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DISCUSSIONS

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Advanced nanoelectrochemistry implementation: from concept to application: general discussion

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DOI: 10.1039/d2fd90004a

Yi-Tao Long opened a general discussion of the paper by Paul W. Bohn: The electrochemical nanoconfinement makes a major impact on controlling the mass transport. Could you please comment on the multi-phase effects on the mass transport under nanoconfinement. What's the next breakthrough application we could expected in controlling the mass transport in nanoconfinement.

Paul W. Bohn responded: You are correct that we see evidence of altered mass transport under conditions of confinement. Although we have not implemented a comprehensive study, we can say some general things about the factors that influence mass transport under these conditions. Across many different experiments, it appears that permselectivity is a dominant factor in determining transport behaviour in nanopores. Beyond this, ion migration appears to play an important role. This is especially true and accentuated by the alteration in ion shielding at small dimensions, which impacts the magnitude of ion migration currents. Lastly, diffusion is an important mass transport mechanism at the small scale, principally because transport between electrodes that are only a few tens to hundreds of nm apart can occur on the micro second time scale and thus be quite efficient. A final thought in this regard concerns a potential role for surface hopping processes. It is widely appreciated that the micro channels can be dominated by surface production processes when the size of the channel becomes small enough, and this becomes a player at the nanoscale as well, but it is a mechanism that we have not yet tried to exploit explicitly.

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Yi-Tao Long added: In the last two *Faraday Discussions*, we discussed the well-defined nanointerface. We could see that there is progression, achieving good development. But still, developing a well-defined nanointerface is a challenge. Could you please comment on the future of nanointerfaces?

Paul W. Bohn answered: I think there are two important parts to answering your excellent question. First, is the question of being able to reproducibly fabricate architectures with sub-nanometer control over spatial positioning and orientation of functional components. For the parts of the structure that are amenable to traditional methods of nanofabrication, we are actually very close, as can be ascertained from some of the cross-sectional SEM images from our laboratory and others. When we move from fabrication strategies developed to support microelectronics to those that are more chemically oriented, for example the self-organization of block copolymers utilized in the structures from our laboratory, then the control is less precise. As an example, although we draw cartoons in which the P4VP nanodomains are all oriented perpendicular to the planer interface, in fact we have good cross-sectional SEM images illustrating that these cylindrical domains are oriented over a range of angles relative to the interface. This is just one example where the molecular approaches to bottom-up fabrication present real challenges. The last point with respect to this part of the question has to do with the incorporation of molecules as functional elements. For example something as well studied and straightforward as enzyme immobilization still produces layers with a large degree of structural heterogeneity, both within the enzyme itself and among neighboring enzyme molecules in a layer.

The second, and perhaps even more challenging, part of the question concerns the ability to translate the high precision nanoscale construction capabilities addressed above to very large macroscopic areas. The tools that we have at our disposal are well-designed for μm-scale architectures with nm-scale precision. However, we will need to develop new approaches that allow us to develop structures at the millimetre and centimetre scale in order to see the full implementation of nano-interface-enabled applications. This is a major challenge for the nanoscience field as a whole, not just nanoelectrochemistry.

Frederic Kanoufi continued: As mentioned by Yi-Tao Long, you have given this nanoelectrode array very interesting developments. In earlier work, you also proposed that those nanoelectrode arrays could be monitored optically. Could it be a complementary way to dynamically visualize the wetting/dewetting of the vestibule, or to probe single molecule activity within the vestibule? The strategy presented by Valavanis *et al.* (DOI:10.1039/d1fd00063b) in session 3, monitoring change in the reflecting properties of the bottom electrode could also be interesting.

Paul W. Bohn answered: Your question is right on the mark. In these experiments, we have constructed hierarchically organized membrane systems in which the BCP is located 0.5–1.0 μm from the floor of the nanopore, which places it sufficiently far away so that we could not use our electrochemical zero mode waveguide strategy directly. However, it should not be too hard to reduce the thicknesses of the NEA nanopore metal–insulator–metal structures such that the BCP membrane would be optically accessible through the NEA nanopore. With

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these altered (thinner) structures, it should be possible to capture fluorescence from the probe species within the nanocylindrical P4VP domains of the BCP under conditions in which these pores are wetted.

Si-Min Lu added: To continue on the comment from Yi-Tao Long, what do you think the next avenue should be for nanopore electrochemistry in terms of single entity measurements? Especially for the precise measurements of the transient electron transport of single entities in Faradaic processes.

Paul W. Bohn replied: In order to address single entities in nanopore electrochemistry, it is first important to define the nature of the single entity – they could be molecules, nanoparticles, viruses, or even bacteria (although at that point one is really talking about microscale structures not nanostructures). The reason it is important to define the problem is that we are still in a regime where addressing 1 single electron transfer event in single molecules by purely electrochemical means is extraordinarily difficult. At this level, the two major routes to measurement, both of which are inherently nanoscale processes, involve some type of amplification process, such as redox cycling, or the conversion of electrochemical events into spectroscopic processes, such as those that occur in electrofluorogenic reactions. If one confines the question of transient electron transport, then spectroscopic approaches have many advantages. Specifically, a fluorescent redox molecule can be cycled through the excited state many times, thus creating an amplified signal for a single redox turnover event. In addition spectroscopic processes can be studied under conditions that are not always limited, and detectors are routinely capable of measuring single photons. The trick, of course, is defining a redox system that has the requisite electrofluorogenic character. If the single entities of interest are larger, such as nanoparticles, viruses, and other microorganisms, then direct electrochemical study becomes more feasible, since the entities can be capable of transferring a large number of electrons in a single interaction. However, for the most demanding experiments, such as the transient electron transport processes that you have in mind, I think spectroelectrochemical approaches will for the near future, be a superior choice to purely electrochemical studies.

Popular Pandey asked: Thank you for the great talk. Of the 160 nm and 89 nm BCP membranes, the 89 nm BCP membrane exhibited a 2-fold increase in transport properties (main text Fig. 3 in your article, DOI: 10.1039/d1fd00048a). Is this due to the thinner BCP membrane? Also, how stable are the BCP membranes over the range of applied potential?

Paul W. Bohn replied: Yes, we assign the increase in transport rate to the membrane thickness being smaller, which should decrease the transport resistance in a general sense. As for the stability of the BCP membranes, we have not observed any overt signs of mechanical or chemical degradation over the range of potentials used in our experiments. Presumably there are limits to the potentials which can be applied without degradation, but we have not explored that question in detail.

