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Conference Report on the Nobel Symposium #168
"Visions of bio-inorganic chemistry: Metals and the molecules of life" held in Lejondal Castle, Sweden, from May 29th – June 1st, 2022

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Abstract: There is usually only one Nobel Symposium per discipline and year, but COVID has also had an effect on this rule. Initially planned to take place in 2020, this Nobel Symposium lined up a unique group of speakers and just about the same number of observers, as well as three members of the scientific press, all by invitation.

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Katharina M. Fromm is professor for chemistry at the University of Fribourg since 2006 and Vice-Rector for research and innovation since 2020. She received her PhD from Karlsruhe University in 1994 and did a postdoc among others with Jean-Marie Lehn in Strasbourg before rejoining the University of Geneva for a habilitation in 1998. She held an SNSF professorship at the University of Basel from 2003 to 2006. Over the past years, she became an expert in the bioinorganic chemistry of silver.

From May 29 to June 1st, 2022, I had the great opportunity to participate in the Nobel Symposium #168 on Bioinorganic Chemistry along with *ca.* 60 speakers, observers and a few representives from the scientific press.

The first top-speaker ***Alison Butler*** (Fig. 1) from UCSB presented an excellent lecture on marine bioinorganic chemistry and how metal ions are taken up by microbes. In particular, she presented amphiphilic siderophores of marine bacteria, responsible for iron uptake. It seems that the chirality of the amino acids not only influences the stereochemistry around the metal ion, but also affects the growth and viability of the microbes. Next to tris-catecholate and β-hydroxyaspartate siderophores, diazeniumdiolate siderophores not only are able to coordinate iron ions, but seem to release two moieties of NO upon irradiation, which is speculated to play a role in quorum sensing.

Lucia Banci spoke about metal trafficking in the cell and how one can and should combine the atomic resolution with the cellular dimension. With an impressive line-up of the newest NMR machines available to her on the CERM campus in Florence, combined with other high-end analytical equipment such as EPR and cryo-EM, she is involved in the genome screening of different species in order to find and identify new metal-binding proteins. She highlighted the investigation of a protein which is folded or not and zinc-binding or not, depending on whether it is *in vitro* or *in vivo*. She also highlighted tailored NMR spectra, combined with EPR, to study iron-sulfur clusters in proteins *in vivo*.

In his talk, ***Nigel Robinson*** from Durham addressed the question how metal-ion binding proteins 'select' their metal ions from their environment, highlighting the fact that many metal-binding proteins can coordinate to several metal ions. For example, MncA, a Mn-folding protein, binds copper about 1000 times better than manganese, this as a consequence of the Irving-Williams series. To prevent wrong metallation, cells are able to control intracellular metal ion availability using *e.g.* DNA-binding metal-sensing transcriptional regulators. As a result, cytosolic metal ion concentrations can be maintained at the inverse of the Irving-Williams series. A metallation calculator is available and its functioning explained under https://mib-nibb.webspace.durham.ac.uk.

Roland Lill from Marburg presented his newest insights into the molecular mechanisms of iron-sulfur protein biogenesis in eukaryotes, focusing first on $[Fe_2S_2]^{2+}$-clusters, then on their reductive assembly into $[Fe_4S_4]^{2+}$-moieties. He presented among others the role of Tyr35, which is universally conserved in all iron-sulfur-cluster mitochondrial scaffold proteins ISCU, and mutations of this amino acid seem to lead to cell death. Given the fact that Tyr easily forms a radical, Tyr-Tyr binding cannot be excluded. Also, a cysteine acts as sulfur source, being itself transformed into alanine.

The next superstar speaker was ***Jackie Barton*** from Caltech, one of the pioneers in understanding long-distance electron transfer through DNA. She explained that more and more DNA-binding proteins involved in genome maintenance, polymerases, were found to possess iron-sulfur clusters. DNA strands attached to a gold electrode *via* a sulfide linker are very sensitive for the electrochemical detection of such new proteins and can even give indication on the redox states of the metal ions. In an oxidized state, such iron-sulfur clusters seem to inhibit replication, while this process is reversible. Mismatches in DNA can be detected *via* this method as well and proteins seem to use this mechanism to direct repair proteins to such sites.

