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Metal complex catalysis in living biological systems

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This feature article discusses synthetic metal complexes that are capable of catalyzing chemical transformations in living organisms. Photodynamic therapy exemplifies what is probably the most established artificial catalytic process exploited in medicine, namely the photosensitized catalytic generation of cell-damaging singlet oxygen. Different redox catalysts have been designed over the last two decades to target a variety of redox alterations in cancer and other diseases. For example, pentaazamacrocyclic manganese(II) complexes catalyze the dismutation of superoxide to O_2 and H_2O_2 *in vivo* and thus reduce oxidative stress in analogy to the native enzyme superoxide dismutase. Recently, piano-stool ruthenium and iridium complexes were reported to influence cellular redox homeostasis indirectly by catalytic glutathione oxidation and catalytic transfer hydrogenation using the coenzyme NADH, respectively. Over the last few years, significant progress has been made towards the application of non-biological reactions in living systems, ranging from the organoruthenium-catalyzed cleavage of allylcarbamates and a gold-catalyzed intramolecular hydroarylation to palladium-catalyzed Suzuki–Miyaura and Sonogashira cross-couplings within the cytoplasm or on the surface of living cells. The design of bioorthogonal catalyst/substrate pairs, which can passively diffuse into cells, combines the advantages of small molecules with catalysis and promises to provide exciting new tools for future chemical biology studies.

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Introduction

The last decade has witnessed the development of increasingly powerful chemical tools for the modulation, sensing, and imaging of biological processes in living systems.[1] Today, synthetic molecules based on diverse chemical scaffolds and discovered through sophisticated strategies are capable of highly selectively interfering with individual enzyme functions or protein–protein interactions. At the same time, progress with the design of bioorthogonal reactions permits the controlled intracellular execution of synthetic chemistry without interference from the myriad of surrounding biological components. One might wonder if there is any other untapped chemistry imaginable that could serve as the next generation of powerful chemical tools for modulating biology. We believe this is indeed the case, namely the transition from mainly exploiting stoichiometric events or reactions to applying catalytic processes. Many attractive applications can be envisioned, ranging from turning over protected, caged substrates multiple times, catalytically labeling or deactivating target biomolecules, to catalytically activating prodrugs at their cellular destination. The advantage of signal amplification through catalytic turnover has been successfully exploited already in the area of enzyme-triggered bioimaging and nucleic-acid-based sensing.[2,3] However, the design of bioorthogonal synthetic catalyst/substrate pairs which can passively diffuse into cells for use as tools in chemical biology studies – thus combining the advantages of small molecules with catalysis – is a highly formidable challenge that has only recently started to come to fruition. We here review and discuss progress in this exciting area of catalytic chemistry at the interface with biology and use catalysis in living systems as our yardstick (Fig. 1).

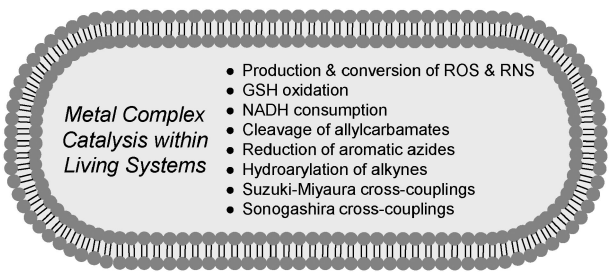

Fig. 1 Overview of metal-complex-catalyzed reactions in living biological systems. ROS = reactive oxygen species, RNS = reactive nitrogen species, GSH = glutathione.

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Redox catalysis

The concept of exploiting catalysis for the amplification of a biological effect is actually not new and has been clinically implemented with photodynamic therapy (PDT) for the treatment of cancer and a few other diseases (*e.g.* skin lesions and age-related macular degeneration).[4] In PDT, nontoxic organic or metal-containing photosensitizers are employed as catalysts for the light-induced generation of highly reactive singlet oxygen ($^1O_2$), which then initiates cell-damaging oxidation reactions. Recently, a photosensitizer prodrug was reported that becomes activated by a specific endogenous ribonucleic acid.[5] The reactivity of singlet oxygen has also been exploited for the chromophore-assisted light inactivation of individual proteins and nucleic acids in living cells.[6,7]

