JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

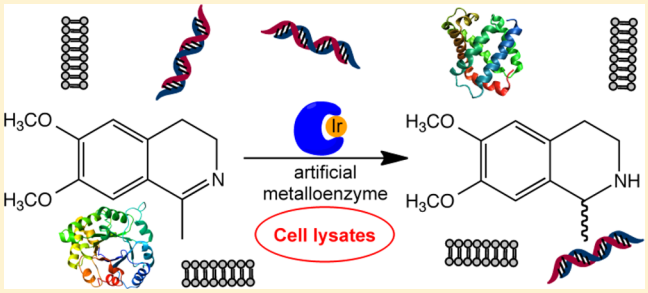
Neutralizing the Detrimental Effect of Glutathione on Precious Metal Catalysts

Yvonne M. Wilson,[‡,†] Marc Dürrenberger,[‡] Elisa S. Nogueira, and Thomas R. Ward*

Department of Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Supporting Information

ABSTRACT: We report our efforts to enable transition-metal catalysis in the presence of cellular debris, notably *Escherichia coli* cell free extracts and cell lysates. This challenging goal is hampered by the presence of thiols, mainly present in the form of glutathione (GSH), which poison precious metal catalysts. To overcome this, we evaluated a selection of oxidizing agents and electrophiles toward their potential to neutralize the detrimental effect of GSH on a Ir-based transfer hydrogenation catalyst. While the bare catalyst was severely inhibited by cellular debris, embedding the organometallic moiety within a host protein led to promising results in the presence of some neutralizing agents. In view of its complementary to natural enzymes, the asymmetric imine reductase based on the incorporation of a biotinylated iridium pianostool complex within streptavidin (Sav) isoforms was selected as a model reaction. Compared to purified protein samples, we show that pretreatment of cell free extracts and cell lysates containing Sav mutants with diamide affords up to >100 TON's and only a modest erosion of enantioselectivity.



INTRODUCTION

In many respects, organometallic- and enzymatic catalysis can be regarded as complementary. Over the past four decades, these two disciplines have by-and-large evolved independently. In recent years, however, there has been an increasing interest in exploiting organometallic catalysis in the context of chemical biology.[1] To achieve this ambitious goal, however, the compatibility of organometallic catalysts within the sea of functionality present in a cell must be ensured.

In recent years, there have been a handful of reports on precious-metal organometallic catalysis within living cells or in the presence of cell lysates.[2] To the best of our knowledge, however, the catalytic efficiency, as reflected by the very high catalyst loading used, remains modest in most cases.[3]

With the aim of improving the catalytic performance in the presence of cellular components, we have identified two potential challenges that may limit the versatility of organometallic catalysis in a cellular environment: (i) the organometallic catalyst and the enzymes present in a cell suffer from mutual inhibition;[4] and (ii) the reduced form of glutathione (GSH hereafter), present in millimolar concentration in cells cultivated under aerobic conditions, may poison the precious transition metals catalysts.

To overcome the mutual inhibition challenge, which may lead to inhibition of both organometallic catalyst and enzyme, we have exploited the potential of artificial metalloenzymes. Such hybrid catalysts result from the incorporation of an organometallic moiety within a protein environment, thus conferring a shielding second coordination sphere, reminiscent of natural enzymes.[5−7] Inspired by the pioneering work of Wilson and Whitesides,[6a] we rely on the biotin−streptavidin technology to incorporate a biotinylated d^6-Ir pianostool complex [Cp*Ir(biot-*p*-L)Cl] within streptavidin (Sav hereafter) to yield an artificial transfer hydrogenase. Genetic optimization revealed that mutation at position S112 offered an attractive means to improve (and revert) the enantioselectivity: [Cp*Ir(biot-*p*-L)Cl] ