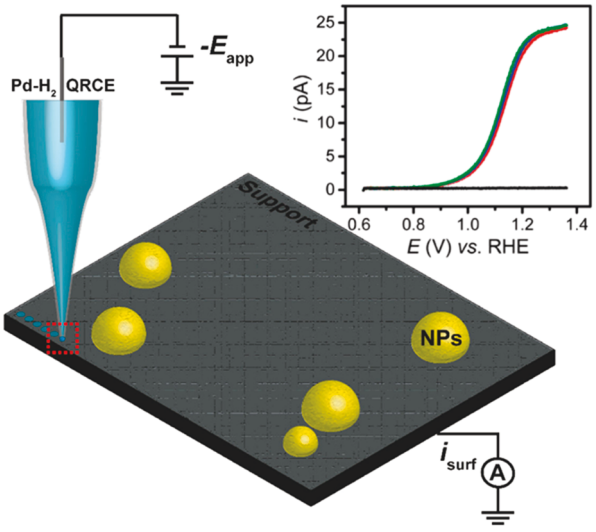

Figure 5. Nanoscale electrochemical imaging. Schematic of a scanning electrochemical cell microscopy experiment for SEE measurement of nanoparticles (NPs). In this experiment, a nanoscale meniscus makes electrical contact with discrete points on the surface, allowing extraction of electrochemical information at the nanoscale. Example linear sweep voltammograms recorded from five individual nanoparticles for the electrocatalytic oxidation of $[N_2H_5]^+$. Figure adapted from ref 37 with permission from the American Chemical Society.

individual nanoparticles were reported (Figure 5).[37] That electrochemical imaging at scales on the order of 10−100 nm is approaching routine, is especially exciting as measurements that focus on chemical characterization at these scales are rapidly emerging. Imaging via scanning electrochemical microscopy, scanning electrochemical cell microscopy, scanning ion conductance microscopy, electrochemical atomic force microscopy and variants of scanning tunneling microscopy have all made significant strides in probe design, instrument development and application to SEE measurements. A number of recent reviews present compelling details that underscore the case for resurgence in the field of nanoscale electrochemical imaging.[38−41]

Single Molecule Electrochemistry. Since the initial single-molecule redox cycling experiments of Bard, significant advance in fabrication of reproducible nanogaps, mostly through the use of micro/nanolithography, has resulted in more reproducible and detailed studies of single molecule redox cycling. In particular, Lemay has greatly advanced both the experimental approach and models used to describe such SEE studies (Figure 6).[42] In addition to nanogap devices, a

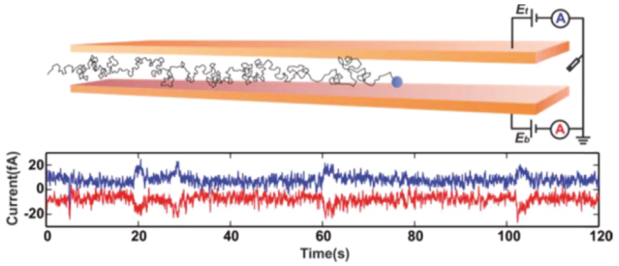

Figure 6. (top) Cartoon of a nanogap device for SEE measurements of single molecules. (bottom) Current−time traces simultaneously measured at the top (blue) and bottom (red) electrodes as single ferrocene derivatives flow through the nanogap and are oxidized and reduced at the top and bottom electrodes, respectively. Figure adapted from ref 42 with permission from the Royal Society of Chemistry.

number of other experimental approaches for SEE studies at the molecular level have been developed. These include electrodes that trap adsorbed molecules, scanned probes with more well-defined control of the small gap, and electrified nanopore formats. Such methods and more are considered in detail in recent comprehensive reviews of single molecule research which provide excellent perspectives on the field.[42−49]

Nanoparticle Impact Experiments. SEE measurements based on collision of nano and micro entities at an electrode surface have surged dramatically since the initial description of the concept.[50] These measurements are more flexible than one might first assume. Blocking experiments, where insulating micro/nanoparticles physically hinder flux of a redox species to a surface were initially investigated. Subsequently, catalytic methods, where the particle turns over a chemical reaction for signal amplification (e.g., Figure 7)[51] or transformative methods, where the particle is physically transformed (e.g., oxidized) have expanded the scope and interest in particle

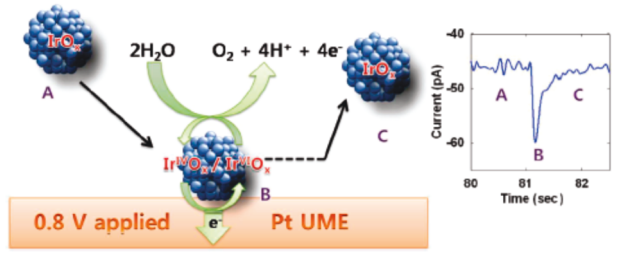

Figure 7. (left) Schematic of single iridium oxide nanoparticle collision event at a platinum ultramicroelectrode (UME) and current enhancement observed (right) by electrocatalytic water oxidation at the nanoparticle. Adopted from ref 51 with permission from the American Chemical Society.

