Review—Electrochemistry's Potential to Reach the Ultimate Sensitivity in Measurement Science

Sondrica Goines [ORCID][1] and **Jeffrey E. Dick** [ORCID][1,2,z]

[1]*Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA*
[2]*Lineberger Comprehensive Cancer Center, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA*

A holy grail in analytical chemistry is the specific quantification of a single entity, be that entity an atom, a molecule, a nanoparticle, a virus, or a circulating tumor cell. Analytical chemistry is entering an era where this sensitivity can be achieved. In these experiments, the limit of detection is 1, and the limit of quantitation is not only measured in units of concentration but units of time: the waiting time before positively identifying a single entity. Electrochemistry is front and center in single entity experiments with hundreds of publications per year detailing observations of various entities colliding with micro- and nano-electrodes. These types of experiments are referred to as 'stochastic electrochemistry' due to the stochastic nature of the collision of an entity with an electrode surface. If methods of detection can be made specific, simple, inexpensive, and robust, the techniques hold the promise of transforming diagnostics (i.e., the specific detection of a single cancerous cell in blood). This review will critically analyze the stochastic electrochemistry literature and focus on reports detailing methods to ensure the response at the electrode surface is specific to the colliding entity. The topic of specificity, and strategies to achieve it, are highlighted.

Analytical chemistry is entering a digital era, where the detection of a single entity is possible. By digital, we mean the entity is either there or it is not (i.e. 1 or 0), and several positive identifications can be made during the time course of a single experiment, increasing precision. By entity, we mean anything from a single atom to an enzyme to a nanoparticle or virus to a single, living (or dead) cell. These experiments are often termed stochastic experiments due to the random nature of the entity detection as collisions are observed following time-dependent mass transport to the electrode surface. A fair question follows: Why is the study of a single entity important?[1] Imagine never having heard of fireflies. Your colleague places millions of fireflies into three large jars, and she asks you to come to some general conclusion regarding the light given off by the fireflies. As a scientist, you observe that the intensity of light coming out of the jars scales linearly with the number of bugs in each jar. From this observation, one may come to the conclusion that fireflies continuously bioluminesce. This is not the fault of the observer, for what is the probability that the millions of fireflies will blink on and off in synchrony? Nearly 0. Not until scientists like WE Moerner[2] began studying the fluorescence of single proteins did we know that proteins blink off into a metastable dark state. Moerner and co-workers used this blinking phenomenon to develop a novel form of microscopy capable of overcoming the diffraction limit of light,[3–5] for which he shared the 2014 Nobel Prize in Chemistry[6] with Eric Betzig and Stefan Hell. Therefore, single entity experiments have exciting ramifications. First, they allow one to study variations in populations, like that of fireflies, that may not be obvious from measurements taken over ensemble amounts of entities. Thus, we may learn new truths of nature concerning the chemical and physical properties of single entities, which in turn may be exploited to the benefit of mankind. Second, and most relevant to this review, is that these experiments take analysis to the ultimate sensitivity in measurement science: a limit of detection of 1.
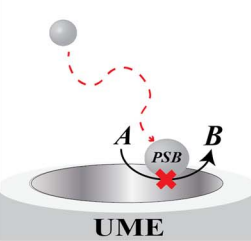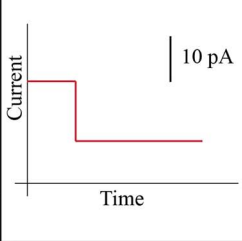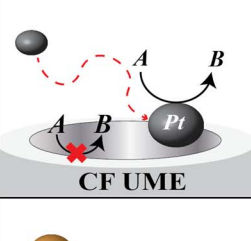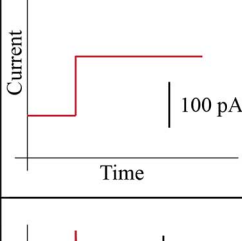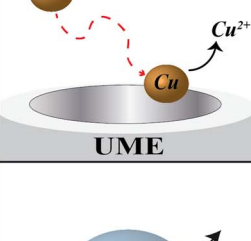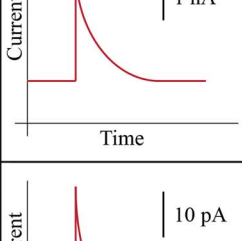
One may more specifically wonder why a limit of detection of 1 is important. While we have chosen to focus our efforts on critically reviewing specific entity detection in stochastic electrochemistry, these types of experiments also allow for basic research with the purpose of answering fundamental questions (understanding, for example, single entity physicochemical properties). In terms of electrocatalysis, for instance, measurements over ensemble amounts of nanoparticles

only reports on the average electrochemical behavior of a nanoparticle. These experiments have limited efficacy since typical synthetic means to create these nanoparticles leave a distribution of size and morphology. Understanding how the size, morphology, and nanoparticle stabilizing agent (i.e., stabilizing ligand) affect electrocatalysis can better direct synthetic methods to optimize reactivity. As mentioned above, due to heterogeneities in nanoparticle shape and size following synthesis, the ideal way to understand nanoparticle reactivity is by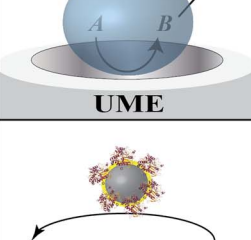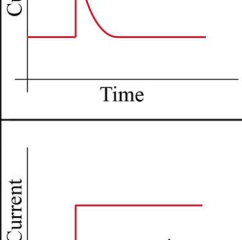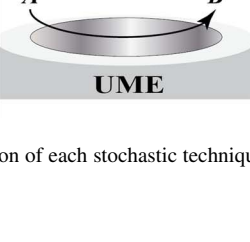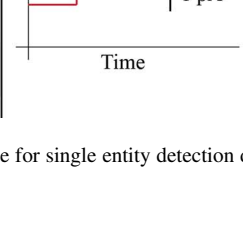 studying single nanoparticles. Electrochemistry is a valuable tool to achieve this understanding over optical measurements, which rely on electrocatalysis of a fluorogenic reaction to diagnose reactivity.[7–12] Direct electrochemical measurements allow the study of more industrially-relevant reactions, such as hydrogen oxidation and proton reduction, oxygen reduction and water oxidation, methanol oxidation and carbon dioxide reduction, and so on.[9,13–25]

In terms of diagnostic technology, early detection of very dilute species will improve patient outcomes and, potentially, diminish or even eradicate the progression of disease. For instance, one example where stochastic electrochemical detection becomes relevant is the specific detection of a circulating tumor cell in the blood.[26,27] At the earliest onset of metathesis, circulating tumor cells manifest on the order of one part-per-billion. Developing strategies to selectively detect one in a billion is difficult, but it would ensure detection before tumors have a chance to metathesize and grow elsewhere in the body. Similarly, stochastic electrochemistry may be used to generate calibration curves at the sub-femtomolar level ($\leq 10^{-15}$ M). For instance, there are extremely dilute species of interest in the blood, such as amyloid plaques or fetal micro-RNA,[28,29] that could be useful in diagnosing Alzheimer's[30] or tracking the health of a fetus throughout pregnancy. These dilute species are not one in a billion, but quantification of sub-nanomolar concentrations is commonplace in the single entity literature. Bard and co-workers have even reported calibration curves at the femtomolar level using concepts derived from stochastic electrochemical studies.[31–36]

The first report of single molecule electrochemistry belongs to Fan and Bard;[37,38] however, the field of stochastic electrochemistry as currently discussed (aka collision electrochemistry, impact electrochemistry, nano-impact electrochemistry) was born in earnest after Lemay's report in 2004 regarding the collision of single latex microspheres on ultramicroelectrodes (UMEs).[39] A discussion of these techniques and others that have evolved out of this first study is given below. Since that time, the field of stochastic electrochemistry has flourished. Several methods have been developed to observe the stochastic collision

[z]E-mail: jedick@email.unc.edu

Technique	Schematic	Amperometric Response & Current Magnitude	Benefits	Limitations
Blocking		10 pA	- Robust - Size-selective on hemispherical UME - Wide variety of entities give response	- Uneven current distribution on disk UME - Specific only to insulating or kinetically unfavorable entities - Prone to false-positives
Electrocatalytic Amplification		100 pA	- Highly selective - Low background current - Can use solvent to amplify signal	- Requires inner-sphere reactant for signal - High dependence on electrode/particle material - Particles may deactivate
Material Dissolution		1 nA	- Highest S/N - Does not require other reactants - Independent of UME material	- Limited to metals that easily oxidize - Oxidation product cytotoxicity - Particles may deactivate before fully reacting
Droplet, Vesicle, Micelle		10 pA	- Reactions may be confined to sub-femto-liter enviroments - Wide range of sizes - Simple to generate	- Must consider charge balance mechanism - S/N is low - Difficult to use as tags for biomarkers
Enzymatically Enhanced		1 pA	- Entity specific detection - ELISA literature precedence	- Low S/N - Electrode fouling/ enzme non-specific adsorption - Complex mechanism

Figure 1. Schematic representation of each stochastic technique for single entity detection discussed in this review as well as the typical response, benefits, and limitations of each technique.

of single entities, and the vast majority of these methods use amperometry to make the observation. There are also reports of single entity electrochemistry using potentiometry[40] and voltammetry.[41,42] The driving force behind developing new strategies to observe single entities is the quantification of different materials based on insulating/conductive and hard/soft properties. While these methods have held the promise of learning a new truth of nature at the single entity level, translation of this science to diagnostic practices has been scarce. Below is a brief summary of each major method developed. Figure 1 gives a general schematic representation of each collision experiment discussed, the typical response in the amperometric *i-t* trace with the magnitude of the event observed, and details benefits and limitations of each technique. Below, we describe the potential for specificity and whether or not specificity was demonstrated in some fashion. If no specificity was demonstrated, but there is a conceivable mechanism for specificity, methods to demonstrate and enhance specificity are discussed.

Specific Detection Strategies, Signal-To-Noise, and Methods to Enhance Specificity

In the methods described below, each technique is performed on an UME or a nanoelectrode due to a combination of low background noise and diminished double-layer capacitance. This dynamic combination allows one to observe minute changes in signal due to the collision of a single entity on the surface of an electrode. Fabrication of UMEs has become routine in most laboratories. However, fabrication of nanoelectrodes can be difficult and irreproducible requiring significant optimization of parameters for different materials on different instruments.[25,43–52] The size of the electrode should be comparable in size to the colliding entity to make a positive identification as shown in Schematic 1. Bard and Boika recommend the size of the entity be roughly 10% the size of the electrode surface.[53] Three electrodes are generally placed in a solution of about 10 mL; however, Hilterbrand and co-workers were able to perform collision experiments in only 100 μL of mouse urine.[54] The reference electrode is a Ag/AgCl
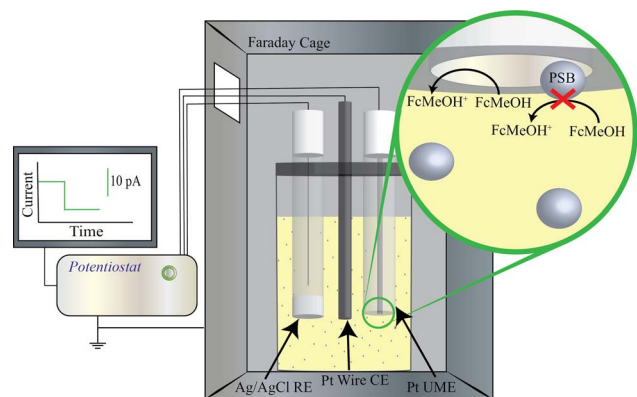
Schematic 1. Experimental schematic for a typical blocking experiment, in which a Pt UME is biased at +0.4 V vs. Ag/AgCl to oxidize ferrocene methanol to ferrocenium methanol. When the electrode is blocked by an insulating polystyrene bead (PSB) a decrease in current is observed.

reference electrode, and the counter electrode is either a platinum wire or a carbon rod. Users should be careful using platinum counter electrodes when studying electrocatalysis, as platinum has been hypothesized to dissolve while oxidizing water[55] or reducing oxygen.[34] Certainly on nanoelectrodes, a two-electrode system is sufficient since small currents are passed, ensuring the integrity (equilibrium) of the reference electrode remains intact. A background within the solution is taken (i.e., an amperometric *i-t* curve will be collected for several hundred seconds to ensure no collisions occur within the background).

