

Concluding remarks: single entity electrochemistry one step at a time

Richard M. Crooks

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This article provides a summary of the Faraday Discussion on single entity electrochemistry held in York, U.K., in early September, 2016. The introduction provides some context for thinking about electrochemical studies of single entities. The next four sections follow the themes of the meeting as they relate to single-entity electrochemistry: (1) nanoparticles, nanotubes, and nanowires; (2) nanopores and nanofluidics; (3) complex surfaces and reactions at the nanoscale; and (4) molecular electroanalysis. Each paper presented at the Discussion is summarized, and some personal thoughts as to the significance of the findings, the technical advances that made the experiments possible, and common themes between articles are interspersed. Finally, at the end, I round-up my impressions and provide them in a succinct list.

Introduction

At the invitation of the meeting chairman, Dr Patrick Unwin, these remarks represent my personal reflections on the many outstanding papers, posters, and presentations, and the ensuing discussions, that comprised Faraday Discussion no. 194. This Discussion, which many participants, myself included, proclaimed as one of the most intellectually stimulating of their career, focused on “Single-Entity Electrochemistry”. Many aspects of this forward-looking topic were discussed at the meeting, including (very broadly defined): nanomaterials, nanopores, and electrochemical detection. The unifying theme tying together the vast range of materials and methods that engaged the participants was to step out of the world of ensemble electrochemistry and into the realm of single particles, molecules, reactions, and, well, “entities”!

The meeting was held on the campus of the University of York in North Yorkshire, England, in September, 2016. York is an ancient city, founded by the Romans in 71 A.D. The central role of York in the affairs of the U.K. over the last 200 or so years, and its vibrant tourism industry, are largely due to George Hudson, a railway entrepreneur, who established York as a hub. Though Mr

Department of Chemistry, The Texas Materials Institute, The University of Texas at Austin, 105 E. 24th St., Stop A5300, Austin, TX 78712-1224, USA. E-mail: crooks@cm.utexas.edu; Tel: +1-512-475-8674

Hudson went bankrupt building his railroad empire, I can report that York is still one of the most accessible and diverse cities in the U.K. thanks to his efforts. York is truly a beautiful city too, and anyone would be well-advised to put it on their bucket list of places to visit.

Although the temptations of York were alluring, the Discussion itself was downright stimulating. I thought the most interesting question that was discussed was “why study single entities using electrochemical methods?”, which really was the main theme of the meeting. It’s premature to get into specifics now, but we will get there by the end of this article. At the outset, however, I will say that part of the reason is now “we can” and before “we couldn’t”, and the difference between then and now has a lot to do with advances in instrumentation and simply thinking about electrochemistry in a different way.

For electrochemists, I think a case can be made that the 1960s and 1970s were the decades of mechanistic organic electrochemistry, and this area of research had very little, if anything, to do with single molecules. There were three major developments in the 1980s, however, that began to change the world-view of electrochemists: the ultramicroelectrode (UME),¹ typically a conductive disk having a diameter of around 5–25 μm , the scanning tunneling microscope (STM),² and the atomic force microscope (AFM).³ The UME got the community thinking about doing electrochemistry in small volumes, like rat brains,¹ while the STM and AFM gave electrochemists their first look at the atomic-level detail of electrode surfaces. The invention of the scanning electrochemical microscope (SECM) in the mid-1980s,^{4,5} which has greatly influenced electrochemical imaging⁶ and measurements in small volumes, was a direct consequence of the appearance of the STM and AFM.

In the 1990s, new electrochemical techniques that involved pores on the order of 2 nm, rather than electrodes, were reported, and this made it possible to analyze nanomaterials, and even molecules, one at a time as they passed through the pore.

Like the farm boy who sees the big city lights, there was no going back: the race was on to probe ever smaller volumes and numbers of molecules with ever smaller electrodes and pores. Nowadays electrodes having diameters of less than 10 nm are fairly commonplace, and even smaller ones have been reported by the experts.⁷ These tiny electrodes open up amazing possibilities for analyzing the properties of single entities.

Given this brief historical context, I will do my best in these concluding remarks to summarize the content of the wide-ranging Faraday Discussion on single-entity electrochemistry. The stage was set for the meeting by N. J. Tao who provided an outstanding overview of single-entity electrochemistry, a field which he has had a major hand in inventing. The remainder of the Discussion was organized around four themes: (1) nanoparticles (NPs), nanotubes, and nano-wires; (2) nanopores and nanofluidics; (3) complex surfaces and reactions at the nanoscale; and (4) molecular electroanalysis. I’m going to follow this same general flow, but there is quite a bit of overlap in these themes and, as with all good science, it is sometimes difficult to pigeon-hole a particular experiment. At the end of the article I will put into perspective some of the challenges electrochemists face moving forward.

