Faraday Discussions

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DISCUSSIONS

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Nanopores: general discussion

Andrew Mount, Minkyung Kang, David Fermin, Tim Albrecht, J. Gooding, Richard Crooks, Nongjian Tao, Wolfgang Schmickler, Julie MacPherson, Shengli Chen, Paolo Actis, Olaf Magnussen, Lane Baker, Philip Bartlett, Sanli Faez, Jan Clausmeyer, Bradley Thomas, Philip A. Ash, Frederic Kanoufi, Yitao Long, Patrick Unwin, Marc Koper, Serge Lemay, Andrew Ewing, Zhongqun Tian, Robert Johnson, Michael Eikerling and Mark Platt

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Serge Lemay opened a general discussion of the paper by Wolfgang Schmickler: Since a nanotube effectively forms a Faraday cage, it is not possible to generate a longitudinal DC electric field inside the nanotube. Any externally applied potential difference between the two reservoirs at the ends of the tube would thus cause the electric field to be localized near the two ends only. Can this behavior be accounted for in the present description?

Wolfgang Schmickler answered: Yes, in our calculations the surface of the tube has a constant potential throughout.

Changing the electrode potential changes the electrochemical potential of the ions inside the tube with respect to a bulk electrolyte. Transport inside the tube is by diffusion. Migration can play a role at the entrance to the tube.

Bradley Thomas asked: First, could you possibly shed more light on your comments concerning the lack of difference between conducting and semiconducting nanotubes? Within the literature, there are experimental examples that show a large difference between the two types. A prime example of this would be work recently published by colleagues in my own group.[1] Here, a new coulombic procedure is developed to experimentally determine the electronic structure of guest@nanotube materials, with fundamentally different behaviour seen between the two nanotube types. Your reasoning seems to be based upon previous calculations and the observed phenomenon that lithium atoms undergo direct electron transfer with the host semiconducting nanotubes on encapsulation. But surely the fact that this occurs shows how different conductive and semiconductive nanotubes are? Rather than suggesting they are the same, as the produced material is electronically comparable to that produced from encapsulating lithium cations into a conducting nanotube, they are fundamentally different. If lithium cations are encapsulated into a semiconducting nanotube, this electron transfer surely cannot occur as the ions no longer have the required

electron to donate. Therefore, for the nanotube to then become conducting you would require an external source of electrons (presumably from the electrical circuit) – something that would not be required in the conducting example.

Secondly, you discussed, both in the manuscript and in your discussion with the delegates, the solvation energy of ions in solution, yet the references you cite in the manuscript for the formation of one-dimensional salts (*e.g.* J. Sloan, ref. 18 of your manuscript) use molten salts primarily due to problems faced in achieving such uniform ionic arrays using solution filling. Have you taken these issues, such as the preferential filling of one ion, into account beyond simply determining the loss of solvation energy upon encapsulation when performing your calculations?

1 R. L. McSweeney, T. W. Chamberlain, M. Baldoni, M. A. Lebedeva, E. S. Davies, E. Besley and A. N. Khlobystov, *Chem. – Eur. J.*, 2016, **22**, 13540.

Wolfgang Schmickler responded: Of course, semiconducting and conducting CNTs behave differently.

However, upon inserting a cation or an anion into the tube, the corresponding image charge flows onto the tube. This shifts the Fermi level near the ion, and the tubes become conductive locally.[1]

There are various ways of filling the tubes. Loss of solvation energy occurs when the ions are inserted from an electrolyte solution. An alternative is molten salts, where the two kinds of ions often have very different sizes. This may entail that only one kind of ion can be inserted. I have discussed this in a previous publication.[2]

1 L. Mohammadzadeh, A. Goduljan, F. Juarez, P. Quaino, E. Santos and W. Schmickler, *ChemPhysChem*, 2016, **17**, 78.
2 W. Schmickler, *Electrochim. Acta*, 2015, **173**, 91.

Philip Bartlett commented: In applications, for example in a supercapacitor, the nanotubes can be in bundles or with solvent and ions in contact with the outside of the carbon nanotube as well as with ions inside the tube. Can you say something about how the environment on the outside of the tube would affect the behavior?

Wolfgang Schmickler answered: This is a good question. At the moment, we are investigating if an ion adsorbed outside of the tube interacts with an ion inside. Please ask me again in a few weeks.

Patrick Unwin addressed Wolfgang Schmickler: In studying electrochemistry at single-walled carbon nanotubes, the main interest is in the double layer external to the nanotube and what effect this might have on the electrochemistry. Especially in the context of Chen's paper, could you comment on whether you would expect such double layer effects to be significant? Rate constants and the voltammetric characteristics for several redox couples at approximately 1 nm diameter single-walled carbon nanotubes[1,2] appear to be broadly similar to those on larger radius (although still nanoscale) platinum disc electrodes.[3]

1 I. Heller, J. Kong, H. A. Heering, K. A. Williams, S. G. Lemay and C. Dekker, *Nano Lett.*, 2005, **5**(1), 137–142.

2 A. G. Güell, K. E. Meadows, P. V. Dudin, N. Ebejer, J. V. Macpherson and P. R. Unwin, *Nano Lett.*, 2014, **14**(1), 220–224.
3 P. Sun and M. V. Mirkin, *Anal. Chem.*, 2006, **78**(18), 6526–6534.

Wolfgang Schmickler answered: Our main interest is in nanoconfinement, *i.e.* in ions inside the tube. Outside the tube the electric field will be strong and affect the double layer properties.[1] However, the effect on electron transfer reactions should be minor.

1 E. Leiva, P. Velez, C. Sanchez and W. Schmickler, *Phys. Rev. B*, 2006, **74**, 035422.

David Fermin remarked: Have you performed calculations of protons or acid incorporation into nanotubes and does this follow the trends observed for NaCl and LiCl?

Wolfgang Schmickler answered: The insertion of a proton is on our list of things to do. We shall model it as a $H_7O_2^+$. However, the energy of ionization of the hydrogen atom is quite high, so we are not sure that DFT will give us a real proton. This is the reason why we started with alkali ions, whose ionization energies are much lower. A previous attempt to model a proton in front of a planar surface was not successful.[1]

1 P. Quaino, N. B. Luque, G. Soldano, R. R. Nazmutdinov, E. Santos, T. Roman, A. Lundin, A. Groß and W. Schmickler, *Electrochim. Acta*, 2013, **105**, 341.

Philip Bartlett said: Carbon nanotubes often have defects of different types. Can you say something about the effects of these defects on the ions inside the nanotube?

Wolfgang Schmickler responded: We have first results for nitrogen-doped nanotubes, but it is too early to comment on them.

Marc Koper asked: How should we think about the interaction of water with the inside of these pores? How would that affect your calculations?

Wolfgang Schmickler responded: We are investigating this question at the moment. We have data, but we still have to evaluate them. Obviously, the ions will lose a part of their solvation shell. It is a competition between solvation and image interaction.

Philip Bartlett opened a general discussion of the paper by Michael Eikerling: In your paper you refer to results for planar platinum (ref. 12 of the manuscript, Huang *et al.*) showing a non-monotonic charging behavior. Can you briefly summarize this work for us?

Michael Eikerling responded: The article referred to is centred on the charging relation of platinum, the catalyst of choice for PEM-based electrochemical technologies such as fuel cells and water electrolysers. For details of the physical-mathematical model as well as the solution, I would like to kindly ask you to look up the original article. The main features are:

Firstly, by revealing the non-monotonic charging behavior of Pt from first principles, the presented theoretical work solves a long-standing puzzle in electrochemistry. It takes the theory of electrified interfaces a decisive step beyond the concept of potential of zero charge (pzc), famously coined by A. N. Frumkin in 1928 and called "one of the most fundamental ideas in electrochemistry".[1]

Secondly, the presented model for the electrified interface at the Pt surface incorporates oxide and ordered water layers. The model can be adapted for other oxide-forming metal surfaces with a plethora of applications in electrocatalysis, semiconductor electrochemistry, and nanofluidics.

Thirdly, the presented work represents a framework for deeper forays into the field of first principles electrochemistry that must account self-consistently for the coupling of metal charging phenomena and vital descriptors of electrocatalyst activity and stability.

1 J. M. Bockris, M. A. Devanathan and K. Muller, *Proc. R. Soc. London, Ser. A*, 1963, **274**, 55–79.

Tim Albrecht asked: Could your model be adapted to include hydrodynamic and bipolar effects in small Pt pores that connect two liquid compartments? If so, can you speculate about the effect those processes might have on the charge distribution inside the pore channel, and potentially rectification effects in the current? Several papers might add some experimental context to the question,[1,2] and in regards to bipolar effects.[3]

1 M. Ayub, A. Ivanov, J. Hong, P. Kuhn, E. Instuli, J. B. Edel and T. Albrecht, *J. Phys.: Condens. Matter*, 2010, **22**(45), 454128.
2 A. Rutkowska, J. B. Edel and T. Albrecht, *ACS Nano*, 2012, **7**(1), 547–555.
3 A. Rutkowska, K. Freedman, J. Skalkowska, M. J. Kim, J. B. Edel and T. Albrecht, *Anal. Chem.*, **87**(4), 2337–2344.

Michael Eikerling replied: Solution of the model, as presented in the Faraday Discussions article, gives the equilibrium distributions of the solution phase potential and proton density in the water-filled nanochannel. These distributions are controlled by the free surface charge density of the metal, which is a non-monotonic function of the metal-phase potential. We use these equilibrium distributions to calculate the activity of the nanochannel for the oxygen reduction reaction (ORR). This approximative treatment is based on the assumption that the finite proton flux and the consumption of protons, required to maintain the ORR current, cause negligible perturbation of the equilibrium proton density and potential. We have verified the validity of this assumption in a previous article,[1] where we compared the analytical solution of the simplified set of equations (assuming equilibrium distributions) with the numerical solution of the fully coupled dynamic problem (based on Poisson–Nernst–Planck equation). The reason for the weakness of hydrodynamic coupling in our case is that rates of proton transport and proton consumption, required to maintain the ORR current, are very small. We only consider one mobile charged species in the channel (protons); thus the model system considered is not suitable to study current rectification effects. However, the non-linear and non-monotonic charging relation of the metal surface, shown in Fig. 6 of the manuscript, should give rise to interesting ionic current rectification effects in systems containing mobile cations and anions.

1 K. Chan and M. Eikerling, *J. Electrochem. Soc.*, 2011, **158**, B18–B28.

Wolfgang Schmickler said: Your charge–potential curves look quite interesting. Could you explain them in greater detail? Is there a potential of zero charge, or a change of local pH?

Michael Eikerling replied: Please consider the curves shown in Fig. 5b and Fig. 6 of our related work in *J. Phys. Chem. C*[1] as a reference for the charge–potential curves shown in our *Faraday Discussions* article. As seen in that work, the charging relation of an extended metal surface forming an interface with a concentrated electrolyte exhibits consecutive transitions from negative to positive and again to negative free surface charge upon increasing potential (in agreement with experimental findings of Frumkin and Petrii,[2] and Garcia-Araez *et al.*[3]). The non-monotonic behaviour and transition to a negative charging region at high potential represents a paradigm change in comparison to the monotonic behaviour found in classical double layer models.[4] The peculiar aspect of the model considered in the Faraday Discussions article is that no explicit electrolyte is added to the solution filling the pore. The only source of excess ions, protons in this case, is the PEM that forms an interface with the pore at one of its openings. This situation forces the metal charging relation of the water-filled nanopore to remain in the negative domain at all potentials. Fig. 7c shows the excess proton concentration as a function of potential. The pore is highly protophilic at small potentials. With increasing potential it becomes less protophilic; however, the proton concentration remains high compared to that of free bulk water. The spatial distribution of potential and proton density in the axial direction of the nanopore is negligible (except for a steep decline in a double layer region at the pore opening to the PEM which is very thin compared to the length of the pore). The water-filled nanopore does not possess a potential of zero charge, as evident from Fig. 7a. In the case of an extended Pt surface interfacing with an explicit electrolyte, a potential of zero charge exists (in the double layer region of the metal), but the charging phenomena are more complex than what could be described by a pzc and a constant double layer capacitance alone.

1 J. Huang, A. Malek, J. Zhang and M. Eikerling, *J. Phys. Chem. C*, 2016, **120**(25), 13587–13595.
2 A. N. Frumkin and O. A. Petrii, *Electrochim. Acta*, 1975, **20**, 347–359.
3 N. Garcia-Araez, V. Climent and J. Feliu, *J. Phys. Chem. C*, 2009, **113**, 9290–9304.
4 J. O'M. Bockris, M. A. V. Devanathan and K. Muller, *Proc. R. Soc. Lond. A*, 1963, **274**, 55–79.

Philip Bartlett commented: Using electrodeposition from lyotropic liquid crystal templates, it is possible to make platinum and other metal films with uniform diameter pores in the range 1.8 to around 3.5 nm diameter with control over the pore diameter.[1,2] Birkin *et al.*[3] and Jiang and Kucernak[4,5] have studied oxygen reduction on this type of mesoporous platinum electrode.

Can you extend your model to smaller pores and to include the presence of electrolyte in order to compare to these experiments?

1 G. S. Attard, P. N. Bartlett, N. R. B. Coleman, J. M. Elliott, J. R. Owen and J. H. Wang, *Science*, 1997, **278**, 838.
2 J. M. Elliott, P. R. Birkin, P. N. Bartlett and G. S. Attard, *Langmuir*, 1999, **15**, 7411.
3 P. R. Birkin, J. M. Elliott and Y. E. Watson, *Chem. Commun.*, 2000, 1693.
4 J. Jiang and A. Kucernak, *Electrochem. Solid State Lett.*, 2000, **3**, 559.
5 J. Jiang and A. Kucernak, *J. Solid State Electrochem.*, 2012, **16**, 2571.

Michael Eikerling replied: Our continuum modeling approach can be expected to give meaningful results down to pore radii in the range of 2 nm. For smaller radii, specific molecular effects at interfaces will become dominant which must be treated by molecular simulations.

Serge Lemay opened a general discussion of the paper by Sanli Faez: The longitudinal electric field is applied by capacitive coupling using external electrodes. A rough estimate of the capacitance between these electrodes and the solution yields approximately 10^{-13} F. Combined with a channel resistance of approximately 2×10^{12} Ohms for a 0.1 mM solution, this yields an RC time of approximately 0.2 s, which is long enough for the electric field not to be screened on the time scale of the experiment. If the solution had physiological salt concentrations (~0.1 M) on the other hand, the electric field would be screened by ions on the sub-ms time scale. Will this limit the technique to low salt concentrations only?

Sanli Faez replied: Indeed, without a major improvement in the measurement speed, increasing the salt concentration is problematic. However, measuring with microsecond time resolution is quite feasible by using a faster camera or a linear array photo detector.

Olaf Magnussen remarked: You assumed in the comparison of your simulations with the experimental data that the particle rapidly samples all axial positions with equal probability and that the measured velocity thus is the average value over the tube diameter. Is this a good assumption? There is evidence that Brownian motion is not uniform, but that particles have an increased residence time near walls. Because of the large differences in the liquid flow near the walls and in the center of the tube, this might have large effects. Is there a way one can experimentally measure the residence times at different axial positions?

Sanli Faez answered: The smallest time step we have considered is 10 ms which is enough for the particle to diffuse the entire cross section, so averaging can be justified. The hydrodynamically hindered diffusion only occurs at distances from the wall comparable with the particle size, so considering that the channel diameter is roughly ten times larger than the particle diameter, for most parts, the diffusion is similar to bulk. Furthermore, the particle and the channel wall are both negatively charged and the Debye layer is relatively long due to low ionic concentration.

Because of the special shape of the optical mode profile, the radial position of the particle determines the instantaneous scattering intensity. By rapidly collecting the intensity statistics, one can obtain the residence time at each radial position.

Philip Bartlett commented: In the video you showed the particles appear as two bright spots rather than one. Can you explain this for us?

Sanli Faez replied: This is due to the optical aberration caused mainly by the cylindrical interfaces of the fiber and imperfect index matching to the immersion oil.

Nongjian Tao asked: How does the detected light scale with the size of the particle? Is it with the square of the volume? What is the smallest size of particle

can you detect? Is the detection limit determined by some sort of background scattering? How does your approach compare with zeta potential measurement?

Sanli Faez replied: Yes, the scattering intensity is proportional to the volume squared. For such small particles, the Rayleigh scattering formula is a very good description. The smallest detectable size depends on the refractive index of the material as well. The smallest detectable scattering signal from mobile particles is limited by the shot-noise and technical noise of the background scattering from the inherent roughness of the channel surface. By implementing interferometric detection and active intensity stabilization, this limitation can also be overcome.

By tracking particles that are actuated in an external electric field, one can measure the electrophoretic mobility and from there determine the zeta potential considering a proper model for the charge double layer.

Philip A. Ash remarked: You mention that labelling with fluorescent tags will be necessary to extend the tracking technique to biomolecules. Would you be able to expand on this comment? Many proteins contain cofactors that already have relatively intense absorbances (and sometimes naturally fluoresce) in the UV-visible region; iron-sulphur centres, haem groups, flavin centres etc. What is the specific requirement of these absorbances that will allow direct application of your technique to biomolecule samples? If the requirement is simply a large scattering cross section is it possible, for example, that the intense Soret absorbance of cytochromes (many of which catalyse important reactions) or the fluorescent or light sensing properties of green fluorescent protein or blue light sensing BLUF domain proteins might be directly applicable to your technique with no labelling necessary?

Sanli Faez responded: The determining factor for tracking smaller entities with their elastic scattering signal is their polarizability at the excitation frequency. For optically active molecules the peak in absorbance is often close to the peak in the scattering cross section in the spectrum. The Lorentz oscillator is often a very good model for describing such a dipole resonance. For a large scattering signal, in the semi classical picture, decay from the excited state should be mostly radiative. For fluorescent molecules this translates to saying that the quantum efficiency should be high.

Zhongqun Tian asked: For your interesting approach, what is the smallest diameter you can reach in the channel of the optical fibre? Can you fix a nanoparticle at the end of the optical fibre robustly? What is the best way to increase the success rate? The reason I am asking is that my group is developing a SPR tip (a nanoparticle-based tip) method for local imaging and reaction.[1] Fig. 1 illustrates that the plasmonic nanoparticle, such as a gold nanoparticle, is fixed at the sharp end of fibre/pipette, and the gap between the nanoparticle and substrate surface can be precisely controlled by the piezo with the feedback signal of the scattering light from the nanogap (hotspot) formed by the nanoparticle and the surface. The SPR peak wavelength and light intensity depend critically on the gap width, *i.e.*, the strong coupling can result largely in red-shift resonance and strongly enhanced intensity.[2] This method could be utilized to investigate the heterogeneous surface and interfacial properties such as the electrochemical

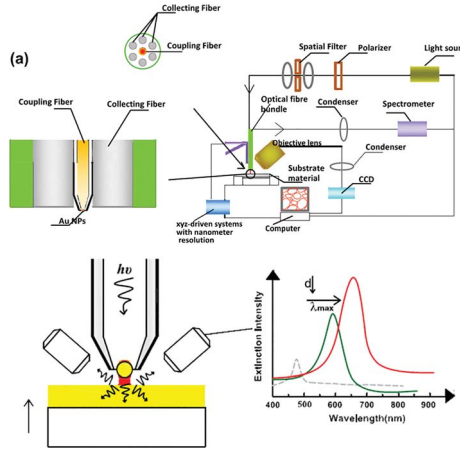

Fig. 1 The schematic illustration of working principle and setup of tip-enhanced dark field microscopy.

double-layer structure and SPR mediated reaction. It may also be applied to study the photoelectrochemistry of single dye sensitized nanoparticles as well as for nanoparticle impact electroanalysis because the particle can be mechanically controlled to hit and leave the electrode surface precisely with or without light illumination.

1 Z. Q. Tian, F. F. Wang, D. P. Zhan and J. Z. Zhou, 'Tip-enhanced dark field microscopy, electrochemical measurement and leveling device', Chinese Patent CN 102798735 B, March 2015.
2 F. F. Wang, J. Z. Zhou, S. Y. Ding, D. P. Zhan and Z.Q. Tian, in preparation.

Sanli Faez responded: The smallest channel diameter that can be controlled for an extended length of the fiber is roughly 100 nm. It is possible to locally shrink the inner diameter even further by post-processing.

Immobilizing a gold nanoparticle inside or at the end of the fiber is also possible.

Tim Albrecht remarked: As a comment, I would like to add that you might consider small heme-containing proteins as model systems for your work, for example cytochrome c, cytochrome c4 or cytochrome c3. These are small proteins in the low-nanometer size range, which feature a different number of heme (iron porphyrin) groups. Such heme groups would act 'intrinsic' staining agents, enhance the scattering cross sections of the protein, and may be relatively easy to study. In contrast to nanoparticle samples, proteins typically have a well-defined molecular weight, often the crystal structure is available (for the above small metalloproteins anyway), and are otherwise well-characterised (charge, surface properties *etc.*). They might also be modified using site-directed mutagenesis, if required.

Shengli Chen returned to the paper by Wolfgang Schmickler: In the charge difference diagram, can you see charge transfer between the tube walls and ions besides the induced image charge?

By the way, what does Pauli repulsion between the nanotube wall and ions mean here?

Wolfgang Schmickler replied: In the case of a single ion, a unit charge is transferred to the walls of the tube. The result is an ion inside the tube and an image charge on the walls of the tube. There is no other charge transfer. Pauli repulsion is a consequence of the Pauli principle, which states that no two electrons can be in the same state. Therefore electronic overlap is unfavourable, unless a chemical bond is formed. In the van der Waals potential, Pauli repulsion is represented by the repulsive branch.

Julie MacPherson said: You state in your paper that ion insertion results in the same charge screening effect be the tube metallic or semiconducting. However, is this a consequence of the way the model works *i.e.* you assume the ion enters as an atom and then transfers an electron to become charged which results in a change in the position of the Fermi level? What would happen if you modelled the process as an ion entering and not an atom?

Wolfgang Schmickler responded: In the calculation we place an atom inside the tube, and DFT converts this into an ion: the charge is transferred to the tube where it forms the image charge.

In an electrochemical experiment an ion enters the tube. Since the tube must stay neutral, charge flows through the external circuit onto the tube and forms the image charge.

The final state is the same in both cases.

In the slab configuration, which we use to model infinite tubes, DFT works for neutral systems only. If we place an ion inside the tube, DFT automatically compensates this by a uniform background charge of opposite sign. The result would be an artificial charge distribution.

Sanli Faez said: If we assume the ion on the axis is in the ground state, what would be the energy in the first excited state of the ion orbiting the tube axis? At what temperatures can one expect to observe quantum confinement effects?

Wolfgang Schmickler responded: I assume you are not talking about electronic excitations. The ion has three degrees of freedom, two of which correspond to vibrations perpendicular to the axis of the tube. These vibrations are quantized. The third corresponds to motion along the axis. If there is only a single ion, this motion has the nature of a translation, but it does experience the corrugation of the surrounding tube. If the tube is filled with ions, motion along the axis is also vibrational. This is in the classical regime, and the frequencies depend on the filling of the tube. Some of these effects have been discussed in a previous publication.[1]

1 W. Schmickler, *Electrochim. Acta*, 2015, **173**, 91.

Shengli Chen asked: In your calculation, the neutral metal atom inserted in a narrow carbon nanotube can be ionised, which should be because of the strong image interaction between metal ions and the tube wall. What do you think it

happens if you put the metal atom outside of the tube? Will it be ionised? If you don't have water outside the tube, I don't think the metal atom will be ionised.

Wolfgang Schmickler responded: You are right, for a particle outside the tube the image interaction is weaker, and ionization, within DFT, is more difficult.

Corresponding calculations are running...

See also our recent article.[1]

1 P. Quaino, N. B. Luque, G. Soldano, R. R. Nazmutdinov, E. Santos, T. Roman, A. Lundin, A. Groß and W. Schmickler, 2013 *Electrochim. Acta*, **105**, 341.

Zhongqun Tian said: Your approach is very interesting and I wonder if your studies can be further extended to metallic atoms/clusters/nanoparticles inside carbon nanotubes (CNTs) or underneath graphene. Deng *et. al.* encapsulated Fe clusters/nanoparticles within pod-like CNTs for the oxygen reduction reaction.[1] The direct contact of nonprecious metal particles with harsh environments including acid media, oxygen, and sulfur contamination is avoided. It does not impede the high activation of O_2 but has long-term stability even in the presence of SO_2 poison. However, it's not easy to use CNTs to encapsulate various metals with a high density of active sites. It's much more feasible to introduce metal clusters/nanoparticles between graphene layers and electrode substrates.[2,3] More importantly, all these systems may provide a defined model for understanding the synergetic (nanoconfinement) effect of metal–CNTs and metal–graphene for tuning them to have a better electronic structure of the localized active sites for electrocatalysis. May I have your comments on the relevant theoretical modeling and calculation?