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impact studies. Modes that operate amperometrically, measuring transient changes in current at small electrodes as nanoparticles encounter the surface, are most common, although measurements based on fast-scan cyclic voltammetry have also been realized.[52] While collision experiments are relatively new and still have considerations to work out, for example aggregation effects, significant depth exists for future work in this area. Nanoparticle analysis, catalytic reactions and details of interfacial charge and electron transfer at the SEE level are all accessible by these innovative measurements. Recent reviews of electrochemical collision experiments provide further descriptions of this rapidly evolving area.[46,53−56]

Nano/micropores. SEE measurements based on pores have advanced greatly since Coulter's initial report. Resistive-pulse measurements akin to the Coulter Counter but at the level of single molecules have been realized in different synthetic pore formats and in a number of protein pores. By controlling chemistry at the surface of synthetic pores or targeting protein channel modification by site-directed mutagenesis, elegant chemical selectivity has been demonstrated in a variety of nanopore sensing formats. One particularly compelling application that has served as a major driver for the field has been sequencing nucleic acids by nanopores,[57−62] which has resulted in astounding advances. Initial nanopore sequencing efforts focused on the α-hemolysin protein pore. Pores derived from other proteins, such as the bacterial amyloid section channel CsgG, have since been employed in this rapidly developing field (Figure 8).[62,63] In addition to

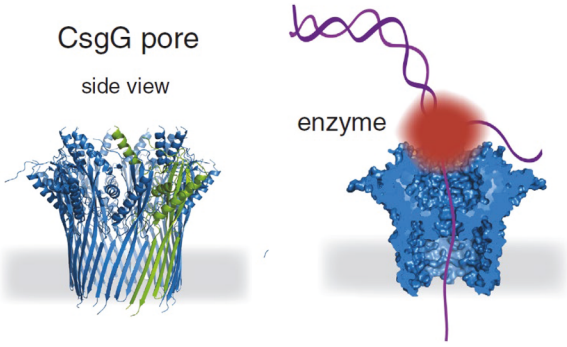

Figure 8. Nucleic acid sequencing by SEE nanopore measurements with a CsgG protein nanopore. (left) Structural depiction of CsgG pore (side view). (right) An enzyme-modified CsgG pore threads nucleic acid through the central channel of CsgG, where blockages of current allow for sequence read-out of the translocating strand. Adapted from ref 62 with permission from Elsevier.

resistive-pulse measurements, simple current−voltage interrogation or other more complicated nanofluidic designs have been able to take advantage of the control provided by single pores to enable new measurement approaches. Recent comprehensive reviews of the state of nano/micropore research are excellent sources for additional detail.[45,49,64−69]

Optoelectrochemical Imaging. Coupling optical measurements with electrochemical processes is an especially compelling approach to SEE. For many techniques, the transduction of electrochemical events by optical means improves measurement. For instance, optical methods often do not require physical movement of an electrode, but instead can record multipixel images, or can quickly raster a light source, which greatly accelerates measurement. The field of optoelectrochemical imaging is advancing rapidly, both with more standard techniques like dark-field or fluorescence imaging, and with developing optical methods related to plasmonic imaging or super resolution imaging. Such methods promise to generate new information at higher resolution than what is otherwise unattainable. SEE studies of metallic nanoparticles for catalysis are a prime example of optical SEE measurements that have recently been developed which provide compelling demonstrations of the power of light-based techniques. Recent reviews of optoelectrochemical imaging give detailed overviews of the field and the influence on SEE measurements (Figure 9).[2,70−75]

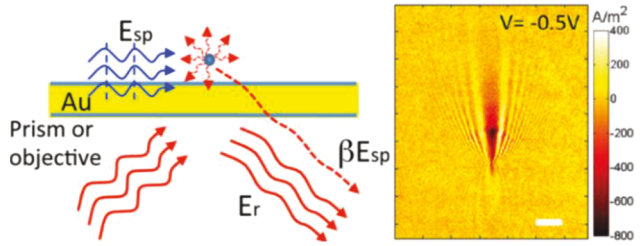

Figure 9. Optoelectrochemical imaging. (left) Scattering model of surface plasmon resonance (SPR) imaging showing that a nanoparticle image contrast arises from the interference of scattered surface plasmon and reflected light, which is sensitive to changes in the medium induced by local electrochemical processes. (right) Plasmonics-based electrochemical current microscopy (PECM) current density image of a single Pt nanoparticle at −0.50. Scale bar: 3 μm. Figure adapted from ref 73 with permission from the American Chemical Society.

Electrochemical Electron Microscopies. A very different instrumental approach has recently gained traction for examining SEE systems. Addition of ultrathin windows to vacuum tight fluidic cells has allowed for *in situ* experiments to be carried out under observation of electron microscopes. Addition of an electrode to the fluidic cell allows investigation of the individual electrochemical response of single nanoscale structures (particles, wires, etc.) (Figure 10).[76] As the electron

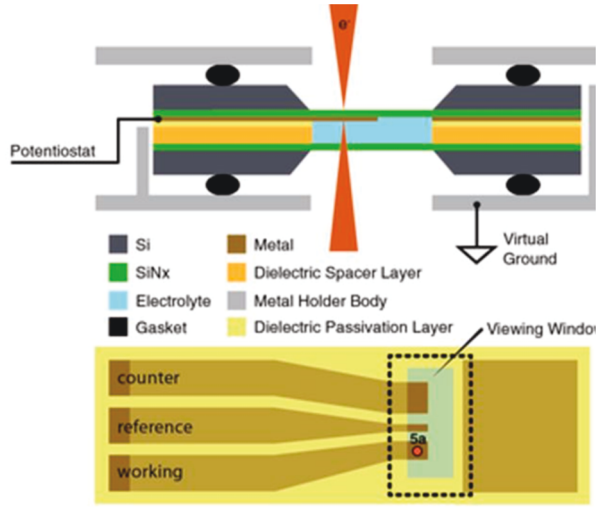

Figure 10. (top) Cross-sectional and (bottom) plan view schematics of a cell with the electrolyte confined primarily to the viewing area. Black dotted lines indicate liquid filled area. Figure adapted from ref 76 with permission from the Electrochemical Society.