Fig. 1. Top: Alison Butler, Lucia Banci, Nigel Robinson, Roland Lill, Ed Solomon; bottom: Jackie Barton, Steve Lippard, Serena DeBeer, the three organizers Pernilla Wittung-Stafshede, Martin Högbom and Erik Johansson.

Steve Lippard from MIT was the last, but definitely not the least speaker of the first day. He presented four stories about

the role of zinc ions in the brain related to sensory perception. Indeed, zinc accumulates in glutamatergic vesicles near the synapses. These vesicles release their cargo after fusion with the membrane, and the freed zinc ions can enter then *via* calcium channels. Be it in the hippocampus, the dorsal cochlear nucleus, the olfactory bulb or the retina, zinc always seems to downregulate the amplitude of the response following stimulation of the different sensory organs, *e.g.* ear, nose or eye. It may be that this effect is important in the relative perception of several sounds, smells or sights and possibly could also be related to autism.

Harry Gray was announced initially as first speaker of the second day for the session on electron transfer, but he could not travel to Sweden due to a sore leg. He nevertheless joined the conference for a short online greeting in the evening. ***Serena DeBeer*** from the Max Planck Institute in Mühlheim and initially planned as last speaker, took the stage instead of him, highlighting how to use different X-ray spectroscopic methods to obtain high resolution information about the redox states of iron-sulfur clusters and to study electron transfer processes in the P-cluster and the FeMoCo cluster in which nitrogen is reduced to ammonia. This [$MoFe_7S_9C$]-cluster has four Fe(III) and three Fe(II) as well as one Mo(III) in the ground state. It is capable of binding CO. Treatment with KSeCN shows the displacement of one sulfur in equatorial (belt) position by selenium. Two-color XES is a method that allows to follow several elements at a time.

Ed Solomon from Stanford discussed correlations between non-heme iron metalloenzymes and heterogeneous catalysis, comparing geometric and electronic structural contributions to the iron-oxygen reactivity. Variable temperature and field magnetic circular dichroism and nuclear resonance vibrational spectroscopy were used to define the electronic and geometric structures, respectively, of Fe(IV)=O intermediates and how their Frontier Molecular Orbitals (FMOs) control reactivity. As model compounds, metallozeolites were studied in the context of transforming methane to methanol.

As ***Dan Nocera*** was struck by COVID, ***Akif Tezcan*** (Fig. 2) presented his talk earlier than initially planned in the program. He discussed the self-assembly of proteins using concepts from supramolecular chemistry based on metal ion binding reminiscent of terpyridine coordination. Introducing three nearby histidine moieties on each protein, octahedral coordination can be expected for many divalent metal ions. Offering several such binding sites per protein and linking two proteins *via* cystine moieties, he nevertheless finds different coordination behaviors for copper compared to zinc or nickel ions.

Bernd Giese from Fribourg, Switzerland, reminded the audience of how his seminal work on radicals led to his discovery of the electron (and hole) hopping mechanism in DNA and proteins. Using live cells of Gram-negative *Geobacter sulfurreducens*, he showed how extracellular metal ions trigger extracellular electron transfer (EET) from NADH through the inner membrane, the periplasm and the outer membrane. EET rates are regulated by the Fe(II) to Fe(III) heme ratios in *c*-type cytochromes. Each cell transports nearly half a million e^-/s through the cell in a constant electron flow, which is independent of the concentration and the type of extracellular metal salts. This leads to constant respiration rates and ATP homeostasis. While electrons in EET travel about 40 nm through the cells, the next challenge is to understand cm-long ET, as it occurs through the cell filaments of cable bacteria.

The Monday afternoon session was dedicated to photosynthesis and kicked off with ***Jian-Ren Shen*** from Okayama as one of the pioneers in X-ray crystallography of the photosystem II and the related elucidation of the mechanism of water oxidation. A 1.9 Å resolution was obtained for the Mn_4CaO_5-cluster as the catalytic center. Upon irradiation, one of the O-atoms, O5, shows an elongation, indicative of its involvement in the oxygen formation. A sixth O-atom from a water molecule is found close to O5 after a second light pulse. The calcium ion is first 7- and then 8-coordinated, and several amino acids in vicinity of the cluster also undergo changes in H-bond formation. Are these the structures picturing the S1 to S3 states of the enzyme?