Nanoparticles, nanotubes, and nanowires

I have a dream. I dream that it will be possible to characterize single, complex, electrocatalytically active NPs in the 1–2 nm size range, like the one shown in Fig. 1,⁸ *in operando*. The reason for this is that my research group has been studying well-defined metallic and bimetallic NPs for 25 years^{9,10} and there is still far more that we don't know about these materials than we do know. In fact, the more we learn about them, the more we realize how little we know! The problem is that these materials are really small: on the order of 30–200 atoms, and that makes them very difficult to characterize at all, much less one at a time in an electrocatalytic environment.

The bigger the particle, the easier it is to characterize, so why not just forget about 1–2 nm particles and focus on materials that are an order of magnitude larger? There are two reasons. First, the most interesting changes in materials properties occur in the <3 nm size range. Larger sizes are dominated by facets rather than edge and corner atoms, and in part for this reason they display more-or-less bulk properties. Second, my group's focus is on correlating first-principles calculations to experimental measurements, and for particles containing more than 200–300 atoms this is not feasible with the present level of computing power. Clearly, it would be wonderful to be able to calculate catalyst structures that yield just the right specificity and turnover rate, but until calculations are tested against experimental measurements on models that correlate directly to the theoretical constructs, that's not going to be possible.

Our approach, and that of most other groups, for correlating theory and experiment is to devise really good synthetic methods that make it possible to synthesize billions of identical NPs, characterize the resulting ensemble, and then extrapolate back to the structure of individual NPs. In many respects this is a fool's errand, because there is no way to make billions of identical particles like that shown in Fig. 1. I used to envy biochemists who study enzymatic catalysis, because after all every enzyme of a particular type is synthesized using the same DNA template. Therefore, it is possible to study ensembles and deduce the function of individual enzymes, right? Wrong. Even enzymes having exactly the same molecular structure have different properties (Zhan *et al.*, DOI: 10.1039/c6fd00061d). What this means is that we electrochemists have to break away

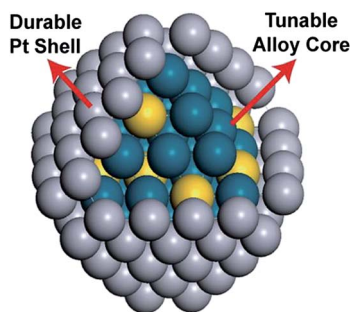


Fig. 1 An illustration of a complex NP consisting of an alloy core covered with a single monolayer shell.

from the ensemble model and begin to focus on individual entities. That's part of what this Discussion was about, and it was quite evident that amazing progress is being made characterizing single NPs, nanotubes, and nanowires.

One of the most interesting methods for analyzing single NPs was first reported by Lemay and coworkers in 2004,¹¹ and the impact of their approach was evident during the Discussion. In their "collision experiment", a microelectrode was held at a potential that maintained a steady-state current (arising from a reversible redox couple). When polymeric beads having diameters as small as 300 nm were added to the solution, quantized decreases in current were observed periodically. The authors correctly interpreted this result in terms of sticking collisions between the beads and the electrode surface. Specifically, individual NPs blocked part of the electrode surface, thereby reducing the surface area, and this is why the current decreased.

A few years after Lemay's groundbreaking publication, Xiao and Bard showed that a related type of experiment, which they called electrocatalytic amplification (ECA), could yield information about significantly smaller metallic NPs.¹² In their experiment, a catalytically inactive electrode was held at a potential at which little or no current flowed, even though a kinetically slow redox molecule was present in solution. When catalytic metal NPs were added to the solution, however, quantized increases in current were observed. These results were interpreted in terms single nanoparticles striking the electrode and acting as individual electrocatalytic sites that sped up the kinetics of the redox molecule. Each time another NP struck the electrode, another current pulse or step was observed.

These ECA experiments seem to have potential for realizing part of my dream for characterizing individual NPs during electrocatalytic reactions, but at this stage in their development many problems remain. The most significant of these, which has yet to be resolved, is that such experiments provide no structural information about the colliding nanoparticle. In fact, as Stevenson and coworkers showed in their Discussion paper (DOI: 10.1039/c6fd00121a), the situation is even worse than that. Because characterization is electrochemical, relatively high ionic strength solutions are required for implementation, and this usually leads to NP aggregation. In other words, even if NPs having a fairly well-defined size and composition are used for ECA experiments, by the time collisions occur it is likely they will have morphed into new configurations. One might think it possible to coat the NPs with a stabilizer, such as DNA, but it turns out that this kills their catalytic properties¹³ so no signal is observed.

