ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2018, **54**, 11945

Received 25th July 2018,
Accepted 27th September 2018

DOI: 10.1039/c8cc06040a

rsc.li/chemcomm

Low catalytic activity of the Cu(II)-binding motif (Xxx-Zzz-His; ATCUN) in reactive oxygen species production and inhibition by the Cu(I)-chelator BCS†

Alice Santoro,‡[a] Gulshan Walke, ‡§[ab] Bertrand Vileno,[ac] Prasad P. Kulkarni,[b] Laurent Raibaut[a] and Peter Faller *[a]

The catalytic redox activity of Cu(II) bound to the motif NH_2–Xxx-Zzz-His (ATCUN) with ascorbate and H_2O_2/O_2 is very low and can be stopped *via* Cu(I)-chelation. This impacts its application as an artificial Cu–enzyme to degrade biomolecules *via* production of reactive oxygen species in a Cu(I)-chelator rich environment like the cytosol.

Copper (Cu) is an essential element for most living organisms being involved in various biological functions, mainly by playing a redox catalytic role (essentially as Cu(I) and Cu(II)) in enzymes. Free or loosely bound Cu ions are very efficient catalysts in the production of reactive oxygen species (ROS), including the Fenton type reaction.[1,2] Hence, Cu-metabolism is tightly controlled and almost all Cu ions are tightly bound to proteins or peptides. Also during transport or storage, Cu is bound to proteins.[3,4] Serum albumin is implicated in the transport of Cu in the blood stream *via* Cu(II)-binding to the N-terminal sequence Asp-Ala-His (for humans). This Cu(II)-site is an example of the so called amino-terminal Cu and Ni-binding (ATCUN) motif, with the general sequence H_2N–Xxx-Zzz-His (XZH).[5–7]

Cu(II) is bound to XZH in a square planar complex coordinated by four nitrogens, the N-terminal amine, the two deprotonated N of the amide (between X-Z and Z-H), and the N(Pi) of the imidazole (Fig. 1).[8,9] The conditional dissociation constants at pH 7.4 for Cu(II) are sequence dependent but are in the range of 10^{-12} to 10^{-15} M^{-1}.[10] Thus, by adding just three amino acids (XZH) to any peptide/protein at the N-terminus, or by mutating the third amino acid to a His, a strong Cu(II)-binding site can be introduced. Indeed, this strategy has been used by several groups in different contexts for anticancer or antimicrobial applications. The Cu(II)–XZH motif was introduced to add a ROS catalytic unit playing the role of an artificial enzyme to degrade biomolecules (such as DNA, RNA, proteins or sugars).[11–14]

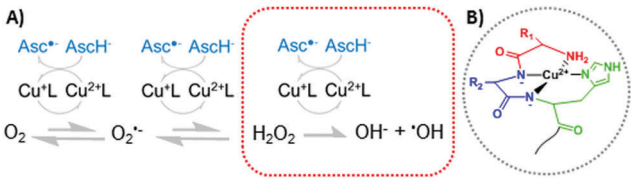

Fig. 1 (A) Mechanism for Cu catalysed ROS production in the presence of ascorbate ($AscH^-$) and dioxygen. The electron flow is from the $AscH^-$ to the oxygen species, catalyzed by Cu. Step 1: Cu undergoes redox cycling, classically between reduction of Cu(II) to Cu(I) through the oxidation of $AscH^-$ to $Asc^{\bullet-}$. Step 2: dioxygen is reduced in one electron events by Cu(I) to superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and finally hydroxyl radicals (HO•). In the presence of $AscH^-$ and H_2O_2 the reaction is mainly limited to the last part highlighted by a red frame. (B) Schematic structure of a Cu(II)–XZH complex. In the present study their activity as catalysts for ROS production is studied.

