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DISCUSSIONS

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Emerging electrochemical methods at the nanointerface: general discussion

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Alain Walcarius opened a general discussion of the paper by Dimitrios Valavanis: Thank you for your nice talk. In relation to what you did to evidence electrochemically induced calcium carbonate precipitation, do you think that you could combine your system with a quartz crystal microbalance to follow mass variation simultaneously? (It would be interesting to follow the kinetics of other electrochemically induced growth processes).

Dimitrios Valavanis replied: Thank you for your question. While a scanning pipette probe method (and SECCM in particular) can be used to approach and scan over a quartz crystal microbalance (QCM) electrode, IRM is more restrictive to the type of media in the optical path. In a reflected light configuration, light from the objective would first reach a glass layer, and then the absorbing layer (in our case indium tin oxide).[1] A QCM electrode in this arrangement would allow the acquisition of IRM images.

[1] S. Campidelli, R. Abou Khachfe, K. Jaouen, J. Monteiller, C. Amra, M. Zerrad, R. Cornut, V. Derycke and D. Ausserré, *Sci. Adv.*, 2017, **3**, e1601724.

Katherine (Kallie) Willets remarked: I noticed that you use ITO from SPI Supplies as your substrate for these studies. Our group and others have found significant heterogeneity in the performance of these substrates as supporting electrodes. Have you noticed this heterogeneity with your technique? In particular, have you seen inactive or "dead" regions within a sample or noted variations in the reaction kinetics across a single substrate or as you move from substrate-to-substrate?

Dimitrios Valavanis replied: Indeed, there are reports of this particular ITO substrate's heterogeneity from both our collaborator's group[1] and from our group (manuscript in preparation). During our work with the proposed SECCM-IRM technique, we did notice that substrates with an ITO thickness of 70 nm (rated 70–100 Ω sq.$^{-1}$ sheet resistance) offered a more uniform optical response, compared to substrates with an ITO thickness of 700 nm (rated 8–12 Ω sq.$^{-1}$ sheet resistance). In terms of the varied electrochemical activity, while this can affect local reaction rates, the precipitation, and the growth of crystals, we believe that the variation did not hinder our experiments at the scale investigated. A high frame rate IRM imaging of a large SECCM meniscus area (tens of μm in diameter) under controlled atmospheric conditions would allow us to closely follow the spatial distribution of events, and in turn record localised fluxes and surface heterogeneities.

[1] P. Ciocci, J.-F. Lemineur, J.-M. Noël, C. Combellas and F. Kanoufi, *Electrochim. Acta*, 2021, **386**, 138498.

Qianjin Chen observed: The study demonstrated a powerful technique by hybridizing SECCM and IRM. I remember the study by Bo Zhang on the fluorescence microscopy of single gas nanobubbles on ITO during electrolysis in a neutral solution. They observed that a fluorescence signal arose ~500 mV before HER occurred. Did you ever observe a similar phenomenon in the KNO_3 solution using IRM? Were there any optical intensities which arose before HER on ITO?

Dimitrios Valavanis responded: Thank you, Qianjin. We did not observe any local change in the optical intensity before the electrochemical HER in KNO_3 solution. We did observe some change at more negative potentials ($E \approx -1.5$ V *vs.* Ag/AgCl), however this is more likely to be indium oxide reduction into metallic indium, considering that the centroid position of the features did not vary at all, as opposed to what is expected from nanobubble formation.[1]

[1] P. Ciocci, J.-F. Lemineur, J.-M. Noël, C. Combellas and F. Kanoufi, *Electrochim. Acta*, 2021, **386**, 138498.

Popular Pandey remarked: Thank you Dimitrios for the nice presentation. How is the SECCM-IRM method advantageous over existing methods for tracking phase formation, as well as imaging the SECCM meniscus?

Dimitrios Valavanis answered: Thank you, Popular. In terms of imaging the SECCM meniscus, IRM offers a direct, *in situ* visualisation. Other methods perform indirect measurements,[1] *ex situ* imaging,[2] or require the addition of fluorescent dyes.[3] Here, the meniscus can be imaged directly during any possible SECCM experiment that can be conducted on optically appropriate substrates. Furthermore, SECCM-IRM uniquely allows the viewing of events within the confined meniscus cell (which is easily tuneable in size) and very close to the working substrate. These characteristics, along with the inherent capacity – owing to the scanning probe architecture – for effortless repeats of the same experiment, make the hybrid technique well-suited to tracking phase formation.

1 C. L. Bentley, M. Kang, F. M. Maddar, F. Li, M. Walker, J. Zhang and P. R. Unwin, *Chem. Sci.*, 2017, **8**, 6583–6593.
2 N. Ebejer, A. G. Güell, S. C. S. Lai, K. McKelvey, M. E. Snowden and P. R. Unwin, *Annu. Rev. Anal. Chem.*, 2013, **6**, 329–351.
3 M. E. Snowden, A. G. Güell, S. C. S. Lai, K. McKelvey, N. Ebejer, M. A. O'Connell, A. W. Colburn and P. R. Unwin, *Anal. Chem.*, 2012, **84**, 2483–2491.

Steven Linfield asked: Does the SECCM droplet experience any evaporation (or flow from within the pipette) during the course of the $CaCO_3$ crystallisation? If so, how much does flow within the droplet contribute to the observed precipitation near the meniscus edge? It seems from the COMSOL simulations that longer tip–substrate distances allow for more uniform O_2 saturation at the substrate surface, so perhaps longer precipitation times at further distances could reveal the contribution of internal flow to the meniscus deposition.

Dimitrios Valavanis replied: We do not expect that limited evaporation or flow from within the pipette would directly result in precipitation because the initial pH conditions are so far from the supersaturation point – indeed, significant currents are required to perturb the pH sufficiently to observe precipitation. We propose that the buffering effect on the pH due to diffusion from inside the pipette can explain the delayed precipitation around the centre of the wetted area; in combination with some effect from the atmosphere surrounding the meniscus (*e.g.*, CO_2 dissolution), contributing to the distinct display at the very edge of the wetted area. Both of those effects would hinder the precipitation. In terms of evaporation in general, we do not observe that taking place significantly in the $CaCO_3$ system, compared to other systems that we have investigated. Based on images just after pipette retraction and at later times, the meniscus residue is not decreasing by any considerable amount.

Yi-Tao Long opened a general discussion of the paper by Hang Ren: Have you considered the capacitance at the water (droplet)/substrate interface in the SECCM experiment? Could you include the effect of interface capacitance in your model? Could you please comment on the future spatial resolution that SECCM could achieve?

Hang Ren responded: In the SECCM experiment, the steady state is readily achieved within the timescale of a typical experiment. Therefore, in our paper (DOI: 10.1039/d1fd00046b), we focused on the steady-state behavior. Therefore, the capacitive behavior is not important.

We have previously estimated the capacitance of the water (droplet)/substrate interface in SECCM experiments and obtained a capacitance of $\sim$50 μF cm^{-2} on a Pt electrode in 10 mM $HClO_4$.[1] The spatial resolution of the SECCM is increasing over time. It will be fundamentally limited by the smallest droplet that can be formed stably due to the capillary force. This has been theoretically estimated to be as wide as 5 water molecules (1.9 nm).[2] However, we are not there yet.

1 Y. Wang, E. Gordon and H. Ren, *Anal. Chem.*, 2020, **92**, 2859–2865.

2 J. Jang, G. C. Schatz and M. A. Ratner, *Phys. Rev. Lett.*, 2004, **92**, 085504.

Gabriel N. Meloni commented: Usually, we can control the electrolyte concentration and in your work these are very low (lower than the electroactive species). At a 1 : 2 ratio (electrolyte to electroactive species), there is almost no ohmic drop in the CVs, which is interesting, meaning that for the *IR*-drop's sake, there is no need for much electrolyte, but as mass transport changes a bit migration starts to be important. What is the reason for these concentrations? Is that due to the applications you are envisaging? Also, as the largest resistance in the system is the pipette opening, small changes in the solution composition due to electrochemical reactions for the system proposed (low electrolyte) would impact the *IR* drop in the system a lot.