Yanfang Wu said: Thanks a lot for the inspiring talk. Could you please provide some more detail/insight into the change in pore size of the BCP membrane from

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hydrophilic to hydrophobic? What is the rationale in terms of the pore size of the BCP membrane to perform potential-induced wetting and dewetting? This is a very interesting study and one day the system may be able to fulfil operations with liquid samples like loading, filtering and analytical analysis afterwards.

Paul W. Bohn responded: This is an interesting, but still open, question. There have been a large number of publications from other laboratories addressing the nature of the pH-dependent change in properties of the nanocylindrical P4VP domains, but I do not think there is at present a definitive answer to the question of the change in pore size above and below the pK_a.

The rationale for exploring potential-induced wetting and de-wetting came from observations of similar phenomena in other polymer nanopore systems. In particular, I would point to the work of Smirnov and coworkers, for example.[1] We certainly agree with your assessment that these phenomena may be capable of enabling operations like liquid sampling, loading, and analytical determinations within the nanopores of our structures.

1 S. N. Smirnov, I. V. Vlassiouk and N. V. Lavrik, *ACS Nano*, 2011, **5**, 7453–7461.

Frederic Kanoufi asked: To continue on the suggestion made by Serge Lemay yesterday (DOI: 10.1039/d2fd90003c): one way to capture and analyze single molecules is to isolate, concentrate then analyse them. You showed a promising way to isolate and analyze molecules into these nanosized vestibules, maybe soon at the single entity level. What about concentrating them into the vestibule? Could you use the polymer layer as an affinity preconcentration layer for this purpose, or use the electric field, *etc.*...?

Paul W. Bohn replied: This is an excellent observation. In fact, this is exactly the strategy we had in mind when preparing Fig. 1(d) in our manuscript and the data presented at the end of the oral presentation. It should be possible to manipulate potential as the BCP membrane can be used as a potential-gated valve to allow the capture and trapping of analyte species within the vestibule of the NEA nanopores. Once trapped they can be subjected to a variety of chemical and electrochemical manipulations in order to enhance their utility in chemical sensing. For example, one can immobilize enzymes within the NEA nanopores and utilize these to convert non-redox active substrates into redox-active products that can then be detected with high sensitivity using the redox cycling capabilities of the two-electrode nanopores discussed in the paper. In fact, we just published an early version of this concept.[1]

1 J. Jia, S.-R. Kwon, S. Baek, V. Sundaresan, T. Cao, A. R. Cutri, K. Fu, B. Roberts, J. D. Shrout and P. W. Bohn, *Anal. Chem.*, 2021, **93**(43), 14481–14488.

Zhu Zhang commented: The insulating layer between two Au layers is SiN_x and the top insulating layer is SiO_2, is the reason that the SiN_x is better than SiO_2 in terms of insulation? Could you give a explanation on that?

Paul W. Bohn responded: In the early days of these experiments, we tried both silicon nitride and silicon oxide insulating layers between the two metallic electrode layers. In our hands, we had better yield employing plasma enhanced CVD

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deposited silicon nitride. For future applications where we want to reduce the size of the insulating layer – for example to enhance mass transport between the two electrodes in redox cycling experiments – it may be advantageous to utilize atomic layer deposition (ALD) of alternative dielectrics. We have had success in depositing both HfO_2 and TiO_2 by this approach, but we have not yet done a careful comparison of these materials to silicon nitride.

Zhu Zhang continued: Do you see any etching of the insulation layer during the experiments?

Paul W. Bohn replied: We have not obtained any evidence of etching of the insulating layers, either during fabrication or the experiments themselves. If etching were to occur, in the silicon nitride layer, it would produce under cutting of the top electrode, while etching of the silicon oxide top layer would likely just reduce the overall dimensions of that layer.

Zhu Zhang further questioned: How do you remove the Ga ions from your nanopore?

Paul W. Bohn responded: We use a nitric acid post-milling etch procedure described previously.[1]

1 S. P. Branagan, N. M. Contento and P. W. Bohn, *J. Am. Chem. Soc.*, 2012, **134**, 8617–8624.

Zhu Zhang commented: The membrane transport from another substrate to nanopores array – how do you check the membranes have good contact?

Paul W. Bohn answered: This is an important and experimentally challenging part of our procedure. First, we do a visual check by optical microscopy, then when we immerse the structure in the target solution, we look for the presence of any non-specific transport into the NEA nanopores. Although these procedures are not fool proof, they certainly identify samples where there is large scale failure, and make it possible to move forward with structures in which we have confidence of their structural integrity.

Zhu Zhang added: You introduced some fluorescent proteins in your nanopores, how do you introduce the singly fluorescent proteins?

Paul W. Bohn answered: Since we do not have a fool proof procedure to introduce exactly one protein molecule into every nanopore, we rely on a statistical distribution (Poisson) of enzyme molecules produced by carrying out the reactions under low loading conditions. By biasing the distribution so that the majority of pores have zero enzyme molecules, the number which exhibit a single enzyme molecule is maximized relative to pores that have multiple enzymes. Determining the conditions under which this is true is done experimentally, and painstaking measurements are typically required to identify the optimum conditions, *i.e.* enzyme concentration, buffer concentration, reaction time, nanopore pre-treatment, *etc.*

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Alain Walcarius queried: How do you interpret the ionic strength-dependence of wetting/dewetting in the BCP membranes? Is there something to do with the thickness of the electrical double layer (on charged membrane) relative to the membrane pore size?

Paul W. Bohn replied: There are likely two major contributions to the ionic strength dependence. The first, as you suggest, likely has to do with the altered structure of the electrical double layer relative to the membrane nanopore at different ionic strengths. The average pore size of the block copolymer membranes was ~12 nm, placing them exactly in the range of Debye lengths applicable at the lower ionic strengths. The other likely contributor is the detailed spatial potential distribution, which would be altered at different ionic strengths, specifically the potential dropped across the thickness of the block copolymer membrane. These two factors are likely not completely separate, and we are currently performing detailed simulations to try to understand the effects we observe experimentally.

Dominik Duleba opened a general discussion of the paper by Shelley Minteer: How are you able to fill these long conical nanochannels with the surface modification reagents?

