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A Photoactive Carbon-Monoxide-Releasing Protein Cage for Dose-Regulated Delivery in Living Cells

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Abstract: *Protein cages can serve as bioinorganic molecular templates for functionalizing metal compounds to regulate cellular signaling. We succeeded in developing a photoactive CO-releasing system by constructing a composite of ferritin (Fr) containing manganese–carbonyl complexes. When Arg52 adjacent to Cys48 of Fr is replaced with Cys, the Fr mutant stabilizes the retention of 48 Mn–carbonyl moieties, which can release the CO ligands under light irradiation, although wild-type Fr retains very few Mn moieties. The amount of released CO is regulated by the extent of irradiation. This could reveal an optimized dose for cooperatively activating the nuclear factor κB (NF-κB) in mammalian cells and the tumor necrosis factor α (TNF-α). These results suggest that construction of a CO-releasing protein cage will advance of research in CO biology.*

Self-assembled protein architectures have received much attention and are an important bio-nanomaterial.[1–3] Assemblies, such as cages, tubes, and frameworks show great potential for use as molecular scaffolds and for development of catalysts, optical materials, and magnetic materials when metal complexes or metal ions are accumulated in the unique environments by metal coordination and chemical modification of amino acids.[4–7] These composites, which have large numbers of metal compounds appropriately aligned on or within the scaffolds, show a high reactivity when externally stimulated, for example, by light irradiation.[8–10] Protein assemblies containing metal compounds are biocompatible and have been recently used for both in vitro and in vivo applications.[11–13] Metal-based drugs, imaging reagents, and metal nanoparticles can be encapsulated within protein assemblies, and delivered into living cells since the proteins retain their coordination structures within the in vivo environment.[13–15] Although several stimulus-responsive composites have been investigated in biological applications at the nanoscale, there have been few reports of functionalizing the composites in living cells.[16,17]

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Delivery of carbon monoxide (CO) into living cells represents a challenging task[18] and could possibly be achieved by applying protein assemblies involving metal complexes. Metal carbonyl complexes have been used as carbon-monoxide-releasing molecules (CORMs) for both in vitro and in vivo delivery of CO for the purpose of investigating the function of CO as an intracellular signaling molecule.[19–21] $[Ru(CO)_3Cl_2]_2$ (CORM-2) and $Ru(CO)_3Cl$(glycinate) (CORM-3), which are commonly used CORMs, provide cytoprotective and vasoactive functions in living cells.[18–21] Since CO is spontaneously released from the Ru–carbonyl complexes as a result of ligand exchange reactions with nucleophilic molecules in the cell,[22] it is difficult to determine the details of the mechanisms of CO-induced cellular functions. To address this issue, stimulus-responsive CORMs have been developed as photo-,[23,24] enzyme-,[25] and pH-active[26] compounds. Photoactive CORMs are expected to have a potential to control the timing of release and the dose of released CO by an external light stimulus although they are only applicable to accessible surfaces of living cells and model animals.[23,27] Since Motterlini reported $Mn_2(CO)_{10}$ (CORM-1) as a first photoactive CORM,[28] several research groups have reported on the development of improved ligands that enable Mn, Fe, Ru, and Re–carbonyl complexes to be used as photoactive CORMs.[24,29] Several carrier systems, such as nanoparticles,[30,31] peptides,[32–34] and dendrimers,[35] have also been constructed to improve the stability of CORMs in aqueous solution. However, there have been few reports on the effects of CO released into cells and tissues by light irradiation of the photoactive CORMs.[36–38] To adapt the reactivities of photoactive CORMs to cellular environments, it is essential to design scaffolds with improved biocompatibility such as proteins because several proteins have great potential to deliver CORMs.[39–43]

In this work, we developed a photoactive CO-releasing protein based on ferritin (Fr), an iron storage protein. Fr has a cage structure with an outer diameter of 12 nm formed by 24 self-assembling polypeptide subunits (Figure 1a and b).[44] The cage has been used to accumulate metal complexes and metal nanoparticles to demonstrate their catalytic reactions.[45–48] Several Fr composites can be functionalized in living cells because the receptor-mediated endocytic uptake process permits cellular internalization of the Fr composites.[49] We synthesized a composite of Fr with Mn–carbonyl complexes by designing a Fr mutant to stabilize the photoactive Mn moieties in the cage. A crystal structure of the composite shows the importance of cysteinyl ligation to retain the coordination structures of the photoactive Mn moieties. The dose control of CO release from the composite was