1 D. H. Deng, L. Yu, X. Q. Chen, G. X. Wang, L. Jin, X. L. Pan, J. Deng, G. G. Sun and X. H. Bao, *Angew. Chem. Int. Ed.*, 2013, **52**, 371–375.
2 R. T. Mu, Q. Fu, L. Jin, L. Yu, G. G. Fang, D. Tan and X. H. Bao, *Angew. Chem. Int. Ed.*, 2012, **51**, 4856–4859.
3 D. H. Deng, K. S. Novoselov, Q. Fu, N. F. Zheng, Z. Q. Tian and X. H. Bao, *Nat. Nanotech.*, 2016, **11**, 218–230.

Wolfgang Schmickler replied: Many thanks for your interesting comments. One of my coauthors has started to perform calculations for iron encapsulated in carbon nanotubes. I think she was inspired by the articles you mention. It is too early to comment on her results.

Bradley Thomas commented: This comment is in relation to a discussion you had with another delegate on the need for simulations to determine whether electron transfer, as seen with lithium metal encapsulation, can still occur with the metal electron source outside the nanotube. Within the literature this has been somewhat answered already.[1,2] To give just a couple of many examples, one of the most favoured functionalisation methods for carbon nanotubes involves adding nanotubes to a solution of sodium (or lithium) metal dissolved in liquid ammonia. The sodium is oxidised in this process, with the nanotubes acting as an electron sink. In this electron-rich state the nanotubes are able to act as nucleophiles in reactions with molecules containing groups such as organic halides. The fact that this functionalisation works regardless of whether the nanotubes have been "opened" suggests encapsulation is not required.

1 D. Wunderlich, F. Hauke and A. Hirsch, *J. Mater. Chem.*, 2008, **18**, 1493–1497.
2 J. J. Stephenson, A. K. Sadana, A. L. Higginbotham and J. M. Tour, *Chem. Mater.*, 2006, **18**(19), 4658–4661.

Wolfgang Schmickler responded: The energies of ionization of Li and Na are so low that they are expected to be ionized when they get into contact with a CNT, no matter whether they enter the tube or stay outside. The previous discussion was about DFT, which does not always give the correct charge. When the Li or Na ion is inside the tube, the image energy is larger, and hence DFT gives the correct charge for small tubes. When they are outside, the image energy is lower, and DFT may have difficulties in obtaining the correct charge.

Philip A. Ash asked: I am interested in proton coupled electron transfer reactions in biomolecules, which often involve transfer of both H^+ and H_2O *via* a series of hydrophobic or hydrophilic interactions. The two 'nanopore' systems you have studied seem to represent models for both hydrophobic and hydrophilic channel environments within proteins. A recent paper by Voth and coworkers[1] suggests a new mechanism for proton transport through hydrophobic channels *via* the creation of temporary water wires by hydrated excess protons. Hydrated protons were found to induce wetting within a hydrophobic nanotube, vastly reducing the free energy barrier to proton tunneling (*via* a Grotthuss mechanism) through the nanotube. Conversely, other monatomic cations were found to have the opposite effect and wetting of the nanotube was disfavoured. Would you be able to comment on proton mobility through such confined hydrophobic or hydrophilic channels and how it might be affected by the presence of other ions?

1 Y. Peng, J. M. J. Swanson, S.-g. Kang, R. Zhou and G. A. Voth, *J. Phys. Chem. B*, 2015, **119**, 9212.

Wolfgang Schmickler answered: I think that the wetting is induced by the strong interaction of the proton with its image charge. This induces the creation of favorable proton transfer channels along the wall. This favorable arrangement can be disturbed by monatomic cations, which form strong solvation shells, and thus inhibit the formation of favorable water channels.

Andrew Mount addressed Wolfgang Schmickler and Michael Eikerling: The calculations of the stabilities of the systems of inserted ions (and the progress to calculating different systems with and without added water in the pores) in nanotubes are extremely interesting. These give insight into the most thermodynamically stable system *e.g.* for each cation after ion insertion but I suspect that for the applications envisaged (*e.g.* supercapacitor charge storage with associated ion insertion), the practical charging rate determines which structure is in fact formed, as this is likely to be kinetically controlled (likely determined by the size of the activation barrier *e.g.* of paying the energy penalty of at least partial ion desolvation prior to ion insertion at the nanotube end). Given the high energy price to pay for full ion desolvation, is this not likely to mean that partially desolvated ions are likely to be inserted initially, followed by a subsequent relaxation (solvent equilibration and potential ejection from the pore) as this metastable state relaxes towards the thermodynamically most stable state? How can your ion and/or solvent calculations be extended to give insight into such relaxation processes?

Wolfgang Schmickler replied: The ion loses at least a part of its solvation sphere, which costs energy. It gains the image energy, and it experiences the potential drop between the solution and the nanotube, so a suitable potential can drive the ions into the tube. I agree that probably at first the ions will enter in a partially hydrated form – this was discussed in one of our papers.[1]

We are performing calculations for solvated ions in nanotubes, but the results are not yet ready for publication.

I agree that the entry of an ion into the tube may require an activation energy; at the same time as the ion starts to lose a part of its solvation shell it begins to feel the image force, and the activation energy will be determined by the balance as the ions enter. We have an idea on how to model this, but, again, it is too early to talk about it. Our results for ions can be applied to ionic liquids, where solvation is not an issue.[2]

1 A. Goduljan, F. Juarez, L. Mohammadzadeh, P. Quaino, E. Santos and W. Schmickler, *Electrochem. Comm.*, 2014, **45**, 48.
2 W. Schmickler, *Electrochim. Acta*, 2015, **173**, 91.

Frederic Kanoufi communicated: In Fig. 7 of your manuscript you are suggesting the intercalation of ions in a nanotube will be accompanied by an optical phonon. Could you comment a bit more on that? Do you mean a way to evidence the extent of such intercalation could be probed spectroscopically, typically the IR based on the frequency you are reporting?

Wolfgang Schmickler communicated in reply: In principle these optical phonon branches could be detected by IR spectroscopy. I do not know if this is feasable experimentally. If it is, it will give information about the filling of the tube.

Frederic Kanoufi communicated a question regarding the paper by Sanli Faez: This is an interesting platform to monitor a single nanoparticle in an electric field. To complement on the comment of Professor Tian regarding the potential of your system for electrochemistry at a single nanoparticle, I was wondering to what extent one could use it for performing bipolar electrochemistry at a single nanoparticle level. This would actually require increasing the applied electric field by 1 to 2 orders of magnitude, is it experimentally feasible?

Richard Crooks communicated in reply: I think it is possible to perform bipolar electrochemistry using nanopores, but as you say some redesign of the system would be required. In fact, I believe there are people working on this experiment.

Sanli Faez communicated in reply: Thank you for the interesting suggestion. One can apply as an high electric field as silica glass can withstand without breakdown.

Yitao Long communicated a question for Wolfgang Schmickler: Firstly, how do you ensure that a one-dimensional salt is embedded in a tube, rather than separated anions and cations in the tube?

Secondly, does the insertion of ion result from free diffusion or electric field stress?

Wolfgang Schmickler communicated in reply: A one-dimensional salt is the energetically stable form. Separated anions and cations would be highly unfavorable because of the Coulomb repulsion.

The insertion results from a gradient of the electrochemical potential.

Sanli Faez opened a general discussion of the paper by Tim Albrecht: Is the circuit design for your special amplifier available as open-source hardware? If so, under which license?

Tim Albrecht answered: We are more than happy to collaborate in this regard and make the designs available as much as possible.

Paolo Actis asked: How is this amplifier different than Chimera Instruments VC100?