Fig. 2. Top: Akif Tezcan, Bernd Giese, Jian-Ren Shen, Junko Yano, Wolfgang Lubitz; bottom: Kara Bren, Per Siegbahn, Marc Fontecave, Angela Casini, Larry Que.

Junko Yano from the Lawrence Berkeley National Lab in Berkeley by and large confirms these findings using X-ray spectroscopy at the XFEL. Looking at the entire protein, she identified a water cluster containing five molecules close to the calcium ion at the end of water channel 1 of the photosystem II protein, and claims that the Cl1-channel could be used for proton release during oxygen formation.

Wolfgang Lubitz, also from Mühlheim's Max Planck Institute for Chemical Energy Conversion convinced the plenum that multifrequency EPR and advanced pulse EPR/NMR techniques such as ENDOR are excellent tools to study the paramagnetic states of photosystem II, their protonation/deprotonation events as well as the above described mechanistic steps towards triplet dioxygen release. Based on his very didactic presentation, many of the later speakers took advantage of referring to his explanations of the method.

Kara Bren from Rochester then discussed bioinorganic approaches to artificial photosynthesis, using Co(II) complexes on one hand, and quantum dots based on CdSe on the other, with ascorbate as sacrificial electron donor. As a new exciting approach, she replaced the ascorbate by the electron-producing bacteria *Shewanella oneidensis*. Whether these bacteria uptake the quantum dots or not during the catalytic process remains to be understood.

Per Siegbahn from Stockholm University completed this discussion around photosystem II from a theoretical standpoint and showed that he was able to predict all the current findings already since 2006. While photosystem II had a very positive potential of 1.25 V to be reached, nitrogenase needs to go as low as –1.6 V. He compared the four activation steps of Fe(III) to Fe(II) of the initially [$Mo(III)Fe(III)_4Fe(II)_3$] to [$Mo(III)Fe(II)_7$] with a vanadium variant [$V(III)Fe(III)_4Fe(II)_3$], which would undergo five activation steps to [$V(II)Fe(II)_7$]. Experimentalists would now need to prove the binding affinity of N_2 to such clusters.

Marc Fontecave from Collège de France was the first speaker on Tuesday, taking a bioinspired approach of metal catalysis, with the aim at making fuels from CO_2. One approach was inspired from the active site of carbon monoxide dehydro-

genase, synthesizing analogs to the molybdopterin cofactor. He also reported on the synthesis of heme-inspired solid catalysts with copper in the N4-sites of N-doped carbon materials. It turned out that copper clusters form during this process, able to yield ethanol, while all other metal ions tested in this system yielded rather methanol.

Angela Casini from TU Munich reported on the use of supramolecular coordination cages which are able to transport drugs like cis-platin or the theranostic agent pertechnetate. While many drugs are otherwise not able to cross the blood-brain barrier, their encapsulation combined with the connection of a target protein allows to bring drugs to specific places, including the brain. Furthermore, cis-platin turned out to be less toxic when encapsulated compared to its 'free' form.

Larry Que from Minnesota explored the nonheme high-valent iron-oxo landscape, presenting a series of synthetic $[Fe_2(\mu\text{-}O)_2]$ diamond type complexes and studying their structure as a function of the oxidation states of iron, Fe(IV) or Fe(III). Such entities are relevant in the Q-state of the soluble methane monooxygenase hydroxylase (sMMOH) enzyme. An interesting discussion ensued regarding the metal-metal distance in the enzyme as various experiments and methodologies point in different directions, to either *ca.* 2.7 Å or >3Å. Thus, the riddle about the sMMOH will certainly continue.

The next speaker before lunch was ***Michelle Chang*** from Berkeley (Fig. 3). She talked about the discovery and application of bioinorganic enzymes for biocatalysis and focused in particular on radical hydrogenases. For example, the chlorination of lysine can occur in 4- or 5-position, depending if the used enzyme is BesD or HalB. Other functional groups like azide, bromide, hydroxide or fluoride can also be introduced at defined positions.

Doug Rees from Caltech discussed the mechanism of nitrogenase, focusing on the sulfur atoms bridging the $[MoFe_7C]$-cluster. For example, if a sulfur in belt position is replaced with selenium, a scrambling of positions can be observed, reminiscent of a Bailar twist mechanism. Reacting the co-factor with CO showed its insertion near the Fe6-position, while the corresponding sulfur S2B is displaced to the glutamine Q176, indicating that Fe6 could be the active site also for isoelectronic nitrogen binding.