The cleavage of biomolecules catalyzed by Cu is a redox dependent mechanism (Fig. 1) involving Cu(I) and Cu(II) (Cu(III) was also suggested[15]), and needs a reducing agent (like ascorbate ($AscH^-$)) and an oxidant (mostly O_2 or H_2O_2).[12]

However, the same motif XZH was also used in chelation therapy based on the redox silencing of Cu.[8,16] For instance peptides/proteins with an XZH motif have been used to suppress the ROS production by Cu-amyloid-β in the presence of $AscH^-$ and O_2.[17] This is based on the retrieval of Cu from amyloid-β and strong stabilization of Cu(II) bound to XZH. Also, the motif was used for ^{64}Cu imaging, with the idea of a redox-inert Cu(II)–XZH complex.[18]

Hence, there seems to be a discrepancy. On the one hand Cu(II)–XZH is used to produce ROS, for which an efficient redox

[a] *Institut de Chimie, UMR 7177, CNRS-Université de Strasbourg, 4 rue Blaise Pascal, 67000, Strasbourg, France. E-mail: pfaller@unistra.fr*
[b] *Bioprospecting Group, Agharkar Research Institute, Savitribai Phule Pune University, Pune-411004, India*
[c] *French EPR Federation of Research (REseau NAtional de Rpe interDisciplinaire (RENARD), Fédération IR-RPE CNRS #3443), Strasbourg, France*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8cc06040a

‡ Contributed equally to the work.

§ Present address: Department of Chemistry, Faculty of Exact Science, Bar Ilan University, Ramat Gan, 5290002, Israel.

cycling of Cu is warranted, on the other hand, the XZH motif is used to redox silence Cu, based on an arrest of its redox cycling once Cu is bound to XZH. In order to gain insight into this discrepancy, we studied three common variants of XZH, *i.e.* DAHK (the motif from serum albumin[19]), KGHK (one of the most used and most efficient to perform cleavage of biomolecules[11]) and FRHD (the motif found in a truncated variant of amyloid-β with a redox-silencing activity[20]). The redox activity and ROS production of these Cu(II)–XZH complexes were evaluated under the most classical conditions, *i.e.* with O_2, $AscH^-$ and/or H_2O_2. Even under the most favorable conditions, the ROS production is very low.

First, the HO• production catalysed by the three Cu(II)–XZH complexes was measured (for complex formation see Fig. S1, ESI†). The HO• production was monitored by following the kinetics of fluorescence of 7-HO-CCA (7-hydroxycoumarin-3-carboxylic acid). Coumarin-3-carboxylic acid (CCA) reacts with HO• to produce 7-HO-CCA. Fig. 2A shows the kinetics of 7-HO-CCA fluorescence

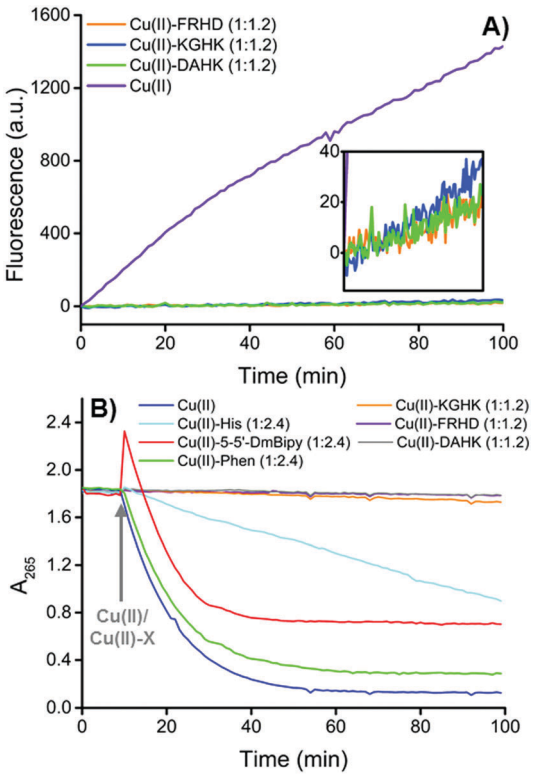

Fig. 2 (A) Time course of HO• production: evolution of fluorescence of the HO• adduct of CCA (HO–CCA; excitation: λ = 390 nm; emission λ = 450 nm) as a function of time in the presence of $AscH^-$ and H_2O_2. Cu(II)–XZH (H–DAHK–OH, H–KGHK–OH, H–FRHD–NH_2) complexes were pre-formed at the desired ratio (Cu(II) : peptide, 1 : 1.2 to avoid the presence of free Cu in phosphate buffer (50 mM, pH 7.4)). The final concentrations of Cu(II), peptide/ligands, $AscH^-$, H_2O_2 and CCA used were 25 μM, 30 μM, 250 μM, 250 μM and 0.5 mM respectively in 50 mM phosphate buffer, pH 7.4. (B) Time course of $AscH^-$ oxidation: evolution of the $AscH^-$ absorption (λ_{max} = 265 nm) as a function of time after exposure to free Cu(II), Cu(II)–XZH, Cu–His_2, Cu(II)–5,5′-$DmBipy_2$, and Cu(II)–$Phen_2$ complexes with H_2O_2. $AscH^-$ oxidation was started by the addiction of free Cu(II) or the preformed Cu(II)–X complexes after 10 min. The final concentrations of Cu(II), peptide/ligands, $AscH^-$ and H_2O_2 were respectively 10 μM, 12 μM/24 μM, 100 μM and 100 μM in 50 mM phosphate buffer, pH 7.4.