Another challenge for ECA and related experiments is that the nature of the collision between the NP and the electrode is not understood. For example, sometimes the resulting electrocatalytic reaction is quenched very quickly, other times less so. This could be due to the presence of impurities in solution, nanobubbles, or something else. One thing is clear, however, if the phenomena that give rise to the collision current signature are not understood, it is going to be very difficult to use this approach to correlate NP structure and function. Having said that, Stevenson's work has shown that the kinetics of aggregation can be measured using this method. In addition, interesting work by Zhang and coworkers has shown that fast scan cyclic voltammetry can be used to study how NPs evolve in time during and just after collisions.¹⁴

While there is still a long road ahead, ECA-type experiments do hold out promise for *in operando* analysis of NPs. For example, in their Discussion,

Tschulik and coworkers (DOI: 10.1039/c6fd00112b) described ambitious collision experiments, similar to those first reported by Compton,¹⁵ aimed at analyzing bimetallic AgAu alloy NPs (most other collision studies have been performed using simple monometallic NPs). A recurring theme of the Discussion was that the information density of single-entity experiments can be increased by coupling electrochemistry to other analytical methods. Toward this goal, Alpuche-Aviles and coworkers showed that dye-sensitized semiconductor NPs also give rise to currents when they collide with electrodes (DOI: 10.1039/c6fd00100a). The idea of coupling spectroscopy to collision experiments was taken a step further by Kanoufi and coworkers (DOI: 10.1039/c6fd00098c), who showed that dark-field imaging could be used to follow the dissolution of individual AgNPs upon striking an electrode surface. Even more interestingly, through the use of some very clever chemistry, they were able to couple spectroscopy to the electrochemical experiments to distinguish between individual AgNPs and agglomerates. In a similar vein, Long and coworkers (DOI: 10.1039/c6fd00069j) coupled electrochemical measurements with dark-field microscopy, and they showed that individual Au nanorods exhibit differences in activity for peroxide oxidation. Continuing with the theme of coupling other analytical methods to electrochemistry to derive a better understanding of NPs, Kranz and coworkers (DOI: 10.1039/c6fd00128a) used SECM coupled with AFM to better understand the relationship between the structure and function of AuNPs having complex topographies.

Although not there yet, the types of experiments described in this section hold out the promise of correlating NP structure with electrochemical function. The drivers that have enabled this degree of progress are clearly clever experimental design, advances in instrumentation, and coupling of other analytical methods to the electrochemical measurements. These three approaches likely light the path forward as well.

Nanopores and nanofluidics

Electroanalytical chemists have historically confined their thinking about electrochemistry to something one does with an electrode made of Hg, Pt, Au, C, a few semiconductors like titania, and a handful of other solid materials. One of the most interesting aspects of the Discussion, however, was the innovative ways in which hollow pores of various sorts were used to probe nanoscale volumes, individual particles, and even single molecules. In this section, therefore, I want to take a little historical detour so that we can see how this field evolved and what led to the most seminal advances. Let me say at the outset, just as I did in the Introduction, that improvements in instrumentation, creative thinking, and a strong desire to probe smaller and smaller volumes and numbers of molecules are at the heart of the nanopore field.

Consider the Coulter counter. This device, depicted in Wallace H. Coulter's original 1953 patent in Fig. 2 and more schematically in Fig. 3, consists of two compartments separated by a thin tube or single-pore membrane. The compartments are filled with an electrolyte solution, and then a driving electrode is placed into each compartment. When a voltage is applied between the electrodes, an ionic current flows through the tube. Now, when entities having a size on the order of the tube diameter (blood cells in Coulter's original device) are