Tim Albrecht replied: The noise performances of our device(s) and the Chimera amplifier are quite comparable – we believe our setup does a little better at high frequencies. It also allows for higher input currents (up to 100 nA, rather than 50 nA) and the AC/DC channel splitting means that translocation events in the AC channel can be analysed without complex baseline correction. A proper, point-by-point comparison would be necessary to address this question in full though. Some characterisation of our devices is shown in our contribution to this Faraday Discussion and in our previous paper in Nanoscale[1](http://pubs.rsc.org/en/content/articlehtml/2016/nr/c5nr08634e), cf. Supporting Information. There are also references to the electronics design, which was published by the Milan group separately.

1 R. L. Fraccari, P. Ciccarella, A. Bahrami, M. Carminati, G. Ferrari and T. Albrecht

J. Gooding inquired: With non-biological nanopores, can we use surface modification to slow down the translocation through the pores to alleviate issues related to the translocation speed? Controlling surface chemistry so it proceeds inside the pore only has been achieved in a few ways.[1,2] Can you illuminate us on the merits versus the fast amplifier? I agree that there are issues with regards to silanization and stability.

1 K. A. Kilian, T. Böcking, K. Gaus and J. J. Gooding, *Angew. Chem. Int. Ed.* 2008, **47** 2697–2699.

2 B. Guan, S. Ciampi, G. Le Saux, K. Gaus, P. J. Reece and J. J. Gooding, *Langmuir*, 2011, **27** 328–334.

Tim Albrecht responded: Tuning the surface properties of a solid-state nanopore sensor can be beneficial to the sensing performance. Careful control of the interaction strength is of paramount importance, however, since otherwise pore clogging or crowding of the analyte can occur. An interesting special case seems to be the translocation of protein/DNA complexes, where the protein appears to adsorb to the sensor surface much more strongly than the DNA itself (see examples from our own work involving p53/dsDNA, low-noise nanopore chips[1]

and SSB/ssDNA, nanopore chips[2]). Hence, controlling both at the same time might add additional challenges. We have also recently shown theoretically that surface friction may affect the scaling law between DNA length and translocation time.[3] The work of Michael Mayer *et al.* around lipid-bilayer-coated solid-state nanopore sensors offers another elegant approach for controlling the translocation process (; protein translocation).

Generally, a well-controlled translocation process combined with high-performance detection electronics will enable new capabilities, depending on the specific application (better resolution, smaller analytes etc.).

1 P. Nuttall, K. Lee, P. Ciccarella, M. Carminati, G. Ferrari, K-B. Kim and T. Albrecht, *J. Phys. Chem. B*, 2016, **120**(9), 2106–2114.
2 D. Japrung, A. Bahrami, A. Nadzeyka, L. Peto, S. Bauerdick, J. B. Edel and T. Albrecht, *J. Phys. Chem. B*, 2014, **118**(40), 11605–11612.
3 R. L. Fraccari, P. Ciccarella, A. Bahrami, M. Carminati, G. Ferrari and T. Albrecht, *Nanoscale*, 2016, **8**, 7604–7611.
4 E. Yusko, J. M. Johnson, S. Majd, P. Prangkio, R. C. Rollings, J. L. Li, J. Yang and M. Mayer, *Nat. Nanotech.*, 2011, **6**, 253–260.

Yitao Long asked: You did show the detection of the short DNA in nanopipettes using the high-bandwidth CMOS current amplifier, what about applying this system in a biological nanopore? Can the bandwidth go higher, and what is the highest bandwidth it can get to?

Tim Albrecht answered: The best achievable performance depends on the input capacitance during the measurement and the magnitude of the current modulation, relative to the background current noise (in the AC channel in our case). These are specific to each experiment, which would have to be optimized accordingly. At present I do not see any reason why the setup cannot be used in conjunction with a biological pore, provided the DC current does not exceed 100 nA and the input capacitance is sufficiently low.

I should add that the internal bandwidth of the amplifier is 3.5 MHz, and our analog filter is currently limited to 2 MHz. However, in measurements we have used up to 400 kHz so far, at S/N ratios similar to the one reported in the paper (long dsDNA translocation in nanopipettes) – these results are unpublished.

Patrick Unwin commented: I'd like to comment on the reliability of eqn 1 in your paper to estimate the aperture size of nanopipettes. While this is widely used, we have tested this equation recently and carried out a through geometric and mass transport analysis of nanopipettes.[1] There are significant changes in the local taper angle with distance into pulled nanopipettes, and the internal and external angles can differ significantly. Such effects need to be taken into account to understand in detail the ion current response of these devices and this information can be obtained very nicely with TEM analysis.

1 D. Perry, D. Momotenko, R. A. Lazenby, M. Kang and P. R. Unwin, *Anal. Chem.*, 2016, **88**(10), 5523–5530.

Tim Albrecht responded: I think your study in *Analytical Chemistry* is very important in this context. It will be interesting to investigate the effect of these parameters on the translocation characteristics of DNA or proteins, for example.

We usually take the conductance as a parameter to assess the similarity of different pipettes (pulled using the same programme), but being a composite quantity this does not rule differences in the actual pore diameter, shape of the channel and so forth. Unfortunately, we are not in a position to perform TEM imaging on every pipette we use (ideally before and after!) Having said this, using the conductance criterion typically provides good reproducibility of the translocation characteristics in our studies. The errors given in our Discussions paper as well as in our previous publication in Nanoscale are normally the standard errors (of the most probable translocation time, for example), based on three independent measurements (hence three different pipettes). These are relatively small, perhaps a reflection of the fact that the very end of the pipette typically has the largest effect on the overall conductance (*i.e.* the sensing region).

Philip Bartlett said: In your paper you say that the translocation of the DNA through the pore can be slowed down by using LiCl electrolyte rather that KCl. Why is that? Has anyone looked at using double-stranded DNA–PNA rather than DNA–DNA duplexes, as this would also be a way to change the charge on the duplex?

Tim Albrecht replied: The reason for the slowing down of DNA translocation in LiCl (and some other electrolytes), compared to KCl, has been associated mainly with differences in cation interactions with the negatively charged DNA backbone (ion pairing). Ref. 45 and 46 in our manuscript constitute rather detailed studies in this regard. Our ref. 48 highlights differences in the transport mechanism, namely flux- *vs.* barrier-limited translocation.

Meller *et al.* used PNA hybridized to dsDNA in tagging/bar coding experiment[1], which is closest to what is suggested in the question, as far as I am aware.

1 A. Singer, M. Wanunu, W. Morrison, H. Kuhn, M. Frank-Kamenetskii and A. Meller, *Nano Lett.*, 2010, **10**(2), 738–742.

Minkyung Kang remarked: As others have said, we also often experienced the change of the glass pipette (diameter < 50 nm) by measuring the *I–V* curve within a day. This change could be significant to the experiment with nanopore devices as the geometry of the tip affects the current responses, as followed by our previous work.[1]

1 D. Perry, D. Momotenko, R. A. Lazenby, M. Kang and P. R. Unwin, *Anal. Chem.* 2016, **88**(10), 5523–5530.

Tim Albrecht replied: Yes, indeed, we also observe equilibration effects in some nanopipettes. For example, in some cases we detect rectification, which tends to disappear after a few bias cycles. For translocation experiments we normally use nanopipettes that show linear and stable *I–V* characteristics (over hours), as shown in the manuscript (ESI).

Richard Crooks opened a general discussion of the paper by Robert Johnson: Proteins like α-hemolysin are structurally dynamic, they misfold, and they are often mobile when in bilayers. How do these types of effects affect the noise and reproducibility of the types of measurements you make? What is the effect of the charges inside the barrel of the protein on translocation?

Robert Johnson responded: The α-hemolysin pore is structurally dynamic. In a typical experiment, we add monomer units that are commercially available and wait for these to diffuse into the lipid bilayer and form a protein channel. The α-hemolysin pore should comprise seven monomer units when correctly formed. However, we do often see mis-folding and incorrect formation during our experiments. Identification of the correctly formed protein channel is achieved through measuring the conductance, which is *ca.* 1 nS at 25 °C in 1 M KCl. With our system, we have the added advantage of pressure control over the protein insertion rate and if a mis-folded protein forms in the lipid bilayer then lowering the pressure can be used to remove it without destroying the bilayer. Measurements with the correctly formed protein channel are highly reproducible.

Charges inside the protein pore are important in governing the interactions between the internal protein walls and DNA held confined within the channel. For example, the latch constriction of α-hemolysin comprises a ring of positively charged lysine groups. Interactions between these groups and the DNA are likely involved in modulating the base-flipping effect that we observe for a DNA mismatch site situated at the latch constriction.

