

## DISCUSSIONS

1

### Physical methods for mechanistic understanding: general discussion

Yutaka Aoki, Matthias Bauer, Thomas Braun, Jamie Cadge, David Davies, Derek Durand, Odile Eisenstein, Daniel Ess, Ian Fairlamb, Natalie Fey, Simone Gallarati, Mike George, Megan Greaves, Meghan Halse, Alexander Hamilton, Jeremy Harvey, Anthony Haynes, Ulrich Hintermair, Alison Hulme, Youichi Ishii, Martin Jakoobi, Vidar R. Jensen, Pierre Kennepohl, Shigeki Kuwata, Aiwen Lei, Guy Lloyd-Jones, Jennifer Love, Kevin Lovelock, Jason Lynam, Stuart Macgregor, Todd Marder, Evert Jan Meijer, Patrick Morgan, Robert Morris, Joseph Mwansa, David Nelson, Aaron Odom, Robin Perutz, Markus Reiher, Joseph Renny, Jana Roithová, Laurel Schafer, David Schilter, Samuel Scott, John Slattery, James Walton, Jonathan Wilden, Chun-Yuen Wong, Tolga Yaman and Tom Young

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## DISCUSSIONS

## Physical methods for mechanistic understanding: general discussion

Yutaka Aoki, Matthias Bauer, Thomas Braun, Jamie Cadge, David Davies, Derek Durand, Odile Eisenstein, Daniel Ess, Ian Fairlamb, Natalie Fey, Simone Gallarati, Mike George, Megan Greaves, Meghan Halse, Alexander Hamilton, Jeremy Harvey, Anthony Haynes, Ulrich Hintermair, Alison Hulme, Youichi Ishii, Martin Jakoobi, Vidar R. Jensen, Pierre Kennepohl, Shigeki Kuwata, Aiwen Lei, Guy Lloyd-Jones, Jennifer Love, Kevin Lovelock, Jason Lynam, Stuart Macgregor, Todd Marder, Evert Jan Meijer, Patrick Morgan, Robert Morris, Joseph Mwansa, David Nelson, Aaron Odom, Robin Perutz, Markus Reiher, Joseph Renny, Jana Roithová, Laurel Schafer, David Schilter, Samuel Scott, John Slattery, James Walton, Jonathan Wilden, Chun-Yuen Wong, Tolga Yaman and Tom Young

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(1:[1]1) **Derek Durand** opened a general discussion of the introductory lecture by Robert Morris: Your lecture discusses Paul Chirik's work on cobalt(0/II) catalysts that can potentially replace or improve upon cationic Rh analogues for asymmetric hydrogenation, but am I right in thinking that they've now actually reported the exact cationic Co(I) analogue of the Rh catalysts? I think I recall reading it in *Angewandte* recently.<sup>1</sup>

1 H. Zhong, M. R. Friedfeld and P. J. Chirik, *Angew. Chem. Int. Ed.*, 2019, **58**, 9194–9198.

**Robert Morris** responded: Thank you for providing this recent reference. This article indeed describes the exact analogue of the rhodium system. I look forward to these researchers reporting the nature of the hydride species involved in this diamagnetic Co(I)-based catalyst and the paramagnetic Co(0)-based system to which I was referring.

(2:[2]2) **Odile Eisenstein** said: There is one question which could apply to all speakers during the entire meeting. When an intermediate of a reaction is proposed on an experimental basis, what is the proof that this intermediate is on the pathway for the reaction being studied? Seeing a species is not a proof of its reactivity.

(3:[3]3) **Ulrich Hintermair** remarked: On the question of how to ascertain whether an observed intermediate is in-cycle (product-forming) or off-cycle

(dormant): this is indeed a very important point that can be difficult to address, but experimental methods to do this do exist, not just theoretical ones. For instance, we have previously used polarisation transfer experiments (EXSY NMR) to show that a metal-hydride dynamically exchanges with substrates and products.<sup>1</sup> Nikitin recently published an excellent review on this technique for mechanistic investigations,<sup>2</sup> and Ruth Gschwind *et al* have used saturation transfer experiments (CEST NMR) to follow chemical exchange between intermediates that aren't even detectable in 1D NMR spectra.<sup>3</sup>

1 A. M. R. Hall, P. Dong, A. Codina, J. P. Lowe and U. Hintermair, *ACS Catal.*, 2019, **9**, 2079–2090.

2 K. Nikitin and R. O'Gara, *Chem. - Eur. J.*, 2019, **25**, 4551–4589.

3 N. Lokesh, A. Seegerer, J. Hioe and R. M. Gschwind, *J. Am. Chem. Soc.*, 2018, **140**, 1855–1862

(4:[4]4) **Robin Perutz** addressed Robert Morris: Your paper brought out wonderful information about hydrogenation which also has implications for proton reduction – electrocatalytic proton reduction is key to water splitting and there's a strong relationship between proton reduction and hydrogenation, which is not really brought out in the literature with the exception of a few authors who are engaged in both. Nevertheless, hydrogenation mechanisms have wide implications for proton reduction.

(5:[5]5) **Stuart Macgregor** said to Robert Morris: One of the outcomes of your work is that there appears to be a limit on how activated a hydride within a *trans*-H-Fe-H moiety can be, compared to analogous *trans*-H-Ru-H structures. However, the computed transition states often have the protic N-H bond acting as a 'hook' that pulls in the carbonyl substrate, making it susceptible to nucleophilic attack by hydride. Which is the more important component, the N-H bond or the Fe-H bond, and to what extent can the reactivity of the N-H group be tuned? For example, could deprotonation of the N-H bond be promoted by installing a *trans*-CO ligand that would then stabilize the amide formed by push-pull effects?

**Robert Morris** replied: I agree that the N-H 'hook' is important in activating the polar bond to nucleophilic attack. Our transfer hydrogenation catalysts have carbonyl *trans* to hydride and they effectively attack ketones and activated imines *via* the FeH and NH on the ligand. Similarly the H<sub>2</sub> hydrogenation catalysts with *trans* dihydrides have the NH and the CO ligand *trans* to each other and can attack the same types of substrates with FeH and NH. It will be interesting to see how much the 'acidity' of the NH can be tuned to increase the activating effect of the substrate. One consideration is that changing ligands, other than the one containing the amine, directly affects the nucleophilicity of the hydride one bond away, but the change to the NH acidity or hydrogen-bond donating capacity is attenuated since it is two bonds removed from the change. On the other hand, as you say, the stabilisation of the amide form is also key for some catalysts and the carbonyl ligand is efficient in doing just that. Effects like this could well be used to overcome the poorer inherent nucleophilicity of the 3d metal hydrides that I referred to.

(6:[6]6) **Patrick Morgan** opened a general discussion of Meghan Halse's paper: How feasible is it to use this pump-probe technique for everyday use? Does it require any specialised set-up or expertise?

1 **Meghan Halse** answered: In terms of specialised equipment, a source of *para*-  
enriched H<sub>2</sub> gas is required. 50% enrichment is relatively straightforward to  
achieve using liquid-N<sub>2</sub>-cooled apparatus, and commercial units are available.  
5 The ability to irradiate the sample with the laser within the NMR spectrometer is  
another requirement. In this work, we use mirrors to direct the beam up the bore  
of the magnet and then through a hole drilled into the side of the NMR probe.

(7:[7]7) **Tom Young** asked: Can you vary the temperature to be able to always  
access the intermediate regime?

10 **Meghan Halse** replied: In principle, variable temperature experiments are  
possible. However, the level of *para*-hydrogen hyperpolarisation is temperature  
dependent and therefore we find that there are practical signal-to-noise limits to  
the range of temperatures that can be explored. In addition, at higher tempera-  
15 tures there will be competition between thermal reactivity and photo-chemical  
reactivity, which complicates the analysis of the results.

(8:[8]8) **Matthias Bauer** remarked: Compared to classical, optical pump-probe  
experiments I'm wondering if the term pump-probe is really correct. Are you  
20 really probing optically excited states? Or are you just illuminating the sample,  
triggering a reaction?

25 **Meghan Halse** responded: In contrast to classical optical pump-probe exper-  
iments, in this method we are not probing optically excited states. We are using  
the pumping step to trigger a reaction in order to do a time-resolved NMR  
experiment where we selectively observe the NMR response from the product(s) of  
the photo-chemically initiated reaction.

30 (9:[9]9) **Jamie Cadge** asked: This technique appears to be extremely powerful  
for the investigation of metal dihydride complexes. Could a similar technique be  
applied to molecular dihydrogen complexes?

35 **Meghan Halse** replied: The NMR signal enhancement that comes from  
breaking the magnetic symmetry of the <sup>1</sup>H nuclei in *para*-hydrogen is necessary  
for the success of this method. In the case of metal dihydride complexes, the  
magnetic symmetry is broken upon oxidative addition at the metal centre by  
introducing a difference in either chemical shift or scalar coupling between the  
40 two hydrides. In a molecular dihydrogen complex, if the symmetry of the <sup>1</sup>H  
nuclei in *para*-hydrogen is not broken, no hyperpolarisation will be observed.  
Therefore this method cannot be used.

(10:[10]10) **David Davies** inquired: As a follow-up to the previous question  
-could this apply to H<sub>2</sub> addition to a frustrated Lewis pair?

45 **Meghan Halse** answered: In principle, a frustrated Lewis pair can be used to  
generate *para*-hydrogen-induced polarisation. This has been shown previously by  
Koptug and co-workers,<sup>1</sup> who used molecular tweezers to activate dihydrogen  
and generate enhanced NMR signals.  
50

1 V. V. Zhivonitko, V.-V. Telkki, K. Chernichenko, T. Repo, M. Leskelä, V. Sumerin and I. V. Koptiyug, *J. Am. Chem. Soc.*, 2014, **136**, 598–601.

(11:[11]11) **Mike George** said: My question has two parts. The first relates to the discussion that the NMR signal from organometallic dihydrogen complexes would not be enhanced using *para*-hydrogen – I wondered whether there may be enhancement in the NMR signal of the dihydrogen complex if the dihydrogen complex was in rapid equilibrium with a dihydride complex, as has been observed for  $\text{CpNb}(\text{CO})_3(\text{H}_2)/\text{CpNb}(\text{CO})_3\text{H}_2$  and related complexes.<sup>1</sup> The second part of the question is: how much enhancement is expected if hyperpolarised Xe is used instead of *para*-hydrogen for enhancement of NMR signals in organometallic Xe complexes?

1(a) M. T. Haward, M. W. George, P. Hamley and M. Poliakoff, *J. Chem. Soc. Chem. Commun.*, 1991, 1101–1103; (b) M. W. George, M. T. Haward, P. A. Hamley, C. Hughes, F. P. A. Johnson, V. K. Popov and M. Poliakoff, *J. Am. Chem. Soc.*, 1993, **115**, 2286–2299; (c) G. I. Childs, D. C. Grills, S. Gallagher, T. E. Bitterwolf and M. W. George, *J. Chem. Soc. Dalton Trans.*, 2001, 1711–1717.

**Meghan Halse** responded: For the first part of your question: the ability to observe *para*-hydrogen enhancement in the situation you describe, where there is rapid exchange between a dihydride and a dihydrogen complex, depends on a number of factors. First, dihydrogen complexes are known to have very short NMR relaxation times. Therefore it is likely that even if some signal enhancement was generated, it would decay too quickly to be observed. Second, the residence time in the dihydride form would need to be long enough for appreciable evolution between the NMR-silent singlet state and the observable triplet nuclear spin states to occur. The timescale of this evolution would depend on the extent to which the magnetic symmetry between the hydrides was broken in the dihydride complex (*e.g.* the difference in chemical shift). On balance, I think it is unlikely that significant NMR enhancements would be observed in this case.

In answer to the second part of the question: in principle, it should be possible to observe NMR signal enhancements in organometallic Xe complexes if they were formed using hyperpolarised Xe. There are too many experimental considerations to confidently predict the levels of signal enhancement that could be achieved. Some relevant factors include reaction timescales, NMR relaxation rates and Xe hyperpolarisation levels. However, hyperpolarised Xe-based molecular sensors have been demonstrated by Pines and co-workers.<sup>1</sup> This suggests that substituting *para*-hydrogen with hyperpolarised Xe in our time-resolved NMR method could be feasible. While *para*-hydrogen has the benefit that it is NMR-silent and so only the product NMR signals are observed in our method, Xe has the benefit of a very large chemical shift range and so it should be possible to selectively probe the product complex without perturbing the hyperpolarisation of the bulk Xe.

1 P. D. Garimella, T. Meldrum, L. S. Witus, M. Smith, V. S. Bajaj, D. E. Wemmer, M. B. Francis and A. Pines, *J. Am. Chem. Soc.*, 2014, **136**, 164–168.

(12:[12]12) **Robert Morris** commented: In your presentation you documented a slow regime, and in your article you write about the addition of dihydrogen to



the iridium complex: “By contrast, the rate of H<sub>2</sub> addition is on the order of 2–5 s<sup>−1</sup> over the range of H<sub>2</sub> pressures studied... Therefore this is within the slow regime.” If there were a dihydrogen intermediate before oxidative addition that typically has a *T*<sub>1</sub> of 15–40 ms at 300 MHz at room temperature, would this affect the lower bound of rates observable in such cases? One might expect the short *T*<sub>1</sub> to result in a loss of coherence.