Oct. 20, 1953

W. H. COULTER

2,656,508

MEANS FOR COUNTING PARTICLES SUSPENDED IN A FLUID

Filed Aug. 27, 1949

2 Sheets-Sheet 1

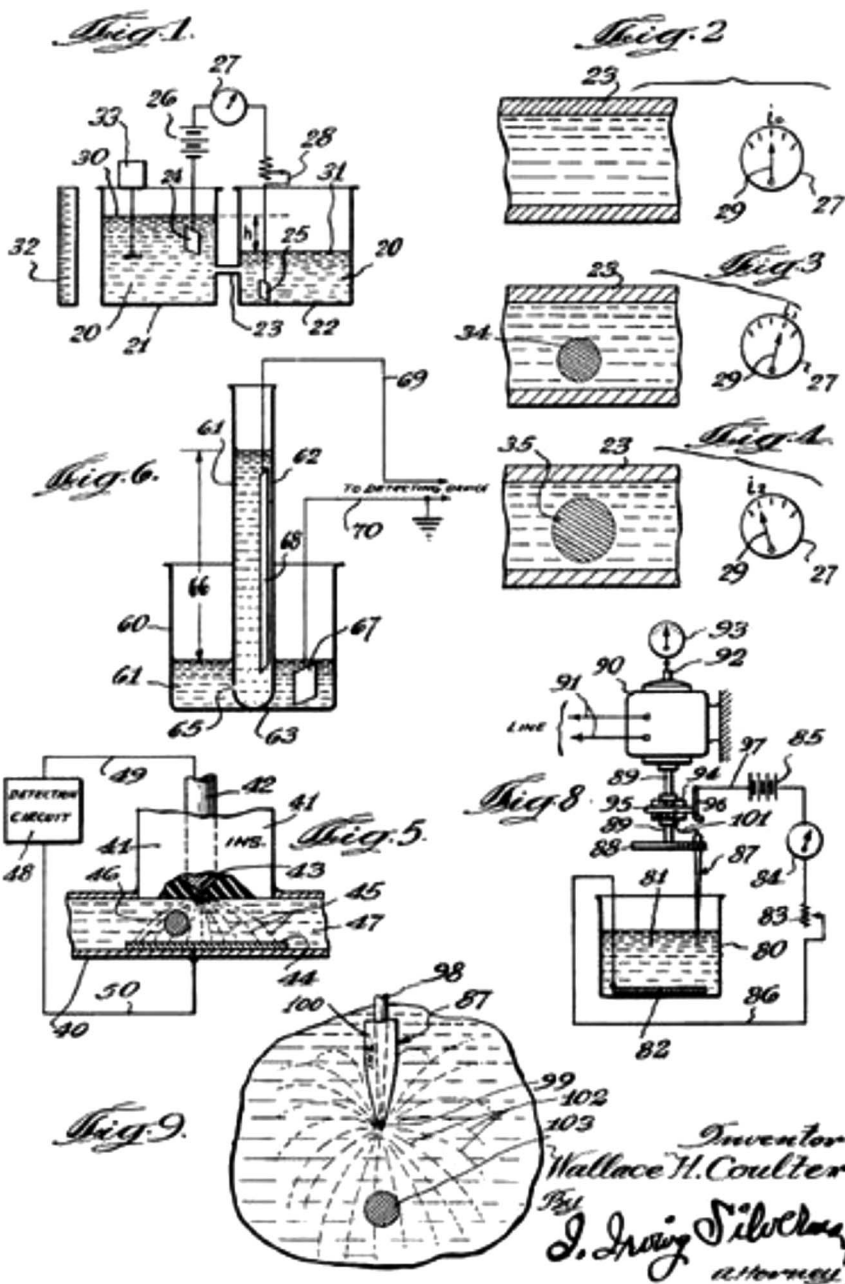


Fig. 2 A diagram from W. H. Coulter's original 1953 Coulter-counting patent.

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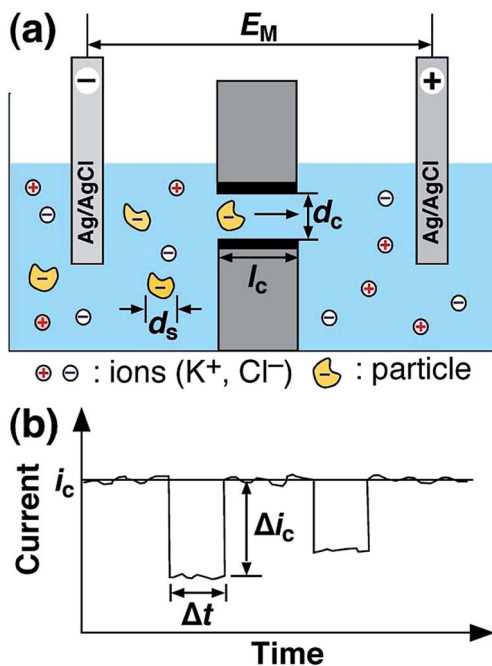


Fig. 3 (a) The general experimental approach for Coulter counting experiments. A membrane containing a single channel divides two chambers containing an electrolyte solution. When an appropriate potential is applied across the membrane, an ionic current is driven through the channel. If particles of an appropriate size and charge are present, they will enter the channel and reduce the ion current. (b) Coulter counter data consist of a series of current pulses associated with the presence of particles within the channel. The height of the pulse, Δi_c , is related to particle size and the width, Δt , corresponds to the particle transit time. In favorable cases, data such as these can provide information about the size, charge, and concentration of the particles. Reprinted from: R. R. Henriquez; T. Ito; L. Sun; R. M. Crooks "The Resurgence of Coulter Counting for Analyzing Nanoscale Objects" *The Analyst* 2004, **129**, 478–482.

placed into one of the compartments, they periodically find their way into tube. The entity then represents a volume constriction in the tube, and leads to a corresponding decrease in ionic current. By counting the resulting current pulses, one can back out the concentration of, for example, blood cells. Moreover, Coulter found that the magnitude of the pulses was closely related to the size of the cells. Pure genius: simple, effective, information dense, and still in daily use worldwide more than 60 years later.