The type of collision that is observed depends on solution conditions and electrode material. Some detection schemes take advantage of using a redox reporter molecule. This molecule can be oxidized or reduced at the electrode surface, and variations in the flux of this redox molecule (ultimately manifesting in the overall current response) indicate the collision of the entity with the electrode surface. Other detection schemes necessitate taking advantage of differences in heterogeneous kinetics on different materials. This is generally accomplished by driving the electrochemistry of an inner-sphere heterogeneous reaction.[56] For inner-sphere reactions, a prerequisite to electron transfer is the adsorption of the molecule on the electrode surface. This generally implies that the potential where electron transfer begins is highly dependent on the type of material used. For instance, the example given below takes advantage of the fact that proton reduction occurs much more quickly on platinum than on carbon. This is due to more favorable adsorption of hydride on platinum, making the heterogeneous kinetics of proton reduction on platinum much faster than on carbon. In contrast, outer-sphere electrochemistry does not depend on electrode material, as the electron transfer occurs via a tunneling mechanism through the inner Helmholtz plane (the first layer of solvent and adsorbed ions on the electrode surface).

Blocking (electrode area-decrease).—The blocking experiment (i.e., electrode area-decrease, where the area of an electrode decreases with the collision of an entity), which was the first stochastic experiment reported by Lemay in 2004,[39] requires a heterogeneous redox reaction to be driven at the electrode surface. When an insulating object collides with the UME surface, the flux of the redox reporter to the UME is locally blocked, and step-wise decreases in current can be observed as shown in Figure 2. The size of the step-wise decrease in current depends on the ratio of the size of the entity to the size of the electrode. In these experiments, it has been difficult to achieve size analysis of the colliding entity.[57] The difficulty lies in the inhomogeneous flux of the reporter molecule to the UME surface. Because radial diffusion on a typical disc UME dominates at the edges, there will be a higher flux of the reporter molecule at the edge of the UME (referred to as the edge effect).[58] Thus, if an insulating entity collides on the edge of the UME, it will block more flux than entities that collide near the center. This may lead one to believe they have a
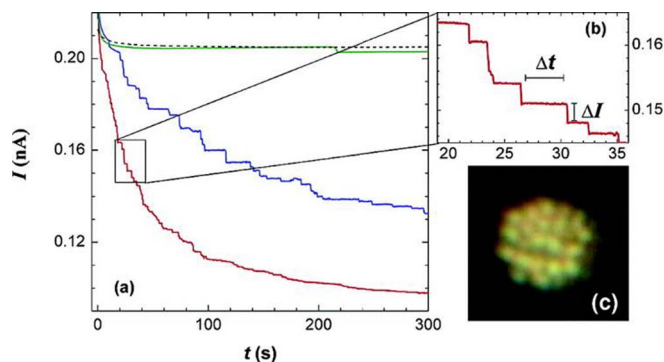

Figure 2. (a) Current−time (*I*−*t*) transients for the diffusion-limited oxidation of 0.31 mM FcMeOH (black line) at a disk Au microelectrode (r_e = 2.5 mm) showing discrete current step decreases in the presence of dispersed beads (r_b = 0.5 mm, c_{bead} = 4 × 10^8 cm^{-3}) at 0.5 (red line), 5 (blue line), and 50 mM (green line) KCl supporting electrolyte concentrations. (b) Detail of panel a. (c) Ex situ optical micrograph taken after the amperometric measurements showing the electrode surface covered by beads. Reprinted with permission from Quinn, B. M.; van 't Hof, P.; Lemay, S. G., Time-resolved electrochemical detection of discrete adsorption events. *J. Am. Chem. Soc.* **2004**, *126* (27), 8360–8361. Copyright 2004 American Chemical Society.

polydisperse system when using a monodisperse sample. Crooks has quantified the edge effects in collisions of insulating microspheres by optically correlating the collision to amperometry using fluorescently-tagged microspheres (Figure 3).[59] To overcome the hindrance of edge effects observed when using disc UMEs, Renault and co-workers
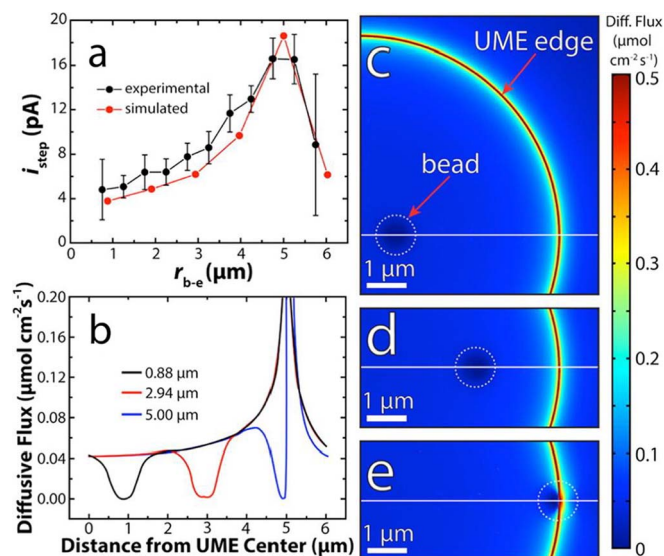
Figure 3. (a) Plot of i_{step} vs. r_{b-e}. The black symbols represent data that were binned into increments of 0.5 μm for simulation comparison. The error bars represent the 95% confidence interval for collisions that occurred within the indicated bin. The red circles represent simulated data for the difference in steady-state current at a UME having an insulating spherical particle (1.03 μm) centered at the indicated distance from the center of the UME vs. the steady-state current at the bare electrode. (b) Simulated diffusive flux profile across the length of the radius of the UME for different values of r_{b-e}. The maximum flux was 4.2 μmol cm^{-2} s^{-1}, but the color scale was truncated to more clearly show the effect of the insulating beads. (c–e) Surface plots of diffusive flux over a portion of the surface of the UME for r_{b-e} = 0.88 μm (c), 2.94 μm (d), and 5.00 μm (e). The location of the spheres is indicated by the dashed white circle, and the white line represents the radius of the UME. Reprinted with permission from Fosdick, S. E.; Anderson, M. J.; Nettleton, E. G.; Crooks, R. M., Correlated Electrochemical and Optical Tracking of Discrete Collision Events. *J. Am. Chem. Soc.* **2013**, *135* (16), 5994–5997. Copyright 2013 American Chemical Society.

(A) [graph]

(B) [graph]
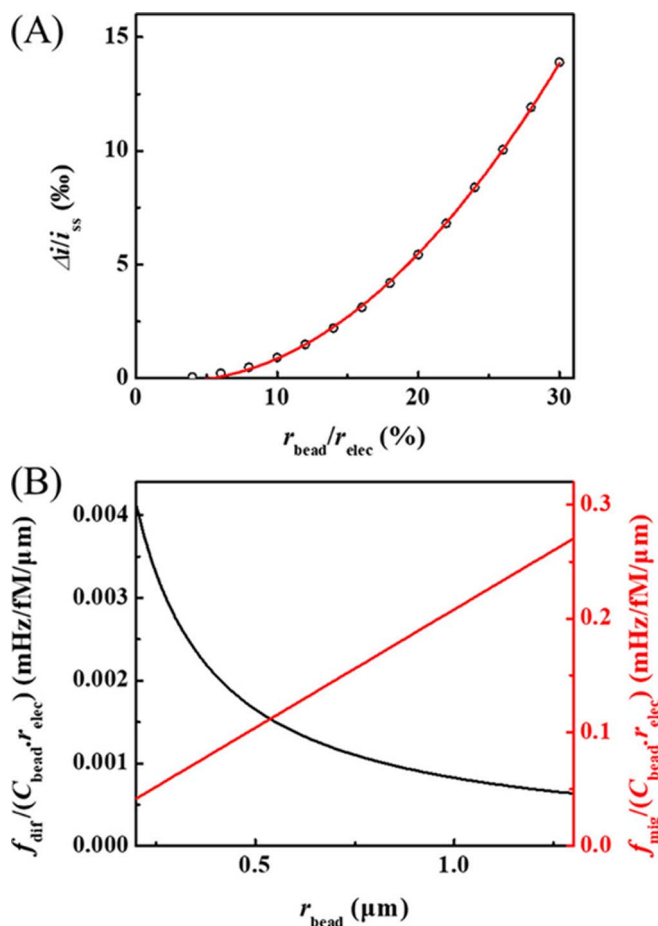
Figure 4. (A) Simulated calibration curve of the step size vs. bead size. The red line corresponds to a least-squares fitting of a second order polynomial: $Ax^2 + Bx$. The coefficients A and B equal 0.0187 and -0.101, respectively ($R^2 = 0.999$). (B) Normalized collision frequencies as a function of the radius of the bead. The black and red lines correspond to the diffusion and migration components, respectively. Frequencies calculated based on specified experimental parameters and equations listed in the reference. Reprinted with permission from Deng, Z.; Elattar, R.; Maroun, F.; Renault, C., In Situ Measurement of the Size Distribution and Concentration of Insulating Particles by Electrochemical Collision on Hemispherical Ultramicroelectrodes. *Anal. Chem.* **2018**, *90* (21), 12923–12929. Copyright 2018 American Chemical Society.

recently used hemispherical UMEs, where the flux profile is consistent across the UME surface, in blocking experiments to report quantitative sizing of entities.[60] This method has also been used to observe the collision of single protein molecules on UME surfaces[61] as well as tracking polystyrene bead aggregation due to the presence of murine cytomegalovirus.[62]

Size of the response.—The magnitude of the current step observed depends on the ratio of the size of the entity to the size of the electrode. Generally, these events are on the order of tens of picoamperes. Figure 4 displays the exponential relationship between the size of the response, or step size, and the size of the entity derived recently by Renault and co-workers.[60]

Potential for specificity.—Unfortunately, any insulator colliding with and sticking to the electrode surface will block flux, which makes complex matrices difficult to study. One can imagine, however, discriminating by size, as Renault and co-workers showed was possible with hemispherical UMEs, to differentiate between entities in complex systems.[60] This could be particularly useful in a complex matrix of different virus particles, which vary greatly in size from Zika
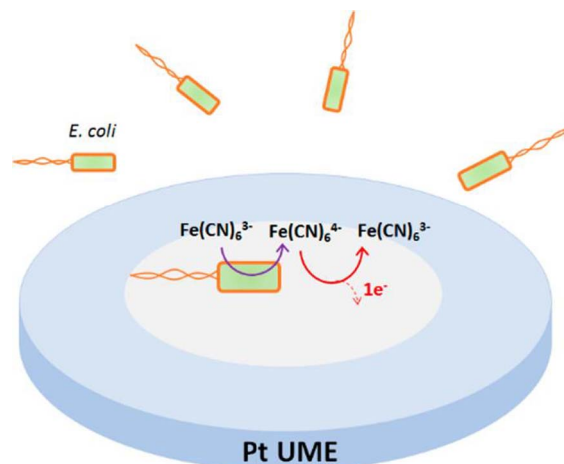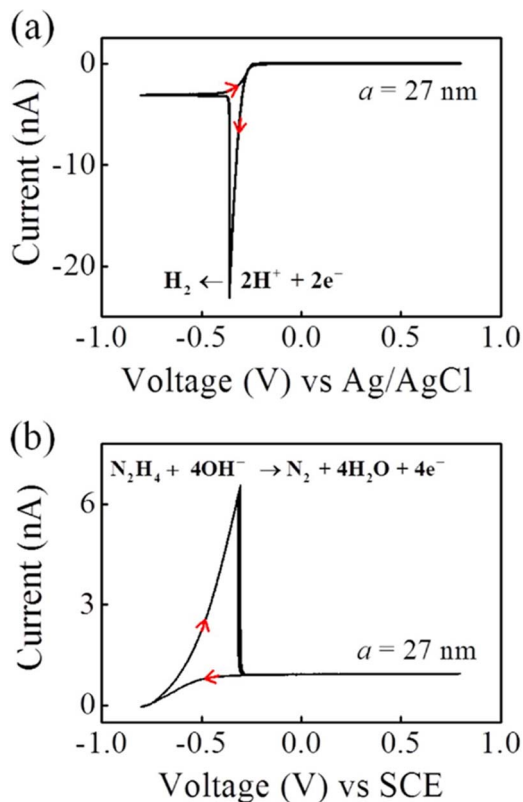

Schematic 2. Experimental schematic of label-free detection of *E. coli* by electrochemical collision technique. Reprinted with permission from Gao, G. W., Denchao; Brocenschi, Ricardo; Zhi, Jinfang; Mirkin, Michael V., Toward the Detection and Identification of Single Bacteria by Electrochemical Collision Technique. *Anal. Chem.* **2018**, *90* (20), 12123–12130. Copyright 2018 American Chemical Society.

