DOI: 10.1002/cbic.201100719

Catalytic Azide Reduction in Biological Environments

Pijus K. Sasmal,[a] Susana Carregal-Romero,[b] Alice A. Han,[c] Craig N. Streu,[c] Zhijie Lin,[a] Kazuhiko Namikawa,[d] Samantha L. Elliott,[e] Reinhard W. Köster,[d] Wolfgang J. Parak,[b] and Eric Meggers*[a]

In the quest for the identification of catalytic transformations to be used in chemical biology and medicinal chemistry, we identified iron(III) *meso*-tetraarylporphines as efficient catalysts for the reduction of aromatic azides to their amines. The reaction uses thiols as reducing agents and tolerates water, air, and other biological components. A caged fluorophore was employed to demonstrate that the reduction can be performed even in living mammalian cells. However, in vivo experiments in nematodes (*Caenorhabditis elegans*) and zebrafish (*Danio rerio*) revealed a limitation to this method: the metabolic reduction of aromatic azides.

Introduction

The last two decades have witnessed a steadily growing interest for metal complexes in medicinal chemistry and chemical biology.[1] Unique properties such as structural complexity, unusual reactivities, adjustable ligand-exchange kinetics, fine-tuned redox activities, photo-reactivity, the availability of radioisotopes, and distinct spectroscopic signatures render metal complexes highly attractive scaffolds for the modulation, sensing, and imaging of biological processes.[2] Surprisingly, the important ability of metal-containing compounds to catalyze chemical transformations has not yet been exploited thoroughly for applications in the molecular life sciences, but promises to point at an exciting future, in which catalytic rather than stoichiometric events trigger biological processes in an amplified fashion due to turnover capability. It can be envisioned that such catalysts could be employed to amplify signals by turning over substrates multiple times, catalytically label or deactivate target biomolecules, and catalytically activate prodrugs. For example, the group of Francis developed a series of novel metal-catalyzed protein modifications,[3] Cowan and co-workers reported a metal-catalyzed oxidative inactivation of enzymes,[4] whereas Suh et al. used cobalt(III)–cyclen complexes as mediators for the hydrolytic cleavage of peptide backbones.[5,6] However, the use of catalytic metal complexes in living systems is limited. Bradley and co-workers recently reported that Pd^0 nanoparticles trapped within polystyrene microspheres can enter cells and mediate a variety of Pd^0-catalyzed reactions, such as, Suzuki–Miyaura cross-couplings,[7] whereas our group disclosed a Ru^{II}-catalyzed cleavage of allylcarbamates within living mammalian cells, albeit with low turnover numbers.[8,9]

Clearly, designing catalysts that retain their catalytic activity in a biological environment is a highly formidable challenge owing to the presence of millimolar concentrations of nucleophilic thiols that are prone to deactivate metal-containing catalysts, especially under protic and aerobic conditions.[10] We here wish to introduce a novel catalyst/substrate system that enables high turnover numbers and might become a valuable tool for signal amplification in chemical biology.

Results and Discussion

Iron–porphyrin-catalyzed reduction of aromatic azides

We decided to seek a robust catalyst for the reduction of organic azides to their amines, as azides are absent from biological systems and, thus, fulfill the requirement of bio-orthogonality, as has been demonstrated for the well-established in vitro and in vivo reactions involving organic azides, such as click chemistry and the Staudinger ligation.[11,12] Furthermore, as aromatic amines are important functional groups in many fluorophores and drugs, so the catalytic reduction of azides to primary amines opens potential applications for employing azides as catalytically activatable caging groups.[13] In fact, in the course of screening a variety of metal complexes, we discovered that the 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (TPP)-containing complex, [Fe(TPP)]Cl, smoothly catalyzes the reduction of aromatic azides to their respective amines in the combined presence of aliphatic thiols, protic solvents, and air.[14–17] For example, the reaction of *p*-chlorophenylazide (**1a**) with five equivalents of β-mercaptoethanol as reducing agent and 1 mol% of [Fe(TPP)Cl] in dichloromethane/methanol (95:5) under air at 30 °C provided *p*-chloroaniline (**2a**) in 86% isolated yield (Scheme 1A). This reduction works similarly well for elec-

[a] Dr. P. K. Sasmal, Z. Lin, Prof. Dr. E. Meggers
Fachbereich Chemie, Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35043 Marburg (Germany)
E-mail: meggers@chemie.uni-marburg.de