Over the last decade, increased efforts have been devoted towards targeting redox alterations in cancer and other diseases.[8] For example, tumors frequently exhibit high oxidative stress with an increased production of reactive oxygen species (ROS). The consequential increased vulnerability of cancer cells to modulations of the ROS homeostasis has therefore been exploited with oxidative stress promoting redox catalysts such as gadolinium texaphyrins, anthracyclines, iron chelators, and nanoparticles,[8,9] but also organometallic compounds such as the multifunctional redox catalyst organotelluride LAB027 (**1**) (Fig. 2).[10,11] In certain oxidative stress related diseases, such as reperfusion injury (*e.g.* which occurs after stroke) and inflammatory processes (*e.g.* arthritis), the production of the highly reactive superoxide anion is enhanced, resulting in oxidative cell damage. Pentaazamacrocyclic Mn^{II}([15]aneN$_5$) complexes have demonstrated high activity and biocompatibility as superoxide dismutase (SOD) mimics with excellent potential for treating a variety of diseases associated with oxidative stress.[12] For example, the manganese(II) complex M40403 (**2**) catalyzes the dismutation of superoxide to O_2 and H_2O_2 with a rate approaching the native Mn SOD enzyme and, revealingly, the injection of M40403 into rat models of inflammation and ischemia-reperfusion injury protected the animals against tissue damage.[13] A recent study demonstrated an additional protective effect of this class of SOD mimics by completely scavenging nitric oxide and peroxynitrite.[14] Peroxynitrite is formed through the diffusion controlled reaction of superoxide with nitric oxide and itself constitutes a potent inducer of cell death.[15] Metalloporphyrins and derivatives with the metals manganese and iron, some of which are currently developed for clinical applications, catalytically decompose peroxynitrite and have been demonstrated to attenuate the toxic effects of peroxynitrite *in vitro* and *in vivo*.

The cellular redox homeostasis can also be targeted in a more indirect fashion. For example, glutathione (GSH) is a major reductant in cells, inactivating reactive oxygen species and serving as a cofactor for redox modulating enzymes. The depletion of GSH could therefore affect the ability of cancer cells to cope with oxidative damage. Intriguingly, Sadler and co-workers have recently reported that ''piano-stool'' ruthenium arene complexes such as [(η^6-biphenyl)Ru(2-(*p*-*N*,*N*-dimethylaminophenylazo)pyridine)I]PF_6 (**3**) are highly cytotoxic to human ovarian and lung cancer cell lines despite their inertness towards hydrolysis.[16] The authors demonstrated that such organoruthenium complexes are catalysts for the oxidation of GSH to glutathione disulfide (GSSG) and this redox catalysis might, at least in part, be responsible for the anticancer activity of this class of catalytic drugs. The proposed catalytic cycle involves ligand-centered reduction of the azo group to a hydrazine, initiated by a nucleophilic addition of GSH to the azo group, followed by the oxidative regeneration of the azo group with molecular oxygen. Interestingly, the same group recently disclosed a family of ruthenium and iridium arene complexes that are capable of catalyzing transfer hydrogenation using the coenzyme NADH as a cofactor and they demonstrated that iridium complex **4** affects the NAD^+/NADH ratio within human A2780 ovarian cancer cells, thus potentially interfering with the function of NADH-dependent redox enzymes.[17]

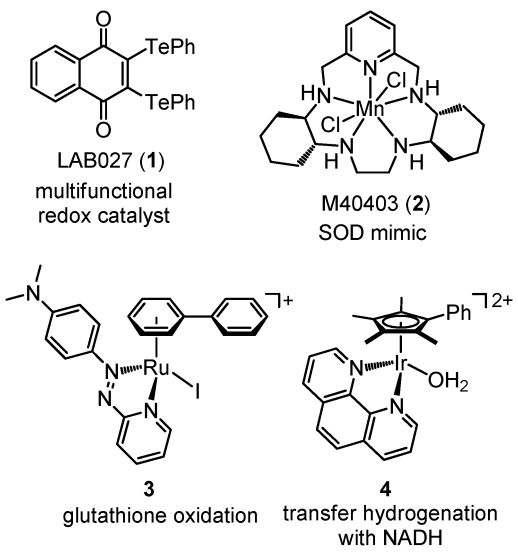
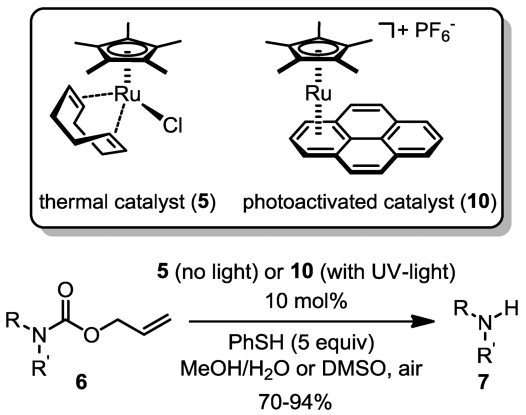


Fig. 2 Structures of some reported redox catalysts.

Catalytic functional group conversions

The catalytic conversion of functional groups such as the removal of protection groups is an attractive objective because it permits the design of catalytically activatable prodrugs or signal amplification with masked imaging probes. Our laboratory was the first to report an example of a protection group which can be removed catalytically within a living biological system.[18,19] We found that the ruthenium(II) half-sandwich complex [Cp*Ru(COD)Cl] (**5**), with Cp* = pentamethylcyclopentadienyl and COD = 1,5-cyclooctadiene, is capable of cleaving allylcarbamates (**6**) to their respective primary amines (**7**) in the presence of water, air, and thiols (Scheme 1). Thiols, preferably aromatic thiols, are actually required for the catalytic cleavage. To evaluate the Ru-catalyzed allylcarbamate cleavage in more relevant biological environments such as living mammalian cells, we utilized the caged, non-fluorescent *N*,*N*-bis-allyloxycarbonyl-protected rhodamine 110 (**8**) as a cellular probe, which can be converted to green fluorescent rhodamine 110 (**9**) upon cleavage of both allylcarbamate moieties (Fig. 3). Accordingly, HeLa cells were first incubated with the caged fluorophore **8**, followed by



Scheme 1 Thermal and photoactivated ruthenium catalyst ($\lambda \geq 330$ nm) for the cleavage of allylcarbamates under bio-relevant conditions.

washing with fresh medium in order to ensure that the caged fluorophore was only present within the living cells. Fig. 3 demonstrates that subsequent addition of the ruthenium complex **5** and thiophenol resulted in a rapid increase in fluorescence as monitored by confocal fluorescence microscopy. This fluorescence development was more pronounced in the presence of thiophenol reaching a 10-fold increase within a time period of 15 min within the cytoplasm of the HeLa cells. We have recently extended this system by reporting the ruthenium complex [Cp*Ru(η^6-pyrene)]PF_6 (**10**) (Scheme 1) as a photoactivatable catalyst for the cleavage of allylcarbamates and demonstrated its utility in HeLa cells.[20]

In the quest for other biocompatible protection groups, our laboratory recently envisioned the use of organic azides as "masked amines" since amines are important functional groups in fluorophores and drugs, making possible a variety of applications for employing organic azides as catalytically activatable caging groups.[21] Furthermore, organic azides are absent from biological systems and thus fulfill an important requirement of bioorthogonality which is a key feature of click chemistry.[1a,g] In the course of screening a variety of metal complexes, we found that [Fe(TPP)Cl], with TPP = 5,10,15,20-tetraphenylporphine, smoothly catalyzes the reduction of aromatic azides to their respective amines in the combined presence of aliphatic thiols, protic solvents, and air. For example, the reaction of *p*-chlorophenylazide (**11**, with R = Cl) with 5 equivalents of β-mercaptoethanol as the reducing agent and 1 mol% of [Fe(TPP)Cl] in CH_2Cl_2 : MeOH 95 : 5 under air at 30 °C, provided *p*-chloroaniline (**12**, with R = Cl) in 86% isolated yield (Scheme 2).[22] This reduction works similarly well for electron-poor and electron-rich aromatic azides and was demonstrated to proceed with very low catalyst loading of just 0.05 mol% [Fe(TPP)Cl]. To evaluate the iron porphyrin catalyzed azide reduction under more biologically relevant conditions, we used the bisazide **13** and monitored its [Fe(TPP)Cl]-catalyzed conversion to the green fluorescent rhodamine 110 (**9**) under varying reaction conditions (Fig. 4). As expected, the catalytic reduction relies on the presence of thiols, which apparently serve as the reducing agent, with aromatic thiols giving superior results to aliphatic thiols, and benefiting from an additional presence of the reducing agent ascorbate. Thus, the existing reducing conditions within cellular cytosol should be ideally suited for the [Fe(TPP)Cl]-catalyzed reduction of aromatic azides. In fact, when we loaded cultured HeLa cells with the bisazide **13** (incubation at 100 μM for 25 min followed by

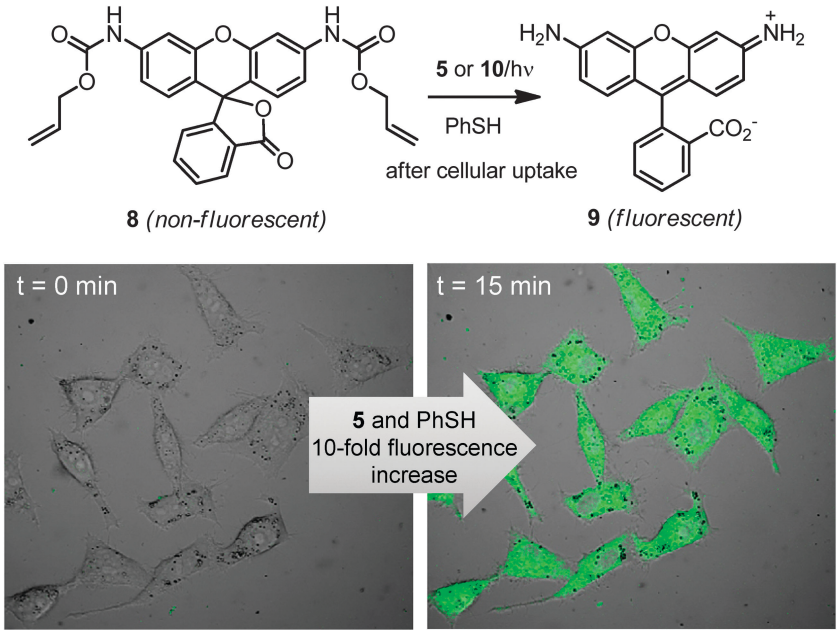
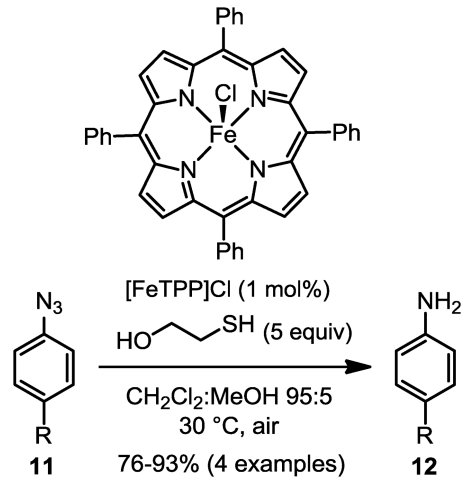