(~40 nm) to Ebola (~1 μm). The blocking experiment has also been applied to single bacteria. Previously, Kwon and co-workers have reported the label-free detection of single *E. Coli* bacteria colliding with UME surfaces.[63] It should be noted here critically that 'label-free' does not constitute specificity, as the bacteria were simply blocking a heterogeneous reaction at the UME surface, as shown in Schematic 2. Recently, Mirkin and co-workers reported that single bacteria colliding with UME surfaces can generate current.[64] This is a much more exciting line of inquiry that may lead to specificity of gram-positive and gram-negative bacteria at the single bacterium level.

Electrocatalytic (kinetic) amplification.—Electrocatalytic, or kinetic, amplification experiments involve taking advantage of differences in heterogeneous kinetics between different materials, which is most relevant in inner-sphere electrochemistry as discussed above. For instance, proton reduction is slow on carbon but is fast on platinum. Imagine a carbon fiber UME is placed in an acidic solution. If the electrode is biased at a potential where proton reduction occurs on platinum but not carbon, and a platinum nanoparticle collides with the carbon UME surface and sticks, a change in the amperometric current will be observed. The oxidation of hydrazine is a similar case, where the potential for oxidation is lowest on platinum and highest on carbon. In each of these two cases, one can choose a potential to discriminate heterogeneous reaction kinetics to observe collisions of nanoparticles.

Size of the response.—Generally, these events are on the order of tens to hundreds of picoamperes depending on the reaction kinetics, analyte concentration, and entity size. The significant challenge in enhancing the signal is that generally, inner-sphere reactions are driven to differentiate the colliding nanoparticle. To date, only inner-sphere reactions that produce gases have been used, and large concentrations of these reactants will cause bubbles to form and ultimately cut off electrocatalysis. White and co-workers have studied nanobubble formation on small electrode surfaces extensively.[65–71] In these experiments, nanoelectrodes are fabricated by etching a microwire to a nanometer-sized tip, which is then insulated in a glass capillary. Upon mechanical polishing, a nanometer-sized disc is exposed, and electrochemistry can be performed on these discs. These experiments are performed in various concentrations of hydrogen, peroxide, or hydrazine, where the oxidation or reduction produces a hydrogen, oxygen, or nitrogen bubble, respectively. Typical *i-V* responses for experiments of this nature are shown in Figure 5. These studies indicate that proton reduction

Schematic 3. Experimental schematic of detection of miRNA *via* ECA. Scheme 3a shows a PtNP that has been surface modified with a monolayer of single-strand DNA (ssDNA). ssDNA coating hinders production of current transients upon collisions with the electrode surface. In Scheme 3b, miRNA, which is complementary to the immobilized DNA, is introduced to a solution containing the PtNP@ssDNA conjugate. This results in hybridization. Next, the PtNP@ssDNA:miRNA conjugate is incubated with duplex specific nuclease (DSN), which cuts ssDNA when it is hybridized to complementary nucleic acids, and if the conjugate is a DNA:RNA hybrid, it leaves the original RNA intact so that it can bind to another immobilized ssDNA. This process continues until sufficient ssDNA is removed from the PtNP that current transients are observed when the particles strike the electrode. Reprinted with permission from Castaneda, A. D.; Brenes, N. J.; Kondajji, A.; Crooks, R. M., Detection of microRNA by Electrocatalytic Amplification: A General Approach for Single-Particle Biosensing. *J. Am. Chem. Soc.* **2017**, *139* (22), 7657–7664. Copyright 2017 American Chemical Society.

Figure 5. *i-V* responses of (a) a 27 nm radius Pt nanoelectrode immersed in 0.5 M H_2SO_4 for H_2 nanobubble formation, and (b) the same Pt nanoelectrode immersed in 1.0 M N_2H_4 for N_2 nanobubble formation, both at a scan rate of 200 mV/s. The voltammograms shown are for two cycles each to demonstrate the reproducibility of bubble formation, as well as bubble dissolution at slow voltammetric scan rates. Data reproduced from German, S. R.; Chen, Q. J.; Edwards, M. A.; White, H. S., Electrochemical Measurement of Hydrogen and Nitrogen Nanobubble Lifetimes at Pt Nanoelectrodes. *J. Electrochem. Soc.* **2016**, *163* (4), H3160-H3166.

will form a hydrogen bubble when the concentration of proton is on the order of 80 mM and hydrazine oxidation will form a nitrogen bubble when the concentration of hydrazine is on the order of 300 mM.[69] Importantly, this implies that the signal in electrocatalytic amplification has a limit unless an inner-sphere reaction is driven, and the product of the reaction is not a gaseous species. Furthermore, deactivation of the response is often seen in the literature even without gas-forming conditions.[69,72]

For proton reduction, even before reaching proton concentrations where bubble formation is favorable, the high ionic strength associated with this concentration increase is deleterious to nanoparticle stability. Nanoparticles are generally charged, and this electrostatic repulsion ensures that nanoparticles do not agglomerate. Upon increasing the ionic strength, the surface charge of the nanoparticle is diminished, and the stability of nanoparticles decreases (i.e., the rate of agglomeration increases). One potential method of mitigating this is by performing electrocatalytic amplification experiments with weak acids. Due to the relatively small acid dissociation constants, the ionic strength of the solution will not increase. However, as the nanoparticle begins to consume acid at the electrode surface to produce hydrogen gas, the concentration gradient will push the equilibrium of the weak acid toward deprotonation. With this method, the stability of the colloidal suspension can be maintained, and an amplification for the collision response can also be obtained. Stability can only be maintained so long as the aggregation kinetics are influenced only by ionic strength, which is not always the case if an ion within the salt reacts with the nanoparticle surface. To the authors' knowledge, this experiment has not yet been performed, but it can solve the problem surrounding nanoparti-

cle stability while maximizing the signal-to-noise ratio before bubble formation diminishes the signal.

Potential for specificity.—It is unlikely that one could discriminate simply based on kinetics of the collisions of 20 different nanoparticles in a complex mixture. However, in order to gain specificity, groups have taken advantage of functionalizing different materials with, for instance, DNA, RNA, or antibodies. Therefore, electrocatalytic amplification cannot be used to discriminate separate nanoparticles, but functionalization techniques can ensure electrocatalytic amplification can be used as a detection scheme for biologically interesting entities. These techniques are remarkably sensitive, as nanoparticles down to 5 nm in size have been reported. Bard previously showed that one can attach a strand of DNA onto platinum nanoparticles to make them specific to the complementary strand, and Crooks has been able to detect strands of micro-RNA with a similar detection scheme (Schematic 3).[73–76] Using concepts around electrocatalytic amplification, one could also conceive of an electrocatalytic depression experiment, where a kinetically unfavorable material would block the heterogeneous electron transfer of an inner-sphere reaction. Furthermore, Bard and co-workers have used these techniques to study electrocatalysis of isolated atoms and clusters on nanoelectrodes and have reported calibration curves for trace metals at the femtomolar level.[21,32–36,77] Zhang and co-workers recently introduced the concept of label-free protein sensing using aptamer-based binding on single nanoparticles (Figure 6).[78] In this experiment, binding of the protein liberates the aptamer from the surface of the nanoparticle, allowing the signal (i.e., the collision response) to be observed in the amperometric *i-t* trace. The authors were able to detect protein-derived growth factor in rat cerebrospinal fluid; however, the technique necessitated introducing hydrazine into the system. This technique requires a kinetic difference, and sluggish reactions that may be deleterious to biological samples (i.e., hydrazine) to observe the response. Inherently, the technique only discriminates between relatively inert and electrocatalytic materials, which covers a broad range of materials. This may result in unfavorable responses due to other electroactive biomolecules within the sample.
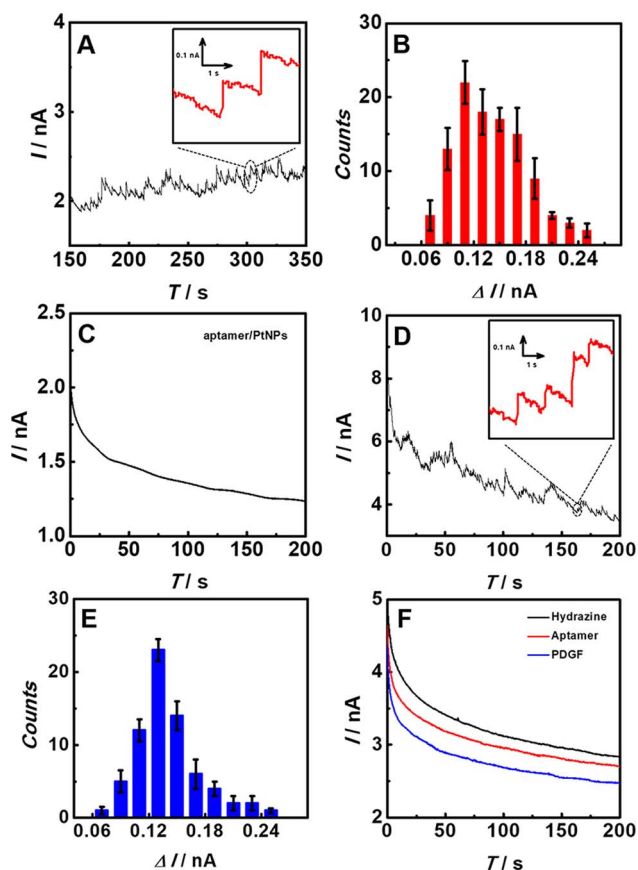
Figure 6. (A) Typical amperometric response recorded with CFME in 10 mM phosphate buffer containing 10 mM N_2H_4 and 0.5 pM PtNPs. (B) Histogram of step current resulting from PtNPs. Typical amperometric response of aptamer/PtNPs before (C) and after (D) being incubated with 0.5 nM PDGF for 30 min. (E) Histogram of step current resulting from aptamer/PtNPs after being incubated with 0.5 nM PDGF. (F) Typical amperometric response recorded with CFME in 10 mM phosphate buffer containing 10 mM N_2H_4 (black curve) in the presence of 0.5 nM aptamer (red curve) or 0.5 nM PDGF (blue curve). Applied potential: +0.10 V vs. Ag/AgCl. Reprinted with permission from Zhang, Y. M., Jinpeng; Ji, Wenliang; Feng, Taotao; Fu, Zixuan; Zhang, Meining; Mao, Lanqun, Collision of Aptamer/Pt Nanoparticles Enables Label-Free Amperometric Detection of Protein in Rat Brain. *Anal. Chem.* **2019**, *91* (9), 5654–5659. Copyright 2019 American Chemical Society.

Material dissolution.—Compton and co-workers first reported the oxidation of single silver nanoparticles on UMEs that was subsequently extended to the oxidation of copper.[79–82] In these experiments, the UME surface is biased at a potential where the oxidation of the metal nanoparticle will occur upon collision with the UME surface. Because one can pack many atoms within the lattice of a single nanoparticle, the faradaic response associated with this type of collision experiment is very large (among the largest in the literature). This technique exploits the high-density packing of metal atoms to obtain a high signal-to-noise ratio, whereas most techniques rely on a solution species being sufficiently concentrated.

Size of the response.—As the current response is driven by the oxidation of metal species on the colliding nanoparticle, the magnitude is highly dependent on the nanoparticle material (i.e. oxidation of Ag to Ag^+ will generate half the signal of Cu to Cu^{2+} oxidation). Furthermore, the response is proportional to the number of metal atoms in the nanoparticle, typically >1 nanoampere for a particle with a radius of 20 nm.

Potential for specificity.—This has not yet been demonstrated. Compton and co-workers have non-specifically adsorbed silver

nanoparticles onto bacteria to detect single bacteria collisions with UMEs. For specificity, one must consider biocompatibility between the entity and the nanomaterial to obtain a large signal-to-noise ratio. Within this experimental set up, the electrochemical signal is driven by the surface oxidation of the nanoparticle into bulk solution, making an additional redox probe unnecessary, potentially widening the range of entities and systems that may be explored using this methodology.

Enzymatically-enhanced.—Oxidoreductase enzymes have been studied widely in electroanalysis and the electrochemical sensing of glucose, and there are a few reports in the literature claiming observation of single enzyme catalysis at UMEs. The amount of current generated by a single enzyme is given by

$$i_{cat} = qnk_{cat} \qquad [1]$$

where q is the charge on an electron, n is the number of electrons transferred, and k_{cat} is the catalytic rate specific for the enzymatic process assuming infinite substrate concentration. Here, the concentration of the substrate must be infinite compared to the concentration of the enzyme to ensure constant saturation of the active site. Fast enzymes (i.e., glucose dehydrogenase) have k_{cat} values of $\sim 1500 \text{ s}^{-1}$, which yield sub-femtoampere currents. Considering the peak-to-peak noise in most electrochemical experiments is, at best, tens of femtoamperes in a well-grounded Faraday cage, single enzyme turnover simply cannot be achieved with high bandwidth measurements, where high bandwidth measurements are sampling rates on the order of kHz or better. Despite this simple analysis, there are reports in the literature on single enzyme collisions passing tens of pA of current.[83–88] These reports continue to be debated in the literature.

One can use another entity as a scaffold around which enzymes can be pre-concentrated, and this line of thought can yield a strategy for both amplification and specificity. Dick and co-workers demonstrated that glucose oxidase enzymes can be linked to a primary antibody that is specific to a glycoprotein on the surface of murine cytomegalovirus.[54] Thus, the virus acts as a scaffold around which enzymes can preconcentrate. When the virus particles collide with an UME, where the oxidation of a well-characterized redox reporter, ferrocene methanol, produces ferrocenium methanol, the ferrocenium methanol is converted back to ferrocene methanol by the enzyme in a deaerated solution. Use of one-electron mediators as enzyme substrates has been previously studied by Saveant and Demaille.[89] This conversion creates a catalytic loop, and the collision results in a stepwise increase in current. Importantly, the collision of a single enzyme will not give a false negative, and enzymes were not found to nonspecifically adsorb to virus particles. Compton and co-workers have also applied these techniques to modifying silica nanoparticles with enzymes.[87] Each of these experiments are still technically enzyme-linked immunosorbent assays (ELISAs), and they have been termed Digital ELISAs.

Size of the response.—Generally, the current response in an enzymatically-enhanced single entity collision is less than 10 picoamperes. One can imagine, however, incorporating materials dissolution strategies to the detection mechanism. Instead of attaching enzymes on antibodies, one could develop methods to attach silver nanoparticles onto antibodies. There are obvious challenges, such as maintaining the integrity of the antibody upon silver nanoparticle attachment, but these have been previously overcome for different systems.[90]

Potential for specificity.—Demonstrated to be highly specific in complex matrices (i.e., urine of infected mice). The greatest drawback in these experiments is the low signal-to-noise ratio for the collision (~ 15); however, other preconcentration schemes (i.e., using silver nanoparticles instead of enzymes) can be imagined to enhance the signal-to-noise ratio.

Droplet, vesicle, and micelle.—*Emulsion droplets.*—Perhaps the most famous emulsion is milk, where fat droplets are suspended in water. These droplets are large, and so scattering of light occurs, rendering

milk a familiar white color. These nanometer-sized droplets (droplets with radii on the order of hundreds of nm have sub-femtoliter volumes, where fL is 10^{-15} L) can act as confined volumes, and electrochemistry can be performed using concepts based on collision experiments. Several experiments have been devised to study electrochemistry in confined volumes. The two phases that comprise these systems are the continuous phase and the dispersed phase. The continuous phase is the solvent in which droplets are suspended, and the dispersed phase is the solvent that makes up the droplets. For instance, one can trap a redox molecule in a water droplet suspended in oil (or vice-versa) based on solvation (i.e., the redox reporter will only be soluble in the droplet or dispersed phase). Upon collision with the UME surface biased sufficiently positive or negative to react the analyte in the droplet, the reactivity can be monitored in the amperometric response. Perhaps most exciting about these experiments is that one can track in the amperometric response the electrolysis of the droplet contents, and Faraday's Law can be used to extract the volume of the droplet and, hence, its radius:

$$V = \frac{4}{3}\pi r^3 = \frac{Q}{nFC^*} \quad [2]$$

where V is the droplet or vesicle volume, r is the droplet or vesicle radius, Q is the charge obtained by integrating under the current-time response, n is the number of electrons associated with the electrolysis, F is Faraday's constant, and C^* is the concentration of analyte species in the droplet or vesicle. One can also use the collision response to fit the transient collision decay to bulk electrolysis theory:

$$i(t) = i_0 e^{\frac{-mA}{V}t} = i_0 e^{\frac{-4Dr}{V}t} \quad [3]$$

where $i(t)$ is the current as a function of time, i_0 is the initial current, m is the mass transfer coefficient, A is the area of the electrode, and t is time. If we consider the mass transfer coefficient to a disc electrode ($m = 4D/\pi r$), the equation above can be further simplified, and r can be interpreted as the contact radius. Fits of this kind have yielded contact radii of tens of nm, implying the droplet collision experiment is effectively a nanoelectrode experiment where the volume is confined.

Bard and co-workers first reported the collision of toluene droplets suspended in water by reacting ferrocene trapped in toluene.[91–97] Dick and co-workers,[98–102] Ahn and co-workers,[103] and Tschulich and co-workers[104] have been working toward using these principles to electrodeposit nanoparticles onto conductive substrates. Recently, Dick and co-workers demonstrated that these types of collisions can be used in materials synthesis of high entropy metallic glass nanoparticles, and they demonstrated applications to designer, multifunctional electrocatalysis (Figure 7).[105]

Vesicles and micelles.—This type of experiment has also been used to study contents within vesicles[26,106] and micelles[107] and may be used in controlled synthesis of molecular or materials libraries in tiny volumes. For vesicle analysis, the dispersed phase is extruded with lipids to form vesicles, which have a narrow size distribution. These vesicles are then separated by size-selective chromatography to be used in collision experiments. The equations given above in this section still hold for these different types of entities.
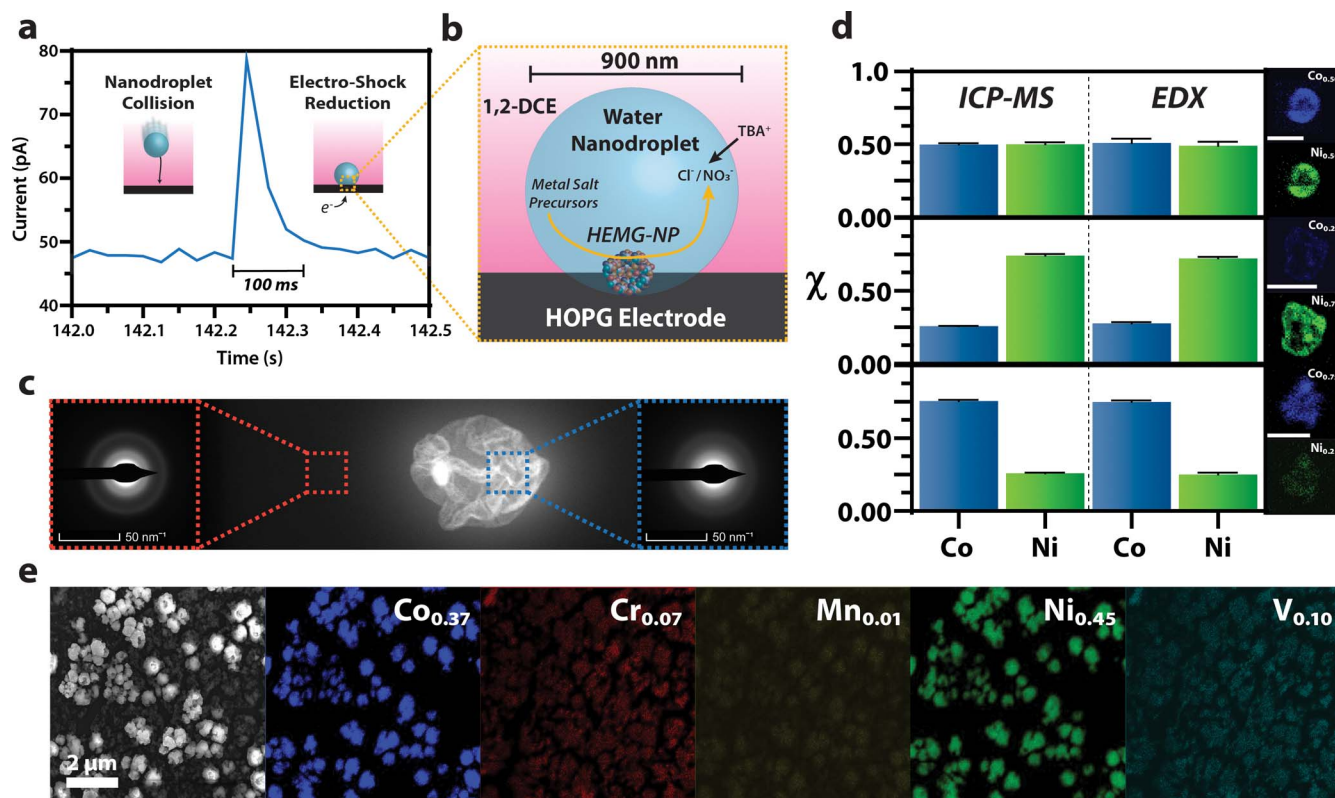
Figure 7. Nanodroplet-mediated electrodeposition overview for controlling NP stoichiometry and microstructure. a) Current transient corresponding to the collision of a single nanodroplet onto a carbon fiber UME ($r_{UME} = 4\,\mu m$) biased at −0.4 V vs. Ag/AgCl. Nanodroplet contents are fully (>98%) reduced within 100 ms, facilitating disordered co-deposition of various metal precursors. b) Representation of a nanodroplet collision event highlighting the rapid NP formation at the water/substrate interface and the charge balance ensured by the transfer of TBA+ across the oil/water interface. c) An amorphous microstructure is confirmed by a lack of crystallinity at high resolution and the presence of diffuse rings on the SAED pattern. d) Correlated ICP-MS and EDX results on Co0.5Ni0.5, Co0.25Ni0.75, and Co0.75Ni0.25 MG-NPs confirming precise control over NP stoichiometry. e) Alloy film electrodeposited from aqueous solution of equimolar metal salt precursors demonstrating phase and stoichiometric heterogeneity. Reprinted from Glasscott, M. W.; Pendergast, A. D.; Goines, S.; Bishop, A. R.; Hoang, A. T.; Renault, C.; Dick, J. E., Electrosynthesis of high-entropy metallic glass nanoparticles for designer, multi-functional electrocatalysis. *Nature Communications* **2019**, *10*. Copyright 2019 Nature Publishing Group. https://creativecommons.org/licenses/by/4.0/.

Table I. Collision Frequency Considerations (Diffusion).

Number of Entities per Liter	Concentration (M)	Time (s)
1	1.7E-24	20,321,709,950,394
10	1.7E-23	2,032,170,995,039
100	1.7E-22	203,217,099,504
1,000	1.7E-21	20,321,709,950
10,000	1.7E-20	2,032,170,995
100,000	1.7E-19	203,217,100
1,000,000	1.7E-18	20,321,710
10,000,000	1.7E-17	2,032,171
100,000,000	1.7E-16	203,217
1,000,000,000	1.7E-15	20,322
10,000,000,000	1.7E-14	2,032
100,000,000,000	1.7E-13	203
1,000,000,000,000	1.7E-12	20

Size of the response.—The size of the response is on the order of tens of picoamperes.[108]

Potential for specificity.—At this point, it is difficult to imagine using a droplet, vesicle, or micelle as a tag for biological detection. From a materials perspective, Bard and co-workers have been able to trap molecules that upon reacting with an electrode, generate light (electrogenerated chemiluminescence).[96–97] The addition of light may add specificity; however, the use of droplets, vesicles, or micelles as tags has yet to be demonstrated.