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beam is highly energetic and structures with some degree of crystallinity are more easily analyzed, *in situ* electrochemical transmission electron microscopy has become an appealing route to investigate nano/microscale electrochemical processes in batteries and energy storage materials. Interaction of the high energy electron beam with electrochemical processes remains a caveat and details of the subtleties of these interactions are still being investigated in this rapidly evolving field.[76−80]

Nanoscale Phases. Nucleation and growth at the nanoscale has been extensively studied and has become more prevalent recently for electrochemists in the realm of single nanobubbles. White and co-workers have recently demonstrated that nanoelectrodes provide a unique platform to study the formation of ultrasmall, transient bubbles (Figure 11).[81]

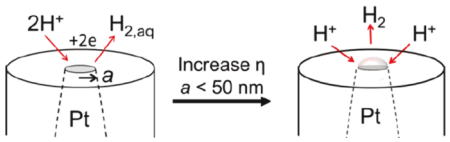

Figure 11. Nanoelectrode for SEE studies of nanobubble nucleation and growth. Schematic of electrochemical formation of an individual nanobubble at a platinum nanodisk electrode with a radius of $a < 50$ nm. Figure adapted from ref 81 with permission from the American Chemical Society.

Catalytic electrode materials are typically used to generate a local change in the concentration of molecules, for example through proton reduction to dihydrogen, which leads to formation of a bubble that then interacts with local electrochemical properties (transport to the electrode, capacitance, etc.) as a measurable property. Nanoscale electrodes are key to the fast time scales and small bubble sizes for these experiments. These fundamental studies have proven especially stimulating for energy applications, as effects from nano and microbubbles are important areas of research in fuel cells or energy storage schemes. Recent reports from the nascent field of SEE nanobubble research as related to electrochemistry provide deeper context.[82−88]

■ PROSPECTUS

Pushing the limits of instrumentation, theory and our fundamental understanding of electrochemistry are some of the most exciting aspects of SEE. These are also some of the most significant challenges, as SEE truly ventures into unknown territory. A recent, vigorous debate over the data interpretation and the possibilities of measuring the kinetics of single enzymes with nanoparticle impact experiments underscores the opportunities and possible pitfalls of SEE.[89−97] The debate, which includes original research, comments and replies, is an excellent introduction to consideration of instrumental limits for fast, low-current measurements, aspects of theoretical differences in measurements of single-entities vs ensembles, and design of both experiments and models to investigate SEE systems. In particular, this example underscores the importance of defining and understanding limits of models employed and in clearly reporting complete experimental set-ups and data treatment protocols of SEE.

Statistics and High-Throughput SEE. A significant present challenge for SEE that is being addressed, but requires additional attention is understanding the relevance of many SEE measurements. That is to say, if we measure the electrochemical response of a single entity, that measurement might prove a significant technical achievement, but can the results be put in context that makes it (more) meaningful outside of that experiment? For most instances, limited scale/scope SEE measurements will be important, but context, through statistical analysis of signals, modeling of measurements, and, if appropriate, comparison to ensemble measurements will be necessary to fully realize the impact of SEE. For the example given in Figure 1, of measuring a neurotransmitter in a biological setting, population statistics are routinely implemented, with statistical criteria applied to aid in determining relevance. Further, for SEE measurements that truly address discrete events, Poisson statistics, as opposed to Gaussian, are necessary to accurately address data analysis. These kinds of tests can be, but are not always, applied to SEE measurements outside of biological settings (with full acknowledgment that statistics are not infallible). There is no reason why more stringent comparative statistics cannot be considered for studies aimed at SEE measurements in other fields like materials science or organic electrochemistry. For instance, if one measured the catalytic activity of a large number of single nanoparticles with voltammetry, could this data be used to reconstruct or extrapolated to what more typical ensemble voltammetric measurements would record? If this can be done, the impact of such studies becomes much more significant outside of the immediate SEE community. The importance of these considerations in SEE measurements often requires careful considerations of probability and statistical treatment of data for detailed analysis. Several studies have made efforts to consider the place of SEE in ensemble measurements and this is an important area for future study that can be expected to grow as we improve techniques and are able to more rapidly generate replicant measurements.

This challenge, to increase data collection is even more daunting for dynamic SEE measurements that look to follow entities at high time resolution.[98] While good examples exist, improving the chance to follow a single entity from beginning to end through a chemical process will be a future area of significance.[8] The idea of collecting more data also brings ideas of multiplexed data collection to mind, where arrays of electrodes or probes could significantly increase the amount of data that can be collected.

The above discussion of increasing throughput for SEE measurements, also leads us directly to so-called "big data" efforts, an area of intense interest in diverse scientific fields. One might expect increasing data collection abilities on robust SEE measurements to generate huge data sets could provide real utility and extend what is possible. A perfect example of this is nanopore sequencing, where the ability to sequence a single strand of DNA is an amazing technical achievement, but put that SEE measurement in the context of multiple reads to generate more complete data sets, coupled with the genetics of the individual in then the larger picture of the genetics of a population of individuals, and the importance of the result is amplified enormously. This would seem a compelling model for other areas of inquiry as well. For instance, SEE measurements could provide the critical bridge between first-principles calculations of new materials and molecules (e.g., a catalyst, a molecular switch, etc.) to larger multientity micro or macroscale devices. As a more detailed level of theory can be applied to small systems, single-entity models can benefit from the reduced or simplified computational costs. These studies

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can then be used to inform measurements of larger populations or averaged measurements.

Instrumental Advances. As highlighted in this Perspective, instrumentation advances have been pivotal to SEE and this is expected only to increase. Methods to position nanoscale electrodes for SEE have evolved rapidly in the past decade and it is now feasible to consider employing typical macroscale electrochemical measurement approaches to nanoscale SEE measurements. Areas of research outside of electrochemistry, for instance in high performance computing, are expected to provide new tools that electrochemists can utilize. This is the case with field programmable gate arrays, which were developed in the 80s and 90s for computing, that have now found wide application in user-friendly formats for developing flexible, powerful and fast electrochemical instrumentation. An obvious question is: how might more recent cutting-edge computational paradigms, like machine learning or quantum computing, impact electrochemical measurements? The step to applying these fields is not as far as it may seem. Some of the most advanced measurements in electrochemical science happen in SEE related research, so we should be on the lookout for new ways to take advantage of these rapidly developing technologies.

