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DISCUSSIONS

Make – underpinning concepts of the synthesis of systems where non-covalent interactions are important: general discussion

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Eva Meeus opened a general discussion of the paper by Thomas R. Ward: In the work that you have nicely outlined in your presentation, the focus lies on the precise localization of the catalyst inside the protein (**https://doi.org/10.1039/d3fd00034f**). In line with the work of *e.g.*, Professor Costas (**https://doi.org/10.1039/d2fd00177b**), have you considered mutating the protein scaffold towards pre-organization of the substrate as well? Or the other way around, would it be interesting to modify the substrate to enable an interaction with one of the close-lying amino-acids to facilitate its pre-organization with respect to the catalyst?

Thomas R. Ward answered: We are indeed in the process of engineering the substrate to increase its weak contacts with the protein. One avenue that we are pursuing is peptide derivatization. Indeed, in the single-chain dimer construct that I presented, one of the biotin-binding sites has been engineered not to bind to biotin but to bind to strept-tag II (an octapeptide). We hope that this will allow us to carry out site-specific peptide modification for peptides bearing a strep-tag II either at their C- or N-terminus.

Wei-Chun Liu said: Have you considered adjusting the pH value of the system to enhance selectivity, given that your catalyst is sensitive to pH?

Thomas R. Ward responded: We typically apply Design of Experiments to optimize experimental parameters for a given reaction. I should mention that

artificial metalloenzymes based on the biotin-streptavidin technology have been reported over a wide range of pHs: from pH 2.5 (sulfoxidation) to 11.5 (allylic alkylation).

Mark Greenhalgh remarked: In your approach to catalyst design you said you first varied the 'metal cofactor', in combination with the wild type protein, to find the best 'lead' catalyst before performing directed evolution to improve activity and selectivity. Does this approach always lead to the best final catalyst, or is it possible that performing directed evolution on a less promising 'lead' catalyst may provide a better final catalyst? Do you know of any examples where you (or someone else) has looked at this?

Thomas R. Ward responded: Your question is perfectly justified. When optimizing a multidimensional problem, one is never able to scan the entire landscape of possibilities. When reaching a given figure or merit (turnover number, ee, rate *etc.*), it is most probably not the global minimum: it is a value that one is satisfied with.

Accordingly, during the chemogenetic optimization procedure, one makes arbitrary decisions: screen chemical diversity with a few streptavidin mutants and then select the most promising cofactor. After that, perform directed evolution with that cofactor and "never look back". In some instances, we have looked back at other cofactors which were not pursued in the first round of evolution. As one would expect, these have shown some promise, but require starting the directed evolution campaign from scratch. You may remember we have focused primarily on a *meta*-biotinylated aminosulfonamide ligand for transfer-hydrogenation. In the original paper (PNAS 2005), the *ortho*-analog was quite promising. I am convinced that we could evolve activity and selectivity for that cofactor. Other mutants would then be identified as the best.

Tatjana N. Parac-Vogt asked: For the genetic optimisation, did you try any modelling before you started any trials?

Thomas R. Ward answered: Yes, more and more we rely on docking or quantum mechanics/molecular mechanics to design the spacer between the biotin anchor and the metal cofactor. This determines the nature of the close-lying amino acid residues, which in turn affect second coordination sphere interaction.

As an experimentalist, however, we always test a few cofactors as the first coordination sphere is critical. The first coordination sphere is always inspired by the best organometallic cofactor for a given reaction. In a nutshell, we use computation to design the spacer.

Joost N. H. Reek highlighted: You focused on enantioselectivity and turn over numbers in the optimisation of your artificial enzymes. When you look at the second coordination spheres interaction with the catalyst and substrate, how does the second coordination sphere affect the rate of the reaction?

Thomas R. Ward responded: We systematically perform Michaelis–Menten kinetics for artificial metalloenzymes. Most often, we observe increased kcat/km

(or rates). The highest increase that we have observed so far is >100 fold increase in rate when comparing the free cofactor *vs.* the cofactor embedded in the host protein. It was for a Rh-catalyzed C–H activation reaction proceeding *via* a concerted metallation–deprotonation mechanism.

Joost N. H. Reek added: By looking at various mutants you get information on the effect of functional groups on these artificial active centers. Would it be possible to do assays to understand how these functional groups affect the second coordination sphere, and use this as a blue print for the design of synthetic homogeneous catalysts that have the same type of interactions?

Thomas R. Ward answered: Thanks for asking. The traditional way of probing the effect of functional groups provided by amino acid residues is site-directed mutagenesis. If canonical amino acids do not allow you to resolve the question convincingly, you can resort to non-canonical amino acids. However, providing a black-or-white answer is exceptionally challenging since we are talking about weak interactions between the host protein and the transition state.

Pierre Kennepohl remarked: You noted that these systems can work in at least a few different solvents. Given that the catalytic pocket is seemingly quite open to the solvent environment, it would seem that one might expect to observe relatively large solvent effects. Is this true?

Thomas R. Ward responded: I agree that the active site is quite solvent-exposed and must admit that we have not investigated systematically the influence of the solvent on catalytic performance. Current efforts include engineering chimeric streptavidin with a hydrophobic lid to shield the active site.

Miquel Costas queried: Have you ever investigated the limits of your hydroxylation and nitrogen transfer system with respect to the bond dissociation energy, especially with regards to the possible intramolecular reactions with amino acid residues of the protein?

Thomas R. Ward responded: We have looked at the limits of accessible BDEs. Using a second generation TAML ligand, we could hydroxylate cyclohexane.

Concerning intramolecular reactions with amino acid in the proximity, we have not seen any change in HR-MS after the reaction. We should look into this in more detail however, *i.e.*, protein sequencing after the reaction to identify any "post-translational" modification.

Kamran T. Mahmudov said: What types of interactions are involved, only hydrogen bonding?

Thomas R. Ward answered: For the transfer hydrogenase, computation of the transition state revealed a critical interaction between lysine 121 and the electron rich aromatic moiety on the substrate. A typical cation–π interaction.

For the nitrene insertion, the X-ray structure of the evolved artificial metalloenzyme revealed an interaction between isoleucine 112 and the amidoquinoline ligand of the cofactor.

Matthew Gyton opened a general discussion of the paper by Michael R. Buchmeiser: Please comment on the speciation of catalyst in the ionic liquid phase and whether any carbene exchange is observed (**https://doi.org/10.1039/d2fd00152g**).

Michael R. Buchmeiser replied: The catalyst stays intact, no carbene exchange is observed under the chosen conditions.

Robin N. Perutz asked: How does the role of the temperature affect the *cis*/*trans* isomer selectivity?