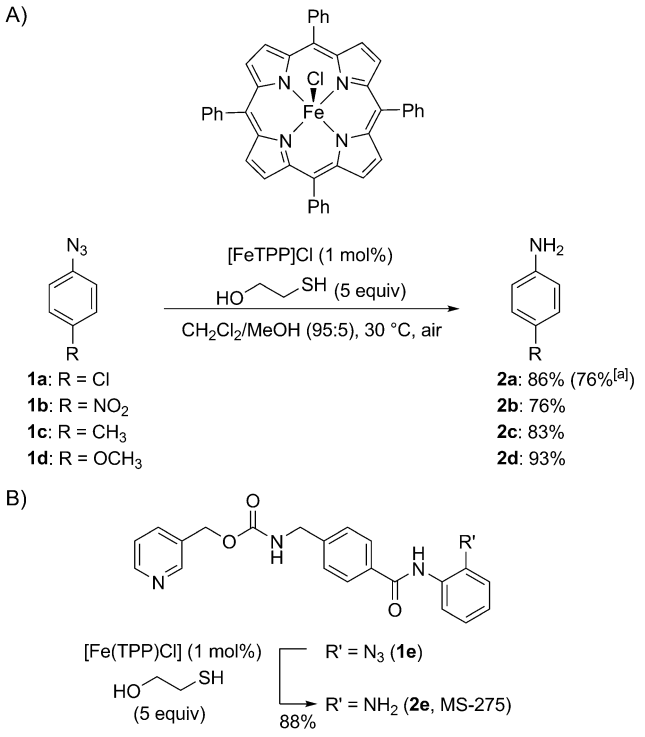
[b] Dr. S. Carregal-Romero, Prof. Dr. W. J. Parak
Fachbereich Physik and WZMW, Philipps-Universität Marburg
Renthof 7, 35037 Marburg (Germany)

[c] A. A. Han, Prof. C. N. Streu
Department of Chemistry and Biochemistry, St. Mary's College of Maryland
St. Mary's City, MD 20686 (USA)

[d] Dr. K. Namikawa, Prof. Dr. R. W. Köster
Zoologisches Institut, Technische Universität Braunschweig
Spielmannstrasse 8, 38106 Braunschweig (Germany)

[e] Prof. S. L. Elliott
Department of Biology, St. Mary's College of Maryland
St. Mary's City, MD 20686 (USA)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201100719.



Scheme 1. [Fe(TPP)]Cl-catalyzed reduction of aromatic azides to amines with thiols. A) Reduction of simple aromatic azides. B) [Fe(TPP)]Cl-catalyzed formation of the anticancer drug candidate MS-275. [a] Only 0.05 mol% [Fe(TPP)]Cl used in the reaction.

tron-poor (**1b**→**2b**, 76%) and electron-rich (**1c**→**2c**, 83%; **1d**→**2d**, 93%) aromatic azides, and even with a reduced catalyst loading of just 0.05 mol% [Fe(TPP)Cl] (**1a**→**2a**, 76%), reflecting a turnover number of 1520. This catalytic azide reduction is also applicable to more complicated substrates, as revealed by the smooth reduction of **1e** to the anticancer drug candidate MS-275 (**2e**)[18] (Scheme 1B).

It is worth noting that other tested metalloporphyrins of Cu^{II}, Co^{II}, Mn^{III}, and Ru^{II} did not work as catalysts under these biologically relevant reaction conditions as displayed in Figure 1, whereas electron-deficient derivatives of [Fe(TPP)Cl] at least gave inferior results. In contrast, the reduced iron(II) porphyrin [Fe(TPP)] (1 mol%) catalyzed the conversion of **1a** to **2a** under argon with the same yield (86%) compared to the corresponding oxidized iron(III) complex [Fe(TPP)Cl]. This strongly indicates that the Fe^{II}-containing complex is the active catalyst and reacts with the arylazide to form an intermediate Fe^{IV}–nitrene complex,[14] which is subsequently converted to the reduced amine by thiols. Nevertheless, we prefer the usage of the iron(III) complex, [Fe(TPP)Cl], due to its favorable solubility properties, especially, in polar and protic solvents. Thus, it can be concluded that the [Fe(TPP)Cl]-catalyzed conversion of aromatic azides to their respective amines is a very efficient and mild method that tolerates biologically relevant reaction conditions, such as, the combination of protic solvent, thiols, and air.