Tim Albrecht commented: The discussion of the advantages and disadvantage of biological *vs.* solid-state pores is a complex one and cannot be exhaustive here. Biological pores have probably no match in the solid-state world, when it comes to well-defined surface properties and the reproducibility of the pore dimensions. They are also very sensitive, in that even small chemical changes inside the pore channel can be detected and studied in detail. They can also be modified by site-directed mutagenesis. A disadvantage can be that the pore size cannot be changed dramatically. So while α-hemolysin and MspA pores are able to pass single-stranded DNA, translocation of larger analytes, such as double-stranded DNA, many proteins or protein/DNA complexes, is not possible without structural alterations (such as separation of the strands in dsDNA). Biological pores in their membrane environment can also stable enough for operation in the field, as demonstrated by Oxford Nanopore Technologies recently in the context of the Ebola epidemic in Africa.[1]

1 Quick *et al.*, *Nature*, 2016, **530**, 228–232.

Philip Bartlett said: I think that it is very impressive that you can carry out kinetic measurements on single DNA molecules and that you can carry out elegant temperature dependence studies to extract activation energies. From your results the base flipping is driven by interaction between the flipped out base and the wall of the latch region. How does this compare with what is happening in the solution? I noted from your paper that there is some disagreement in the literature between the results from NMR and from fluorescence.

Robert Johnson answered: While the activation energies and timescales for a base flipping out of the DNA helix are similar to those published for DNA in bulk solution (between 10 and 20 kcal mol^{-1}), the energies and time scales for flipping a base back into the helix are not. This strongly suggests that the extra-helical state is in someway stabilized relative to what would be observed in bulk solution. At the latch constriction of α-hemolysin, there is a ring of positively charged lysine

groups and it is highly likely that these are able to interact with an extra-helical base through electrostatic and hydrogen-bonding interactions. Some recent ionic-strength dependent studies that we have done in our lab support this, and do show that ionic strength does effect the base-flipping kinetics within the pore. We plan to publish this work shortly.

In a biological context, base-flipping is understood to occur both passively and actively. In the active case, enzymes may stabilize the extra-helical conformation through binding and this type of interaction may be similar to what we observe when the base-flipping occurs at the latch constriction of alpha-hemolysin.

Andrew Ewing asked: When you look at summary data and get the variance from the data, if you do that in one channel *vs* several channels, is the variation the same? I think all proteins are not the same; crystal structures don't tell the whole story and proteins fold differently depending on how they are made. So a little variability can occur, so purification process must pick the same variation. How do the commercial sources make their protein that you use?

Robert Johnson said in reply: The α-hemolysin protein can be purchased commercially as monomer units that are secreted from *Staphylococcus aureus* and subsequently isolated. We place these monomer units into a buffer solution that is in contact with the lipid bilayer. Subsequent diffusion into the bilayer, followed by the combination of seven monomer units, form the heptameric protein used for ion channel recordings. At 25 °C, this pore has a conductivity of 1 ns $\pm$ 0.04 ns in 1 M KCl.[1] Occasionally, the monomers units do not form the correct protein channel and when the conductance of the open channel is out of this range we reject the channel.

1 J. J. Kasianowicz, E. Brandin, D. Branton and D. W. Deamer, *Proc. Natl. Acad. Sci.*, 1996, **93**, 13770.

Lane Baker asked: Can you comment on use of this approach to measure single base changes in unknown samples?

Robert Johnson replied: This is a really interesting point. The approach that we are presenting relies of knowing what single-base change you are looking for. In an application, one would design a probe DNA strand to bind to a target DNA sequence such that the base site of interest is aligned with the alpha-hemolysin latch constriction. Then, DNA molecules can be analyzed within the pore for the presence or absence of this base change one molecule at a time. Actually, the real power in our approach is in counting how many DNA molecules within a population have a known base change. This is important for determining heterogeneity within a gene sample. Nanopores have a real advantage here, because of their inherent ability to measure single entities of DNA one at a time rather than relying on averaging across hundreds or thousands of molecules.

Philip Bartlett opened a general discussion of the paper by Mark Platt: You mention in the Conclusions to your paper that an advantage of the restive pulse assay is that it can be multiplexed. How can this be achieved and what is a realistic value for the number of different analytes you could detect simultaneously in a multiplexed measurement?

Mark Platt answered: Multiplexed assays have been done with two analytes by the group previously.[1] You need particles of narrow size distributions.

1 E. R. Billinge and M. Platt, *Biosens. and Bioelectron.*, 2015, **68**, 741.

J. Gooding asked: You present to us two assay formats where the particles are the sensing element or where modification of the pore is used. Why would you do the latter? What's the advantage of doing this when with the particle assay you can sample a lot more solution space?

Mark Platt responded: There were two motivations for developing the second – *i.e.* the aptamer modified pore wall experiment. The first was to continue to explore the nature of the rectification behavior of these larger pores. Having seen some increase in current rectification behavior we were curious to how and if DNA and aptamer walls changed the current flow, in the presence of the analyte. This had a natural lead into some other research in the group looking at blood assays where we might want to simply drop some sample into the upper fluid cell and confirm the presence of an analyte by monitoring the current flow and not have to use particles. The second was in our work looking at environmental samples for example heavy metal ions in water. Again the sample could be dropped into the upper fluid cell for analysis, but long term we are exploring adding this into fluid chips with automated sample handing and the pores might serve as a means to provide a rapid read out.

Philip Bartlett questioned: One of the questions that has already come up in the Discussion is about the effects and extent of overlap of the double layers of adjacent surfaces. In this case in your work you have a very large pore (> 700 nm) and yet you see current rectification when you might not expect to. Have you some idea why this should be?

Mark Platt replied: I think the early literature suggested the pore opening had to be of similar size to the double layer, but there is a growing number of papers where larger pores are still producing rectification behavior, and even biphasic pulses in resistive pulse measurements are seen on the same system.[1] This all suggests that the double layer does not need to be comparable to the pore size. H. White *et. al* have suggested the electroosmosis plays a larger role then initially suspected and I think in our pores we will see a much larger contribution of this,

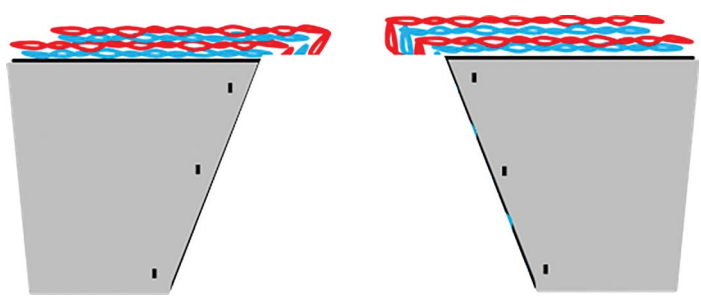

Fig. 2 Schematic of the layer-by-layer assembly on the top pore surface.

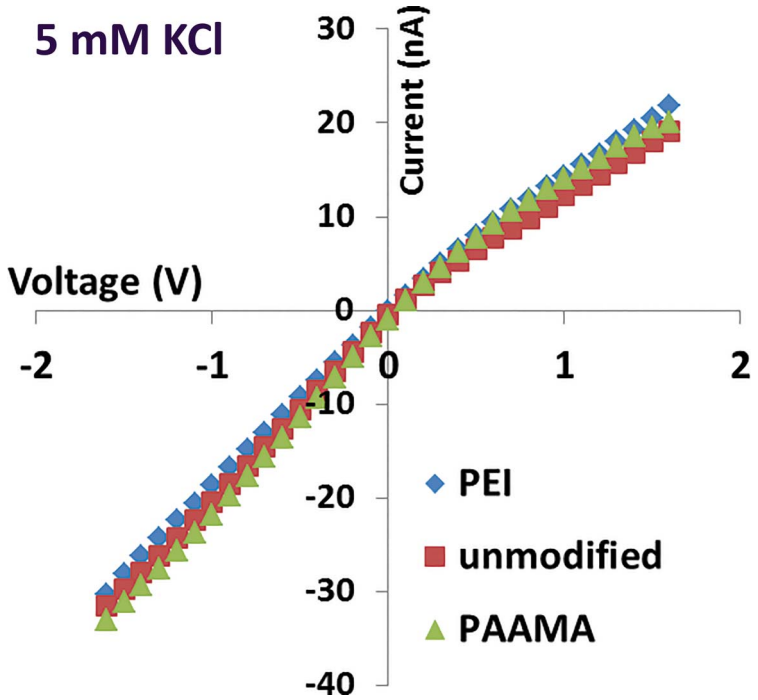

Fig. 3

but modelling is required for me to say that with certainty. We did hypothesize in the paper that the pore top surface might contribute to the behavior, but studies done by us since the submission of the paper (Fig. 2 and 3 and Table 1 below) shows this is not the case.