Fig. 3 Ruthenium-catalyzed uncaging of the bisallylcarbamate-protected rhodamine 110 (**8**) after uptake by HeLa cells. Shown are superimposed phase contrast and fluorescence images. Cells were preincubated with 100 μM of **8** for 30 min, washed with PBS buffer, and then treated with 20 μM [Cp*Ru(COD)]Cl (**5**) and 500 μM thiophenol. Shown images are taken immediately after this addition (a) and after 15 min (b). Analogous results were obtained with the catalyst **10** upon activation with UV-light. See ref. 18 and 20 for more details.



Scheme 2 [Fe(TPP)]Cl-catalyzed reduction of aromatic azides to amines with thiols as reducing agents.

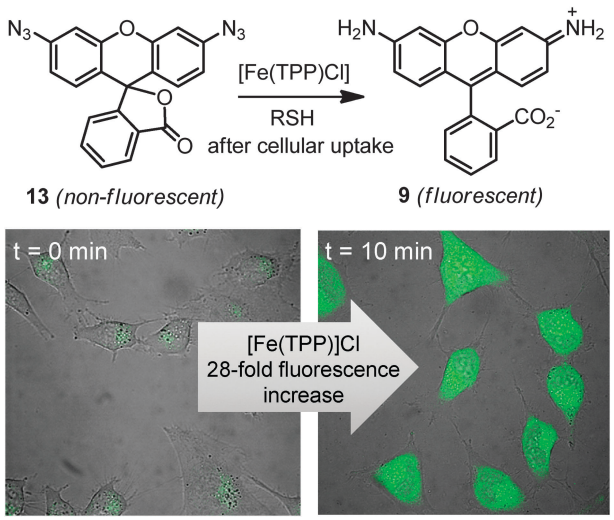


Fig. 4 [Fe(TPP)Cl]-catalyzed reduction of bisazide **13** to green fluorescent rhodamine 110 (**9**) within living HeLa cells. Shown are superimposed phase contrast and confocal fluorescence images in which HeLa cells were preincubated with 100 μM of bisazide **13** for 25 min, washed with PBS buffer, and subsequently treated with 10 μM [Fe(TPP)Cl]. See ref. 22 for more details.

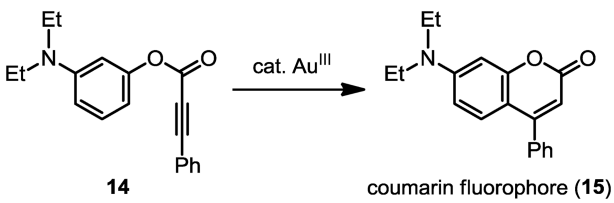
washing the cells with PBS buffer and the subsequent addition of fresh medium), the addition of [Fe(TPP)Cl] to the medium (10 μM) resulted in a rapid development of green fluorescence as monitored by live cell imaging with a confocal fluorescence microscope, with a 28-fold increase in fluorescence over a time period of just 10 min. (Fig. 4). Thus, the mild and efficient [Fe(TPP)Cl]-catalyzed reduction of aromatic azides to their respective amines uses thiols as the reducing agent and tolerates water, air, and other biological components, rendering it a rare example of a catalytic reaction that can be performed under physiological conditions such as in living mammalian cells. However, we found that in the nematode *Caenorhabditis elegans* as well as the zebrafish (*Danio rerio*) the bisazide **13** is metabolised quickly into rhodamine 110 without the need for [Fe(TPP)Cl], thus revealing a limited bioorthogonality of aryl azides.[22]