Fast forward to 1970. A pair of scientists working at General Electric in Schenectady, New York, R. W. DeBlois and C. P. Bean, reduced the size of the pore separating the compartments of the electrochemical cell from the 10–100 μm used by Coulter, to just 450 nm by using track etched polycarbonate as a membrane material.¹⁶ This made it possible for them to detect polystyrene particles as small as 90 nm. A few years later DeBlois used the same sort of device to detect individual viruses!^{17,18}

In 2000, my own group, building on the Coulter concept, used a multiwall carbon nanotube as the pore, and we were able to determine the concentration

and size of particles having diameters around 50 nm.^{19,20} Although this isn't much smaller than the particles used by DeBlois and Bean, there were some structural aspects of the carbon nanotube pore, primarily its uniformity and absence of permanent surface charge on the interior, that improved the resolution of the measurements and also made it possible to quantitatively assess the surface charge of individual particles. We were able to visualize DNA translocation through a carbon nanotube pore using fluorescence microscopy,²¹ but the pore was too big to make Coulter-type measurements and we didn't know how to make a smaller pore. We learned two other things during the period we were carrying out these experiments that are still relevant today. First, it is surprising that the pores, especially those having nanometer-scale diameters, don't get clogged up by impurities in solution. The fact is, however, that clogging is rare. Second, Coulter-type experiments detect one entity at a time, but high entity concentrations are required or else the time between pulses is too long for practical applications. In other words, these devices are useful for single-entity analysis, but many entities are required to make measurements.

At about the same time our experiments were going on, Kasianowicz and coworkers were taking a very different approach for studying translocation of DNA.²² Instead of using artificial pores, they were using a protein, called α -hemolysin, which has a hole in its center, as the pore. The protein assembles in a lipid bilayer, which is the high-resistance membrane in this experiment. The magic of this approach is threefold. First, the pore in α -hemolysin is very small: just 1.4 nm, so one can use it in a Coulter counting format to detect the passage of individual molecules. Second, because it is a protein, every α -hemolysin is structurally well defined and nearly identical. Third, the chemical structure and amino acid sequence of α -hemolysin is known, and therefore it is possible to change it using the tools of biotechnology. This means it is possible to introduce specificity into the pore.²³

This trail of advances, one way or another, led to some remarkable discussions in York. While it is not 100% clear that it will be possible to routinely and accurately sequence DNA using α -hemolysin pores, they are being used to study the details of its molecular structure and dynamics (White *et al.*, DOI: 10.1039/c6fd00058d). Advances in the experimental design of nanopores is also leading to new understanding and applications. For example, faster electronics exhibiting lower noise have led to significant advances in contemporary translocation measurements (Albrecht *et al.*, DOI: 10.1039/c6fd00109b). Likewise, pores made of novel materials and fitted out with biorecognition elements (Platt *et al.*, DOI: 10.1039/c6fd00072j) to introduce specificity will likely lead to useful sensors for real-life applications.

Another important message from the Discussion is that theory can be of great value for understanding interactions between pores and the fluids they contain (Schmickler *et al.*, DOI: 10.1039/c6fd00076b; Eikerling *et al.*, DOI: 10.1039/c6fd00094k). Indeed, much work has been done using classical theories for this purpose, but for pores, like single-walled carbon nanotubes or α -hemolysin, it doesn't make sense to think of water as a continuum fluid. Rather, more advanced first-principles theory is required to obtain a molecular level interpretation of experimental results. Most electrochemists are familiar with classical theories of ion solvation, the electrical double layer, and so forth, but we would do well to

collaborate with our theory colleagues to better understand the structure of water and ions in nanoscopic pores and other volume elements.