Methods to Decrease Limit of Quantitation

As alluded to in the abstract, the limit of quantitation can be easily thought of as the amount of time one wishes to wait for the detection of a single entity to occur. Because of the stochastic nature of single entity experiments, the concentrations are almost always below 1 pM. Consider an Olympic-sized pool with a pole at its center, stretching from the bottom of the pool through the water and piercing the air above the water. If a billion ping pong balls are introduced into the pool, the random collision of a ball with the pole is highly probable. However, if only one ping pong ball is placed in the pool, one would have to wait a very long time for the random collision of the ball with the pole. One can think of different ways to increase the collision rate of the ball with the pole by increasing the diameter of the pole or introducing convection within the pool. Similarly, by placing a small UME into a solution of entities, the frequency with which entities collide with the UME surface is dictated by mass transfer and the size of the UME. Unfortunately, one sacrifices measurement sensitivity by increasing the electrode size – small entities (i.e., single proteins) simply cannot be observed colliding with millimeter-sized electrodes. To highlight the necessity for other modes of mass transfer, let us consider the amount of time one has to wait to observe the collision of an entity considering a certain number of entities in a liter of solution. Table I indicates that 20 trillion seconds are necessary to observe the collision of one entity suspended in a liter of solution; however, when a trillion entities are in a liter of solution, only 20 seconds are necessary to make the observation. This corresponds to picomolar concentrations of entities, and the diffusion coefficient was calculated using the Stokes-Einstein approximation considering an entity with a radius of 100 nm.

Collisions by diffusion.—The frequency with which entities collide with an electrode surface is given by

$$f_{diffusion} = A m_O C N_A \quad [4]$$

where A is the electrode area, m_O is the mass transfer coefficient, C is the concentration of colliding entity, and N_A is Avogadro's Number. While the majority of the stochastic electrochemistry literature has focused on the collision frequency of entities, it is likely more appropriate to describe these experiments as a collision rate. This is

especially important in fundamental studies of a single entity colliding with an electrode surface multiple times, where the frequency with which the single entity collides with the electrode can be quantified with high-bandwidth measurements.[109–113] Equation 4 is simply a form of Fick's Law and predicts the collision frequency for freely diffusing nanoparticles, where m_O depends on the electrode geometry (i.e., m_O for an inlaid disc is $\frac{4D}{\pi r}$, where r is the electrode radius). This equation gives access to the concentration of colliding species assuming unit probability of entities sticking to the UME surface, which may not always be the case.[99] However, the use of this equation necessitates more than one collision to establish a frequency. It should be noted that the diffusion coefficient of the colliding species is always calculated using the Stokes-Einstein approximation[114] and assuming spherical geometry:

$$D = \frac{k_B T}{6\pi\eta r_{entity}} \quad [5]$$

where k_B is Boltzmann's constant, T is temperature, η is the kinematic viscosity of the solution, and r_{entity} is the radius of the entity.

Boika has previously reported a better measurement of concentration based on the time of first arrival of the entity with the UME surface. This allows a single collision to be used to calculate the concentration of colliding species assuming different mass transfer of single entities. If the mass transfer is governed mainly by diffusion, one simply has to take the inverse of Equation 4 as the average time between collisions. There are experimental difficulties, however, with regard to the time of first arrival of an entity with an UME surface. For instance, the RC time constant associated with the potential step may convolute collision results, rendering collisions difficult to observe at short times. Once entities are introduced in solution, homogenization must occur before the measurement to ensure accurate characterization.

Despite these limitations, one of the most powerful aspects of single entity electrochemistry is that, over time, the measurements increase in precision. Importantly in these studies, one should take care to ensure only a few collisions are analyzed. As collisions occur, the electrode size changes. Since frequency of collision and concentration depend on electrode size, only the first few collisions can be used in quantitative analysis.

Enhancing mass transfer by electrophoretic migration.—As Boika reports, one can also take advantage of electrophoretic migration.[53,115–117] In these experiments, the charge of the entity can be exploited to enhance mass transfer. For instance, if a carboxylated polystyrene bead is diffusing in solution, it will move toward the electrode surface by electrophoretic migration once the UME is polarized. The frequency with which these entities collide with an UME is given by

$$f_{migration} = \frac{i_{ss} C \mu}{e} \sum \frac{1}{C_i \mu_i} \quad [6]$$

where e is the charge on an electron and μ is the mobility. Whereas the diffusion of an entity is given by the Stokes-Einstein equation, the mobility of an entity is given by

$$\mu = \frac{2\sigma r_{entity}}{3\eta} \quad [7]$$

where σ is the charge density of the entity. There is an important implication in considering the differences in Equations 4 and 6: Smaller beads will diffuse more quickly under diffusion control but migrate less quickly under electrophoretic migration control. Larger beads will diffuse more slowly under diffusion control but migrate more quickly under electrophoretic migration control. This implies that one can use these physical differences to separate entities of different size and gain further specificity. Schematic 4 illustrates how entity size and charge density will affect diffusion and migration during stochastic experiments. It is important to note that groups have also found success using dielectrophoresis techniques.[118–120]
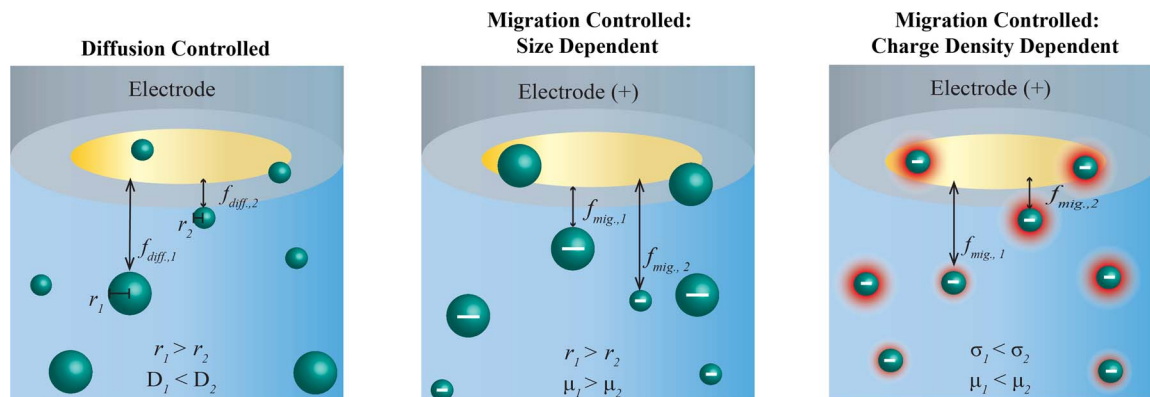
Schematic 4. Experimental schematics for stochastic experiments in which the collision frequency (*f*) is determined by the radius (*r*) or charge density (σ) of an entity under diffusion control or electrophoretic migration control. Charge density is indicated by the intensity of the red disk surrounding a sphere.

Other methods to enhance mass transfer.—There are, of course, other methods to enhance mass transfer other than migration in an electric field. For instance, Stevenson and co-workers specially synthesized iron oxide nanoparticles with platinum islands to observe electrocatalytic amplification and increase flux to the electrode surface by magnetism.[121] Crooks and co-workers have demonstrated that the collision frequency of magnetic microbeads will be enhanced drastically in the presence of a magnetic field focused on the tip of the UME.[122] The limit of quantitation in these experiments was pushed down to 20 aM – according to Table I, one would have to wait for over 200 million seconds (over six years!) to observe a positive response by diffusion. This proof-of-principle experiment used silver-nanoparticle-decorated-magnetic microspheres, where the material dissolution of silver gave a response upon collision with an UME surface.

Boika and co-workers have also shown that by using alternating current to selectively heat the UME surface, mass transfer can be enhanced (Figure 8).[123] This 'hot-tip' approach has been shown to enhance the collision frequency of nanoparticles on the UME surface by a factor of 10^3. While mass transfer is enhanced, the temperature gradient at the electrode surface may be deleterious to biological entities of interest.

Drawbacks and Limitations

Despite the exciting possibilities that have been discussed throughout this review, it is important to remark on drawbacks and limitations. As with all sensors based on electrochemical measurements,
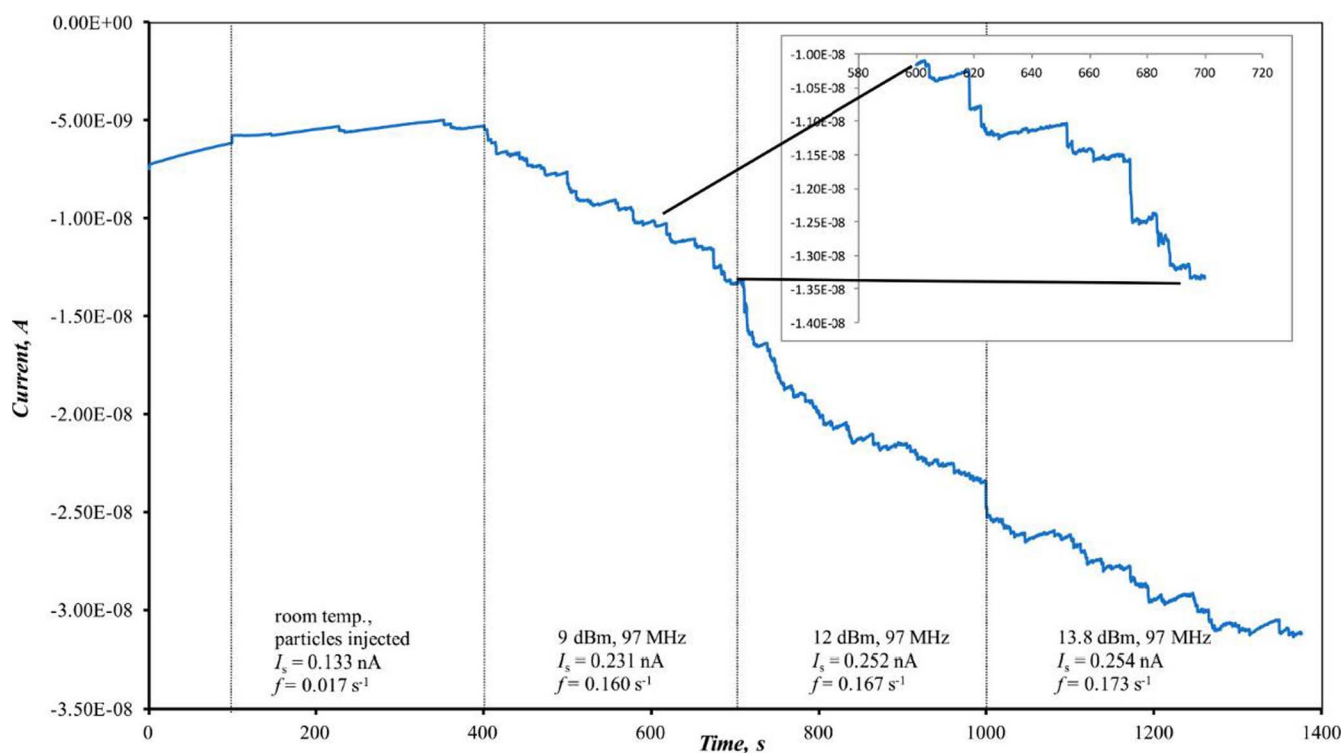
Figure 8. Chronoamperometric curve obtained in an experiment involving the detection of Pt NPs (33 nm diameter) in 15 mM hydrazine, 50 mM sodium phosphate buffer solution (pH 7.5). Working electrode: 10 μm gold disk, applied potential +0.05 V vs. Ag/AgCl. Pt NP concentration: 1 pM. Particles were injected at *t* = 100 s, and at *t* = 400 s the ac waveform was applied (frequency 97 MHz, power 9–13.8 dBm). The results of data analysis (average current step, I_s, and frequency of collisions, *f*) for each observation period are included in the graph. Reprinted with permission from Bonezzi, J. L., Tulashi; Boika, Aliaksei, Electrokinetic Manipulation of Silver and Platinum Nanoparticles and Their Stochastic Electrochemical Detection. *Anal. Chem.* **2017**, *89* (17), 8614–8619. Copyright 2017 American Chemical Society.

one important issue to address is the rate of fouling. Stevenson and co-workers have addressed the fouling issue.[124,125] This rate is accelerated on microelectrodes and nanoelectrodes given the mass transfer coefficient is inversely proportional to the radius of the electrode (see section above regarding droplet, vesicle, and micelle collisions). Incidentally, this also implies that fouling rates are enhanced on single nanoparticle sensors. Fouling is a frequent issue within direct electrochemical detection. To prevent this, coatings that are employed to make a method of detection more specific may act as barriers to the electrode surface. Nanoparticles and conducting polymers are typically used as coatings to improve signal-to-noise; modified carbon nanotubes are employed for the direct electrochemical detection of sulfhydryl compounds.[126,127] Though the use of coatings has proven advantageous, we can also find ourselves limited by instable or weakly adsorbed materials. Networks of enzymes on the surface of an electrode, for example, may be unstable because the immobilization of the enzyme on the electrode surface may change the pH activity and structural identity of the enzyme resulting in limited detection.[127] On the other hand, the use of bare electrode material may be kinetically limited due to slow adsorption attributed to inner-sphere mechanisms at the electrode surface resulting in low sensitivity. Detection may also be impeded by adsorption of non-electroactive material within biological samples; this is termed "poisoning" of the electrode surface. The high cost of metal materials used to fabricate electrodes is a common concern when fabricating biosensors.[128] Those preparing sensors based on the use of stochastic electrochemical detection must consider these drawbacks and limitations. Another point to consider is the rate of particle aggregation, which can greatly affect the signal. Citrate-stabilized nanoparticles are typically used in these studies but aggregate in solutions when ionic strength is greater than 30 - 50 mM. This aggregation may limit the biological buffers one can employ. Using hydrazine oxidation as the electrocatalytic reaction is also problematic. Aggregation due to hydrazine was first brought to light by Koper. Stevenson and Crooks showed in a following study that single nanoparticles (not aggregates) are detectable if colloidal stability is maintained by lowering the ionic strength and hydrazine concentration,[129] demonstrating a good match with the predicted collision rate according to analytical solutions for diffusion-limited transport. Importantly, this implies that groups should be careful in reporting results under the aforementioned conditions to ensure singe particles are being detected instead of particle aggregates. Furthermore, these issues should be seriously considered in building a robust sensor using stochastic electrochemical methods. Importantly, future reports regarding sensors developed using these concepts should definitively demonstrate collisions of single nanoparticles, instead of large aggregates of nanoparticles.

Conclusions and Final Comments

Making an observation of an entity colliding on an UME is rather trivial and has become commonplace. The grand challenge lies in making this collision specific to the colliding entity. Only then has the ultimate sensitivity in measurement science been achieved, where sensors can be developed to detect single analyte species, one at a time.

In our opinion, the greatest strategy for sensing lies in the materials dissolution system, and the greatest strategy for decreasing the limit of quantitation is using magnetism. These systems present the highest signal reported with the lowest limits of quantitation. One potential drawback can be highlighted by considering an example. Say one wishes to detect a single virus in the urine of an infected mouse, and silver nanoparticles are conjugated with primary antibodies to make the measurement specific. If incomplete binding occurs, free-floating silver nanoparticles will complicate the amperometric signal since single nanoparticles can be observed rather easily in the amperometric trace. One can, however, develop strategies to eliminate this background response by introducing a centrifugation step. Silver nanoparticles have also been shown to non-specifically adsorb to bacteria, which introduces a further complication that would necessitate experimental mitigation.

As we explore these ideas, we recognize the value of stochastic electrochemistry within this new frontier for single entity biosensors. This review attempts to offer a comprehensive understanding of the field thus far and details opportunities for improvement.

ORCID

Sondrica Goines https://orcid.org/0000-0002-1562-5084
Jeffrey E. Dick https://orcid.org/0000-0002-4538-9705

References

1. L. A. Baker, "Perspective and Prospectus on Single-Entity Electrochemistry." *J. Am. Chem. Soc.*, **140**(46), 15549 (2018).
2. E. William, Moerner - Biographical. *The Nobel Foundation: The Nobel Prizes*, 2014.
3. W. E. Moerner, R. M. Dickson, and D. J. Norris, "Single-molecule spectroscopy and quantum optics in solids." *Advances in Atomic, Molecular, and Optical Physics*, **38**, 193 (1997).
4. W. E. Moerner, "Polymer luminescence - Those blinking single molecules." *Science*, **277**(5329), 1059 (1997).
5. R. M. Dickson, A. B. Cubitt, R. Y. Tsien, and W. E. Moerner, "On/off blinking and switching behavior of single molecules of green fluorescent protein." *Nature*, **388**(6640), 355 (1997).
6. W. E. Moerner, "Nobel Lecture: Single-molecule spectroscopy, imaging, and photocontrol: Foundations for super-resolution microscopy." *Reviews of Modern Physics*, **87**(4), (2015).
7. G. Q. Chen, N. M. Zou, B. Chen, J. B. Sambur, E. Choudhary, and P. Chen, "Bimetallic Effect of Single Nanocatalysts Visualized by Super-Resolution Catalysis Imaging." *Acs Central Science*, **3**(11), 1189 (2017).
8. P. Chen, X. C. Zhou, H. Shen, N. M. Andoy, E. Choudhary, K. S. Han, G. K. Liu, and W. L. Meng, "Single-molecule fluorescence imaging of nanocatalytic processes." *Chemical Society Reviews*, **39**(12), 4560 (2010).
9. K. S. Han, G. K. Liu, X. C. Zhou, R. E. Medina, and P. Chen, "How Does a Single Pt Nanocatalyst Behave in Two Different Reactions? A Single-Molecule Study." *Nano Lett.*, **12**(3), 1253 (2012).
10. J. B. Sambur and P. Chen, "Distinguishing Direct and Indirect Photoelectrocatalytic Oxidation Mechanisms Using Quantitative Single-Molecule Reaction Imaging and Photocurrent Measurements." *J. Phys. Chem. C*, **120**(37), 20668 (2016).
11. J. B. Sambur, T. Y. Chen, E. Choudhary, G. Q. Chen, E. J. Nissen, E. M. Thomas, N. M. Zou, and P. Chen, "Sub-particle reaction and photocurrent mapping to optimize catalyst-modified photoanodes." *Nature*, **530**(7588), 77-+ (2016).
12. H. Shen, W. L. Xu, and P. Chen, "Single-molecule nanoscale electrocatalysis." *Phys. Chem. Chem. Phys.*, **12**(25), 6555 (2010).
13. H. S. Ahn and A. J. Bard, "Single-Nanoparticle Collision Events: Tunneling Electron Transfer on a Titanium Dioxide Passivated n-Silicon Electrode." *Angew. Chem. Int. Ed.*, **54**(46), 13753 (2015).
14. T. M. Alligrant, M. J. Anderson, R. Dasari, K. J. Stevenson, and R. M. Crooks, "Single Nanoparticle Collisions at Microfluidic Microband Electrodes: The Effect of Electrode Material and Mass Transfer." *Langmuir*, **30**(44), 13462 (2014).
15. A. J. Bard, H. J. Zhou, and S. J. Kwon, "Electrochemistry of Single Nanoparticles via Electrocatalytic Amplification." *Israel Journal of Chemistry*, **50**(3), 267 (2010).
16. A. D. Castaneda, T. M. Alligrant, J. A. Loussaert, and R. M. Crooks, "Electrocatalytic Amplification of Nanoparticle Collisions at Electrodes Modified with Polyelectrolyte Multi layer Films." *Langmuir*, **31**(2), 876 (2015).
17. J. T. Cox and B. Zhang, "Nanoelectrodes: Recent Advances and New Directions." In *Annual Review of Analytical Chemistry*, Vol **5**, R. G. Cooks; E. S. Yeung, Eds. 2012; Vol. **5**, pp 253.
18. R. M. Crooks, "Concluding remarks: single entity electrochemistry one step at a time." *Faraday Discussions*, **193**, 533 (2016).
19. R. Dasari, D. A. Robinson, and K. J. Stevenson, "Ultrasensitive Electroanalytical Tool for Detecting, Sizing, and Evaluating the Catalytic Activity of Platinum Nanoparticles." *J. Am. Chem. Soc.*, **135**(2), 570 (2013).
20. B. Fu, C. Van Dyck, S. Zaleski, R. P. Van Duyne, and M. A. Ratner, "Single Molecule Electrochemistry: Impact of Surface Site Heterogeneity." *J. Phys. Chem. C*, **120**(48), 27241 (2016).
21. J. Kim, J. E. Dick, and A. J. Bard, "Advanced Electrochemistry of Individual Metal Clusters Electrodeposited Atom by Atom to Nanometer by Nanometer." *Accounts of Chemical Research*, **49**(11), 2587 (2016).
22. S. J. Kwon, F. R. F. Fan, and A. J. Bard, "Observing Iridium Oxide (IrOx) Single Nanoparticle Collisions at Ultramicroelectrodes." *J. Am. Chem. Soc.*, **132**(38), 13165 (2010).

23. S. J. Kwon, H. J. Zhou, F. R. F. Fan, V. Vorobyev, B. Zhang, and A. J. Bard, "Stochastic electrochemistry with electrocatalytic nanoparticles at inert ultramicroelectrodes-theory and experiments." *Phys. Chem. Chem. Phys.*, **13**(12), 5394 (2011).

24. Y. X. Li, J. T. Cox, and B. Zhang, "Electrochemical Responses and Electrocatalysis at Single Au Nanoparticles." *J. Am. Chem. Soc.*, **132**(9), 3047 (2010).

25. T. Sun, Y. Yu, B. J. Zacher, and M. V. Mirkin, "Scanning Electrochemical Microscopy of Individual Catalytic Nanoparticles." *Angew. Chem. Int. Ed.*, **53**(51), 14120 (2014).

26. J. E. Dick, "Electrochemical detection of single cancer and healthy cell collisions on a microelectrode." *Chem. Commun.*, **52**(72), 10906 (2016).

27. J. J. Gooding, "Single Entity Electrochemistry Progresses to Cell Counting." *Angew. Chem. Int. Ed.*, **55**(42), 12956 (2016).

28. M. M. Barchitta, "Andrea; Quattrochchi, Annalisa; Agrifoglio, Ottavia; Agodi, Antonella, The Role of miRNAs as Biomarkers for Pregnancy Outcomes: A Comprehensive Review." *International Journal of Genomics* (2017).

29. E. E. R. Winger, L. Jane, Xuhuai Ji, and Kypros Nicolaides, "Peripheral blood cell microRNA quantification during the first trimester predicts preeclampsia: Proof of concept." *PlosOne* (2018).

30. A. Nabers, L. Perna, J. Lange, U. Mons, J. Schartner, J. Guldenhaupt, K.-U. Saum, S. Janelidze, B. Holleczek, D. Rujescu, O. Hansson, K. Gerwert, and H. Brenner, "Amyloid Blood Biomarker Detects Alzheimer's Disease." *EMBO Mol. Med.*, '**0**(5), e8763 (2019).

31. M. W. Glasscott and J. E. Dick, "Direct Electrochemical Observation of Single Platinum Cluster Electrocatalysis on Ultramicroelectrodes." *Anal. Chem.*, **90**(13), 7804 (2018).

32. M. Zhou, J. E. Dick, K. K. Hu, M. V. Mirkin, and A. J. Bard, "Ultrasensitive Electroanalysis: Femtomolar Determination of Lead, Cobalt, and Nickel." *Anal. Chem.*, **90**(2), 1142 (2018).

33. M. Zhou, J. E. Dick, and A. J. Bard, "Electrodeposition of Isolated Platinum Atoms and Clusters on Bismuth-Characterization and Electrocatalysis." *J. Am. Chem. Soc.*, **139**(48), 17677 (2017).

34. S. J. Percival, J. E. Dick, and A. J. Bard, "Cathodically Dissolved Platinum Resulting from the O-2 and H2O2 Reduction Reactions on Platinum Ultramicroelectrodes." *Anal. Chem.*, **89**(5), 3087 (2017).