SEE measurements also need to be very careful in instrumental applications and how data is processed with more conventional techniques as well. Recent reports have underscored the need to carefully consider instrument limitations and filtering effects on single nanoparticle impact measurements.[99,100] White and co-workers convincingly describe the need to combine modeling approaches to truly understand the limits in SEE measurements, an approach that should be considered more widely.[99]

Chemical Amplification Schemes. A recurring theme in SEE measurements has been chemical amplification schemes, which can overcome signal-to-noise and instrument limitations. For instance, single-molecule redox cycling and catalytic nanoparticle impact studies both take advantage of clever chemical amplification schemes. Such amplification schemes might be expected to find wider application, and even signal conversion processes from electrons/ions to photons through techniques like electrogenerated chemiluminescence provide fertile ground to enhance SEE studies.

Improving Selectivity and Heterogeneity. Selective measurement is an issue that can prove difficult for macro or microscale electrochemistry, and this can be exacerbated at the level of single entities. The classic example of selective requirements in an electroanalysis is identification of different species in brain chemistry, where quantitation of dopamine, serotonin, ascorbate, etc., by electrochemical means has required significant attention. Tricks and techniques such as development of selective coatings and potential waveforms have overcome many of these issues for applications of microelectrodes in the brain. In other electroanalytical measurements, ion selective electrodes provide exquisite selectivity in discrimination of different ionic species, but can be limited in temporal resolution. The ability to measure individual entities in a background of similar entities is a significant challenge that is matrix dependent and is especially important in analysis of real samples. When coupled with possible needs to address location or to measure dynamics of a single entity, such tasks become even more daunting. Methods to improve selectivity might include incorporation of biological paradigms (e.g., lessons from ion channels) or incorporation of orthogonal techniques for measurement (considered more below).

Hybrid Techniques. As described above, hybrid approaches are poised to revolutionize electrochemical measurements. Optical techniques have extremely wide adoption and have rapidly improved in resolution, speed and sensitivity in recent years. These techniques are beginning to bleed into electrochemical studies and SEE techniques are at the forefront of this intersection.

Mass spectrometry also provides another interesting area for advance in SEE measurements. This field is rapidly evolving, especially with respect to the mass analyzer, in particular with ion traps allowing the measurement of smaller and smaller quantities of material. Many of the tools used for SEE can be utilized in mass spectrometry, especially for sampling approaches at the level of single cells and smaller. A number of studies at this intersection of mass spectrometry and electrochemistry have been carried out recently.[101,102] A caveat to these measurements is even if single entities can be analyzed, transmission efficiency, or how many entities are not collected for mass analysis after ionization, remains an area in need of significant improvement.

There are a number of complementary areas, for instance molecular biology, organic synthesis, that are in a prime position to contribute to SEE measurements as well, it remains to be seen where overlaps will emerge, but one would expect that spreading the SEE concept to new arenas will be prevalent in the near future.

■ CONCLUSIONS

Single-entity electrochemistry has nucleated as a special area of inquiry at the forefront of chemical measurement and analysis. This Perspective provides context of the history and inspiration of SEE studies, and how these initial seeds have grown to modern studies that push the limits of instrumentation, theory and imagination. A prospectus of where we might go in the future, and where the author sees important challenges is also provided. Because of the difficulty in fast, low-current measurements, advances in instrumentation and theory are critical to advance chemical insight from experiments, more so than most typical areas of electroanalysis. Statistical analysis is of special important for SEE, where probabilistic treatments must be considered and subtleties in data analysis become even more pronounced as SEE moves to dynamic measurements at ever-shrinking time scales. As SEE experiments that are focused on electrochemistry become more mature, we can expect hybrid measurements that use spectroscopic or spectrometric techniques can provide orthogonal approaches to complement electrochemical measurement. With acknowledgment that the diversity and breadth of SEE studies makes prognostication difficult, the horizon for SEE surely goes well beyond these speculations. A hallmark of SEE studies has been clever experiments and an open-minded approach to area of study, which are sure to continue in the future.