This brings up another important theme from the conference that I mentioned earlier: theory is no better than the experimental models used to test it. Therefore, it is incumbent on experimentalists to devise nanopore systems that as closely as possible mimic theoretical models. Carbon nanotubes probably represent such a model, but evaluating the structure of water or of small numbers of ions in such an environment is going to be tricky. We're getting there, however, as evidenced by the elegant experiments of Faez and coworkers (DOI: 10.1039/c6fd00097e), who discussed a well-defined capillary system for tracking individual (60 nm) gold nanoparticles. Their approach relied on light scattering for detection, and this brings up another important outcome of the meeting that I want to reinforce: it is profitable to couple non-electrochemical methods with electrochemistry to gain a fuller understanding of nanoscale phenomena. The key point is that if experimental models like this can be reduced in dimension by an order of magnitude, then we will be able to better understand and optimize many important types of energy conversion and storage devices where nanopores may well dominate function.

Complex surfaces and reactions at the nanoscale

One of the first publications dealing with single entity electrochemistry was published in 1969.²⁴ This work was carried out in Newcastle upon Tyne, just an hour up the road from York. In their article, Giles and coworkers showed that catalytic reduction of protons could be carried out on individual catalytic nuclei of different metals grown on Hg electrodes. One of the key points the authors made is that Hg is a great electrode material for studying the electrochemistry of electrocatalytic nuclei, because Hg itself is not catalytically active for hydrogen evolution and, more important, the surface of mercury is much more uniform than that of solid electrodes. This is a really interesting paper, but amazingly it has only been cited an average of once per year since its publication. This is a further indication that electrochemists weren't, until recently, paying attention to nanoscale reactions. Fortunately, we've made up for the delay with a range of remarkable experiments, discoveries, and emergent observations, some of which were discussed in York.

As Giles pointed out 50 years ago, a good approach for simplifying single-entity electrochemistry is to confine the entity to a surface: this makes it easier to find. The state of the art in this regard may be results reported at the Discussion by Magnussen and coworkers (DOI: 10.1039/c6fd00086j). They used STM to obtain high-resolution (both spatial and temporal) video during Bi electrodeposition onto single-crystal Au surfaces. The striking thing about their images were just how far the field has come since the pioneering work of Binnig and Rohrer. Surface confined structures were also discussed by Krischer and her colleagues (DOI: 10.1039/c6fd00115g). In their case, simulations and experiments were combined to better understand molecular domain formation.

Koper's article described the interesting phenomenon of cathodic corrosion (DOI: 10.1039/c6fd00078a). The process by which cathodic corrosion occurs is not well understood, but individual corroding defects certainly count as an important single entity. By combining their exacting electrochemical and microscopy results

with density functional theory, they concluded that adsorbed ions play a central role in cathodic corrosion.

An emerging single entity of interests to electrochemists and others is nanobubbles.²⁵ White and his coworkers have been working on understanding these materials for a few years, and they presented their most recent findings at the Discussion (DOI: 10.1039/c6fd00099a). The question they addressed was the nature of electrogenerated nanobubbles of H₂, N₂, and O₂ at a Pt UME, and specifically the number of molecules required for nucleation of individual stable bubbles. The results showed that just a few thousand molecules are required. This study was a particularly good example of how the study of a single entity, a nanobubble in this case, can provide quantitative insights into bubble formation in important industrial processes ranging from the chloralkali production to energy conversion and storage.

Clausmeyer, Schuhmann, and their colleagues reported on the oxygen reduction reaction (ORR) at the three-phase boundary (DOI: 10.1039/c6fd00101g). The beauty of this experiment is its design, which consists of a well-defined model for studying the very complex electrochemical processes that occur in, for example, the gas diffusion electrodes used in fuel cells. As mentioned earlier in this article, these types of well-defined model systems, stripped to their essence, are required to disentangle the complexity of critically important industrial processes. This same general theme was found in the discussion of the work of Chen and coworkers (DOI: 10.1039/c6fd00087h), who reported on finite size effects of ions in the electric double layer.

Molecular electroanalysis: from single molecules to single cells

In 1996, Bard and coworkers reported the electrochemistry of single molecules in solution, and I think this publication really kicked off the modern era of single-entity electrochemistry.²⁶ Bard's experiment was carried out by trapping small numbers of molecules (1–10) between a sharpened metal tip and a planar electrode. The authors explained that the molecules were confined by a sheath of wax, and sufficient current for detection arose from redox recycling of the trapped molecules. A clear message from this work is that if one wants to study small numbers of molecules electrochemically, they have to be confined near the electrode. Another important message was that it is challenging to measure the very small currents associated with the electrochemistry of small number of molecules. This article is important background for another reason: the system was not very well-defined, and that meant that the experiment was difficult to reproduce. During the past 20 years the situation has gotten much better (in fact, as we will see shortly, there were some outstanding examples in this regard at the Discussion), but it is still difficult to construct well-defined volume elements for electroanalysis of small numbers of molecules.