Shelley Minteer responded: The solution was injected into the glass nanopore with a microloader. Then, we centrifuged the glass nanopore for 5 min at around 1878 *g* for the removal of air bubbles in the nanopipette. For this step, we placed the nanopipette with the tip facing down into a homemade holder within a 2 mL centrifuge tube. For more detail refer to our previous papers.[1,2]

1 R. Gao, Yao Lin, Y.-L. Ying, Y.-X. Hu, S.-W. Xu, L.-Q. Ruan, R.-J. Yu, Y.-J. Li, H.-W. Li, L.-F. Cui and Y.-T. Long, *Nat. Protoc.*, 2019, 2015–2035.
2 R. Gao, L.-F. Cui, L.-Q. Ruan, Y.-L. Ying and Y.-T. Long, *J. Vis. Exp.*, 2019, **145**, e59003.

Steven Linfield asked: Control experiments with FeSII modified nanopores seem more like typical translocations through a conically shaped pore; a sharp decrease in current as the protein travels through the pore mouth (greatest sensing zone), followed by a relatively gradual return to background current. Translocations through a Pdx modified nanopore have a stranger shape; a sharp decrease in current followed by a further decline during the translocation event. What is the explanation for the shape of the P450cam translocations through Pdx modified nanopores in the context of protein–protein interactions? Also, did you record rectification in FeSII modified nanopores? Was it similar to Pdx modified nanopores or could some change in surface charges/ion exclusion zones contribute to differences in translocation speed?

Shelley Minteer replied: The further decline during the translocation of P450cam in the nanopore is because the P450cam undergoes multiple and frequent associations and disassociations with Pdx on the surface of the nanopore. We did not record rectification in FeSII modified nanopores.

Ping Yu questioned: how could you sort out the information on protein–protein interaction from the nanopore results?

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Shelley Minteer responded: In our experiment, the Pdx is modified on the inner surface of the glass nanopore while the P450cam was driven into the glass nanopore under bias potential. The interaction between P450cam and Pdx produced a characteristic signal with a long statistic duration of 1.1 ms. In order to confirm that the protein–protein interaction indeed gives the long duration, we performed control experiments by replacing Pdx with FeSII (Shethna) protein. Note that FeSII is the same size as Pdx (Fig. S1 in the ESI of our article, DOI: 10.1039/d1fd00042j), but does not interact with P450cam. The results revealed that a single P450cam molecule undergoes a rapid translocation through the FeSII modified glass nanopore with a statistic duration of 0.1 ms at 200 mV. Therefore, the interaction between P450cam and Pdx prolongs the duration of the blockage.

Ndrina Limani commented: I was wondering if there is a way to check before the measurement if the protein was indeed immobilized in the nanopore? Also, is it stable enough over time so it does not impact the measurement?

Shelley Minteer answered: In our experiments, we compared the *I–V* responses before and after modification to confirm the immobilization of Pdx. As shown in Fig. 2c in our article, the rectification ratio at $\pm$0.8 V decreased from 0.54 to 0.32 after modification. The exposed carboxyl group on the Pdx leads to a change in the surface charge density of the nanopore, giving the low rectification ratio. The open pore current traces were stable and consistent (Fig. S2 in the ESI of our article), which suggest the good stability of our modified nanopore during the measurement.

Hui Wei said: Since your proteins have His tags, have you tried to attach your protein to the Au with the His tag? Would the dissolved O_2 in protein solutions affect the measurement?

Shelley Minteer responded: The proteins with a His tag can not be immobilized on the Au surface directly. The Au surface would need to be modified by a nickel and copper chelating agent. So, we did not immobilize Pdx on the Au surface with the His tag. We did not investigate the effect of dissolved O_2 on the measurement, but we would not expect it to make a difference in the P450cam/Pdx protein system. However, if we were looking at protein–protein interactions where O_2 causes a conformational change in the protein (*i.e.* the Shethna protein and nitrogenase), then we would expect to see an effect of dissolved O_2 on the nanopore measurement.

Hui Wei added: It seems that when you measure/monitor the protein–protein interaction, you apply different voltages. Therefore, I am wondering whether there is possible electron transfer between your proteins and the gold layer?

Shelley Minteer replied: The potentials applied in these systems are small compared to the potentials needed for the bioelectrochemistry of the protein or bioelectrocatalysis catalyzed by the protein.

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Ben Slater highlighted: (1) The method you refer to in ref. 23,[1] for the thiol modification, uses a different thiol, mercaptoacetic acid, to that in your work. Was there a reason for the change to the undecanoic acid in your work?

(2) On page 5 you state "Due to the negatively charged carboxyl group, the MUA modified nanopore shows an obvious ionic current rectification" where is this result? There are only *I–V* curves for the gold and Pdx nanopipettes (Fig. 2c in your article).

(3) On page 5 you state "After modification, the exposed carboxyl group on the Pdx leads to a change in the surface charge density of the nanopore" – what is the difference in carboxylate density between the MUA modified nanopipette and the PDX modified nanopipette? Can you show a comparison of all of the current rectifications please? Adding to the two shown in 2c.

1 Y.-L. Ying, R.-J. Yu, Y.-X. Hu, R. Gao and Y.-T. Long, *Chem. Commun.*, 2017, **53**, 8620–8623.

Shelley Minteer replied: (1) The modification procedure of mercaptoundecanoic acid (MUA) is the same as mercaptoacetic acid. According to a previous report, long-chain ($n > 10$) can form stable, close-packed, and well-ordered self-assembled monolayers on gold surface (DOI: 10.1021/jp054290n) therefore may act as a more friendly substrate for protein immobilization. That is one reason we chose MUA in this work. Another reason is that we had MUA in the lab and did not have to purchase mercaptoacetic acid.

(2) The modification was carried out according to a previous protocol, which included the step-by-step procedure and characterization, except mercaptoacetic acid was replaced MUA.[1] We agree that further characterization is needed and we will compare the modification difference between mercaptoacetic acid and mercaptoundecanoic acid on the nanopipette in our future work.

(3) In this experiment, we focused on the nanopore recognition of protein–protein interactions. More details about the modification can be found in ref. 1 and 2. In our future studies, we will do the suggested experiment to compare the modification difference between mercaptoacetic acid and mercaptoundecanoic acid on the nanopipette.

1 Y.-L. Ying, R.-J. Yu, Y.-X. Hu, R. Gao and Y.-T. Long, *Chem. Commun.*, 2017, **53**, 8620–8623.
2 Q. Li, Y.-L. Ying, S.-C. Liu, Y. Lin and Y.-T. Long, *ACS Sens.*, 2019, **4**(5), 1185–1189.

Seung-Ryong Kwon said: Could you differentiate current signals between a single binding event of P450cam to Pdx and multiple binding events of P450cam to several Pdx while P450cam translocates through the nanopore?