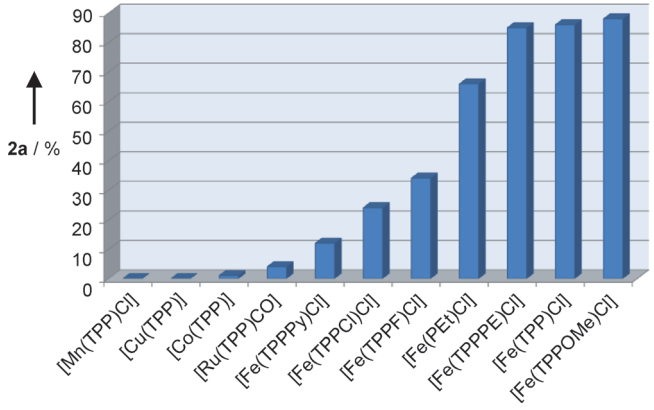
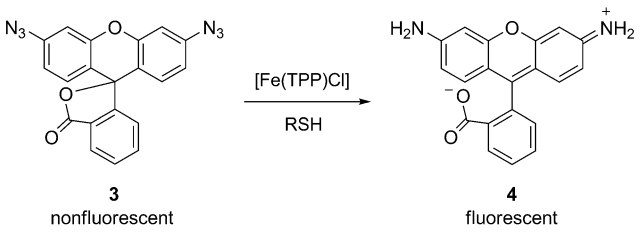


Figure 1. Comparison of different metalloporphyrins for the catalytic reduction of aromatic azides with thiols. Shown are isolated yields of **2a**. TPPPy: 5,10,15,20-tetra(*N*-methyl-4-pyridyl)porphine, TPPCl: 5,10,15,20-tetra(*o*-dichlorophenyl)-21*H*,23*H*-porphine, TPPE: 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine modified with ethylene glycol chains, TPPF: 5,10,15,20-tetra(pentafluorophenyl)-21*H*,23*H*-porphine, PEt: 2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine, TPPOMe: 5,10,15,20-tetra(4-methoxyphenyl)-21*H*,23*H*-porphine. Reaction conditions: azide **1a** (1 M), β-mercaptoethanol (5 equiv), and metalloporphyrin (1 mol%) were reacted in CH_2Cl_2/MeOH (95:5) for 30 min at 30 °C. See the Supporting Information for more details.

Reduction under biologically relevant conditions

To evaluate the iron–porphyrin-catalyzed azide reduction under more biologically relevant conditions, we synthesized bisazide **3**, which upon reduction of the two azides can be converted to the well-known fluorophore, rhodamine 110 (**4**), thus allowing a simple fluorescence readout for the determination of yields and turnover numbers (Scheme 2).[8,19] For example, the incubation of **3** with 5 mol% [Fe(TPP)Cl] in dimethyl sulfoxide/phosphate buffer (1:1, 20 mM, pH 7.25) at 37 °C for 1 h gave no fluorescence signal (0.2±0.1%; Table 1, entry 1), whereas the addition of 5 mM L-cysteine (Cys) led to an increased fluorescence signal and corresponding yield (13±2%; Table 1, entry 2). These results indicate that the thiol is needed as reducing agent for the iron-catalyzed reduction of the azide. Consistently, the additional presence of the reducing agent, ascorbate (10 mM), or a higher Cys concentration (15 mM) increased the yield further to 40±3% and 77±5%, respectively (Table 1, entries 3 and 4). A stronger reducing agent such as thiophenol in the presence of ascorbate even led to an almost complete reduction of **3** to rhodamine 110 (85±5%) with only



Scheme 2. Conversion of rhodamine bisazide **3** to rhodamine 110 (**4**) by [Fe(TPP)Cl]-catalyzed azide reduction.