As mentioned earlier, if one wants to study small numbers of molecules, then one has to know where to find them. There are two ways to do this: either trap the molecules within a small solution volume, as Bard did, or confine them to a surface. As he discussed at the meeting, Lemay and coworkers (DOI: 10.1039/c6fd00075d) implemented the basic Bard experiment but refined the

confinement volume into a well-defined geometry using the tools of micro-fabrication. Specifically, they engineered an electrochemical cell having two parallel electrodes a little over $1\ \mu\text{m}$ long but separated by only $40\ \text{nm}$ (Fig. 4). Their interest ran more deeply than simply detecting molecules in this gap; rather, they were interested in measuring the dynamics of mass transport as the molecule moved from one end of the nanochannel to the other. As in Bard's experiment they relied on redox recycling to obtain measureable currents, but as with many of the experiments described at the Discussion advanced electronics (high sensitivity and fast rise times) were critical to success.

There are similarities between Lemay's approach and that of Bohn and his colleagues (DOI: 10.1039/c6fd00062b). Both studies rely on small volumes to confine small numbers of molecules, both involve difficult and exacting micro-fabrication to create well-defined volume elements, and both rely on redox cycling to generate measureable currents. However, there is one big difference: the Lemay approach involves a single electrochemical cell, whereas Bohn's system uses on a large array of electrochemical cells having effective volumes on the order of $1\ \text{aL}$. The average occupancy of a single cell in these arrays was less than one molecule: an amazing achievement.

As these examples show, with a good cleanroom and a few tens of millions of British Pounds worth of high-tech equipment, one can make a really small electrochemical cell. It turns out Nature is also pretty good at making small-volume containers: vesicles and liposomes are examples. Ewing described his group's efforts to better understand how these containers rupture in the presence of carbon electrodes. Unlike microfabricated devices, however, these systems are a little more akin to the original Bard experiment in that their structure and

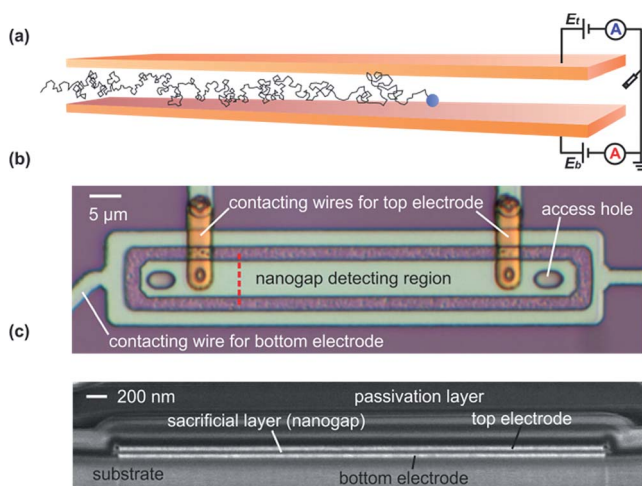


Fig. 4 (a) Schematic illustration of Lemay's nanogap device (DOI: 10.1039/c6fd00075d). (b) Optical microscope image (top view) of the device. The cyan rectangle in the center corresponds to the top electrode, below which lies the detection region. The remaining structures serve as electrical interconnects to the top and bottom electrodes (yellow and cyan lines, respectively). (c) Scanning electron microscopy image of the cross section of a device from a 52° viewing angle. To make this image the device was cut open using a focused ion beam along the line indicated by the dashed red line in (b).

dynamics are not well-defined. Actually, this is the whole point of these experiments: to better understand how molecules move in and out of natural systems. A lively discussion at the meeting emphasized the difficulty of drawing conclusions about such complicated systems using just electrochemical measurements.

Another way to confine a single molecule of electrochemical interest to a defined location is to attach it to a pair of electrodes. Higgins *et al.* (DOI: 10.1039/c6fd00080k) presented this approach, and showed that it could be used to distinguish between tunneling and redox hopping.

For electrochemical imaging,⁶ SECM has been the go-to method for many years. Recently, however, the scanning ion conductance microscope (SICM)²⁷ has been adopted as a companion method for measuring and imaging ion fluxes. One of the leaders in this field is Lane Baker, and he presented remarkable findings in which one or more natural ion channels were placed over the tip of a glass capillary using the patch clamp method (DOI: 10.1039/c6fd00133e). This resulted in a kind of ionic transistor in which the current through the ion channel “gate” is controlled by a Ca^{2+} flux (akin to the gate voltage in a field-effect transistor). The key point, however, is that this method provides a means for understanding single ion channels and also for introducing specificity to SICM tips.