Shelley Minteer answered: In our stochastic analysis, the P450cam induced blockage current amplitude (ΔI) in FeSII modified glass nanopore is comparable to Pdx modified glass nanopores (Fig. 3c and d). This result demonstrates that each event represents a single P450cam interacting with Pdx modified glass nanopores. The current fluctuation during the translocation of P450cam is mainly due to the multiple and frequent associations and disassociation between P450cam and Pdx on the surface of the nanopore.

Hui Wei commented: It seems that both reduced and oxidized forms of Pdx proteins are used. Are both forms stable and easily prepared?

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Shelley Minteer commented in reply: Only oxidized Pdx was used in this study. The oxidized Pdx is stable and easily prepared. As for the reduced Pdx, it can be reduced using NADH as the electron donor and ferredoxin reductase (Pdr) as an electron transport protein to reduce the iron–sulfur center of Pdx. It is very difficult to evaluate the stability of the reduced Pdx as there are many factors that affect its stability, such as the presence of O_2 and the surrounding electric field environment, *etc.*

Weilin Xu suggested: Is it possible to use nanopores to study your cascade enzyme catalysis? Thanks.

Shelley Minteer replied: This depends on the enzyme cascade system. Some of our cascades form metabolons (supercomplexes of the enzymes in the cascade – *i.e.* Kreb's cycle cascade) and they could be studied with the nanopore. However, some of our enzyme cascades involve enzymes that have no intermolecular interactions with each other (*i.e.* alcohol dehydrogenase, aldehyde dehydrogenase, and formate dehydrogenase) and, therefore, would not be easily studied using nanopores.

Yi-Tao Long asked: Could you please give some comments on the future of single enzyme electrochemical analysis? Should we go for biophysics-like studies or for sensors? Thanks!

Shelley Minteer answered: In the future, this technique could be used to study electrostatic, hydrogen bonding, and other intermolecular interactions controlling protein–protein complexation in electron transport chains, substrate channelling, molecular motors, and disease progression. By developing a suitable algorithm for analyzing the noise in the blockage current, the transient conformational changes of the protein could be further resolved during the protein–protein interactions inside a nanopore.

Popular Pandey remarked: How do you ensure the formation of monolayer Pdx on the inner wall of the nanopipette? What is the electric field effect on the stability of the Pdx enzyme? Is there any influence of the P450cam protein as well as Pdx enzyme orientation on the observed current blockade characteristics? Additionally, in Fig. 3a in your article, why does the blockade current have a slow drop and fast increase shape as P450cam translocates from the bath into the nanopipette? While the usual translocation signal features a fast current drop and slowly increases as it traverses through the narrow channel of the pipette.

Shelley Minteer answered: After modification, the exposed carboxyl group on the Pdx leads to a change in the surface charge density of the nanopore. As a result, the rectification ratio of the positive current value, $I+$, to the negative current values $I-$, at ± 0.8 V decreased from 0.54 to 0.32 (Fig. 2c in our article). Moreover, the stable time-series current trace from the Pdx-modified nanopore further confirms the stability of the Pdx layer under bias potential, as shown in Fig. S2 in the ESI of our article. During the association between P450cam protein and Pdx layer, the orientation of the complex could affect the current fluctuations. The further decreases in the diameter of the nanopore would be beneficial to

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revealing the orientation of the complex. Comparing the outcome from P450cam and FeSII, the current fluctuation during the translocation of P450cam in the nanopore is mainly due to the multiple and rapid associations and disassociation between P450cam and Pdx on the surface of the nanopore.

Samuel Confederat said: Is the stability of the Pdx-modified nanopore limited by the range of the potential applied?

Shelley Minteer replied: Pdx is a small protein and is very stable at room temperature. Moreover, the Pdx is immobilized on the surface of the nanopore which means the local concentration of Pdx is very high in this nanoscale space. The high local concentration helps maintain protein stability. It is also important to note that the protein is more stable on the surface of the nanopore than it would be at the surface of an electrode because we are not applying a potential directly to the gold surface that the protein is modified on.

Bing-Wei Mao commented: The nanopore electrode technique has played important roles in a variety of studies, including single molecule detection. Such detection is based on the blockage of the current flow, *i.e.* with detection of the reduced current. Could there be any possibilities in the future development of nanopore techniques that detections are based on current enhancement mechanism for further improving detection sensitivity?

Shelley Minteer replied: Thank you very much for your comments and suggestions. The ionic current flow through the glass nanopore is regulated by a series of competing effects including volume exclusion of transported molecules and current enhancement from the charged analyte at the tip. In the low salt concentration, counterions screen the glass surface charge within a region further from the pore. Therefore, the introduction of mobile counterions carrying the analyte outweighs the current reduction from the volume exclusion effects. Then, the enhanced signals could be observed upon translocation of a charged analyte. This mechanism has been applied in glass nanopore sensing including for DNA and peptides.[1,2] Especially, the nanobubble-induced current enhancement was applied in the wireless nanopore electrode for the detection of single redox molecules/ions, analyzing the metabolism of single cells, and discrimination of single nanoparticles in a mixture.[3–6] In the future, the further combination of volume exclusion and electrochemical charge enhancement at the nanopore confinement could benefit to produce a fingerprint current pattern for single-molecule sensing.

1 V. Wang, N. Ermann and U. F. Keyser, *Nano Lett.*, 2019, **19**(8), 5661–5666.
2 R.-J. Yu, S.-M. Lu, S.-W. Xu, Y.-J. Li, Q. Xu, Y.-L. Ying and Y.-T. Long, *Chem. Sci.*, 2019, **10**, 10728–10732.
3 Y.-L. Ying, Y.-X. Hu, R. Gao, R.-J. Yu, Z. Gu, L. P. Lee, and Y.-T. Long, *J. Am. Chem. Soc.*, 2018, **140**(16), 5385–5392.
4 R. Gao, Y.-L. Ying, Y.-X. Hu, Y.-J. Li and Y.-T. Long, *Anal. Chem.*, 2017, **89**(14), 7382–7387.
5 R. Gao, Y. Lin, Y.-L. Ying, X.-Y. Liu, X. Shi, Y.-X. Hu, Y.-T. Long and H. Tian, *Small*, 2017, 13(25), 1700234.
6 R. Gao, Y.-L. Ying, Y.-J. Li, Y.-X. Hu, R.-J. Yu, Y. Lin and Y.-T. Long, *Angew. Chem., Int. Ed.*, 2018, **57**(4), 1011–1015.

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Dongfei Chen asked: What if the translocation of the proteins occurs from the inside of the nanopore to the outside (*i.e.*, bulk solution)? Will we expect to see any changes from that?

Shelley Minteer replied: If the P450cam adds to the inside of the glass nanopore, the multiple binding between several P450cam proteins and modified Pdx layer is expected. To avoid multiple binding interference, the P450cam was driven from outside of a glass nanopore into the side lumen. Therefore, each event represents a single P450cam interaction with the Pdx.

Ping Yu opened a general discussion of the paper by Jill Venton: How about the stability of the CNS electrode when we continuously cycle the electrode.

Jill Venton answered: CNSs are very stable in PBS buffer conditions. In Fig. 4F in our article (DOI: 10.1039/d1fd00053e), we continuously tested the electrodes for 4 hours, and we don't see an obvious current drop. They have a very long shelf life as well, as we tested them many weeks after being made and they are still good.

Andrew Ewing said: Why do you not see DA electrochemistry on Nb? That seems interesting.

Jill Venton answered: Yes that is interesting. It is known that gold and platinum are sensitive to dopamine, but in our previous work, we found some metals or alloys are not sensitive to dopamine, including niobium, tantalum, stainless steel, *etc.* This is probably due to the adsorption kinetics of dopamine on the metal surface.

Andrew Ewing said: Can you take something that's normally fast, for example ruthenium hexamine?

Jill Venton replied: I don't exactly remember where this comment came from in the discussion. We use ruthenium hexamine as an outer sphere test compound in our research to test surface insensitive processes. Most of the improvement in electrocatalytic effects we see are with inner sphere compounds that are surface sensitive.

Andrew Ewing remarked: I will bet the carbon layer is peeling off at high potentials for W?

Jill Venton responded: That may be true. However, the tungsten wire itself has electrochemical activity for dopamine and higher background charging capacitance. Thus, we need the wires to be fully covered or the exposed tungsten may interfere with the measurement. We have not checked the wires to see if the carbon is peeling off but it may be seen with long term measurements. They are stable for shorter term measurements if they are made fully coated.

Andrew Ewing added: I find it fascinating that Nb has no effect.

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Weilin Xu asked: Did you try any other carbon materials with a porous structure as the electrode materials?

Jill Venton responded: We have been studying carbon nanotube yarn electrodes. These electrodes are porous so they can trap analytes. We have also used simulations to study the electrochemical process at the rough carbon electrode surface.

Ping Yu queried: Does dopamine still adsorb onto CNS? How could you conclude this? Thanks.

Jill Venton replied: Yes, dopamine adsorbs onto the CNS surface. We have demonstrated this in Fig. 3C in our article, where the dopamine current is linear to the scan rate. Edge-plane carbon can adsorb dopamine, and CNSs have rich edge-plane sites.

Peisan E asked: Have you looked at how the neurotransmitters may foul the CNS surface? Is there any functional groups at the CNS surface that may reduce the fouling effect?

Jill Venton replied: We haven't studied the antifouling properties in this work. CNSs are slightly doped by nitrogen, and they have dense nanostructures, which may reduce the fouling effect.

Ashley Ross commented: I was just curious about why enhanced redox cycling for DA isn't observed on the CNS coated electrodes (because of the apparent porosity of the surface)? And also, I was just curious if you can control the surface functionality of the CNS surface during the plasma enhanced CVD method when growing on metal wires?

Jill Venton commented in reply: Redox cycling is not observed because the surface roughness isn't high enough to trap the molecules. CNSs have a surface roughness of a few hundred nanometers, but roughness of over a micron is needed to see the redox cycling. With plasma-enhanced CVD we do not see enhanced surface functionality on the CNSs that we know of. Other plasma treatments in oxygen plasma can enhance oxygen groups, but we don't see that here because of the low oxygen.

Xu Liu remarked: Could you please explain a bit about the potential applications of carbon nanoelectrodes in other fields, in addition to measurements of neurotransmitters?

Jill Venton responded: As other application examples, the CNS nanoelectrodes would be useful as scanning electrochemical microscopy (SECM) tips in electrochemical imaging or for investigating catalytic responses of atom clusters.

Xiangkun Elvis Cao commented: Nice work on using carbon nanospike coated nanoelectrodes for neurotransmitter measurements. Your paper stated that CNS nanoelectrodes are a promising strategy for the mass fabrication of nanoelectrode sensors for neurotransmitters. Would you be able to share more insights over

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challenges your group encountered or envisioned for the scale-up process for mass fabrication and the potential pathways to solve these challenges?

Jill Venton responded: The most challenging thing we encountered is to uniformly control the size of the nanoelectrodes. Although we mounted a batch of metal wires onto the same conductive substrate, so that the same potential and time is applied to each sample, the size of the etched tips still varied, especially for niobium wires. In future work, we will continue to optimize the methods to etch metal wires. Finally, while our process is a few steps, we have to put the electrodes into several instruments. True mass manufacturing would integrate all these steps into a process line.

Bing-Wei Mao mentioned: It is not very easy to distinguish processes having a slightly different time constant from Nyquist plots. A more sensitive way could be to plot the EIS data in a complex capacitance plane (CCP or Cole–Cole plot) representation that it is easier to judge whether there is one or more kinds of capacitance.

Jill Venton replied: Thank you for the suggestions. The complex capacitance plane would better show the capacity difference. Here we used a more common complex impedance plot, because we also wanted to compare with the impedance plots of other carbon electrodes in the literature, such as carbon fiber microelectrodes and carbon nanotube electrodes.

Ali Reza Kamali commented: This is a very nice work; have you characterised carbon nanospikes in terms crystallinity, crystalline structure and surface area? Also, can you comment on the nature of the bonding between CNTs and the metal substrate, please? Thank you.

Jill Venton communicated in reply: The surface area and crystal structures can be found in previous work.[1,2] CNSs are grown layer by layer onto the metal substrates, so the carbon atoms will fit into the vacancies of the metal and form stable bonds.[3]

1 L. B. Sheridan, D. K. Hensley, N. V Lavrik, S. C. Smith, V. Schwartz, C. Liang, Z. Wu, H. M. Meyer and A. J. Rondinone, *J. Electrochem. Soc.*, 2014, **161**, H558–H563.
2 Q. Cao, D. K. Hensley, N. V. Lavrik and B. J. Venton, *Carbon*, 2019, **155**, 250–257.
3 H. L. Zhuang, G. P. Zheng and A. K. Soh, *Comput. Mater. Sci.*, 2008, **43**, 823–828.