Hang Ren said: You are correct that when you have sufficient electrolytes compared with the electroactive species, the *iR* drop is not significant. We have chosen redox couples with fast outer-sphere electron transfer rates, so that the deviation in the voltammogram can be associated with the *iR* drop. The application is towards electrocatalysis, where the determination of the Tafel slope has been widely used to probe electrode mechanisms, requiring a very accurate determination of the Tafel slope. Indeed, deviation from 30 mV dec^{-1} to 40 mV dec^{-1}, which can easily be caused by an uncompensated *iR* drop, can change the interpretation of the mechanism. There are scenarios in electrocatalysis where the electrolyte concentration is lower than the electroactive species, for example, the electrolysis of water, *i.e.* the oxygen evolution reaction and hydrogen evolution reaction from water, where the water concentration is 55 M.

Popular Pandey remarked: Thank you for the beautiful presentation. What is the contribution of the electric double layer at the inner wall of the nanopipette, as well as the ion concentration polarization near the pore opening, to the *iR* drop?

Hang Ren answered: It depends on the size of the nanopipette opening relative to the thickness of the electrical double layer (EDL), *i.e.*, the Debye length. We do not specifically consider the effect of EDL from the charged glass wall of the nanopipette. When the Debye length is much shorter than the size of the opening of the nanopipette, our model is expected to be accurate. This is often true in practical systems, in which the electrolyte concentration is typically >1 mM and the radius of the nanopipette is typically >100 nm. If the EDL at the opening of the nanopipette overlaps, the local conductance will change, which is observed as ion current rectification.

Xiangkun Elvis Cao asked: This is really interesting work to estimate and compensate for the *iR* drop. I was wondering whether you could provide a high-level summary of comparing your method with other local electrode kinetics measurement approaches?

Hang Ren replied: There are other local electrochemical kinetic measurements which have been developed, for example, some optical methods based on converting electrochemical measurements to an optical signal (*i.e.*, electron to photon). However, quantitative analysis of the transduction to reveal the true local electrode kinetics sometimes can be convoluted, and might not be generally applicable for all electrochemical systems. Scanning electrochemical microscopy (SECM), a popular scanning electrochemical probe technique, is also very good at measuring local electrode kinetics. Scanning electrochemical cell microscopy (SECCM), which our work is focused on, can isolate a small nanointerface to be interrogated, and the current is directly associated with the interface isolated by the nanodroplet.

Martin A. Edwards remarked: This is nice, clear work. Firstly, a comment. In your work, you assume uniform concentration. It is possible to analytically determine resistances with changes in concentration with ET and migration and/or diffusion. The poster by Kamsy Anderson from my group (#5 at this meeting), which we are presently writing up, details these expressions. I think this could be quite easily included in your model. I don't anticipate dramatic changes in the interpretation, but it would extend it to a wider range of scenarios, *e.g.*, insufficient supporting electrolyte.

Second, a question. Do you think that evaporation at the end of the pipette could cause enhanced concentration at/near the meniscus, altering the conductivity? Would you notice that? Or is mass transport so darn fast on those length scales that it wins out?

Hang Ren answered: Thanks. We considered integrating the concentration as a function of distance into the analytical expression, but decided to go with simplicity: a simple equation that captures the major effects from the geometry and the bulk electrolyte concentration. We look forward to your work. Evaporation is a good point. The concentration near the liquid–air interface could be higher, but we haven't experimentally verified whether it would dramatically change the conductivity. We do keep the experiments at a controlled humidity so that the evaporation rate in a given experiment is stable. The fact that the numeric model predicts the limiting current very well suggests that the role of evaporation is insignificant.

Mark A. Buckingham opened a general discussion of the paper by Caleb Hill: You use this technique to probe the charge carriers in photoelectrochemical excitation, can you also use this technique for thermo-excited carriers, as in thermoelectrics instead of photovoltaics? Do you imagine that there would be a difference in the results between the two excitation mechanisms? Both applications are obviously hugely topical for metal chalcogenides/dichalcogenides, so have you also looked at metal mono-chalcogenides?

Caleb Hill replied: We do suspect that the methodology would work similarly well, providing that a suitable probe can be used to locally heat the sample. As for the metal mono-chalcogenides, not yet, but we look forward to applying this approach to a wide variety of materials such as these!

Steffi Krause said: Could you please comment on the difference in the excitation profile between the bulk sample (Fig. 2b of the paper, DOI: 10.1039/d1fd00052g) and the bilayer (Fig. 3b of the paper)? It looks as though both samples display a narrow excitation profile at 0.45 V, while the profile for the bulk sample significantly expands at higher potentials of 0.60 V and 0.75 V. Do you have comparable data for the bilayer sample at higher potentials? As for the anomalously broad photocurrent profile in Fig. 4b of the paper, is it possible that light scattering at defects in the complex WSe_2 sample could have caused the photocurrents at a greater distance from the excitation point than expected?

Caleb Hill replied: Comparable data can be found in the accompanying photocurrent movies. Unlike in the bulk samples, the profile does not significantly expand at the stated potentials. We attribute the unique responses observed at bilayer systems to the formation of interlayer excitons which are effectively repelled from the pipet interface. As for the second question, the defects mentioned could certainly scatter light. However, the source in these experiments is a tightly focused (<1 μm spot size) laser, making it possible to excite carriers within a pristine region of the sample.

Yi-Lun Ying remarked: In bulk n-WSe_2, have you considered the carrier recombination, as the carriers seem to exhibit long diffusion lengths?

Caleb Hill responded: In the bulk measurements, we quantify the diffusion length, which itself depends on both the diffusion constants of the carriers, as well as their lifetimes:

$$\mathrm{L} = \sqrt{D\tau}$$

The carrier lifetime will be set by the bulk recombination rate. In order to determine the recombination rate independently, a time-resolved variant of the described technique would need to be employed.

Qianjin Chen asked: These are very elegant measurements using a carrier generation-tip collection mode SECCM technique. What is your laser power and beam size used to initiate the photoelectrochemical reaction? Will these cause any significant increases in the local temperature and background current noise? Would this be a issue?[1]

[1] R. Hao, Y. Fan, M. D. Howard, J. C. Vaughan and B. Zhang, *Proc. Natl. Acad. Sci. U.S.A.*, 2018, **115**, 5878–5883.

Caleb Hill responded: The laser power was <1 μW and the beam spot size was <1 μm in diameter. While the resulting local intensity is high, the resulting temperature fluctuations one would expect are actually rather small (<0.1 K) due to efficient thermal transport away through the substrate.

Serge G. Lemay remarked: This is a rather open-ended question that was prompted by one of the answers during the discussion. In thin ion-sensitive field-effect transistors made with, *e.g.*, graphene, 1/*f* noise usually dominates the noise spectrum at low concentrations. This has been attributed to gate noise from the gating electrical double layer. I am curious as to whether the same mechanism dominates in your system, or if instrumental fluctuations (from either the light source or the readout circuitry) render this inaccessible.

Caleb Hill responded: Excellent question. Because these CG-TC experiments involve the detection of photogenerated carriers, the stability of the light source employed, with respect to both the intensity and spatial position, will dominate the apparent noise. This would not be associated with the measurement itself, but fluctuations in the concentration profile generated *via* excitation.

Ali Reza Kamali remarked: This is a very nice work. I am wondering how WSe_2 flakes could be characterised in order to identify the monolayer regions? What was the minimum thickness of the flakes? Thank you very much!

Caleb Hill replied: Transition metal dichalcogenides exhibit thickness-dependent changes in the band structure which make it possible to identify monolayers optically. In particular, monolayers possess direct bandgaps which enable strong photoluminescence (PL) to be generated. The strength of this PL, as well as its characteristic spectrum, allow monolayers to be easily identified.

Alain Walcarius opened a general discussion of the paper by Serge G. Lemay: Very nice work! What would be the best resolution (lowest microdroplet size) achievable with this technique? Would it be "simply" related to the characteristics of the nanoelectrode array (the nanoelectrodes' diameters and distance between them) or something else?

Serge G. Lemay responded: You are correct, as far as we understand it is that "simple". More specifically:

For *imaging* particles, the pitch of the nanocapacitor array is the defining parameter. This is, in turn, dictated by the CMOS process employed. Feature sizes have decreased exponentially with time, and a higher nanocapacitor density could be achieved today compared to the 90 nm technology that was used to fabricate the chips used in these measurements.

For *detecting* particles that are smaller than the array pitch, the key parameters are the analyte-to-electrode size ratio and the sensitivity (background noise) of the readout AD converters. As an example of what has been possible so far, we have previously published a measurement of single 40 nm dielectric particles.[1]

[1] F. Widdershoven, A. Cossettini, C. Laborde, A. Bandiziol, P. P. van Swinderen, S. G. Lemay and L. Selmi, *IEEE Transactions on Biomedical Circuits and Systems*, 2018, **12**, 1369–1382.