The original scanning probe method, STM, was also well represented at the Discussion and provided an example of studying single molecules by confining them to a two-dimensional surface. By using well-defined, single-crystal substrates and coupling atomically resolved STM with voltammetry, Ulstrup, Zhang, and coworkers showed that it is possible to correlate structure and function of a well-defined array of DNA quadruplexes (DOI: 10.1039/c6fd00091f). Although not strictly a single-entity method, the message here is that if one can be certain that every molecule in an array is identical, then one can learn as much as would be possible by studying a single molecule. This is an interesting idea, but I think there are limits in drawing this correlation, particularly for large biological molecules which often seem to have a life of their own.

Summary and conclusions

Up to this point I have described the findings discussed in York, and in the process of doing so have tried to include some general themes relevant to single-entity electrochemistry. I'm a list-maker, however, and so I want to call those out here explicitly. Before doing so, however, I promised in the Introduction to try to answer the question “why study single entities”. I expect there are more answers to that question than there were discussants in York. Almost anyone who has ever been in love knows the answer to this question, however. One usually doesn't fall in love with the entire human race, one falls in love with a single entity (or in some cultures a small group of single entities). A lifetime probably isn't enough time to really understand that single entity (or small group of entities), but one sure can't complain about investing time in the study if they find an interesting entity (or entities)!

OK, here's my list.

- One has to locate single entities before one can study them electrochemically. The methods for doing this include: using large numbers of entities so that it is easy to find one of them; confining small numbers of entities to small volumes; tacking the entity down to a surface (preferably a nanoelectrode),

thereby reducing the dimensionality of the search space; or using relatively large entities like microbeads.

- The heterogeneity of solid surfaces, which often changes as a function of time, is a real problem, because the entity under study is affected by, for example, the nature of an electrode surface. Impurities represent a kind of heterogeneity, and even atomic-level heterogeneity can affect measurements and result in incorrect conclusions. Smaller surfaces may reduce the impact of this effect. Biological pores and those based on perfect nanotubes may not suffer as much from this problem. Maybe we need all-bio electrodes that can be constructed by DNA programming?

- Many of the systems described in this Discussion volume were characterized only by electrochemical methods, and this inevitably results in some mechanistic guesswork. Coupling spectroscopy or microscopy to electrochemistry *in operando* can address this issue, but that is often difficult or impossible to do. New analytical methods are needed for coupling to electrochemistry.

- More effort should be put into coupling experiments with theory, and particularly for single entities first principles theory is an important companion to classical approaches. It might be productive to think of theory as yet another analytical tool. Developing experimental electrochemical models that very closely approximate constructs used in first-principles calculations is critical for improving theoretical approaches of any kind.

- Even if one can detect the presence of a single entity, it might not be possible to correlate its structure and function. This is a particularly serious shortcoming of most methods.

- Electrochemical measurements of single entities often require precision electrochemical cells, and fast, sensitive electronics. These refinements are evolving at a rapid rate, and it will be interesting to see how far they can be pushed.



Fig. 5 A cowpoke on his horse surveying a field of single entities. Imagine how difficult it would be to round up them doggies (that means "cattle" in Tex-speak) if they were in three dimensions!

At this Faraday Discussion we heard about advances in all of the areas mentioned above, but in many cases there is still too much complexity, too many unknowns, and too much guesswork. I reckon that if progress in addressing these issues proceeds as quickly during the next 20 years as it has during the past 20 the next Faraday Discussion on single entity electrochemistry is really going to be something to behold. I sure hope I'm around to see what the next generation of electrochemists cooks up!

Before closing, I'd like to add a personal reflection about my visit to England. I viewed York, and the parts of England I visited on my way there, through the eyes of a Texan (Fig. 5). For reading material en route, I was advised by an English friend to take along P. G. Wodehouse. He assured me that this would help to prepare me for the people I would meet on my journey. Imagine my surprise when I didn't hear a single "What ho!", did not run into any private clubs like the "Drones", did not have to "dress" for dinner, and was not greeted upon arrival at my lodgings by Jeeves! The other advice I received was to listen to Gary P. Nunn, a legendary Texas songwriter, and particularly his song "London Homesick Blues". This was also not helpful, because I made a lot of new friends (I didn't have to rely on my "only friends" being "a smoke and a cheap guitar"), no one stared at my boots ("And them Limey eyes, they were eyein' the prize that some people call manly footware"), and I will definitely not "substantiate the rumor that English sense of humor is drier than the Texas sand". It is true, however, that by the end of this excellent meeting old Gary P. had it right: my heart was "longin' to be home in a Texas bar".

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