for Cu(II), Cu(II)–DAHK, Cu(II)–KGHK and Cu(II)–FRHD with 250 μM $AscH^-$ and H_2O_2. The measurements with $AscH^-$ or H_2O_2 only are shown in Fig. S2 (ESI†). Several observations can be made. (i) Cu in the buffer is much more efficient in HO• generation than Cu(II)–XZH complexes (by about 2 orders of magnitude), (ii) in line with the literature the HO• production is more efficient with H_2O_2 and $AscH^-$, about twice as fast as with $AscH^-$ alone. H_2O_2 alone produced HO• very slowly, at least an order of magnitude slower than $AscH^-$ alone (Fig. S2, ESI†). Note, the concentrations of hundred μM used here are physiologically relevant for $AscH^-$,[21] but H_2O_2 is normally much lower.[22] (iii) Although Cu bound to the XZH peptides showed very little activity, in all repetitions of the experiment Cu(II)–KGHK was slightly more active than Cu(II)–DAHK and Cu(II)–FRHD. However, this difference was around the statistical error, and hence is just a tendency.

We confirmed the low HO• production of Cu(II)–KGHK compared with Cu in the buffer at different ratios of Cu(II) : peptide (1 : 1, 1 : 1.2, 1 : 2, 1 : 3), by EPR through a spin-trapping investigation in the presence of $AscH^-$ and H_2O_2 (Fig. S3, ESI†).

The measurement of HO• production *via* CCA or POBN (α-(4-pyridyl *N*-oxide)-*N*-*tert*-butylnitrone) used to evaluate the redox activity of Cu(II)–XZH complexes is indirect since it needs the trapping of HO•. HO• can also be trapped by other molecules in the solution (*e.g.* peptides), thus the measurement is not quantitative. Hence, we also measured the consumption of the substrate, *i.e.* $AscH^-$, *via* absorbance spectroscopy at λ_{max} = 265 nm.

Indeed, Fig. 2B and Fig. S4 (ESI†) show that Cu in the buffer oxidizes $AscH^-$ rapidly (with or without H_2O_2) with an initial rate of 12.3 ± 1.9 μM min^{-1} under the given conditions (Table 1). In contrast Cu bound to the XZH peptides almost completely blocked the $AscH^-$ oxidation, with rates of 0.08–0.11 μM min^{-1} (Table 1). These rates are similar to the background of $AscH^-$ oxidation (in which no Cu and peptide are present). This parallels the HO• trapping experiments shown above. Moreover, Cu(II)–KGHK was slightly more active than DAHK/FRHD. The $AscH^-$ oxidation rate of Cu(II)–KGHK at a 1 : 1.2 ratio was the same in phosphate buffer and HEPES buffer (Fig. S9 and S10, ESI†), although Cu alone showed higher activity in HEPES than phosphate (18.9 μM min^{-1}).

For comparison, we also selected well known redox active Cu(II)-complexes, *i.e.* Cu(II)$DmBipy_2$ (5,5′-DmBipy: 5,5′-dimethyl-2,2′-dipyridyl), and Cu(II)$Phen_2$ (Phen: 1,10-phenantroline) that

Table 1 Molar $AscH^-$ oxidation rate (μM min^{-1}), calculated for the different Cu(II)-complexes tested in this work (Fig. 2B)

Cu(II)-complex	r_{obs}[a] (μM min^{-1})
Background	0.11 ± 0.06
Cu(II)	12.3 ± 1.9
Cu(II)–KGHK (1 : 1.2)	0.11 ± 0.01
Cu(II)–FRHD (1 : 1.2)	0.06 ± 0.02
Cu(II)–DAHK (1 : 1.2)	0.08 ± 0.03
Cu(II)–His (1 : 2.4)	0.89 ± 0.02
Cu(II)–5,5′-DmBipy (1 : 2.4)	6.16 ± 1.07
Cu(II)–Phen (1 : 2.4)	8.29 ± 0.61