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■ REFERENCES

(1) *Single Entity Electrochemistry: Faraday Discussion 193*; Royal Society of Chemistry, 2016.
(2) Wang, Y. X.; Shan, X. N.; Tao, N. J. Emerging tools for studying single entity electrochemistry. *Faraday Discuss.* **2016**, *193*, 9−39.
(3) Crooks, R. M. Concluding remarks: single entity electrochemistry one step at a time. *Faraday Discuss.* **2016**, *193*, 533−547.
(4) Actis, P.; Bentley, C. L.; Edwards, M. A.; Jacobse, L. Highlights from the Faraday Discussion on Single Entity Electrochemistry, York, UK, August-September 2016. *Chem. Commun.* **2016**, *52*, 13934−13940.
(5) Gooding, J. J. Single Entity Electrochemistry Progresses to Cell Counting. *Angew. Chem., Int. Ed.* **2016**, *55*, 12956−12958.
(6) Long, Y. T.; Unwin, P. R.; Baker, L. A. Single-Entity Electrochemistry: Fundamentals and Applications. *ChemElectroChem* **2018**, *5*, 2918−2919.
(7) Lemay, S.; White, H. Electrochemistry at the Nanoscale: Tackling Old Questions, Posing New Ones. *Acc. Chem. Res.* **2016**, *49*, 2371−2371.
(8) Edwards, M. A.; Robinson, D. A.; Ren, H.; Cheyne, C. G.; Tan, C. S.; White, H. S. Nanoscale electrochemical kinetics & dynamics: the challenges and opportunities of single-entity measurements. *Faraday Discuss.* **2018**, *210*, 9−28.
(9) Coulter, W. H. Means for counting particles suspended in a fluid. US Patent 2,656,508A, 1953.
(10) Beckman Coulter Life Sciences. https://www.beckman.com/cell-counters-and-analyzers (accessed October 15, 2018).
(11) Auerbach, A.; Sachs, F. Patch Clamp Studies of Single Ionic Channels. *Annu. Rev. Biophys. Bioeng.* **1984**, *13*, 269−302.
(12) Cahalan, M.; Neher, E. Patch Clamp Techniques - An Overview. *Methods Enzymol.* **1992**, *207*, 3−14.
(13) Neher, E.; Sakmann, B. Single-channel currents recorded from membrane of denervated frog muscle-fibers. *Nature* **1976**, *260*, 799−802.
(14) Dunlop, J.; Bowlby, M.; Peri, R.; Vasilyev, D.; Arias, R. High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. *Nat. Rev. Drug Discovery* **2008**, *7*, 358−368.
(15) Obergrussberger, A.; Goetze, T. A.; Brinkwirth, N.; Becker, N.; Friis, S.; Rapedius, M.; Haarmann, C.; Rinke-Weiss, I.; Stolzle-Feix, S.; Bruggemann, A.; George, M.; Fertig, N. An update on the advancing high-throughput screening techniques for patch clamp-based ion channel screens: implications for drug discovery. *Expert Opin. Drug Discovery* **2018**, *13*, 269−277.
(16) Leszczyszyn, D. J.; Jankowski, J. A.; Viveros, O. H.; Diliberto, E. J.; Near, J. A.; Wightman, R. M. Nicotinic receptor-mediated catecholamine secretion from individual chromaffin cells - chemical evidece for exocytosis. *J. Biol. Chem.* **1990**, *265*, 14736−14737.
(17) Finnegan, J. M.; Borges, R.; Wightman, R. M. Comparison of cytosolic Ca^{2+} and exocytosis responses from single rat and bovine chromaffin cells. *Neuroscience* **1996**, *71*, 833−843.
(18) Bucher, E. S.; Wightman, R. M. Electrochemical Analysis of Neurotransmitters. *Annu. Rev. Anal. Chem.* **2015**, *8*, 239−261.
(19) Li, X. C.; Dunevall, J.; Ewing, A. G. Quantitative Chemical Measurements of Vesicular Transmitters with Electrochemical Cytometry. *Acc. Chem. Res.* **2016**, *49*, 2347−2354.
(20) Bard, A. J.; Fan, F. R. F.; Pierce, D. T.; Unwin, P. R.; Wipf, D. O.; Zhou, F. M. Chemical imaging of surfaces with the scanning electrochemical microscope. *Science* **1991**, *254*, 68−74.
(21) Fan, F. R. F.; Bard, A. J. Electrochemical detection of single molecules. *Science* **1995**, *267*, 871−874.
(22) Chen, R.; Hu, K. K.; Yu, Y.; Mirkin, M. V.; Amemiya, S. Focused-Ion-Beam-Milled Carbon Nanoelectrodes for Scanning Electrochemical Microscopy. *J. Electrochem. Soc.* **2016**, *163*, H3032−H3037.
(23) Wilde, P.; Quast, T.; Aiyappa, H.; Chen, Y.-T.; Botz, A.; Tarnev, T.; Marquitan, M.; Feldhege, S.; Lindner, A.; Andronescu, C.; Schuhmann, W. Towards Reproducible Fabrication of Nanometre-Sized Carbon Electrodes: Optimisation of Automated Nanoelectrode Fabrication by Means of Transmission Electron Microscopy. *ChemElectroChem* **2018**, *5*, 3083−3088.
(24) Perry, D.; Momotenko, D.; Lazenby, R. A.; Kang, M.; Unwin, P. R. Characterization of Nanopipettes. *Anal. Chem.* **2016**, *88*, 5523−5530.
(25) Morris, C. A.; Friedman, A. K.; Baker, L. A. Applications of nanopipettes in the analytical sciences. *Analyst* **2010**, *135*, 2190−2202.
(26) Clausmeyer, J.; Schuhmann, W. Nanoelectrodes: Applications in electrocatalysis, single-cell analysis and high-resolution electrochemical imaging. *TrAC, Trends Anal. Chem.* **2016**, *79*, 46−59.
(27) Cox, J. T.; Zhang, B. Nanoelectrodes: Recent Advances and New Directions. *Annu. Rev. Anal. Chem.* **2012**, *5*, 253−272.
(28) Fan, Y. S.; Han, C.; Zhang, B. Recent advances in the development and application of nanoelectrodes. *Analyst* **2016**, *141*, 5474−5487.
(29) Ying, Y. L.; Ding, Z. F.; Zhan, D. P.; Long, Y. T. Advanced electroanalytical chemistry at nanoelectrodes. *Chem. Sci.* **2017**, *8*, 3338−3348.
(30) Li, Y. T.; Zhang, S. H.; Wang, L.; Xiao, R. R.; Liu, W.; Zhang, X. W.; Zhou, Z.; Amatore, C.; Huang, W. H. Nanoelectrode for Amperometric Monitoring of Individual Vesicular Exocytosis Inside Single Synapses. *Angew. Chem., Int. Ed.* **2014**, *53*, 12456−12460.
(31) Actis, P.; Maalouf, M. M.; Kim, H. J.; Lohith, A.; Vilozny, B.; Seger, R. A.; Pourmand, N. Compartmental Genomics in Living Cells Revealed by Single-Cell Nanobiopsy. *ACS Nano* **2014**, *8*, 546−553.
(32) Actis, P. Sampling from Single Cells. *Small Methods* **2018**, *2*, 1700300.
(33) Ying, Y. L.; Hu, Y. X.; Gao, R.; Yu, R. J.; Gu, Z.; Lee, L. P.; Long, Y. T. Asymmetric Nanopore Electrode-Based Amplification for Electron Transfer Imaging in Live Cells. *J. Am. Chem. Soc.* **2018**, *140*, 5385−5392.
(34) Lovric, J.; Li, X. C.; Ewing, A. G. *Quantitative Chemical Measurements of Vesicular Transmitters with Single Cell Amperometry and Electrochemical Cytometry*. In Compendium of In Vivo Monitoring in Real-Time Molecular Neuroscience; Wilson, G. S., Michael, A. C. Eds.; 2015, 181−200.
(35) Zhang, J. J.; Zhou, J. Y.; Pan, R. R.; Jiang, D. C.; Burgess, J. D.; Chen, H. Y. New Frontiers and Challenges for Single-Cell Electrochemical Analysis. *ACS Sensors* **2018**, *3*, 242−250.
(36) Robinson, D. L.; Hermans, A.; Seipel, A. T.; Wightman, R. M. Monitoring rapid chemical communication in the brain. *Chem. Rev.* **2008**, *108*, 2554−2584.
(37) Bentley, C. L.; Kang, M.; Unwin, P. R. Nanoscale Structure Dynamics within Electrocatalytic Materials. *J. Am. Chem. Soc.* **2017**, *139*, 16813−16821.
(38) Bentley, C. L.; Kang, M. Y.; Unwin, P. R. Scanning electrochemical cell microscopy: New perspectives on electrode processes in action. *Current Opinion in Electrochemistry* **2017**, *6*, 23−30.
(39) Kai, T. H.; Zoski, C. G.; Bard, A. J. Scanning electrochemical microscopy at the nanometer level. *Chem. Commun.* **2018**, *54*, 1934−1947.
(40) Kang, M. Y.; Momotenko, D.; Page, A.; Perry, D.; Unwin, P. R. Frontiers in Nanoscale Electrochemical Imaging: Faster, Multifunctional, and Ultrasensitive. *Langmuir* **2016**, *32*, 7993−8008.
(41) Chen, C. C.; Zhou, Y.; Baker, L. A. Scanning Ion Conductance Microscopy. *Annu. Rev. Anal. Chem.* **2012**, *5*, 207−228.