Yi-Lun Ying addressed Jill Venton, Paul W. Bohn and Shelley Minteer: The topic of this session is "Advanced nanoelectrochemistry implementation: from concept to application". Could you please comment on the future application of your new techniques? How to bring the nanoelectrochemistry into the reality? What's the major gap?

Jill Venton responded: The future application of the electrodes will be the mass-manufacturing of carbon nanoelectrode sensors. Compared with other fabrication methods of carbon nanoelectrodes, CNS nanoelectrodes have a larger surface area to volume ratio, so they show larger currents for sensing. Both the etching of metal wires, and the growth of CNSs, are done in batch. The major gap would be controlling the quality of the nanoelectrodes in mass fabrication. For *in vivo* applications, these will enable localization in the synapse and better synaptic measurements.

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Paul W. Bohn added: Thank you for this forward-looking question. I believe that work in our laboratory and many others have made significant progress in fleshing out the concepts that can be imported from nanoscience to enable advanced applications, such as high sensitivity chemical sensors. The single most important roadblock to further progress, in my opinion, lies in developing the fabrication and chemical manipulation strategies that can produce architectures with nanoscale functional properties over large, macroscopic distances. While there are tools capable of producing structures which are many square μm's, these need to be extended to produce structures which are square mm–cm's in area. Perhaps there are opportunities to develop reel-to-reel processes for direct printing of such structures. This would do more than anything else I can think of to drive the implementation of the structures in real-world applications.

Shelley Minteer said: In the future, our technique could be used to study intermolecular interactions controlling protein–protein complexation in supercomplexes, substrate channelling, molecular motors, and disease progression caused by protein–protein interactions. However, for optimal quantitative evaluation of binding constants for a single protein–protein interaction, the gap is in making a nanopore that only contains one immobilized protein (*i.e.* one binding site).

Ben Slater opened a general discussion of the paper by Jin He: I am interested in the centrifugation method for the nanopipettes (assuming it is to remove bubbles), are there limitations to this method, *e.g.*, maximum speed before it breaks? Can the centrifugation remove surface modifications? Do you think this would also successfully remove bubbles in solid state nanopores such as SiN?

Jin He replied: The centrifugation method was used previously to enrich the nonentities at the tip of the nanopipette.[1] For the experiments in our work here, the nanopipette was centrifuged at 2000 rpm for 2 min. Higher centrifugation speed and longer centrifugation time either damage or cause clogging issues during delivery. If you can wait, a lot of nanoentities we tested can also diffuse to the nanopipette tip overnight without using the centrifugation method.

To effectively remove the air bubbles at the nanopipette tip, we used the heating method. The nanopipettes were first filled with solution and then placed in the 120 °C oven for 30 s. With heating, we do not have air bubble issues. Both centrifugation and heating may not work with the SiN nanopore because there is no long fluidic channel for the SiN thin membrane based nanopore. We worked with SiN nanopores before; you may first fill the nanopore with pure ethanol, then mix it gradually by increasing the concentration of water.

1 R.-C. Qian, J. Lv and Y.-T. Long, *Anal. Chem.*, 2018, **90**(22), 13744–13750.

Samuel Confederat asked: In terms of the fabrication of these multi-functional probes, how do you control the pyrolytic carbon deposition on one barrel in such a way that it does not constrain the pore size of the other barrel (the open barrel used for translocations)? Furthermore, is there a large variation between the fabricated probes with regards to the carbon-deposited barrel?

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Jin He answered: Thanks for the questions! The details of the fabrication process can be found in our previous publication.[1] In brief, we carefully controlled the pressure of the carbon source, butane gas, as well as the protection gas, N_2. The deposition time is also very short, only about 20 s. Based on the ionic current measurements, the pore size is still reduced after the carbon deposition. For nanopipettes with smaller pores, below 20 nm, the size reduction is generally small and no more than a few nm. The size reduction is sometimes beneficial for protein measurements. It should be mentioned that the nanopore barrel is still easy to fill after the carbon deposition, suggesting the carbon deposition at the inside surface of the nanopore barrel is very limited. Otherwise, the nanopipette tip inner surface becomes more hydrophobic, preventing the filling of aqueous solution. Based on the steady-state CV measurements of the fabricated CNE, there are relatively large variations of the effective surface area, which can be more than several times. However, in the low-resolution SEM images, we did not find such large variations in the CNE geometries. We believe a thin layer of carbon may deposit to the outer surface of the nanopipette tip, which contributes to the larger variation in the CV derived effective surface area. For each experiment, the students typically prepared 5–10 nanopipettes, which are enough to obtain several nanopipettes with similar geometries (based on ionic current and electrochemical current). We are fine with this yield. The potential signals are also less sensitive to the surface area variation of the CNE. However, the large variation still limits us in conducting high precision quantitative measurements.

1 N. Panday, G. Qian, X. Wang, S. Chang, P. Pandey and J. He, *ACS Nano*, 2016, **10**(12), 11237–11248.

Dominik Duleba said: How do you set up the centrifuging of the pipettes considering how extremely fragile they are? Secondly, according to your manuscript, Navier–Stokes was not included in the FEM simulations, do you think the electroosmotic flow has a negligible effect on the particle delivery?

Jin He responded: Please also see the reply to Ben Slater's opening question above. First, the quartz nanopipette is actually not that fragile, as shown in our recent papers.[1,2] Second, we still need to control the centrifuging speed and time to avoid tip damage. The nanopipette is placed in a typical 5 ml centrifuge tube. The backend of the nanopipette is secured by a PDMS slab, which cannot move in the centrifuge tube. To simplify the calculations, we did not consider the fluid velocity and ignore the electroosmotic flow. Indeed, the electroosmotic flow may play a non-negligible effect for particle delivery. It depends on the applied bias, size and surface charges of nanopore and nanoparticles. Also, large local ion and nanoparticle concentration change becomes more common at the nanopipette tip in bias regulated nanopipette delivery. The depletion of ion or the accumulation of charged entities may also induce stronger electroosmotic flows. In our experiments, at the higher applied nanopore bias, we often observed both upward and downward current spikes, suggesting the presence of electroosmotic flow. We will investigate these phenomena in follow-up work.

1 J. Guo, A. S. Rubfiaro, Y. Lai, J. Moscoso, F. Chen, Y. Liu, X. Wang and J. He, *Analyst*, 2020, **145**(14), 4852–4859.