**Meghan Halse** answered: In order to observe hyperpolarisation in these experiments, it is necessary for the relaxation rates for the relevant spin states to be sufficiently long for the initial NMR-silent singlet state of *para*-hydrogen to convert to the observable states. This timescale will depend on the difference in chemical shift between the *para*-hydrogen-derived protons in the product. While most systems we have observed have had coherence lifetimes of tens to hundreds of ms, we have successfully studied one system with an apparent coherence lifetime of only a few ms.<sup>1</sup> We have not observed hyperpolarisation for a dihydrogen complex, probably because the coherence lifetimes are too short. The presence of a dihydrogen intermediate with a short lifetime can also limit the level of hyperpolarisation observed for the final product(s) by converting the *para*-hydrogen in solution to normal hydrogen. In this case, the *para*-hydrogen in solution needs to be refreshed between each step of the pump–probe experiment in order to maintain a reproducible level of hyperpolarisation throughout.

<sup>1</sup> O. Torres, B. Procacci, M. E. Halse, R. W. Adams, D. Blazina, S. B. Duckett, B. Eguillor, R. A. Green, R. N. Perutz and D. C. Williamson, *J. Am. Chem. Soc.*, 2014, **136**, 10124–10131.

(13:[13]13) **Guy Lloyd-Jones** asked: Are there any spin manipulations that you can make use of during – or immediately after – the laser pulse, but before the rf-pulse, that would have the effect of moving the boundary for the fast regime into the medium regime?

**Meghan Halse** replied: The <sup>1</sup>H nuclei in *para*-hydrogen are in a nuclear singlet state. Therefore they are NMR-invisible and invariant under RF irradiation. It is only once the magnetic symmetry of the *para*-hydrogen-derived nuclei is broken in the product molecule that the NMR-silent singlet state evolves into observable NMR signals. The timescale that controls this evolution is determined by the difference in either coupling or chemical shift between the *para*-hydrogen-derived nuclei. The only way to increase this timescale (and so move the boundary for the fast regime into the medium regime) is to introduce a bigger difference between the nuclei in some way. It cannot be done by RF irradiation during or immediately after the laser pulse.

(14:[14]14) **Jeremy Harvey** remarked: I have a question about the timescales that you can access with your method. In the paper, it appears that the key is that the pseudo-first order rate constant of the rate should fall within a certain range of the NMR *para*-hydrogen dephasing time. But the reactions you look at are for addition of hydrogen. The pseudo-first order rate constant includes the concentration of H<sub>2</sub>. So in principle, in the example you gave where reaction is too fast, if you dropped the H<sub>2</sub> pressure, could you slow it down? Is that conceivable?

**Meghan Halse** answered: It is possible to slow down the rate of H<sub>2</sub> addition by reducing the H<sub>2</sub> pressure. Indeed we have studied the rate of H<sub>2</sub> addition to IrI(CO)(PPh<sub>3</sub>)<sub>2</sub> as a function of H<sub>2</sub> pressure in Ref. 11 of the paper. The only practical limitation is the signal-to-noise ratio due to the level of hyperpolarisation achieved.

(15:[15]15) **John Slattery** commented: You have talked about optimising the 'probe' part of the experiment in terms of signal-to-noise. Is there any scope for improving the 'pump' part – for example, by increasing the concentration of the photoactivated complex? Are there any specific requirements for your complexes that limit the 'pump' part?

**Meghan Halse** replied: The main limitation on the complex concentration is the requirement for it to work under optically dilute conditions. For the systems we have studied, we have had to work at very low concentrations in order to meet this requirement. Therefore there is limited scope to increase signal-to-noise by increasing the concentration of the complex. This is why the *para*-hydrogen hyperpolarisation is crucial for providing sufficient signal-to-noise in these experiments. More generally the requirements are that the complex needs to absorb at the operating wavelength and display some sort of reactivity that involves or leads to the pair-wise insertion of *para*-hydrogen into the target species.

(16:[16]16) **Joseph Mwansa** opened a general discussion of the paper by Ulrich Hintermair: With respect to the proposed mechanism, if you look at structure **4** which is chiral, once you lose the hydride you form an achiral complex. Is it possible then for the resultant hydride complex to exist as distinct diastereoisomers?

**Ulrich Hintermair** replied: The octahedral [(arene)Ru(bidentate)X] complexes are indeed chiral-at-metal, and with enantiopure TsDPEN as the bidentate it may form two diastereoisomers depending on which side the hydride is added to the achiral-at-metal unsaturated amido complex. This possibility has been noted by Noyori and Ikariya as well as a few others since (including Martin Wills and Neil Strotman). However, the importance of the configuration at Ru in enantioselective hydrogenation with these complexes is still unclear. We have observed both diastereoisomers of the active Ru–H complex during asymmetric transfer hydrogenation catalysis with FlowNMR spectroscopy, and a manuscript looking into their interconversion and their involvement in the catalytic cycle is currently in preparation.

(17:[17]17) **Megan Greaves** asked: How did you determine where **2** appeared in the UV-Vis if, as you said, none of this species was formed (in measurable amounts) during the reaction?

**Ulrich Hintermair** answered: As described in the supplementary information of our paper (page S3) and shown in the insert of Fig. 9 in the paper, we have been able to synthesize this intermediate and measure a UV-vis spectrum of the pure compound for comparison. From its molar extinction coefficient at  $\lambda_{\text{max}}$  we can

1 then deduce the lower limit of its concentration under reaction conditions to be  
less than 2% of the total Ru loading.

5 (18:[18]18) **Thomas Braun** said: You see H<sub>2</sub> evolution before the catalytic  
reaction and during it too. Does it go back, with the H<sub>2</sub> being consumed? Do you  
see CO<sub>2</sub> hydrogenation? Could you hydrogenate different substrates?

10 **Ulrich Hintermair** responded: Although we have not repeated the experiment  
to verify it ourselves, Noyori and Ikariya reported in their 1996 JACS paper (ref. 7 in  
our paper) that running the transfer hydrogenation of acetophenone from  
HCOOH under an atmosphere of D<sub>2</sub> did not yield any deuterated product but only  
15 <sup>1</sup>H phenylethanol. Thus, under normal reaction conditions the two reactions  
seem to occur in parallel. As they are mediated by the same catalyst system they  
are interconvertible however, and under high-pressure conditions it is indeed  
possible to reduce CO<sub>2</sub> to HCOOH with H<sub>2</sub> using these catalysts (as described by  
Jessop and Noyori in the late 1990s).

20 (19:[19]19) **Robert Morris** said: You comment when writing about Fig. 6 of your  
article that the maximum in the chemical shift of the carboxylic acid resonance in  
the <sup>1</sup>H-NMR spectrum is indicative of the maximum acidity of the system. What is  
the significance of this for catalysis?

25 **Ulrich Hintermair** answered: As I said previously, we don't know yet what the  
exact H-bonding situation is in the mixture at the point of maximum chemical  
shift and minimal peak width (shown in Fig. 6 of our paper), but it does correlate  
nicely with transfer hydrogenation activity of the catalyst. To test this hypothesis  
we have run a reaction with the optimum concentration of formic acid present  
30 from the start (all other things being equal) and indeed observed much lower H<sub>2</sub>  
co-production and maximum *T*<sub>Hyd</sub> rates without any induction period. These  
recent results, which we hope will prove useful for applied catalysis, will be added  
to the paper following the meeting.

35 (20:[20]20) **Aaron Odom** remarked: I have a few related questions I was hoping  
to get your thoughts on. In your proposed mechanism, you have a coordinated  
formate that needs to, in essence, β-hydride eliminate to give CO<sub>2</sub> and a metal  
hydride. There are a couple of different mechanisms that could apply. In one, the  
formate de-coordinates from the metal center, and the hydrogen swings around  
40 to the metal in a tight ion-pair. In fact, I think Prof. Morris showed this transition  
state during his introductory lecture. In the second mechanism, the hydrogen is  
abstracted similarly to that in an ethyl group, while still being coordinated to the  
methyl. In this, and other potential transition states and intermediates, you could  
potentially have a polar system that would be affected by the polarity of the  
45 medium. Have you looked at the effect of solvent polarity on your system? Do you  
think the polarity of the medium is changing substantially as the reaction  
proceeds, since you have a very polar reagent in excess (formic acid) that is con-  
verted to nonpolar products and by-products? Could this be affecting the evolu-  
tion of the reaction you observe, from unproductive H<sub>2</sub> formation to useful  
50 hydrogenation?

**Ulrich Hintermair** replied: There are indeed alternative pathways. If the O-bound formate complex was in-cycle as drawn, then there could be various degrees of dissociation in this global  $\beta$ -H elimination step that should be sensitive to solvent polarity, as you suggest (and cation effects as discussed in DOI: 10.1039/C9FD00055K). We have not investigated different solvents ourselves, but the system is known to work well in a range of solvents from methylene chloride up to methanol. I would caution, however, that this apparent insensitivity to solvent polarity is masked by the fact that high (several molar) concentrations of the polar FA/TEA mix are typically used. In fact, some applications run the catalysis in neat FA/TEA mixtures, so any organic solvent added is more of a co-solvent rather than the main reaction medium. How much the global polarity of these mixtures changes over the course of the reaction as FA is being consumed and ketone transformed into alcohol is not an easy question to answer (experimentally or theoretically) but we have observed characteristic shifts of the  $\text{HCOO}^-\text{H}$  during the reaction (Fig. 6 in our paper). I am not sure how much we can interpret this time-averaged signal of the catalytic mixture, but stoichiometric reactions in dilute solution could be more informative.

(21:[21]21) **David Nelson** said: I have two brief questions about this work. Firstly, a back-pressure regulator was used to ensure 7 bar of pressure in the line and therefore reduce bubble formation within the loop. Does this affect any aspect of the performance of the reaction?

Secondly, has a crossover experiment been used to verify the origin of the hydrogen from the parasitic reaction? The use of a mixture of  $\text{HCO}_2\text{H}$  and  $\text{DCO}_2\text{D}$  ought to lead to a mixture of HH, HD, and DD if the proposed mechanism is correct. This might, however, depend on whether a mixture of  $\text{HCO}_2\text{H}$  and  $\text{DCO}_2\text{D}$  leads to the formation of  $\text{DCO}_2\text{H}$  and  $\text{HCO}_2\text{D}$ .

**Ulrich Hintermair** responded: With a residence time in the FlowNMR loop of less than a minute and  $\sim 80\%$  of the reaction volume residing in the open flask, only a small amount of sample will experience the increased pressure conditions for a short amount of time. While in principle this could lead to a change in chemical equilibria involving  $\text{H}_2$  and  $\text{CO}_2$  release, control experiments (included in Fig. 3 of our paper) have shown that the influence is negligible in this case. As the stoichiometric test experiments of protonating the hydride complex with  $\text{HCOOH}$  to yield  $\text{H}_2$  and a formate complex were conclusive enough for our purposes, we have not performed the suggested deuteration experiments (which could potentially be complicated by H–D scrambling, as suggested).

(22:[22]22) **John Slattery** commented: As a follow-up to the previous question, can you vary the back pressure that you use in the experiments to suppress bubble formation? If so, when you vary it do you see any changes in the kinetics?

**Ulrich Hintermair** replied: That should in principle be possible with a pressure-tunable BPR and a high-pressure pump, but we have not tried this experimentally so far.

(23:[23]23) **Anthony Haynes** asked: Since there is gas formation in the system you describe, are the spectroscopically observed species in equilibrium with the

1 gas in the headspace of the reaction vessel? What is the circulation time of the  
flow system and does this have an influence on your measurements?

5 **Ulrich Hintermair** answered: Yes, similarly to my response to Dr Nelson, there  
could in principle be pressure effects on H<sub>2</sub> and CO<sub>2</sub> release caused by the short  
residence time in the sample loop, but under the conditions applied we have not  
noticed any significant implications from comparison with control experiments. The  
mean round-trip residence time at the applied flow rate of 4 mL min<sup>-1</sup> was about  
10 one minute, with ~30 s delay for half way between the reactor and NMR detection.  
Thus, all data recorded is accurate with a 30 s offset from the real time axis.

15 (24:[24]24) **Jeremy Harvey** said: I have a question about the mechanisms and  
competition between the two cycles, as shown in Fig. 11 in your paper, in relation  
to the reaction time series for formic acid shown, for example, in Fig. 4 of the  
paper. Looking at those kinetics, you seem to have a very sharp transition from  
the first regime, with very rapid consumption of formic acid but formation of H<sub>2</sub>  
rather than product, to the regime where product formation dominates (with slow  
20 H<sub>2</sub> formation). Looking at the mechanism in Fig. 11, there shouldn't be such  
a sharp transition, as you're varying the concentration of formic acid by roughly  
a factor of two only. I find this surprising, but I suspect the answer is to do with  
the data shown in Fig. 6 of the paper, showing a change in speciation of formic  
acid over the time of the reaction. Does this imply the mean chemical structure of  
the formic acid is changing; or in other words, does the *activity* of free formic acid  
25 change more than its overall concentration does? Is that how I should read your  
Fig. 11?