Table 1. Influence of biorelevant reaction conditions on the catalytic reduction of rhodamine bisazide **3** by [Fe(TPP)Cl].[a]

	[Fe(TPP)Cl] [mol%]	Thiol	Ascorbate [mM]	Yield [%][b]
1	5	–	–	0.2±0.1
2	5	Cys (5 mM)	–	13±2
3	5	Cys (5 mM)	10	40±3
4	5	Cys (15 mM)	10	77±5
5	5	PhSH (5 mM)	10	85±5[d]
6	5	Cys (5 mM)	10[c]	41±2
7	–	Cys (5 mM)	10	2.3±0.5

[a] Bisazide **3** (0.5 mM) was treated with the indicated amounts of [Fe(TPP)Cl] in DMSO/phosphate buffer (1:1, 20 mM, pH 7.25) at 37 °C for 1 h. [b] Standard deviations from three independent experiments. [c] Reaction performed in DMEM instead of phosphate buffer. [d] In the absence of [Fe(TPP)Cl] the yield is only 1%.

5 mol% [Fe(TPP)Cl] (Table 1, entry 5). Gratifyingly, [Fe(TPP)Cl] is quite tolerant towards other biological components commonly present in culture medium: entry 6 in Table 1 demonstrates that the replacement of phosphate buffer by cell culture medium did not reduce the yields. It is important to note that aromatic azides are not significantly reduced in the presence of biologically relevant concentrations of organic thiols (Table 1, entry 7). Thus, it can be concluded that [Fe(TPP)Cl] is a highly suitable catalyst for the reduction of aromatic azides in a biological environment.[20]

Experiments in living mammalian cells

Encouraged by these results, we next used caged fluorophore **3** to investigate the [Fe(TPP)Cl]-catalyzed reduction of azides in living mammalian cells. Accordingly, cultured HeLa cells were incubated with **3** (100 μM) for 25 min and washed with phosphate-buffered saline (PBS); subsequent addition of fresh medium ensured that **3** is located only within the cells and not in the culture medium. Following the addition of [Fe(TPP)Cl] (10 μM) to the medium, cellular fluorescence was monitored by live-cell imaging with a confocal fluorescence microscope (Figure 2A). Indeed, Figure 2B reveals that the cellular green fluorescence of rhodamine 110 developed quickly with a 28-fold increase in fluorescence over a time period of 10 min.

In vivo experiments in nematodes and zebrafish

Next, we investigated the scope of this method in vivo. Unfortunately, experiments with nematodes, *Caenorhabditis elegans*, as well as the zebrafish, *Danio rerio*, revealed that caged fluorophore **3** develops a fluorescence signal even in the absence of [Fe(TPP)Cl] catalyst. For example, adult *C. elegans* incubated with bisazide **3** (25 μM) for 20 min revealed strong fluorescence intensities in intestinal cells surrounding the lumen (Figure 3A). Similarly, the incubation of 2.5-day-old transparent zebrafish embryos with bisazide **3** (100 μM) for 30 min led to a strong fluorescence in head and trunk regions (Figure 3B). In both nematode and zebrafish, the addition of catalyst had no significant effect on the fluorescence. Apparently, bisazide **3** is quickly metabolized to rhodamine 110 in vivo without the need for [Fe(TPP)Cl]. This observation is consistent with other reports on the reductive metabolism of aromatic azides in vivo.[21]

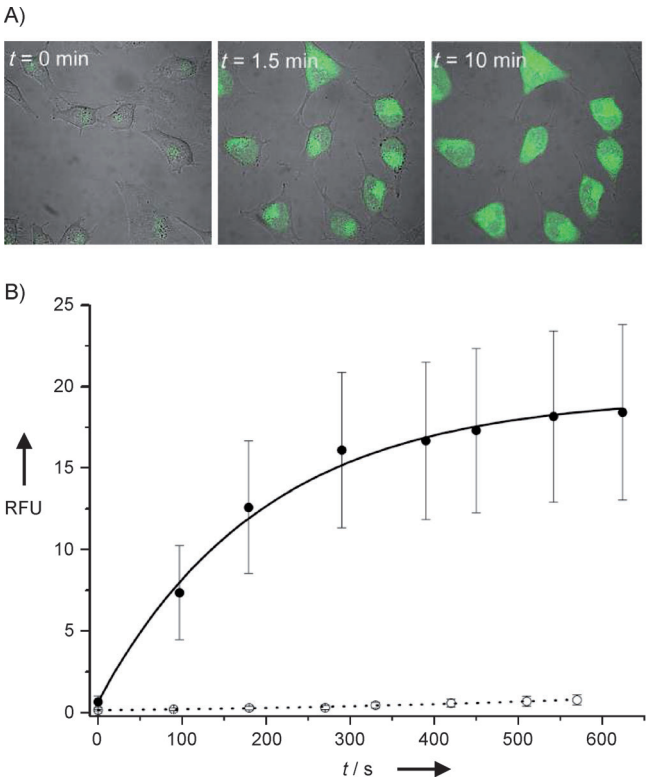


Figure 2. [Fe(TPP)Cl]-induced reduction of bisazide **3** to fluorescent rhodamine 110 inside HeLa cells. A) Superimposed phase contrast and confocal fluorescence images. HeLa cells were pre-incubated with bisazide **3** (100 μM) for 25 min, washed with PBS, and subsequently treated with [Fe(TPP)Cl] (10 μM). See the Supporting Information for more details. B) Quantified time-dependent increase of fluorescence emission within the cellular cytosol of HeLa cells upon [Fe(TPP)Cl] addition (●) and the corresponding time-dependent fluorescence in the absence of catalyst (○). The image analysis was performed on regions of interest (ROIs) for eight individual HeLa cells with the software ImageJ. The fluorescence evolution was analyzed for eight different HeLa cells. The data for the catalytic reaction were fitted to an exponential equation with the exponent being the rate constant [s^{-1}] of the intensity increase.

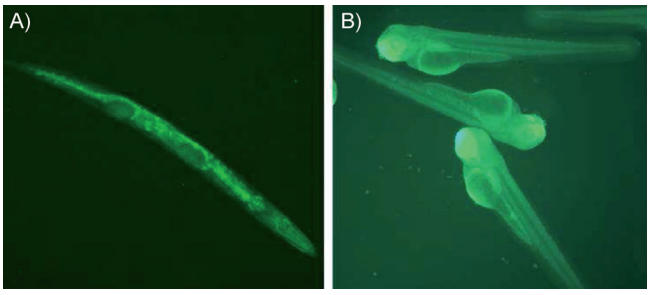


Figure 3. Activation of bisazide **3** in vivo. A) Adult *C. elegans* nematode incubated with bisazide **3** (25 μM) in M9 buffer with 1% DMSO for 20 min, before being pelleted, washed with M9 buffer (1 mL), concentrated to 100 μL by decanting, placed on a slide, and treated with one drop of 10% azide, followed by fluorescence imaging. B) 2.5-day-old transparent zebrafish embryos incubated with bisazide **3** (100 μM) for 30 min followed by fluorescence imaging.

Conclusion

We here reported the discovery of the efficient and mild [Fe(TPP)Cl]-catalyzed reduction of aromatic azides to their respective amines. This reaction uses thiols as the reducing agent and tolerates water, air, and other biological components, thus rendering it a rare example of a catalytic reaction that can be performed under physiological conditions and even in living mammalian cells. However, the apparent in vivo metabolic reduction of aromatic azides reveals a limitation of this method. The application of this new catalytic reaction to cellular imaging and catalytic prodrug release are underway in our laboratory.

Experimental Section

Synthesis: Azide substrates 1-azido-4-chlorobenzene (**1a**), 1-azido-4-nitrobenzene (**1b**), 1-azido-4-methylbenzene (**1c**), and 1-azido-4-methoxybenzene (**1d**), and poly(ethylene glycol)-modified iron(III) porphyrin [Fe(TPPE)Cl] were synthesized according to literature reports.[22,23] See the Supporting Information for the synthesis of substrate **1e** and bisazide **3**.

Reduction of arylazides in organic solvents: Azide **1a** (60 mg, 0.392 mmol), [Fe(TPP)Cl] (2.76 mg, 0.0039 mmol, 1 mol%) and β-mercaptoethanol (137 μL, 1.96 mmol) were dissolved in CH_2Cl_2/MeOH (95:5, 0.4 mL) and stirred for 0.5 h at 30 °C. Next, the reaction mixture was subjected to silica gel chromatography (hexane/EtOAc, 4:1→3:1) to provide amine **2a** (43 mg, 86%). With a reduced catalyst loading of 0.05 mol%, the reaction was stirred at 30 °C for one week to give amine **2a** (76%). See the Supporting Information for additional examples with different substrates.