1 E. Weatherall and G. R. Willmott, *J. Phys. Chem. B*, 2015, **119**(16), 5328–5335.

Patrick Unwin commented: The membrane you use to make the pore is rather thick. I noticed that with unmodified pores, the current-voltage response was close to ohmic and that significant rectification was seen with modified pores. Is that correct? Could the rectification in modified pores be magnified by sub-surface constrictions from polymer strands or plugs? Further, do these polymeric pores change their dimensions over time?

Table 1 Table showing the current rectification ratios (measured at ± 1.6V) for each modification of the pore surface

[KCl] / mM	Unmod	PEI	PAAMA
5	1.64	1.38	1.65
10	1.12	1.00	1.10
50	1.05	0.93	1.04
137	0.97	0.94	1.06

Mark Platt replied: The standard unmodified pores gave a small rectification behavior. If a smaller pore size is used this is increased; however we opted to use a larger pore opening for the study as it's the same size we use for the resistive pulse experiments. The increase in rectification behavior with the polymers could be due to the polymer forming islands or protrusions in the pore, but with two bilayers Ithink this would still be only a few nm in size. However given that we are never sure of the exact features in each pore, more work has to be done to characterize the cause of behavior in the *I–V* curves. One of the areas of research we are just starting is the production of bespoke pores, with more controlled features, and this might help us understand the mechanism.

The pores do change dimensions over time by becoming more flexible, and as we continue to stretch the polymer over the course of weeks we often find that the baseline current increases as the pore openings become larger. We only used the pores in this study a few times, in some cases only once (each DNA experiment was performed on a new pore).

Tim Albrecht asked: In order to explain the occurrence and magnitude of current rectification effects in pore channels (including nanopipettes), it might be necessary, under some conditions, to consider hydrodynamic (electroosmotic) effects as well (see, *e.g.*Keyser *et al.*)[1]

1 N. Laohakunakorn, V. V. Thacker, M. Muthukumar and U. F. Keyser, *Nano Lett.* 2015, **15**, 695–702.

Mark Platt answered: Thanks for the comment, I agree the contribution of the EOF may well be unknown here. White and co-workers also looked at this and whilst we alluded to their work to indicate that EOF may be important, we have no idea yet as to the extent of its contribution. This looks like an excellent reference to add to the search and our future work hopes to look at pores of differing geometries via modelling and through experimental work.

Patrick Unwin addressed Tim Albrecht: Apologies for stating the obvious, but while the Debye length might be a reasonable rule of thumb for the size of the double layer, compared to, say, the diffusion layer at most of the electrodes used in electrochemistry, when dealing with nanoscale devices, such as nanopipettes, it is important to consider the entire potential–distance profile. Several people at this Discussion, including us, have looked at this and current–voltage curves are predicted that are closely in line with what is seen experimentally.[1–5]

1 H. S. White and A. Bund, *Langmuir*, 2008, **24**, 2212–2218.
2 W.-J. Lan, C. Kubeil, J.-W. Xiong, A. Bund and H. S. White, *J. Phys. Chem. C*, 2014, **118**, 2726–2734.
3 N. Sa, W.-J. Lan, W. Shi and L. A. Baker, *ACS Nano*, 2013, **7**, 11272–11282.
4 K. McKelvey, S. L. Kinnear, D. Perry, D. Momotenko and P. R. Unwin, *J. Am. Chem. Soc.*, 2014, **136**, 13735–13744.
5 D. Perry, D. Momotenko, R. A. Lazenby, M. Kang and P. R. Unwin, *Anal. Chem.*, 2016, **88**(10), 5523–5530.

Tim Albrecht replied: I fully agree – considering the charge distribution will implicitly include the actual geometry of the channel, which is obviously important, too. It is also important, however, to differentiate between preferential

ion transport *(i.e.* differences in the transport numbers) and current rectification (bias asymmetry of the current) – the former does not automatically lead to the latter. The point I was trying to make was that electroosmotic effects can lead to such a current asymmetry, which is perhaps not as widely appreciated as electrostatic effects.

Serge Lemay commented: While current rectification was originally often described in terms of double-layer overlap, more general modeling has shown that it is sufficient to have spatially inhomogeneous transference numbers for cations and anions along the length of the channel. This can be the result of double-layer overlap but also of, for example, surface conduction.

J. Gooding asked Mark Platt: You talked about the unexpected rectification for a large nanopore and I was wondering whether the open and porous nature of the layer by layer films, which swell in solution, may contribute to this effect considering there may be strands of polymer floating around in solution and possibly close to the pore entrance? I appreciate it is hard to modify a pore but perhaps something like plasma reacting where the chemistry is maintained very close to the surface might help answer such a question.

Mark Platt replied: An excellent point and to understand the true mechanism of the rectification behavior we will have to use a more specific chemistry to ensure we do not have any polymer extending out from the surface. In the supplementary material we do use a lower molecular weight PEI solution (although the same MW PAAMA) and observe similar behavior, although the rectification is smaller. This was initially attributed to the PEI forming islands before completely coating the pore wall, and we can not exclude the fact that the polymer may extend out and swell. I would comment that we have not optimized the layer-by-layer assembly, in fact other groups often place the polyelectrolytes down in specific pH, high ionic strength solutions to ensure they polymers go down in a dense coating and we might observe much stronger and varied behavior if we varied this. For resistive pulse measurements a swollen polymer would prohibit particle translocations, but such a mechanism might be very powerful for other types of assays. Only by knowing the actual mechanism can we exploit this and more work is needed.

Paolo Actis returned to discussion of the paper by Tim Albrecht: In the last slide of your presentation you proposed a detection mechanism very similar to the one published by Bell and Keyser.[1] How is your approach different?

1 N. A. W. Bell and U. F. Keyser, *Nat. Nanotech.*, 2016, **11**, 645.

Tim Albrecht replied: The idea is very similar, but perhaps more akin to Plesa *et al.*,[1] where dsDNA overhangs on a long dsDNA track are being detected. The purpose of the latter study was to investigate velocity fluctuations during translocation, whereas our current idea is to use ssDNA overhangs to 'fish' for particular target sequences in a sample. This is similar to a DNA assay, hence the term 'DNA assay on a string'. The idea of detecting features incorporated in or bound to DNA is obvious not new, see for example Singer *et al.*,[2] , for PNA/DNA complexes.

1 C. Plesa, N. van Loo, P. Ketterer, H. Dietz and C. Dekker, *Nano Lett.*, 2015, **15**(1), 732–737.
2 A. Singer, M. Wanunu, W. Morrison, H. Kuhn, M. Fank-Kamenetskii and A. Meller, *Nano Lett.*, 2010, **10**(2), 738–742.

Richard Crooks addressed Tim Albrecht, Robert Johnson and Mark Platt: I have two questions. First, how come the nanopores rarely seem to clog up? There must be a very high concentration of "junk" in the solution. Second, an expert in nanopore DNA sequencing told me there was no way that it would ever be possible to sequence DNA due to the noise level. Yet, it seems to be that this is what people are doing. Can you reconcile these two points of view?

Tim Albrecht responded: First, they do! The more contaminated the electrolyte solutions, the more likely they clog. Sometimes this clogging is reversible, *i.e.* it can be removed by a large reverse voltage pulse ('zapping'), sometimes it is not (and the device may have to be re-cleaned or discarded).