Yi-Lun Ying asked: Is there any crosstalk between the nanoelectrodes? Is it possible to fabricate a nanoelectrode array with a smaller diameter? Can you comment on what the size limitation of the electrode is?

Serge G. Lemay replied: Cross-talk between the electrodes contributes to the background (stray) capacitance. This is not a problem *per se*, as neighboring electrodes already serve as the counter-electrode to the electrode row being actuated. In principle, changing the solution impedance at an electrode will also redistribute the coupling to neighboring electrodes, but we have no experimental evidence of this so far.

The electrodes are fabricated using a mostly standard CMOS process, the devices used in the present work being based on a 90 nm low-power node from

TSMC. Roughly speaking, the electrode diameter scales with the minimal metal half pitch, which means that substantial further decreases in size are already possible today. It is important to keep in mind, however, that in most sensing applications it is insufficient to decrease the size of the electrode. To retain an analytically relevant number of receptor sites, one must simultaneously scale up the number of nodes so as to retain sufficiently effective binding kinetics. We address this point further in ref. 1.

1 S. G. Lemay and T. Moazzenzade, *Anal. Chem.*, 2021, **93**, 9023–9031.

Ben Slater asked: (1) Please can you detail the method and elapsed time from the emulsion preparation with sonication to the measurement of an image? What is the process for incubation? What does successful incubation refer to? Some images in the paper (DOI: 10.1039/d1fd00044f) are recorded at $t = 0$; what is $t = 0$ defined as?

(2) Regarding the preparation of emulsions with more than one component, specifically referring to 3B – are nitrobenzene and silicone oil miscible? How can you be sure that the droplets are individual components?

(3) On page 9, you state "The FWHM of these Gaussian-like profiles is typically 5–10 mm", what is the significance of the FWHM of the lines?

Serge G. Lemay replied: (1) As stated in the article (DOI: 10.1039/d1fd0044f), the emulsions were prepared by sonication and the chip was incubated within less than a minute. The incubation process entailed loading the emulsion into a syringe and injecting it by hand into the microcavity above the chip through a narrow capillary. No in-line emulsion preparation was employed, although this could be implemented if desired.

(2) We make no specific claim about possible coalescence of the different components. These were exploratory experiments of the nanocapacitor array platform and we did not attempt to characterize the emulsions independently.

(3) The FWHM gives an idea of the size of the object being imaged. For micron-sized dielectric particles, this scales approximately with the size of the particle (see, for example, ref. 1). The situation is more complicated, however, as the sensitivity to the sub-parts of a larger object falls off nonlinearly with distance from the array. This (so far unresolved) complexity is highlighted by the discrepancy between the two numerical methods for particle sizing investigated in the present article. It is fair to say that a full solution to the problem of how best to determine particle size using this method remains to be determined.

1 C. Laborde, F. Pittino, H. A. Verhoeven, S. G. Lemay, L. Selmi, M. A. Jongsma and F. P. Widdershoven, *Nat. Nanotechnol.*, 2015, **10**, 791–795.

Xu Liu enquired: For the physicochemical characterization that you have mentioned, can you compare it with other similar methods, such as SEM?

Serge G. Lemay responded: There is no doubt that SEM and its derivatives, such as EDX, are far more powerful and versatile tools than impedance spectroscopy for material characterization, even using dense arrays as we do here. The one virtue of chip-based impedance methods is that they provide a route to rather sophisticated information in a very cheap way (if the chips are mass-produced).

The costs of a readout board are a fraction of those of a SEM. A somewhat oversimplified comparison (but certainly on the side of fair) is to think in terms of the relative advantages and disadvantages of an optical microscope *versus* a miniaturized photodiode array. The microscope is much better at imaging, but there are many experiments and applications where you would rather have the cheap, compact and versatile photodiodes.

Gabriel N. Meloni commented: I was curious if the lack of agreement in the silicone oil permittivity comes down to a frequency limitation due to the smaller capacitance of the system. Is the frequency limiting you to a few systems?

Serge G. Lemay answered: In principle no, insofar as the total charge that is transferred per cycle does not become much smaller than that from the background capacitance and leakage so as to become undetectable. That said, we do need to correct for the properties of the readout circuit when the effective capacitance and the corresponding average current deviate significantly from the conditions under which the system was calibrated. Such large differences appear with emulsions because of the large differences in the dielectric constants, and this might introduce systematic errors that we have not yet fully understood. These corrections were addressed in the context of measurements at different frequencies, which also lead to large changes in the average current, in ref. 1.

We, however, also consider the possibility that the discrepancy stems from the limitations of our modeling. At the moment, we do not model any electrical double layers and the associated relaxation when interpreting experimental data. But this is a crucial component in interpreting electroosmotic data, for example, and it is likely to be relevant here as well.

1 F. Widdershoven, A. Cossettini, C. Laborde, A. Bandiziol, P. P. van Swinderen, S. G. Lemay and L. Selmi, *IEEE Transactions on Biomedical Circuits and Systems*, 2018, **12**, 1369–1382.

Frédéric Kanoufi asked: To follow up on Gabriel N. Meloni's question, could the disagreement in the permittivity measurement when multiple emulsions are used be due to an uptake (partitioning) of the second organic solvent by the silicone droplets? I guess that the water used for the emulsion is saturated with the organic solvent, which may then be able to be captured by the silicone droplets. It is actually an interesting analytical platform, like liquid nanochromatography, where the silicone can as a stationary phase to concentrate and detect a wide range of analytes. As you also mentioned the dynamics aspect of this high throughput technique, would you be able to track the dynamics of whatever happens to the droplets? For example, would it enable a new picture of liquid–liquid interface electrochemistry dynamics, where ion transfer at these interfaces generally has faster kinetics than electron transfer?

Serge G. Lemay answered: I agree that partitioning may play a role. We have not attempted this analysis, as there are also some open questions about the overall calibration of the method when the effective capacitance varies over a large range (as mentioned in the article, DOI: 10.1039/d1fd00044f). It will be interesting to return to this question when we are absolutely confident about the absolute accuracy; until then we only claim a qualitative discrimination capability.

Furthermore, using the platform to monitor the dynamics of partitioning is an exciting suggestion. At the moment this would be difficult; our present time resolution for imaging is about 200 ms, which is barely comparable to the time scale for diffusive processes on the ∼10 μm length scale. One could imagine achieving this in the future, but we are unfortunately not yet in a position to do so.

Yi-Tao Long commented: Single entity characterization *via* CMOS-based nanocapacitor arrays is amazing. I am wondering if this array could be used to measure redox nanoparticles?

Serge G. Lemay responded: In principle, absolutely, as we can measure the charge/discharge signals of a few electrons per cycle. But this would require fast electrochemical kinetics: the particle should be oxidized and reduced on the time scale of the cycling period, which is only 20 ns at the frequency where we normally operate.

Hang Ren said: What is the strategy for measuring such a small capacitance? To measure attofarad capacitance, is it because of much lower stray capacitance, or is it because it is measuring a difference and the stray capacitance is constant during the measurement? What can be done to measure even smaller capacitance changes?

Serge G. Lemay replied: The former. Implementing the readout circuit on a chip allows a decrease of the stray input capacitance to about 1 fF. Also important is that the $1/f$ noise from the modulating transistors does not contribute to the overall noise level; discharge is basically a passive process where the transistor is not actively gated, as would occur in an ISFET. This may seem like a detail, but note that the $1/f$ noise dominates at very high frequencies in nanoscale transistors. In the future, some gains are possible by improving the A/D converters in the readout circuitry.

Steffi Krause asked: Could you please show a schematic of your chip architecture and explain how you address individual electrodes? It would also be interesting to see how you extract the capacitance from the measurements, as the use of CMOS technology implies the use of an array of field effect capacitors, the capacitance of which would be mostly dependent on the insulator and the space charge layer in the semiconductor, but not necessarily dependent on what happens on the solution side.

Serge G. Lemay replied: This is a good question. Regarding addressing individual electrodes, this is explained in detail in ref. 1. Regarding the second part of your question, our use of the term ‘effective capacitance’ can lead to confusion. The effective capacitance is defined as the amount of charge induced on the nanocapacitor during one charge/discharge cycle, divided by the applied voltage step amplitude. At low frequencies or in situations where ion migration is negligible, this corresponds to the actual capacitance of the electrode in solution plus the stray capacitance. We believe that this is the case that applies in most of the *Faraday Discuss.* data (DOI: 10.1039/d1fd00044f). In situations where migration is relevant (at significant salt concentrations, such as in biological samples, for example), the effective 'capacitance' is in fact determined by the solution resistance. In addition, note that the transistor gate capacitance does not really enter the problem. This is because we do not use the gate charge as the signal, which is very different from the mode of operation of an ISFET.