Reduction of rhodamine bisazide 3 under biologically relevant conditions

[Fe(TPP)Cl] as catalyst: Bisazide **3** (0.5 mM), [Fe(TPP)Cl] (0.025 mM), (+)-sodium L-ascorbate (10 mM), and PhSH (5 mM) were mixed thoroughly with a micropipette in DMSO/phosphate buffer (1:1, 20 mM, pH 7.25) and shaken for 1 h at 37 °C and 50 rpm in a KEM-Lab Vortex Mixer (J-KEM Scientific, St. Louis, MO, USA). [Fe(TPP)Cl] (0.025 mM) was added as a DMSO stock solution (1 mM), which was preheated to 70 °C for 5 min for complete dissolution. The yield of rhodamine 110 (85±5%) was determined by measuring the generated green fluorescence.

[Fe(TPPE)Cl] as catalyst: Bisazide **3** (0.5 mM), [Fe(TPPE)Cl] (0.025 mM), (+)-sodium L-ascorbate (10 mM), and PhSH (5 mM) were mixed thoroughly with a micropipette in DMSO/phosphate buffer (1:3, 20 mM, pH 7.25) and then shaken for 1 h at 37 °C and 50 rpm using a KEM-Lab Vortex Mixer (J-KEM Scientific). The yield of rhodamine 110 (91±4%) was determined by measuring the generated green fluorescence. See the Supporting Information for more details and related experiments according to Table 1.

Reduction of bisazide 3 in HeLa cells: HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma–Aldrich) supplemented with 10% fetal bovine serum (FBS, Biochrom), glutamine (2 mM), and penicillin/streptomycin (100 μg mL^{-1}). One day prior to experiments, approximately 1×10^5 cells were plated in medium (2 mL) on standard-bottom dishes (35/12/1.5 mm). Rhodamine bisazide **3** (10 μL, 20 mM DMSO stock) was added to HeLa cells, briefly agitated to ensure a homogeneous final concentration (100 μM) and incubated for 25 min at 37 °C. After removal of the medium, cells were washed with PBS (2×1 mL) and taken-up in fresh medium (1 mL). [Fe(TPP)Cl] (10 μL, 1 mM DMSO stock, preheated for 5 min at 70 °C for complete dissolution) was added to reach a final concentration of 10 μM, and the mixture was agitated moderately for a few seconds before live-cell imaging.

Fluorescence microscopy of cellular experiments: All confocal laser scanning microscope (CLSM) images were recorded with a LSM 510 META confocal microscope (Zeiss, Oberkochen, Germany). Images were acquired from phase-contrast and green fluorescence channels (488 nm excitation wavelength and BP 505–530 nm emission wavelength filter). Time series of images were recorded in order to analyze the increase in green fluorescence intensity in the cellular cytosol of HeLa cells. Images of the cells before the addition of catalyst were taken as a control.

Image analysis of cellular experiments: CLSM images of the green fluorescence channels were recorded at various times after catalyst addition and in the absence of catalyst (control). The images were analyzed with the software ImageJ (version 1.44p) as recently published.[24] Briefly, the cellular area was drawn in the same position for eight cells in every CLSM image. Each drawn area defines a region of interest (ROI), which was analyzed with the program after the final image was acquired. After selecting a ROI in the first image at time t_1, this ROI was then transferred to the next image at time t_2 until t_n using a ROI manager, in this way keeping the size and position of the ROI constant for every image of the time series. A background value was measured in an area of the same size in the surrounding medium outside of the cellular cytosol. The background intensity was found to be negligible. The net intensities were used to study the time dependence of the green fluorescence emission in the cell cytosol after catalyst addition.

In vivo experiments in nematodes and zebrafish: See the Supporting Information for details.

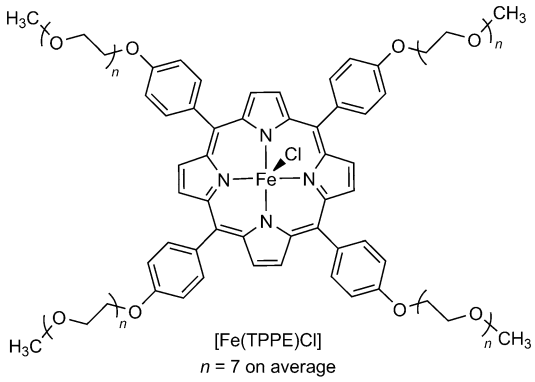
Acknowledgements

We thank the Philipps-University Marburg (Germany) for financial support. Z.L. thanks the China Scholarship Council for a stipend. S.C.R. is grateful to the Junta Andalucia (Spain) for a fellowship. Parts of this work were supported by the German Research Foundation (FOR630, grant no. ME-1805/3-1 to E.M.; SPP1313, grant no. PA794/4-1 to W.J.P.; grant no. KO1949/4-1 to R.W.K.).