Regarding the second question, I think it depends on how one defines the question or rather the term 'nanopore DNA sequencing'. To this date, no-one has demonstrated DNA sequencing with a plain, unmodified solid-state device. The DNA bases pass too quickly, and the differences between the bases are indeed drowned in the noise. On the other hand, it has been shown that modified biological pores can successfully be used. It is necessary to control (slow down) the translocation process (*e.g. via* a processive motor enzyme, such as a helicase) and to enhance the specificity of the 'read head' inside the pore (for example, by adding a cyclodextrin using site-directed mutagenesis). Combining these with the appropriate sample processing, electronics development and other factors will result in a functional sequencing device, as demonstrated for ebola by Oxford Nanopore Technologies[1] or for that demonstrated for sequencing in space.[2] The latter is based on alpha-hemolysin. The Gundlach group at the University of Washington have been developing an alternative technology around the MspA pore (apparently with involvement from Illumina). The Kasianowicz group at NIST, in collaboration with a group at Cornell, have developed a very interesting, nanopore-based 'sequencing by synthesis' method, which may not be quite as far developed towards a commercial product, but does provide very good differentiation between bases (since it is actually a PEG tag that's being detected, not the base itself).

However, this does not mean that this technology is necessarily the best for every application. At present, it seems that nanopore-based technologies possess higher error rates than other technologies (partly because they read more than one base at a time consecutively and the actual composition must be reconstructed using appropriate statistics), but they are very compatible with field- or 'point of care' operation. The answer given here cannot be comprehensive in a sense that it captures every aspect of this diverse and rapidly developing field; it is to the best of the author's kowledge at this stage.

1 Quick *et al.*, *Nature*, 2016, **530**, 228–232.
2 https://www.nasa.gov/mission_pages/station/research/news/dna_sequencing

Robert Johnson replied: First, occasionally we see clogging with protein nanopores. We do take care to remove possible contaminants by filtering our buffer solutions and using HPLC to purify DNA samples prior to measurement.

Secondly, with the alpha-hemolysin pore, the noise level of our measurements tends to be down around 1 pA or less, and we can observe dynamic changes in a DNA molecule held within the pore that give rise to current changes of just 1–2 pA. Indeed, noise can actually be beneficial to us in our measurements. For example, we have found that the noise associated with the blocking current when dsDNA is inside the pore to be dependent on the stability of the DNA.[1] We have speculated that the noise associated with the blocking current may be a result of short timescale motions of the DNA duplex inside the pore, such as breathing and bubble formation.

1 R. P. Johnson, A. M. Fleming, Q. Jin, C. J. Burrows and H. S. White, *Biophys. J.*, 2014, **107**(4), 924–931.

Mark Platt answered: Dealing with blocked pores would be the a typical week for the group and for the larger PU pores the lifetime is correlated to how quickly we block them with particles. If we have a high concentration of nanomaterials then the pores block within seconds and can't be recovered. This can mean you need some experience/information on the sample you are analysing to ensure you don't block the pore immediately, and this of course is not always possible.

Jan Clausmeyer addressed Tim Albrecht: The presented electronic amplifier might be very useful as a tool to address questions related to nanoparticle electrochemistry. For experiments exploiting collisions of nanoparticles with electrode surfaces (nanoimpacts), the dilemma is that one only has a very short time to obtain information about individual particles. In most cases, some kind of transient current signal is detected upon collision of the particle but it is very unlikely to find that very particle again. Often, one must decide whether to acquire information regarding the particle size OR regarding electrocatalytic activity of particles. However, it would be very valuable to correlate the structure of individual particles with their activity.

The fast and automatic switching of the applied voltage would be very useful to get more information out of nanoparticle impact experiments. One may test several electrochemical reactions at different electrode potentials (for instance electrocatalysis followed by coulometry for particle sizing) during the very short residence time of the particle. However, electrode capacitance would certainly be a problem for that. Do you think it is possible to implement such an electronic amplifier for a typical experimental setup used for nanoimpact measurements? Which factors need to be taken into account?

Tim Albrecht responded: In fact, we have just begun to explore those possibilities, *i.e.* to try to use the nanopore setup for nanoimpact experiments. However, some previous nanoimpact studies in the literature have been performed with very powerful electronics, too, so we will have to see whether we can improve on this aspect further. It will be key to minimize the capacitance of the electrochemical cell as much as possible, ideally to significantly below 10 pF, since this is important for maximizing the time resolution also with our nanopore setup. If further performance enhancement can be achieved, it remains to be seen whether the improved time resolution provides significant new insight into the nanoimpact process. Nevertheless, this is currently of great interest to us.

Switching experiments, *e.g.* trying to recapture particles after bouncing off the electrode surface, are a more complicated matter, because the switching time will depend on the RC time constant. Hence, it is not clear yet whether fast enough switching can be achieved (even if C is very small, R may be significant, too).

Olaf Magnussen asked: One of the very fundamental questions in single entity electrochemistry is the ultimate limit of charge transfers that can be measured at electrochemical interfaces. Currently, the limit seems to be on the order of femtocoulombs. Is there a chance to go to significantly lower values, *e.g.* one or two orders of magnitude lower? Otherwise, every measurement will always have to rely on strong amplification effects, which strongly limits the scope of these measurements.

Tim Albrecht replied: Indeed, in our experiments on 200 bp dsDNA we obtain a most-probable translocation time of about 20 μs at event currents of 60 pA – this yields 1.2 fA. A single electron would be 0.16 aF, so that's still a significant way off.

Single-nanoparticle capacitances of approximately 1 aF have been measured at the single-particle level using electrochemical STM,[1] but in those experiments the tunnelling current acts in some way as an amplifier. Moreover, the configuration is rather specific.

1 T. Albrecht, S. F. L. Mertens and J. Ulstrup, *J. Am. Chem. Soc.*, 2007, **129**(29), 9162.

Patrick Unwin inquired: How are you generating the AC signal? I could not find many details in the paper. Could you explain this in a bit more detail?

Tim Albrecht answered: A full explanation of how the pipette current is handled through the different stages of the detection electronics would be difficult to provide here and a separate, electronics-focused publication is currently in preparation. However, we did describe the basic principle in our Nanoscale paper,[1] (see figure 1 in particular) and provide further references therein. An important point is that current amplification is achieved without (noisy) feedback resistors, but rather by matched transistor/capacitor circuits, combined with a transimpedance amplifier. This part of the setup is described in detail in reference 25 in our Nanoscale paper.[2] On the other hand, the large DC current is handled by a low-frequency DC feedback network, as described by P. Ciccarella *et. al.*[3]

In effect, a fast path is provided by the current amplifier/transimpedance amplifier, which amplifies the short pulses given by the pore blockade irrespective of the DC current (and low-frequency fluctuations therein). The DC current is determined by the voltage drop across a known resistor prior to the DC feedback network.

1 R. L. Fraccari, P. Ciccarella, A. Bahrami, M. Carminati, G. Ferrari and T. Albrecht, *Nanoscale*, 2016, **8**(14), 7604–7611.
2 G. Ferrari, M. Farina, F. Guagliardo, M. Carminati and M. Sampietro, *Electron. Lett.*, 2009, **45**, 1278–1280.
3 M. Carminati, G. Ferrari, R. Fraccari and A. Bahrami, An Integrated Low-Noise Current Amplifier for Glass-Based Nanopore Sensing, *10th Conference on Ph.D. Research in Microelectronics and Electronics (PRIME 2014)*, Grenoble, France, June 29–July 30, 2014, 1–4.

Paolo Actis asked: Nanopipettes can be integrated with nanomanipulators to comprise a scanning ion conductance microscope. Are you planning to use your protocols to deliver short nucleotides into living cells?

Tim Albrecht replied: I imagine they could be used in this context, but we do not have any immediate plan to do so.

Yitao Long communicated a question for Robert Johnson: First, when focusing on Fig. 1 of your manuscript, the control experiments show that the CC9 mismatch contributes to two well-defined residual current states (I1 and I2). However, how can you make sure that well-defined residual current states were not caused by dsDNA with CG9 base pair?

Secondly, according to Fig. 4 of your manuscript, why is $\tau_{\text{intra-helical}}$ less dependent on temperature?

Robert Johnson communicated in reply: Regarding the first question, three experiments were run with either just a fully complementary duplex, just a CC mismatch containing duplex and both the complementary and mismatch containing duplexes. Modulation between I1 and I2 states is observed only for the CC containing duplex, and all capture events for this duplex exhibit a modulating signature.

Regarding the second question, this is an indication that the activation energy of the two processes are different. As shown in Fig. 5 of the manuscript, the activation energy required to flip the base into the helix is higher, and so, the extra helical lifetime changes more with temperature.