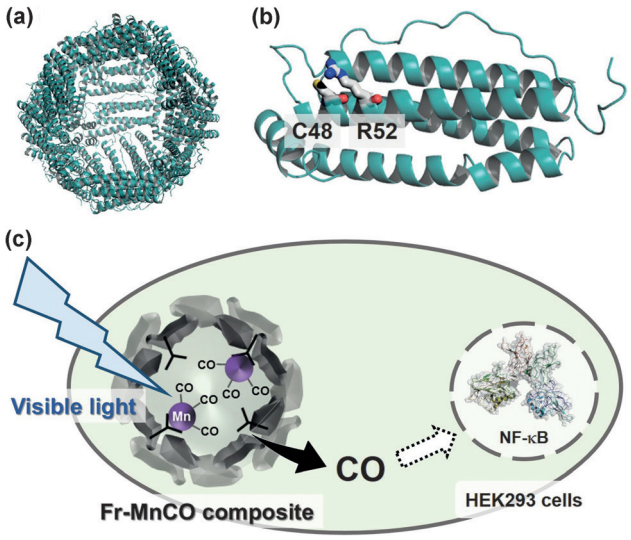

Figure 1. An intracellular photoactive CO-releasing system based on ferritin (Fr). a) Crystal structure of Fr for the whole structure with 24-mer polypeptides (PDB code; 1DAT), b) and the monomer structure in which the two residues at positions 48 (Cys) and 52 (Arg) are represented as stick models. c) The release of CO from MnCO coordinated within the Fr cage is triggered by visible-light irradiation.

achieved by changing the length of time that the composite is irradiated with visible light. The CO released from the composite activates nuclear factor κB (NF-κB), a transcriptional factor in HEK293 cells with dose-dependency associated with the irradiation period (Figure 1c). NF-κB, which is a key transcriptional regulator of a variety of genes including pro-inflammatory and anti-apoptoticgenes, is considered one of the therapeutic targets of CO.[50] Although it was reported that the NF-κB activity is cooperatively modulated by cytokines and CO,[51,52] the effect of exogenously delivered CO has not been elucidated in detail using previously employed photoactive CORMs. Thus, the photoactivatable protein cage system for CO release has the potential to provide photoactive control of cellular signal transduction by CO.

To design coordination sites of Mn–carbonyl (MnCO) moieties in the Fr cage, we employed the mutant apo-R52C-recombinant L-chain apo-ferritin from horse liver (apo-R52C-rHLFr). The mutant has two cysteine residues at positions 48 and 52 (C48, R52C), which are located close to each other on the accumulation center (Figure 1b).[41] The residues are expected to coordinate to a MnCO moiety with S, S-bidentate ligation as reported for a photoactive Mn CORM.[53] A composite of apo-R52C-rHLFr with photoactive MnCO moieties (MnCO·apo-R52C-rHLFr **1**) was prepared by adding a MeOH solution of $Mn(CO)_5Br$ (50 equiv) to a buffer solution of apo-R52C-rHLFr (50 mM Tris/HCl (pH 8.0), 0.15 M NaCl). The number of Mn atoms in the Fr cage (44 ± 2) was determined by inductively coupled plasma mass spectrometry (ICP-MS) and a bicinchoninic acid (BCA) assay. Compound **1** has an absorption band at 365 nm which is assigned as the metal-to-ligand charge transfer (MLCT) band of the photoactive MnCO moiety as reported previously (see Figure S1 in the Supporting Information).[54] The attenuated total reflectance infrared (ATR-IR) spectrum of **1** includes three peaks at 2028, 2011, and 1917 cm^{-1} in the CO-stretching vibrational region (Figure S2a, black line). The peaks were assigned to a *fac*-$Mn(CO)_3$ structure. Although we attempted to prepare a composite of wild-type Fr (apo-WT-rHLFr) and $Mn(CO)_5Br$ under the same conditions, very few Mn atoms were accumulated in the cage (3 ± 1 Mn/apo-WT-rHLFr). ATR-IR spectroscopy confirmed that no CO ligands exist in the composite of apo-WT-rHLFr and $Mn(CO)_5Br$ (Figure S2a, blue line).[53] Thus, apo-R52C-rHLFr was deemed to be more appropriate for accumulating the MnCO moieties than apo-WT-rHLFr.