[a] Measurements were performed in triplicate with different solutions at different days and the average values of r_{obs} (μM min^{-1}) with standard deviations are reported.

have been used to produce ROS *via* a Cu dependent mechanism (Fig. 2B).[23,24] Both Cu(II)–5,5′-DmBipy$_2$ and Cu(II)–Phen$_2$ were about as active as free Cu(II) in AscH$^-$ oxidation. Also Cu(II)–His$_2$ was more active than Cu(II)–XZH. All three complexes were one or two orders of magnitude faster than Cu(II)–XZH (Table 1). If one subtracts the background of AscH$^-$ oxidation the difference is even larger. Hence comparison with these Cu(II)-complexes confirms that Cu(II)–XZH complexes are very slow catalysts for ROS production, even under the most favorable conditions. Even in the most active case, *i.e.* Cu(II)–KGHK in the presence of AscH$^-$ and H_2O_2, less than 7 μM AscH$^-$ was consumed over 1 h (Fig. 2B). This corresponds to a maximal turnover rate of about 0.7 per hour with 100 μM AscH$^-$ and H_2O_2. Subtracting the background AscH$^-$ oxidation yields even lower activity.

This very low reactivity was also confirmed by measuring the effect of AscH$^-$ or H_2O_2 on the Cu(II) d–d bands of Cu(II)–KGHK, Cu(II)–DAHK and Cu(II)–FRHD. In the case of a strong reactivity with the substrate one would expect the disappearance of the d–d bands, either due to reduction to Cu(I) by AscH$^-$ or oxidation to Cu(III) by H_2O_2. But even at a 100 times excess, no significant changes in the d–d bands typical for Cu(II) could be observed (V Fig. S5 and S6, ESI†).

Next, we investigated the redox state(s) of Cu that is/are involved in the slow catalytic reaction of Cu(II)–XZH with AscH$^-$ and/or H_2O_2. In the presence of AscH$^-$, it is generally assumed that the ROS production by Cu(II)–XZH complexes takes place *via* a redox cycling between Cu(II) and Cu(I), as AscH$^-$ is a reducing agent. It is known from electrochemical studies that Cu(II)–XZH is very difficult to reduce and that Cu(I) does not bind to the XZH motif, as soft Cu(I) is not acidic enough to deprotonate amides and it prefers a tetrahedral and not a square planar coordination geometry.[10] Hence, we hypothesized that if the HO$^\bullet$ production of the Cu(II)–XZH complexes passes *via* Cu(I), this Cu(I) is not strongly bound and could be retrieved from the peptide. To address this, we used bathocuproinedisulfonate (BCS), a well-known Cu(I)–ligand/chromophore (Fig. 3). Adding BCS to Cu(II)–KGHK, Cu(II)–DAHK or Cu(II)–FRHD did lead to a very small increase of less than 0.003 corresponding to the typical Cu(I)–BCS$_2$ complex (λ_{max} = 483 nm) (left inset Fig. 3). This corresponds to 0.2 μM Cu(I)–BCS$_2$ formed after 1 h. However, upon AscH$^-$ addition the band at 483 nm increased steadily due to the Cu(I)-binding to BCS. This indicates that the formation of Cu(I) is directly linked to HO$^\bullet$ production. Analogous experiments were conducted with H_2O_2 alone. As H_2O_2 is an oxidant, we were surprised, that Cu(I)–BCS$_2$ was also formed and clearly more than the background (right inset Fig. 3), at least for Cu(II)–KGHK. Again, the amount of Cu(I)–BCS$_2$ formed paralleled the HO$^\bullet$ production efficiency. This indicates that also in the case of H_2O_2 only, a strong oxidant, the HO$^\bullet$ production takes place *via* Cu(I) and not Cu(III). Cu(III) could have been expected, because the square planar coordination in XZH (Fig. 1) is well adapted for Cu(III), but not for Cu(I).[1,2,10] However, this seems not to be the case as the BCS test shows the formation of Cu(I)–BCS$_2$. An explanation, in line with the very slow kinetics, is that H_2O_2 is reducing Cu(II)–XZH, as it is known for Cu in the buffer.[25] Moreover, it is