Michael R. Buchmeiser responded: So far, reactions have been run at 55 °C. After an initiation period, the *cis*/*trans* ratio is constant (no isomerization). We neither tried higher temperatures since that would result in the evaporation of the methyl *tert*-butyl ether (MTBE), nor lower temperatures to avoid a high viscosity or even solidification of the IL.

Rens Ham opened a general discussion of the paper by Tatjana N. Parac-Vogt: When looking at your fluorescence quenching studies (**https://doi.org/10.1039/d2fd00161f**), it strikes me that the polyoxometalate cluster (POM)–organic linker complexes, quench about the same as the free POM + organic linker in the case of the methyl and amine linkers. However, in the biotin case, the quenching seems much stronger when the POM–biotin complex is used. How can you explain this?

Tatjana N. Parac-Vogt responded: Indeed AE-Biot showed the greatest quenching of tryptophan fluorescence emission, which is most likely due to the more complex organic functionality which can interact with protein residues *via* both hydrogen bonding and hydrophobic interactions – in contrast to AE-NH_2 and AE-CH_3. Moreover, the co-crystal structure of AE-Biot with Hen Egg White Lysozyme (HEWL) showed, in addition to hydrogen bonding interactions between the POM core and HEWL residues, a network of hydrogen bonding interactions between the biotin moieties and Arg14, His15, Thr89, Arg128 and Asn93 protein residues. In the case of AE-CH_3, only hydrogen bonding interactions between the POM core and HEWL residues were observed in the co-crystal structures. However, the higher binding affinity of AE-CH_3 is most likely due to the more hydrophobic character of the methyl group compared to –NH_2, which can be seen from binding site 3 of co-crystal HEWL/AE-CH_3 A, in which the CH_3 group is positioned 3–5 angstroms from the hydrophobic residue Leu75. Moreover, in aqueous solutions the AE-CH_3 is potentially more labile and more likely to also interact with the protein in the vicinity of the hydrophobic Trp residues, causing greater quenching of the fluorescence (with K_a for AE-CH_3 one order of magnitude higher than for AE-NH_2). Hydrogen bonding between the amine ligand and Asp18 is observed in the co-crystal structure of AE-NH_2 with HEWL, however the lack of hydrophobic interactions between AE-NH_2 and HEWL accounts for the weaker quenching effect of AE-NH_2. Based on the obtained tryptophan fluorescence results, the K_a of the unfunctionalized AE-Al POM is likely to be two orders of magnitude lower than for AE-NH_2, which had the lowest K_a of all the hybrid POMs (HPOMs). Consequently, the combination of the POM core with the organic

ligands results in synergistic contributions towards enhanced interactions with HEWL.

Bartosz Lewandowski said: You mentioned that the 'naked'/unfunctionalized polyoxometalates bind more weakly to the protein, but how much weaker is this interaction?

Tatjana N. Parac-Vogt responded: Since the interaction of the unfunctionalized aluminum-centered POM with HEWL is so weak, the association constant (K_a) could not be easily determined using the Stern–Volmer equation, in line with previous reports for the quenching interactions of $[Fe(OH)_6Mo_6O_{18}]^{3-}$ and $[TeW_6O_{24}]^{6-}$ Anderson–Evans POMs with different proteins.[1,2] However, based on the obtained tryptophan fluorescence results, the K_a is likely to be two orders of magnitude lower than for AE-NH_2, which had the lowest K_a of all the HPOMs (2.8×10^3 M^{-1}, with the highest K_a being 292.6×10^3 M^{-1} for AE-Biot).

1 L. Vandebroek, H. Noguchi, K. Kamata, J. R. Tame, L. Van Meervelt, T. N. Parac-Vogt and A. R. Voet, *Chem. Commun.*, 2020, **56**(78), 11601–11604
2 E. Al-Sayed, A. Blazevic, A. Roller and A. Rompel, *Chem.–Eur. J.*, 2015, **21**, 17800–17807.

Bartosz Lewandowski commented: You suggest that introducing amino groups allows to take advantage of additional hydrogen bonding interactions with the protein. Do you think other interactions, *e.g.* electrostatic or coulombic are also possible?

Tatjana N. Parac-Vogt answered: The ability of the amine group in the AE-NH_2 POM to become protonated in solution means that charge–charge interactions can indeed also be tuned through the use of this functional group and by varying the pH of the solution. Furthermore, functionalization of the POM with anionic or cationic groups, although synthetically more challenging, can be achieved and could be exploited to induce additional charge–charge interactions.[1]

1 V. Tagliavini, C. Honisch, S. Serratì, A. Azzariti, M. Bonchio, P. Ruzza and M. Carraro, *RSC Adv.*, 2021, **11**, 4952–4957.

Kamran T. Mahmudov asked: In the title of your manuscript you used 'non-covalent interactions', I think in your system, the positive charge assisted hydrogen bonding is dominant, in order to justify the title, did you observe another type of non-covalent interaction? Moreover, you have studied the oxo-metal complexes, can the metal centre of oxo-metal complexes participate in intermolecular non-covalent interactions?

Tatjana N. Parac-Vogt answered: Our work strongly suggests that hydrophobicity is one of the key factors in HPOM–protein interactions. The higher association constant of AE-CH_3 with HEWL compared to AE-NH_2, as determined through tryptophan fluorescence spectroscopy, is likely due to the more hydrophobic nature of AE-CH_3. Furthermore, refinement of the co-crystal structures of these HPOMs with HEWL (which were obtained under identical conditions) revealed that AE-CH_3 co-crystallized with greater occupancy (57%) compared to AE-NH_2 (40%), likely due to the higher binding affinity of AE-CH_3 to HEWL. The

importance of hydrophobic interactions is especially shown at binding site 3 of HEWL/AE-CH_3 A, in which the CH_3 group is positioned 3–5 angstroms from the hydrophobic residue Leu75. However, the metal centers in the POM core, Mo and Mn, are all coordinatively saturated with oxo ligands, therefore they are not directly involved in non-covalent interactions. Such involvement of the metal has only been reported with metal-substituted POMs, where a metal with labile coordinated waters is present that can coordinate to residues of the protein.[1]

1 L. Vandebroek, L. Van Meervelt and T. N. Parac-Vogt, *Acta Crystallogr.*, 2018, **C74**, 1348–1354.

Kamran T. Mahmudov added: Can pH change increase the strength of the interactions?