Catalysis of carbon–carbon bond formations

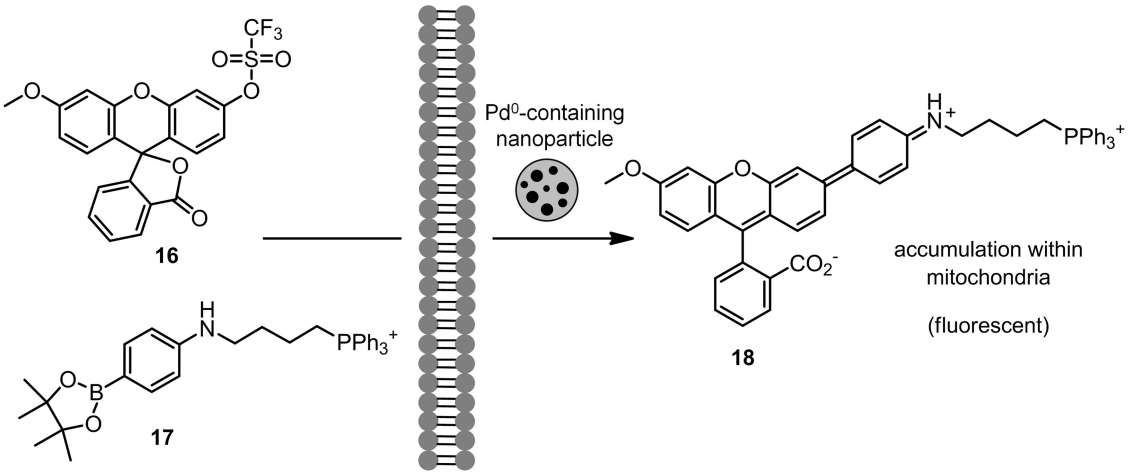
The importance of the development and application of methods for the formation of C–C bonds is manifested by the recently awarded Nobel Prize for Pd-catalyzed cross-coupling reactions, one of the most versatile tools for the generation of bonds between carbon atoms. Whereas organic chemists are predominately focusing on developing methods under highly defined reaction conditions and in organic solvents, the capability to apply mild C–C bond formation methods at will within a living biological system opens up exciting future opportunities in chemical biology and medicinal chemistry, such as the bioorthogonal tagging of biomolecules or the assembly of drugs from two or more components at its final cellular destination. Here we will highlight examples of metal-catalyzed C–C bond formation reactions that have been tested in cellular experiments.

A recent surge in homogeneous gold-catalyzed transformations has exploited gold ions as mild and carbophilic Lewis acids for the activation of carbon–carbon π-bonds and in particular alkynes.[23] This ''alkynophilicity'' has been used to design fluorescence sensors for the detection of gold(III) and gold(I) ions in biological systems and even in living mammalian cells.[24] However, most systems are reported to rely on stoichiometric amounts of gold ions. In contrast, Kim, Kim, Yoon, and co-workers recently revealed a turn-on fluorescent sensor for gold(III) based on a catalytic intramolecular hydroarylation resulting in formation of a fluorescent coumarin dye (**14** → **15**, Scheme 3).[25] The authors applied this sensor to the fluorescence microscopic detection of gold ions in fixed human keratinocyte cells.

Palladium catalysis in living biological systems was first reported for the hydrogenation of membrane lipids of cyanobacteria, plant, and mammalian cells.[26] Li and Chen recently reviewed newer developments regarding Pd-mediated chemistry in biological systems.[27] We will here highlight only Pd-catalyzed C–C bond formations that have been performed in living biological systems. Most notably, Bradley and co-workers recently reported palladium nanoparticles trapped within polystyrene microspheres as heterogeneous Pd^0-catalysts with the ability to enter mammalian cells and catalyze an intracellular Suzuki–Miyaura cross-coupling (Scheme 4).[28,29] To this end, HeLa cells were incubated with Pd-microspheres for 24 hours, followed by intensive washing of the cells in order to eliminate any extracellular palladium. Subsequently, the triflate **16** (20 μM) and the boronic ester **17** (20 μM) were added to the medium and



Scheme 3 Gold(III)-catalyzed cyclic hydroarylation reported by Kim, Kim, Yoon, and co-workers.[25] The formation of coumarin fluorescence was applied to verify the gold(III)-catalyzed hydroarylation in fixed mammalian cells.