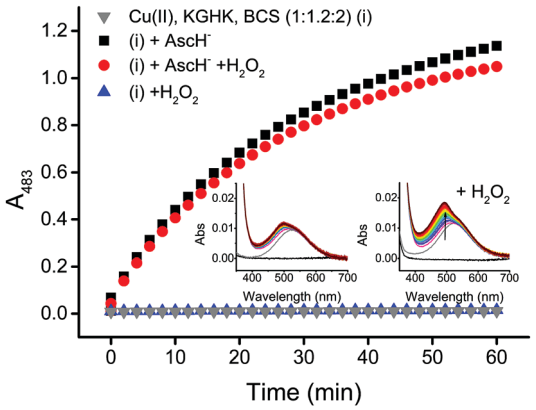

Fig. 3 Kinetics representing the tendency of formation of free or loosely bound Cu(I) from Cu(II)–KGHK in the presence of (i) AscH$^-$ (black squares profile), (ii) AscH$^-$ and $H_2O_2^-$ (red circles profile), (iii) H_2O_2 (blue triangle profile), and (iv) blank, *i.e.* no AscH$^-$ and H_2O_2 (inversed grey triangle) using BCS as a Cu(I) chelator (λ_{max} [Cu(I)–BCS$_2$] = 483 nm). Inset: Corresponding UV-vis spectra for (iv) (left) and (iii) (right) conditions: concentrations of Cu(II), peptide, AscH$^-$, H_2O_2 and BCS were respectively 100 μM, 120 μM, 10 mM, 10 mM and 200 μM; 50 mM phosphate buffer, pH 7.4.

important to note that BCS can complex the formed Cu(I), indicating that Cu(I) is not strongly bound and accessible for a ligand.

To confirm the relation of Cu(I) formation to the HO$^\bullet$ production, we measured HO$^\bullet$ in the presence of BCS. If the formed Cu(I) is the key species for the HO$^\bullet$ formation, in the presence of BCS no HO$^\bullet$ should be produced, because it is known that Cu(I)–BCS$_2$ is very redox inert and hence does not react with oxygen under aerobic conditions. As BCS could interfere with CCA fluorescence because of its absorption at the same spot we used EPR Spectroscopy. Fig. 4 shows the HO$^\bullet$ production by Cu(II)–KGHK measured using POBN as a spin trap. After 4 hours of incubation using AscH$^-$ and H_2O_2, a signal originating from trapped HO$^\bullet$ could be detected. However, in the presence of BCS, after 4 h no signal was detected. This supports the mechanism that indeed when the formed Cu(I) from Cu(II)–XZH is chelated by BCS no HO$^\bullet$ is detected anymore.

In conclusion, our data indicate that the ROS production of Cu(II)–XZH is very slow, with KGHK being tentatively the most active peptide, and that in the presence of both AscH$^-$ and H_2O_2 it is less than 0.7 turnover per hour under the present conditions. Other well known Cu(II)-complexes (Cu(II)–DmBipy$_2$/Cu(II)–Phen$_2$) or free Cu had two orders of magnitude higher initial turn-over rates. Moreover, the test with the Cu(I) specific chelator BCS suggests that Cu(I) is involved in the mechanism, indicating that the redox couple Cu(I)/Cu(II) is predominant and not Cu(II)/Cu(III). This is also supported by the much lower HO$^\bullet$ production activity in the presence of H_2O_2 alone. If Cu(III) would easily be reached, a fast reaction of Cu(II) + H_2O_2 → Cu(III) + HO$^\bullet$ could occur (at least one turnover). This is not observed. This is also in line with the redox potential of around 1 V (NHE) for Cu(II)–XZH (H_2O_2/HO$^\bullet$ with 0.32 V).