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2 L. Yu, Z. Wang, H. Chen, J. Guo, M. Zhang, Y. Liu, J. He and S. Chang, *ACS Appl. Nano Mater.*, 2020, **3**(4), 3410–3416.

Jill Venton queried: What do you plan to use the carbon nanoelectrode for in the electrode? Will you detect the electrochemical payload you deliver or another change in something redox active in the cell?

Jin He replied: Thanks for the question! In general, we are interested in using the multifunctional nanopipette for single-cell analysis. We are still learning about applications to cellular biology and conducting literature surveys to better decide the direction. One initial direction is to use the carbon nanoelectrode to conduct intracellular electrophysiology, such as the recording of action potentials of excitable cells. Also, we are interested in using the carbon nanoelectrode to manipulate the delivered particles. Your suggestion is certainly another interesting direction.

Martin A. Edwards said: Thanks for the interesting talk and paper. In the work, you repeatedly mentioned 'single-entity delivery'; however, the data you present seem to show 'countable delivery' – that is, while you identify each single entity, you don't deliver just one and then stop. Have you considered triggering off the resistive pulses and then reversing the driving force? We have demonstrated this to be a feasible approach to control nanoparticles and nanorods exiting/entering a nanopipette using either mechanical pressure[1] or electrokinetic phenomena[2,3] and are even able to manipulate a nanoparticle so it is passed back and forth many times. If you are able to implement this within your system, it would offer the opportunity to deliver either one single entity or a defined number of entities.

I would also like to draw your attention to other similar work using nanopipettes to deliver single entities to interfaces.[4–6] The results of these papers broadly support the interesting results you presented in your work.

1 S. R. German, T. S. Hurd, H. S. White and T. L. Mega, *ACS Nano*, 2015, **9**(7), 7186–7194.
2 M. A. Edwards, S. R. German, J. E. Dick, A. J. Bard and H. S. White, *ACS Nano*, 2015, **9**(12), 12274–12282.
3 Y. Zhang, M. A. Edwards, S. R. German and H. S. White, *J. Phys. Chem. C*, 2016, **120**, 20781–20788.
4 X.-W. Zhang, A. Hatamie and A. G. Ewing, *J. Am. Chem. Soc.*, 2020, **142**(9), 4093–4097.
5 K. McKelvey, M. A. Edwards and H. S. White, *J. Phys. Chem. Lett.*, 2016, **7**, 3920–3924.
6 Y. Wang, H. Cai and M. V. Mirkin, *ChemElectroChem*, **2**(3), 343–347.

Jin He replied: Thanks for the great suggestion! Indeed, it is very interesting to conduct repeat measurements of the same nanoparticle inside the cell. In this way, we can better and more accurately probe the changes of the nanoparticle and also the surrounding intracellular environment. Also, thanks for the references. We will look at them carefully.

Hang Ren said: Is there any cross-talk between ionic current and open circuit potential (OCP)? Since the current is passed through the quasi-counter/reference Ag/AgCl electrode, its potential will change. Is this change what is being observed in the OCP measurement?

Also, what is the effect of the cell membrane junction for the ionic current and potential measurement? Since the ionic current is measured to be significant, does that mean the cell membrane is leaking? The current has to pass between

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the Ag/AgCl inside the pipette to the outside of the reference electrode. Does the current spike you saw come from the opening and closing of the ion channel? Can you put the reference electrode in the second barrel of the pipette so that there is no effect from the cell membrane potential?

Jin He answered: Thanks for the great questions. We have conducted a lot of control experiments. The change of Ag/AgCl electrode only induces the overall drift of the potential baseline. The fast transient potential changes are induced by the particle movements. These fast changes are also clearer in the first derivative of the potential time traces. The nanopore resistance we used for this work is typically in the giga or 10 s of giga Ohms range. Compared with the high resistance of the nanopore, the cell membrane is leaky. Therefore, the current of the equivalent circuit is dominated by the pore resistance. As shown in our current traces (Fig. 3b and 4c in our article, DOI: 10.1039/d1fd00057h), the current change is very small during the nanopipette tip cellular penetration process. For the smaller nanopipette in Fig. 4c in our article, the current change is less than 10 pA. In the control experiments of nanopipettes without loading nanoparticles, we did not observe the current spikes. The chance to observe current changes from individual membrane ion channels is expected to be low. We have measured the potential changes in the self-beating cardiac cell using these nanopipettes. The signals are also different. We will conduct the suggested experiment using a double-barrel nanopipette (without filling the second barrel with carbon).

Shuai Chang commented: You have mentioned that each ionic current spike corresponds to the delivery of a single NP, how did you quantify that? Are there any clustered NPs? And does the size of NPs matter in the quantification of the ionic current signals?

Jin He responded: The spike is generally determined by the shape, magnitude and time duration of the transient current spikes (or potential spikes). For larger entities such as GNPs (whose size is comparable to the nanopore size), the magnitude of the current spikes is usually quite uniform (see Fig. 5 in our article). The clustered NPs appeared as closely-spaced current spikes. So we know they are from individual NPs. For the same size nanopore, the size change of the NP will obviously affect the ionic current signals. The smaller NP moves faster and induces smaller current changes.

Jill Venton opened the discussion of the paper by Ashley Ross: Why do you think there are differences between adenine and guanine purines? Is it shape with the roughness – because adenine is bigger? Chemistry?

Ashley Ross responded: That's a great question. I do think it has to do with the shape in a sense. These differences could be due to the steric effect from the ribose group. The steric effect is thought to be different for guanine-based purines compared to adenine-based purines.

The literature suggests that where the intramolecular hydrogen-bonding between the NH_2 group of the guanine moiety and the OH group of the ribose at the 5′-position occurs, changes the shape of the nucleoside. Possibly these subtle differences in shape have an impact on how they approach and interact at the electrode surface.

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Luke Gundry asked: My question is on the COMSOL and simulation, is it fair to assume that the 2E electron transfer is fully reversible with such a fast scan rate and how does the simulation compare to experimental results?

Ashley Ross answered: The simulation results are a bit different than what we observe experimentally. Experimentally, at such fast scan rates, the 2E transfer is not fully reversible; however, COMSOL simulations don't capture this in the model.

Andrew Ewing asked: Did you look to see if the type of C–O functional groups change on the carbon surface with the Ar etching? (Can they change the adsorption?)

Ashley Ross answered: Yes, we did further evaluation of the XPS data to analyze specific oxide functionality and did not observe any differences between the bare carbon fiber and the argon plasma-treated carbon fiber.