To confirm the binding site of the Mn atoms of **1**, the X-ray crystal structure was refined to 1.42 Å resolution (Figure 2, and Table S1). Anomalous peaks of two Mn atoms (Mn1 and Mn2) were observed only at the accumulation center in the monomer of **1** (Figure 2b). Although a total of 48 Mn binding sites were identified in the whole structure, the occupancies of Mn1 and Mn2 were 0.80 and 0.60, respectively. Since the total number of Mn estimated from ICP-MS and the BCA assay was 44 ± 2, the binding sites seem to be partially unoccupied. Mn1 is bound to the Sγ of Cys48 and the Sγ of Cys52 with bond distances of 2.09 and 2.20 Å, respectively (Figure 2c). The distances of Mn–S bonds are within the typical range of MnCO complexes.[55] The S-Mn-S angle is 155.73°, which is larger than the typical values (78.4–82.6°)[53] because of the restricted positions of Cys48 and Cys52. Mn2 is bound to a water molecule (W1) with a bond distance of 2.01 Å. This coordination is stabilized by a hydrogen bond between W1 and Oε of Asp38 (2.62 Å) and a weak interaction with Sγ of Cys48 with a distance of 2.82 Å (Figure 2c). Although there is no electron density

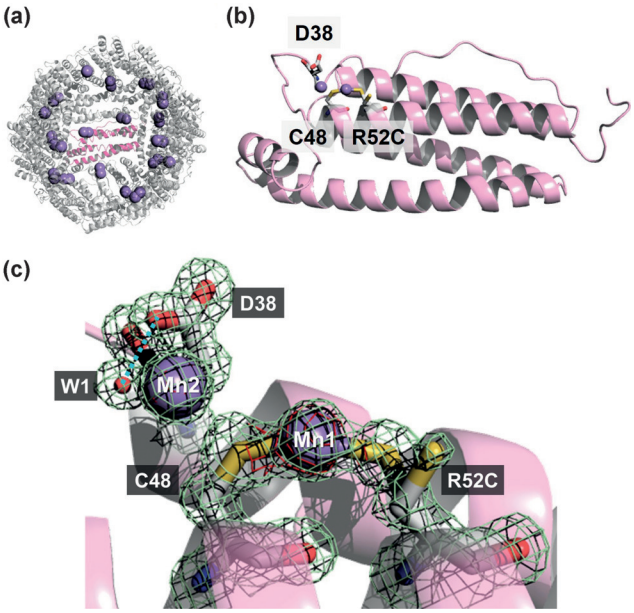

Figure 2. X-ray crystal structure of MnCO·apo-R52C-rHLFr (**1**). a) The whole structure consisting of 24 protein monomers and b) the monomer structure with Mn binding sites. c) The Mn coordination structures at the accumulation center. The Mn and the O atoms of water molecules are shown as purple and red spheres, respectively. The hydrogen bond is shown as a blue dotted line (D38-W1). Anomalous difference Fourier maps at 4.0 σ indicate the positions of Mn atoms (red). The $2|F_o| - |F_c|$ electron density maps at 1.0 σ are shown in green.

assignable to CO ligands in the crystal structure, as described above, three peaks arising from the CO-stretching frequencies of **1** indicate the presence of CO ligands with the *fac*-$Mn(CO)_3$ coordination structure in **1** (Figure S2a). Moreover, the CO coordination was confirmed by ATR-IR spectroscopy of **1** before and after crystallization in a precipitation buffer in the dark (Figure S2b). The dark condition had been kept until starting the data collection at a synchrotron facility. Although we compared electron densities of the Mn-binding sites by processing with the initial 30 data sets and the last 30 data sets, we could not find the densities of the CO ligands, and there was no difference in the Mn coordination structures between the initial and the last data sets (Figure S3). Thus, we conclude that there was no X-ray damage leading to CO loss during the data collection. These results imply that each monomer in the mutant cage is coordinated to MnCO moieties in different orientations. Thus, the electron densities of the CO ligands were not observed as unambiguous densities in an averaged structure of the monomers of the cage.