Overall this indicates that the cleavage of biomolecules by Cu(II)–XZH with AscH$^-$ and H_2O_2/O_2 is catalytically not very

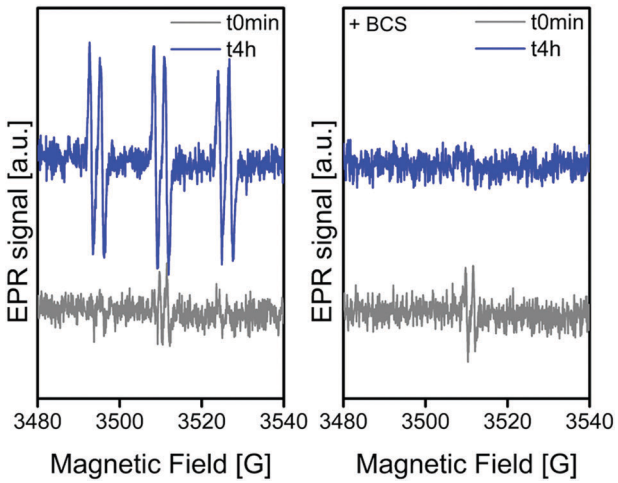

Fig. 4 Indirect evidence of HO• production by Cu(II)–KGHK measured by EPR spin trapping with POBN in the presence (right panel) and absence (left panel) of BCS. A POBN–CH_3 spin adduct (g = 2.0056, A_H = 2.7 G, A_N = 16 G) was observed after 4 h of mixing Cu(II)–KGHK (at 1 : 1.2 ratio) with $AscH^-$ and H_2O_2. It results from the reaction between the spin trap and a carbon centred radical originating from the decomposition of EtOH with HO•. EtOH was added here as an efficient hydroxyl scavenger (5% v/v) to enhance the detection threshold and thus the EPR S/N. The two lines observed at t = 0 are ascribed to the ascorbyl radical. Experimental conditions: KGHK 120 μM, Cu(II) 100 μM (1.2 : 1), $AscH^-$ 1 mM, H_2O_2 1 mM, PB 100 mM, pH 7.4, POBN 50 mM, ETOH 5%, ±BCS 300 μM.

efficient, but is possible. However, a real limit for applications could be the fact that a Cu(I) chelator (here BCS) would be able to retrieve Cu(I) during the redox cycle and abolish such ROS production. This is in line with the fact that the XZH motif is not adapted for Cu(I)-binding. In the case of Cu(II)–DAHK, no electrochemical reduction of Cu(II) to Cu(I) was observed.[8] As in a cell strong and abundant Cu(I) chelators are present (such as glutathione[26] and metallothionein[27,28]), this could hence totally supress the ROS production catalysed by the Cu(II)–XZH complexes. Indeed, a recent study showed that glutathione at cytosolic and nuclear relevant concentrations is able to reduce Cu(II) bound to FRHD and retrieve it.[29] Thus, considering the slow rate of ROS production by Cu(II)–XZH and the reductive Cu-dissociation by physiologically relevant reducing agents, it seems very difficult to use Cu(II)–XZH efficiently in catalysis for targets such as DNA or proteins in the cytosol or nucleus.

Concerning the other applications, where a redox inertness of Cu(II) is warranted, such as the redox silencing of loosely bound Cu or for imaging, the XZH motif is quite efficient in keeping Cu(II) redox stable, but not completely, as a small activity in ROS production remains. Hence, XZH peptides were able to suppress efficiently, but not completely, the ROS production of Cu-peptides related to neurodegenerative diseases.[30] As shown above, the ROS production activity of XZH might be sequence dependent, but maximal in a modest way (Fig. 2). Nevertheless, there might be space to further improve the redox inertness of Cu(II)–XZH by changing X and Z and the amino acids after the His at position 3.