DOI: 10.1021/jacs.8b09747
J. Am. Chem. Soc. 2018, 140, 15549−15559

(42) Kang, S.; Nieuwenhuis, A. F.; Mathwig, K.; Mampallil, D.; Kostiuchenko, Z. A.; Lemay, S. G. Single-molecule electrochemistry in nanochannels: probing the time of first passage. *Faraday Discuss.* **2016**, *193*, 41−50.
(43) White, H. S.; McKelvey, K. Redox cycling in nanogap electrochemical cells. *Current Opinion in Electrochemistry* **2018**, *7*, 48−53.
(44) Nazmutdinov, R. R.; Zinkicheva, T. T.; Shermukhamedov, S. A.; Zhang, J. D.; Ulstrup, J. Electrochemistry of single molecules and biomolecules, molecular scale nanostructures, and low-dimensional systems. *Current Opinion in Electrochemistry* **2018**, *7*, 179−187.
(45) Lin, Y.; Ying, Y. L.; Long, Y. T. Nanopore confinement for electrochemical sensing at the single-molecule level. *Current Opinion in Electrochemistry* **2018**, *7*, 172−178.
(46) Fan, Y.; Anderson, T. J.; Zhang, B. Single-molecule electrochemistry: From redox cycling to single redox events. *Current Opinion in Electrochemistry* **2018**, *7*, 81−86.
(47) Nichols, R. J.; Higgins, S. J. Single molecule electrochemistry in nanoscale junctions. *Current Opinion in Electrochemistry* **2017**, *4*, 98−104.
(48) Mathwig, K.; Aartsma, T. J.; Canters, G. W.; Lemay, S. G. Nanoscale Methods for Single-Molecule Electrochemistry. *Annu. Rev. Anal. Chem.* **2014**, *7*, 383−404.
(49) Fu, K. Y.; Bohn, P. W. Nanopore Electrochemistry: A Nexus for Molecular Control of Electron Transfer Reactions. *ACS Cent. Sci.* **2018**, *4*, 20−29.
(50) Quinn, B. M.; van 'tHof, P.; Lemay, S. G. Time-resolved electrochemical detection of discrete adsorption events. *J. Am. Chem. Soc.* **2004**, *126*, 8360−8361.
(51) Kwon, S. J.; Fan, F. R. F.; Bard, A. J. Observing Iridium Oxide (IrOx) Single Nanoparticle Collisions at Ultramicroelectrodes. *J. Am. Chem. Soc.* **2010**, *132*, 13165−13167.
(52) Guo, Z. H.; Percival, S. J.; Zhang, B. Chemically Resolved Transient Collision Events of Single Electrocatalytic Nanoparticles. *J. Am. Chem. Soc.* **2014**, *136*, 8879−8882.
(53) Laborda, E.; Molina, A.; Batchelor-McAuley, C.; Compton, R. G. Individual Detection and Characterization of Non-Electrocatalytic, Redox-Inactive Particles in Solution by using Electrochemistry. *ChemElectroChem* **2018**, *5*, 410−417.
(54) Stevenson, K. J.; Tschulik, K. A materials driven approach for understanding single entity nano impact electrochemistry. *Current Opinion in Electrochemistry* **2017**, *6*, 38−45.
(55) Peng, Y. Y.; Qian, R. C.; Hafez, M. E.; Long, Y. T. Stochastic Collision Nanoelectrochemistry: A Review of Recent Developments. *ChemElectroChem* **2017**, *4*, 977−985.
(56) Mirkin, M. V.; Sun, T.; Yu, Y.; Zhou, M. Electrochemistry at One Nanoparticle. *Acc. Chem. Res.* **2016**, *49*, 2328−2335.
(57) Reiner, J. E.; Balijepalli, A.; Robertson, J. W. F.; Campbell, J.; Suehle, J.; Kasianowicz, J. J. Disease Detection and Management via Single Nanopore-Based Sensors. *Chem. Rev.* **2012**, *112*, 6431−6451.
(58) Maglia, G.; Heron, A. J.; Stoddart, D.; Japrung, D.; Bayley, H. Analysis of Single Nucleic Acid Molecules with Protein Nanopores. In *Single Molecule Tools, Pt B: Super-Resolution, Particle Tracking, Multiparameter, and Force Based Methods*; Walter, N. G., Ed.; Methods in Enzymology, 2010; Vol. *475*; pp 591−623.
(59) Kasianowicz, J. J.; Robertson, J. W. F.; Chan, E. R.; Reiner, J. E.; Stanford, V. M. Nanoscopic Porous Sensors. *Annu. Rev. Anal. Chem.* **2008**, *1*, 737−766.
(60) Deamer, D. W.; Branton, D. Characterization of nucleic acids by nanopore analysis. *Acc. Chem. Res.* **2002**, *35*, 817−825.
(61) Branton, D.; Deamer, D. W.; Marziali, A.; Bayley, H.; Benner, S. A.; Butler, T.; Di Ventra, M.; Garaj, S.; Hibbs, A.; Huang, X. H.; Jovanovich, S. B.; Krstic, P. S.; Lindsay, S.; Ling, X. S. S.; Mastrangelo, C. H.; Meller, A.; Oliver, J. S.; Pershin, Y. V.; Ramsey, J. M.; Riehn, R.; Soni, G. V.; Tabard-Cossa, V.; Wanunu, M.; Wiggin, M.; Schloss, J. A. The potential and challenges of nanopore sequencing. *Nat. Biotechnol.* **2008**, *26*, 1146−1153.