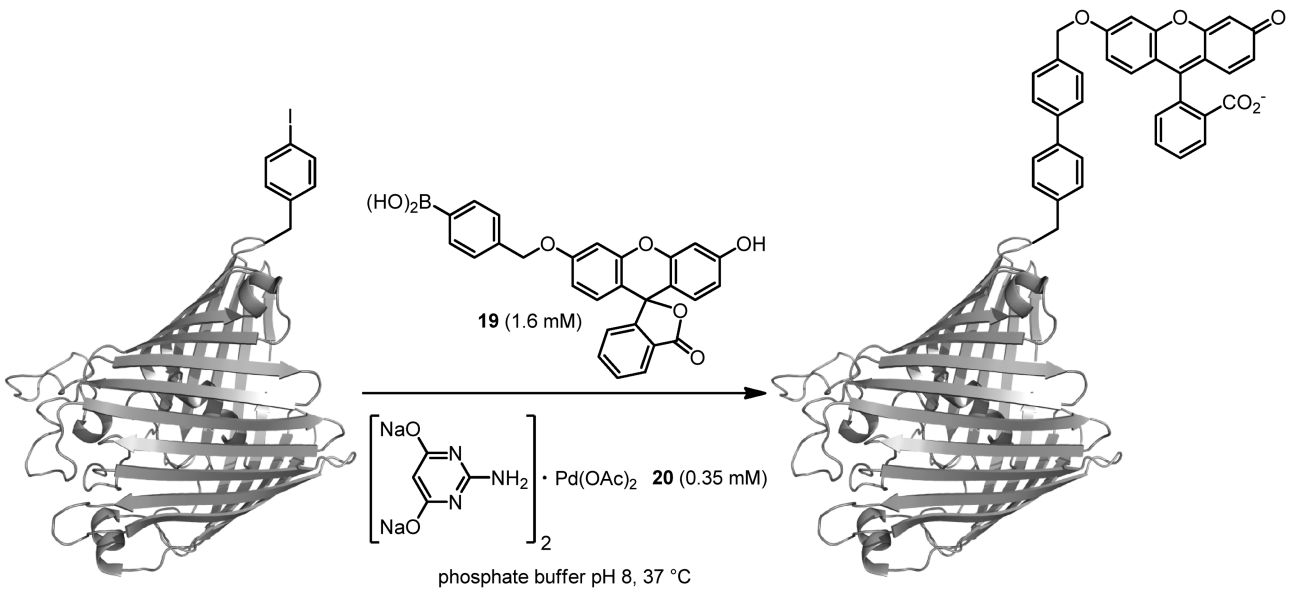


Scheme 4 Intracellular Suzuki–Miyaura cross-coupling with a cell-penetrating Pd^0-based heterogeneous catalyst by Bradley *et al.*[28,29]

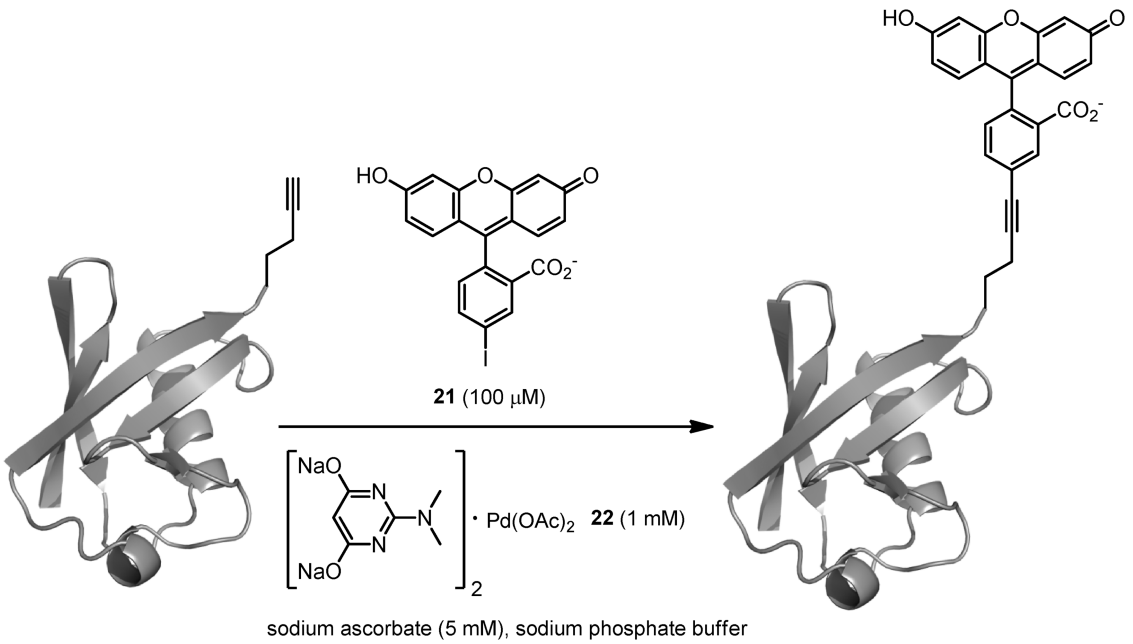
incubated for 48 hours. Confocal fluorescence microscopy revealed the development of mitochondria-localized fluorescence, which could be traced back to the anthofluorescein dye **18** by extraction from cells, and confirmation of its identity by HPLC and mass spectrometry. Interestingly, in control experiments the authors found that the triflate **16** was remarkably robust so that the development of fluorescence from hydrolyzed triflate **16** could be ruled out. Thus, from this study it was concluded that intracellular Pd-nanoparticles catalyzed the aryl–aryl coupling between the aryl triflate of **16** and the aryl boronic ester moiety of **17**. Opening of the lactone then restored fluorescence by generation of a delocalized π-system (**18**) and the lipophilic triphenylphosphonium moiety caused the accumulation within mitochondria.

Davis and co-workers recently reported the Pd-catalyzed labeling of the cell surface of *Escherichia coli*.[30] For this, the unnatural amino acid *p*-iodophenylalanine was incorporated into the loop region of the channel protein OmpC *via* amber stop codon suppression. The OmpC protein folds into a β-barrel and assembles into a trimer within the bacterial membrane and the incorporation of the *p*-iodophenylalanine into the extracellular loop region (loop 5) at position 232 was shown to be most effective. In a typical experiment, genetically modified *Escherichia coli* cells harboring *p*-iodophenylalanine-containing OmpC porin channels were treated with the boronic acid **19** (1.6 mM) and the previously designed phosphine-free, water-soluble Pd^{II}-catalyst **20**[31] (0.35 mM) for 1 h at 37 °C in pH 8 buffer, affording blue fluorescent *Escherichia coli* cells by covalent labeling of OmpC with a fluorophore (Scheme 5). The Pd-catalyst **20** was shown to be effective at concentrations for which no significant cell toxicity was observed under the reported reaction conditions.