Brian Hoffman from Northwestern discussed the hydrogen binding of the same enzyme, proposing hydride formation rather than reduction of the core metal ions. This could then lead to hydrogen release, while two electrons remain on the cluster. Replacing molybdenum with iron led to a similar reactivity, again indicating iron as the active site. He interestingly compared the CFe_6-moiety to an excerpt of cementite. Nevertheless, molybdenum seems to play an essential role as well, as stated by Per Siegbahn in the following discussion.

Joan Broderick from Montana State reported exciting results about the mechanism of radical initiation in radical S-adenosyl-L-methionine (SAM) enzymes. One of the first observed intermediates was the organometallic Ω form, in which C5' is directly connected to iron, reminiscent of the adenosylcobalamin cofactor in B12. This Fe–C bond is then cleaved homolytically to release the 5'-deoxyadenosyl radical 5'-dAdo•. Utilizing rapid freeze quenching and applying EPR and ENDOR, she could trap, identify and study this long hypothesized radical 5'-dAdo•, which can then attach substrates in a regio- and stereoselective manner.

Judy Hirst from Cambridge, UK, presented a talk on the use of cryo-EM to solve structures of the respiratory complex I as a basis for understanding its mechanism of catalysis. Mutations in the subunits of this mitochondrial enzyme responsible for powering ATP synthesis by oxidative phosphorylation, impact on its structure and function and may cause mitochondrial diseases.

Cathy Drennan from MIT, in collaboration with JoAnne Stubbe, gave an enthusiastic talk about how she was able to finally get more insights into the functioning of ribonucleotide reductase RNR (Class Ia) of *E. coli* by trapping the active state of the enzyme using cryo-EM. Initially thought to be a symmetrical dimer of the α- and β-subunits, the active state of RNR shows that only one of the β-entities interacts with the α-moiety at a time, suggesting an alternating waggling interaction. Both of these talks nicely showed the impressive resolution available *via* cryo-EM.

Fig. 3. Top: Michelle Chang, Doug Rees, Brian Hoffman, Judith Klinman, Judy Hurst; bottom: Joan Broderick, Cathy Drennan, Paul Walton; Lejondal Castle.

The final day of this symposium came much too quickly and was kicked off by ***Judith Klinman*** from Berkeley. In a very didactic manner, she shared her observations from deuteration experiments of lipoxygenase, mutations thereof as well as versions with extrinsically and intrinsically attached fluorescent probes. Temperature-dependent measurements revealed then a first-time observation of a correlation between pico-second heat transfer through proteins, leading to protein quakes and subsequent opportunities for hydrogen tunneling or protein rearrangement that occur at a much slower time-scale.

Last but not least, ***Paul Walton*** from York University, UK, took the stage. He is not only a gifted entertainer in his talks, but also gave an exciting story about cellulose degrading species, using copper-dependent lytic polysaccharide monooxydases (LPMOs). By using different methods such as stopped-flow spectroscopy, targeted mutagenesis, EPR, high-resolution XRF and DFT-calculations, he was able to propose the individual steps involved in this mechanism, for example the radical formation on a histidine coordinated to the copper ion and subsequent radical transfer to a nearby tyrosine, giving a Cu(II)-tyrosyl radical pair.

I can only join Serena and Akif in saying what a great and welcoming atmosphere there was. After 2.5 years of COVID-related isolation, this was for so many of us a privileged first occasion to meet again in person. And of course, to get the latest news from bioinorganic chemistry research. A very big THANK YOU goes to the three 'musketeers' who have organized this Nobel Symposium since *ca.* 2019 with all the ups and downs in between. So, cheers to Pernilla Wittung-Stafshede, Martin Högbom and Erik Johansson as well as all chairpersons for putting such an exquisite program together with plenty occasions to exchange around lunch, dinner, coffee breaks, a glass of wine or a billiards game.

For those who got hooked on the fascinating ideas presented during this Nobel Symposium (Fig. 4), you should look out for a special issue of *FEBS* later this year, in which the original papers of the speakers will be gathered.

Fig. 4: Speakers and observers as well as press representatives at the Nobel Symposium #168 on Bioinorganic Chemistry.

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