The time course of photoactivated CO release was determined using a myoglobin assay and the data correction method with consideration for the CORM unevenly absorbing at the Q-bands in both deoxy-Mb and Mb-CO (Figures 3 and S4).[28,56] A phosphate-buffered saline (PBS) solution (10 mM, pH 7.4) containing deoxy-Mb (24 μM) with dithionite (10 mM) under Ar atmosphere was used for the assay as previously reported.[41] The PBS solution containing deoxy-Mb was irradiated with visible light at 456 nm (15 $mW\,cm^{-2}$) for defined periods after addition of **1** (final concentration of [Mn] = 6 μM, in 10 mM PBS buffer of pH 7.4). The absorption spectrum of the deoxy-Mb solution clearly changes to that of carbonmonoxy-Mb (MbCO) with light irradiation for 1–20 minutes (Figure S3). The amount of CO released per Mn atom was calculated from the amount of MbCO produced according to the previous report.[57] When the solution was irradiated with visible light for 1, 5, and 10 minutes, 0.23 ± 0.03, 0.58 ± 0.08, and 0.70 ± 0.08 equivalents, respectively, of CO per Mn were captured by deoxy-Mb. The amount of CO released without light irradiation was very low (Figure 3a). The half-life ($t_{1/2}$) for CO release from **1** under light exposure was 2.5 ± 0.2 minutes (Figure 3b). The rapid response of **1** to the light irradiation shows a $t_{1/2}$ value expected for *fac*-$Mn(CO)_3$ complexes.[24] The value of $t_{1/2}$ is smaller than that of CORM-1 ($t_{1/2} = 11.4 \pm 0.8$ minutes; Figure S5). The amounts of CO released from **1** could be modulated by light irradiation in a time-dependent manner as for CORM-1 (Figure S5).

These results suggest that the responsiveness of MnCO moieties bound in the Fr mutant cage to visible light is appropriate for use as a compound for cellular photoactivated CO release.

Intracellular CO release from **1** was confirmed by confocal microscopy with a CO probe-1 (COP-1).[57] HEK293/κB-Fluc cells containing **1** ([Mn] = 50 μM, in 0.1M sodium phosphate (pH 7.0), 0.15M NaCl) were exposed to 456 nm light for 10 minutes to release CO in the cells (Figure S6). Green fluorescence was observed which is derived from cellular COP-1 as a result of the reaction with intracellular CO from **1** (Figure S6b). Since the control experiments without the irradiation and with CORM-1 (Figure S6c and S6d, respectively) showed less green fluorescence compared with that of **1**, **1** has a higher efficiency on intracellular release of CO. The cellular uptake of Mn binding to **1** and CORM-1 was measured using ICP-MS when the 10 μM of each sample was added to HEK293/κB-Fluc cells. The uptake ratio of **1** and CORM-1 into the cells are found as 0.35% (1.76×10^{-10} μmol/cell) and 0.33% (1.64×10^{-10} μmol/cell), respectively, after incubation for 6 h. No cytotoxic effects of **1** and CORM-1 on HEK293/κB-Fluc cells after 12 h incubation were observed according to the results of a 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay (Figure S7).[41]

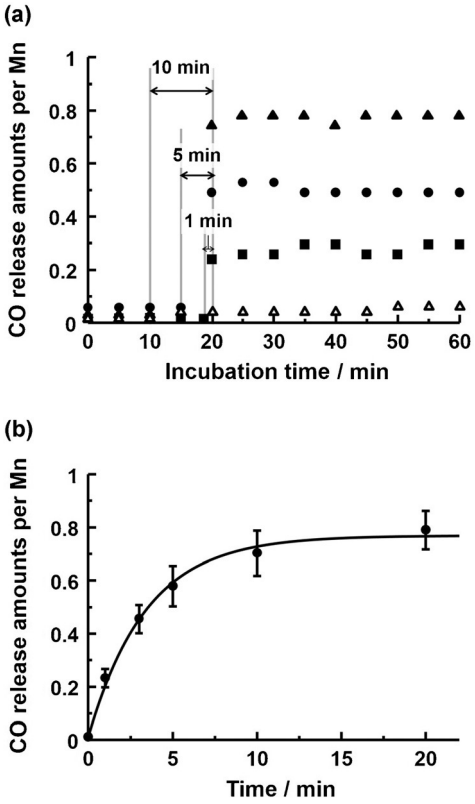

Figure 3. Amounts of released CO per Mn atom in **1** detected using the Mb assay. a) Plots of the amounts of released CO per Mn atom of **1**. The symbols represent the light irradiation time: 10 minutes (black triangle), 5 minutes (black circle), 1 minute (black square), and 0 (white triangle). The horizontal line shows the incubation time of the assay. b) Change of the time-dependent amounts of released CO per Mn atom. The horizontal line shows the time of light irradiation. The amounts of released CO were determined according to the previously reported calculation by monitoring the changes of the absorption spectrum of deoxy-Mb (24 μM, in 10 mM PBS buffer (pH 7.4)) reduced with sodium dithionite (10 mM). The deoxy-Mb solution was reacted with **1** (6 μM Mn, in 10 mM PBS buffer (pH 7.4)). After incubation for several minutes, the mixture was irradiated with 456 nm light for the indicated time period (1–20 minutes) after mixing of **1** with deoxy-Mb. The Mb solution and PBS buffer were degassed by saturation with Ar for 30 minutes prior to initiation of the assay. The mixture was incubated for 60 minutes at room temperature. Each assay was performed three times and the error bars represent the standard error of the mean (SEM).