1 F. Widdershoven, A. Cossettini, C. Laborde, A. Bandiziol, P. P. van Swinderen, S. G. Lemay and L. Selmi, *IEEE Transactions on Biomedical Circuits and Systems*, 2018, **12**, 1369–1382.

Zhu Zhang addressed Selvaraj Chinnathambi and Serge G. Lemay: Since the electrodes are very sharp, and the frequency of the applied potential is about 50 MHz, I was wondering if the nanoelectrode generates a dielectrophoresis force that can attract or repel single particles or molecules? If so, during the periodic switch on/off of the nanoelectrode rows, the particles could bounce between rows of electrodes, which would be very interesting.

Serge G. Lemay and Selvaraj Chinnathambi answered: We agree that dielectrophoresis can generate significant forces at nanoelectrodes. For example, under our operation conditions, we estimate a (negative) force of order 10 fN for a 10 nm radius particle at a distance of 100 nm from an electrode, assuming a purely dielectric particle with a low dielectric constant and a frequency above f_2. This corresponds to a drift velocity of $\sim$5 μm s^{-1} (!). The force, however, decreases to 10 aN for a 1 nm radius entity and falls off at the fifth power of distance, so the effect is mostly relevant for larger nanoparticles and very locally at the electrode, where the field gradient is strongest. Note, however, that these estimates are based on the conventional Clausius–Mossotti factor treatment, ignoring the electrical double layer of the particle, and are therefore coarse approximations. To our knowledge, the description of dielectrophoretic forces under conditions where the particle size, the electrode size and/or the electrical double layer thickness are comparable has not yet been fully elucidated (see, *e.g.*, ref. 1).

1 E.-S. Yu, H. Lee, S.-M. Lee, J. Kim, T. Kim, J. Lee, C. Kim, M. Seo, J. H. Kim, Y. T. Byun, S.-C. Park, S.-Y. Lee, S.-D. Lee and Y.-S. Ryu, *Nat. Commun.*, 2020, **11**, 2804.

Martin A. Edwards communicated: With your chips, is it possible to zoom-in, *i.e.*, on only a sample or subset of the electrodes? In doing so, it might be possible to gain higher temporal resolution while maintaining the spatial resolution. Hopefully, this would allow you to see more dynamics of your system. Obviously, this would limit the area you are looking at and so you would have to be lucky that the region that you are looking at contains a droplet (unless one wanted to take on the challenge of making some sort of real-time adaptive strategy!)

Serge G. Lemay communicated in reply: You are absolutely correct. Since the array is read out in a mostly sequential manner, it is possible to zoom-in on subregions so as to trade a smaller array size in exchange for higher speed. There are 8 AD converters on chip, so in principle one could push this idea as far as measuring only 8 electrodes with a concomitant 8192× increase in speed. However, implementing this zoom-in capability would require a substantial reprogramming of the readout board for the chip, which is not something our lab has delved into yet.

Hong-Yuan Chen commented: A very innovative concept, "Electronic Chemistry" (or "Electron Chemistry"), is presented for the topic of next generation nanoelectrochemistry, which is the study of the electrochemistry of single entities down to the nanometer or sub-nanometer scale. The fundamental theories and rules of classic "electrochemistry", such as the Cottrell equation and Coulomb's law, are appropriate to macroscopic chemical processes, but no longer apply in the limited conditions of single nanoparticle and single molecule measurements. These theories and practical measurements are confined to the macroscopic and continuous systems of study, and lose sight of the granularity on the meta-microscale. These discrete and stochastic processes have attracted much attention for the nanoelectrochemical analysis of single entities. This new understanding, "Electronic Chemistry", could expand the electrochemical theories approaching the limit of temporal and spatial resolutions and highlights the discontinuous granularity and multiscale nature of electrochemical study in the quantum regime. Guided by this science, next-generation nanoelectrochemistry and molecular electronics could be characterized more accurately.

Si-Min Lu replied: I totally agree with you that the electrochemical process is stochastic and discrete at the microscale. The ions and single entities cannot be regarded as the mass point in single entity electrochemistry. There is an urgent demand to develop high-bandwidth instrumentation and advanced data algorithms to track the transient electron transfer process. Also, a comprehensive theoretical modelling framework (a combination of Marcus theory, the random walk model, Simmons model, *etc.*…) is needed to simulate the mass transfer and electron transfer kinetics at the nanoconfined interface. And single entity electrochemistry at the nanoconfined interface is actually a interdiscipline with the combination of quantum physics, physical chemistry, organic chemistry…

Yi-Lun Ying responded: It is a very interesting concept; the transport of charge, electrons and ions at the nanointerface and underconfinement conditions needs to have new models.

Yi-Tao Long said: In this session, we look deep into the nanointerface under confined conditions, which has raised more questions than we expected but provides more chances to get close to the 'truth'. For example, the correlations should be well understand to bridge the characteristics we found in the nanointerface with the sensing/catalytic performance on a large scale interface. Moreover, massively parallel measurements are expected on the nanointerface. Any comments on the future development of nanoelectrochemical methods and their applications are welcome.

Weilin Xu answered: For the study of confined nanointerfaces, in the future, it probably will go deep into the interface on the electrode, especially the nanointerface between the electrode and single entities. Some questions, such as the ET process between them, are very interesting.

Stefano Fornasaro responded: I believe that in the future, research at the nanointerface will have to take into account the increasing use of simulations and data processing algorithms, including machine learning, in designing experiments to optimise performance or test hypotheses more effectively. These methods are currently reshaping how researchers collect, analyse, and interpret data. Their impact on nano-electrochemistry is still not fully realised.

Shelley Minteer replied: In the field of bioelectrocatalysis, there is a great interest in nanoconfinement for enzyme cascades (*i.e.* Fraser Armstrong's recent work[1]), but there are challenges in developing single entity measurements for evaluating these systems. I hope that as we see more advances in single enzyme measurement techniques, we will be able to learn more about nanoconfinement in enzyme cascades.

1 G. Morello, C. F. Megarity and F. A. Armstrong, *Nat. Commun.*, 2021, **12**, 340.

Li Xiao commented: I hope that the research and method development of nanoelectrochemistry can help us understand more problems encountered in practical devices. For example, we would like to see the transport path of ions in polymer electrolytes, and how ions transfer and participate in the reaction at the electrode/polyelectrolyte interface. I hope that this can be realized by nano-electrochemical methods.

Zhongqun Tian opened a general discussion of the paper by Bing-Wei Mao: *In situ* atomic force microscopy is a powerful surface characterization technique, and your work (DOI: 10.1039/d1fd00043h) has demonstrated that it can be used to study the initial stage of Li deposition and dissolution, and provide insights on the SEI influence on the reversibility of Li deposition–dissolution cycle. In your presentation, you gave a perspective, in which combined *in situ* AFM and SECM is recommended as a nanoelectrode-based scanning probe microscopic technique. Could you comment on what additional merits beyond the capability of ECAFM can be expected from the AFM-based SECM for the nanoscale study of Li deposition?

Bing-Wei Mao answered: Thank you for the good question. AFM can provide a multitude of nanoscale information based on its imaging and beyond imaging capabilities. And one of the most important merits of AFM in electrochemistry is that it can work in electrochemical environments. However, AFM itself lacks chemical and electrochemical identification ability, and not all measurements can be performed in liquids by conventional AFM. For example, measurements of electric properties, such as conductivity and surface potential, in a liquid environment are difficult if not impossible because faradaic processes occurring at a bare conducting probe would cause significant interferences. Incorporating nanoelectrodes into AFM probes to form AFM-based SECM can enable important issues to be studied under electrochemical control, which would otherwise be difficult by conventional AFM. Dedicated AFM-based SECM is already commercially available. By utilizing such an AFM-based SECM, many scientific problems in Li deposition can be explored. For example, the conductivity of the SEI could be measured during Li deposition, or the local electrochemical reactivity of a porous SEI could be probed. Also, the interface formed between the SEI and electrolyte could be studied by combined surface potential measurements at the SEI side and force curve probing of the structure at the electrolyte side. Such a study would not only provide important information about Li ion transport across the interface, but also extend our understanding of the electric double layer in an interface formed by two ionic conductors involving a SEI.