Keywords: azide reductions · catalysis · cellular chemistry · fluorescence · iron porphyrins

[1] a) U. Schatzschneider, N. Metzler-Nolte, *Angew. Chem.* **2006**, *118*, 1534–1537; *Angew. Chem. Int. Ed.* **2006**, *45*, 1504–1507; b) T. W. Hambley, *Dalton Trans.* **2007**, 4929–4937; c) E. J. Merino, A. K. Boal, J. K. Barton, *Curr. Opin. Chem. Biol.* **2008**, *12*, 229–237; d) A. Mukherjee, P. J. Sadler, *Wiley Encyclopedia of Chemical Biology*, Wiley, Hoboken, **2009**, 1–47; e) C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391–401; f) E. Meggers, *Chem. Commun.* **2009**, 1001–1010; g) E. A. Hillard, G. Jaouen, *Organometallics* **2011**, *30*, 20–27; h) G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25; i) W.-H. Ang, A. Casini, G. Sava, P. J. Dyson, *J. Organomet. Chem.* **2011**, *696*, 989–998.
[2] K. L. Haas, K. J. Franz, *Chem. Rev.* **2009**, *109*, 4921–4960.
[3] J. M. Antos, M. B. Francis, *Curr. Opin. Chem. Biol.* **2006**, *10*, 253–262.
[4] L. Hocharoen, J. A. Cowan, *Chem. Eur. J.* **2009**, *15*, 8670–8676.
[5] T. Y. Lee, J. Suh, *Chem. Soc. Rev.* **2009**, *38*, 1949–1957.

[6] For pioneering work in this direction, see also a) A. Schepartz, B. Cuenoud, *J. Am. Chem. Soc.* **1990**, *112*, 3247 – 3249; b) D. Hoyer, H. Cho, P. G. Schultz, *J. Am. Chem. Soc.* **1990**, *112*, 3249 – 3250.
[7] R. M. Yusop, A. Unciti-Broceta, E. M. V. Johansson, R. M. Sánchez-Martín, M. Bradley, *Nat. Chem.* **2011**, *3*, 239 – 243.
[8] C. Streu, E. Meggers, *Angew. Chem.* **2006**, *118*, 5773 – 5776; *Angew. Chem. Int. Ed.* **2006**, *45*, 5645 – 5648.
[9] For recent examples of catalytically active metal complexes in medicinal chemistry see: a) D. Salvemini, Z.-Q. Wang, J. L. Zweier, A. Samouilov, H. Macarthur, T. P. Misko, M. G. Currie, S. Cuzzocrea, J. A. Sikorski, D. P. Riley, *Science* **1999**, *286*, 304 – 306; b) S. J. Dougan, A. Habtemariam, S. E. McHale, S. Parsons, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11628 – 11633.
[10] It is our experience that not the individual components, but rather the combination of thiols, protic solvent and air causes serious problems for most metal-containing catalysts.
[11] E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108 – 7133; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974 – 6998.
[12] For a recently reported photochemical Ru^{II}-catalyzed azide reduction, see: Y. Chen, A. S. Kamlet, J. B. Steinman, D. R. Liu, *Nat. Chem.* **2011**, *3*, 146 – 153.
[13] For recent applications based on the reduction of aromatic azides, see: a) H. Abe, J. Wang, K. Furukawa, K. Oki, M. Uda, S. Tsuneda, Y. Ito, *Bioconjugate Chem.* **2008**, *19*, 1219 – 1226; b) Z. Pianowski, K. Gorska, L. Oswald, C. A. Merten, N. Winssinger, *J. Am. Chem. Soc.* **2009**, *131*, 6492 – 6497; c) A. R. Lippert, E. J. New, C. J. Chang, *J. Am. Chem. Soc.* **2011**, *133*, 10078 – 10080; d) M. Röthlingshöfer, K. Gorska, N. Winssinger, *Org. Lett.* **2012**, *14*, 482 – 485.
[14] For the interaction and activation of organic azides by transition metal complexes, see: a) S. Cenini, E. Gallo, A. Caselli, F. Ragaini, S. Fantauzzi, C. Piangiolino, *Coord. Chem. Rev.* **2006**, *250*, 1234 – 1253; b) T. G. Driver, *Org. Biomol. Chem.* **2010**, *8*, 3831 – 3846.
[15] In Co^{II} – porphyrin-catalyzed nitrene transfer reactions with aromatic azides, the reduction of the azides to their respective amines was reported as a side reaction. For an example, see: F. Ragaini, A. Penoni, E. Gallo, S. Tollari, C. Li Gotti, M. Lapadula, E. Mangioni, S. Cenini, *Chem. Eur. J.* **2003**, *9*, 249 – 259.
[16] For an efficient reduction of organic azides with dithiols, see: a) I. L. Cartwright, D. W. Hutchinson, V. W. Armstrong, *Nucleic Acids Res.* **1976**, *3*, 2331 – 2339; b) H. Bayley, J. R. Standring, J. R. Knowles, *Tetrahedron Lett.* **1978**, *19*, 3633 – 3634.
[17] Aliphatic azides are not reduced under these conditions as has been tested, for example, for 2-phenethylazide.
[18] T. Suzuki, T. Ando, K. Tsuchiya, N. Fukazawa, *J. Med. Chem.* **1999**, *42*, 3001 – 3003.
[19] E. A. Novikova, L. F. Avramenko, T. F. Grigorenko, I. D. Laznikova, V. G. Syromyatnikov, L. I. Khmil, *Russ. J. Org. Chem.* **1998**, *34*, 1762 – 1767.
[20] The better water-soluble poly(ethylene glycol)-functionalized catalyst [Fe(TPPE)Cl] can be used with a reduced DMSO content, although some DMSO is necessary to dissolve the rhodamine bisazide **3** substrate. See the Experimental Section for more details.