(62) Ayub, M.; Bayley, H. Engineered transmembrane pores. *Curr. Opin. Chem. Biol.* **2016**, *34*, 117−126.
(63) Goyal, P.; Krasteva, P. V.; Van Gerven, N.; Gubellini, F.; Van den Broeck, I.; Troupiotis-Tsaïlaki, A.; Jonckheere, W.; Péhau-Arnaudet, G.; Pinkner, J. S.; Chapman, M. R.; Hultgren, S. J.; Howorka, S.; Fronzes, R.; Remaut, H. Structural and mechanistic insights into the bacterial amyloid secretion channel CsgG. *Nature* **2014**, *516*, 250.
(64) Ying, Y. L.; Gao, R.; Hu, Y. X.; Long, Y. T. Electrochemical Confinement Effects for Innovating New Nanopore Sensing Mechanisms. *Small Methods* **2018**, *2*, 1700390.
(65) Long, Z.; Zhan, S. S.; Gao, P. C.; Wang, Y. Q.; Lou, X. D.; Xia, F. Recent Advances in Solid Nanopore/Channel Analysis. *Anal. Chem.* **2018**, *90*, 577−588.
(66) Shi, W. Q.; Friedman, A. K.; Baker, L. A. Nanopore Sensing. *Anal. Chem.* **2017**, *89*, 157−188.
(67) Experton, J.; Wu, X. J.; Martin, C. R. From Ion Current to Electroosmotic Flow Rectification in Asymmetric Nanopore Membranes. *Nanomaterials* **2017**, *7*, 445.
(68) Albrecht, T. Progress in single-biomolecule analysis with solid-state nanopores. *Current Opinion in Electrochemistry* **2017**, *4*, 159−165.
(69) Bayley, H. Nanopore Sequencing: From Imagination to Reality. *Clin. Chem.* **2015**, *61*, 25−31.
(70) Yuan, T. L.; Wang, W. Studying the electrochemistry of single nanoparticles with surface plasmon resonance microscopy. *Current Opinion in Electrochemistry* **2017**, *6*, 17−22.
(71) Jing, C.; Reichert, J. Nanoscale electrochemistry in the ″dark-field″. *Current Opinion in Electrochemistry* **2017**, *6*, 10−16.
(72) Bouffier, L.; Doneux, T. Coupling electrochemistry with in situ fluorescence (confocal) microscopy. *Current Opinion in Electrochemistry* **2017**, *6*, 31−37.
(73) Fang, Y. M.; Wang, H.; Yu, H.; Liu, X. W.; Wang, W.; Chen, H. Y.; Tao, N. J. Plasmonic Imaging of Electrochemical Reactions of Single Nanoparticles. *Acc. Chem. Res.* **2016**, *49*, 2614−2624.
(74) Brasiliense, V.; Berto, P.; Combellas, C.; Tessier, G.; Kanoufi, F. Electrochemistry of Single Nanodomains Revealed by Three-Dimensional Holographic Microscopy. *Acc. Chem. Res.* **2016**, *49*, 2049−2057.
(75) Kanoufi, F. Editorial: Innovative Methods in Electrochemistry. Seeing electrochemistry with new eyes. *Current Opinion in Electrochemistry* **2017**, *6*, 1−3.
(76) Fahrenkrug, E.; Alsem, D. H.; Salmon, N.; Maldonado, S. Electrochemical Measurements in In Situ TEM Experiments. *J. Electrochem. Soc.* **2017**, *164*, H358−H364.
(77) Yang, Y. J.; Liu, X. Z.; Dai, Z. H.; Yuan, F. L.; Bando, Y.; Golberg, D.; Wang, X. In Situ Electrochemistry of Rechargeable Battery Materials: Status Report and Perspectives. *Adv. Mater.* **2017**, *29*, 1606922.
(78) Luo, C.; Wang, C. L.; Wu, X.; Zhang, J.; Chu, J. H. In Situ Transmission Electron Microscopy Characterization and Manipulation of Two-Dimensional Layered Materials beyond Graphene. *Small* **2017**, *13*, 1604259.
(79) Hodnik, N.; Dehm, G.; Mayrhofer, K. J. J. Importance and Challenges of Electrochemical in Situ Liquid Cell Electron Microscopy for Energy Conversion Research. *Acc. Chem. Res.* **2016**, *49*, 2015−2022.
(80) Ramachandramoorthy, R.; Bernal, R.; Espinosa, H. D. Pushing the Envelope of In Situ Transmission Electron Microscopy. *ACS Nano* **2015**, *9*, 4675−4685.
(81) Luo, L.; White, H. S. Electrogeneration of Single Nanobubbles at Sub-50-nm-Radius Platinum Nanodisk Electrodes. *Langmuir* **2013**, *29*, 11169−11175.
(82) Soto, A. M.; German, S. R.; Ren, H.; van der Meer, D.; Lohse, D.; Edwards, M. A.; White, H. S. The Nucleation Rate of Single O-2 Nanobubbles at Pt Nanoelectrodes. *Langmuir* **2018**, *34*, 7309−7318.
(83) Perera, R. T.; Arcadia, C. E.; Rosenstein, J. K. Probing the nucleation, growth, and evolution of hydrogen nanobubbles at single catalytic sites. *Electrochim. Acta* **2018**, *283*, 1773−1778.