Tatjana N. Parac-Vogt answered: Our work has demonstrated the role of electrostatic interactions between (H)POMs and proteins, which can be tuned by varying the pH. For example, co-crystals obtained at pH 4.6 of AE-CH_3 with HEWL were shown to contain 2 POMs per asymmetric unit, as opposed to 1 POM per asymmetric unit for co-crystals obtained under more neutral conditions. Moreover, 4 additional binding interactions were observed for the co-crystals obtained under the more acidic crystallization conditions, which is likely due to the more favourable electrostatic attraction between the protein surface (since the pI of HEWL is approximately 11) and the negatively charged HPOM at lower pH. Additionally, it has been previously shown that the binding affinity of all-inorganic POMs increases as the pH is made more acidic.[1] However, in the case of AE-NH_2, below pH 7.0 the $-NH_2$ group becomes protonated thus lowering the overall charge of the HPOM, resulting in weaker electrostatic interactions with the protein. Hence, although lowering the pH generally favors POM–protein electrostatic interactions, the nature of the functional groups of the HPOM should be carefully considered when employing pH as a tool to tune the strength of HPOM–protein interactions.

1 K. Stroobants, D. Saadallah, G. Bruylants and T. N. Parac-Vogt, *Phys. Chem. Chem. Phys.*, 2014, **16**, 21778–21787.

Eva Meeus remarked: What I have understood from your work, is that your POMs are targeting proteins in a site-selective manner *via* electrostatic interactions, eventually forming a host–guest system. Given that you are also introducing organic linkers to your POMs, it comes very close to artificial metalloenzymes. In this light, I was curious to hear whether you could actually use the protein as a secondary coordination sphere while performing catalytic reactions with the POMs? Could there be an anticipated effect of this secondary coordination sphere on the catalytic outcome of the reaction and would this be of interest?

Tatjana N. Parac-Vogt responded: Indeed, combining HPOMs with proteins is a potentially interesting strategy for the design of artificial metalloenzymes due to the catalytic properties of the POM core. Furthermore, if the HPOM binds within a deep pocket of the protein structure where it is surrounded by different amino acid residues, it is possible that these residues may play a key role in catalytic reactions that take place within that pocket, thereby involving both the POM and

the protein as a secondary coordination sphere. This is indeed something our group is interested in looking into in the future.

Kamran T. Mahmudov continued the general discussion of the paper by Michael R. Buchmeiser: How do the non-covalent interactions affect the selectivity?

Michael R. Buchmeiser responded: Selectivity is believed to be governed by non-covalent interactions between the catalyst and the IL and the catalyst and MTBE; with the metal center being in the IL phase and the N-heterocyclic carbene (NHC) in/close to, the MTBE phase. This places the active center in the IL phase while hindering the rotation of the ligands allowing for the formation of the *Z*-isomer (hypothesis, to be confirmed by calculations).

Basheer Chanbasha† asked: How stable is the coating in porous medium?

Michael R. Buchmeiser answered: The amount of the IL is such that it is not removed under continuous flow.

Basheer Chanbasha† added: Have you tried any gaseous reactions?

Michael R. Buchmeiser responded: Not with this system but earlier we tried with related ones.[1]

1 C. Lee, B. Sandig, M. R. Buchmeiser and M. Haumann, *Catal. Sci. Technol.*, 2018, **8**, 2460–2466.

Mark Greenhalgh highlighted: In your paper (**https://doi.org/10.1039/d2fd00152g**) you suggest that the *E*/*Z* selectivity may be related to a steric clash between the (*Z*)-silylvinylidene Rh intermediate and the *N*-mesityl group. Did you try changing the steric hindrance of this N-substituent (2,4,6-triisoproylphenyl, Ph, Me, *etc.*) to provide some experimental support for the importance of the suggested steric contact?

Michael R. Buchmeiser answered: Good point. Not yet.

Joost N. H. Reek commented: The charges in your catalyst system play an important role in the orientation with respect to the surface. Have you considered altering the charges in the catalyst, but putting various charged groups at different locations of the catalyst, and evaluate the effect on the selectivity?

Michael R. Buchmeiser answered: Very good point. We have to keep the overall charge of the catalyst positive in order to keep it selectively in the IL. However, adding an additional positive charge to the ligand might indeed provide useful insights.

Bartosz Lewandowski remarked: When investigating the effect of decreasing the content of ionic liquid on the selectivity, you observed a more rapid increase

† Dr Chanbasha could not be contacted to confirm the text of the question before going to print.

in selectivity when you went down to 10 μL, why do you think that was? Could this be somehow related to aggregation of catalyst molecules? What happens if you go even lower with the ionic liquid content?

Michael R. Buchmeiser answered: First, we cannot go lower since the amounts of IL used are already very small. The more pronounced increase of confinement effect with decreasing thickness of the IL layer is in line with findings for organometallic catalysts immobilized in mesoporous supports[1]. There, confinement effects also strongly increase when reducing the pore diameter from 6 down to 2.5 nm. And yes, it is probably about restricted mobility, aggregation, solidification of the IL. Calculations are ongoing to get a more quantitative picture.

1 F. Ziegler, H. Kraus, M. J. Benedikter, D. Wang, J. R. Bruckner, M. Nowakowski, K. Weißer, H. Solodenko, G. Schmitz, M. Bauer, N. Hansen and M. R. Buchmeiser, *ACS Catal.*, 2021, **11**, 11570–11578.

Calum Ferguson said: Can you please comment on the mass-transport of substrate into the pore and how this is affected by the pore size? Do you think that you are using all the pore or just the entrance.

Michael R. Buchmeiser responded: We use only the pore entrance (estimation is the first 20 nm). Generally, the smaller the pores, the lower mass transport is. However, the porous system used here results in quite a large intersurface area between the IL and the MTBE phases, resulting in sufficiently fast mass transport, as evidenced by 50% conversion within 40 hours under supported IL conditions compared to 50% conversion within 10 hours under non-supported biphasic conditions (see the ESI of our article).

Rafael Gramage-Doria queried: Did you consider studying the stability of the Rh complexes before, during and after the catalysis?

Michael R. Buchmeiser answered: We did. They are quite stable allowing for TONs of several thousand, at least.[1]

1 P. K. R. Panyam, B. Atwi, F. Ziegler, W. Frey, M. Nowakowski, M. Bauer and M. R. Buchmeiser, *Chem.–Eur. J.*, 2021, **27**, 17220–17229.

Rafael Gramage-Doria further queried: Did you also consider studying the catalytic system *in situ* in order to identify which active species form? Perhaps some spectroscopy characterization would provide some answers.

Michael R. Buchmeiser replied: No. This would require the study of transition states and reactive intermediates in an IL and thus probably a beam line.