35. J. E. Dick and A. J. Bard, "Toward the Digital Electrochemical Recognition of Cobalt, Iridium, Nickel, and Iron Ion Collisions by Catalytic Amplification." *J. Am. Chem. Soc.*, **138**(27), 8446 (2016).

36. J. E. Dick and A. J. Bard, "Recognizing Single Collisions of PtCl62- at Femtomolar Concentrations on Ultramicroelectrodes by Nucleating Electrocatalytic Clusters." *J. Am. Chem. Soc.*, **137**(43), 13752 (2015).

37. F. R. F. Fan, J. Kwak, and A. J. Bard, "Single molecule electrochemistry." *J. Am. Chem. Soc.*, **118**(40), 9669 (1996).

38. F. R. F. Fan and A. J. Bard, "Electrochemical Detection of Single Molecules." *Science*, **267**(5199), 871 (1995).

39. B. M. Quinn, P. van't Hof, and S. G. Lemay, "Time-resolved electrochemical detection of discrete adsorption events." *J. Am. Chem. Soc.*, **126**(27), 8360 (2004).

40. J. H. Park, H. J. Zhou, S. J. Percival, B. Zhang, F. R. F. Fan, and A. J. Bard, "Open Circuit (Mixed) Potential Changes Upon Contact Between Different Inert Electrodes-Size and Kinetic Effects." *Anal. Chem.*, **85**(2), 964 (2013).

41. S. J. Percival and B. Zhang, "Fast-Scan Cyclic Voltammetry Allows Determination of Electron Transfer Kinetic Constants in Single Nanoparticle Collision." *J. Phys. Chem. C*, **120**(37), 20536 (2016).

42. J. L. Zhang, J. Rector, J. Q. Lin, J. H. Young, M. Sans, N. Katta, N. Giese, W. D. Yu, C. Nagi, J. Suliburk, J. S. Liu, A. Bensussan, R. J. DeHoog, K. Y. Garza, B. Ludolph, A. G. Sorace, A. Syed, A. Zahedivash, T. E. Milner, and L. S. Eberlin, "Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system." *Science Translational Medicine*, **9**(406), (2017).

43. Y. L. Ying, Z. F. Ding, D. P. Zhan, and Y. T. Long, "Advanced electroanalytical chemistry at nanoelectrodes." *Chem. Sci.*, **8**(5), 3338 (2017).

44. L. H. Han, W. Wang, J. Nsabimana, J. W. Yan, B. Ren, and D. P. Zhan, "Single molecular catalysis of a redox enzyme on nanoelectrodes." *Faraday Discussions*, **193**, 133 (2016).

45. J. Velmurugan, J. M. Noel, and M. V. Mirkin, "Nucleation and growth of mercury on Pt nanoelectrodes at different overpotentials." *Chem. Sci.*, **5**(1), 189 (2014).

46. J. Kim, B. K. Kim, S. K. Cho, and A. J. Bard, "Tunneling Ultramicroelectrode: Nanoelectrodes and Nanoparticle Collisions." *J. Am. Chem. Soc.*, **136**(23), 8173 (2014).

47. J. H. Park, S. N. Thorgaard, B. Zhang, and A. J. Bard, "Single Particle Detection by Area Amplification: Single Wall Carbon Nanotube Attachment to a Nanoelectrode." *J. Am. Chem. Soc.*, **135**(14), 5258 (2013).

48. S. G. Lemay, S. Kang, K. Mathwig, and P. S. Singh, "Single-Molecule Electrochemistry: Present Status and Outlook." *Accounts of Chemical Research*, **46**(2), 369 (2013).

49. B. K. Jena, S. J. Percival, and B. Zhang, "Au Disk Nanoelectrode by Electrochemical Deposition in a Nanopore." *Anal. Chem.*, **82**(15), 6737 (2010).

50. Y. X. Li, D. Bergman, and B. Zhang, "Preparation and Electrochemical Response of 1–3 nm Pt Disk Electrodes." *Anal. Chem.*, **81**(13), 5496 (2009).

51. R. W. Murray, "Nanoelectrochemistry: Metal nanoparticles, nanoelectrodes, and nanopores." *Chemical Reviews*, **108**(7), 2688 (2008).

52. F. J. M. Hoeben, F. S. Meijer, C. Dekker, S. P. J. Albracht, H. A. Heering, and S. G. Lemay, "Toward Single-Enzyme Molecule Electrochemistry: NiFe - Hydrogenase Protein Film Voltammetry at Nanoelectrodes." *ACS Nano*, **2**(12), 2497 (2008).

53. A. Boika, S. N. Thorgaard, and A. J. Bard, "Monitoring the Electrophoretic Migration and Adsorption of Single Insulating Nanoparticles at Ultramicroelectrodes." *J. Phys. Chem. B*, **117**(16), 4371 (2013).

54. J. E. Dick, A. T. Hilterbrand, L. M. Strawsine, J. W. Upton, and A. J. Bard, "Enzymatically enhanced collisions on ultramicroelectrodes for specific and rapid detection of individual viruses." *Proc. Nat'l. Acad. Sci. USA*, **113**(23), 6403 (2016).

55. X. Deng, Frederica Galli, and Marc T. M. Koper, "In Situ Electrochemical AFM Imaging of a Pt Electrode in Sulfuric Acid under Potential Cycling Conditions." *J. Am. Chem. Soc.*, **140**(41), 13285 (2018).

56. A. J. Bard, "Inner-Sphere Heterogeneous Electrode Reactions. Electrocatalysis and Photocatalysis: The Challenge." *J. Am. Chem. Soc.*, **132**(22), 7559 (2010).

57. J. Bonezzi and A. Boika, "Deciphering the Magnitude of Current Steps in Electrochemical Blocking Collision Experiments and Its Implications." *Electrochim. Acta*, **236**, 252 (2017).

58. A. J. B. A. L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2 ed.; John Wiley & Sons: New York, New York, 2001.

59. S. E. Fosdick, M. J. Anderson, E. G. Nettleton, and R. M. Crooks, "Correlated Electrochemical and Optical Tracking of Discrete Collision Events." *J. Am. Chem. Soc.*, **135**(16), 5994 (2013).

60. Z. Deng, R. Elattar, F. Maroun, and C. Renault, "In Situ Measurement of the Size Distribution and Concentration of Insulating Particles by Electrochemical Collision on Hemispherical Ultramicroelectrodes." *Analytical Chemistry*, **90**(21), 12923 (2018).

61. J. E. Dick, C. Renault, and A. J. Bard, "Observation of Single-Protein and DNA Macromolecule Collisions on Ultramicroelectrodes." *J. Am. Chem. Soc.*, **137**(26), 8376 (2015).

62. J. E. Dick, A. T. Hilterbrand, A. Boika, J. W. Upton, and A. J. Bard, "Electrochemical detection of a single cytomegalovirus at an ultramicroelectrode and its antibody anchoring." *Proc. Nat'l. Acad. Sci. USA*, **112**(17), 5303 (2015).

63. J. Y. Lee, Byung-Kwon Kim, Mijeong Kang, and Jun Hui Park, "Label-Free Detection of Single Living Bacteria via Electrochemical Collision Event." *Scientific Reports*, **6** (2016).

64. G. Gao, Denchao Wang, Ricardo Brocenschi, Jinfang Zhi, and Michael V. Mirkin, "Toward the Detection and Identification of Single Bacteria by Electrochemical Collision Technique." *Anal. Chem.*, **90**(20), 12123 (2018).

65. H. Ren, S. R. German, M. A. Edwards, Q. J. Chen, and H. S. White, "Electrochemical Generation of Individual O-2 Nanobubbles via H2O2 Oxidation." *J. Phys. Chem. Lett.*, **8**(11), 2450 (2017).

66. Y. W. Liu, M. A. Edwards, S. R. German, Q. J. Chen, and H. S. White, "The Dynamic Steady State of an Electrochemically Generated Nanobubble." *Langmuir*, **33**(8), 1845 (2017).

67. S. R. German, M. A. Edwards, Q. J. Chen, Y. W. Liu, L. Luo, and H. S. White, "Electrochemistry of single nanobubbles. Estimating the critical size of bubble-forming nuclei for gas-evolving electrode reactions." *Faraday Discussions*, **193**, 223 (2016).

68. S. R. German, Q. J. Chen, M. A. Edwards, and H. S. White, "Electrochemical Measurement of Hydrogen and Nitrogen Nanobubble Lifetimes at Pt Nanoelectrodes." *J. Electrochem. Soc.*, **163**(4), H3160 (2016).

69. Q. J. Chen, H. S. Wiedenroth, S. R. German, and H. S. White, "Electrochemical Nucleation of Stable N-2 Nanobubbles at Pt Nanoelectrodes." *J. Am. Chem. Soc.*, **137**(37), 12064 (2015).

70. Q. J. Chen, L. Luo, H. Faraji, S. W. Feldberg, and H. S. White, "Electrochemical Measurements of Single H-2 Nanobubble Nucleation and Stability at Pt Nanoelectrodes." *J. Phys. Chem. Lett.*, **5**(20), 3539 (2014).

71. L. Luo and H. S. White, "Electrogeneration of Single Nanobubbles at Sub-50-nm-Radius Platinum Nanodisk Electrodes." *Langmuir*, **29**(35), 11169 (2013).

72. Z. P. Xiang, H. Deng, P. Peljo, Z. Y. Fu, S. L. Wang, D. Mandler, G. Q. Sun, and Z. X. Liang, "Electrochemical Dynamics of a Single Platinum Nanoparticle Collision Event for the Hydrogen Evolution Reaction." *Angew. Chem. Int. Ed.*, **57**(13), 3464 (2018).

73. A. D. Castaneda, N. J. Brenes, A. Kondajji, and R. M. Crooks, "Detection of microRNA by Electrocatalytic Amplification: A General Approach for Single-Particle Biosensing." *J. Am. Chem. Soc.*, **139**(22), 7657 (2017).

74. A. D. Castaneda, D. A. Robinson, K. J. Stevenson, and R. M. Crooks, "Electrocatalytic amplification of DNA-modified nanoparticle collisions via enzymatic digestion." *Chem. Sci.*, **7**(10), 6450 (2016).

75. T. M. Alligrant, E. G. Nettleton, and R. M. Crooks, "Electrochemical detection of individual DNA hybridization events." *Lab on a Chip*, **13**(3), 349 (2013).

76. T. M. Alligrant, R. Dasari, K. J. Stevenson, and R. M. Crooks, "Electrocatalytic Amplification of Single Nanoparticle Collisions Using DNA-Modified Surfaces." *Langmuir*, **31**(42), 11724 (2015).

77. J. E. Dick, "Studies in the Electrochemistry of Single Atoms, Molecules, and Nanoparticles." *Dissertation, The University of Texas at Austin* (2017).

78. Y. Zhang, Jinpeng Mao, Wenliang Ji, Taotao Feng, Zixuan Fu, Meining Zhang, and Lanqun Mao, "Collision of Aptamer/Pt Nanoparticles Enables Label-Free Amperometric Detection of Protein in Rat Brain." *Anal. Chem.*, **91**(9), 5654 (2019).

79. B. Haddou, N. V. Rees, and R. G. Compton, "Nanoparticle-electrode impacts: the oxidation of copper nanoparticles has slow kinetics." *Phys. Chem. Chem. Phys.*, **14**(39), 13612 (2012).

80. Y. G. Zhou, N. V. Rees, and R. G. Compton, "Electrode-nanoparticle collisions: The measurement of the sticking coefficient of silver nanoparticles on a glassy carbon electrode." *Chemical Physics Letters*, **514**(4–6), 291 (2011).

81. N. V. Rees, Y. G. Zhou, and R. G. Compton, "The Aggregation of Silver Nanoparticles in Aqueous Solution Investigated via Anodic Particle Coulometry." *Chemphyschem*, **12**(9), 1645 (2011).

82. Y. G. Zhou, N. V. Rees, and R. G. Compton, "The Electrochemical Detection and Characterization of Silver Nanoparticles in Aqueous Solution." *Angew. Chem. Int. Ed.*, **50**(18), 4219 (2011).