Using a related Pd-catalyst, Lin and co-workers recently reported a copper-free Sonogashira cross-coupling in bacterial cells.[32]



Scheme 5 Pd-catalyzed Suzuki–Miyaura cross-coupling of a phenyliodide-modified pore protein with a boronic acid on the surface of *Escherichia coli*.[30]



Scheme 6 Pd-mediated Sonogashira cross-coupling between an alkyne-functionalized ubiquitin protein and an iodofluorescein in *Escherichia coli*.[32]

Genetically modified *Escherichia coli*, which overexpresses a modified ubiquitin protein that metabolically incorporates homopropargylglycine at the C-terminus, was used for this study. Treatment of such genetically engineered cells with iodofluorescein **21** (100 μM) and the phosphine-free, water soluble Pd^{II}-complex **22** (1 mM) in the presence of 5 mM sodium ascorbate in sodium phosphate buffer, followed by growing at 37 °C for 4 hours, resulted in green fluorescent cells (Scheme 6). Lysis of the cells and workup allowed the isolation of fluorescent ubiquitin, which the authors traced back to a Sonogashira cross-coupling between the terminal alkyne on the ubiquitin and the iodofluorescein inside of the *Escherichia coli* cells. However, this study relied on the use of an excess of Pd catalyst and the authors explain this to be necessary as a result of the nonspecific sequestration of the Pd complex by cellular proteins. This effect highlights one of the major challenges inherent in using Pd catalysis efficiently in biological systems: the lability of coordinative bonds to Pd combined with the affinity of Pd to highly abundant, soft thiol nucleophiles within biological systems.

Summary and outlook

This review has outlined the state of the art of metal complex catalysis in living biological systems, ranging from therapeutically relevant redox catalysts to the development of catalytic functional group conversions and catalyzed C–C bond formations. Moving bioorthogonal chemistry from merely stoichiometric reactions to catalytic cycles offers exciting future opportunities exploiting signal amplification. However, the challenges are formidable due to the desired concomitant characteristics of substrate-catalyst bioorthogonality and high catalyst turnover numbers. The latter has not been reported for any catalytic system operating in a living biological system but we expect these numbers to be modest. It is our experience from screening dozens of metal-based catalysts for their use in biological systems that it is especially the combination of water, air, and thiols that eventually deactivates the metal catalysts. Reaching the efficiency of enzymes with small molecule catalysts in complex biological systems – particularly considering a toxicity-related desirable restriction to micromolar catalyst concentrations – is therefore a considerable challenge and we predict that the upcoming years will reveal not only solutions to these challenges but subsequently also useful applications for such catalysts.

References

1 (*a*) J. A. Prescher and C. R. Bertozzi, *Nat. Chem. Biol.*, 2005, **1**, 13–21; (*b*) S. L. Schreiber, *Nat. Chem. Biol.*, 2005, **1**, 64–66; (*c*) B. N. G. Giepmans, S. R. Adams, M. H. Ellisman and R. Y. Tsien, *Science*, 2006, **312**, 217–224; (*d*) D. W. Domaille, E. L. Que and C. J. Chang, *Nat. Chem. Biol.*, 2008, **4**, 168–175; (*e*) X. Wu and P. G. Schultz, *J. Am. Chem. Soc.*, 2009, **131**, 12497–12515; (*f*) J. L. Major and T. J. Meade, *Acc. Chem. Res.*, 2009, **42**, 893–903; (*g*) E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974–6998; (*h*) E. Meggers, *Angew. Chem., Int. Ed.*, 2011, **50**, 2442–2448.
2 G. Zlokarnik, P. A. Negulescu, T. E. Knapp, L. Mere, N. Burres, L. Feng, M. Whitney, K. Roemer and R. Y. Tsien, *Science*, 1998, **279**, 84–88.
3 S. K. Silverman, *Wiley Encycl. Chem. Biol.*, 2009, **3**, 450–466.
4 T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan and Q. Peng, *J. Natl. Cancer Inst.*, 1998, **90**, 889–905.
5 D. Arian, E. Cló, K. V. Gothelf and A. Mokhir, *Chem.–Eur. J.*, 2010, **16**, 288–295.
6 L. M. Wittenhagen, J. R. Carreon, E. G. Prestwich and S. O. Kelley, *Angew. Chem., Int. Ed.*, 2005, **44**, 2542–2546.
7 J. Lee, P. Yu, X. Xiao and T. Kodadek, *Mol. BioSyst.*, 2008, **4**, 59–65.
8 D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery*, 2009, **8**, 579–591.
9 W. J. Stark, *Angew. Chem., Int. Ed.*, 2011, **50**, 1242–1258.
10 R. Coriat, W. Marut, M. Leconte, L. B. Ba, A. Vienne, C. Chéreau, J. Alexandre, B. Weill, M. Doering, C. Jacob, C. Nicco and F. Batteux, *Cell Death Dis.*, 2011, **2**, e191.