The effects of CO released from **1** on NF-κB in HEK293/κB-Fluc cells were evaluated using a luciferase reporter assay system.[41] Several NF-κB-based assay systems have been

applied to evaluate cellular CO functions because it is believed that the nuclear factor is essential for processes against inflammation and apoptosis and for cell proliferation by CO.[25,51,52,57] The HEK293/κB-Fluc cells were pre-incubated for 1 h with **1** or CORM-1 (final concentration of [Mn] = 10 μM) before addition of tumor necrosis factor-α (TNF-α). Then, the cells were incubated for 12 h at 37 °C in 5 % CO_2. The cells were irradiated by 456 nm light at six setup time points (0.5 h before and 0.5 h, 1 h, 3 h, 6 h, and 9 h after the addition of TNF-α; Figure 4a and b). The HEK cells were irradiated with visible light for 0, 1, 5, and 10 minutes to evaluate the NF-κB activity as a function of the CO dose (Figure 4c). Activation of NF-κB was observed for the cells treated with **1** and subjected to light exposure with appropriate timing. The highest activity of NF-κB was obtained when light was irradiated 0.5 h before addition of TNF-α. In addition, as a CO molecule is released from a single Mn atom in **1** after light irradiation for 10 minutes (Figure 3), this time is required to release enough CO to activate NF-κB in living cells. The CO released from **1** cooperatively activates NF-κB with TNF-α because no effects were observed with respect to activation in the absence of TNF-α (Figure S8). These results suggest that the rapid photoactivated CO release and the stable deliver of MnCO moieties into living cells cooperatively contribute to the activation of NF-κB because CORM-1 had no effect despite of the almost identical to **1** uptake efficiency and the lager amount of released CO relative to **1** (Figures 4 and S5). The relationship between the timing of CO release and stimulation with TNF-α on NF-κB activation is being investigated in detail.

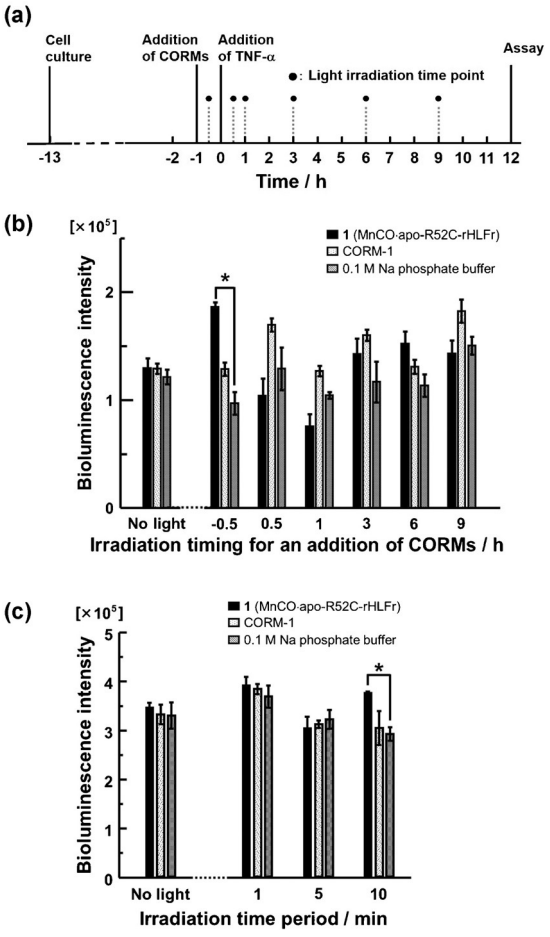

Figure 4. Bioluminescence intensity of the luciferase reporter assay for evaluating the NF-κB activity of HEK293/κB-Fluc cells. a) Experimental procedure of the assay. The cells were cultured for 12 h, then **1** and CORM-1 were added to the cells with pre-incubation for 1 h before stimulation with TNF-α (1.0 ng mL^{-1}). The cells were irradiated by 456 nm light before and after the addition of TNF-α. The black circles indicate each time point for the light irradiation of the cells. The standard point (0 h) was set when TNF-α was added. b) The bioluminescence intensities with 456 nm light irradiation for 10 min at each irradiation time. c) The bioluminescence intensities after 456 nm light irradiation for 0, 1, 5, and 10 minutes and 0.5 h before stimulation with TNF-α. *$P < 0.05$. All data are raw bioluminescence intensities derived from the cells. The experiments were performed three times and the error bars represents the SEM.

In summary, we constructed a photoactive CO-releasing protein cage based on a mutant of Fr. This represents the first example of a photoactive CORM conjugated to a protein. The cellular uptake and the light-induced CO-releasing properties of **1** cooperatively contribute to the activation of NF-κB. The results show that protein cages have great potential for use as CORM carriers. Moreover, the strategy used here can be extended to other protein assemblies with various structures and sizes. In order to clarify the roles of CO gas in living cells in detail, we are developing dose-, time-, and space-controlled CO-releasing systems using other protein assemblies for fine-tuning the cellular uptake ratio and localizing the release of CO.

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[1] F. Yu, V. M. Cangelosi, M. L. Zastrow, M. Tegoni, J. S. Plegaria, A. G. Tebo, C. S. Mocny, L. Ruckthong, H. Qayyum, V. L. Pecoraro, *Chem. Rev.* **2014**, *114*, 3495–3578.
[2] P. A. Sontz, W. J. Song, F. A. Tezcan, *Curr. Opin. Chem. Biol.* **2014**, *19*, 42–49.
[3] T. Ueno, H. Tabe, Y. Tanaka, *Chem. Asian J.* **2013**, *8*, 1646–1660.
[4] A. Fu, R. Tang, J. Hardie, M. E. Farkas, V. M. Rotello, *Bioconjugate Chem.* **2014**, *25*, 1602–1608.