Yi-Lun Ying enquired: Would the type of solid electrolyte give different types of SEI formation? Could you please comment on the correlation and difference between nanoscale measurements and large scale performance in SEI studies?

Bing-Wei Mao responded: Thank you for your good questions. If the electrochemical window of a solid electrolyte is insufficiently large, SEIs will be formed at the interface. Their compositions are expected to be different from those formed from liquid organic electrolytes. Since AFM is a surface technique, it is unfavorable for studying the SEI formation taking place at the electrode and solid electrolyte interfaces. Nevertheless, it is possible to study the interface if a section of the interface can be exposed. Such investigations are still very limited.

Regarding the correlation and difference, nanoscale measurements by AFM, for example, are to characterize the formation and properties of SEIs from the microscopic point of view. The obtained information is helpful for understanding phenomena observed in the macroscopic performance measurements. The differences lie in that SEI-related issues are complex and the macroscopic performance is usually an averaged result of many influencing factors, while microscopic measurements are usually based on model systems and emphasize some discrete aspects. A thorough understanding of the related processes requires a multitude of *ex situ* and *in situ* characterizations by microscopic and spectroscopic techniques and, if possible, under *operando* conditions.

Frédéric Kanoufi remarked: It is a very interesting study showing what *in situ* AFM can bring to the study of the initial growth steps of the SEI during Li deposition. The diversity in the SEI behavior is very interesting. Looking at them quickly, it seems that they have similar chemical structures and comparable thicknesses; maybe the SEI-Li is the stiffest, even though it is the one that allows the more reversible Li dissolution/deposition. I wonder how you picture these deposition/dissolution processes? To be effective, Li ions have to enter or be expelled from these protected Li metal spheres. This suggests that quite large amounts of Li ions should be transported through the SEI. Is the SEI acting as an homogeneous ion-transport layer? Do you have an estimate of such ion transport through the different SEI layers?

One could also invoke a mechanism where small ion channels/pores within this SEI shell are allowing the growth/dissolution of Li metal. This is maybe more consistent with the evolution of the shape of the SEI shell during Li dissolution. As the SEI shape seems to change, does it mean that the SEI always adheres to the underlying Li metal or does it just behave as a deformable shell/scaffold (a deflating balloon)? The latter behavior is suggested in different NP conversion mechanisms implying inorganic shells. For example, the dissolution of nanoparticles (by galvanic exchange reactions) under precipitating conditions generates a scaffold SEI-like inorganic layer and the dissolution proceeds through multiple pitting channels.[1]

[1] J. G. Smith, X. Zhang and P. K. Jain, *J. Mater. Chem. A.*, 2017, **5**, 11940–11948.

Bing-Wei Mao responded: Thank you for your critical questions. The differences of the two SEIs (SEI-Cu-step and SEI-Li) formed by potential stepping to the potential where Li overpotential deposition takes place are indeed small. But it is worth noting that their thickness difference is *ca.* 25%, which is not minor. Also, the small amount of Li_3N contained in the SEI-Li is not present in SEI-Cu. Nevertheless, I agree that the mechanism which explains the opening of segmented SEI-shells formed by the two kinds of SEI requires further explorations.

Because an ideal SEI is electron insulator and Li ion conductor, Li deposition and dissolution have to proceed underneath the SEI. It is right that Li ions have to transport in and out of the SEI layer during deposition and dissolution, and SEI does act as an ion-transport layer, but probably an inhomogeneous layer. The estimated diffusion coefficient of Li ions in SEIs is in the range of $\sim 5\text{–}8 \times 10^{-8}$ $cm^2\ s^{-1}$.[1] Regarding the mechanism of small ion channels/pores, the formation of the SEI is a self-limiting process; once a complete SEI is formed, it is expected to be an electron insulator, and pores and channels can only exist in the inner region of the SEI. So, it is possible that Li deposits fill in the pores and channels of the SEI, but this should be unlikely to cause changes of the shape of the SEI unless Li dendrite growth occurs. The evolution of the shape of the SEI is due to further formation of the SEI on the growing nucleus. Finally, for the preformed SEI-Cu-lsv, inorganic rich components dominate the thick inner layer. Such an SEI has insufficient elasticity to accommodate the growth of the Li nucleus by solely deforming itself. Even for the two SEIs (on Cu and on Li created after potential stepping) with inorganic–inorganic hybrid structures, it is unlikely that SEIs can be stretched to accommodate Li deposition. Rather, SEIs would always continually form on the growing nucleus.

1 J.-W. He, Y. Ge, W.-W. Wang, J.-H. Wang, Z.-B. Chen, H.-Y. He, Q.-H. Wu, J.-W. Yan and B.-W. Mao, *ChemElectroChem*, 2021, **8**, 62–69.

Svetlana Menkin enquired: How can the under-potential deposition of lithium be distinguished from the SEI formation?

Bing-Wei Mao responded: Thank you for the good question. Li underpotential deposition (UPD) can be distinguished from the SEI formation by comparing CVs of different cycles (which are not shown in this paper (DOI: 10.1039/d1fd00043h), but can be found in our previous paper[1]). The SEI formation is almost completed after the first cathodic linear sweep of potential, so that both the main peak at *ca.* 1.3 V and the background current in the lower potential region from the electrolyte reduction nearly disappear on subsequent CV curves, but the peak at *ca.* 0.5 V always persists. So, we can safely distinguish Li UPD from SEI formation. Nevertheless, the underpotential deposited Li layer may somehow interact with the partially formed SEI, as is mentioned in ref. 1, which could cause compositional rearrangement of the inner layer of the SEI. But we believe that such a kind of influence is different from that of the bulk Li deposit.

1 J.-W. He, Y. Ge, W.-W. Wang, J.-H. Wang, Z.-B. Chen, H.-Y. He, Q.-H. Wu, J.-W. Yan and B.-W. Mao, *ChemElectroChem*, 2021, **8**, 62–69.

Frédéric Kanoufi said: There is a further very nice concept that you are showing in your work: controlling the SEI chemistry and its formation dynamics allows the production of a self-limiting growth of the Li metal. Such homogeneous SEI formation actually explains the Li spherical shape growth rather than a dendritic one. However, this could also mean that the amount of deposited Li will also be limited. How far have you been able to grow the Li deposit? How thick the Li can be grown? In the extent of large capacity, do you end up with dendrites anyway or does this chemically-controlled SEI prevent the dendritic growth regime by a more controlled Li^+ ion release?

Bing-Wei Mao responded: Thank you for your good questions. In our paper (DOI: 10.1039/d1fd00043h), our efforts are to follow the nucleation and growth processes of the initial stage of Li deposition. This is partly because AFM is more suitable for working with a smoother surface, with several micrometers being the upper limit. The highest capacity we reached was 0.5 mA h cm^{-2} (*i.e.* 1.8 C cm^{-2}), which is equivalent of a 2.5 μm thick dense Li deposit. Since we did not perform experiments of long deposition and dissolution cycles and to sufficiently high capacitance, dendritic growth was not observed, even at the capacitance of 0.5 mA h cm^{-2}. But I wish to mention that the shell-opening phenomenon or creation of holes and defects have also been observed by the group of Wen and Wan,[1] as well as by Shiraishi and coworkers,[2] in PC-based electrolytes. So, it is reasonable to believe that the shell-opening phenomenon generally exists regardless of the solvent environment, and may be accounted for by the model of segmented SEI-shells. Such a phenomenon is different from dendritic growth, which is more associated with the inhomogeneity of the SEI and depletion of Li ions in a later stage, especially after long cycling, but it is also a negative effect that degrades the Li deposition and dissolution reversibility.

1 Y. Shi, G.-X. Liu, J. Wan, R. Wen and L.-J. Wan, *Sci. China Chem.*, 2021, **64**, 734–738.
2 S. Shiraishi and K. Kanamura, *Langmuir*, 1998, **14**, 7082–7086.

Svetlana Menkin asked: How is the “dead” SEI defined? Does it have different mechanical properties?

Bing-Wei Mao responded: Thank you for your good questions. “Dead” SEIs are defined as SEIs that are no longer usable as an ordinary ionic conductor for accommodating Li deposition. By nature, such SEIs are detached from the substrate so that Li deposition does not proceed by Li ions being transported through the SEI. In our paper (DOI: 10.1039/d1fd00043h), the dead SEIs arise from the opening of segmented SEI-shells during Li dissolution. We measured the mechanical properties of the SEIs and the results show that segmented SEI-shells do not necessarily bear very different Young’s moduli from those of the integrated SEI-shells. It is the discontinuity of the SEI-shell kinds that is a more important factor leading to dead SEIs. Our work about the mechanical properties of SEIs will be published elsewhere.

Si-Min Lu opened the discussion of the paper by Kazue Kurihara: Could you please discuss the difference of the redox reaction in a confined space and bulk solution? Where does the difference come from? Is it from the aspects of mass transport or electrode kinetics?

Kazue Kurihara answered: In the bulk system, redox species diffuse from the bulk to the electrode, so we may say that this is the diffusion controlled reaction. When the redox species are in a confined space, they cannot diffuse far away, so are cycled between the cathode and anode, resulting in an enhanced redox reaction (an increase in the current with the decreasing separation distance between the electrodes). What we observe in this study is that the redox current sharply increases when the space becomes narrower. We think that the redox species are organized or linked like polymers in the narrower space to form redox chains, thus the current sharply increases.

Patrick Unwin commented: Thank you very much for your interesting paper. Evidently, the SFA has considerable potential (excuse the pun!) to probe the EDL at electrodes under operation. You discuss the approach curve (longer distance) before the sudden onset in current to redox cycling in the gap. Have you analysed the shape of the curve and magnitude of the current in terms of a simple thin layer cell model?

Kazue Kurihara responded: Thank you for your interest. We have not analyzed the current curve. Because the current in the longer distance range increased with the increasing redox concentration, we think that the current increase in the longer distance range is due to well-known redox cycling in the gap between electrodes. So, we should be able to analyze it by a thin layer cell model, although our interest was more in the short range component. Thank you for your suggestion.

Patrick Unwin said: In the very high current region at close distances, if the “chain reaction of electron transfer between redox species” you state in your paper (DOI: 10.1039/d1fd00060h) is operating, it would be expected to be sensitive to the particular redox species (homogeneous self-exchange rate constant). Have you tried these experiments with other redox couples?

Kazue Kurihara replied: We agree with this comment, although we have not tried other redox couples.

Justin Gooding asked: The hysteresis in this sudden current increase is very interesting. Is it possible that in the experiment Prussian blue is being formed, which then bridges between the two electrodes? Then, upon withdrawal, polymer strands extend and maintain the effective electrochemical short between the two electrodes?

Kazue Kurihara replied: When we stopped the separation of the electrode at the middle of the largest current range, the current gradually decreased. So we think that the bridging is a temporary phenomenon in our case. We do not know the stability of Prussian blue polymer chains.

Justin Gooding said: In light of the possibility of ferricyanide forming Prussian blue polymer chains, the suggestion of Professor Pat Unwin to try a different redox species that cannot form polymeric materials seems like a good idea.

Kazue Kurihara answered: We completely agree with this comment. Thank you.

Qianjin Chen asked: While the 10 mM $KClO_4$ solution gave a consistent sudden current increase between the approach and separation, the redox molecule $Fe(CN)_6^{3-/4-}$ gave a large hysteresis. Are you able to extract/obtain the cyclic voltammetry at each of the electrodes at different distances (redox cycling)? This may help understand the contribution from mass transport within the nanogap?

Kazue Kurihara responded: Yes, we can perform cyclic voltammetry at different distances, though we did not do such measurements for this case. I agree with this comment. The measurement will provide useful information. The observation which convinced us of the presence of organized redox molecules is that the current gradually decreased when we stopped separation at the middle of the separation. We think that the organized structures finally disappear by separation.

Shuai Chang said: You have shown force distance curves in your presentation. What is the difference between your force measurement instrument and regular AFM?

Kazue Kurihara replied: There are several advantages of SFA when compared to AFM as follows.

(1) More defined geometry in the case of SFA, which employs a crossed cylindrical geometry. Thus, the measured force can be converted to the interaction between flat surfaces, so quantitative theoretical treatments are easy. In case of AFM, the shape of AFM tips usually varies, so this is not easy. There is a colloidal probe AFM method, but it may not be so easy to find a suitable probe and control their surface potential by applying the voltage.

(2) The size of the surface is relatively large, *i.e.* 30 μm in diameter at contact, so it is possible to monitor multiple molecules like real systems. If the ultimate goal of AFM is a single molecular measurement, this difference is important since a real system consists of many molecules.

(3) Because of the large surface area, it is possible to measure weaker forces.

(4) The large surface enabled us to combine the force measurement with other complementary techniques for surface characterization, including cyclic voltammetry, fluorescence spectroscopy, and X-ray diffraction.

(5) It is possible to employ the measurement for a wider variety of samples. We can modify surfaces using most surface modification techniques, including silane coupling agents, polymer coating and metal sputtering.

Shuai Chang enquired: In what kind of media can you perform your experiments? Liquid, air, organic solvents?

Kazue Kurihara responded: We usually perform the measurements in liquids (water and organic solvents), but sometimes in air (or gas).

Si-Min Lu opened the discussion of the paper by Shuai Chang: I am interested in the tunneling distance in the stochastic collision electrochemistry. Have you estimated the tunneling distances of the CBA molecule, FC molecule, and AFC molecule?

Shuai Chang answered: CB7 is a structurally rigid molecule with a molecular height of 0.91 nm between two portal rims. The tunneling distance between the gold NP and the gold nanoelectrode at collision can be estimated to be close to 0.91 nm. For experiments with guest molecules (FC and AFC) present, the guest molecules are complexed inside the CB7 cavity and the tunneling distance is still dominated by the CB7 height.

Alain Walcarius asked: Did you observe any effect of the NP size on the response time (as it might influence the extent of the host–guest interactions)?

Shuai Chang replied: We have performed the collision experiments with different GNPs, *e.g.*, 10 nm and 40 nm GNPs, in the study of a different molecule (4-aminothiophenol) using the same setup.[1] No electrochemical current changes were detected, but the event rate appears to be higher with the smaller sized GNPs. In this work (DOI: 10.1039/d1fd00054c), we did not carry out experiments with different sized NPs, and we expect a similar effect of the size of the gold NPs, which should have some effect on the response time but little effect on the electrochemical current amplitude.

1 J. Guo, J. Pan, S. Chang, X. Wang, N. Kong, W. Yang and J. He, *Small*, 2018, **14**, 1704164.

Alain Walcarius enquired: Perhaps you could also think about functionalizing the nanoparticles to tune their interactions with the macrocyclic ligand?

Shuai Chang answered: Thanks for the great suggestion. Functionalizing the nanoparticles with different ligands could definitely tune the interactions and allow more complex measurements.

Wenrong Yang said: The electrochemical sensing of host–guest chemistry triggered by nanoparticle collisions is an very interesting approach, which could be used to study the dynamics of other noncovalent (weak) interactions. Could the authors discuss if pH would play an important role in such noncovalent (weak) interactions?

Shuai Chang responded: Thanks for the comments. In the current study (DOI: 10.1039/d1fd00054c), the experiments were carried out at a neutral pH (pH = 7.4) and the pH effect on the NP collisions and the corresponding electrochemical current changes was not studied. But we do expect that pH would play an important role in affecting such noncovalent interactions because the electron rich portal of CB7 and the properties of the negatively charged gold nanoparticles can be greatly affected by the local environmental pH, thus the colliding interactions (distance and frequency) between the molecule and NP can be impacted.

Ben Slater commented: (1) Is the inclusion complex a 1 : 1 or 1 : 2 complex? In your methods, you describe it as a 1 : 2 complex, but the diagrams show a 1 : 1 complex. You also report a final concentration of 0.5 mM; how did you verify that you have 100% yield?

(2), did you separate excess guest molecules or do you have mixture of 0.5 mM of the inclusion complex and 0.5 mM of guest Fc? No method to separate the excess from inclusion complex is reported.

(3) Apart from the electrochemical measurements, do you have any evidence for the formation of the inclusion complex? Did you characterise the inclusion complex by other methods, for example NMR?

(4) Another report sonicates for 1 day; how can you be sure that the complex has been formed in around 30 minutes?[1]

(5) Please can you comment on the difference in the baseline conductivity of Fig. 2B of the paper (DOI: 10.1039/d1fd00054c)? CB[7]-Fc is around 3× higher than CB[7]AFc, why is this?

1 C. I. R. Magalhães, A. C. Gomes, A. D. Lopes, I. S. Gonçalves, M. Pillinger, E. Jin, I. Kim. Y. H. Ko, K. Kim, I. Nowik and R. H. Herber, *Phys. Chem. Chem. Phys.*, 2017, **19**, 21548–21555.

Shuai Chang replied: (1) Two guest molecules, FC and AFC, can bind to CB[7] in a 1 : 1 stoichiometry with a high binding constant. In this work, we prepared the host–guest solution at a 1 : 2 ratio to facilitate the complete occupation of the CB[7] cavity by guest molecules. The final concentration is 0.5 mM. It may not be a 100% yield because the host–guest complex is in a dynamic binding equilibrium. Based on the 0.5 mM and 1 mM mixture of CB7 and FC, together with a binding constant of 4×10^5 M, we calculated that the binding yield is ~99.5%. AFC has a higher binding affinity than FC toward CB7 and the yield will be even higher.

(2) We did not separate excess guest molecules from the inclusion complex, but used it directly for the electrode modification.

(3) Yes, we also characterized the inclusion complex using other methods, such as NMR[1] and surfaced enhanced Raman spectroscopy.[2]

(4) Both FC and AFC have a high binding affinity toward CB7, and we found that 30 minutes is enough for the complexed state to be formed, as characterized in the above referenced articles.

(5) The difference in the baseline conductivity of Fig. 2B of the paper (DOI: 10.1039/d1fd00054c) could be associated with the variation of the exposed electrode area.

1 M. Huang, M. Sun, X. Yu, S. He, S. Liu, W. M. Nau, Y. Li, T. Wu, Y. Wang, S. Chang and J. He, *J. Phys. Chem. C*, 2020, **124**(29), 16143–16148.

2 Q. Ai, J. Zhou, J. Guo, P. Pandey, S. Liu, Q. Fu, Y. Liu, C. Deng, S. Chang, F. Liang and J. He, *Nanoscale*, 2020, **12**(32), 17103–17112.

Steven Linfield commented: You showed that the two current levels observed during nanoparticle collisions with a CB7-Fc modified nanoelectrode were the result of the ferrocene molecule's rotational state. However, some L-type events are stable for tens of seconds, even ~100 s in one case. Do you ever see changes between the two rotational states (and therefore current level) during an L-type event? If the two rotational states are interchangeable, why are currents related to this observed more during the shorter S-type events than in the longer L-type events? And what could the ratio of the ferrocene configurations (horizontal and vertical) observed in the current tell us about the stability of ferrocene in the host?

Shuai Chang responded: In both S and L types of current switching signals, we could see two current states, which we attributed to the two rotational states of FC in a CB7 cavity. This can be seen in the histogram displayed in Fig. 2C and D of the paper (DOI: 10.1039/d1fd00054c). We do see some events showing the interchange of two states in an individual current switching spike, but these are quite rare. The S and L types of signals should be predominantly related to the properties of CB7 molecules on the nanoelectrode surface. We see both S and L types of signals in the NP impact experiments on CB7, CB7-FC and CB7-AFC, and find that the addition of guest molecules does not show an obvious impact on the appearance rate of S *versus* L signals. The rotation of FC inside the CB7 cavity is induced by the interaction between the gold tip and the FC.[1] Different rotational geometries could stay stable at different gap distances between two gold surfaces. In the current distribution, the two peaks for the S type events are not as well defined as those of the L type. We have reasoned that this is because in the L types of signals, FC is more stabilized because NP is transiently trapped on the molecular surface and this serves better to stabilize the geometry, as discussed in the manuscript. And for your last question, the ratio of the ferrocene configurations (horizontal and vertical) observed in the current may not be directly correlated to the stability of ferrocene in the host, because both horizontal and vertical states of FC can be stable depending on the gap distance between the two gold electrodes. Thus, if we dig further, the ratio of the ferrocene configurations could possibly reflect the statistically weighted gap conditions in various NP colliding events. One thing to note here is that the NP impact experiments are very different from the exact single molecule conductance measurements because the collision motion between NP and the electrode in the NP impact experiment is a random Brownian motion, where the tunneling distance at the collision can not be manipulated. While the single molecule conductance experiments could precisely control the tunneling distance between two electrodes.

1 M. Huang, M. Sun, X. Yu, S. He, S. Liu, W. M. Nau, Y. Li, T. Wu, Y. Wang, S. Chang and J. He, *J. Phys. Chem. C*, 2020, **124**(29), 16143–16148.

Yanfang Wu queried: Can you comment on the possible effect of the size of gold nanoparticles on the amplitude of the switching current signals (or electrochemistry activity) from ferrocyanide for the situation when gold nanoelectrode was modified with CB7 (or CB7 with a guest molecule) in your system? Also, how important do you think the homogeneity of gold nanoparticles may be in your study?

Shuai Chang answered: In a previous study of a different molecule (4-aminothiophenol) using the same setup,[1] we have performed the collision experiments with different GNPs, *e.g.*, 10 nm and 40 nm size GNPs. No electrochemical current changes were detected, but the event rate appears to be higher with the smaller sized GNPs. In this work (DOI: 10.1039/d1fd00054c), we did not carry out experiments with different sized NPs, and we expect a similar effect of the size of gold NPs. Similar for the homogeneity of gold NPs, we assume that it does not have a significant impact on the electrochemistry activity in our measuring system.

1 J. Guo, J. Pan, S. Chang, X. Wang, N. Kong, W. Yang and J. He, *Small*, 2018, **14**, 1704164.

Yi-Lun Ying opened the discussion of the paper by Weilin Xu: What about the homogeneity of the nanoparticles? Would you please comment on what is the major challenge in future nanoscale electrochemical measurement for studying nanoparticles in catalysis?

Weilin Xu responded: In the case, the homogeneity of the nanoparticle is not perfect, actually these particles are heterogeneous in both size and performance. The major challenge in future nanoscale electrochemical measurement for studying nanoparticles in catalysis can be summarized into the following two points: (i) how to monitor or analysis the electrocatalysis-induced structure variation of nanoparticles precisely; (ii) how to correlate the structure of a nanoparticle with its electrocatalytic performance precisely at single particle level.

Yi-Tao Long queried: About the platinum NPs, how did you characterize them? TEM or HRTEM?

Weilin Xu responded: As for the characterization of the Pt NPs, in this work, these nanoparticles were characterized by multiple types of tools, such as TEM, HRTEM, XRD, XPS, to reveal the physico-chemical properties of these particles, while for the electrocatalytic properties, CV, CO-stripping and electrochemical impedance spectroscopy (EIS) were adopted to characterize their electrocatalytic properties.

Ali Reza Kamali asked: Very nice work! The core/shell nanostructure that you suggest, with copper located at the center of the nanoparticles and Pt just on the surface, is very interesting, and may find many applications in different fields. However, it might be difficult to come to this conclusion based on Fig. 1 presented in the article (DOI: 10.1039/d1fd00047k). Have you conducted further characterization to further support this suggestion? Thank you.

Weilin Xu answered: Thanks for your comment here. Indeed, Fig. 1 of the paper (DOI: 10.1039/d1fd00047k) shows that the Cu may be surrounded by Pt skin, while the further XPS results shown in Fig. 2b of the paper, which show that the only metallic Cu(0) can be detected with no Cu oxide, can further support that the Cu atoms are in the core with a Pt skin outside. Of course, more direct evidence could be obtained with some other complex tools, such as STEM-EELS, through which we probably can directly see its core/shell structure. Thanks for your comment. We will try this in future if such tool is available.

Ali Reza Kamali commented: As a comment, further characterisation such as STEM-EELS analysis might be helpful. Thanks.

Weilin Xu responded: Yes, more complex tools, such as EEL, can be adopted to characterize the microstructure of the alloy nanoparticles. If it can be done deeply, more precise information can be revealed for a better understanding of its morphology control. Indeed, the precise structure of the Pt nanoparticles could be characterized more clearly *via* tools such as STEM-EELS. We will try this in future if such tools are available. While in the present, based on some other results obtained, such as those shown in Fig. 1 and 2 of the paper (DOI: 10.1039/d1fd00047k), we still can tell the possible structure of the particles we obtained in this work.

Hui Ma asked: Thank you for your wonderful talk. In this work, you provide a great method to fabricate Pt–Cu nanoparticles. Could this method be extended to other materials? And how can the thickness of the shell be controlled?

Weilin Xu replied: Thanks for your comment on our work. Basically, such a method can be extended to the synthesis of some other nanomaterials with different metals, for example, with the same protocol, we have synthesized PtFe and PtCo alloy nanoparticles with similar structures. For the thickness of the Pt shell, in the present, we found that it can be tuned or controlled simply by varying the mass ratio between Pt and Cu.

Yi-Tao Long opened a general discussion of the paper by Ayumi Hirano-Iwata: Have you tried different materials for supporting the bilayer? Could you please provide more details about the bilayer formation?

Ayumi Hirano-Iwata responded: We have tried SiN/Si, Teflon, and porous Al_2O_3 as materials for suspending BLMs. All of them were useful for BLM formation. BLMs in SiN/Si chips were highly stable, but channel incorporation was sometimes very difficult. BLMs in Teflon chips were fragile, and they were often broken by mechanical shocks, including solution exchanges. However, the incorporation of biological channels was more frequently observed. BLMs in porous Al_2O_3 exhibited an intermediate nature between the SiN/Si and Teflon systems. BLMs were formed by the monolayer-folding method as follows. The membrane supporting material was placed in a Teflon recording chamber so that the supporting material separated the two compartments. A buffer solution was added to each compartment. The level of the buffer surface was set to below the aperture. A lipid solution was then carefully spread on the buffer solution in each compartment. After evaporation of the solvent, a BLM was formed by gradually raising the buffer level until it surpassed the aperture. A more detailed procedure was described in our previous paper.[1] For the Teflon and porous Al_2O_3 systems, pretreatment of the aperture edge with *n*-hexadecane was necessary before adding the buffer solution.

[1] D. Yamaura, D. Tadaki, S. Araki, M. Yoshida, K. Arata, T. Ohori, K. Ishibashi, M. Kato, T. Ma, R. Miyata, H. Yamamoto, R. Tero, M. Sakuraba, T. Ogino, M. Niwano and A. Hirano-Iwata, *Langmuir*, 2018, **34**, 5615.

Hui Ma commented: I notice that the noise of the chip at different potentials is quite different. I am curious about what caused this phenomenon?

Ayumi Hirano-Iwata answered: This is a difficult question to answer. As shown in Fig. 5 of the paper (DOI: 10.1039/d1fd00045d), the noise level varied with the lateral voltage in an unsystematic manner. We noticed that the noise currents at the lateral voltage of 1 V were larger than those at different lateral voltages. But we do not know the reason for the unsystematic noise level.

Hui Ma said: In Fig. 5 of your paper (DOI: 10.1039/d1fd00045d), the noise of your system under light is greater than that in dark. What caused this phenomenon? Is it because the lipid bilayer is unstable? Will this influence the current recording?

Ayumi Hirano-Iwata replied: There is still a lot we do not know about noise. As you commented, we often observed higher noise currents under the light than in the dark. However, we sometimes observed the opposite phenomena, for example, for PCBM-doped BLMs at lateral voltages of 0 and 1 V. At the moment, we have no clear answer to the unsystematic noise level. But the noise current was still lower than the photo-induced current, which enabled us to detect the photoresponse.

Yi-Lun Ying enquired: The structure of the electrode-equipped Si chip plays an important role in the formation of the bilayer. Is there any simulation model available for the design of the chip?

Ayumi Hirano-Iwata replied: Unfortunately, we do not have a good simulation model for the chip design. At the moment, we designed the shape of the SiN aperture edge based on the stability of the BLMs and SiN septa; the thinner the edge angle of the SiN aperture, the more stable the BLMs, but the SiN aperture itself becomes more fragile. As a result of this trade-off, the edge angle is currently 45°.

Shelley Minteer responded: Not that I am aware of.

Popular Pandey said: Thank you for the nice presentation. Why was an electrode-equipped Si chip used for PCBM-doped BLMs, and an electrode-equipped Teflon chip used for BLMs incorporated with voltage-gated sodium channels? Also, could you please comment on why the current noise level for the two BLMs systems are very different at applied AC/DC voltages?

Ayumi Hirano-Iwata replied: BLMs formed in the electrode-equipped Si chips were more stable with tolerance to a high transmembrane voltage of ±1 V, while BLMs in the electrode-equipped Teflon chips ruptured at ±1 V. For sensor applications like PCBM-doped BLMs as a light-sensitive membrane, the higher applied transmembrane voltage is preferable to obtain larger responses. Therefore, we used the Si chip platform for the PCBM-doped BLMs. On the other hand, for recording channel activities, the probability of ion channel incorporation was sometimes more important than the stability of the BLMs, especially for ion channels with low open probability, like voltage-gated sodium channels (Na_v channels). From our experience, incorporation of the $Na_v1.5$ channel was more frequently observed for BLMs in the electrode-equipped Teflon chips than those in the electrode-equipped Si chips. We chose the Teflon chip platform for recording ion channel activities.

Regarding the current noise level for the two BLM systems, the pronounced differences lie in the flexibility of the supporting material (Teflon *vs.* Si_3N_4) and the necessity of *n*-hexadecane to be present around the aperture, as discussed in the paper in detail (DOI: 10.1039/d1fd00045d). In brief, we tentatively assume that the BLMs formed in the flexible Teflon films are likely to vibrate mechanically due to the Coulomb force from the AC lateral voltage, generating a displacement current noise near the BLM surface. On the other hand, the noise would be suppressed in the Si chip due to its mechanical stability. At the same time, because it is necessary to apply *n*-hexadecane near the aperture to facilitate BLM formation on Teflon chips, the edge of the lateral electrodes around the aperture would be in indirect contact with the BLM. A DC lateral voltage would not induce an overly large electric field around the edge of the lateral electrodes. In the Si chip systems, *n*-hexadecane is not required to form BLMs. The BLM is in direct contact with the SiO_2-protected electrodes at the edge of the aperture. We speculate that a larger electric field would be generated at the sharp edges of the lateral electrodes under the lateral DC voltage, resulting in high noise.

Shane O'Neill asked: Do you know if the size of the lipid bilayer has an influence on the lateral voltage effect on the transmembrane voltage or current?

Ayumi Hirano-Iwata answered: Since we used *n*-hexadecane for the pretreatment of the aperture edge, the BLMs were surrounded by the *n*-hexadecane torus in the aperture. The size of the BLMs varied from membrane to membrane due to the *n*-hexadecane torus, even if they were formed in the same aperture. Nonetheless, from our experience, the lateral voltage was most efficiently applied to BLMs when the BLMs were formed in the apertures of 120 ± 20 μm. When we used apertures larger than 150 μm, the effect of the lateral voltage was rarely observed. With apertures smaller than 100 μm, *n*-hexadecane often clogged them. Based on these observations, we usually used an aperture size in the range of 100–140 μm.

Martin A. Edwards asked: Do you know the mechanism of the behavior you observed? Did you investigate how the behavior varies as a function of the PCBM concentration? Perhaps there is a critical concentration required to observe this behavior, or you will gain some other insight from doing this.

Ayumi Hirano-Iwata replied: The mechanism of the photo-induced current observed for BLMs doped with PCBM is still under investigation. We have examined the PCBM concentrations of 1, 2, and 4 mg mL^{-1} in the lipid solution to form the BLMs. Much higher responses were observed at the PCBM concentration of 2 mg mL^{-1} (present study, DOI: 10.1039/d1fd00045d) compared to at 1 mg mL^{-1}. However, the photoresponse was quite unstable at 4 mg mL^{-1} due to the formation of the PCBM aggregates.

Yi-Tao Long said: Nature uses ion channels for ion transports. The crystal size of ion channel protein is around or under 1 nm. Usually, the channel surface is made from one molecule. Grouping the amino acids together provides the confined chemical and physical environments for single entity sensing. Could you please comment on perspective of the artificial bilayer and channel system?

Ayumi Hirano-Iwata responded: There would be two possible directions for the artificial lipid bilayer systems; one is a platform for single-molecule sensing, including DNA sequencing, the other is a platform for the functional analysis of membrane proteins, including ion channels. So far, BLM systems with reconstituted ion channels have mainly been used for the single-channel analysis of bacterial channels and have provided insight into the channel gating mechanism. On the other hand, for human ion channel analysis, the whole-cell patch-clamp method has exclusively contributed. However, single-channel analysis is essential for further understanding human channel functions and the pharmaceutical and medicinal applications. For this purpose, new factors are necessary that can extend the regulation of the highly complex human channel gating mechanisms. We believe that the lateral voltage has a high potential as that new regulatory factor. Now that cell-free expression systems have made it possible to synthesize various ion channels, the combination of synthesized human channels and lateral voltages opens up a new frontier in ion channel science.

Conflicts of interest

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