30 **Ulrich Hintermair** replied: It is correct that there are several things changing  
over the course of the reaction, not only the amount of free formic acid but also its  
H-bonding situation (which affects its activity), as can be seen from the shift of the  
O–H proton shown in Fig. 5 of the paper. This goes along with a shift in the  
catalyst distribution to more hydride at the expense of formate, in combination  
causing the increasing rate of transfer hydrogenation of the ketone and reduced  
35 H<sub>2</sub> co-production. We have not been able to elucidate the H-bonding network of  
the system in more detail yet, but we will have another look at it.

40 (801:[25]25) **Odile Eisenstein** communicated: Several mechanisms have been  
proposed for hydrogen transfer between a metal complex with the functionality  
H–M–X–H where X is often a nitrogen but can also be oxygen. Originally the  
transfer involved only the alcohol and the 16 e<sup>-</sup> Ru=X complex. Then assistance by  
the solvent was proposed for the transfer of the proton. There is a review by Pavel  
Dub on the topic.<sup>1</sup> One should also not forget the ion pair based mechanism  
proposed in related problems by Faraj Hasanayn.<sup>2</sup>

45 1 P. A. Dub and J. C. Gordon, *ACS Catalysis*, 2017, 7, 6635–6655.

2 See, for instance: a) F. Hasanayn and R. H. Morris, *Inorg. Chem.*, 2012, 51, 10808–10818; b)  
F. Hasanayn and H. Harb, *Inorg. Chem.*, 2014, 53, 8334–8349; c) F. Hasanayn, L. M. Al-Assi,  
R. N. Moussawi and B. S. Omar, *Inorg. Chem.*, 2016, 55, 7886–7902.

50 (25:[26]26) **Robin Perutz** remarked: We have seen from Bob Morris's presen-  
tation the importance of hydrogen bonding in organometallic reaction

mechanisms, including hydrogenation and transfer hydrogenation. Other presenters have emphasised the role of H-bonds throughout the meeting. The strength of hydrogen bonds to metal-bound ligands varies widely according to the metal and spectator ligands as well as the ligand directly forming the H-bond. We have measured the strength of a set of related group 10 square planar metal(II) monofluoride complexes and a set of group 4 complexes of the type  $\text{Cp}_2\text{MF}_2$ , where fluorine is the H-bond acceptor. We have shown by  $^{19}\text{F}$  NMR measurements that the equilibrium constants of the nickel fluoride complexes for forming the hydrogen bond vary with the H-bond donor by at least a factor of 1000, from 15 for  $\text{Ph}_2\text{NH}$  to 15000 for hexafluoroisopropanol (in toluene solvent). Moreover, by using H-bond scales, we are able to compare the H-bond acceptor abilities of the metal fluorides to those of organic H-bond donors. The square planar complexes  $\text{M}(\text{PR}_3)_2(\text{Ar}^{\text{F}})\text{F}$  have an H-bond acceptor ability comparable to or even greater than the strongest organic H-bond acceptors such as  $\text{Me}_3\text{NO}$  while the H-bond ability of the group 4 complexes is much weaker and comparable to acetonitrile.<sup>1</sup> I would strongly encourage experimentalists to measure H-bond strengths where possible using NMR or IR spectroscopy, and computational chemists to calculate H-bond strengths in order to establish the role of the H-bond more quantitatively.

1 D. A. Smith, T. Beweries, C. Blasius, N. Jasim, R. Nazir, S. Nazir, C. C. Robertson, A. C. Whitwood, C. A. Hunter, L. Brammer and R. N. Perutz, *J. Am. Chem. Soc.* 2015, **115**, 11820–11831.

**Odile Eisenstein** answered: It would be of great interest to computational chemistry to have more quantitative information on weak interactions. They are often challenging to reproduce, as relatively weak interactions in general, and thus can be used for method and/or model improvements. Additionally, one should keep in mind that weak interactions between molecules suggested by X-ray analysis on a crystal may not be well modelled *in silico* by a small number of molecules set in a vacuum. Other types of calculations adapted to represent a crystal structure (without forgetting dispersion effects) may be necessary. For a related problem on  $\sigma$ -bonded alkane to transition metal complexes see the work by S. A. Macgregor and A. Weller.<sup>1</sup> In this work, periodic calculations and dynamic studies have been carried out.

1 For example: F. M. Chadwick, N. H. Rees, A. S. Weller, T. Krämer, M. Iannuzzi and S. A. Macgregor, *Angew. Chem. Int. Ed.*, 2016, **55**, 3677–3681.

**Todd Marder** added: This is very important. In our Ni–F system, although H-bonding is not involved, the similar property of the Ni–F bond as a donor to boron is likely to be critical to its effectiveness in the transmetalation process.

**Ulrich Hintermair** said: This comment by Prof. Perutz is much appreciated; however, I would like to add that in many cases such measurements are complicated by dynamic exchange processes occurring in a catalytic reaction mixture. This should not be taken as an excuse not to try and measure these important interactions, but rather to highlight the difference between model systems and applied catalysis, where NMR spectroscopy would show a time-average of all interactions in solution with little contribution from the catalyst present in low concentrations.

1 (26:[27]27) **Thomas Braun** commented: When talking about the hydrogen bond strength, the nature of the bond could be more covalent or ionic, *etc.* Can you comment on this? I think it would affect the properties and reactivity.

5 **Robin Perutz** responded: I think that the strength of the hydrogen bond will affect the reactivity. The ionic/covalent nature of the bond is not relevant to the change of reactivity unless we are referring to very strong H-bonds that are largely covalent.

10 (27:[28]28) **Odile Eisenstein** remarked: The nature of the H bond has been the topic of a large number of studies. There are many components to the hydrogen bond. They are (in no specific order) electron transfer, electrostatic, polarization, *etc.* The relative percentages of these components depend on the system. It should be pointed out that it is important to remember the quantum  
15 nature of the hydrogen bond. See, for instance, Li's *et al*'s paper,<sup>1</sup> in which it is written, "The small mass of hydrogen means that they are inherently quantum mechanical in nature, and effects such as zero-point motion and tunneling must be considered, though all too often these effects are not considered. As a prominent example, a clear picture of the impact of quantum nuclear effects on the strength of hydrogen bonds and consequently the structure of hydrogen  
20 bonded systems is still absent." Also, consider that this quantum nature is also essential to the representation of the isotope (H/D) fractionation (see the work of Markland, for instance). These are only two examples, but many more studies exist. Essentially all of them use state-of-the-art quantum and dynamic methods to best represent this bond, but do not necessarily investigate its nature. To end this comment, it should be mentioned that the nature of a chemical bond is still the topic of continuous debate and weak bonds are even more difficult to analyze. Regular scientific meetings are held on this  
25 topic.

30 1 X.-Z. Li, B. Walker, and A. Michaelides, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 6369–6373.

35 (28:[29]29) **Todd Marder** asked: What is L on your slide, and why is the nickel fluoride so effective as a hydrogen bonding acceptor?

**Robin Perutz** responded: Group 10 metal fluoride complexes of the type *trans*-ML<sub>2</sub>(aryl<sup>F</sup>)F (M = Ni, Pd, Pt) are outstandingly effective as hydrogen-bond acceptors. Here L is a monodentate phosphine, usually PEt<sub>3</sub> or PCy<sub>3</sub>. The principal reason may be traced to the absence of pπ–dπ M–F bonding resulting in a highly polar M–F bond. Nevertheless, the strong ligand field means that these complexes adopt square-planar diamagnetic structures and are soluble in non-polar solvents. Of the three group 10 metals, palladium forms the strongest H-bonds both by the equilibrium constant at 300 K and by the enthalpy of dissociation.<sup>1</sup> In contrast, the polarity of the M–F bonds in d<sup>0</sup> systems such as Cp<sub>2</sub>MF<sub>2</sub> (M = Ti, Zr, Hf) is much lower and the hydrogen bonds are much weaker.<sup>1</sup>

50 1. D. A. Smith, T. Beweries, C. Blasius, N. Jasim, R. Nazir, S. Nazir, C. C. Robertson, A. C. Whitwood, C. A. Hunter, L. Brammer and R. N. Perutz, *J. Am. Chem. Soc.*, 2015, **137**, 11820–11831.



(29:[30]30) **Todd Marder** addressed Meghan Halse and Robin Perutz: In a general sense (as we had a question about pump–probe earlier) what is the fastest timescale on which you can observe a process using NMR spectroscopy? Assuming we wanted to do a pump–probe in a Mike George sense and look at the triplet state of an NMR active species – on what kind of timescale could we monitor the photophysical properties? If we had something that lasted more than 100 microseconds, could we look at it using NMR?

**Meghan Halse** answered: The timescale on which we can directly observe an NMR active species is defined by the time it takes to encode the NMR signal with the desired spectral resolution. This is typically on the order of tens to hundreds of ms. Therefore a triplet state that lasts for on the order of 100 microseconds would not be directly observed in a standard NMR spectrum. However, there are indirect methods that could potentially be used to infer the presence of more short lived species. In our pump–probe experiments with *para*-hydrogen, if the hydrogen nuclei from *p*-H<sub>2</sub> have their symmetry broken in a suitable way in an intermediate state with a lifetime on the order of 100 μs this will affect the observed evolution of the enhanced NMR signal of the product as a function of the pump–probe delay. In principle, some information about the identity and lifetime of the intermediate could be inferred from these signals but theoretical modelling and numerical simulations would be required to interpret these effects.

**Robin Perutz** added: Such an effect as described above has been reported by Dobkowski *et al.*<sup>1</sup>

1 J. Dobkowski, A. Gorski, M. Kijak, M. Pietrzak, K. Redekas and M. Vengris, *J. Phys. Chem. A*, 2019, **123**, 6978–6985.

(30:[31]31) **Evert Jan Meijer** commented: Coming back to the H-bond strength and complexity of measuring it, the environment the substrate is in is also important.

For example, if one measures the ‘strength’ of a water–catalyst hydrogen bond in a protic solvent, the ‘strength’ of the hydrogen bond is governed by the hydrogen-bond network that embeds the water–catalyst H-bond. Hence, the H-bond involves not only the local species forming the hydrogen bond, but rather is an extended non-local property.

(100:[100]100) **Martin Jakooobi** opened a general discussion of Jana Roithová’s paper: Are there any other types of macromolecule, such as cucurbiturils or acyclic cucurbiturils, that have been used to verify the role of anions in a reaction mechanism? Additionally, how do your measured anion binding constants in bambusurils compare with those in other macrocycles? Finally, are there any other examples in literature where macromolecules have been used to verify the role of anions in a reaction mechanism?

**Jana Roithová** responded: I am not aware of examples of using macrocycles or macromolecules selectively binding anions to verify the role of anions in reaction mechanisms. The binding constants of bambusurils with anions were



determined by isothermal titration calorimetry.<sup>1</sup> Here, we used a series of NMR experiments to check whether the studied anions are quantitatively encapsulated in the bambusurils (see the Supplementary Materials in the paper). We did not study the binding constants.

1 See, for example: V. Havel and V. Sindelar, *ChemPlusChem*, 2015, **80**, 1601–1606.

(101:[101]101) **Simone Gallarati** asked: Have the bambusuril macrocycles been used to selectively capture counter-ions in other catalytic systems apart from the gold-mediated addition of methanol to 1-phenylpropyne, and have they been proven to actively influence the reaction by altering the nature of the counter-ion?

**Jana Roithová** answered: No, bambusurils were not used previously as probes of counter-ion effects in metal catalysis. This is the first report on this use of bambusurils.

(102:[102]102) **Jamie Cadge** inquired: In your reaction using these macrocycles, you will generate reactive 'Au<sup>+</sup>' species; therefore, how innocent is the bambusuril macrocycle's interaction with these species? The macrocycle has structural motifs which look like they could interact with an 'Au<sup>+</sup>'. Has this reaction been tried with other weakly coordinating ions such as BARF or Krossing's anion as a comparison?<sup>1</sup>

1 ACIE 2004 pp. 2066.

**Jana Roithová** replied: We have not detected any complexes between gold cations and bambusurils. It is likely that NHC- or phosphino-gold cations will form complexes with bambusurils, if no more strongly binding molecules are present in solution. However, we have investigated solution containing alkynes that are strongly coordinating to the ligated gold cations. Moreover, gold cations are involved in the studied reaction and their resting state is in the form of carbon-bound gold intermediates. For an overview of anions that were tested in the studied reaction, see ref. 12 in the paper. The BARF<sup>−</sup> anion is among the studied counter-ions. It performs similarly to BF<sub>4</sub><sup>−</sup> and ClO<sub>4</sub><sup>−</sup>.

(103:[103]103) **Tom Young** asked: Given that you see no effect when adding bambusurils (*i.e.* seemingly the counter ions do not facilitate the deprotonation), what is the counter effect? Would you consider the different rates shown in Fig. 2 of the manuscript in the absence of additive to be within error?

**Jana Roithová** responded: The rate determining step in this reaction (*i.e.* gold(I) mediated addition of methanol to an alkyne) is protodeauration. Hence, changes in the concentration of protons have an enormous effect on the reaction rate. Of course, the anions, being free or encapsulated, affect the acido-basic equilibrium in solution. Hence, the effects are mostly likely associated with the acidity of the conjugated acid. This can be nicely demonstrated by the experiment with anion exchange in the cavity of bambusurils that is described in the paper.

(104:[104]104) **Samuel Scott** said:  $\text{NTf}_2^-$  is a more strongly coordinating anion that has been demonstrated to slow reaction rates in gold chemistry. Have you investigated using  $\text{AgNTf}_2$  to activate the gold complex, and if so, do the bambusurils capture the anion as efficiently as the more weakly coordinating ones? Do you see a faster rate with bambusurils than without? Is the rate comparable to the other anions?

**Jana Roithová** responded: No, we did not investigate  $\text{NTf}_2^-$ . I am also not aware that complexation of  $\text{NTf}_2^-$  with bambusurils would be studied.

(105:[105]105) **Ian Fairlamb** remarked: I'm interested in the sensitivity of these reactions to water, particularly relating to gold hydroxide type species. Did you look at this sensitivity at all?

**Jana Roithová** responded: Yes, we looked into this problem. The reaction with methanol is faster than the reaction with water. There is usually a small amount of water in the reaction mixture, but methanol is always in a large excess and therefore a genuine addition of water could be neglected. We see the presence of water displayed by hydrolysis of ketals, the products of the double addition of methanol, to ketones. The intensity of ketones in the NMR spectra correlates with the intensity of the corresponding ketals and depends on the overall amount of water in the methanol solvent. We did not detect formation of digold hydroxide in the reaction mixtures in methanol.

(106:[106]106) **Aaron Odom** remarked: You see a small effect on addition of the anion encapsulating agent, bambusuril, which you suggest is due to changes in acidity. However, if tight ion pairing is occurring, the bambusuril anions could reside at different places around the cation in the tight ion pair, which is an alternative explanation. Have you used a method like DOSY to examine if the anion and cation have different diffusion constants in solution? Alternatively, you could examine the system with the popular  $\text{B}(\text{Ar}^{\text{F}})_4^-$  anions; there are several. In previous work, we've found that they form tight ion pairs just as often as everything else; however, they are nonselective in where they reside around cations, which can lead to differences in reactivity with many of the anions you employed.<sup>1</sup> Perhaps you can rule out tight-ion pairing using some other method?

1 K. E. Aldrich, B. S. Billow, D. Holmes, R. D. Bemowski, A. L. Odom, *Organometallics*, 2017, 36, 1227–1237.

**Jana Roithová** replied: The counter-ion effect that we were trying to prove or disprove has been described previously.<sup>1</sup> It has been suggested that the anions in a reaction mixture mediate deprotonation of the gold–alkyne–methanol adduct in a tight transition structure. Such a situation would not be possible if the anions were encapsulated in the cavity of bambusurils (the anions are bound by 12 hydrogen bonds inside the cavity). Note that the NMR spectra shown in the Supplementary Materials of the paper clearly show that the anions are inside the cavity. If I understand correctly, you suggest that the gold cations might form tight ion pairs with encapsulated anions and thus the anions could still mediate the deprotonation step. I don't consider this as a probable scenario, with respect to

the structure of bambusuril–anion complexes. Nevertheless, it could be indeed further investigated and the experiments that you suggested could be done.

1 See, for example: D. Zuccaccia, A. Del Zotto and W. Baratta, *Coord. Chem. Rev.*, 2019, **396**, 103–116.

(107:[107]107) **John Slattery** said: I have a comment following on from the other questions. I don't think it's too helpful to think about these macrocycles as 'removing' the anions. Macrocycle coordination changes the nature of the anion, rather than removing it, and this may or may not influence the reactivity that is seen. For example, if chloride is coordinated by the macrocycle, the anion becomes [macrocycle.Cl]<sup>−</sup>. The macrocycle has some Lewis basic functional groups on its surface, which could interact with a Lewis acidic metal centre. I agree with your suggestion that in a protic, coordinating solvent such as methanol the anion may be well solvated regardless of whether it is 'free' or coordinated by the macrocycle, but the anion isn't taken away. In other solvents the macrocycle-coordinated anions may not be so innocent.

**Jana Roithová** replied: The anions are obviously not removed from solution – I am sorry for the simplified language that I perhaps used in stressing my point. Anions get encapsulated in the cavity of the bambusurils as evidenced by NMR spectroscopy. I agree that bambusurils with anions in their cavity can also participate in reactions proceeding in the given solution. For example, they will affect acid–base equilibria. They can also probably interact with metal ions. In any case, the point of our study is that some changes in the reaction kinetics should be observable, if anions play an important mechanistic role and if their form in solution changed (free vs. encapsulated in the cavity of the bambusurils).

(108:[108]108) **Ulrich Hintermair** opened a general discussion of the paper by Alison Hulme: I was wondering about the quantification of your Raman signals. You even showed an example of different peak intensities that didn't correlate with their respective concentrations due to the different oscillator strengths. How do you account for this, and can you actually derive concentration values from species you can't isolate for signal calibration?

**Alison Hulme** responded: Oscillator strengths depend on the polarizability of the oscillator; the effect of this is particularly noticeable in the Glaser–Hay reaction where the diyne product has a Raman intensity which more than an order of magnitude greater than that of the alkyne precursor (*cf.* Fig. S4 in the paper). The experimentally determined intensities of a range of alkyne motifs have been collated.<sup>1</sup> We have shown that DFT calculations can be used to predict the relative intensity of an oscillator<sup>2</sup> and others have used DFT predicted wavenumbers to assign the identity of unknown oscillators.<sup>3</sup>

1 H. Yamakoshi, K. Dodo, A. Palonpon, J. Ando, K. Fujita, S. Kawata and M. Sodeoka, *J. Am. Chem. Soc.*, 2012, **134**, 20681–20689.

2 W. J. Tipping, M. Lee, A. Serrels, V. G. Brunton and A. N. Hulme, *Chem. Sci.*, 2017, **8**, 5606–5615.

3 K. Aljakouch, T. Lehtonen, H. K. Yosef, M. K. Hammoud, W. Alsaïdi, C. Kötting, C. Mügge, R. Kourist and S. F. El-Mashtoly, *Angew. Chem. Int. Ed.*, 2018, **57**, 7250–7254.

(109:[109]109) **Robin Perutz** asked: What is the effect of laser fluence on your reactions and have you measured this effect? Most organometallic chemists recognise that their samples can be heated or photolysed by high laser intensities and laser fluence; they would usually use oscillating samples or sample flow. I would count high intensity as anything where you have a focused laser beam, which you always do with Raman. You have two lasers, one is 1064 nm and the other is shorter wavelength. There are a lot of absorbing compounds in the cell and the 1064 laser will heat these – I would be concerned by this.

**Alison Hulme** answered: In consultation with my co-author, Dr Martin Lee, I can confirm that although the peak fluence in stimulated Raman scattering microscopy is quite high due to the small focal spot used (FWHM xy beam diameter: 0.5  $\mu\text{m}$ ), this is rapidly raster scanned over a large area (500 x 500  $\mu\text{m}$ ) with short dwell times (pixel-dwell: 2–20  $\mu\text{s}$ ) thus dissipating the energy. This approach minimises the effects of local heating that are present in fixed imaging systems, such as a spontaneous Raman system where the same spot is held under the laser for much longer times (pixel-dwell typically >0.5 s). For our SRS microscope ( $\lambda$ : 1064 nm and 868.2 nm) each beam has similar characteristics ( $P_{\text{ave}}$ : 50 mW; repetition rate: 80 MHz; FWHM xy beam diameter: 400 nm; pulse duration: 6 ps; energy per pulse: 0.625 nJ) with a laser peak fluence of 0.345 J cm<sup>-2</sup> and a peak power of 57.4 MW cm<sup>-2</sup>. For specimens consisting mainly of water it has been shown that for a large power,  $P_{\text{ave}} \approx 100$  mW, and  $\lambda < 1070$  nm, the water temperature in the focus will not increase more than 3 K.<sup>1</sup> Indeed, we have shown that in a 13.5 min continuous scan through a liver window<sup>2</sup> there are no visible signs of photodamage or impaired biological function that would occur if local heating was an issue. Notwithstanding this, it is true that SRS microscopy is not generally successful in the presence of strongly absorbing cellular components (e.g. pigments such as melanin found in skin) which can give rise to localised heating, and this will guide our choice of cell lines and tissue in future experiments.

1 A. Schönle and S. W. Hell, *Optics Lett.*, 1998, 23, 325–327.

2 M. Lee, A. Downes, Y.-Y. Chau, B. Serrels, N. Hastie, A. Elfick, V. Brunton, M. Frame and A. Serrels, *IntraVital*, 2015, 4, e1055430.

(110:[110]110) **Guy Lloyd-Jones** added: The control reactions we conducted using IR to measure the kinetics gave the same rate constants as those determined by spontaneous Raman spectroscopy (RS); however, they were not compared with those determined by stimulated-RS, and we appreciate that it is important that they should be, in order to evaluate the thermal or photochemical impact of the laser(s) on the reaction rate/evolution.

(111:[111]111) **James Walton** commented: I really enjoyed reading the paper and listening to the discussion on Raman spectroscopy as a technique to follow the progression of the CuAAC and Glaser–Hay reactions. The paper validates the use of this technique to produce kinetic data, which helps to understand the mechanism of the reactions. After reading the paper, I was interested to ask about whether any cell studies, or studies in cell media had been carried out or planned. In the 5 min presentation of the paper, Prof. Hulme presented some results on

1 this, showing how the CuAAC reaction proceeded differently in different cell lines.  
My question is, have the authors considered how they will carry out the studies in  
the cellular environment to gain kinetic information? In a 'test tube' we can vary  
5 conditions, such as reactant concentration or catalyst/ligand, and see the effect  
upon reaction rate. The issue, of course, arises in the cellular environment where  
you can dose a cell with a given concentration of reagent, but you don't know the  
concentration of that reagent at the location of the reaction (*e.g.*, the nucleus for  
10 EdU). Equally, you can dose with a given metal/ligand complex, but you don't  
know whether that complex is still intact at the site of reaction or whether some  
ligand exchange takes place with endogenous ligands (thiols, GSH, histidine,  
*etc.*). Have the authors thought about how they might address these challenges in  
the cellular environment? My other (perhaps naïve) question during the discus-  
15 sion was, relating to the cell studies that were shown on the slides, does the  
Raman technique give you an idea of the speciation at Cu? If not, are there other  
techniques which could help to elucidate the/an active species – HPLC or LCMS,  
perhaps, taken from a cell media experiment? Thank you again for a nice paper  
and talk. I look forward to reading more in the future!

20 **Alison Hulme** replied: Thank you for your interest! We have indeed conducted  
preliminary studies on the CuAAC reaction in cells, as I showed in my presenta-  
tion. Plotting the decreasing signal intensity observed across the nucleus of  
individual cells (not, as yet, cell lines) allows us to make single-cell kinetic  
25 measurements. Our preliminary data suggests that the  $k_{\text{obs}}$  values obtained vary  
from cell to cell as might be expected in an asynchronous population of cells,  
since cells at different stages of the cell cycle vary considerably in their uptake of  
EdU. In contrast to other Raman techniques (*e.g.* CARS or SERS), SRS is a quan-  
titative technique and hence we are able to estimate the local concentrations of  
30 EdU by using solution phase calibration curves determined on the SRS micro-  
scope. The ability to effectively separate out the alkyne signal from the cellular  
background is one of the key advantages of a Raman-based approach, and makes  
it more attractive than the use of fluorescence to follow intracellular kinetics  
where there is competing background cellular autofluorescence. For the CuAAC  
35 reaction, we are not as yet able to determine local concentrations of the other  
reagents at a sub-cellular level, although these can be determined for the overall  
cell population using ICP-MS, or quantitative ESI-MS. Indeed, this approach to  
the determination of reactive components has been demonstrated in a recent  
investigation of CuAAC reactions on/in live cells.<sup>1</sup> Thus our initial experiments  
40 will be to use SRS microscopy to compare, rather than quantitate, the  $k_{\text{obs}}$  values  
obtained, for example, in the different microenvironments found within a cell, or  
between different cell lines.

Deactivation of copper catalysts by intracellular thiols such as glutathione has  
45 been proposed to be one of the major causes of low yields in CuAAC reactions  
conducted in the cytoplasm of cells.<sup>1</sup> We have not as yet attempted to use Raman  
to determine the speciation of the copper catalyst in an intracellular environment,  
as the local concentrations of species are likely to be too low to be detectable.  
Furthermore, cell media experiments (LC or LC-MS) are unlikely to provide an  
50 accurate assessment of intracellular speciation, as they present a very different  
environment and rapid ligand/species exchange might be expected. Thus this  
represents a considerable challenge in the field.

1 S. Li, L. Wang, F. Yu, Z. Zhu, D. Shobaki, H. Chen, M. Wang, J. Wang, G. Qin, U. J. Erasquin,  
Y. Wang and C. Cai, *Chem. Sci.*, 2017, **8**, 2107–2114.

(112:[112]112) **Todd Marder** said: I'm a little confused – are you monitoring  
a reaction inside the cell? You use HeLa cells here – what are you imaging? From  
your expertise in Raman imaging, are diynes, the product of your Glazer coupling,  
more intense? What's the preference between ions, diynes and internal ions with  
Raman signal?

**Alison Hulme** responded: In our paper we present kinetic data achieved by  
following two bioorthogonal reactions, the CuAAC and Glaser–Hay coupling  
reactions, in solution using spontaneous Raman microscopy (Fig. 4–7 and asso-  
ciated discussion in the paper). The images of HeLa cells (Fig. 3 in the paper) were  
used to show how this is relevant to an intracellular environment by demon-  
strating that we can tune a stimulated Raman scattering (SRS) microscope to  
different wavenumbers to allow us to image proteins (Fig. 3B(i)), lipids  
(Fig. 3B(ii)), or an alkyne such as that found in the thymidine analogue EdU  
(Fig. 3B(iii)). As discussed in response to Dr Walton's question, at the Discussion  
Meeting we were able to present additional preliminary data to show how we  
could convert such images into a kinetic analysis of these reactions in an intra-  
cellular environment. A discussion of the relative strengths of different alkyne  
oscillators has been given in response to Dr Hintermair's earlier question.

(113:[113]113) **Daniel Ess** commented: It seems like the rate constants imply  
the reaction is non-statistical. Could you talk about this in relation to Fig. 6 of the  
paper? Could you predict if there is a non-statistical pathway or a combination of  
statistical and non-statistical pathways?

**Jeremy Harvey** replied: You are correct! Some of the rate constants (all are  
expressed in  $\text{s}^{-1}$ , and in some cases there are pseudo-first-order rate constants  
obtained by folding in the solvent concentrations to bimolecular rate constants)  
are very large indeed. Many of the values in Table 4 of the paper exceed  $10^{12} \text{ s}^{-1}$ ,  
with the largest, the rate constant for ligand loss from the triplet  $\text{CpMn}(\text{CO})_2(-\text{C}_2\text{H}_6)$   
complex, as large as  $3.1 \times 10^{13} \text{ s}^{-1}$ , corresponding to a lifetime of the order  
of 30 fs. Such very large rate constants occur in transition state theory for very  
small barriers, but clearly such large values are not compatible with statistical  
behaviour. In the limit, one can get ballistic, non-statistical behaviour in some  
cases. However, the TST calculations are, in our opinion, still relevant for  
mapping out trends, and also, the actual observed reactivity is in most cases not  
dependant on the very large rate constants – experimentally, the shortest lifetime  
measured for a triplet transient is about 270 ps, which is enough time to have  
roughly statistical behaviour.

(114:[114]114) **Martin Jakooobi** opened a general discussion of Michael  
George's paper: I'm curious about the choice of substrates you have used in the  
study. In the paper it is mentioned that the cyclic alkane complexes are more  
stable than the acyclic ones, and can therefore lead to slightly faster spin cross-  
over. However, at the same time you have compared the triplet lifetime of a cyclic  
PFMCH (perfluoromethylcyclohexane) complex with that of an acyclic heptane

1 complex. Wouldn't it have been better to use MCH (methylcyclohexane) instead of  
heptane, to obtain triplet lifetimes from structurally comparable complexes?

5 **Mike George** responded: There is a dramatic difference in reactivity between  
the organometallic alkane and perfluoro-alkane complexes. We find that per-  
fluoromethylcyclohexane (PFMCH) behaves very similarly to krypton as a solvent  
and as a ligand. In our previous studies we used supercritical krypton as an inert  
solvent and doped in ligands to the solvent to investigate their reactivity. Here we  
10 used the PFMCH as it has similar properties that we require without the need to  
use high pressures and sophisticated equipment.

15 ([115:[115]115) **Odile Eisenstein** remarked: In the earlier study, the coordina-  
tion of the ligand L prior to the intersystem crossing was considered not possible  
because it was assumed that L could not coordinate to the triplet state of the 16  
electron complex, because there was an electron in the main orbital responsible  
for bonding with L. In this study, it is shown that L coordinates to the triplet state,  
which facilitates the inter-system crossing. Is there any way to know if the effect  
of L is felt at a distance longer than 'full coordination'?

20 **Jeremy Harvey** replied: The ligand interacts with the metal complex in the  
triplet state largely through dispersion effects (the dispersion correction makes  
a major contribution to the interaction energy), which do operate at intermediate  
distances significantly longer than that corresponding to the dative interaction  
25 present in the singlet adduct. There is perhaps also some weak two-centre three-  
electron bonding. This type of 'bonding' probably plays the biggest role in  
decreasing the repulsive nature of the interaction in the triplet state compared to  
what would occur if there was a closed-shell:closed-shell repulsion ('two-centre  
four-electron antibonding'), thereby allowing the crossing with the singlet  
30 surface to be lower in energy.

35 ([116:[116]116) **Robin Perutz** said: My question relates to the point you make  
that the solvent is already there. The problem that I often see with these calcu-  
lations and situations is that your measurements and calculations refer to  
a situation outside the range of normal approximations of the activities of solutes,  
which have to be in dilute solutions. Similarly, you are outside the conditions for  
diffusion controlled reactions. Instead, you are looking at reactions of the inter-  
mediate with the solvent. How do you know that you can do valid calculations  
40 under these rather tricky circumstances?

45 **Jeremy Harvey** answered: This is an excellent point. You are referring to some  
assumptions that we make when computing the elementary rate constants con-  
tained in Table 4 of the paper. These assumptions are discussed in the text of the  
paper, near that table. As we state there, we assume that rates involving solvent  
addition go as  $\text{Rate} = k[\text{Solvent}][\text{metal fragment}]$ , even though for some of the  
cases modelled, [solvent] is that of the pure liquid, and there is no potential  
energy barrier for some of the steps, so we assume  $k = k_{\text{diff}}$ , a diffusion-limited  
rate constant. Assuming that the activity of the solvent is linear with the  
50 solvent concentration is a dubious assumption! Assuming that one can talk about  
'diffusion-limited' rate behaviour for addition of a solvent is also dubious.



1 However, these limits, on the one hand, allow us to compute the rate constant  
that we need to compare different mechanisms, and on the other hand, are  
probably more reasonable for these very non-polar solvents than for solvents such  
5 as water. Finally, the aim of our project here is to obtain qualitative insight, rather  
than exact numbers. So despite misgivings concerning these and other approxi-  
mations in our method, we believe that the calculations are 'valid', at least in  
terms of insight.

10 (117:[117]117) **Odile Eisenstein** commented: You study the strength of  
bonding of several ligands to the  $\text{Cp}_2\text{Mn}(\text{CO})_2$ ; these ligands are alkanes, fluo-  
roalkanes and rare gas. However, nothing is mentioned for  $\text{H}_2$ . Would  $\text{H}_2$  bind  
more or less strongly than alkane?

15 **Mike George** replied:  $\text{H}_2$  would bind more strongly than alkane. We did not  
study this here, but have examined it in our previous study on the reactivity of  
 $^3\text{Fe}(\text{CO})_4$ . We plan a much more extensive investigation of a range of ligands with  
various coordination abilities to provide further insight into the spin-crossover  
problem.

20 **Jeremy Harvey** added: In previous work resulting from collaboration between  
our two groups,<sup>1</sup> we examined reactivity of  $\text{H}_2$ . There are also other papers from  
the George group and others looking at dihydrogen addition to triplet metal  
centres. My vague memory is that  $\text{H}_2$  binds roughly equally strongly as alkanes but  
25 I may be mistaken on this.

1 M. Besora, J.-L. Carreón-Macedo, A. J. Cowan, M. W. George, J. N. Harvey, P. Portius, K. L.  
Ronayne, X.-Z. Sun and M. Towrie, *J. Am. Chem. Soc.*, 2009, **131**, 3583–3592.

30 (118:[118]118) **Matthias Bauer** remarked: In order to understand the interac-  
tion of the metal center with the solvent from a solvent perspective, diffuse X-ray  
scattering could be a good choice. Have you already considered this?

35 **Mike George** responded: We did consider this, but these were the first  
measurements of their type to access metal–alkane and metal–Xe bond lengths by  
XAFS, and limitations of beamtime meant that we focussed on the present study.  
We believe these measurements provide a new approach to obtaining insight into  
these weak ligand–metal interactions and we hope further studies using time-  
resolved X-ray methods can unlock information for a wide range of complexes.

40 (119:[119]119) **Stuart Macgregor** said: Your paper highlights differences  
between heptane and perfluorotoluene and links these to their ability to interact  
with  $\text{CpMn}(\text{CO})_2$  in its triplet from. How sensitive is this outcome to the level of  
theory employed, in particular the inclusion (and description) of dispersion  
45 effects? To what extent might these effects be affected by interaction with the bulk  
solvent, which would tend to counter-balance dispersion interactions between the  
substrate and the metal complex?

50 **Jeremy Harvey** answered: The interaction energy of the various molecules/  
atoms with the metal fragment  $\text{CpMn}(\text{CO})_2$  in its triplet and singlet states is



quite strongly dependent on the level of theory. Dispersion obviously plays a big role in the interaction with the triplet form (and indeed with the singlet). The project would not be meaningful when using functionals such as B3LYP which account very poorly for dispersive interactions. Note that we also had to carefully select a functional so as to reproduce the singlet–triplet splitting reasonably well. We did quite a lot of benchmarking that is not described in the paper to try to make a sensible choice of functional in both respects.

In terms of the balance between ligand–solvent, metal fragment–solvent, and metal fragment–ligand complex–solvent dispersive interactions: it is correct that this is a matter of concern. It can be argued that roughly speaking, the weak *Gibbs free energy* of interaction with solvent will cancel out between the reactant ligand + metal fragment and product metal–ligand complex. This is supported by test calculations we did with the SMD continuum model, in which we obtained rather small free energies of solvation for all three species, and a very small change in free energy of solvation. Obviously the *enthalpy* of interaction with the solvent may cancel out less well. But in terms of predicting free energies, I think the procedure we use is roughly correct.

(120:[120]120) **David Nelson** said: Fig. 6 of the paper treats three mechanistic possibilities as distinct reactions. Are these best considered a continuum, where different systems might undergo reaction with different  $L\cdots M$  distances and at different points on the singlet to triplet spin change? This might mirror in some ways the way in which we can consider nucleophilic substitution reactions at carbon in organic chemistry.  $S_N1$  and  $S_N2$  are taught to students as being the two options –with intermediates in which the carbon is five-coordinate being ruled out in all but the most extreme conditions. However, the use of More-O’Ferrall–Jencks diagrams can be used to see these substitution reactions as a continuum, where different systems have transition states with a range of nucleophile $\cdots$ carbon distances and carbon $\cdots$ leaving group distances.

**Jeremy Harvey** replied: The analogy to  $S_N1$  and  $S_N2$  is an excellent one. More generally Fig. 6 of the paper could indeed be viewed as being a form of More O’Ferrall–Jencks diagram, so that all kinds of intermediate behaviour is possible. Inorganic reaction mechanism courses face up to this type of complexity in the context of ligand substitution reactions by introducing, as well as purely associative and dissociative mechanisms, the intermediate-associative and intermediate-dissociative cases. The only difference that should be noted here is that one of the ‘reaction’ coordinates in Fig. 6 (the horizontal axis) is not a bond length or bonding coordinate as in more traditional More O’Ferrall diagrams, but instead a spin-state change coordinate.

(121:[121]121) **Jason Lynam** inquired: I was wondering - with regards to Fig. 6 of the paper, I’ve always thought about these reactions as the route going across the top, going from the singlet and then undergoing solvent coordination. But aren’t all these reactions using the bottom route to different degrees? Ultimately the xenon will get there too, even though it has a weaker interaction than, say, heptane; this is in the equilibrium geometry.

**Jeremy Harvey** replied: Dr Nelson's earlier question referenced Fig. 6 as being a kind of More O'Ferrall-Jencks diagram. Thinking of it that way, it is easy to recognize that depending on conditions and the ligand, one might go through the top right (mechanism **B**), the diagonal (**A**) or the bottom left (**A'**). Our understanding of these reactions has evolved over the years, under the challenge of some of the excellent experimental data coming from Mike George's group and from elsewhere. At first,<sup>1</sup> we thought mechanism **A** might dominate, then we were forced to recognise that in some cases mechanism **B** (initial spin state change, then addition) must be occurring. Then the ligand-dependent reactivities demonstrated here (and to be fair, Mike George has mentioned this dependence to me ever since we started our project) made us realize that mechanism **B** is not enough either; we need to allow for **A'**. It starts to look as though the different cases really do span the whole diagram.

1 J. N. Harvey and M. Aschi, *Faraday Discuss.*, 2003, **124**, 129–143.

(122:[122]122) **Tolga Yaman** addressed Alison Hulme: The whole idea of bio-orthogonal reactions is to keep the cell safe and healthy, but sometimes selectively destroying something hostile is just as beneficial as keeping a cell healthy. Do you know if anyone has ever used your method to analyze such cases?

**Alison Hulme** replied: Various markers associated with cell death can be detected directly by vibrational spectroscopy without the need for additional labelling experiments. Imaging at the vibrational frequency associated with lipids (Fig. 3B(ii) in the paper) can be used to track cell morphology and has been used to image processes, such as blebbing, that are associated with cell death by apoptosis.<sup>1</sup> The vibrational imaging of lipids can also be used to track dynamic processes such as lipid droplet formation<sup>2</sup> and changes in lipid composition,<sup>3</sup> both of which are key markers of bioenergetic and cell stress responses in cancer progression.<sup>4</sup>

1 Y. Fu, H. Wang, R. Shi and J.-X. Cheng, *Opt. Express*, 2006, **14**, 3942–3951.

2 W. J. Tipping, M. Lee, A. Serrels, V. G. Brunton and A. N. Hulme, *Chem. Sci.*, 2017, **8**, 5606–5615.

3 L. E. Jamieson, C. Wetherill, K. Faulds and D. Graham, *Chem. Sci.*, 2018, **9**, 6935–6943.

4 P. Shyu, X. F. A. Wong, K. Crasta and G. Thibault, *Biosci. Rep.*, 2018, **38**, BSR20180764.

(123:[123]123) **Derek Durand** addressed Jana Roithová: With regards to the bambusuril ion traps, have you considered applications that make use of the fact that these are essentially the inverse of the 18-crown-6 ether complexes which form with potassium ions?

**Jana Roithová** answered: This is exactly how we were thinking about these macrocycles. Gold catalysts are usually sold as chloride salts and they have to be activated by an exchange of the anions to non-coordinating anions with a silver salt. We have tried to use bambusurils to strip the anions and liberate the active gold cations. In our test, however, this approach did not work and we did not investigate it further. It is definitively worth investigating this further.

(124:[124]124) **Jason Lynam** said to Alison Hulme: What's the sensitivity when you do the in-cell measurements? Because you've got such a clear spectroscopic window, could you observe the copper alkynyls?

**Alison Hulme** replied: We have been able to detect species using SRS microscopy when cells are incubated with nanomolar concentrations of reagents, but this relies on localised concentration of these species within regions of the cell (*e.g.* within the nucleus, lysosomes, *etc.*), typically estimated as high micromolar to low millimolar concentrations. In our solution phase Glaser–Hay experiments we were able to observe the formation of copper–alkynyl species using spontaneous Raman microscopy, but only in ‘failed’ experiments where copper acetate was used as the catalyst and there was insufficient mixing of the reaction medium caused by a low air bubbling rate. A shift in the alkyne vibrational frequency from 2102 cm<sup>−1</sup> to 1930 cm<sup>−1</sup> was observed, alongside the formation of a precipitate. This shift is in good accord with the Raman spectrum reported for solid phenylethynylcopper.<sup>1</sup> A second additional vibration of much lower intensity was also observed in these experiments at 1971 cm<sup>−1</sup>, but this was not assigned. The formation of inert Cu(I) acetylide polynucleates in ‘failed’ reactions has also been reported in other adaptations of the Glaser reaction for bioconjugation.<sup>2</sup> These results suggest that although the copper alkynyl species falls within the cell silent region, it is unlikely that the local concentrations would be high enough to be observed intracellularly with the current detection limits of the SRS microscope.

1 I. A. Garbuzova, I. R. Golding and A. N. Schegolikhin, *J. Raman. Spectrom.*, 1995, **26**, 391–395.

2 A. P. Silvestri, P. A. Cistrone and P. E. Dawson, *Angew. Chem. Int. Ed.*, 2017, **56**, 10438–10442.

(125:[125]125) **Alexander Hamilton** addressed Jeremy Harvey: The importance of spin states in first row transition metals involved in catalysis is becoming ever-present. Could you comment on state-of-the-art DFT and WFT methodologies for their accurate description?

**Jeremy Harvey** replied: Accurate prediction of relative energies (and properties) of different spin-states of open-shell first-row transition metal compounds remains challenging. A key observation was made some years ago by Markus Reiher<sup>1</sup> that DFT predicted relative energies are often highly sensitive to varying the amount of exact exchange in DFT functionals. This remains a key observation, and suggests an initial strategy when tackling such problems: calculate the relative energies of your target system with a range of different functionals, especially hybrid *vs.* GGA functionals. In cases where high sensitivity is found, experiment can often be used to guide the choice of functional. Another possibility is to use correlated *ab initio* methods to try to derive an absolute benchmark for relative energies. A note of caution here is that it is often seen that correlated *ab initio* calculations, despite their expense, can be less accurate than standard DFT functionals. Reaching the levels of electron correlation treatment and basis set size required to converge relative energies can be surprisingly difficult. New linear scaling correlated methods are not yet fully tested for open-shell first-row

transition metal systems, so some caution is needed to ensure that results obtained with such methods are reliable.

1 M. Reiher, O. Salomon and B. A. Hess, *Theor. Chem. Acc.*, 2001, **107**, 48–55.

(126:[126]126) **Markus Reiher** said: To add to what Jeremy said – a major problem is that you do not know if you have picked the right functional for the specific system you are studying. You need reliable molecular-similarity descriptors and then machine learning to sort the transferability out, but this is hardly ever done. Transferability of density functional accuracy assessed for one or several compounds to similar or even homologous ones cannot be taken for granted (see, for example, ref. 1,2). Also, switching to more accurate wave function based methods is not necessarily a safe haven. We have an accurate reference method available for single-configuration electronic structures, namely explicitly correlated coupled cluster with sufficiently high excitation rank in a triple-zeta basis. This single-reference coupled cluster approach is likely to work for high-spin states, which are often single-configurational, but low- and intermediate-spin states may require multi-configurational approaches, for which no such unique model of comparable accuracy exists (not even within the coupled cluster framework for conceptual reasons). The standard model, namely applying multi-reference perturbation theory, typically in the flavors of CASPT2 or NEVPT2, is, at the end of the day, nothing else but second-order perturbation theory and therefore is intrinsically of limited accuracy. Hence, the situation for calculating spin-state splitting is even worse.

1 G. N. Simm and M. Reiher, *J. Chem. Theory Comput.*, 2016, **12**, 2762–2773.

2 T. Husch, L. Freitag and M. Reiher, *J. Chem. Theory Comput.*, 2018, **14**, 2456–2468.

(200:[200]200) **Megan Greaves** opened a general discussion of the paper by Aiwen Lei: Why did you use NaHMDs for this study, when other (less expensive) bases are used in the relevant coupling reactions?

**Aiwen Lei** replied: We find that the reaction is the most clean and all of the Cu(II) was converted into Cu(I), thus, we considered it as the best model reaction for this study. In fact, before this study, we already knew that NaOBu<sup>t</sup> is able to reduce the Cu(II) to Cu(I). And many bases such as BuLi can reduce the Cu(II) to Cu(I).

(201:[201]201) **Ian Fairlamb** added: Is hexamethyl bisilylazide special in some way, in that it can selectively access the manifold? Could butoxide do a similar thing?

**Aiwen Lei** answered: We are not really sure whether bisilylazide is special. The butoxide is similar but less reactive.

(202:[202]202) **Jason Lynam** said: Your X-ray data show you've identified the copper-based species, but what about the organic radicals? You say you make hydrazine and oxygen radicals – can you determine the fate of those with other methods?

**Aiwen Lei** responded: We did not carefully analyze the decomposition of small molecules (organic radicals) during the early days.

(203:[203]203) **Aaron Odom** remarked: In Scheme 3 you propose a mechanism for the reduction of Cu(II) to Cu(I) by  $\text{NaN}(\text{SiMe}_3)_2$ . In your presentation you showed a series of such mechanisms with various reductants. When working with students in the laboratory, if they see reduction of the type you describe (which is often unwanted) I usually suggest that they switch to a less polar solvent. The thought is that the reduction could be occurring through an outer-sphere electron transfer mechanism in polar solvents, like the DMF solutions you are studying. The reaction sequence would look like this:  $\text{NaN}(\text{SiMe}_3)_2 + \text{CuCl}_2 \rightarrow [\text{NaN}(\text{SiMe}_3)_2]^+ + [\text{CuCl}_2]^- \rightarrow \text{NaCl} + \cdot\text{N}(\text{SiMe}_3)_2 + \text{CuCl}$ . Is there a reason you prefer this mechanism of group transfer followed by radical loss from the metal over the outer sphere electron transfer mechanism?

**Aiwen Lei** answered: For the example of  $\text{CuCl}_2$  with  $\text{NaOBu}^t$ , in low temperatures, such as  $-50^\circ\text{C}$ , we were able to see  $\text{Na}[\text{Cu}(\text{O}^t\text{Bu})_3]$ , but when we increased the temperature, we saw that the Cu(II) was reduced, and  $\text{Na}[\text{Cu}(\text{O}^t\text{Bu})_2]$  was formed. This is not an outer sphere electron transfer pathway.

(204:[204]204) **Jonathan Wilden** remarked: You use the sodium salt for the amide base and the butoxide. There's a lot of work being done with potassium salts, where the potassium ion is critical to the success of the reaction. Is there anything special about the sodium salts in the cases listed in the presentation and paper? If the potassium salts were used, might they have enhanced reactivity?

**Aiwen Lei** replied: We did not try the K-salts.

(205:[205]205) **Ian Fairlamb** asked: I was wondering about the solubility of these 8-complexes you're thinking about. Have you looked at tetrabutyl ammonium salts of these, which would give you that solubility? We talk about how we study these reactions *in situ*, but when you get to synchrotron you're looking at different reaction cells – it's interesting that you'd see this in beamline but not in the lab.

**Aiwen Lei** responded: We did not have chance to investigate the tetrabutyl ammonium salts, which should have better solubility.

(207:[206]206) **David Schilter** commented: This was a great presentation that shows how, using modern analytical approaches, we can still learn more about some of the simplest, oldest and most ubiquitous chemical systems. Your work on acetylacetone mixtures containing Cu(II) and hexamethyldisilazide piqued my interest in particular. Of course, Cu(II) salts of basic anions, such as acetate, will readily react with acetylacetone to afford the classical square-planar complex bis(acetylacetonato)copper(II). I was amazed that you found that when Cu(II) is in the form of a salt of a non-basic anion like bromide, the presence of acetylacetone and hexamethyldisilazide actually results in reduction of Cu(II) to Cu(I). I guess this arises, in no small part, due to the redox-activity of bromide?

**Aiwen Lei** replied: It is unclear currently whether the reduction is due to the redox-activity of bromide or not. One guess is that it's functioning as a weak ligand, which can easily come off the Cu(II)-metal center so the acetylacetonate/hexamethyldisilazide will have a chance to get closer to the Cu(II); then the electron-transfer will have a chance to take place.

(208:[207]207) **Stuart Macgregor** opened a general discussion of the paper by Matthias Bauer: Many of the systems you look at have essentially no symmetry. Does that have any effect on the selection rules? To what extent does orbital mixing affect the outcome?

**Pierre Kennepohl** answered: Symmetry plays a huge role in determining the intensity of spectroscopic features in X-ray absorption spectroscopy. For core spectroscopies, we typically find that electric dipole allowed transitions ( $\Delta l = 1$ ) are about 100 times more intense than corresponding electric dipole allowed transitions ( $\Delta l = 2$ ). As an example, we would therefore expect a pure Ni  $1s \rightarrow 4p$  transition to be 100 times more intense than a pure Ni  $1s \rightarrow 3d$  transition (assuming the same number of possible transitions). But in lower symmetry, these guidelines break down through mixing. For example, the Ni  $1s \rightarrow 3d$  transition in octahedral Ni(II) complexes is very weak, yet those of Ni(II) tetrahedral complexes are significantly more intense. This is because of the same symmetry breaking that causes ligand field transitions for  $T_d$  complexes to be more intense than corresponding  $O_h$  complexes: d/p mixing. In the  $T_d$  complex, the valence 3d orbitals have the same symmetry as the higher-lying valence 4p orbitals, thus allowing for some amount of mixing between the two. Note that even very small amounts of mixing can have a massive impact in such a situation. Given the 100-fold intensity difference between the dipole and quadrupole intensity mechanisms, a 1% mixing of 4p character into the 3d orbitals will essentially double the intensity of the transition. In such cases, the amount of dipole-allowed character becomes critical. In our case, this is one of the main reasons for moving to L-edge spectroscopy to probe the 3d final states. We now have a dipole allowed  $2p \rightarrow 3d$  transition and mixing into the final states will be a small perturbation on what we are trying to evaluate.

(209:[208]208) **Natalie Fey** addressed Matthias Bauer: You have talked about the role calculations play in the deconvolution of spectra. What do you do if you have no idea which complexes are present? That is, how sensitive is the deconvolution/fitting to the calculation results, and if you've considered the wrong complex, will this come out in the fitting?

**Matthias Bauer** replied: Indeed, we need some chemical knowledge about the system we are measuring. For EXAFS it is true that the fitting procedure to extract the data could allow misinterpretation, as the number of parameters used in the fit is typically quite large. However, there are some measures that allow one to judge how good one structural model is compared to another. The problem is that you then have to use several models, which is mandatory for a sound analysis, but not everyone does this.

For the emission and HERFD-XANES calculations using DFT the situation is different, since there no fitting is applied. Here one will realize more immediately

whether a model is good or not. Since typically a geometry optimization is carried out beforehand, this already gives an estimation for the quality of the model in terms of convergence.

(210:[209]209) **Robert Morris** inquired: The deconvoluted peak for the hydride is a fairly small feature. When one thinks of an IR spectrum, you can introduce deuterium and see an isotopic shift of the signal. I was wondering about the corresponding test for the X-ray experiment. Could you substitute that hydride with another X ligand?

**Matthias Bauer** answered: Yes, we tried to prepare complexes substituting the hydride with a halide. But on top of that we investigated other hydride complexes, containing up to four hydride ligands in  $[\{^5\text{CpFe}\}_2(\mu\text{-H})_4]$ , and the particular hydride ligand emission so far always appears at the same position.<sup>1</sup> Thus we are pretty sure that the assignment is correct.

1 L. Burkhardt, C. Mueller, O. A. Groß, Y. Sun, H. Sitzmann and M. Bauer, *Inorg. Chem.*, 2019, **58**, 6609–6618.

(211:[210]210) **John Slattery** said: I have several related questions regarding your computational methodology. How sensitive is your work to the type of electronic structure method used to model the complexes? How much benchmarking do you have to do in order to work out which DFT functional to use? Do you think that wavefunction-based methods would improve the description of the system?

**Matthias Bauer** responded: Indeed the functional does affect the outcome of the analysis, but not in a sense that everything can be simulated ‘under’ the data. Benchmarking is surely important, but we rely mostly on published work on complexes using the same metal in the core of the complex. But surely methods to identify the optimal functional would be highly beneficial for future work. We have applied wavefunction methods on certain occasions.<sup>1</sup> They offer certain benefits, but are computationally much more expensive.

1 N. J. Vollmers, P. Müller, A. Hoffmann, S. Herres-Pawlis, M. Rohrmüller, W. G. Schmidt, W. Gerstmann and M. Bauer, *Inorg. Chem.*, 2016, **55**, 11694–11706.

(212:[211]211) **Odile Eisenstein** asked: Is there an influence of the vibrations on the spectra? In other words, is there an influence of the temperature on the spectra?

**Matthias Bauer** replied: Typically, calculations are zero Kelvin calculations, and the effect of temperature on valence emission calculations are typically not addressed. In EXAFS analysis, temperature is accounted for by the Debye–Waller factor, which reflects in a homogeneous solution the harmonic oscillator behaviour of the bond under investigation.

(213:[212]212) **Vidar R. Jensen** remarked: You have an impressively complete set of X-ray spectroscopic methods available. I am curious as to the capability of this toolbox in general. If, for example, you were to use your toolbox to determine

the oxidation state of organometallic compounds, how confident would you be in your oxidation state assignment for the individual compounds?

**Matthias Bauer** responded: Very confident. XANES spectra can be used if references with the same types of ligand in different oxidation states are used in combination with the unknown sample. Core to core emission provides a less comparative measure on the d-electron multiplicity and thus also the oxidation state. Here a large degree of covalency might cause misinterpretations.

(214:[213]213) **Markus Reiher** said: I need to comment again on what methods to choose for the calculations. I would never trust any statement on which functional is best. It is fine to treat, for instance, DFT calculations as a black box, but then one should not rely on the way we have come to do benchmarking. These benchmarks are, to a certain degree, arbitrary and were not scrutinized with advanced stochastic consistency checks, although they are simple and easy to carry out (and well used in fields of computational science other than computational chemistry). Starting from the data that you have already collected (experimental related to DFT), you could apply bootstrapping to create synthetic data sets out of it and figure out what the uncertainty in the DFT predictions actually is or, more importantly, will be. Then, you can apply jackknifing to carve out data points (one at a time) in order to figure out their importance in the data set (you might want to check ref. 1 for a description of these approaches (with an application to theoretical Moessbauer spectroscopy). If you do not want to enforce a specific physical-model relation between reference data (from experiment) and calculated data, then confidence intervals from machine learning models such as neural networks or Gaussian processes can be helpful to figure out the actual uncertainty of a DFT model for a specific model under consideration.<sup>2</sup> Furthermore, I would like to emphasize that a wavefunction calculation is not necessarily better than a DFT calculation, just because it is expensive. Take, for example, multi-reference perturbation theory on a multi-configurational wave function with a (too) small active space that doesn't represent the electronic structure well; here the result deteriorates, but was computationally demanding to obtain (see ref. 3 for an example and ref. 4 for tracing the problem back to the too-small orbital space).

1 J. Proppe and M. Reiher, *J. Chem. Theory Comput.*, 2017, **13**, 3297–3317.

2 G. N. Simm and M. Reiher, *J. Chem. Theory Comput.*, 2018, **14**, 5238–5248.

3 C. J. Cramer, M. Włoch, P. Piecuch, C. Puzzarini and L. Gagliardi, *J. Phys. Chem. A*, 2006, **110**, 1991–2004.

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(215:[214]214) **Patrick Morgan** opened a general discussion of Pierre Kennepohl's paper: Commenting on your question to the audience during your presentation: surely whether the electrons came from the metal or the ligand would have differing effects, as it would result in a change of the electron distribution of the complex.

**Pierre Kennepohl** responded: I personally agree that the electronic distribution – and specifically the electron density at the metal centre – should be extremely important in determining the chemical properties of that species, including its



1 reactivity. It remains an open question as to how we might experimentally probe  
the differences between the effect of metal-based and ligand-induced back-  
bonding on the reactivity of the resultant species. We have, as yet, not identified  
5 a mechanism by which we could control the relative importance of ligand and  
metal donation into the  $\pi$  ligand but feel that this is worthy of continued study.

(216:[215]215) **Derek Durand** said: This looks to me like a descriptor, in the  
sense that you're systematically describing where your electron density sits. Here  
10 you've modified the alkene or carbonyl ligand in order to determine how electron  
deficient the metal becomes. Have you thought about using a range of bidentate  
phosphine ligands other than 1,2-bis(ditertbutylphosphino)ethane, to maximise  
that deficiency at the metal?

15 **Pierre Kennepohl** replied: Our next step is to explore the specific role of the  
ancillary ligands (in this case, the diphosphine) in controlling the degree of  
backbonding. This requires a library of diphosphine ligands with similar steric  
constraints but different electronics at the phosphorus atom. The bite angle may  
also play an important role in electron donation and thus the geometry of the  
20 different species must remain as similar as possible. We are working towards  
using a series of bis-*N*-heterocyclic carbene ligands with varying remote electronic  
substituents to create a well-defined series of complexes with varying electron  
donation. This will allow us to explore how much control we might have on the  
degree of backbonding in such systems.

25 (217:[216]216) **Aaron Odom** said: There are some semantic issues brought up  
with studies like this. We know there is a difference between formal oxidation  
state and charge, but terms like d-electron count also have formal definitions  
coming from Ligand Field Theory. When you say a diphosphine olefin complex is  
30 'd<sup>9</sup>', how are we supposed to think about that electronically in what I'm assuming  
is a diamagnetic system? Do we need new names for concepts, like pulling elec-  
tron density out of the d-manifold, that don't conjure the same consequences as  
d-electron count does?

35 **Pierre Kennepohl** answered: This brings up an important point: that nomen-  
clature matters. We have used the term d-electron count to describe total electron  
density in valence d-states. In the context of this study, this is what matters as our  
goal was to quantify the degree of direct metal involvement in backbonding. Any  
40 decrease in the d-electron count (from a maximum of 10) represents electron  
density that has been transferred to the  $\pi$  ligand. As you correctly point out,  
however, this concept differs markedly from what I would term the metal d<sup>n</sup>  
configuration (its electronic configuration), as used in ligand field theory. The  
problem is that there isn't a consistent way that the community describes these  
45 very different things. In a sense, the nomenclature already exists: the electron  
*configuration* is a formal description whereas the electron *count* is an accounting  
of total electron distribution. Current literature is inconsistent, however, and we  
should work to improve clarity.

50 (218:[217]217) **David Nelson** asked: Are these effects particular to complexes of  
bidentate phosphine ligands? How would one expect perturbation of the bite

angle to affect this phenomenon? Some extreme examples may be the use of monodentate phosphines or the use of rigid *trans*-spanning ligands such as spanphos or xantphos, which might give an almost linear P–Ni–P arrangement. It would be very interesting if there were a threshold bite angle at which these effects were reduced to the point that they prevent the complex from preferring a square planar geometry.

**Pierre Kennepohl** replied: These are all very interesting points that would be interesting to evaluate more fully. We have, so far, focused our attention on one aspect of the problem. Now that we have a framework for understanding these complexes, I would agree with you that we should extend these to probe specific aspects of the ancillary ligands to see how they may affect the degree of ligand-induced backbonding.

(219:[218]218) **Kevin Lovelock** commented: XANES is often used to probe the unoccupied molecular orbitals. Are you interested in probing the occupied orbitals indirectly by probing the unoccupied orbitals using XANES? Or does this method work very well for understanding bonding by probing only the unoccupied orbitals? You use the energy of peak B to compare to other data on the formal Ni oxidation state. Your assignment of A and B in your XANES spectra for the four compounds – does that rely on TD-DFT calculations, or can that assignment be made solely using measured experimental data? Could P 1s XANES be of use for these compounds, to try to more directly determine the contribution of the P-based ligand to the bonding?

**Pierre Kennepohl** responded: There are advantages and disadvantages to probing occupied and unoccupied states, both experimentally and depending on the questions that you are asking. Experimentally, probing empty states is easier because we can quite easily detect photons (either *via* transmission or fluorescence detection). Experiments involving filled states (typically) requires electron detection and measurement of their kinetic energy (*e.g.* photoelectron spectroscopy), which is technically more of a challenge. In our case, for example, we were seeking to know the degree of electron depletion in the 3d manifold and thus measuring the unoccupied states (holes) is actually better from a conceptual standpoint as well as the intensity of the feature directly tracking with electron depletion in the d manifold.

The detailed interpretation of the L-edge data is model-dependent since there are a number of possible final states (Ni 3d, 4s, 4p, charge transfer states) that may be present. There are both molecular orbital strategies (TD-DFT) and atomic multiplet approaches to modelling such data. A model-independent approach would be to integrate the total intensity of the spectroscopic features in the L3-edge, which has been shown to correlate well with oxidation states. In our case, the TD-DFT modelling is highly consistent across both the Ni K- and Ni L-edges and is in good agreement with multiplet simulations (not included in the manuscript).

Ligand K-edge spectroscopy has been used very effectively to investigate metal–ligand covalency and thus would be of interest in these cases. Unfortunately, one of the challenges of using phosphorus K-edge spectroscopy is that there is a very strong contribution from the P–C bonds in the spectra that obscures potential

contributions from the P–M bonds. This makes accurate comparisons potentially challenging. We have some data and have not pursued it extensively but others have recently shown that careful analysis is possible. We plan to look more carefully into the possibilities in the near future.

(220:[219]219) **Odile Eisenstein** remarked: The proposal that back-bonding could come from the ligand orbital and not from the metal is very important and is, in my opinion, a general concept. For instance, this was also proposed in several papers.<sup>1,2</sup> In fact, the electrons are very democratic and come from where they can, since the orbitals are delocalized over the whole molecule. It is very nice to have experimental proof that electron density can be provided by the ligands.

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**Pierre Kennepohl** replied: Thank you very much for making me aware of this previous literature that also suggests the same phenomenon. We had missed these papers but will clearly reference them moving forward. I will admit to being particularly intrigued by the situation described in your own 2009 *Organometallics* paper, where there isn't very much coupling through the metal but it is sufficient to allow for global electronic changes in the molecule. By the way, I love the idea of democratic electrons!

(221:[220]220) **Stuart Macgregor** commented: I was wondering about the geometries of the alkene and arene complexes, **1** and **2**, compared to those of the carbonyl adducts, **3** and **4**. The latter are presumably much less symmetric and so how does this affect your analysis? Could substituted alkenes be studied to probe these effects?

**Pierre Kennepohl** answered: The carbonyl complexes are definitely much more asymmetric in their geometry and we can see major effects of this in the calculated electronic structures. For example, whereas the alkene complexes have a well-defined ring critical point in an atoms-in-molecules (AIM) analysis of the electron density map, the carbonyl complexes do not. The carbonyl complexes show differences in their structure that suggest that one might wish to describe these systems as having oxygen to metal s-donation and metal to carbon s-backdonation. We have described this in a prior publication (ref. 9 in our paper). Substituted alkenes are of great interest, as these would not involve such large asymmetry yet would still provide control over the  $\pi$  acidity of the ligands.

(222:[221]221) **Ian Fairlamb** remarked: It's very important to think about regioselectivity in metal catalysis.

(223:[222]222) **Robin Perutz** addressed Pierre Kennepohl: You talk in your conclusions about bonding, from these X-ray techniques in isolation from other techniques, so please can you tell us how your conclusions fit with the information from other techniques such as IR spectroscopy (the CO stretches), <sup>13</sup>C NMR,

1 or X-ray diffraction structures, or any other technique you wish to bring to bear on  
this problem?

5 **Pierre Kennepohl** responded: Our initial study included evaluations of the  
structural characteristics of the  $\pi$  ligand as well as the commonly used  $^{31}\text{P}$  NMR  
coupling constants in complexes with asymmetry. With respect to the NMR coupling  
constants, we find an extremely strong correlation between them and Ni K-edge X-  
ray absorption pre-edge energies, suggesting that both methods are reporting on  
10 similar properties of the metal complex – namely electron density at the metal  
centre. We do not have full experimental data sets on IR or  $^{13}\text{C}$  NMR, although it  
would be very useful to see how well these would correlate with our results.

15 (224:[223]223) **Ian Fairlamb** asked: Were all your measurements done in  
solution, or were they in the solid state?

20 **Pierre Kennepohl** replied: All of the measurements were done in the solid state  
for the nickel L-edges. However, we know from previous nickel K-edge XAS data  
that there is very little difference between the solid state electronic structure and  
that in a solution of toluene (at a 5 mM concentration). Solvation in low dielectric  
solvents therefore doesn't seem to have an important impact on the electronic  
distribution in these complexes.

25 (225:[224]224) **John Slattery** commented: Following on from Robin's point, one  
of the techniques that Odile Eisenstein and Christophe Copéret have pioneered is  
to use solid-state NMR (SSNMR) spectroscopy to extract chemical shift tensors for  
organometallic complexes. This can give really useful information about metal-  
ligand bonding, observing what is going on at the ligand directly. This type of  
approach would nicely complement your XAS data, which looks at what is  
30 happening at the metal. We've found this type of SSNMR approach to be very  
useful for understanding bonding in unusual, fluorinated organometallic  
complexes.

35 **Pierre Kennepohl** responded: I would love to follow up on this. SSNMR would  
provide insights from the perspective of the ligands that could be extremely  
useful.

40 (226:[225]225) **Jennifer Love** remarked: In terms of Robin's question about  
other methods (*e.g.*  $^{13}\text{C}$  coupling constants) that report on the same information  
as the K-edge data, you don't always have NMR-active nuclei. We thought it was  
important to establish that the K-edge data correlate with coupling constants. For  
example, if you have a bisamine ligand, which doesn't have a convenient NMR  
handle, you can still determine the formal oxidation state using the K-edge data.

45 (227:[226]226) **Robert Morris** addressed Aiwen Lei: In your paper you refer to  
linear bisamido copper(I), but is it possible to obtain the angle from your X-ray  
data?

50 **Aiwen Lei** replied: No. It is not possible for XAFS to resolve the angle directly.  
However, according to Prof. Edward I. Solomon and Prof. Keith O. Hodgson's

early finding,<sup>1</sup> the XANES on Cu provides structural information by comparing the white line intensity. In our case, the Cu XANES shows an obvious sp hybridized characteristic, which is why we claim a linear structure. Besides, an alternative technique, polarized X-ray absorption spectroscopy (PXAS), has the ability to provide angle information if the sample is crystal, membrane or fiber.<sup>2</sup>

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(228:[227]227) **Ulrich Hintermair** said to Matthias Bauer: Thinking about using these advanced X-ray techniques for *operando* investigations and reaction monitoring (rather than on static samples), what level of temporal resolution is possible at the sensitivity required for dilute samples? As a specific example, if you had a reaction catalysed by a mono-nuclear transition metal complex at a few millimolar concentration in organic solvent, could you monitor what the catalyst is doing, say, every minute, or would there be a trade-off between structural information and kinetic profiling?

**Matthias Bauer** answered: Generally, all time scales down to the femtosecond regime are possible with the advent of X-ray free electron lasers. For *operando* measurements, typically milliseconds are sufficient and here low concentrations in the millimolar range are indeed possible, if the X-ray flux is large enough (which is normally the case even at bending magnet beamlines). Different measurement techniques are available here: dispersive XAS in combination with stopped flow, or QEXAFS using a continuously moving monochromator. With particular properties of the sample, measurements become more complicated and a high time resolution becomes more challenging, for example for highly absorbing solvents. Moving to faster measurements, like microseconds, the trigger to start a reaction has to be short enough, otherwise such a high time resolution makes no sense.

(229:[228]228) **Odile Eisenstein** addressed Aiwen Lei: You provide some calculated free energy values in your Scheme 3. It would be nice to have some more details on the way the calculations have been carried out and on the way the experimental chemical system was represented. This could be important, since these highly polar or ionic systems are in fact rather challenging to represent. In the present case, the calculations indicate that one starts with the endoergic formation of  $\text{Cu}(\text{HMDS})_3\text{Na}$ . Can this aspect be commented on?

**Aiwen Lei** replied: We did not carry out further calculations and experiments on this reaction.

(230:[229]229) **Stuart Macgregor** said: Speciation in organometallic chemistry is particularly important and one classic example is phosphine dissociation from  $\text{Pd}(\text{PR}_3)_4$  species. Depending on the  $\text{PR}_3$  substituent or solvent, there will be cases where no ligand loss occurs, or tris- or bis- $\text{Pd}(\text{PR}_3)_n$  species may be formed or complex equilibria set up. Are these sorts of problems amenable to study using the techniques described?

**Ian Fairlamb** responded: In complexes of the type,  $\text{Pd}(\text{PR}_3)_4$ , we expect ligand dissociation to be a dominant process in solution. The famous complex is  $\text{Pd}(\text{PPh}_3)_4$ , which is an 18-electron palladium complex which can only (reasonably) lose phosphine ligands. Even in mildly polar solvents, but especially in polar aprotic solvents, this can occur to give  $\text{Pd}(\text{PPh}_3)_3$  and  $\text{Pd}(\text{PPh}_3)_2$  species in solution, in equilibrium with  $\text{Pd}(\text{PPh}_3)_4$ . The unsaturated species are likely solvated in solution. If we accept that it is  $\text{Pd}(\text{PPh}_3)_2$  that undergoes oxidative addition with organohalides ( $\text{R-X}$ ) to give  $\text{PdR}(\text{X})(\text{PPh}_3)_2$  type complexes, then it is this species that is the reactive one in cross-coupling catalyst cycles. Moreover, the excess  $\text{PPh}_3$  will play a role in metering out  $\text{Pd}(\text{PPh}_3)_2$  species (less stable) when the organohalide ( $\text{R-X}$ ) is very reactive and the next step in catalysis is slow (*e.g.* transmetallation, often rate-determining when  $\text{X} = \text{Br}$  or  $\text{I}$ ). That can be a positive intervention, as high concentrations of  $\text{PdR}(\text{X})(\text{PPh}_3)_2$  in solution can lead to side reactions (*e.g.* protodehalogenation/ $\text{R-X}$  homocoupling reactions, for example). Addition of additives such as  $\text{CuI}$  or similar metal salts, capable of scavenging  $\text{PPh}_3$ , can perturb the position of the equilibrium between  $\text{Pd}(\text{PPh}_3)_4$ ,  $\text{Pd}(\text{PPh}_3)_3$  and  $\text{Pd}(\text{PPh}_3)_2$ , leading to higher concentrations of the latter in solution. This is also solvent dependent. Phosphine oxidation to give  $\text{O=PPh}_3$  is likely in the presence of trace air, thus the equilibrium described above would be expected to be altered on exposure of any reaction to air (*e.g.* cross-coupling). While restriction of trace air in an academic laboratory is feasible, for industrial scale-up processes trace air is likely to be present. Thus catalyst speciation is anticipated, and changes will occur as a reaction converts substrate to product. It is important to recognise this process, as it provides one pathway (there are likely several) for aggregation of  $\text{Pd}(\text{PPh}_3)_n$  species to give small Pd clusters, nanoparticles and eventually large particles (that precipitate from the reaction, *e.g.* moribund Pd black). We are aware that Pd clusters and nanoparticles are competent catalyst species (with the implications this has in terms of catalyst efficacy and product selectivity).

There are further reports of  $\text{Pd}(\text{PR}_3)_1$  species being important for catalysis, especially where the phosphine is a bulky electron-rich ligand. However, as is the case for all strongly ligating 2-electron phosphine donor ligands, they can be activated as well, either by oxidation (mentioned above), organo-moiety chemical exchange (from P to Pd for example), P-C bond cleavage (leading to phosphido pseudohalide anionic ligands) or cyclopalladation (5-membered and 4-membered cyclopalladation is possible for certain phosphines). The role of Pd clusters and nanoparticles again becomes an important question in this context.

To sum up, catalyst speciation is critical when using Pd complexes such as  $\text{Pd}(\text{PPh}_3)_4$ . There are many caveats, as briefly highlighted above. Going forwards, aggregated species from ' $\text{Pd}(\text{PR}_3)_n$ ' (where  $n = 1$  or  $2$ ) ought to be considered in mechanistic studies and the findings used to guide future catalyst design strategies.

**Pierre Kennepohl** added: This is a particularly challenging problem! A limitation of using extended X-ray absorption fine structure (EXAFS) data in such cases is that it will always give an average scattering pattern of everything in the sample (with the nucleus of interest). With pure species, it is possible to model metal-metal scattering from other metal atoms within 500 pm or less, but even in those cases, identifying the number of such neighbours can be challenging. It is

1 therefore relatively easy to determine whether small clusters are being formed,  
but effectively impossible to identify exact cluster sizes – particularly in mixtures.

5 The solution, though, is not to rely on a single technique but to use a combination of methods to provide supporting information that can allow for holistic analysis. In the case of speciation of clusters, aggregates, and nanoparticles, small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) can be used to great effect to ascertain the size and shape of well-defined species and/or the overall distribution of such species.

10 **Matthias Bauer** added: Yes, X-ray techniques can help here because they provide either a spectroscopic signature or a measure for the number of phosphine ligands. Indeed equilibria are a challenge. But with well-selected references (their synthesis itself might be a challenge), this problem can be solved as well. XANES data can be analyzed with what is called a LC-XANES fit following a principle component analysis. Valence emission and prepeaks are influenced by the symmetry of a complex and thus also provide important tools here. But their application has to be explored in detail in the future.

20 (231:[230]230) **Robin Perutz** addressed Matthias Bauer and Pierre Kennepohl: The three of you restricted yourselves to first row transition metal X-ray spectroscopy. I recently wanted to do some X-ray spectroscopy on iodine compounds and learned that, contrary to my expectations for a heavy atom like this, it was far from ideal. Can you tell us a bit more about the limitations of the different elements you might want to concentrate on? Clearly the very light elements are inappropriate, although from Pierre's remarks, this might not be true?

30 **Pierre Kennepohl** replied: There are several factors that enter into this. In principle, X-ray spectroscopy is feasible for any element except perhaps the very lightest of the elements (H, He). In practicality, there are two competing factors that must be considered: (1) the mean free path of the photons at different X-ray energies, and (2) the lifetime of the excited states that are being probed.

35 The first of these is an experimental limitation on experiments at lower photon energies (1eV). Newer methods allow us to somewhat circumvent the Heisenberg limit through what has become known as high energy resolution fluorescence detected (HERFD) XAS. Such methods are not without their difficulties but they afford much higher resolution spectra for elements that would otherwise not provide good enough resolution for meaningful data analysis.

40 Note that when resolution is good, the experiment is difficult – and conversely, when the experiment is easy, the data are generally poor. The K-edge of the first row transition metals represents a Goldilocks energy regime, where meaningful data can be obtained with few(er) experimental limitations. This explains why first row transition metals are clearly over-represented in X-ray spectroscopy of organometallic species at this time.

45 With respect to iodine, near-edge spectra can be obtained but they are very broad (due to lifetime broadening) and thus electronic information is limited. HERFD experiments would be the way to go to overcome this limitation.

50 **Matthias Bauer** added: XAS on halides in metal–organic compounds are challenging. One reason is the lack of a sufficient number of backscattering atoms

to yield a proper signal-to-noise ratio. At the metal centers the number of next neighbours in the complex amounts typically to three or more, which causes intense oscillations in the X-ray absorption coefficient. Halides often have just the metal or even just the solvent molecules as next neighbours. With the heavier halides, the absorption signal suffers from a severe lifetime broadening, which smears out a lot of spectral signatures and makes the analysis more difficult.

But in general, with either L-edges or the K-edge at hand, most elements can be measured, depending on the energy range of the beamline used.

## Conflicts of interest

There are no conflicts to declare.