83. A. N. Sekretaryova, M. Y. Vagin, A. P. F. Turner, and M. Eriksson, "Electrocatalytic Currents from Single Enzyme Molecules (vol 138, pg 2504, 2016)." *J. Am. Chem. Soc.*, **139**(3), 1344 (2017).

84. A. N. Sekretaryova, M. Y. Vagin, A. P. F. Turner, and M. Eriksson, "Correspondence on "Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts"." *Acs Catalysis*, **7**(5), 3591 (2017).

85. A. N. Sekretaryova, M. Y. Vagin, A. P. F. Turner, and M. Eriksson, "Electrocatalytic Currents from Single Enzyme Molecules." *J. Am. Chem. Soc.*, **138**(8), 2504 (2016).

86. E. Katelhon, L. Sepunaru, A. A. Karyakin, and R. G. Compton, "Reply to Comment on "Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts"." *Acs Catalysis*, **7**(5), 3594 (2017).

87. C. Chan, L. Sepunaru, S. V. Sokolov, E. Katelhon, N. P. Young, and R. G. Compton, "Catalytic activity of catalase-silica nanoparticle hybrids: from ensemble to individual entity activity." *Chem. Sci.*, **8**(3), 2303 (2017).

88. E. Katelhon, L. Sepunaru, A. A. Karyakin, and R. G. Compton, "Can Nanoimpacts Detect Single-Enzyme Activity? Theoretical Considerations and an Experimental Study of Catalase Impacts." *Acs Catalysis*, **6**(12), 8313 (2016).

89. C. Bourdillon, C. Demaille, J. Moiroux, and J. M. Saveant, "Analyzing product inhibition and pH gradients in immobilized enzyme films as illustrated experimentally by immunologically bound glucose oxidase electrode coatings." *J. Phys. Chem. B*, **103**(40), 8532 (1999).

90. K. Scida, "Microfluidics for bioanalytical research: transitioning into point-of-care diagnostics." *Dissertation, The University of Texas at Austin* (2014).

91. H. Q. Deng, J. E. Dick, S. Kummer, U. Kragl, S. H. Strauss, and A. J. Bard, "Probing Ion Transfer across Liquid-Liquid Interfaces by Monitoring Collisions of Single Femtoliter Oil Droplets on Ultramicroelectrodes." *Anal. Chem.*, **88**(15), 7754 (2016).

92. J. E. Dick, E. Lebegue, L. M. Strawsine, and A. J. Bard, "Millisecond Coulometry via Zeptoliter Droplet Collisions on an Ultramicroelectrode." *Electroanalysis*, **28**(10), 2320 (2016).

93. Y. Li, H. Q. Deng, J. E. Dick, and A. J. Bard, "Analyzing Benzene and Cyclohexane Emulsion Droplet Collisions on Ultramicroelectrodes." *Anal. Chem.*, **87**(21), 11013 (2015).

94. J. E. Dick, A. Poirel, R. Ziessel, and A. J. Bard, "Electrochemistry, Electrogenerated Chemiluminescence, and Electropolymerization of Oligothienyl-BODIPY Derivatives." *Electrochim. Acta*, **178**, 234 (2015).

95. B. K. Kim, A. Boika, J. Kim, J. E. Dick, and A. J. Bard, "Characterizing Emulsions by Observation of Single Droplet Collisions-Attoliter Electrochemical Reactors." *J. Am. Chem. Soc.*, **136**(13), 4849 (2014).

96. J. E. Dick, C. Renault, B. K. Kim, and A. J. Bard, "Electrogenerated Chemiluminescence of Common Organic Luminophores in Water Using an Emulsion System." *J. Am. Chem. Soc.*, **136**(39), 13546 (2014).

97. J. E. Dick, C. Renault, B. K. Kim, and A. J. Bard, "Simultaneous Detection of Single Attoliter Droplet Collisions by Electrochemical and Electrogenerated Chemiluminescent Responses." *Angew. Chem. Int. Ed.*, **53**(44), 11859 (2014).

98. M. W. Glasscott, A. D. Pendergast, S. Goines, A. R. Bishop, A. H. Hoang, C. R. Renault, and J. E. Dick, "Electrosynthesis of high-entropy metallic glass nanoparticles for designer, multi-functional electrocatalysis." *Nature Communications* (2019).

99. M. W. Glasscott, A. D. Pendergast, M. H. Choudhury, and J. E. Dick, "Advanced Characterization Techniques for Evaluating Porosity, Nanopore Tortuosity, and Electrical Connectivity at the Single-Nanoparticle Level." *ACS Applied Nano Materials*, **2**(2), 819 (2019).

100. M. W. Glasscott and J. E. Dick, "Fine-Tuning Porosity and Time-Resolved Observation of the Nucleation and Growth of Single Platinum Nanoparticles." *ACS Nano*, **13**(4), 4572 (2019).

101. M. W. Glasscott and J. E. Dick, "Direct Electrochemical Observation of Single Platinum Cluster Electrocatalysis on Ultramicroelectrodes." *Anal. Chem.*, **90**, 7804 (2018).

102. M. W. Glasscott, A. D. Pendergast, and J. E. Dick, "A Universal Platform for the Electrodeposition of Ligand-Free Metal Nanoparticles from a Water-in-Oil Emulsion System." *ACS Applied Nano Materials*, **1**(10), 5702 (2018).

103. Y. E. Jeun, Bongkwan Baek, Myoung Won Lee, and Hyun S. Ahn, "Surfactant-free electrochemical synthesis of metallic nanoparticles via stochastic collisions of aqueous nanodroplet reactors." *Chem. Commun.*, **54**(72), 10052 (2018).

104. M. V. Evers, Miguel Bernal, Beatriz Roldan Cuenya, and Kristina Tschulik, "Piece by Piece-Electrochemical Synthesis of Individual Nanoparticles and their Performance in ORR Electrocatalysis." *Angew. Chem. Int. Ed.*, **58**(24), 8221 (2019).

105. M. W. Glasscott, A. D. Pendergast, S. Goines, A. R. Bishop, A. T. Hoang, C. Renault, and J. E. Dick, "Electrosynthesis of high-entropy metallic glass nanoparticles for designer, multi-functional electrocatalysis." *Nature Communications*, **10**, (2019).

106. E. Lebegue, C. M. Anderson, J. E. Dick, L. J. Webb, and A. J. Bard, "Electrochemical Detection of Single Phospholipid Vesicle Collisions at a Pt Ultramicroelectrode." *Langmuir*, **31**, 11734 (2015).

107. H. S. Toh and R. G. Compton, "Electrochemical detection of single micelles through 'nano-impacts'." *Chem. Sci.*, **6**, 5053 (2015).

108. B. K. Kim, J. Kim, and A. J. Bard, "Electrochemistry of a Single Attoliter Emulsion Droplet in Collisions." *J. Am. Chem. Soc.*, **137**(6), 2343 (2015).

109. D. A. Robinson and H. S. White, "Electrochemical Synthesis of Individual Core@Shell and Hollow Ag/Ag2S Nanoparticles." *Nano Lett.*, **19**(8), 5612 (2019).

110. D. A. Robinson, M. A. Edwards, H. Ren, and H. S. White, "Effects of Instrumental Filters on Electrochemical Measurement of Single-Nanoparticle Collision Dynamics." *Chemelectrochem*, **5**(20), 3059 (2018).

111. K. McKelvey, D. A. Robinson, N. J. Vitti, M. A. Edwards, and H. S. White, "Single Ag nanoparticle collisions within a dual-electrode micro-gap cell." *Faraday Discussions*, **210**, 189 (2018).

112. D. A. Robinson, Y. W. Liu, M. A. Edwards, N. J. Vitti, S. M. Oja, B. Zhang, and H. S. White, "Collision Dynamics during the Electrooxidation of Individual Silver Nanoparticles." *J. Am. Chem. Soc.*, **139**(46), 16923 (2017).

113. S. M. Oja, D. A. Robinson, N. J. Vitti, M. A. Edwards, Y. W. Liu, H. S. White, and B. Zhang, "Observation of Multipeak Collision Behavior during the Electro-Oxidation of Single Ag Nanoparticles." *J. Am. Chem. Soc.*, **139**(2), 708 (2017).

114. A. Einstein, "Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen." *Annalen der Physik*, **322**(8), 549 (1905).

115. A. Boika and A. J. Bard, "Time of First Arrival in Electrochemical Collision Experiments as a Measure of Ultra low Concentrations of Analytes in Solution." *Anal. Chem.*, **87**(8), 4341 (2015).

116. A. Boika and A. J. Bard, "Electrophoretic Migration and Particle Collisions in Scanning Electrochemical Microscopy." *Anal. Chem.*, **86**(23), 11666 (2014).

117. J. H. Park, A. Boika, H. S. Park, H. C. Lee, and A. J. Bard, "Single Collision Events of Conductive Nanoparticles Driven by Migration." *J. Phys. Chem. C*, **117**(13), 6651 (2013).

118. K. J. Freedman, L. M. Otto, A. P. Ivanov, A. Barik, S. H. Oh, and J. B. Edel, "Nanopore sensing at ultra-low concentrations using single-molecule dielectrophoretic trapping." *Nature Communications*, **7**, 9 (2016).

119. F. R. Madiyar, L. U. Syed, C. T. Culbertson, and J. Li, "Manipulation of bacteriophages with dielectrophoresis on carbon nanofiber nanoelectrode arrays." *Electrophoresis*, **34**(7), 1123 (2013).

120. B. P. Nadappuram, P. Cadinu, A. Barik, A. Ainscough, M. J. Devine, M. Kang, J. Gonzalez-Garcia, J. T. Kittler, K. R. Willison, R. Vilar, P. Actis, B. Wojciak-Stothard, S. H. Oh, A. P. Ivanov, and J. B. Edel, "Nanoscale tweezers for single-cell biopsies." *Nature Nano.*, **14**(1), 80-+ (2019).

121. D. A. Robinson, J. J. Yoo, A. D. Castaneda, B. Gu, R. Dasari, R. M. Crooks, and K. J. Stevenson, "Increasing the Collision Rate of Particle Impact Electroanalysis with Magnetically Guided Pt-Decorated Iron Oxide Nanoparticles." *ACS Nano*, **9**(7), 7583 (2015).

122. J. J. Yoo, J. Kim, and R. M. Crooks, "Direct electrochemical detection of individual collisions between magnetic microbead/silver nanoparticle conjugates and a magnetized ultramicroelectrode." *Chem. Sci.*, **6**(11), 6665 (2015).

123. J. Bonezzi, Tulashi Luitel, and Aliaksei Boika, "Electrokinetic Manipulation of Silver and Platinum Nanoparticles and Their Stochastic Electrochemical Detection." *Anal. Chem.*, **89**(17), 8614 (2017).

124. K. J. Stevenson and K. Tschulik, "A materials driven approach for understanding single entity nano impact electrochemistry." *Current Opinion in Electrochemistry*, **6**(1), 38 (2017).

125. R. Dasari, K. Tai, D. A. Robinson, and K. J. Stevenson, "Electrochemical Monitoring of Single Nanoparticle Collisions at Mercury-Modified Platinum Ultramicroelectrodes." *ACS Nano*, **8**(5), 4539 (2014).

126. V. Tsakova and R. Seeber, "Conducting polymers in electrochemical sensing: factors influencing the electroanalytical signal." *Analytical and Bioanalytical Chemistry*, **408**(26), 7231 (2016).

127. M. Scampicchio, A. Bulbarello, A. Arecchi, M. S. Cosio, S. Benedetti, and S. Mannino, "Electrospun Nonwoven Nanofibrous Membranes for Sensors and Biosensors." *Electroanalysis*, **24**(4), 719 (2012).

128. X. H. Niu, L. B. Shi, H. L. Zhao, and M. B. Lan, "Advanced strategies for improving the analytical performance of Pt-based nonenzymatic electrochemical glucose sensors: a minireview." *Analytical Methods*, **8**(8), 1755 (2016).

129. D. A. Robinson, A. M. Kondajji, A. D. Castaneda, R. Dasari, R. M. Crooks, and K. J. Stevenson, "Addressing Colloidal Stability for Unambiguous Electroanalysis of Single Nanoparticle Impacts." *J. Phys. Chem. Lett.*, **7**(13), 2512 (2016).