A. S. and G. W were supported by the IDEX PhD program (University of Strasbourg) and an EMBO short-term fellowship (ASTF 505-2016), respectively. The Frontier Research in Chemistry Foundation of Strasbourg (P. F.), the University of Strasbourg Institute for Advanced Study (USIAS) (P. F.), IDEX attractivity program of the University of Strasbourg (L. R.) and the Reseau NAtional de Rpe interDisciplinaire (RENARD, Fédération IR-RPE CNRS #3443) are acknowledged. P. F. would like to thank Dr C. Hureau (LCC, Toulouse) for helpful discussions. P. K. would like to acknowledge funds from the Department of Science and Technology, India (EMR/2014/001235). We would like to thank Dr Romain Ruppert (UMR7177 – Institut de Chimie, Strasbourg) for the gift of the Phen and DmBipy compound.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 B.-E. Kim, T. Nevitt and D. J. Thiele, *Nat. Chem. Biol.*, 2008, **4**, 176.
2 P. Delangle and E. Mintz, *Dalton Trans.*, 2012, **41**, 6359.
3 J. H. Kaplan and E. B. Maryon, *Biophys. J.*, 2016, **110**, 7.
4 H. Öhrvik, J. Aaseth and N. Horn, *Metallomics*, 2017, **9**, 1204.
5 C. Harford and B. Sarkar, *Acc. Chem. Res.*, 1997, **30**, 123.
6 R. Sankararamakrishnan, S. Verma and S. Kumar, *Proteins*, 2005, **58**, 211.
7 W. Bal, M. Sokołowska, E. Kurowska and P. Faller, *Biochim. Biophys. Acta, Gen. Subj.*, 2013, **1830**, 5444.
8 C. Hureau, H. Eury, R. Guillot, C. Bijani, S. Sayen, P.-L. Solari, E. Guillon, P. Faller and P. Dorlet, *Chem. – Eur. J.*, 2011, **17**, 10151.
9 H. Kozłowski, W. Bal, M. Dyba and T. Kowalik-Jankowska, *Coord. Chem. Rev.*, 1999, **184**, 319.
10 P. Gonzalez, K. Bossak, E. Stefaniak, C. Hureau, L. Raibaut, W. Bal and P. Faller, *Chem. – Eur. J.*, 2018, **24**, 8029.
11 Y. Jin and J. A. Cowan, *J. Am. Chem. Soc.*, 2005, **127**, 8408.
12 Z. Yu and J. A. Cowan, *Chem. – Eur. J.*, 2017, **23**, 14113.
13 M. D. J. Libardo, V. Y. Gorbatyuk and A. M. Angeles-Boza, *ACS Infect. Dis.*, 2016, **2**, 71.
14 C. M. Agbale, M. H. Cardoso, I. K. Galyuon and O. L. Franco, *Metallomics*, 2016, **8**, 1159.
15 A. N. Pham, G. Xing, C. J. Miller and T. D. Waite, *J. Catal.*, 2013, **301**, 54.
16 D. Bar-Or, L. T. Rael, E. P. Lau, N. K. R. Rao, G. W. Thomas, J. V. Winkler, R. L. Yukl, R. G. Kingston and C. G. Curtis, *Biochem. Biophys. Res. Commun.*, 2001, **284**, 856.
17 L. Perrone, E. Mothes, M. Vignes, A. Mockel, C. Figueroa, M.-C. Miquel, M.-L. Maddelein and P. Faller, *ChemBioChem*, 2010, **11**, 110.
18 T. Miyamoto, Y. Fukino, S. Kamino, M. Ueda and S. Enomoto, *Dalton Trans.*, 2016, **45**, 9436.
19 J. P. Laussac and B. Sarkar, *Biochemistry*, 1984, **23**, 2832.
20 M. Mital, N. E. Wezynfeld, T. Frączyk, M. Z. Wiloch, U. E. Wawrzyniak, A. Bonna, C. Tumpach, K. J. Barnham, C. L. Haigh, W. Bal and S. C. Drew, *Angew. Chem., Int. Ed.*, 2015, **54**, 10460.
21 M. E. Rice, *Trends Neurosci.*, 2000, **23**, 209.
22 B. Chance, H. Sies and A. Boveris, *Physiol. Rev.*, 1979, **59**, 527.
23 P. Deschamps, P. P. Kulkarni, M. Gautam-Basak and B. Sarkar, *Coord. Chem. Rev.*, 2005, **249**, 895.
24 M. Pitié and G. Pratviel, *Chem. Rev.*, 2010, **110**, 1018.
25 J. F. Perez-Benito, *J. Inorg. Biochem.*, 2004, **98**, 430.
26 M. T. Morgan, L. A. H. Nguyen, H. L. Hancock and C. J. Fahrni, *J. Biol. Chem.*, 2017, **292**, 21558.
27 G. Meloni, P. Faller and M. Vašák, *J. Biol. Chem.*, 2007, **282**, 16068.
28 E. Atrián-Blasco, A. Santoro, D. L. Pountney, G. Meloni, C. Hureau and P. Faller, *Chem. Soc. Rev.*, 2017, **46**, 7683.
29 A. Santoro, N. Wezynfeld, M. Vasak, W. Bal and P. Faller, *Chem. Commun.*, 2017, **53**, 11634.
30 E. Atrián-Blasco, P. Gonzalez, A. Santoro, B. Alies, P. Faller and C. Hureau, *Coord. Chem. Rev.*, 2018, **371**, 38.