Eva Meeus commented: As you need a very sensitive method to detect your reactive species, preferentially during the reaction, would it be of interest to use on-line mass spectrometry? Would there be a way to apply this to your system, after leaching the compounds out of the pores perhaps?

Michael R. Buchmeiser answered: No. This is a supported system. The catalyst stays inside the column. If it is removed from the column, there will be no way to detect intermediates (by definition).

Joost N. H. Reek remarked: Can you use IR to study the catalyst *in situ*?

Michael R. Buchmeiser replied: No way. The catalyst is supported in a polymeric matrix inside a tube. The catalyst concentration is in the low micromole range.

Andrew S. Weller continued the general discussion of the paper by Tatjana N. Parac-Vogt: Do you see any crystallographic disorder in your POMs?

Tatjana N. Parac-Vogt responded: For all HEWL/HPOM crystal structures, the electron density corresponding to the POM with attached organic linkers could be clearly observed (see Fig. S6 in the ESI of our article, **https://doi.org/10.1039/d2fd00161f**). Moreover, the anomalous difference maps clearly showed the positions of the six molybdate atoms and the central manganese heteroatom, corresponding to the Anderson–Evans archetype (Fig. S7 in the ESI of our article, **https://doi.org/10.1039/d2fd00161f**). Therefore, no crystallographic disorder was observed for these POMs. Moreover, refinement of the crystallographic data was performed using crystallographic restraints files previously reported for AE-NH_2 and AE-CH_3, while the crystallographic restraints file for AE-Biot was generated manually based on the geometry of AE-NH_2. For the co-crystal of HEWL with AE-Biot, the HPOM lies in a special position on an inversion center with two POMs superimposed.

Andrew S. Weller added: How dynamic are these species in solution, do you see exchange or a dynamic equilibrium with a mixture of POMs?

Tatjana N. Parac-Vogt replied: We have previously reported the stability and speciation of these Anderson–Evans POMs in solution.[1] All hybrid POMs were found to dissociate *via* nucleophilic attack by hydroxide ions at the –OCH_2– groups attached to the POM core, causing release of the organic ligand and subsequent collapse of the POM core into $[MoO_4]^{2-}$ and free Mn^{3+}. Moreover, the nature of the organic linker was shown to influence the stability, with AE-NH_2 showing the highest rate of decomposition, while AE-CH_3 showed the lowest rate of decomposition. Therefore, placing less electron-withdrawing (and more sterically hindered) groups on the quaternary carbon of the tripodal anchor to the POM, likely favors higher stability. Furthermore, in PBS buffer at pD 7.4, AE-NH_2 reportedly decomposed by only 35% after 24 hours, therefore under the conditions used for tryptophan fluorescence studies of HEWL with each of the HPOMs, it is highly unlikely that decomposition of the HPOMs occurs. However, the solution dynamics of a mixture of these POMs has not yet been studied and merits further detailed research.

1 D. E. Salazar Marcano, S. Lentink, M. A. Moussawi and T. N. Parac-Vogt, *Inorg. Chem.*, 2021, **60**, 10215–10226.

Robin N. Perutz asked: As you say, phasing of protein X-ray data often depends on isomorphous replacement by heavy atoms. The use of POMs represents one example of this method. When I asked a colleague several years ago whether these structures were available to guide bioinorganic chemists, I was told that their coordinates were not deposited in the Protein Data Bank (PDB). Has there been any change in their availability? This is potentially a rich resource. How can we help to make the resource available and encourage colleagues to use it?

Tatjana N. Parac-Vogt responded: Following single-crystal X-ray diffraction of POMs, the obtained CIF (Crystallographic Information Files), which contains the coordinates of all atoms, is typically deposited on the Cambridge Crystallographic Data Centre (CCDC). Such CIF files are now widely available for most common POM structures and may therefore be used during data processing of protein X-ray data containing POMs. As a result, in recent years, the coordinates of POMs in protein structures deposited in the PDB are typically included. In addition, Bijelic and Rompel have published several excellent reviews on the use of POMs in protein crystallography,[1–3] which should aid in encouraging their use.

1 A. Bijelic and A. Rompel, *Coord. Chem. Rev.*, 2015, **299**, 22–38.
2 A. Bijelic and A. Rompel, *Acc. Chem. Res.*, 2017, **50**, 1441–1448.
3 A. Bijelic and A. Rompel, *ChemTexts*, 2018, **4**, 10.

Paul R. Raithby added: The PDB has been storing atomic coordinates of deposited protein crystal structures for some years now. I agree that some of the early structures that were deposited did not have atomic coordinates included.

Thomas R. Ward continued the general discussion of the paper by Michael R. Buchmeiser: I suspect the ionic liquid is quite viscous, should crowding (in addition to confinement) be taken into consideration?

Michael R. Buchmeiser replied: The IL is indeed viscous and might become even more viscous upon immobilization. This will result in a reduced self-diffusivity of the IL itself, the catalyst dissolved therein and the reactants. Concentration of the reactants and products is comparably low since they preferentially dissolve in the second phase (here MTBE). If "crowding" is meant to describe a situation in which the catalyst and the IL form larger "aggregates", which in turn results in hindered rotation of ligands of the catalyst, then, yes, that is the hypothesis (see Fig. 3 in the paper, **https://doi.org/10.1039/d2fd00152g**).

Odile Eisenstein commented: You are mentioning ongoing molecular dynamic studies. Can you give more information about them?

Michael R. Buchmeiser answered: Sorry Odile, ongoing calculations. I don't like to talk about immature results.

Odile Eisenstein added: Do you know why the reactions are faster? Is it by decreasing the entropic term or by forcing the proximity between chemical species

Michael R. Buchmeiser answered: There is a misunderstanding. Reactions under supported supported ionic-liquid-phase conditions are slower.

Imogen Riddell asked: Does the shape of the pore play a part in the observed reactivity? Is the enhancement a result of the optimised catalyst surface area, possibly due to the pore structure or does the pore uniquely orientate the catalyst/ligand?

Michael R. Buchmeiser replied: The shape of the pore might well play a role, yet, we have not looked into that here. We do not enhance the catalyst area since the catalyst is dissolved in the IL in all cases. While the pore as such does not orient the catalyst (which is dissolved), the catalyst is expected to become oriented in some way (…) at the IL/MTBE interphase. Calculations are ongoing….

Josh Morris asked: Have you considered any additional counter anions beyond BF_4^-, and how do you think anion steric bulk and in particular interaction strength with the cationic Rh catalyst would affect the overall stability and selectivity of the catalyst?