Andrew Ewing commented: It wouldn't take much, 1/2% might be enough to affect adsorption – molecular models may help to figure this out.

Martin A. Edwards remarked: It is possible and relatively straightforward to use COMSOL Multiphysics to model the adsorption/desorption and surface electrochemical processes. Whether this would capture any remaining behaviour that is not captured by your current simulations, or is a worthwhile endeavor, is something I don't know. However, the mechanics of setting up the simulations is not too onerous.

Ashley Ross responded: Thank you Martin!

Popular Pandey asked: Why does Ar-plasma treatment of the electrode impact purine detection more than dopamine? Does the carbon fiber disc microelectrode size also affect detection sensitivity? Have you applied this system to intracellular neurochemical recordings?

Ashley Ross answered: We have not used these Ar-plasma treated electrodes yet for neurochemical recording *in vivo*. In terms of comparing purines *vs.* dopamine, we only really see significantly higher improvement for the adenine-based purines. The guanine-based purines increase similarly to dopamine after Ar-plasma treatment. We speculate that it's probably a complicated combination of size, analyte structure and chemistry. But it is really hard to know for sure without more in-depth analysis of these specific analyte–electrode interactions.

Swathi Naidu Vakamulla Raghu said: After the plasma treatment, the surfaces investigated had varied functional groups. So do you think the type of functional group as a result of (if any) substitution that was measured, results in a difference in signal intensity for A and G purine. And on the purines, is one functional group more favorable for detection?

Ashley Ross responded: Absolutely, we actually published a paper a few years ago investigating the extent to which small changes in functional group type and placement on the purine ring impacts their interaction at the carbon surface. We found that amine-functionalized purines, especially disubstituted purines, interact

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more favorably than carbonyl-functionalized purines and even the placement of the functional group on the purine ring has an impact. So yes, this does influence the interaction at the carbon surface which ultimately leads to changes in detection.

Paul W. Bohn commented: There are likely a very large number of types/geometries/compositions of local sites on the etched electrode surface. Do you have any sense of how the reactivity changes among these – in analogy to SERS do you think there are "hot spots" of unusually high activity that dominate the behaviour for the remainder of the electrode?

Ashley Ross answered: You are correct, that these surfaces are quite heterogeneous. It would be interesting to do SECCM at these electrodes to truly look at these "hot spots" of reactivity. I don't think we would see the same level of reactivity across the surface at all.

Paul W. Bohn addressed Jin He and Ashley Ross: Both Dr He and Dr Ross present complex nanostructures obtained by challenging fabrication protocols. How tightly can you control the structural properties of your materials/devices. What is the dispersion in properties from one run to the next – either in tip shape (Jin He) or surface topography (Ashley Ross).

Jin He replied: Please also see the response to Samuel Confederat above. We have tried our best to control the structural variation in the fabrication process. We have had some problems (charging, tip structure is not stable under higher energy electron beam) to obtain the high-resolution images of the nanopipette tip by transmission electron microscopy. Based on the steady-state cyclic voltammetry measurements, we have relatively large variations in the effective surface area of the carbon nanoelectrode. Obviously, the dispersion in the nanopipette tip prevents us from being more quantitative in our data analysis. So far, most of our studies are still qualitative. For these qualitative studies, we can get reproducible results from one run to another with high yield. However, we cannot use the nanopipette to carry out in-depth studies like other single-molecule biophysics methods, such as protein nanopore, single-molecule force measurements or single-molecule FRET. In the long-term, nanofabricated on-chip nanopore/nanoelectrode by mass-production should help to solve this issue.

Ashley Ross added: The Ar-plasma procedure can be controlled by controlling the flow rate of gas, power, and time of treatment and we have shown that manipulating these parameters produces differences in the average change in surface roughness. However, I think moving to even more controlled methods of controlling the nanostructure of the surface would be beneficial in the future for studying precise analyte–electrode interactions.

Jill Venton asked Jin He and Ashley Ross: Can the speakers comment on what applications these new techniques will enable and where they think their fields will be going for biological measurements.

Jin He responded: Due to the limitation in the geometry and size control of glass/quartz nanopipette tip, the accuracy of the nanopipette nanopore based

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measurements cannot match with other solid state nanopores, such as ion or electron beam drilled silicon nitrite nanopore. Not to mention the atomic level resolution achieved by protein nanopores. However, the nanopipette is extremely versatile, easy and cheap to make and use, and can be conveniently integrated with other techniques. We try to seek the applications of nanopipette based single-entity techniques in cellular biology, especially single-cell analysis. There are already many works and applications of glass micropipettes/microelectrodes in single-cell studies. The nanopipette can extend the earlier work. The nanoscale size and high flexibility of the nanopipette tip induces minimized cellular damage. Therefore, the nanopipette can conduct intracellular measurements for a long time. Also, we are interested in integrating nanoelectrochemistry methods with optical methods for biological measurements.

Ashley Ross replied: I foresee these Ar-plasma treated surfaces being used to: (1) more sensitively detect purines in tissue, but also (2) enable improved fundamental studies on how changes in the nanostructure of the carbon-fiber surface impacts the interaction and detection of various neurochemical analytes. I do foresee the field though moving to more precise methods of controlling the nanostructure of the surface. I think moving beyond carbon-fiber, and to more controllable surfaces will significantly advance not only fundamental electrochemical studies but also push the field to be able to detect traditionally more difficult electroactive biomolecules.

Li Xiao opened a general discussion of the paper by Patrick Unwin: I usually study macro scale catalysts and have not done single particle reaction research before. I want to know, if we gradually let two single particles with different composition come close together, can it help us better understand synergistic effects? For example, PtRu for the hydrogen oxidation reaction, and NiFe for the oxygen evolution reaction, *etc.*

Patrick Unwin responded: There are considerable prospects for understanding and rationalising electrocatalytic processes by studying a spectrum of entity populations, from single entities (particles), to pairs, small aggregates and upwards, including interactions between different entities and groups of particles. A technique such as scanning electrochemical cell microscopy (SECCM) should allow a large number of measurements within these populations, and the structure and arrangement of entities can be determined by complementary microscopy techniques, so that detailed structure–activity datasets are constructed. SECCM is also amenable to multiscale measurements, by changing the tip size. The examples you suggest would be very interesting to look at with this type of approach.

Conflicts of interest

Patrick Unwin is co-inventor of granted patent PCT/GB2011/051518 "Pipets containing Electrolyte and Electrodes", which describes dual-channel SECCM.