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DISCUSSIONS

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Analytical SERS: general discussion

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Jeremy Baumberg, Steven Bell, Alois Bonifacio,
Rohit Chikkaraddy, Paul Dawson, Bart de Nijs,
Volker Deckert, Ines Delfino, Giuliana Di Martino,
Olga Eremina, Karen Faulds, Augustus Fountain,
Sylwester Gawinkowski, Mayte Gomez Castano, Roy Goodacre,
Jennifer Gracie, Duncan Graham, Jason Guicheteau,
Laurence Hardwick, Mike Hardy, Christian Heck,
Lauren Jamieson, Marlous Kamp, Alex Keeler, Christian Kuttner,
Judith Langer, Sumeet Mahajan, Natalia Martín Sabanés,
Kei Murakoshi, Marc Porter, George Schatz, Sebastian Schlücker,
Zhongqun Tian, Ashish Tripathi, Richard Van Duyne and Peter Vikesland

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George Schatz opened a general discussion of the paper by Zhong-Qun Tian: The dependence of Raman intensities with the angle of incidence and angle of scattering is an important issue. This was described for flat surfaces long ago (before SERS) by Greenler and Schlager.[1] How do your results differ?

[1] R. G. Greenler and T. L. Slager, *Spectrochim. Acta, Part A*, 1973, **29**, 193-201.

Zhong-Qun Tian responded: Thank you George very much for reminding us of the pioneering work by Greenler and Slager in 1973. However, our results differ from theirs considerably in three aspects.

(1) What they reported is the optimal incident angle (around 70° from the normal) and collection angle (around 60° from the normal) for a Raman measurement of a thin-film sample on a flat silver or nickel substrate using a 488 nm laser in the absence of plasmonic nanoparticles. Here, we consider the important coupling between the nanoparticles and flat substrate. Nanoparticles with different sizes may have different behavior.

(2) They only reported the metallic substrate materials, such as silver, nickel. Here, we studied not only the representative transition metal substrates such as platinum, but we also did several non-metallic material substrates with emphasis on the materials with a different refractive index, such as alumina. As we know, it is much more difficult to get Raman signals from low-refractive-index surfaces than from high-refractive-index surfaces or metallic surfaces.

(3) In this Discussion, we aim to explore the effective methods to measure Raman signals from weakly SERS-active materials or SERS-inactive materials. The

first idea is to excite at a high angle and collect the signal with high numerical aperture (NA) simultaneously. If a tilted lens is used for side excitation and collection, as usually adopted in TERS, only a backscattering signal will be collected, which is not efficient enough. Here, we suggest an alternative way by designing an upright objective which supports high-angle incidence and supports high NA as well.

Jeremy Baumberg said: For experiments on irradiating SHINERS, despite the silica shell it has been shown[1] that shape changes are possible (he used nanorods, showing that they can easily become spherical under irradiation, despite the silica).

What do you know about the migration of gold atoms in SHINERS? This is not necessarily melting, since it is low energy surface atom migration. The nanoparticle on mirror geometry is very sensitive to morphology.[2,3]

Have you looked at a single SHINER on a surface? There are sphere/cube differences that do not make this go away,[4] so how do you feel that cubes will really help here?

[1] *NanoLett.*, 2016, **16**, 1818–1825.

[2] J. Mertens, A. Demetriadou, R. W. Bowman, F. Benz, M.-E. Kleemann, C. Tserkezis, Y. Shi, H. Y. Yang, O. Hess, J. Aizpurua and J. J. Baumberg, Tracking Optical Welding through Groove Modes in Plasmonic Nanocavities, *Nano Lett.*, 2016, **16**, 5605–5611.

[3] C. Tserkezis, R. Esteban, D. O. Sigle, J. Mertens, L. O. Herrmann, J. J. Baumberg and J. Aizpurua, Hybridization of plasmonic antenna and cavity modes: Extreme optics of nanoparticle-on-mirror nanogaps, *Phys. Rev. A*, 2015, **92**, 053811.

[4] R. Chikkaraddy *et al.*, How Ultranarrow Gap Symmetries Control Plasmonic Nanocavity Modes, *ACS Photonics*, 2017, **4**, 469–475.

Zhong-Qun Tian replied: Thank you for raising this very interesting question and several important papers. One of the reasons that we develop SHINERS is to make the hotspot much more stable. The surface diffusion of gold atoms under laser illumination makes the shape of the bare gold nanoparticles change easily, which may cause the problems related to experimental reproducibility, while the over-coated shell materials will significantly slow down the surface diffusion. However, you are right that even for the core-shell nanoparticles, surface diffusion cannot be fully ruled out. The degree of the surface diffusion depends on many factors, *e.g.* high or low laser power density, a liquid or gas environment, Au or Ag core materials, SiO_2 or Si shell materials, compact or loose shell structure, *etc.* Therefore, when applying the measurement, we can carefully choose the right experimental parameters to avoid the surface diffusion causing changes in the SERS activity of the hotspot.

We can get high-quality single-nanoparticle SHINER spectra on Au flat surfaces. Here I mean the real single nanoparticle rather than single nanoparticle-dimers or aggregated forms. Your question in fact suggests an interesting approach to utilize a SHINERS system to study some special surface diffusion at a solid-solid interface if we locate a single SHINERS particle on a flat Au substrate. The SHINERS signal intensity will change when surface diffusion of the Au atom at the interface of the Au core-shell occurs because it will change the gap of the hotspot. Since the surface diffusion is very sensitive to the morphology of the nanoparticle, we can have a systematic test by changing the nanoparticle from a sphere to cube, bar or other shapes. Our simulation shows that the average enhancement of a single-nanocube SHINERS is always much larger than that of a single-nanosphere SHINERS no matter which materials are used for the substrates, metallic or non-metallic. The relevant simulation on the possible surface diffusion could also be very interesting.

Heike Arnolds asked: Can SHINERS be used to monitor analytes or chemical reactions directly in the liquid phase, when the particles are dispersed in the liquid and not attached to a surface?

Zhong-Qun Tian replied: Thank you for highlighting this important question not only for SHINERS but also for SERS. It is worthy although very challenging to develop shell-isolated nanoparticles to directly monitor analytes or chemical reactions in the liquid phase. It critically depends on whether the analyte or reaction product can be strongly adsorbed onto the surface of Au/Ag nanoparticles for SERS or shell materials for SHINERS. In many cases they are not strong adsorbates, so one should rationally design the linker to modify on the shell to pre-concentrate the analyte selectively. It should be noted that typically, shell-isolated nanoparticles such as Au@SiO_2 cannot aggregate as easily as bare nanoparticles. Here, there could be two ways to expand the application of SHINERS in liquids. First, shell-isolated nanoparticles, *e.g.* Au@SiO_2 such as nanorods, nanocubes, nanobars and nanostars with higher SERS activity, are strongly suggested for the development of single-particle SHINERS in liquids. However, it is challenging to overcoat these nanoparticles with ultrathin shells. Second, shell-isolated nanoparticles with 2D materials such as graphene with inter-particle van der Waals forces are suggested for the development of multi-particle SHINERS in liquids. However, it might be challenging to perform the quantitative SHINERS measurement because the multi-particle electromagnetic coupling from various hotspots is much more complex. In addition, for studying chemical reactions, the 2D shell materials should be chemically inert to avoid any interference to the chemical reaction. The combination of micro-fluidics and SERS/SHINERS could be a good solution for this direction.

Rohit Chikkaraddy asked: The proposed nanoparticle on a plasmonic film has sharp plasmon resonance in the near-IR region, especially at small gaps. Does this limit the enhancement of SERS as it is hard to cover in-coupling light and out-going Raman light into this resonance? Is there any possibility of harvesting two different plasmon resonances, one for coupling-in and another to couple-out? Is there any benefit of doing such SERS experiments?

Zhong-Qun Tian responded: Thank you for this interesting question. We do agree with your comment that bare gold or silver nanoparticles on a flat plasmonic film show sharp resonance in the near-IR spectral range if the nanogap is less than 1 nm or the length of the contacting facet of the nanoparticle is long. However, if the nanoparticle is a shell-isolated nanoparticle such as Au@SiO_2 with a shell thickness of 2–4 nm, then the story is quite different as the coupling between the shell-isolated nanoparticle and the plasmonic film is not strong enough to produce narrow and strong plasmon resonance in the near-IR region. In other words, the isolating dielectric shell can control the particle-film coupling beyond strong coupling. Secondly, our simulation showed that it is possible to design a multiparticle-on-film system such as multiple Au@SiO_2 particles on a flat gold surface (see Fig. 26 and 27 from *Chem Soc Rev*)[1] or a single Ag@SiO_2 nanoparticle on a flat facet on a flat Ag surface for multiple resonance due to the interference between a broad antenna scattering mode and a series of waveguide cavity modes (See *IEEE Transaction on nanotechnology*, Fig. 2).[2] In the second case, side illumination is usually necessary to excite the interfered modes. Overall, the SERS study has to move forward from simple bare Au/Ag sphere nanoparticles on Au/Ag films to more rationally designed nanostructures for the higher efficiency and an optimal spectral region as well as better versatility.

1 S.-Y. Ding, E.-M. You, Z.-Q. Tian and M. Moskovits, *Chem. Soc. Rev.*, 2017, **46**, 4042–4076.
2 B. Joshi, A. Chakrabarty and Q.-H. Wei, *IEEE Trans. Nanotechnol.*, 2010, **9**, 701–707.

Christian Kuttner commented: SHINERS particles have been defined as nanoparticles that exhibit an isolating shell that renders them electrically and chemically inert. Could you comment on the definition of SHINERS particles? Could nanoparticles with soft macromolecular shells (*e.g.* polymers[1] and proteins[2,3]), which are not inherently chemically/electrically inert, be included in the family of SHINERS particles?

1 C. Kuttner, M. Chanana, M. Karg and A. Fery, Macromolecular Decoration of Nanoparticles for Guiding Self-Assembly in 2D and 3D, *Macromolecular Self-Assembly*, 2016, 159–192, ISBN: 978-1118887127.
2 M. Tebbe, C. Kuttner, M. Männel, A. Fery and M. Chanana, *ACS Appl. Mater. Interfaces*, 2015, **7**, 5984–5991.
3 R. P. M. Höller, M. Dulle, S. Thomä, M. Mayer, A. M. Steiner, S. Förster, A. Fery, C. Kuttner and M. Chanana, *ACS Nano*, 2016, **10**, 5740–5750.

Zhong-Qun Tian responded: Thank you for your question and for highlighting those interesting papers. It's better to start this discussion on why we need SHINERS. The advantages of SHINERS are fourfold. First, the ultrathin yet pinhole-free shells separate the cores from the material surface (and the environment), thus ensuring that there is almost no interference from the Au and Ag cores. Second, the chemically inert shell effectively avoids interparticle and particle-metal substrate fusion, which significantly improves the stability of the nanoparticles and the probe structures. Third, the shell thickness can be used to control the nanogap between the Au or Ag core particle and the substrate, and consequently determines the particle-substrate electromagnetic coupling. Last, the Au and Ag cores can boost the local electromagnetic field to enhance the Raman signals from the probe substrate without distorting its structure. Your question is related to how one can expand the SHINERS application. But meanwhile one should also consider how to avoid interference to the studied system by introducing the nanoparticles. It is evident that one of the priorities for any spectroscopic and analytical studies is to avoid the interferences that can mislead the analytical data. The chemically and electrically inert shell can achieve this goal at least to a large extent. We published a detailed discussion on this important issue with several pages in the supporting information of our first SHINERS paper published in *Nature* in 2010.[1] Briefly the idea of SHINERS is to prevent strong chemical interactions such as chemisorption, chemical or photochemical reactions of the analytes which could directly cause contact with the surface of the gold or silver nanoparticles. Any strong chemical or electrical interference will distort the system to be studied and provide misleading spectral information, as shown in Fig. 1.[1] Therefore, the shell materials are strongly recommended to be chemically inert to the system to be studied. The thinnest shell materials are 2D inorganic materials, such as graphene and hexagonal boron nitride (h-BN). Based on the simulation, their SHINERS sensitivity could be further increased by one order of magnitude. However, in practice we have not found a good way to completely avoid creating pinholes in these materials.

Accordingly the shell-isolated strategy can also be extended to 2D organic molecular SAM layers and your soft macromolecular shells. Generally speaking, polymers and proteins are not inherently chemically/electrically inert. They are difficult to form a compact and stable shell being pinhole free to meet the definition of SHINERS in terms of isolation, so they are not the best choice unless these macromolecules can play a special role for some specific purposes such as the linker to catch some specific analytes in live cells. Moreover, it should be noted that the soft macromolecular shell has many more Raman bands in comparison with silica, which may strongly interfere with the Raman signals of the probed molecules.

[1] J. F. Li, Y. F. Huang, Y. Ding, Z. L. Yang, S. B. Li, X. S. Zhou, F. R. Fan, W. Zhang, Z. Y. Zhou, D. Y. Wu, B. Ren, Z. L. Wang and Z. Q. Tian, Shell-isolated nanoparticle-enhanced Raman spectroscopy, *Nature*, 2010, **464**, 392–395, ESI†.

Sebastian Schlücker questioned: The ultra-thin silica shell of the SHINERS is prepared using sol–gel chemistry which involves a condensation reaction. Hydrolysis is the reverse reaction which potentially degrades the shell and

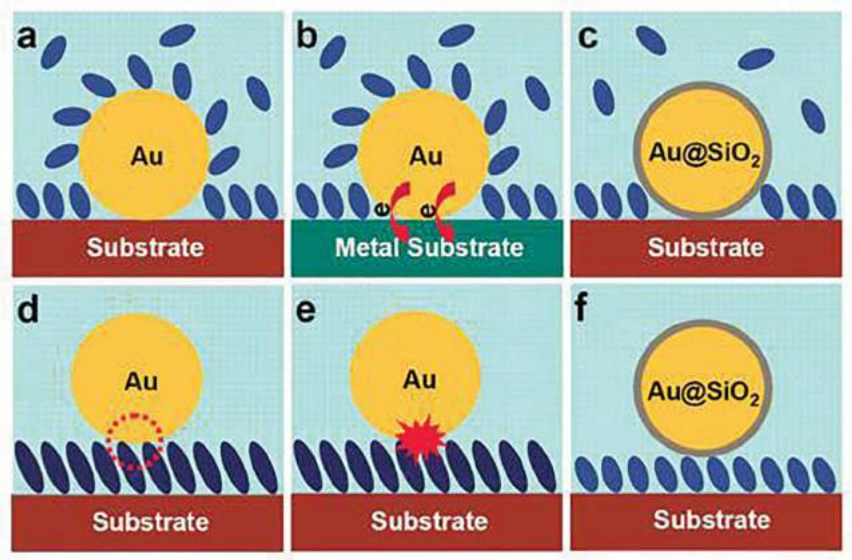

Fig. 1 Schematic illustration of six different surface configurations for SERS or SHINERS measurements[1]

therefore the stability of the nanoparticles. How critical is this and what are the practical implications for the users of SHINERS?

Zhong-Qun Tian answered: Yes, this is what we have found for the reverse hydrolysis reaction on the SiO_2 shell system since we invented the SHINERS technique in 2008. There are several ways to overcome this problem. (1) The simplest way we suggested to other groups is to prepare SHINERS particles that take about one hour, then do the measurement soon after that for best results. (2) If the experiment has to be done later, it's necessary to centrifuge the SHINERS nanoparticles by removing most of the reactant in solution, then storing them in a refrigerator at 4 °C. By doing so the $Au@SiO_2$ particles with a shell thickness of 3–4 nm can be used within 6 months. However, if the shell thickness is below 2 nm, the life time will be considerably shortened. This means that if one wants to gain the highest sensitivity, the thinnest shell is necessary, so option 1 is recommended. (3) We can also first let the pinhole develop then introduce other inert molecules to fill the pinhole. For some experiments, the pinholes are useful because we can introduce molecular linkers to go through the pinholes to stick strongly to the Au core surface. If these linkers are modified on the silica surface, the gap width between two nanoparticles could be too large to create the strong electromagnetic field coupling. (4) It should be pointed out that silica is the first shell material we used for SHINERS, but not the best. In addition to the shell degradation issue discussed above, another drawback is that silica cannot work in high pH systems such as pH > 12, so the term of a chemically inert shell is really environmental dependence. Overall, there is plenty of room to develop different methods on different shell materials to optimize the stability and sensitivity of SHINERS.

Laurence Hardwick communicated: You stated that silica is not an optimum coating for SHINERS particles and there are better coatings. What would you recommend as an alternative?

Zhong-Qun Tian answered: Based on the simulation performed by my colleague, Dr S. Y. Ding, the shell-isolated nanoparticles with shell materials having a high refractive index could improve the detection sensitivity by ten times or more. Accordingly diamond-like carbon, gallium phosphide or silicon could be the best choice in the visible spectral range. We have been working along this direction but it turned out to be extremely difficult in practice to overcoat these hard materials as the shell uniformly.

Mayte Gomez Castano communicated: In order to be able to detect SHINERS enhancement on non-metallic surfaces, you have proposed two alternatives: either using side illumination at high angles or changing your nanoparticle shape to a nanocube. In this context, I was wondering if you have performed any simulations combining these two aspects (high angle of incidence plus a non-spherical shape), and if in these conditions the local field enhancement could be even stronger than for a normal incidence on the single-nanocube system.

In addition, I would like to know if these results for a single shell-isolated nanoparticle could change if we extend them to a non-metallic surface covered by nano-cubes or nano-spheres. Could the order of the entire system, a hexagonal array of nanospheres for instance, play a role and improve the SERS response due to the additional coupling to the lattice?

Zhong-Qun Tian replied: Thanks for your suggestion. The combination of two strategies (design special objective supporting high incident angle and high numerical aperture; using non-spherical shape nanoparticles) will synergistically lead to stronger local field enhancement. Regarding multiparticle SHINERS, yes, we have performed many simulations on various systems. The results clearly show that the additional coupling of arrays of nanoparticles will further increase the SERS enhancement. This strategy will be helpful to gain a better quality Raman signal from a great variety of non-metallic surfaces. For many practical applications, the qualitative study may be sufficient. It's fine to have an ill-defined arrangement or even random distribution of nanoparticles spread over the substrate surface. However, it is challenging to make an ordered and even well-defined arrangement of particles on the substrate surface to be studied. This is an important direction for not only SHINERS but also SERS in order to have quantitative studies.

George Schatz opened a general discussion of the paper by Laurence Hardwick: When you put SHINERS in contact with lithium metal, the possibility arises that you might excite the plasmon excitation in the lithium, and this could influence the SERS measurements. Is this important for your experiment?

Laurence Hardwick responded: Yes, the possibility of exciting the surface plasmon in lithium metal was considered. However, lithium metal is very reductive and so there is a native oxide film on the surface. The thickness of the layer is not determined, but we believe that the thickness is sufficient to exclude the surface enhancement of lithium foil within our experimental conditions. Tang *et al.*[1] did attempt to take advantage of the surface plasmon of nanostructured lithium, but saw only modest enhancements. The enhancement of the Raman signal from the film on lithium metal that we wished to examine using SHINERS particles (SiO_2 coated Au) was in the order of 2–5x. This was sufficient to detect differences in the Raman spectra of the amorphous films after the lithium metal sheets had been exposed to different battery electrolytes, but we were unable to obtain significantly enhanced Raman spectra.

1 Z.-Q. Tang *et al.*, *J. Raman Spectrosc.*, 2016, **47**, 1017–1023.

Richard Van Duyne addressed Laurence Hardwick: Regarding your figures about different oxygen species, can you distinguish adsorbed species, *e.g.* O_2^-, from compounds such as lithium oxides? The signatures are pretty clear for separating peroxo species from superoxo. It would be very useful to do an ^{18}O experiment, as this would allow for a strong handle for separating compounds from adsorbed species.

Laurence Hardwick answered: Yes we can distinguish the various oxygen species. Adsorbed O_2^- tends to have a Raman band at *ca.* 1110 cm^{-1}, where as LiO_2(ad) has a band at 1125 cm^{-1}. Peroxy species, such as Li_2O_2, have a band at 790 cm^{-1}.

We have attempted $^{18}O_2$ experiments, but so far we have found that the gas supplied is not as dry as we would prefer. After bubbling $^{18}O_2$, the water content in the electrolyte is found to be *ca.* 100 ppm H_2O, which is high enough to significantly affect the electrochemistry. Generally we aim to have a H_2O content of below 20 ppm. In the data obtained so far (as yet unpublished) we see smaller shifts in the spectral bands than expected and we are not sure whether this is a result of the water contamination. We are working towards solving these experimental issues.

Richard Van Duyne addressed Laurence Hardwick and Zhong-Qun Tian: The lithium plasmon resonances should be in the blue region, not at around 600 nm. They should be pretty strong. Was this experiment done in a non-aqueous solvent?

Zhong-Qun Tian replied: Thank you Rick for this comment. Indeed, the intrinsic resonances of small lithium particles are normally located in the blue region (around 440 nm), as can be seen in Fig. 2. However, it can also be easily tuned to the red region *via* enlarging the particle size and/or forming the dimer or other particle aggregations. For a single lithium sphere with a radius of 45 nm, the plasmon resonance is red shifted to around 600 nm, but the intensity decreases considerably. The best way is to form the lithium particle dimer or oligomers. As

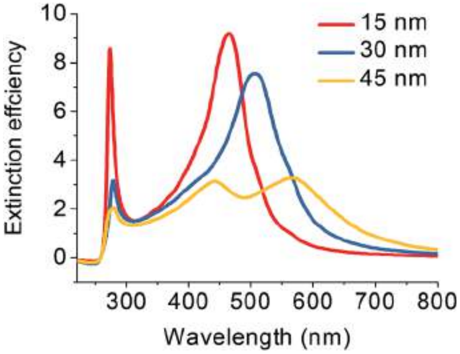

Fig. 2 Extinction spectra of single lithium spheres with various radii in propylene carbonate which is commonly used for Li batteries.

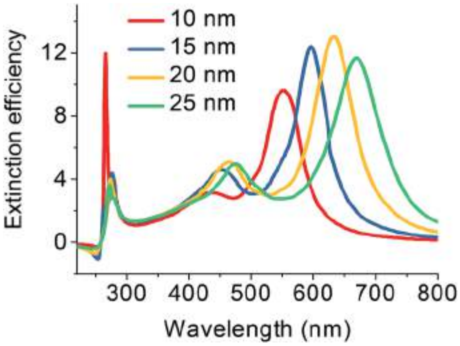

Fig. 3 Extinction spectra of lithium dimers with various radii in propylene carbonate. The inter-particle gap is 2 nm.

shown in Fig. 3, for the dimer with a gap of 2 nm in propylene carbonate which is commonly used in lithium ion batteries, the resonance can be further red tuned whilst still maintaining a good extinction efficiency.

Laurence Hardwick replied: In this experiment we did not intend to use lithium metal as the SERS active substrate - rather than SHINERS particles that were put down upon the lithium surface. The SHINERS particles have a plasmon resonance near 600 nm.

The experiment is conducted in a non-aqueous solvent, either organic carbonate or ether based.

Marlous Kamp asked: Such interesting work! I am not very familiar with battery materials, however I have worked with $LiCoO_2$ before. This made me wonder what other battery materials may be interesting to study, an idea you already mention in the paper's outlook. Can you comment on what results we may expect with, for example, $LiCoO_2$ and LiC_6 batteries? Those materials have the additional advantage that both allow us to deposit the SHINs directly onto the battery material.

Laurence Hardwick answered: One area we are actively working on is to use SHINERS to detect the formation of the solid electrolyte interphase on carbon negative electrodes of Li-ion batteries. Passivation layers on positive electrodes such as $LiCoO_2$ are much thinner, so we thought a good place to begin was to look at the thicker surface layers on negative electrodes. We have looked at other negative electrodes such as $ZnFe_2O_4$ and observed the appearance of bands that we have tentatively assigned to the growth of a polyethylene oxide type layer.

Steven Bell asked: I guess that the signal intensity depends on the number of particles within the area probed by the laser. Do you take special steps to maximise the density of particles packed together on the surface? Do you have any control over that?

Laurence Hardwick answered: Yes, the signal intensity depends of the density of particles at the surface within *ca*. a 2 micron squared area of the laser spot. We have generally used the method of putting an aqueous droplet of a low concentration of SHINERS particles and allowing it to dry slowly either over a few hours or overnight. Fig. 4 and 5 in our paper[1] presented at this Faraday Discussion concern our efforts to understand how the distribution of the SHINs affects the signal intensity. Our work continues in the direction of developing more reproducible methods of evenly dispersing these particles onto substrates.

1 T. A. Galloway, L. Cabo-Fernandez, I. M. Aldous, F. Braga and L. J. Hardwick, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00151g.

Jeremy Baumberg enquired: For lithium carbonate you have indexed these lines. How secure is this assignment? How do you know this is lithium carbonate?

Laurence Hardwick answered: The assignment of the Li_2CO_3 bands appearing at the carbon black surface is based upon them being present at the expected wavenumber regions for Li_2CO_3, and also data (internal or literature) from other techniques such as FTIR and XPS that identify Li_2CO_3 as being present at the lithium-oxygen cell carbon cathode. We are continuing our work in this area particularly in the use of isotopic studies (C^{13} enriched carbon powder) to provide stronger experimental proof of these assignments.

Zhong-Qun Tian commented: So far probably over 90% of SERS studies have focused on species adsorbed or located in the hotspot. Your approach to study reaction intermediates and mechanisms is important but challenging especially for the lithium batteries that have a very complex mechanism. Many different reaction intermediates (some have a short life and some only exist in the special reaction environment) are hard to be distinguished because they may not have existing spectra from standard samples for comparison. Accordingly, high quality theoretical calculations on different intermediates are essential. Without help from theoreticians, it's really hard to tell what the reaction intermediates are and the whole mechanism (*e.g.* series or parallel reactions) is.

Laurence Hardwick responded: Indeed you are correct, we have recognised that the SHINERS generated data requires input from theoretical calculations due to its complexity. We have began in our *in situ* IR studies to complement experimental data with calculated expected bands of intermediates and we are working towards more complex calculations at interfaces.[1,2]

1 J. Padmanabhan Vivek, N. G. Berry, J. Zou, R. J. Nichols and L. J. Hardwick, *In Situ* Surface-Enhanced Infrared Spectroscopy to Identify Oxygen Reduction Products in Nonaqueous Metal-Oxygen Batteries, *J. Phys. Chem. C*, 2017, **121**, 19657–19667.

2 J. Padmanabhan Vivek, N. G. Berry, G. Papageorgiou, R. J. Nichols and L. J. Hardwick, Mechanistic Insight into the Superoxide Induced Ring Opening in Propylene Carbonate Based Electrolytes using *in situ* Surface-Enhanced Infrared Spectroscopy, *J. Amer. Chem. Soc.*, 2016, **138**, 3745–3751.

Kei Murakoshi addressed Zhong-Qun Tian and Laurence Hardwick: Your SHINERS particles are covered by a surface dielectric coating, but it could be possible to allow the penetration of target molecules, leading to the direct absorption of molecules onto the surface of core metals. In this case, the charge transfer (CT) effect may contribute to the enhancement as well as the electromagnetic (EM) effect. Is it possible to quantify the contributions of the respective effects by controlling the penetration, or by changing the choice of materials for the surface coating?

Laurence Hardwick answered: For our application in using the SHINERS particles to detect species at the electrode surface, we do not want to have exposed gold as this will make the analysis of the origin of the Raman peaks unclear as we will not know if the peaks are from the electrode surface or the gold core. Therefore a continuous (*ca.* 2–3 nm thick) pinhole free coating is required. Some applications may benefit from the presence of pinholes to detect absorbed species onto the gold core, but this is not our present motivation.

Zhong-Qun Tian responded: Thank you for your interesting question and suggestion. It's quite possible that the target molecules can penetrate into the shell and directly adsorb at the surface of the Au or Ag core if the shell has some pinholes. Generally speaking, we should avoid such things happening and that is why we emphasize that the shell quality should be high and pinhole free. Nevertheless it's relatively easy to check such a case simply by changing the substrate over-spread by the SHINERS particles, *e.g.* from gold or silver to a carbon or silicon substrate. If the SHINERS signal of the target molecules can still be detected, it reveals the existence of pinholes and confirms the additional chemical enhancement. In fact, if the shell is pinhole free, SHINERS could be one of the best systems to separate the contribution of the EM effect from the chemical enhancement. The compact dielectric shell can completely block charge transfer between the target molecule and Au/Ag surface. One can compare the SERS spectra with the SHINERS spectra of the target molecules, thus quantitatively analyzing the contribution by the chemical enhancement on the SERS spectra. We have not tried to quantify the contributions of the respective effects by changing the choice of materials for the surface coating. However my colleague, J. F. Li, has made another different approach. He used the shell pinholes on purpose as a channel to allocate a specific group of the target molecule to different position in the channel for systematical study.

Heike Arnolds opened a general discussion of the paper by Peter Vikesland: I think there is a great need to find reliable ways to compare surface enhanced Raman spectra across labs and your method can potentially address this. My group uses the value of the background SERS signal at a frequency without molecular peaks as a normalisation constant. In Fig. 4 shown, we have plotted the heights of various vibrational peaks of 4-mercaptopyridine adsorbed on gold as a function of the height of the background signal at 915 cm^{-1}.

The data in the top graph were obtained from a map of 132 spectra in a 5 μm raster across an electrochemically roughened gold surface (785 nm laser, Renishaw, 50x objective) and clearly show that the peak heights are proportional to the SERS background. The bottom graph shows how this can help to compare data from different students (Nungnit Wattanavichean and Ella Casey) and very different surfaces (here: roughened gold *versus* pinhole SHINs deposited on a Si wafer). This normalisation has helped us greatly in comparing data obtained using different laser systems, preparation methods and substrate morphologies, and has helped us to look at the effects of any *ex situ* surface treatments. I look forward to further development of your approach.

Peter Vikesland answered: Thank you for the comment. We have also tried to normalize to the background SERS signal and have found varying degrees of success. Our approach to normalize to the 'pseudo' Rayleigh band near the laser-cut off has continually proven to be more successful. It would be interesting to determine if our approach (obtained using a WITec system) translates to your Renishaw system or it if is system specific. While we have tested our hypothesis using two separate WITec systems, we have yet to try it on others.

Richard Van Duyne asked: You have to be careful of the terminology around photoluminescence and the background. The nature of the SERS background is still murky. It has been described as "junk" from decomposition products, and in clean systems it is thought of as electronic Raman scattering from the metal. It is

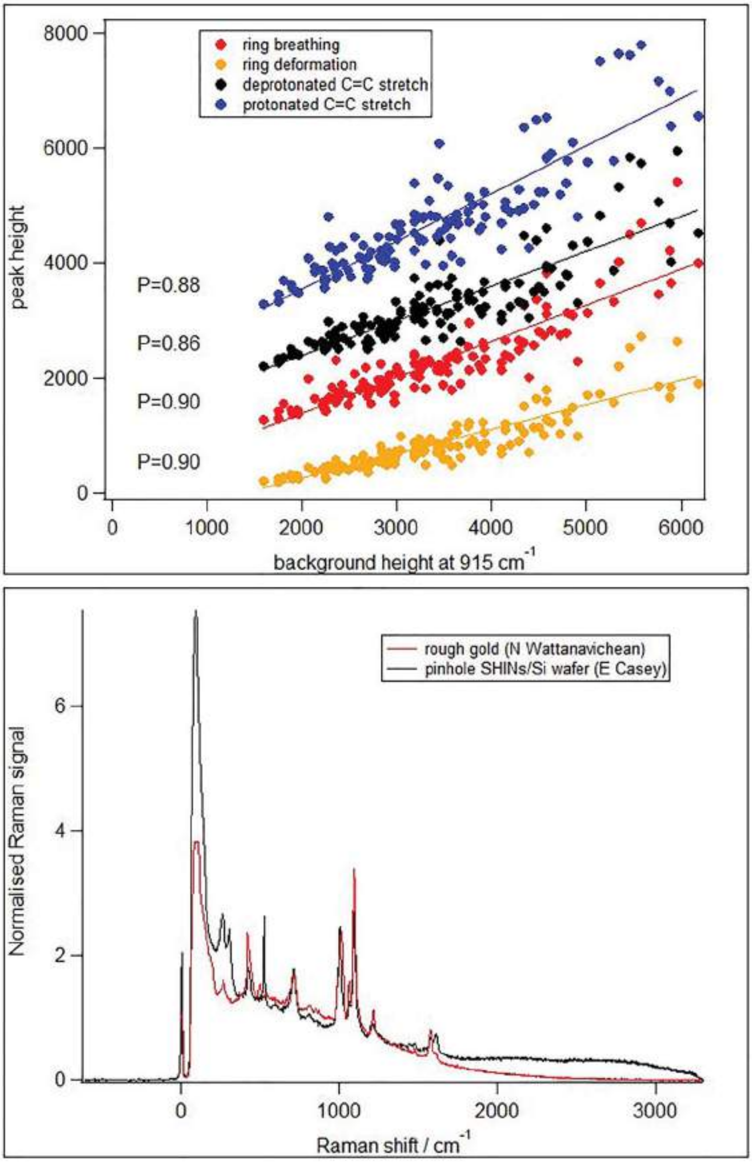

Fig. 4 Top: A plot of the heights of various vibrational peaks of 4-mercaptopyridine adsorbed on gold as a function of the height of the background signal at 915 cm^{-1}. Bottom: Comparison of 4-mercaptopyridine spectra on gold from different researchers and surfaces is made easier after background normalisation.

a real signal that scales with the intensity of the Raman signal. It's sort of a known quantity, even in UHV where you can show there are no other molecules on the surface. It's not luminescence as there's no lifetime to it.

Peter Vikesland replied: The reason we referred the SERS background to photoluminescence was that this term was used to describe SERS background in the recently published literature.[1–4] These papers have described photoluminescence as "the radiative damping of particle plasmons generated from the recombination of sp-band electrons and excited d-band holes". We agree that it is important to be careful when using the term photoluminescence since the processes that affect the SERS background are still under debate.

1 C. Lumdee, B. Yun and P. G. Kik, Gap-plasmon enhanced gold nanoparticle photoluminescence, *ACS Photonics*, 2014, **1**(11), 1224–1230.
2 Z. Guan, N. Gao, X.-F. Jiang, P. Yuan, F. Han and Q.-H. Xu, Huge enhancement in two-photon photoluminescence of au nanoparticle clusters revealed by single-particle spectroscopy, *J. Am. Chem. Soc.*, 2013, **135**(19), 7272–7277.
3 H. Hu, H. Duan, J. K. Yang and Z. X. Shen, Plasmon-modulated photoluminescence of individual gold nanostructures, *ACS Nano*, 2012, **6**(11), 10147–10155.
4 K.-Q. Lin, J. Yi, J.-H. Zhong, S. Hu, B.-J. Liu, J.-Y. Liu, C. Zong, Z.-C. Lei, X. Wang and J. Aizpurua, Plasmonic photoluminescence for recovering native chemical information from surface-enhanced Raman scattering, *Nat. Commun.*, 2017, **8**, 14891.

Volker Deckert remarked: In Fig. 3,[1] the "real" Rayleigh line at 0 cm^{-1} seems to be uniform for the different substrates. However, compared to the respective Rayleigh line used in the manuscript at 84 cm^{-1} the intensity (ratio) is quite different.

(1) Why was the intensity at 0 cm^{-1} not always used for the proposed Rayleigh normalization?

(2) How can the difference in the intensity ratio between the 0 cm^{-1} and the 84 cm^{-1} lines be explained, if both have the same origin (Rayleigh scattering)?

1 H. Wei, A. McCarthy, J. Song, W. Zhou and P. J. Vikesland, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00125h.

Peter Vikesland responded: This is a very good question and one that we have thought about in some detail. As noted by Dr Deckert, the intensity of the "real" Rayleigh line (at 0 cm^{-1}) is uniform, while the band at 84 cm^{-1} (or 126 cm^{-1} depending on the filter cutoff) varies in intensity. The uniformity in the peak at 0 cm^{-1} is due to the fact that we normalized each of the collected spectra to that band using a fixed intensity of 1000 CCD cts. This normalization was done simply

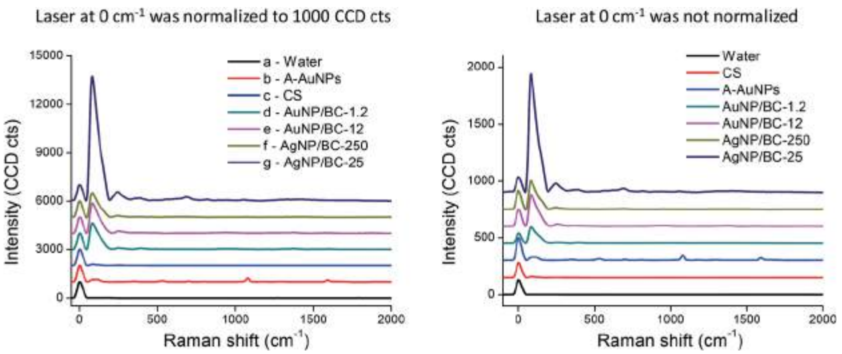

Fig. 5 (Left) Raman spectra of the SERS substrates in the absence of analyte addition (a. water; b. A-AuNPs; c. commercial substrate (CS); d. AuNP/BC-1.2; e. AuNP/BC-12; f. AgNP/BC-250; g. AgNP/BC-25) normalized by setting the peak at 0 cm^{-1} to 1000 CCD cts (NB: This is a replica of Fig. 2a in the presented paper). (Right) The non-normalized results. As shown there is little change in the reported intensity of the band at 84 cm^{-1}.

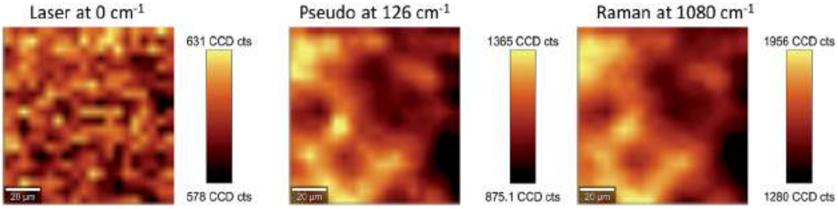

Fig. 6 Raman maps indicating the signal intensity of the 0 cm^{-1} (left panel), 126 cm^{-1} (center panel), and 1080 cm^{-1} (right panel) peaks for a AgNP/BC substrate exposed to 4-MBA. As shown, the Pseudo-band at 126 cm^{-1} correlates well with the SERS band at 1080 cm^{-1}, while the laser signal at 0 cm^{-1} does not correlate.

to account for minor differences in the collected signal (see Fig. 5) across the samples. In addition to using the band at 84 or 126 cm^{-1} we also attempted to use the measured signal at 0 cm^{-1} to normalize our collected data. As shown in Fig. 6, however, the locations where the SERS signal was highest do not correlate with the locations where the 0 cm^{-1} peak intensity was greatest. In contrast, the band at 126 cm^{-1} correlates quite well with the SERS signal across the substrate. We are presently investigating exactly why the band at 0 cm^{-1} does not scale with the SERS signal, while the ‘pseudo-band’ at 126 cm^{-1} (or 84 cm^{-1}) does.

Augustus Fountain said: If this is a real band or ‘pseudo-band’ as you call it, it should appear on the Stokes as well as the Anti-Stokes side of the spectrum. Did you check either side of the laser line to see if this is a real effect? If this is just the tail of the laser, the intensity should be equal on both sides. However, if it is truly inelastic scattering it should be more intense on the Stokes shifted side.

Peter Vikesland answered: We have not conducted any experiments on the other side of the laser line. We do agree, however, that such experiments would be very useful and would help to better delineate the source of this ‘band’.

Javier Aizpurua commented: I would like to elaborate a bit more on how to deal with the normalization of your data regarding the background subtraction. In the paper, one can see spectra already clean. When you subtract/divide the background, this will affect the intensities of all of the vibrational peaks, including the zero Rayleigh peak that you use to normalize. How do you deal with that? Would the particular treatment of the background affect the obtained correlation. My guess is that it would. I would like to call to your attention and to the attention of all of the attendees a procedure that we recently implemented together with the group of Bin Ren, in Xiamen, to retrieve the genuine information (peak intensity) of the vibrational fingerprints.[1] This method consists of dividing the SERS signal by the previously measured scattering of the antenna/substrate, or, alternatively, dividing the SERS signal by the background followed by multiplication by photoluminescence. I am wondering how the application of such a procedure to treat the background could modify the slope of the correlations that you find in your paper.

[1] K.-Q. Lin *et al.*, *Nat. Commun.*, 2017, **8**, 14891.

Peter Vikesland answered: We concur that background normalization can lead to substantial artifacts in the presented data. To that end we utilized a very simple baseline correction protocol as reported by P. H. C. Eilers.[1] It will be very interesting to examine how the protocol in the Lin *et al.* paper[2] affects our collected data.

1 P. H. C. Eilers, *Anal. Chem.*, 2004, **76**, 404–411.
2 K.-Q. Lin *et al.*, *Nat. Commun.*, 2017, **8**, 14891.

Alois Bonifacio asked: Many analytes interact with the metal surface *via* oxygen and nitrogen atoms, forming stable Ag-X bonds (X=N, O) and thus yielding Ag-X stretching bands in the 200–300 cm^{-1} region. Such bands are usually medium or intense, and rather broad. I expect such bands to overlap with the "pseudo-Rayleigh" band, making it difficult to use it as an internal standard for quantitative measurements.

Peter Vikesland responded: We agree that there are numerous bands that occur in the low wavenumber region. While we have not investigated how every potential analyte might overlap with the 'pseudo-Rayleigh' band, we have done a fair amount of work examining the signal from adsorbed halides with Raman bands in the 150–260 cm^{-1} region. As shown in Fig. 7 (excerpted from a paper currently in Press), each of these bands is readily distinguished from the 'pseudo-Rayleigh' band. Clearly, however, additional work needs to be done to ascertain how widely applicable our normalization approach is.

Ashish Tripathi commented: Regarding the slide with the spectra of 4-MBA with different concentrations (Fig. 2 of your paper[1]), the band at 84 cm^{-1} is a low frequency Raman band, also loosely called the THz Raman band. The vibrations

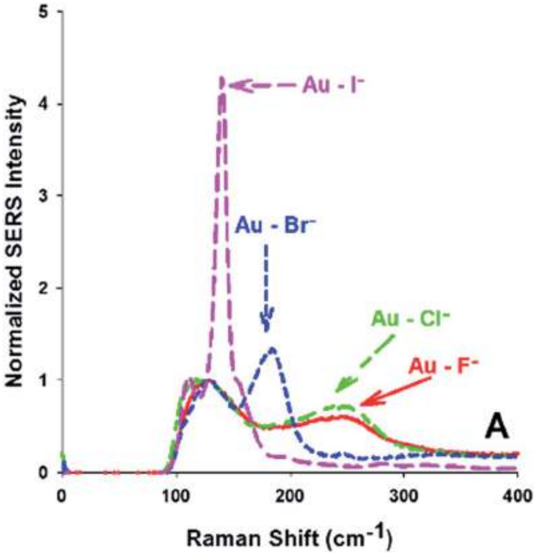

Fig. 7 SERS spectra of Au-X-gold surface interactions. This experiment was performed using aliquots of 1 mM NaX solutions and a commerical substrate.

in this region are reflective of low energy bonds, such as a hydrogen bond. The information at this region could help explain the binding nature of the analyte to the substrate. Features in the low-frequency region could also indicate the clustering or inter-molecular bonding (as in crystals) of the analyte.

1 H. Wei, A. McCarthy, J. Song, W. Zhou and P. J. Vikesland, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00125h.

Christian Kuttner communicated: You employ the pseudo-band near 84 cm^{-1} for normalisation of the SERS data. As a comment, nanoparticle dispersions with surfactants as stabilising agents might possess SERS signals close to this spectral feature. In particular, halide ions absorbed onto the surface of plasmonic nanoparticles have been reported at 176 cm^{-1} for Au–Br[1] and at 250 cm^{-1} for Au–Cl.[2]

1 M. Tebbe, C. Kuttner, M. Männel, A. Fery and M. Chanana, *ACS Appl. Mater. Interfaces*, 2015, 7, 5984–5991.
2 S. Lee, L. J. E. Anderson, C. M. Payne and J. H. Hafner, *Langmuir*, 2011, **27**, 14748–14756.

Peter Vikesland responded: As discussed in one of our prior responses, we are aware that there are a number of analytes that exhibit SERS signals in the very low wavenumber region. We were able to show (see Fig. 7) that halides are not responsible for the ‘pseudo-Rayleigh band’ we report. We see a very strong ‘pseudo-Rayleigh band’ signal in the presence of SERS substrates that have been prepared by a variety of different approaches, and thus at this point we cannot attribute it to any specific ion or other constituent. We note that the amplified spontaneous emission (ASE) field of our laser exhibits a very similar ‘tail’ in the absence of a SERS substrate, but with much, much weaker intensity.

Olga Eremina communicated: According to your findings, can you tell if it will be useful to apply normalized SERS-intensities to Rayleigh-intensities for the validation of surface enhanced Raman spectroscopy as a powerful analytical tool in a wide range of practical fields? For example, for each analysis or everyday calibration?

Peter Vikesland responded: This is an idea that we are actively investigating. Our current results suggest that this normalization protocol makes it possible to quantify analyte surface interactions more readily and we are attempting to see if it can be used for the everyday calibration of our instrumentation.

Richard Van Duyne said: Are these experiments done in water? If so, the substrate is submerged, which is similar to an electrochemical system. You can add a supporting electrolyte that’s inert, such as perchlorate or sulfate, and add very sharp lines that can be used as an internal standard. What you’re getting to here is the notion of an internal standard. This comes along naturally in electrochemistry, and eliminates other issues. With clean electrochemistry, you can use Faraday’s law to quantitate what’s on the surface. With water, did only the low-frequency mode go away, on one spectrum? That’s unusual, if it’s part of the laser signal. It should scale with the laser intensity; it shouldn’t be different.

Peter Vikesland replied: All the experiments except for the experiments with the AuNP clusters (A-AuNPs) were done in air. The substrates were immersed in 4-

MBA solution for 1–3 h and then taken out for Raman scanning following air drying.

We agree that adding inert ions in electrochemical systems as internal standard is a useful idea, but note that it is not a solution that is necessarily applicable to all SERS imaging conditions. Our intent with this work is to investigate alternative approaches for signal normalization that add to the existing armamentarium of choices.

In the spectrum of DI water, our reported low-frequency band does not disappear but its intensity substantially decreased (Fig. 8). As shown in Fig. 8, the low-frequency band was observed both in DI water and with a Si wafer but their intensities were thousands of times lower than those collected with our SERS substrates (AuNP/BC and AgNP/BC). This result is clearly demonstrated in the

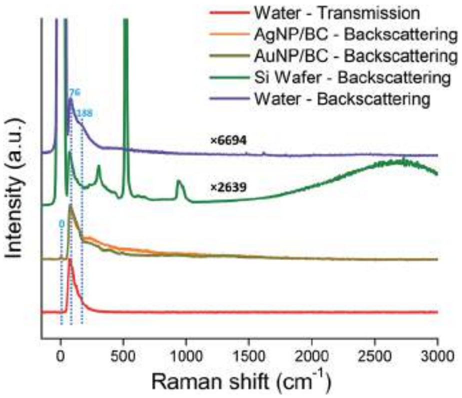

Fig. 8 Raman spectra of two SERS substrates, water, and Si wafer collected using backscattering Raman mode compared to transmission mode laser emission spectra.

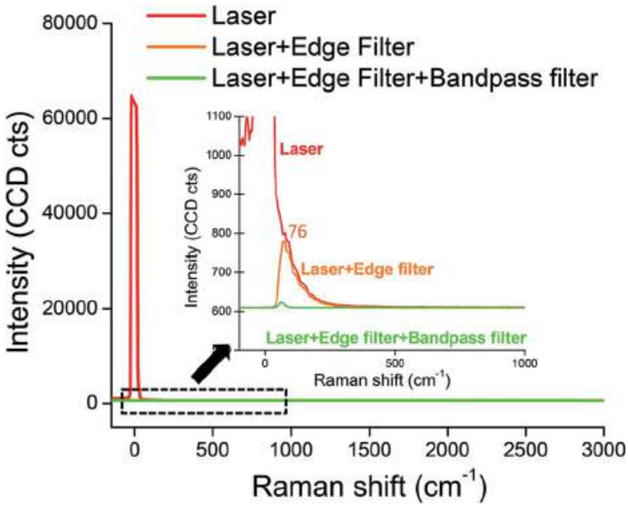

Fig. 9 Laser emission spectra in transmission mode with or without an edge or bandpass filter.

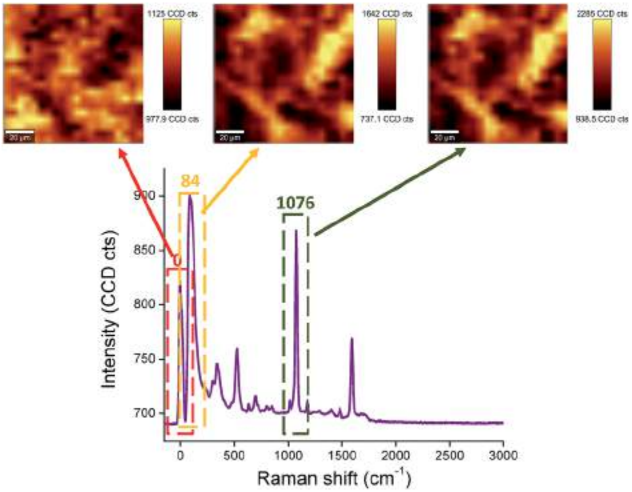

Fig. 10 Maps tracking the laser band at 0 cm^{-1}, Rayleigh band at 84 cm^{-1}, and the Raman band at 1076 cm^{-1}. (The SERS spectrum was collected from the AuNP/BC SERS substrate following immersing in 4-mercaptobenzoic acid solution).

Raman spectrum of DI water in transmission mode (Fig. 9). As shown in Fig. 9, this pseudo band appeared right after the insertion of an edge filter. Based on these reasons, we believe this low frequency band originates from the spontaneous emission of laser and can be significantly enhanced by SERS "hot spots".

The low frequency band does not scale with the laser band at 0 cm^{-1}, but does scale with the Raman band at 1076 cm^{-1} (Fig. 10). As shown in Fig. 10, the map tracking the band at 0 cm^{-1} exhibited an opposite pattern with those tracking the pseudo band at 84 cm^{-1} and the Raman band at 1076 cm^{-1}. It seems that the laser at 0 cm^{-1} excited the LSPR of AuNPs, which in turn enhanced the Raman scattering at 1076 cm^{-1} and the Rayleigh scattering at 84 cm^{-1}. This makes the band at 84 cm^{-1} a great internal standard for the observed SERS bands. We are still working to better understand the mechanism underlying this phenomenon.

Marc Porter enquired: I always worry about how baselines are estimated when analyzing spectral data. The use of the so-called "rubber-band baseline fitting" is but one example and I confess that we have adopted such an approach in some of our own work. You described one of the contributions to the baseline structure. Can you comment on the range and scope of the applicability of your approach to the field and what your thoughts are on other potential contributors to baseline distortion?

Peter Vikesland replied: We agree that there are many inherent challenges associated with baseline estimation. As noted in a separate response, we used a very simple approach for our baseline fitting. This approach was intended to address both the background photoluminescence (PL) of the plasmonic nanostructures employed and the fluorescence emitted by fluorophores near the surface under laser excitation. In the presented work there was minimal fluorescence from either the substrates or the analytes, and the background PL signal was minimized through our use of a 785 nm laser. We have tested our approach across a wide range of different substrates (suspended colloids, commercial substrates such as Klarite, gold and silver nanocomposites) and find it to be broadly applicable. Such a result is encouraging given that these substrates tend to exhibit very different 'SERS backgrounds'. We are currently expanding the range of substrates under consideration and we will see how broad the range and scope of our approach truly is.

Roy Goodacre opened a general discussion of the paper by Bart de Nijs: I'm not sure showing a bottle of Whisky even in Scotland at 9 in the morning is 'motivation', especially after a very nice conference meal last night. Anyway, your methanol detection in water and ethanol is impressive; however that's not in whisky. Have you attempted to do this in the whisky directly as this contains many small molecules and some of these may interact with your cucurbit[*n*]urils-based sequestration?

Bart de Nijs answered: We did explore whisky which indeed presents a more complex set of peaks, though most of the additional peaks come from aromatic compounds that typically tend to contribute to the spectrum at lower wavenumbers than the ROI. It is true, however, that these molecules can potentially competitively interact with the plasmonic cavity. We are now exploring methods for sample pre-treatments that will offer a more robust analysis for such complex analyte solutions.

Richard Van Duyne asked: There are methanol-in-zeolite/MOF/porous solid experiments. A comparison with those might be interesting. Also, you could verify these results with isotopic labelling of the OH group. Have you done anything like that?

Bart de Nijs replied: We agree it would be very interesting to compare our results with different SERS substrates to gain a thorough understanding of the complex processes that might be involved. But we agree isotope labeling is probably one of the most powerful tools at our disposal in order to identify which peaks belong to which vibration. We did try this, however the OD group readily exchanges with the aqueous environment to OH, so we will need to transfer our experiments to a D_2O environment for this to work. Deuterating the CH groups in methanol did result in changes in the observed peaks, indicating a relation between the observed peaks and the deuterated moieties.

Augustus Fountain said: Your spectra are truncated before 2000 cm^{-1}. Did you look out further towards 2800–3000 cm^{-1} for evidence of hydrogen bonds to the -OH group?

Bart de Nijs answered: If we perform extended scans (see Fig. 11 shown for deuterated MeOH in CB[6]) we see peaks at 2080 and indeed around 2800–3000 cm^{-1}. We have not yet attempted to assign these peaks nor have we performed extended scans for our typical experiments as we want to measure the SERS response in quick succession to prevent evaporation of MeOH and EtOH.

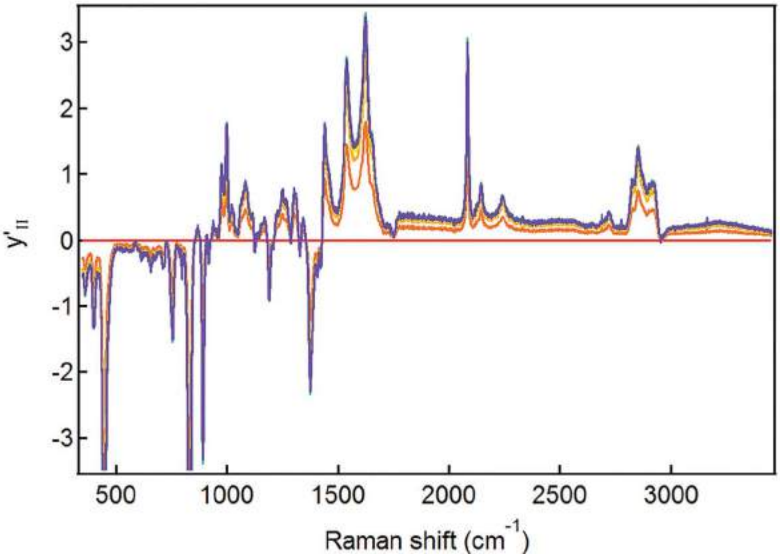

Fig. 11 Eigenspectra with extended range, obtained from principal component analysis. This shows the observed spectral changes in the SERS peaks when adding deuterated MeOH to a CB5-gold aggregates solution.

However, it seems that this indeed would provide extra information and is worth looking into.

Zhong-Qun Tian asked: This is very beautiful work, your CB molecule on a plasmonic Au nanoparticle really provides a special molecular cavity to controllably host various molecules for the SERS study. I wonder if you can make a further approach by combining CB with SHINERS. One can make many pinholes in the shell of core-shell nanoparticles. If one can change the shell materials with a different refractive index and dielectric constant, the location of the plasmonic hotspot could be changed. Moreover, if CB can sit in a different location in the pinhole tube, then host the probed molecules, the distance of the probed molecules and the smooth surface of the Au substrate can be well controlled. It may provide more possibilities to study the distance dependence.

Bart de Nijs responded: We agree, combining CB[*n*]s with SHINERS will provide a large range of possible experiments. One can change the refractive index or thickness of the gap to tune the coupled mode in or out of resonance with a dye molecule in a CB[*n*]. Another possible avenue to explore would be using SHINERS to limit the number of CB[*n*] molecules and with it the number of analytes in the plasmonic hot-spot, aiding in possible single-molecule measurements.

Zhong-Qun Tian remarked: Many groups including ours have tried hard to get a hydrogen bonding signature directly using SERS or normal Raman spectroscopy but it turned out to be very difficult, in spite of the fact that there are so many hydrogen bonds in aqueous solution and we still cannot be successful. The design of your experiment is very good and I wonder which kind of environment it creates and which SERS mechanism it operates? Since hydrogen bonding is rather weak, the temperature influence can be studied and could provide useful information. I wonder if you could study the temperature dependence in particular to lower the temperature to the freezing point, thus to study the spectral features systematically?

Bart de Nijs answered: We agree that the hydrogen bonding is weak making this indeed a rather surprising result. We think that the tight confinement in the plasmonic hot-spot, containing both hydrophobic and hydrophilic moieties, provides an environment that limits the conformational changes a hydrogen-bonded complex can undergo. This results in a narrower set of peaks one would expect for an unconstrained hydrogen bonded complex. Although, this is likely not the only explanation as to why we observed these peaks. Indeed, temperature dependence would be a good method to further explore the properties of these interactions, as both cooling and heating would provide us with more insights into the observed behavior.

Duncan Graham asked: In your paper you mention that you've calculated KDs, and reference the supplementary material but I couldn't find it. What are the dissociation constants of methanol/ethanol? In your models, you don't show any water but presume some is in the cavity? Have you done this in D_2O?

Bart de Nijs answered: To answer the first question regarding the KDs: The dissociation constants (KDs) we found were CB[5]:MeOH 140mM, CB[6]:MeOH 51mM and CB[5]:EtOH 56mM. These are measured as individual analytes, as a mixture will likely affect the KD measured values. For calculation of the vibration spectra, a solvation model was used.

For the calculated interaction between CB[*n*] and the analyte discrete water molecules were included in the model. We have not yet performed measurements in D_2O as we will need to transfer existing nanoparticles to D_2O or synthesize gold nanoparticles in D_2O. We did, however, explore deuterated MeOH (CD_3OH) in water which shows a change in the peaks of interest.

Sebastian Schlücker said: You reported on spectral changes associated with deuterated methanol (HO-CD_3) compared with non-deuterated methanol (HO-CH_3). Does your DFT-computed vibrational spectrum including the animated vibrational mode you displayed already explain this experimental observation qualitatively or even quantitatively?

Bart de Nijs replied: I agree that the coupled vibration between the citrate and the MeOH already explains some of the change in the spectra we see as a result of deuteration. We have not yet done simulations of our deuterated analytes, and indeed this might shine some light on the results we are getting. We will explore this further.

Jennifer Gracie asked: In the paper it states that the CB[*n*]s have excellent molecular recognition and high binding affinities for guest molecules. Are they able to be designed for a specific target *e.g.* a small drug molecule? Or are they more able to distinguish between two similar molecules?

Bart de Nijs responded: The large CB[8] molecules can typically sequester two aromatic molecules, as such host–guest interactions can be used to tune the interaction between a CB[8]-host complex with the desired analyte (guest).[1] For the smaller CB[*n*] molecules this is no longer possible, but they do provide a size selectivity, as shown in the text, for sequestering small molecules in the spacer's cavity. Using the smallest spacer (CB[5]) we can still detect ethanol which should not fit in the molecular cavity. This suggests that the small volume between the nanoparticles formed by the spacers readily incorporates analytes. We think this is a result of an interaction-rich environment that is formed as a result of the presence of citrate, CB[5], water and gold, making this volume more preferential for analytes over the continuous aqueous phase. This means that the CB[5] spacer is not very selective but capable of detecting a wide range of analytes.

1 S. Kasera, F. Biedermann, J. J. Baumberg, O. A. Scherman and S. Mahajan, *Nano Lett.*, 2012, **12**, 5924–5928.

Judith Langer asked: New SERS peaks appear in the range of 1520–1605 cm^{-1} after adding ethanol and/or methanol to the CB[5]- or CB[6]-citrate NP sensing aggregates. We observe a strong peak around 1540 cm^{-1} often when citrate-stabilized Au nanoparticles are aggregated in the absence of CB[*n*], ethanol or methanol. We related that peak to remaining stabilizer molecules close to the surface. Can you say something about the initial aggregation degree of the sample before the analytes were added? Does it change after adding the analyte? Do you see differences between the nanoparticle batches or sizes?

Bart de Nijs replied: Modelling MeOH:citrate shows indeed peaks in this region as well, as presented in Fig. 12 shown, so citrate can play a crucial role in the binding. We do see a few weak peaks in this region prior to adding analytes

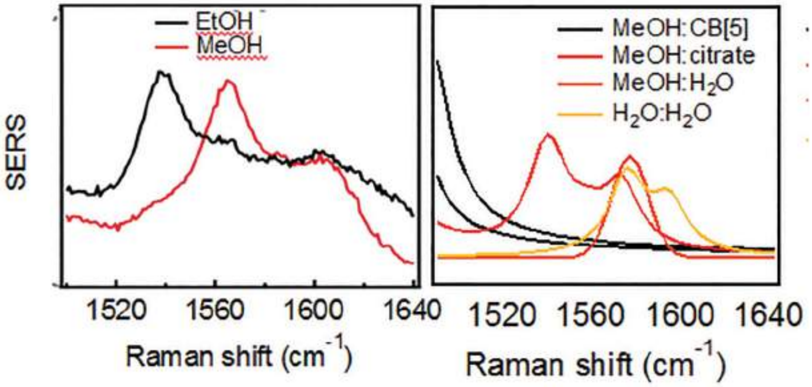

Fig. 12 Observed and calculated Raman spectra. Left: SERS spectra obtained for methanol (MeOH) and ethanol (EtOH) in CB[5] gold-aggregate solutions. Right: Calculated vibrational spectra for potential molecular configurations present in the formed plasmonic cavity, with hydrogen bonded compounds showing peaks in the area of interest.

when we induce self-assembly using CB[5], however, these peaks are absent if we bubble the CB[5] mixture with N_2 beforehand. Combining with the observation that we see different peaks for MeOH and EtOH shows this can not be a peak arising from citrate alone. We let the gold solution with CB[*n*] aggregate for a few minutes prior to adding the analyte. We have not tried different nanoparticle sizes for these experiments, though we do see differences between the different batches of CB[*n*] used, suggesting that subtle differences such as ion concentrations and pH can influence such measurements.

Jeremy Baumberg addressed Laurence Hardwick, Bart de Nijs, Zhong-Qun Tian and Richard Van Duyne: SERS experiments show that we make constructs where we now supposedly know the plasmonic environment very precisely, and the molecules involved. However we often see new lines appearing, and we are still struggling to understand the science. For instance, the CB system shows that we don't know on the nanoscale where other molecules (such as water and citrate) may be, inside or outside the CB, or what is attached to the Au, and in what way. We don't understand how molecules are arranged in the 2D space within the Au gap.

How might we make systematic progress on this problem?

Richard Van Duyne answered: This is indeed a problem. The appearance of new lines can be due to thermal/photo decomposition of the target molecule or it can signal the onset of plasmon-driven chemistry. The observation of molecules with small Raman cross sections such as citrate and water is difficult/not possible. Although the development of TERS is still in its infancy, it does provide a method for spatially locating molecules in the tip sample gap which is analogous to the gap between two nanoparticles or a nanoparticle and flat surface.

Laurence Hardwick responded: The question raises some important points on the experimental challenges of using SERS to study complex systems, such as the electrode/electrolyte interface, and precise interpretation of the data collected.

To make systematic progress on this problem, efforts should focus on more defined electrode surfaces in which the surface chemistry and behavior is better understood. Systematic isotopic labeling of key constituents will allow more confident assignment of the origin of the bands detected. Defined surfaces will allow calculation of the expected bands from computational modelling of the interface.

Bart de Nijs answered: As suggested low-temperature studies and systematic analyte changes such as using D_2O can provide some additional insights into the behavior of molecules in such tight plasmonic hot-spots. One can also limit the study to fewer hot-spots such as a single aggregate, or maybe even a single nanoparticle-on-mirror can reveal some of the dynamics that play a role.

Ideally, a second analysis method would be available to probe the molecular behavior, though most methods probe the bulk of the solution/volume instead of the small cavity between the metal surfaces. Conductivity measurements across a single nanocavity are one of the few techniques that allow probing of the cavity properties.

Zhong-Qun Tian replied: This is indeed a very important question and future work in both fundamental and application research not only for SERS but also for other spectroscopic and imaging techniques of surface chemistry/physics. At the present stage, it's extremely difficult to *in situ* study several different kinds of molecules arranged in the plasmonic nanogap thus to draw a comprehensive picture. It's not sufficient to reach this goal only by analysing the observed SERS bands. The systematical experimental and theoretical study is really necessary.

It is important to take advantage of SERS as a fingerprint spectroscopy method to study not only inter- and intra-molecular interactions but also molecule–surface interactions. Some additional unknown SERS lines could be helpful for us to gain a deeper understanding of the specific molecular arrangement in 2D space. However, the precondition is to avoid any possible impurities, which is essential.

Your CB system could be a good example to be discussed, as the CB molecule can act as a linker to two Au nanoparticles then create a specific chemical environment and construct a well-defined building block in the hotspot. The molecules inside or outside the CB cavity cannot be easily exchanged, which means the different molecules cannot be freely mobile in the 2D space we are capable to distinguish. Let's discuss this in detail and look at several delicate ways to tackle this problem.

(i) To distinguish molecules such as water inside and outside the CB cavity can be realized by changing the temperature. It is quite difficult to directly distinguish water molecules inside and outside the cavity because the water band is very broad at room temperature and its signal is rather weak. However, when the temperature is increased to a certain degree, the water outside the cavity will first leave the Au gap.

(ii) One can also carefully decrease the temperature and monitor the changes in the spectral feature. The freezing point of the water molecules inside and outside must be distinctively different because the SERS band of ice-water is much narrower.

(iii) Another relevant way is to exclude the molecules outside the cavity by using core-shell nanoparticles. If the SiO_2 shell thickness is only around 1–2 nm, the shell has many pinholes. One can deliberately control the size of the pinholes to fit each CB that already contains water or other molecules, so the SERS/SHINERS spectra of these molecules inside can be studied at room temperature.

(iv) One can also try to replace citrate with other molecules or change the solvent when making the comparison. More importantly one can take advantage of the chemical enhancement and/or resonance Raman effect to increase the sensitivity and selectivity. This approach of applying resonance Raman could be more feasible if the laser wavelength is changed to UV light and Al nanoparticles are used for the UV based surface-enhanced resonance Raman effect to selectively increase the detection sensitivity of a certain analyte.

(v) In order to obtain a comprehensive picture about this very complicated molecular arrangement in 2D space, other spectroscopic methods, such as TERS and AFM based nano-IR spectroscopy, are complementary. Electrochemical techniques can apply various potential to modify the molecular arrangement and the impedance analysis may provide additional information from another angle. It should be noted that due to recent developments *in situ* TEM will probably become a very powerful new tool in providing unprecedented spatial resolution at the sub-nanometer scale on solid-liquid interfaces at room temperature by using ultrathin liquid cells in the near future. Moreover, *ab initio* molecular dynamic simulations should be more and more powerful to guide various experiments and understand the molecular structures and arrangement within the hotspot.

Duncan Graham opened a general discussion of the paper by Steven Bell: In your paper you say you won't get sequence information directly. I think you can. If you use a mini-sequencing approach *i.e.* a dideoxy triphosphate you could potentially get single base sequence information?

Steven Bell responded: That's an excellent idea, terminating with a dideoxy triphosphate should give us sequences which differ by a single terminal nucleobase and we have shown that we can identify the additional terminal nucleobase in the paper. This is something we definitely should explore further.

Duncan Graham commented: There is real interest in the effect of environmental carcinogens - *e.g.* tobacco smoke, diesel emissions. A lot of the time this results in new base adducts being formed and I am just wondering if this approach could be used to identify and detect these? They should give a very nice and unique vibrational signature.

Steven Bell responded: That's a very good point. The real strength in the label-free approach is that it does directly probe the chemical structure of the bases. Under conditions where the number of bases is changed but the bases themselves are unmodified there is little opportunity to take advantage of this, since the structure and vibrational signatures of the bases are known and not expected to change. However, if the bases are modified then the spectra do change significantly and this is where the label free approach can be exploited. There are already some published studies on various modifications in which the limit of detection is typically similar to that for substituting conventional bases since the modified bases have similar scattering cross-sections. We have recently been working on nitrated guanine which is exciting because it is very easy to detect. This is due to a combination of the high Raman scattering cross-section of nitrate and the fact that the nitrated bases show large resonance enhancement effects with visible excitation. Indeed, we have been able to detect spectral changes by eye in the case where only 1 in every 1300 bases is nitrated.[1]

[1] S. Dick, S. E. J. Bell, K. J. Alexander, I. A. O'Neil, R. Cosstick, *Chem.– Eur. J.*, 2017, **23**, 10663–10669.

Rohit Chikkaraddy remarked: You mentioned about how the SERS spectra are sensitive to the secondary structure of ss-DNA. Do you think it is possible to manipulate the secondary structure with local heating due to plasmon damping? Have you tried looking at the power dependence of SERS? How can we get better control over causing "proper" damage?

Steven Bell responded: The idea of changing the secondary structure of ss-DNA and then monitoring the change using the same beam is very interesting. Under the conditions we used in our experiments we probably couldn't get the temperature increases required but in principle by redesigning the experiment to reduce the sample volume and/or increase the power density we should be able to induce the changes. The only problem might be balancing the power required to get plasmonic heating against the danger of photochemical sample damage.

Christian Heck asked: 1: Why do you irradiate with a 785 nm laser while using silver nanoparticles?

2: Your excitation power of 160 mW is quite high - do you not see any sample degradation?

Steven Bell replied: We routinely use a 785 nm laser if possible because of the wide availability of low cost portable spectrometers that use this wavelength, which leaves open the possibility of users ultimately being able to read our signals on low cost systems. With aggregated Ag colloid the plasmonic extinction stretches right into the far red region and we get very good enhancement factors with 785 nm.

We can use this laser power (which you correctly point out is quite high) because we have aqueous samples so the water acts as a heat sink. In addition, we are using a system with a > 60 micron beam diameter which penetrates several mm into the sample so the irradiated volume is much larger than that of a dried spot on a solid substrate, which would certainly require much lower power.

Zhong-Qun Tian remarked: With regards to reproducibility, Prof Bell has brought up a key question in this field on whether SERS has good reproducibility or not. He has clearly demonstrated that SERS indeed has very good reproducibility for Au nanoparticles in solution with large volumes. However, the SERS community is quite different from many other communities, with people from many different fields such as chemistry, physics, materials, life sciences/medicine, *etc.* This is because SERS is very attractive and has a very low threshold for people to come in. The way of synthesizing Au and Ag nanoparticles is so simple and getting SERS signals is so easy. It's therefore reasonable to have quite different criteria and different angles on the definition and level of reproducibility from different groups having diverse backgrounds. SERS is similar to most nanostructure-related techniques and products (except IC industries), and is facing up to the challenge of good reproducibility at least to some extent. In most cases the major SERS signal is from limited hotspots that could be changeable at the atomic scale during laser illumination, which is more distinctive when SERS nanoparticles are immobilized on the solid substrate. Another indication is that there have been some SERS-based instruments and techniques on the market but the sales are not good partially due to the reproducibility. Evaluation of the reproducibility could come from users and end customers. Having a Au or Ag core protected with an ultra-thin inert shell could be a way to improve it. I would like to ask how we could further improve the reproducibility, stability and durability.

Steven Bell responded: I do think that the SHINERS approach is potentially very useful in this area. Also, more generally, your idea of asking end users what they consider to be good reproducibility in the context of their particular measurements may be a good way forward, since the requirements may be very different for different groups. For example, in the nicotine work I showed in the talk we had the advantage of being able to average over large numbers of particles and add an internal standard,[1] but in other cases that may not be possible. Also, as Prof. Goodacre has pointed out, many very successful analytical methods require calibration steps to be included as part of the method, so using these approaches in SERS-based procedures may be acceptable to end users in some contexts. Having a clear picture of what end users really need in order to consider routinely using SERS methods in their laboratories should help us to focus on the issues that are stopping the widescale adoption of SERS by the broader analytical chemistry community.

[1] N. Itoh and S. E. J. Bell, High dilution surface-enhanced Raman spectroscopy for rapid determination of nicotine in e-liquids for electronic cigarettes, *Analyst*, 2017, **142**, 994–998.

Roy Goodacre commented: The data you've shown on using isotopologues for the quantification of nicotine are beautiful, and I agree that SERS can be made reproducible with internal standards or with the use of standard addition method. Indeed, one of the poster prize winners, Rachel Kidd, used this method for urinalysis. It's worth pointing out that no detector is quantitative: LC is not quantitative, MS is not quantitative, NMR probably is, but all of these methods can be made quantitative using suitable calibration curves. In SERS we have a very sensitive detector and within the linear range we can consistently achieve excellent quantification, so we should definitely consider SERS as an attractive approach for quantitative analysis.

Steven Bell answered: Absolutely, I do think it is important for us to point out that there are many applications where we can make SERS quantitative. Of course there is a continuum which at one extreme has problems which we can readily quantify using conventional enhancing materials and internal standards (or standard addition as you have shown so nicely), but at the other end has hugely challenging problems, such as quantitative *in vivo* measurements of low concentration compounds in tissue, but it is useful to be clear that we can already work very successfully at one end of this continuum. Indeed Prof Tian's talk was inspirational in showing how we might move further out into solving more difficult analytical problems.

Karen Faulds asked: Do you see differences in the spectra between single and double stranded DNA *i.e.* can you observe when hybridisation has taken place from changes in the spectra?

Steven Bell responded: Our approach using negatively charged particles is not very sensitive to hybridisation. I think if I wanted to see hybridisation I'd use the method from the paper that you published with Luca Guerinni[1] using positively charged particles which are modified with spermine. As that paper shows, DNA interacts differently with these particles than the negatively charged particles we use and this results in them being much better at detecting hybridisation.

[1] A. Torres-Nuñez, K. Faulds, D. Graham, R. A. Alvarez-Puebla and L. Guerrini, *Analyst*, 2016, **141**, 5170–5180.

Ines Delfino asked: Your work is very interesting and the proposed approach very promising. Having information about the number of specific nucleobases in a sequence is crucial for many applications. The point is that often the sequences of interest are longer than the ones investigated in the paper. How optimistic are you about using the method for studying longer sequences?

Steven Bell replied: We did only look at 25-mer or shorter sequences but the signals we obtained had sufficiently high S/N ratios that we could clearly go below the levels we show in the paper, particularly if we start to use multivariate data analysis. I'd estimate we should be able to see a change in a 100 nucleobase sequence without having to redesign the experiment and it might be possible to push it significantly below that level with further refinement. We had no pressing need to go much lower so we did not push the boundaries of sensitivity but if we had a reason for needing to go lower we could probably get some more sensitivity. In fact, point mutations in a 141-mer have been detected using PLS-DA.[1] However, getting better than an order of magnitude improvement over what we have shown would be challenging.

1 J. Morla-Folch *et al.*, *Angew. Chemie*, 2017, **129**, 2421–2425.

Jeremy Baumberg remarked: In some of your single base pair addition experiments (*e.g.* T), you see clear extra peaks (with frequency in the 1400–1600 band) appearing that you don't expect.[1] Where are these coming from?

1 S. Dick and S. E. J. Bell, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00134g.

Steven Bell answered: Although the changes we see are predominantly due to the added base, there is some cross-talk from the other bases, which presumably arises from the small but non-zero end effects we also observe. In the figure (Fig. 3) you mention, the additional bands are adenine bands which did not subtract cleanly from the spectra.

Lauren Jamieson communicated: Have you tried using this technique in a multiplexed manner to measure the signal from multiple different ssDNAs and tried to determine if you can still pick up changes in the individual bases from each of the strands?

Steven Bell responded: We have only tried some preliminary experiments but as you'd expect, with pre-treated samples if the relative proportions of each base changes then we can detect that but if we make mixtures which are adjusted to have the same overall composition their spectra are effectively indistinguishable at our S/N levels.

Natalia Martín Sabanés opened a general discussion of the paper by Giuliana Di Martino: Have you performed cyclic voltammetry (CV) on your system? The hydrogen evolution reaction (HER) should be obvious in the CVs, further confirming your hypothesis. When measuring in different electrolytes and pHs, the HER is expected to appear at different potentials, and therefore your system should respond (spectral changes) at different potential values. Do you observe a correlation between the trends of your scattering peak (intensity, FWHM, position) and the potential where the HER should appear in different environments? Additionally, at high potentials, surface reconstruction effects can alter further your plasmonic response, and should be visible in the CVs. Is surface reconstruction happening within your potential window?

Why are the trends in the peak intensity, position and FWHM so symmetric when you ramp from 0 to −1.2 V and from 0 to 0.3 V? The HER can happen when reducing the potential and will explain the changes observed, however, what is producing the spectral changes when you increase the potential from 0 to 0.3 V?

Giuliana Di Martino replied: Activation of the hydrogen evolution is indeed confirmed in the CV scans, where increasing currents for negative voltages imply that H^+ is continuously provided by the solution. To prove this, we introduce 0.01 μM HNO_3 in 0.1 M $NaNO_3$ and verify that the onset of both the reduction current and the SERS enhancement start at lower applied potentials for this lower pH electrolyte.[1] In the more acidic solution the reduction current starts to appear around −650 mV instead of −900 mV. Similarly the enhancement appears at lower applied potentials, proving the strong link between the SERS enhancement and the reduction process. SAM desorption is reported for higher negative potentials and we always make sure to work within a desorption-free window of potential. According to Thom *et al.*[2] and considering that we use a Pt psuedo-electrode which shifts the potentials reported in this paper, we should not incur SAM desorption. Moreover the processes we see are very reversible, therefore SAM desorption cannot be playing a role in what we observe. For positive voltages the reaction is reversed, with hydrogen reduction happening on the ITO. In this case, charge neutrality requires oxidation on the gold, explaining the symmetry observed in the system. In the CV measurements we see clear oxidation currents when scanning towards +0.3 V even though they are less strong than the reduction current that we see, towards −1.2 V. Normally gold starts oxidizing around +0.9 V with Ag/AgCl as a reference electrode. Oxidation of water starts at even more positive potentials. So what we see seems to be far away from any of these processes, however since we use a pseudo reference electrode, we do not know more precisely what is the actual potential of the electrode. Nevertheless there is no obvious oxidation component (the SAM monolayer is not oxidized because the process is reversible over many cycles). We thus deduce indirectly that it must be (reversible) formation of an ultrathin gold oxide layer and the electrode is at a potential which is sufficiently positive to do so. This concords with our calculations which show that a refractive index change in just the 0.3 nm layer surrounding the nanoparticle is sufficient to account for the spectral shifts that we observe. A monolayer oxidation would thus account for these observations.

1 G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.
2 I. Thom and M. Buck, *Surf. Sci.*, 2005, **581**, 33–46.

Marc Porter remarked: Have you examined the structural integrity of the adlayer after an experiment by wettability, capacitance, or some other surface characterization technique? Passing charge through the adlayer can potentially damage and change its passivation properties with respect to heterogeneous electron-transfer.

Is there any evidence for a change in the adsorbate orientation with applied potential as observed in the work by J. Pemberton and colleagues?[1]

[1] M. H. Schoenfisch and J. Pemberton, *Langmuir*, 1999, **15**, 509–517.

Giuliana Di Martino answered: No torsion, tilt or damage of the thiols is occurring. We are able to rule out torsion of the molecule since the intensity of the Raman line at 1570 cm^{-1} (tangential C=C stretch in the two phenyl rings) should be more affected by torsion compared to the line at 1061 cm^{-1} (Cring–S stretching) while instead we find the same enhancements for different Raman peaks.[1] Additional tilting upon voltage can also be excluded. Self-assembled monolayers are oriented with an average tilt angle of $\theta \sim 30°$ with respect to the Au surface.[2,3] These organic layers are close-packed and constrain molecules with very little flexibility.[4] We performed full DFT computations of different initial conformations and arrangements of BPT relative to the Au layer, and confirm they converge to the same equilibrium geometry showing little conformational flexibility. Changes in the tilt angle would result in a modification of the film thickness and therefore a shift of the plasmonic resonance, *e.g.* $\theta \sim 15°$ would give +10% in film thickness and ~8 nm blueshift. Shifts ≤8 nm are seen for all self-assembled monolayers (conductive and insulating) but we find SERS enhancements only for conductive self-assembled monolayers and not for insulating ones. Hence tilt is not a possible explanation for the observed SERS enhancement. Finally, the processes we see are very reversible, therefore SAM desorption or damage cannot be playing a role in what we observe.

[1] G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.
[2] M. Bryant and J. Pemberton, *J. Am. Chem. Soc.*, 1991, **113**, 8224.
[3] Z. Wang, J. Chen, S. Oyola-Reynoso and M. Thuo, *Coatings*, 2015, **5**, 1034–1055.
[4] R. W. Taylor, F. Benz, D. O. Sigle, R. W. Bowman, P. Bao, J. S. Roth, G. R. Heath, S. D. Evans and J. J. Baumberg, *Sci. Rep.*, 2014, **4**, 5940.

Sylwester Gawinkowski asked: If this molecular hydrogen layer is created on the surface of the nanoparticle, then it should disappear a while after switching off the potential on the electrode. This can be visible as a change of the spectrum back to the shape it had before you applied the voltage. Such behavior supports the conclusion that the spectral changes are caused by the molecular hydrogen layer generation, not by any change of the monolayer on the support surface.

Giuliana Di Martino replied: These effects are indeed fully reversible over many cycles, as reported in *Nano Letters*.[1] As this comment underlines, this finding supports the conclusion of having a hydrogen gas layer on the surface of the nanoparticles.

[1] G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.

Sumeet Mahajan said: While it is indeed possible that hydrogen evolution is occurring in your system, it would be good to clarify and present evidence on why/how this might be happening and whether all other possibilities for the increase in the SERS signals which you observe are really discounted? What is the confirmation that you have good-quality SAMs? How long do you dip the surface in the thiols? Monolayers with small thiols especially with molecules such as BPT are expected to be more disordered, and well-formed compact monolayers for small molecules can take several days. With a very compact SAM, which will be highly insulating (kohm), hydrogen evolution should not be that efficient unless there are pinholes or disorder. Therefore, verification through electrochemical impedance measurements would be desirable. Moreover, at negative potentials, reductive desorption of thiols is well known to take place and can co-occur with hydrogen evolution - it all depends on the quality/nature of the thiol SAM. A good example is shown in *Phys. Chem. Chem. Phys.*, 2014.[1] Even in your *Nano Letters* paper, while there is good reversibility, the presented evidence shows that the SERS intensities do not recover fully and the amperometric reduction current also reduces over 10 cycles. While the differences are small they could be enough in terms of molecules to suggest some desorption, and if there is desorption besides hydrogen evolution there could be orientation changes as well. Experiments with alkyl chain monolayers could provide stronger proof.

1 N. Kemnade, Y. Chen, M. I. Muglali and A. Erbe, *Phys. Chem. Chem. Phys.*, 2014, **16**, 17081–17090.

Giuliana Di Martino replied: A deeper discussion on the hydrogen evolution process can be found in G. Di Martino *et al.*,[1] where we present additional studies on the pH dependence of the SERS signal (in a more acidic solution the reduction current starts to appear at around −650 mV instead of −900 mV and similarly the enhancement appears at lower applied potentials). Moreover, DFT simulations prove that the large applied dc fields shift the electron distribution within each molecule, enhancing their Raman across sections by modulating their static electric dipoles.

Thiolated self-assembled monolayers (SAMs) remain mobile for extended periods of time. Poirier *et al.*[2] reported that after a 14–16 h incubation in a 1 mM butane thiol solution, there is disorder in the SAM even after 2 days. Nevertheless, for smaller thiols similar to what we use, it has been shown that at 1 mM the vast majority of adsorption occurs within the first 5 min.[3] In order to ensure a good quality SAM, we incubated the gold substrates in a 10 mM thiol solution for typically > 12 h, often > 24 h, with no discernible differences in the electrochemical/optical results between overnight or longer incubations.

SAM desorption is reported for higher negative potentials and we always make sure to work within a desorption-free window of potential. According to Thom *et al.*[4] and considering that we use a Pt psuedo-electrode which shifts the potentials reported in this paper, we should not incur SAM desorption. The SERS signal shows good reversibility and over long measurements (100 s) other processes (*e.g.* defocussing or sample drift) would be more likely to induce any reduction of the signal after 10 cycles. Moreover, orientation changes would be clearly evidenced by shifts in the Raman lines, which we never observe.

1 G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.
2 G. E. Poirier and M. J. Tarlov, *J. Phys. Chem.*, 1995, **99**, 10966–10970.
3 D. K. Schwartz, *Annu. Rev. Phys. Chem.*, 2001, **52**, 107–137.
4 I. Thom and M. Buck, *Surf. Sci.*, 2005, **581**, 33–46.

Paul Dawson asked: Fig. 3a[1] shows the modelled scattering intensity as a function of wavelength for various values of ambient refractive index. The caption starts “Plasmonic tuning of scattering and SERS by applied potential…” I assume Fig. 3a is thus intended to indicate what would happen with the evolution of hydrogen which is essentially scenario 4 depicted in Fig. 2d? With that being the connection to the applied potential. Fig. 1b shows the experimental scattering intensity, presumably for a cell filled with electrolyte. (a) Were the results for an ‘empty’ cell collected that would allow you to confirm the low refractive index limit ($n = 1$) of Fig. 3a?

(b) The focus of attention is on changes in the characteristic parameters of the main peak which is due to the coupled plasmon mode. However, there appears to be a parallel set of changes (of similar relative magnitude) monitored in the smaller peak below 600 nm due to the transverse plasmon mode. Would it not be expected that the coupled mode should be rather less sensitive to changes in the ambient refractive index (than the transverse mode) since the associated fields are concentrated heavily in the ~1 nm gap? Also would it not be useful to analyse the peak for the transverse mode? Essentially the transverse mode should offer a useful control case since it preferentially samples (changes on the surface of) the gold nanoparticle remote from the spacer layer. The results from the coupled mode could be referenced to those from the transverse mode.

[1] G. Di Martino, V. A. Turek, C. Tserkezis, A. Lombardi, A. Kuhn and J. J. Baumberg, *Faraday Discuss.*, 2017, DOI: 10.1039/C7FD00130D.

Giuliana Di Martino replied: The simulations in Fig. 3a depict the case of a 1.5 nm shell of refractive index n varying between 1 and 1.33. The refractive index outside this shell is still 1.33 (*i.e.* water). The “empty” cell is a different system where everywhere around the particle has $n = 1$. The coupled mode has the highest signal and the strongest sensitivity to surface, refractive index or conductivity change happening in the gap between the nanoparticle and the mirror, which also includes the electrolyte sitting in the crevices between the nanoparticle and organic layer. We are able to observe 5–10 nm shifts mainly in the coupled mode and no significant change in the transverse mode as reported by G. Di Martino *et al.*, 2017.[1]

[1] G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.

Paul Dawson commented: (a) You depict 4 scenarios of nanoparticle-on-metal (NPoM) reconfiguration, Fig. 2 of your paper.[1] In 3 of which (S2, S3 and S4) there is a modification of the whole NP surface environment and in one (S1) the change is confined to the spacer layer only. Is it the case that the reconfigurations of S2–S4 manifest in both the transverse and coupled modes, while that of S1 is detected only in the coupled mode properties and not the transverse mode since the transverse mode must sample the spacer layer only weakly?

(b) I would like to reiterate my previous query, as to whether it would be useful to monitor the changes in the coupled mode plasmon with reference to those from the transverse mode. Since the behaviour of the former should be dominated by the spacer properties (refractive index and thickness) while the latter should be more sensitive to the electrolytic environment, better distinction could be drawn between the various scenarios of Fig. 2 – the electrolyte (at the Au surface) and spacer properties would appear to vary to differing degrees in S1–S4.

1 G. Di Martino, V. A. Turek, C. Tserkezis, A. Lombardi, A. Kuhn and J. J. Baumberg, *Faraday Discuss.*, 2017, DOI: 10.1039/C7FD00130D.

Giuliana Di Martino answered: We discard the different scenarios by finding contradictions with the experimental data. In the case of S1 being true, we note that the penetration of different sized ions into the SAM should give rise to different Raman signatures. The discussion based on the Raman signal is enough to rule S1 out. In our scattering measurements we focus our attention on the coupled mode since it is the most sensitive to any surface, refractive index or conductivity change, since it is really focused in the hotspot between the particle and the mirror. Any change in that region, being the SAM or the electrolyte nearby, would be highlighted enormously in the scattering spectra of the coupled mode more than the transverse.

Zhong-Qun Tian said: In most cases SERS is closely related to surface chemistry, but I am afraid that some groups might have not paid sufficient attention to this issue. I have a little bit of doubt that the picture you described for the Au electrode/SAM/Au nanoparticle is comprehensive. The interaction of SAM with Au is quite complicated particularly inside the hot spot, where the electromagnetic field is very strong, the density of hot electrons is high and the temperature is considerably higher than the outside. Moreover, in spite of the fact that SAM can stick firmly on the Au substrate, the thio-Au interaction will weaken the Au-Au interaction. It's quite possible that thiol molecules (maybe together with the bonded Au atom) will diffuse out then move to the bare nanoparticles that are overcoated on the SAM layer. To my knowledge the SAM layer on Au will become very ordered, compact and rigid only when the assembled molecules have long alkyl chains with more than 6 carbon atoms. Otherwise there must be some defects and pinholes in the SAM layer. The SAM with pinholes will affect the distribution of the Au nanoparticles over the SAM layer. It means that when the electrochemical potential is applied to this system, the physical picture depends on whether the SAM layer is pinhole free or not. Hydrogen reduction and hydrogen gas formation will happen first on the pinhole area of the SAM. It also determines the electrochemical behavior of the Au nanoparticles. Some nanoparticles may come into contact directly with the Au substrates and some may be separated by the SAM later. Could you please comment on the homogeneity of your Au electrode/SAM/Au nanoparticle system?

Giuliana Di Martino replied: Thiolated self-assembled monolayers (SAMs) remain mobile for extended periods of time. Poirier *et al.*[1] reported that after a 14–16 h incubation in a 1 mM butane thiol solution, there is disorder in the SAM even after 2 days. Nevertheless, for smaller thiols similar to what we use, it has been shown that at 1 mM the vast majority of adsorption occurs within the first 5 min.[2] In order to ensure a good quality SAM, we incubated the gold substrates in a 10 mM thiol solution for typically > 12 h, often > 24 h, with no discernible differences in the electrochemical/optical results between overnight or longer incubations.

Moreover, the presence of the thiol characteristic Raman peaks we record for each particle ensures that thiols are always sitting under the nanoparticle (*i.e.* the particles we observe are never sitting in a pinhole "thiol-free" region).

Damage of the SAM or its diffusion outside the gap due to voltage cannot be playing a role since the processes we see are very reversible.

We never claim understanding of the electrical characteristics at the single particle level. We report optical single particle sensitivity. The potential is applied over ~50 mm^2 surfaces and the current is averaged over thousands of nanoparticles. Any small imperfections (*e.g.* scratches or pinholes) on the SAM elsewhere in the sample contribute to the electrical signature. Indeed, scratches can contribute to the hydrogen gas formation, but it is crucial to underline that at the same voltage the nanoparticles would do it as well (*i.e.* hydrogen reduction happens on scratches and on particles at the same time). Therefore, our optical single particle sensitivity is be able to detect such hydrogen evolution at the single nanoparticle level.

1 G. E. Poirier and M. J. Tarlov, *J. Phys. Chem.*, 1995, **99**, 10966–10970.
2 D. K. Schwartz, *Annu. Rev. Phys. Chem.*, 2001, **52**, 107–137.

Hannah Aitchison commented: Can you rule out the possibility of additional physisorbed adsorbate molecules being present on the SAM surface, which could potentially migrate and bind to the surface of the nanoparticles?

Giuliana Di Martino replied: The gold mirror surface is fully thiolated (*i.e.* no additional binding sites are available), but the nanoparticle surface is not. The presence of physisorbed molecules is indeed the key mechanism for the SERS enhancement, since it allows H$^+$ reduction on the non-thiolated nanoparticle surfaces.

Alex Keeler commented: Why in all of your experiments were you using a Pt pseudo-reference electrode? As you were using aqueous electrolytes, would it not be better to use a reference electrode such as Ag/AgCl? This would provide a more much more stable reference potential, avoiding the normal drift associated with Pt, and allow you to more accurately report the potentials throughout your work.

Giuliana Di Martino replied: A full calibration for the Pt quasi-reference electrode can be found in Kasem *et al.*,[1] where it is shown that the use of a Pt quasi-reference electrode introduces a ~0.3 V shift compared to the Ag/AgCl electrode (or 0.1 V *vs.* SHE). We confirm this and measure a peak shift of ~0.25 V between the Pt and Ag/AgCl reference electrodes. This does not compromise the stability of our measurements.

1 K. K. Kasem and S. Jones, *Platinum Metals Rev.*, 2008, **52**, 100.

Paul Dawson communicated: What is the distribution of potential within the electrochemical cell? In particular what potential difference, if any, is developed between the gold nanoparticle and the gold film substrate across the SAM spacer layer? And if such a potential difference does exist how does it come about?

Giuliana Di Martino replied: The likely reversible process of H^+ reduction to form H_2 gas trapped around the NPs would result in NP charging competing with electron tunnelling through the molecular layer, to allow a non-equilibrium and extremely large electric field to be formed between the NP and the underlying electrode. The large applied dc fields (reaching 107 V cm^{-1}) shift the electron distribution within each molecule. Electrons can reorient along the SAM, changing the SERS intensity as is predicted.[1]

[1] G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.

Kei Murakoshi communicated to Giuliana Di Martino: In your system, the localized surface plasmon (LSP) energy does not change in the double layer region between −0.9 V and −0.1 V, as you reported in your previous report (Fig. S2 in *Nano Letters*[1]). It may imply that the electrochemical potential, *i.e.* the Fermi level *vs.* the vacuum level, of the nanoparticles on the SAM layer is not the same as that of the substrate metal electrode, because it is experimentally established that negative polarization of the electrochemical potential of nanoparticles shows a blue shift of the LSP energy. Even though this is the origin of the electrochemical LSP energy shift, this needs further consideration as discussed during the discussion of our paper.[2] Your result suggests that the SAM layer prevents the tunneling of electrons in the substrate electrode to/from the metal nanoparticles. What do you think about this?

[1] G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.
[2] H. Minamimoto, F. Kato, F. Nagasawa, M. Takase and K. Murakoshi, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00126f.

Giuliana Di Martino answered: In the simple system consisting of a metal electrode coated with an organic self-assembled monolayer (SAM), the rate of electron transfer through the SAM has been shown to decay exponentially as the chain length of the monolayer is increased.[1] Adsorption of metal nanoparticles (NPs) onto such layers results in an overall charge transfer across the modified electrode.[2] Surprisingly, several electrochemical investigations of nanoparticle-mediated electron transfer across the organic layers[2–5] show distance-independent charge transfer between two metals when separated by gaps as wide as 6.5 nm.[3] Charge transfer across the gaps has proven to be much faster than electron transfer between a metal and the dilute redox species in solution (estimates suggest up to 10^{12} times faster through an organic layer compared to redox transfer at the metal surface.[5] In this description, the metal/thin-insulator/metal stack effectively short-circuits when the NPs adsorb,[3] making the NPs an extension of the electrode underneath the organic layer.[5]

Nevertheless the process of H^+ reduction to form H_2 gas trapped around the NPs would result in the NP charging competing with electron tunnelling through the molecular layer, to allow a non-equilibrium and extremely large electric field to be formed between the NP and the underlying electrode. This latter explanation best explains the combined observations, but opposes previous results showing no potential can be electrochemically applied across a NPoM construct. We suggest that non-equilibrium currents are responsible, as these are correlated to the observed enhancements. This also concords with calculations in Fig. 3a in the paper which show that a refractive index change in just the 0.3 nm layer surrounding the nanoparticle is sufficient to account for the spectral shifts that we observe.

1 C. Creutz *et al.*, *J. Phys. Chem. B*, 2003, **107**, 6668–6697.
2 C. R. Bradbury, J. Zhao and D. J. Fermin, *J. Phys. Chem. C*, 2008, **112**, 10153–10160.
3 J. Zhao, M. Wasem, C. R. Bradbury and D. J. Fermin, *J. Phys. Chem. C*, 2008, **112**, 7284–7289.
4 J. Zhap, C. R. Bradbury and D. J. Fermin, *J. Phys. Chem. C*, 2008, **112**, 6832–6841.
5 J.-N. Chazalviel and P. Allongue, *J. Am. Chem. Soc.*, 2011, **133**, 762–764.

Kei Murakoshi communicated to Giuliana Di Martino: SAM layers usually have pits or holes due to their structural imperfection. Thus, it could be possible to evolve hydrogen by proton reduction at the pits or holes on the substrate electrode at −1.2 V. Higher energy shifts of the LSP mode could be due to the injection of electrons from these evolved hydrogen molecules to the nanoparticles, leading to the shift of the Fermi level of the nanoparticles to the negative electrochemical potential direction, becoming closer to the vacuum level. Could you comment on this idea?

Giuliana Di Martino answered: The H^+ reduction can happen at pits and holes, but since the nanoparticles are also an extension of the electrode, the main contribution to the H^+ reduction would be on the particle surfaces, leading to the potential gradient across the SAM we propose in G. Di Martino *et al.*, 2017.[1]

1 G. Di Martino *et al.*, *Nano Lett.*, 2017, **17**, 4840–4845.

Jeremy Baumberg opened a general discussion of the paper by Jason Guicheteau: I am interested in other things that can vary between the drying and Langmuir protocols. When we developed this substrate, we investigated drying (nanofluidics) and where molecules end up on corrugated surfaces upon drying is very peculiar since there is pinning of droplets at the substrate edges.[1]

Why do you get then so close an agreement between the two protocols? How do you average over the spatial inhomogeneity?

1 F. Birembaut, N. Perney, K. Pechstedt, P. N. Bartlett, A. E. Russell and J. J. Baumberg, Sharp-cornered Liquid Drops by Wetting of Nanoscale Features, *Small*, 2008, **4**, 2140–2142.

Jason Guicheteau responded: It is unclear on what you mean by close agreement between the two protocols. From Table 2 in our paper[1] the calculated SEV values differ by two orders of magnitude for BPE and have an even greater difference for thiophenol. Spectrally, from Figure 2, the emergence of a signal for drop and dry doesn't appear until 5×10^{-6} M, when compared to the signal observed at 5×10^{-9} M for the Langmuir protocol. The second point is that the drop and dry technique, while providing the most rapid response for a SERS measurement, is essentially starving the substrate of molecules (Table 1), thus the measurement is more on the laser sampling method. In our case we map a large area of the substrate for each analysis to ensure we acquire a reliable average response of the substrate.

1 J. A. Guicheteau, A. Tripathi, E. D. Emmons, S. D. Christesen and A. W. Fountain III, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00141j.

Marc Porter remarked: Did you rinse the sample between each application in your drop-and-dry deposition process and did you change the reactant concentrations between each deposition step when using the same sample?

Jason Guicheteau replied: We did not rinse the substrate between the drop-and-dry deposition. Starting at the lowest concentration we would perform the analysis, and then deposit another droplet on the same substrate with the next lowest concentration in our experimental procedure (E^{-9} M - E^{-8} M - E^{-7} M *etc.*).

Zhong-Qun Tian commented: I would like to add one observation to your interesting approach. In 2011 Prof. L. B. Yang and coworkers observed an interesting behavior during the drop then dry procedure. Just before the dry state (we called it a metastable state), the SERS signal was increased significantly, two to three orders of magnitude higher than that for the traditional method (ref. 1). This drop and dry protocol may have some correlation with your system and Laurence Hardwick on his transient state. In fact this could be a fundamental issue on the hotspots with unique environments and special dielectric constant distributions with a mixture of metal/water/air (or H_2) phases at the nanoscale. Because this transient state only lasts several to tens of seconds, it's very difficult to study in detail, but it is really interesting to find a way to make this state last much longer or create a similar situation such as a steady state.

1. L. B. Yang, H. L. Liu, J. Wang, F. Zhou, Z.-Q. Tian, J. H. Liu, Metastable state nanoparticle-enhanced Raman spectroscopy for highly sensitive detection, *Chem. Commun.*, 2011, **47**, 3583–3585.

Richard Van Duyne commented: One problem is that these systems, especially with these two molecules, never come to thermodynamic equilibrium. They have Langmuir adsorption kinetics. There is an on rate, but no off rate for thiophenol. It's an activated bond-breaking process that makes the adsorbed molecule, which is irreversible. You can demonstrate this by putting the molecule on, and trying to displace it with an isotopically labelled molecule. There is competitive binding – you can separate equilibrium from non-equilibrium behaviour.

Bart de Nijs asked: I've noticed in one of your concentration series[1] something that I also observe in my concentration experiments where there is a decrease in the peak intensity with the increase in concentration. Do you see this consistently and do you know if there is any explanation for this?

1 J. A. Guicheteau, A. Tripathi, E. D. Emmons, S. D. Christesen and A. W. Fountain III, *Faraday Discuss.*, 2017, DOI: 10.1039/c7fd00141j.

Jason Guicheteau responded: This is an observation we have seen several times with this work. Unfortunately as of yet I'm unable to offer an explanation.

Marc Porter commented: Does your analysis of signal strength assume a constant orientation for the adlayer as it forms? Some of these systems demonstrate an orientation dependence as the surface concentration of the adsorbate changes.

Jason Guicheteau replied: We don't directly assume the constant orientation question in our work. We acquire repeat data in all the protocol analysis to hopefully find any oddities in the spectra (associated error bars in the data). However, we haven't directly related any error to the possibility of changing the orientation. However, we have seen a solvent dependence on orientation and do account for that.

Rohit Chikkaraddy asked: A more general comment regarding the drop and dry experiment – the way the droplet dries can be controlled by controlling the dynamics of the fluid in the droplet. The coffee-ring effect can be manipulated to achieve a uniform deposition of molecules by modulating the substrate temperature, charge and hydrophobicity. This will lead to a homogenous SERS substrate with a more uniform SERS enhancement.

Jason Guicheteau replied: The comment about the issue with drop and dry as it relates to our article does not concern the uniformity of the droplet or the coffee-ring effect. The number of molecules present in the droplet is the concern. The drop and dry technique is useful, but in all cases you are most likely starving your substrate of molecules, due to the concentration and volume of the droplet. Please refer to Table 1 in the paper and accompanying text.

Mike Hardy communicated: Hi Jason, I think it is great to see work that attempts to build on that of Le Ru *et al.*,[1] with an emphasis on moving SERS into the 'application space'.

My simple question is: what do you see as the next step? I have been concerned with many of the more subtle experimental issues that make enhancement factors (EFs) difficult to meaningfully compare. Hence, have you considered an inter-laboratory study (*i.e.* same materials, different SERS set-ups) to experimentally illuminate any other possible barriers towards meaningful comparisons of SERS EFs? Would this be useful? I am conscious of one such study from the Zenobi group[2] looking at TERS reproducibility.

1 E. C. Le Ru, E. Blackie, M. Meyer and P. G. Etchegoin, *J. Phys. Chem. C*, 2007, **111**, 13794–13803.
2 C. Blum *et al.*, *J. Raman Spec.*, 2014, **45**, 22–31.

Jason Guicheteau replied: Exactly right that this builds off Le Ru's work and his discussion on the different enhancement factor calculations for different experimental setups. In 2012 we were part of a DARPA program that did just that in comparing disparate SERS substrates across an inter-laboratory study.[1] This is where we first introduced our initial SEV value equation and is the constant time and volume protocol procedure. I believe it would benefit the community to have a standard base line protocol to compare substrates to each other which may or may not include just reporting the traditional EF value. With regards to next steps, I believe SERS is on the precipice of breakthrough in the application market. Portable Raman instrumentation is at a point where adding front end SERS modules can be accomplished. Substrates both planar and colloidal based are now highly reproducible. I think the key is finding that near term need and application space that demonstrates the sensitivity (trace analysis) of SERS. If there is a commercialization success that really hits a visible market, then I believe that will open the door for the community as a whole.

1 J. A. Guicheteau, M. E. Farrell, S. D. Christesen, A. W. Fountain, P. M. Pellegrino, E. D. Emmons, A. Tripathi, P. Wilcox and D. Emge, *Appl. Spectrosc.*, 2013, **67**, 396–403.

Christian Kuttner addressed Richard Van Duyne and Zhong-Qun Tian: Prof. Tian commented that today, the SERS of water is still not fully understood and H-bonding is quite a mystery in many situations. Could you comment about the implications of this and to what degree the understanding has advanced over time?

Richard Van Duyne replied: So far as I am aware there is no new understanding about the SERS of water. That said it should be noted that in UHV-SERS at low temperatures there is no problem getting a SERS signal from water. Also it was shown by Richard Chang back in the early 1980s that SERS of water could be observed in high concentration electrolyte solutions.

Zhong-Qun Tian answered: Thank you Prof. Van Duyne for the comment but I am afraid that I cannot fully agree with his view. After discovering SERS, one remarkable feature of SERS from electrode surfaces is the absence of water signals although a large fraction of the surface is covered by water molecules. In 1981, M. Fleischmann and I. Hill reported the first SERS of water but only from high concentration halide solutions (*e.g.* KCl, KBr, KI).[1] It was I. Pockrand to report the first ice-like water in UHV-SERS at a low temperature (120 K) in 1982.[2] It should be noted that these two kinds of water are quite special and distinctively different from the normal surface (interfacial) water people most want to study in many fields such as electrochemistry, biology and materials sciences. The first one is solid state water at a very low temperature that is most likely a multilayer structure, which cannot represent the majority of normal interfacial water solid/liquid interfaces we discuss here. Regarding the second kind of water at room temperature, the SERS signal was observed only in the limited potential region and the potential dependency of the SERS intensity of water and the halide ions were found to be parallel.[1] When we move the applied potential from the positive potential to negative potential region, leading to desorption of halide ions, then move the potential back to the original potential region, the SERS signals of the Ag-halide ion and water are irreversibly lost. Therefore, the SERS result of water observed by Fleischmann *et al.* is only associated with some surface complexes consisting of water, silver adatoms, halide ions and cations. These data cannot represent the majority of normal interfacial water explicitly. This discrepancy in fact resulted in the dramatic decline in the number of SERS studies on water in the latter 1980s.

Since 1992 our group has successfully obtained the SERS of water in the potential region of hydrogen revolution, *e.g.* from −1.2 V to −2.0 V (vs SCE), and performed systematic SERS studies on the effects of electrolyte ions (Li^+, Na^+, K^+, Cs^+, ClO^{-4}, $SO_2{}^{-4}$, and OH^-) and electrode substrates (Au, Ag, Cu, Pt, Pd).[3–9] It is of special interest to find that in the potential region of hydrogen evolution, the spectral feature of H_2O is strikingly different from that of normal water. The SERS intensity of the bending vibration is greatly enhanced and just about the same as the stretching one. This is in clear contrast to the fact that normally the Raman intensity of the bending band is about 20 times lower than that of the stretching band for bulk water.[4–6] The intensity of the libration mode of water is also abnormally enhanced in comparison with that of the stretching mode. Based on the fact that the strong H_2O libration band is only obtained from solid states like ice, we assumed that the interfacial water during its electrolysis process may have a relatively ordered structure, enabling it to expedite electron transfer and the mobility of OH^- and H^+ through the unique hydrogen bonding network.[6–8] Although the structural information discerned from the present results is rather qualitative in nature, this preliminary study may stimulate more molecular-level research and descriptions of the interface in the potential region of water reactions.

It is of interest that the SERS signal of water is much stronger at negative potentials and further enhances significantly with negative movement of the potential.[4–9] This trend is in the sequence of Ag > Au $\gg$ Pd and Pt. This abnormal phenomenon seems to not be able to be explained by either the conventional EM or chemical enhancement. As we know the polarizability of metal conduction electrons is also in the sequence of Ag > Au $\gg$ Pd and Pt. According to the classical electronic jellium model, the tail of a conductive electron (*i.e.* jellium electron) penetrates into the electrolyte with lengths of several angstroms upon applying a negative potential on an electron-conductive metal. The spilled jellium tail could be involved in the collective oscillation of the conductive electrons in the metal electrode. Under this condition, the surface water molecules are immersed in the electronic tail, which could further enhance the SERS signal of the interfacial water.[9] This work may shed light on a new understanding of the complicated SERS behavior of water. However, it has still been very challenging to establish a generic way to get the SERS signal of water from a great variety systems without restricted conditions. An understanding of how to further improve the sensitivity of SERS could be a key.

1 M. Fleischmann, P. J. Hendra, I. R. Hill and M. E. Pemble, *J. Electroanal. Chem.*, 1981, **117**, 243.
2 I. Pockrand, *Surf. Sci.*, 1982, **122**, L569.
3 Z. Q. Tian, Y. Z. Lian and T. Q. Lin, *J. Electroanal. Chem.*, 1989, **265**, 277.
4 Z. Q. Tian, Y. X. Chen and B. Ren, *J. Chem. Soc. Farad. Trans.*, 1996, **20**, 3829.
5 S. Z. Zou, Y. X. Chen, B. W. Mao, B. Ren and Z. Q. Tian, *J. Electroanal. Chem.*, 1997, **424**, 19.
6 Y. X. Chen, S. Z. Zou, K. Q. Huang and Z. Q. Tian, *J. Raman Spectrosc.*, 1998, **29**, 749.
7 Z. Q. Tian and B. Ren, *Annu. Rev. Phys. Chem.*, 2004, **55**, 197.
8 D. Y. Wu, J. F. Li, B. Ren and Z. Q. Tian, *Chem. Soc. Rev.*, 2008, **37**, 1025.
9 Y. F. Huang, X. Jin, X. Wang, D. Y. Wu, B. Ren and Z. Q. Tian, in preparation.