Michael R. Buchmeiser answered: The counter anion of the IL has to match the one of the catalyst (and *vice versa*). The list of ILs that are liquid below 50 °C is rather limited. In an IL, the catalyst is considered to be fully dissociated. Increased steric build, *e.g.* provided by the $B(ArF)_4$ anion would require an IL with the same anion, which is not feasible. In general, the catalyst is very stable. The question whether selectivity could be further increased by the use of larger anions is hard to answer due to the lack of suitable ILs.

Albert Poater opened a general discussion of the paper by Miquel Costas: Is the enantioselectivity/yield achieved in your article (**https://doi.org/10.1039/d2fd00177b**) related to the temperature?

Miquel Costas responded: In general, enantioselectivity is favored when temperature is reduced. In the current reactions we don't generate chiral compounds. Changes in yields responding to temp changes depend on the reaction. A general trend can not be deduced.

Albert Poater added: How do you keep the crown ethers under control? I mean, how to ensure that you only have one metal moiety per crown ether, or instead, no polymeric chains form?

Miquel Costas responded: You analyze by bidimensional NMR the nature of the species that form from binding the diamine to the catalysts. NOE interactions observed are only consistent with a 1 : 1 structure.

In addition, catalytic reactions are conducted at different concentrations. Reducing concentrations disfavors higher order structures. No significant differences in selectivity are observed when conducting reactions at low concentrations.

MS analyses are also consistent with a 1 : 1 structure and no higher order structures are observed.

Basheer Chanbasha‡ asked: Is the catalyst suitable for small molecule oxidation, *e.g.* methane to methanol?

Miquel Costas answered: We don't know. We have not been able to test this possibility because oxidizing methane requires working under pressure, and delivering the oxidant to the reaction mixture under these conditions is something we have not been able to test yet. We think it should be possible, because we can oxidize primary alkyl C–H bonds.

Helma Wennemers said: Your work reminds me of work by Breslow and Woggon, who used cyclodextrin-based systems to achieve site-selective oxidations or alkene cleavage. Have you considered combining your crown ether recognition site with a cyclodextrin moiety? Also, Woggon used biphasic systems for the site-selective cleavage of Carotene.[1] Maybe this could be useful for your system.

1 R. R. French, P. Holzer, M. G. Leuenberger and W.-D. Woggon, *Angew. Chem., Int. Ed.*, 2000, **39**, 1267–1269.

Miquel Costas answered: Thank you very much for the insightful comments. Indeed, Breslow's work was the initial inspiration for the design of our system. Thank you for pointing out the work of Woggon. I must admit I somehow missed this work despite the similarity being quite clear. We have considered the use of cyclodextrins but the biphasic media has represented a problem we have not been able to solve so far. When water has been added to our reactions, the chemistry of peroxide activation is severely perturbed.

Bartosz Lewandowski asked: What was your reasoning behind using this specific diamine substrate with a 14-carbon long alkyl chain?

Miquel Costas answered: The reason was the original selectivity observed when using a monoamine, for C8 and C9 positions. We also want to explore C13, C15 and C16 diamines.

Bartosz Lewandowski added: Have you tried using substrates of different lengths and if not would you expect to achieve high selectivity as well?

Miquel Costas replied: We have used steroidal amines.[1] Short amines are not oxidized.

We are currently trying to expand the concept, using substrates where we can tune the length by an amine linker of different length.

1 G. Olivo, G. Capocasa, B. Ticconi, O. Lanzalunga, S. Di Stefano and M. Costas, *Angew. Chem., Int. Ed.*, 2020, 59, 12703–12708.

Bartosz Lewandowski further queried: Could you design a derivative of your system where you would control the distance between the two crown ether moieties by an external stimulus, *e.g.* light, and by doing so alter the selectivity of the oxidation reaction?

‡ Dr Chanbasha could not be contacted to confirm the text of the question before going to print.

Miquel Costas responded: Wow, this is a beautiful idea! We never thought about that. It should be possible since there are several ways we introduce the crown ether in the structure of the ligand. We'll think about that. If we come up with a solution, I will contact you. Many thanks!!!

Joe C. Goodall opened a general discussion of the paper by Torsten Beweries: I note that in all of the ^{19}F magic-angle spinning (MAS) SSNMR spectra of these complexes you see two signals for each of the Ni–F and Ar–F environments (**https://doi.org/10.1039/d2fd00171c**). Do you see the same doubling of the peaks in the $^{13}C\{^{1}H\}$ MAS SSNMR spectra for the Ar–F and carbene environments?

Torsten Beweries replied: We have not recorded $^{13}C\{^{1}H\}$ MAS SSNMR spectra as we were interested in the F environments. My feeling is however, that this doubling of the arene signals should also be observable in the ^{13}C NMR spectra.

Joe C. Goodall added: You reason that the doubling of the peaks that you see in the ^{19}F MAS SSNMR is from disorder which is not observed in single crystal X-ray diffraction structures, have you run any powder X-ray diffraction studies on the bulk material, at the same temperature, to rule out any polymorphism or phase transitions occurring?

Torsten Beweries responded: No, we have not checked for polymorphism or phase transitions using powder X-ray diffraction.

Tatjana N. Parac-Vogt asked: Is your compound paramagnetic or diamagnetic?

Torsten Beweries answered: These complexes are square planar Ni(II) in d8 configuration, hence diamagnetic.

Tatjana N. Parac-Vogt further queried: Do you see anything for your complex in solution-state NMR? Did you consider trying it?

Torsten Beweries responded: I assume you are referring to the complexes/adducts that were analysed by SC-XRD and SS-NMR. We have not checked the solution NMR of these systems. It would however, be interesting to see whether the 1 : 1 or 1 : 2 adducts can also be detected. From solution NMR we know that a pronounced downfield shift of the Ni–F resonance can also be observed at donor–acceptor ratios of 1 : 1 and lower.

Stuart A. Macgregor continued the general discussion of the paper by Miquel Costas: The role of the carboxylate in enhancing selectivity is intriguing. Can you clarify the role played by the carboxylate in the general mechanism of oxidation and comment on the origin of this enhancement?

Miquel Costas responded: Carboxylic acid helps in promoting the heterolytic cleavage of the O–O bond, releasing a water molecule, thus forming a Mn(O)(-carboxylate) reactive species. Being a ligand, the carboxylate modulates the electronic properties of the metal, and helps define the space in proximity to the reactive Mn=O. The improvements we observe with α,α,α-substituted carboxylic

acids are systematic, which to us suggest that the effect may be steric (https://doi.org/10.1039/d2fd00177b). We speculate that a bulky carboxylic acid may create a spacially more constrained reactive site, limiting directions of approach of the substrate.

There is a large degree of speculation in this interpretation. We are looking at modest enhancements, meaning that changes in activation barriers may also be quite modest. Suggestions will be very welcome.

Rafael Gramage-Doria remarked: It appears that the carboxylic acids are co-catalysts in the catalytic reactions, so why is there such an excess of the carboxylic acid per ligand? Can we use sub-stoichiometric quantities?

Miquel Costas responded: Thank you very much for your question. The amount of carboxylic acid necessary depends on the e-donating ability of the pyridine in the catalysts and also the nature of the carboxylic acid. Catalysts with pyridines containing e-donating groups (MeO, NMe_2) operate with 10–15 mol% of the carboxylic acid, and can be reduced down to 3–5 mol% when using phthalimido protected amino acids. Catalysts with e-withdrawing groups require larger amounts of carboxylic acids, 1–15 equiv. with respect to the substrate.

Wei-Chun Liu continued the general discussion of the paper by Torsten Beweries: Have you examined the proton NMR spectra to determine if there is any interaction between the C–H bond and the fluorine atom?

Torsten Beweries answered: Proton NMR spectra show negligible changes in chemical shifts of the surrounding groups upon addition of the halogen bond donor.

Wei-Chun Liu added: Is there any reason you choose di-iodobenzene for co-crystallisation?

Torsten Beweries replied: 1,4-Diiodobenzene serves as a structural model of iodopentafluorobenzene that was used for titration studies. We chose this donor as it shows much better co-crystallisation properties (https://doi.org/10.1039/d2fd00171c).

Pierre Kennepohl addressed Torsten Beweries and Odile Eisenstein: I wonder whether there may be some π-contributions to the bonding in $2IPr_2Im\cdots IC_6F_5$, especially given that the two *trans*-aromatic rings are coplanar. Is it possible that this could be reflected in the observed NMR parameters? It might be possible to explore this from a computational perspective by rotating the IC_6F_5 fragment to 'break' the potential π network.

Torsten Beweries responded: I think you are referring to the related recent (computational) study.[1] In this study the arenes were treated as fully coplanar based on the experimental SC-XRD data published earlier.[2]

For the described Ni carbene complexes, no data of such self-complementary adducts are available. In our SC-XRD data the fluorinated arene acting as a halogen bond donor is not coplanar with the Ni-arene unit. In solution, the situation might be

different. It might be worth looking into the π-contributions computationally, varying the arene unit of the halogen bond donor. Maybe Odile Eisenstein or Robin N. Perutz can comment on whether such things have been considered in ref. 1?

1 A. C. Castro, M. Cascella, R. N. Perutz, C. Raynaud and O. Eisenstein, *Inorg. Chem.*, 2023, **62**, 4835–4846.
2 V. Thangavadivale, P. M. Aguiar, N. A. Jasim, S. J. Pike, D. A. Smith, A. C. Whitwood, L. Brammer and R. N. Perutz, *Chem. Sci.*, 2018, **9**, 3767–3781.

Odile Eisenstein responded: We have not explored this point in the related study of the halogen bond in nickel fluoride complexes,[1] but some test calculations done by changing the nature of how the iodoaryl ligand binds to the fluoride did not show any significant effect.

The strength of the halogen bond interaction, which is an order of magnitude larger for the NHC nickel fluoride complexes than for the corresponding phosphine complexes[2] does not appear to be reflected in the magnitude of the chemical shift of the halogen bond. Interestingly, the fluorine downfield shift due to the halogen bond, and the lack of relationship between halogen bond strength and NMR chemical shift, has already been noted. It is nicely confirmed by this study.[2] To some extent it is not really surprising since the strength of the bond and the NMR chemical shifts are associated with different groups of occupied and molecular orbitals as was presented by Castro *et al.*[1] Another point is that NHC ligands are better σ donors than phosphines and thus they interact more with d(x^2–y^2) which is the main component of σ* (Ni–F) This could account for the fluorine chemical shift being more upfield with NHC (higher lying σ* Ni–F). This is thus probably more a comment than a question.

1 A. C. Castro, M. Cascella, R. N. Perutz, C. Raynaud and O. Eisenstein, *Inorg. Chem.*, 2023, **62**, 4835–4846.
2 V. Thangavadivale, P. M. Aguiar, N. A. Jasim, S. J. Pike, D. A. Smith, A. C. Whitwood, L. Brammer and R. N. Perutz, *Chem. Sci.*, 2018, **9**, 3767–3781.

Torsten Beweries added: I could maybe add that the π back donation of Arduengo carbenes with the metal centre is often less pronounced compared to phosphine groups, making the metal more electron rich, leading to a more polar Ni–F bond, thus enabling stronger interactions of the metal fluoride with halogen or hydrogen bond donors.

Tatjana N. Parac-Vogt opened a general discussion of the paper by Kamran T. Mahmudov: What is the advantage of using selenium over sulfur in your synthesis (**https://doi.org/10.1039/d2fd00160h**)?

Kamran T. Mahmudov responded: The atom polarizability of the chalcogen bond (ChB) donor atom increases in the following order (a.u.): 5.3 (O) < 19.4 (S) < 29.0 (Se) < 38.0 (Te), which indicates a poor or a strong ChB donor ability for the O or the Te atom, respectively. The advantage of using selenium over sulfur is to increase strength and directionality of ChB between tectons.

Wei-Chun Liu commented: How can you assess the acidity of compound **7**, which is a di-imine? Additionally, if you utilize DMF, would you be able to observe coordination to the compound?

Kamran T. Mahmudov answered: Due to the strong ChB acceptor ability of DMF we have observed/isolated compound **7**. If we use $Cu(OAc)_2$ instead of $Cu(NO_3)_2$ we can isolate a new copper complex, which was not included in our paper.

Jorge Echeverria asked: In your presentation, you have shown both single and double-bonded selenium atoms acting as electron density acceptors *via* their σ-holes. Have you observed any difference, *e.g.*, in directionality or interaction strength, between these two?

Kamran T. Mahmudov replied: In these copper(II) complexes the selenium atoms act as mono- or bifurcated chalcogen bond donors between tectons. Yes, we have observed differences in directionality as well as strength of ChB, between these two. Please see Fig. 1 and 2 in our article.

Frances E. Bugden remarked: In Scheme 3 in your article, you start the synthesis from diphenyl diselenide to prepare compound **L1**. Have you considered adding electron-withdrawing groups to the phenyl ring *e.g.* p-CF_3 to increase the potential chalcogen bonding ability of this compound?

Kamran T. Mahmudov responded: Of course, attachment of the electron-withdrawing groups to the phenyl ring *e.g.* p-CF_3 can increase the strength of chalcogen bonding. But, in this work, our idea was to demonstrate the design of the secondary coordination sphere of copper(II) complexes with ChB donor centre(s).

Stuart A. Macgregor commented: The BCP potential energy densities have been used to provide an estimate of how much H-bonding contributes to the association energies within compounds **2**, **3** and **5** (*e.g.* Fig. 3 and 4 in your article). This is less than 10% of the computed association energies. What other factors contribute to these association energies, and how much of this is do you estimate to be due to chalcogen bonding?

Kamran T. Mahmudov answered: The chalcogen bonding energies of compounds **2**, **3** and **5** are:

E(ChB) = −7.1 kcal mol^{-1} for compound **2**, E(ChB) = −5.6 kcal mol^{-1} for compound **3**, E(ChB) = −4.6 kcal mol^{-1} for compound **5**. These energies are estimated using the V_r energy predictor and the formula proposed in ref. 1. So the energies are larger than the HBs. The large binding energies are mainly due to electrostatic forces, as anticipated by the molecular electrostatic potential analysis.

1 A. Bauzá and A. Frontera, *ChemPhysChem*, 2020, **21**, 26–31.

Andrew S. Weller continued the general discussion of the paper by Torsten Beweries: When looking at your Ni–F bond length changes (**https://doi.org/10.1039/d2fd00171c**) can this be interpreted as reducing any d(π)–p(π)-interactions, and therefore this results in the shortening of the bond?

Torsten Beweries responded: This is indeed an interesting observation. Your idea of reducing d(π)–p(π) interactions between Ni and F could play a role for shortening the Ni–F bond upon adduct formation.

For Pd systems with pincer ligands *trans* to the fluoride we have however observed an elongation of the M–F bond by 2–3% upon adduct formation.[1] This system is however, structurally different (different M, different L) to the one in our current *Faraday Discussions* article, so a direct comparison might be difficult, calling for a more detailed theoretical study of this question.

1 M. Joksch, H. Agarwala, M. Ferro, D. Michalik, A. Spannenberg and T. Beweries, *Chem.–Eur. J.*, 2020, **26**, 3571–3577.

Andrew S. Weller further queried: Can you explain why there is no disorder observed in your structure, despite the fact that the solid state NMR shows two different environments?

Torsten Beweries replied: The difference between the X-ray and MAS NMR data may relate to the temperature: the MAS NMR data were obtained at room temperature whereas the crystal structures were determined at 100 K.

Neil R. Champness remarked: Were the solid state NMR and crystallography experiments carried out at the same temperature? If not, might this explain the differences in structural model suggested by the data?

Torsten Beweries answered: This relates to the earlier question from Joe C. Goodall. X-ray analysis was done at 100 K whereas solid state NMR spectra were recorded at room temperature, which could explain the presence of an additional set of signals in some of the NMR spectra.

Andrew S. Weller added: We see significant differences in data between SSNMR and crystallography due to the large differences in temperature the two techniques are routinely carried out at (for example 150 K X-ray crystallography, 298 K SSNMR).

Andrew S. Weller continued the general discussion of the paper by Miquel Costas: Have you ever tried your catalysts (**https://doi.org/10.1039/d2fd00177b**) using phase transfer systems/conditions to see if any increase in performance is observed?

Miquel Costas responded: Thank you for the suggestion. We have not used phase transfer conditions because the chemistry of H_2O_2 activation is perturbed when reactions are performed in water.

Robin N. Perutz remarked: Is your selectivity determined by chain-walking of the carboxylic acid along to the desired reaction site?

Miquel Costas responded: No, there is no evidence that suggests that a chain walking process may be operative. Our understanding is that reactions proceed without organometallic intermediates susceptible to engage in chain walking

processes. Chain walking will require cleaving of other C–H bonds by the first formed carbon centered radical. I am not aware of any chain walking process along an alkyl chain through radical intermediates. So I conclude this is highly unlikely, although it may be an issue to explore in the near future *via* isotopic labelling.

Joost N. H. Reek asked: the selectivity achieved by pre-organisation is already great, but not perfect. The small amount of regioisomeric products that are formed can be a result of: (1) a reaction between an unbound substrate and the catalysts; (2) a reaction between the bound substrate and the catalysts, in which case there is sufficient flexibility in the system to reach the other sites. Can you distinguish between these two? That would help the future design of catalyst systems using supramolecular pre-organization.

Miquel Costas responded: That is a very good point. Thank you for pointing it out. I must admit we never considered it. When we have run competitive oxidations between alkylamine substrates and hydrocarbons we observe preferential oxidation of the amine substrate, but the selectivity towards the bound substrate is never complete. In these cases we always employ substrates that are easier to oxidize (on the basis of BDE) than the aliphatic amine, so the comparison may not be appropriate. We need to run a reaction with a monomethylated amine, which will be electronically very similar, but is not recognized by the crown. Again, thank you for the suggestion.

Would anyone have another experiment in mind to address this question? Any suggestion will be very welcome.

Joost N. H. Reek added: Once the substrate is converted, it can still bind in a ditopic fashion (potentially with the oxygen coordinating the iron). This suggests that product inhibition may play a role. Do you have any information on this aspect?

Miquel Costas answered: We indeed suspect this is happening because ketones are the dominant product, even when reactions are run at low substrate conversion. Avoiding overoxidation of the first formed alcohol in acetonitrile is quite a difficult problem, even for supramolecular binding. Not sure how to address it. Any suggestion will be very welcome. I find the suggestion of embedding the catalyst in a porous material, suggested in Professor Buchmeiser's article (**https://doi.org/10.1039/d2fd00152g**) quite appealing.

Joost N. H. Reek continued the general discussion of the paper by Torsten Beweries: You have reported interesting nickel–fluoride complexes that act as a Lewis base with aryl iodide (**https://doi.org/10.1039/d2fd00171c**). When such complexes form, does it activate the fluoride at the metal center for further reactivity, for example by making the halogen a better leaving group?

Torsten Beweries replied: We have never observed this in our studies. This reflects the generally strong M–F bonding imposing a different chemistry upon the metal-halide than with the heavier halides.

Stuart A. Macgregor addressed Torsten Beweries and Odile Eisenstein: You have described the role of the coupling between the fluorine occupied p-orbitals with the Ni–F σ* orbital on the ^{19}F chemical shift. Can you affect the energy of the Ni–F σ* by varying the nature of the *trans*-fluoroaryl ligand, and then assess the effect (if only computationally) on the chemical shift?

Odile Eisenstein responded: we would have liked to see the relationship that you are describing but the number of orbitals involved in the halogen bond with nickel fluoride complexes is very large. Thus changing the chemical group modifies many contributions to the chemical shift tensors. Nothing really clear arises. However, we were able to verify that the chemical shift tensor (CST) of the fluoride was qualitatively unchanged when replacing the trans fluoroaryl ligand with another ligand. Even replacing the fluoroaryl ligand by hydride does not have a major consequence on the shape of the CST. Clearly the fact that the fluoride is bonded to a d8 metal in a square planar coordination, determines the main characteristics of the ^{19}F CST.

Torsten Beweries added: This is an interesting point. The mentioned complexes, formed by oxidative addition of an aryl fluoride are however synthetically rather difficult to access. Partially fluorinated pyridines could be an alternative as shown by the Perutz group.[1–3] In the past we have shown that group 10 POCOP pincer complexes can be another alternative.[4] In these complexes, the monoanionic arene based pincer ligand *trans* to the halide ligand can be readily tuned prior to cyclometalation at Ni. Subsequent salt metathesis is then used to introduce the fluoride ligand. We are currently looking into the SSNMR spectra of such complexes, combined with computations. I am afraid that for the systems discussed in our present *Faraday Discussions* paper, which are formed by oxidative addition of the fluoroarene, such studies are only possible computationally.

1 S. J. Pike, C. A. Hunter, L. Brammer and R. N. Perutz, *Chem.–Eur. J.*, 2019, **25**, 9237–9241.
2 T. Beweries, L. Brammer, N. A. Jasim, J. E. McGrady, R. N. Perutz and A. C. Whitwood, *J. Am. Chem. Soc.*, 2011, **133**, 14338–14348.
3 T. Braun and R. N. Perutz, *Chem. Commun.*, 2002, 2749–2757.
4 M. Joksch, H. Agarwala, M. Ferro, D. Michalik, A. Spannenberg and T. Beweries, *Chem.–Eur. J.*, 2020, **26**, 3571–3577.

Odile Eisenstein addressed Torsten Beweries and Stuart A. Macgregor: Earlier in the discussion about halogen-bonding, we were discussing the amount of electron transfer between the halogen bond acceptor (the Ni–F bond) and the halogen bond donor (the I–C bond). There is a very small electron transfer between the two chemical entities. In contrast there is significant polarization of the bond. This is quite typical of non-covalent interactions. In fact, the study by Martin Head-Gordon *et al.* of a halogen-bonded system (CX_3I, with X equal to F, Cl, Br, and I) showed that the interaction is the result of the interplay between Pauli repulsion, electrostatic, charge transfer and dispersion, with no dominant influence.[1] It was also shown in this publication that a picture of the halogen bond that excludes charge transfer cannot be completed, and permanent and induced electrostatics do not always provide the dominant stabilizing contributions to halogen bonds. Overall, three universally attractive factors – polarization, dispersion and charge transfer – together with permanent electrostatics, which is

usually attractive, drive halogen bonding, against Pauli repulsion. Similar results were obtained in the study of the halogen bond in Ni(II)fluoride complexes.[2]

1 J. Thirman, E. Engelage, S. M. Huber and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2018, **20**, 905–915.
2 A. C. Castro, M. Cascella, R. N. Perutz, C. Raynaud and O. Eisenstein, *Inorg. Chem.*, 2023, **62**, 4835–4846.

David Powers continued the general discussion of the paper by Miquel Costas: Are there significant through-bond effects that contribute to the selectivity that is seen in your work (**https://doi.org/10.1039/d2fd00177b**)?

Miquel Costas answered: Yes, there are. These can be appreciated when running the reactions with a catalyst that does not contain the crown ether. The first 3 positions adjacent to the protonated amine are deactivated by polar effects. Farther from these positions, the effect vanishes.

Thomas R. Ward remarked: How would you engineer an active site which has preference for your desired product and increase turnover numbers? This would avoid over-oxidation and increase selectivity.

Miquel Costas answered: Maybe an option will be to create highly hydrophobic active centers that would favor substrate (hydrocarbon moiety) binding *via* weak interactions, while also extruding the more polar oxidized product.

Eva Meeus asked: To target the challenges with respect to 'product release' after substrate conversion, it would be interesting to make use of a 'two-step' mechanism in which not only the desired C–H bond is oxidized, for example, but also the 'targeting moiety' is converted in a way that it is released from the recognition site. Did you consider this? Would it be feasible?

Miquel Costas replied: This is a nice idea and we have pursued it with limited success. We have considered using divalent cations to trap alcohols and diols. The trapping of alcohols and ketones resulting from C–H oxidation is a more challenging question for which we don't have a solution. A very powerful strategy is using fluorinated alcohol solvents, they promote rapid detachment of the substrate. However, they are incompatible with the crown ether recognition of the protonated amine moiety.

Michael R. Buchmeiser suggested: To prevent over-oxidation, the primary product must be transported off. Why don't you put everything into a non-polar pore?

Miquel Costas responded: Thank you very much for the suggestion. This is something we never considered. How would you introduce the catalysts within the pore? Could you give me some relevant references?

Michael R. Buchmeiser said: Maybe introduce an anchor at a remote position of the catalyst for immobilization on silica, see ref. 1 and 2.

1 F. Ziegler, H. Kraus, M. J. Benedikter, D. Wang, J. R. Bruckner, M. Nowakowski, K. Weißer, H. Solodenko, G. Schmitz, M. Bauer, N. Hansen and M. R. Buchmeiser, *ACS Catal.*, 2021, **11**, 11570–11578.
2 F. Ziegler, J. Teske, I. Elser, M. Dyballa, W. Frey, H. Kraus, N. Hansen, J. Rybka, U. Tallarek and M. R. Buchmeiser, *J. Am. Chem. Soc.*, 2019, **141**, 19014–19022.

Pinkie Ntola asked: Could you share briefly how the manganese oxidation catalysts were prepared, that is, the synthesis procedure?

Miquel Costas replied: It is quite simple. Mixing ligand and $Mn(OTf)_2$ in acetonitrile, precipitation with ether and then recrystallization from acetonitrile : ether or CH_2Cl_2 : ether.

In the ESI of one of our previous papers, you can find the procedure in detail.[1] Contact me in case you need some clarification.

1 G. Olivo, G. Farinelli, A. Barbieri, O. Lanzalunga, S. Di Stefano and M. Costas, *Angew. Chem., Int. Ed.*, 2017, **56**, 16347–16351.

Conflicts of interest

Dr Chanbasha could not be contacted regarding any possible conflict of interest before going to print. There are no other conflicts to declare.