[21] a) F. Kamali, A. Gescher, J. A. Slack, *Xenobiotica* **1988**, *18*, 1157 – 1164; b) D. Nicholls, A. Gescher, R. J. Griffin, *Xenobiotica* **1991**, *21*, 935 – 943.
[22] a) M. Hu, J. Li, S. Q. Yao, *Org. Lett.* **2008**, *10*, 5529 – 5531; b) S. W. Kwok, J. R. Fotsing, R. J. Fraser, V. O. Rodionov, V. V. Fokin, *Org. Lett.* **2010**, *12*, 4217 – 4219; c) P. S. Branco, A. M. M. Antunes, M. M. Marques, M. P. Chiarelli, A. M. Lobo, S. Prabhakar, *Chem. Res. Toxicol.* **1999**, *12*, 1223 – 1233.
[23] J. W. Long, I. K. Kim, R. W. Murray, *J. Am. Chem. Soc.* **1997**, *119*, 11510 – 11515.
[24] L. L. Del Mercato, A. Z. Abbasi, W. J. Parak, *Small* **2011**, *7*, 351 – 363.

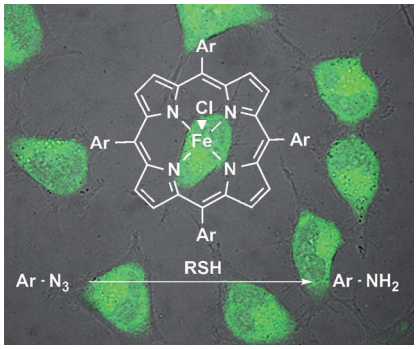
Received: November 15, 2011
Published online on ■■ ■■, 0000

COMMUNICATIONS

*P. K. Sasmal, S. Carregal-Romero, A. A. Han, C. N. Streu, Z. Lin, K. Namikawa, S. L. Elliott, R. W. Köster, W. J. Parak, E. Meggers**

■■ – ■■

Catalytic Azide Reduction in Biological Environments



A tolerant catalyst: The iron *meso*-tetraarylporphin-catalyzed reduction of aromatic azides with thiols is described and analyzed. The discovered system tolerates the presence of water, air, and other biological components, and can even be performed in living mammalian cells, thus providing a novel tool for signal amplification in molecular life sciences.