DOI: 10.1021/jacs.8b09747
J. Am. Chem. Soc. 2018, 140, 15549−15559

(84) German, S. R.; Edwards, M. A.; Ren, H.; White, H. S. Critical Nuclei Size, Rate, and Activation Energy of H-2 Gas Nucleation. *J. Am. Chem. Soc.* **2018**, *140*, 4047−4053.
(85) Ma, W.; Hu, K. K.; Chen, Q. J.; Zhou, M.; Mirkin, M. V.; Bard, A. J. Electrochemical Size Measurement and Characterization of Electrodeposited Platinum Nanoparticles at Manometer Resolution with Scanning Electrochemical Microscopy. *Nano Lett.* **2017**, *17*, 4354−4358.
(86) Liu, Y. W.; Edwards, M. A.; German, S. R.; Chen, Q. J.; White, H. S. The Dynamic Steady State of an Electrochemically Generated Nanobubble. *Langmuir* **2017**, *33*, 1845−1853.
(87) German, S. R.; Edwards, M. A.; Chen, Q. J.; White, H. S. Laplace Pressure of Individual H-2 Nanobubbles from Pressure-Addition Electrochemistry. *Nano Lett.* **2016**, *16*, 6691−6694.
(88) Chen, Q. J.; Luo, L.; Faraji, H.; Feldberg, S. W.; White, H. S. Electrochemical Measurements of Single H-2 Nanobubble Nucleation and Stability at Pt Nanoelectrodes. *J. Phys. Chem. Lett.* **2014**, *5*, 3539−3544.
(89) Bard, A. J. Toward Single Enzyme Molecule Electrochemistry. *ACS Nano* **2008**, *2*, 2437−2440.
(90) Chan, C.; Sepunaru, L.; Sokolov, S. V.; Katelhon, E.; Young, N. P.; Compton, R. G. Catalytic activity of catalase-silica nanoparticle hybrids: from ensemble to individual entity activity. *Chemical Science* **2017**, *8*, 2303−2308.
(91) Han, L. H.; Wang, W.; Nsabimana, J.; Yan, J. W.; Ren, B.; Zhan, D. P. Single molecular catalysis of a redox enzyme on nanoelectrodes. *Faraday Discuss.* **2016**, *193*, 133−139.
(92) Katelhon, E.; Sepunaru, L.; Karyakin, A. A.; Compton, R. G. Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts. *ACS Catal.* **2016**, *6*, 8313−8320.
(93) Katelhon, E.; Sepunaru, L.; Karyakin, A. A.; Compton, R. G. Reply to Comment on ″Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts″. *ACS Catal.* **2017**, *7*, 3594−3596.
(94) Lin, C. H.; Katelhon, E.; Sepunaru, L.; Compton, R. G. Understanding single enzyme activity via the nano-impact technique. *Chemical Science* **2017**, *8*, 6423−6432.
(95) Lin, C. H.; Sepunaru, L.; Katelhon, E.; Compton, R. G. Electrochemistry of Single Enzymes: Fluctuations of Catalase Activities. *J. Phys. Chem. Lett.* **2018**, *9*, 2814−2817.
(96) Sekretaryova, A. N.; Vagin, M. Y.; Turner, A. P. F.; Eriksson, M. Electrocatalytic Currents from Single Enzyme Molecules. *J. Am. Chem. Soc.* **2016**, *138*, 2504−2507.
(97) Sekretaryova, A. N.; Vagin, M. Y.; Turner, A. P. F.; Eriksson, M. Correspondence on ″Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts″. *ACS Catal.* **2017**, *7*, 3591−3593.
(98) Singh, P. S.; Lemay, S. G. Stochastic Processes in Electrochemistry. *Anal. Chem.* **2016**, *88*, 5017−5027.
(99) Robinson, D. A.; Edwards, M. A.; Ren, H.; White, H. S. Effects of Instrumental Filters on Electrochemical Measurement of Single-Nanoparticle Collision Dynamics. *ChemElectroChem* **2018**, *5*, 3059−3067.
(100) Kanokkanchana, K.; Saw, E. N.; Tschulik, K. Nano Impact Electrochemistry: Effects of Electronic Filtering on Peak Height, Duration and Area. *ChemElectroChem* **2018**, *5*, 3000−3005.
(101) Schorr, N. B.; Gossage, Z. T.; Rodriguez-Lopez, J. Prospects for single-site interrogation using in situ multimodal electrochemical scanning probe techniques. *Current Opinion in Electrochemistry* **2018**, *8*, 89−95.
(102) Yuill, E. M.; Baker, L. A. Electrochemical Aspects of Mass Spectrometry: Atmospheric Pressure Ionization and Ambient Ionization for Bioanalysis. *ChemElectroChem* **2017**, *4*, 806−821.

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