[5] M. Dürrenberger, T. R. Ward, *Curr. Opin. Chem. Biol.* **2014**, *19*, 99 – 106.
[6] M. Uchida, M. T. Klem, M. Allen, P. Suci, M. Flenniken, E. Gillitzer, Z. Varpness, L. O. Liepold, M. Young, T. Douglas, *Adv. Mater.* **2007**, *19*, 1025 – 1042.
[7] B. Maity, K. Fujita, T. Ueno, *Curr. Opin. Chem. Biol.* **2015**, *25C*, 88 – 97.
[8] Z. Varpness, J. W. Peters, M. Young, T. Douglas, *Nano Lett.* **2005**, *5*, 2306 – 2309.
[9] Y. S. Nam, A. P. Magyar, D. Lee, J. W. Kim, D. S. Yun, H. Park, T. S. Pollom, Jr., D. A. Weitz, A. M. Belcher, *Nat. Nanotechnol.* **2010**, *5*, 340 – 344.
[10] N. Yokoi, Y. Miura, C. Y. Huang, N. Takatani, H. Inaba, T. Koshiyama, S. Kanamaru, F. Arisaka, Y. Watanabe, S. Kitagawa, T. Ueno, *Chem. Commun.* **2011**, *47*, 2074 – 2076.
[11] W. J. Song, F. A. Tezcan, *Science* **2014**, *346*, 1525 – 1528.
[12] Y. M. Wilson, M. Durrenberger, E. S. Nogueira, T. R. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 8928 – 8932.
[13] M. Uchida, H. Kosuge, M. Terashima, D. A. Willits, L. O. Liepold, M. V. Young, M. V. McConnell, T. Douglas, *ACS Nano* **2011**, *5*, 2493 – 2502.
[14] K. Fan, C. Cao, Y. Pan, D. Lu, D. Yang, J. Feng, L. Song, M. Liang, X. Yan, *Nat. Nanotechnol.* **2012**, *7*, 459 – 464.
[15] Z. Zhen, W. Tang, C. Guo, H. Chen, X. Lin, G. Liu, B. Fei, X. Chen, B. Xu, J. Xie, *ACS Nano* **2013**, *7*, 6988 – 6996.
[16] M. Brasch, A. de La Escosura, Y. Ma, C. Uetrecht, A. J. Heck, T. Torres, J. J. Cornelissen, *J. Am. Chem. Soc.* **2011**, *133*, 6878 – 6881.
[17] Z. Zhen, W. Tang, Y.-J. Chuang, T. Todd, W. Zhang, X. Lin, G. Niu, G. Liu, L. Wang, Z. Pan, X. Chen, J. Xie, *ACS Nano* **2014**, *8*, 6004 – 6013.
[18] S. García-Gallego, G. J. Bernardes, *Angew. Chem. Int. Ed.* **2014**, *53*, 9712 – 9721; *Angew. Chem.* **2014**, *126*, 9868 – 9877.
[19] R. Motterlini, L. E. Otterbein, *Nat. Rev. Drug Discovery* **2010**, *9*, 728 – 743.
[20] B. E. Mann, *Organometallics* **2012**, *31*, 5728 – 5735.
[21] C. C. Romão, W. A. Blattler, J. D. Seixas, G. J. Bernardes, *Chem. Soc. Rev.* **2012**, *41*, 3571 – 3583.
[22] S. McLean, B. E. Mann, R. K. Poole, *Anal. Biochem.* **2012**, *427*, 36 – 40.
[23] U. Schatzschneider, *Inorg. Chim. Acta* **2011**, *374*, 19 – 23.
[24] I. Chakraborty, S. J. Carrington, P. K. Mascharak, *Acc. Chem. Res.* **2014**, *47*, 2603 – 2611.
[25] S. Romanski, B. Kraus, U. Schatzschneider, J. M. Neudorfl, S. Amslinger, H. G. Schmalz, *Angew. Chem. Int. Ed.* **2011**, *50*, 2392 – 2396; *Angew. Chem.* **2011**, *123*, 2440 – 2444.
[26] R. Motterlini, P. Sawle, S. Bains, J. Hammad, R. Alberto, R. Foresti, C. J. Green, *FASEB J.* **2005**, *19*, 284 – 286.
[27] M. A. Gonzales, P. K. Mascharak, *J. Inorg. Biochem.* **2014**, *133*, 127 – 135.
[28] R. Motterlini, J. E. Clark, R. Foresti, P. Sarathchandra, B. E. Mann, C. J. Green, *Circ. Res.* **2002**, *90*, 17e – 24e.
[29] R. D. Rimmer, A. E. Pierri, P. C. Ford, *Coord. Chem. Rev.* **2012**, *256*, 1509 – 1519.
[30] G. Dördelmann, H. Pfeiffer, A. Birkner, U. Schatzschneider, *Inorg. Chem.* **2011**, *50*, 4362 – 4367.
[31] G. Dördelmann, T. Meinhardt, T. Sowik, A. Krueger, U. Schatzschneider, *Chem. Commun.* **2012**, *48*, 11528 – 11530.
[32] J. B. Matson, M. J. Webber, V. K. Tamboli, B. Weber, S. I. Stupp, *Soft Matter* **2012**, *8*, 2689 – 2692.
[33] S. Pai, K. Radacki, U. Schatzschneider, *Eur. J. Inorg. Chem.* **2014**, 2886 – 2895.
[34] F. Zobi, O. Blacque, R. A. Jacobs, M. C. Schaub, A. Y. Bogdanova, *Dalton Trans.* **2012**, *41*, 370 – 378.
[35] P. Govender, S. Pai, U. Schatzschneider, G. S. Smith, *Inorg. Chem.* **2013**, *52*, 5470 – 5478.
[36] H. Pfeiffer, A. Rojas, J. Niesel, U. Schatzschneider, *Dalton Trans.* **2009**, 4292 – 4298.
[37] M. A. Gonzales, H. Han, A. Moyes, A. Radinos, A. J. Hobbs, N. Coombs, S. R. J. Oliver, P. K. Mascharak, *J. Mater. Chem. B* **2014**, *2*, 2107.
[38] A. E. Pierri, P. J. Huang, J. V. Garcia, J. G. Stanfill, M. Chui, G. Wu, N. Zheng, P. C. Ford, *Chem. Commun.* **2015**, *51*, 2072 – 2075.
[39] I. S. Albuquerque, H. F. Jeremias, M. Chaves-Ferreira, D. Matak-Vinkovic, O. Boutureira, C. C. Romao, G. J. Bernardes, *Chem. Commun.* **2015**, *51*, 3993 – 3996.
[40] M. Chaves-Ferreira, I. S. Albuquerque, D. Matak-Vinkovic, A. C. Coelho, S. M. Carvalho, L. M. Saraiva, C. C. Romao, G. J. Bernardes, *Angew. Chem. Int. Ed.* **2015**, *54*, 1172 – 1175; *Angew. Chem.* **2015**, *127*, 1188 – 1191.
[41] K. Fujita, Y. Tanaka, T. Sho, S. Ozeki, S. Abe, T. Hikage, T. Kuchimaru, S. Kizaka-Kondoh, T. Ueno, *J. Am. Chem. Soc.* **2014**, *136*, 16902 – 16908.
[42] H. Tabe, K. Fujita, S. Abe, M. Tsujimoto, T. Kuchimaru, S. Kizaka-Kondoh, M. Takano, S. Kitagawa, T. Ueno, *Inorg. Chem.* **2015**, *54*, 215 – 220.
[43] H. Tabe, T. Shimoi, K. Fujita, S. Abe, H. Ijiri, M. Tsujimoto, T. Kuchimaru, S. Kizaka-Kondo, H. Mori, S. Kitagawa, T. Ueno, *Chem. Lett.* **2015**, *44*, 342.
[44] E. C. Theil, R. K. Behera, T. Tosha, *Coord. Chem. Rev.* **2013**, *257*, 579 – 586.
[45] S. Abe, J. Niemeyer, M. Abe, Y. Takezawa, T. Ueno, T. Hikage, G. Erker, Y. Watanabe, *J. Am. Chem. Soc.* **2008**, *130*, 10512 – 10514.
[46] S. Abe, K. Hirata, T. Ueno, K. Morino, N. Shimizu, M. Yamamoto, M. Tanaka, E. Yashima, Y. Watanabe, *J. Am. Chem. Soc.* **2009**, *131*, 6958 – 6960.
[47] Z. Wang, Y. Takezawa, H. Aoyagi, S. Abe, T. Hikage, Y. Watanabe, S. Kitagawa, T. Ueno, *Chem. Commun.* **2011**, *47*, 170 – 172.
[48] T. Ueno, M. Suzuki, T. Goto, T. Matsumoto, K. Nagayama, Y. Watanabe, *Angew. Chem. Int. Ed.* **2004**, *43*, 2527 – 2530; *Angew. Chem.* **2004**, *116*, 2581 – 2584.
[49] L. Zhang, L. Laug, W. Munchgesang, E. Pippel, U. Gosele, M. Brandsch, M. Knez, *Nano Lett.* **2010**, *10*, 219 – 223.
[50] M. P. Soares, A. Usheva, S. Brouard, P. O. Berberat, L. Gunther, E. Tobiasch, F. H. Bach, *Antioxid. Redox Signaling* **2002**, *4*, 321 – 329.
[51] J. Megías, J. Busserolles, M. J. Alcaraz, *Br. J. Pharmacol.* **2007**, *150*, 977 – 986.
[52] H. Song, C. Bergstrasser, N. Rafat, S. Hoger, M. Schmidt, N. Endres, M. Goebeler, J. L. Hillebrands, R. Brigelius-Flohe, A. Banning, G. Beck, R. Loesel, B. A. Yard, *Br. J. Pharmacol.* **2009**, *157*, 769 – 780.
[53] R. Mede, V. P. Lorett-Velasquez, M. Klein, H. Gorls, M. Schmitt, G. Gessner, S. H. Heinemann, J. Popp, M. Westerhausen, *Dalton Trans.* **2015**, *44*, 3020 – 3033.
[54] M. A. Gonzalez, S. J. Carrington, N. L. Fry, J. L. Martinez, P. K. Mascharak, *Inorg. Chem.* **2012**, *51*, 11930 – 11940.
[55] N. Begum, Md. I. Hyder, S. E. Kabir, G. M. G. Hossain, E. Nordlander, D. Rokhsana, E. Rosenberg, *Inorg. Chem.* **2005**, *44*, 9887 – 9894.
[56] A. J. Atkin, J. M. Lynam, B. E. Moulton, P. Sawle, R. Motterlini, N. M. Boyle, M. T. Pryce, I. J. Fairlamb, *Dalton Trans.* **2011**, *40*, 5755 – 5761.
[57] U. Hasegawa, A. J. Vilies, E. Simeoni, C. Wandrey, J. A. Hubbell, *J. Am. Chem. Soc.* **2010**, *132*, 18273 – 18280.
[58] B. W. Michel, A. R. Lippert, C. J. Chang, *J. Am. Chem. Soc.* **2012**, *134*, 15668 – 15671.

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