11 V. Jamier, L. A. Ba and C. Jacob, *Chem.–Eur. J.*, 2010, **16**, 10920–10928.
12 D. P. Riley, *Chem. Rev.*, 1999, **99**, 2573–2587.
13 D. Salvemini, Z. Q. Wang, J. L. Zweier, A. Samouilov, H. Macarthur, T. P. Misko, M. G. Currie, S. Cuzzocrea, J. A. Sikorski and D. P. Riley, *Science*, 1999, **286**, 304–306.
14 M. R. Filipović, A. C. W. Koh, S. Arbault, V. Niketić, A. Debus, U. Schleicher, C. Bogdan, M. Guille, F. Lemaître, C. Amatore and I. Ivanović-Burmazović, *Angew. Chem., Int. Ed.*, 2010, **49**, 4228–4232.
15 C. Szabó, H. Ischiropoulos and R. Radi, *Nat. Rev. Drug Discovery*, 2007, **6**, 662–680.
16 S. J. Dougan, A. Habtemariam, S. E. McHale, S. Parsons and P. J. Sadler, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 11628–11633.
17 S. Betanzos-Lara, Z. Liu, A. Habtemariam, A. M. Pizarro, B. Qamar and P. J. Sadler, *Angew. Chem., Int. Ed.*, 2012, **51**, 3897–3900.
18 C. Streu and E. Meggers, *Angew. Chem., Int. Ed.*, 2006, **45**, 5645–5648.
19 This method has recently been applied to the site-specific generation of N^{ε}-methyllysine in proteins from genetically encoded N^{ε}-allyloxycarbonyl-N^{ε}-methyl-L-lysine: H.-w. Ai, J. W. Lee and P. G. Schultz, *Chem. Commun.*, 2010, **46**, 5506–5508.
20 P. K. Sasmal, S. Carregal-Romero, W. J. Parak and E. Meggers, *Organometallics*, 2012, **31**, 5968–5970.
21 For recent applications based on the reduction of aromatic azides, see: (*a*) H. Abe, J. Wang, K. Furukawa, K. Oki, M. Uda, S. Tsuneda and Y. Ito, *Bioconjugate Chem.*, 2008, **19**, 1219–1226; (*b*) Z. Pianowski, K. Gorska, L. Oswald, C. A. Merten and N. Winssinger, *J. Am. Chem. Soc.*, 2009, **131**, 6492–6497; (*c*) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078–10080; (*d*) M. Röthlingshöfer, K. Gorska and N. Winssinger, *Org. Lett.*, 2012, **14**, 482–485.
22 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 and E. Meggers, *ChemBioChem*, 2012, **13**, 1116–1120.
23 (*a*) A. S. K. Hashmi, *Chem. Rev.*, 2007, **107**, 3180–3211; (*b*) D. J. Gorin, B. D. Sherry and F. D. Toste, *Chem. Rev.*, 2008, **108**, 3351–3378.
24 (*a*) M. J. Jou, X. Chen, K. M. K. Swamy, H. N. Kim, H.-J. Kim, S.-g. Lee and J. Yoon, *Chem. Commun.*, 2009, 7218–7220; (*b*) Y.-K. Yang, S. Lee and J. Tae, *Org. Lett.*, 2009, **11**, 5610–5613; (*c*) J.-B. Wang, Q.-Q. Wu, Y.-Z. Min, Y.-Z. Liu and Q.-H. Song, *Chem. Commun.*, 2012, **48**, 744–746.
25 J. H. Do, H. N. Kim, J. Yoon, J. S. Kim and H.-J. Kim, *Org. Lett.*, 2010, **12**, 932–934.
26 (*a*) L. Vigh, F. Joó and Á. Cséplö, *Eur. J. Biochem.*, 1985, **146**, 241–244; (*b*) S. Benkö, H. Hilkmann, L. Vigh and W. J. van Blitterswijk, *Biochim. Biophys. Acta*, 1987, **896**, 129–135; (*c*) L. Vigh, D. A. Los, I. Horváth and N. Murata, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 9090–9094; (*d*) I. Horváth, A. Glatz, V. Varvasovszki, Z. Török, T. Páli, G. Balogh, E. Kovács, L. Nádasdi, S. Benkö, F. Joó and L. Vigh, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 3513–3518.
27 J. Li and P. R. Chen, *ChemBioChem*, 2012, **13**, 1728–1731.
28 R. M. Yusop, A. Unciti-Brocetal, E. M. V. Johansson, R. M. Sánchez-Martín and M. Bradley, *Nat. Chem.*, 2011, **3**, 239–243.
29 A. Unciti-Broceta, E. M. V. Johansson, R. M. Yusop, R. M. Sánchez-Martín and M. Bradley, *Nat. Protocols*, 2012, **7**, 1207–1218.
30 C. D. Spicer, T. Triemer and B. G. Davis, *J. Am. Chem. Soc.*, 2012, **134**, 800–803.
31 J. M. Chalker, C. S. C. Wood and B. G. Davis, *J. Am. Chem. Soc.*, 2009, **131**, 16346–16347.
32 N. Li, R. K. V. Lim, S. Edwardraja and Q. Lin, *J. Am. Chem. Soc.*, 2011, **133**, 15316–15319.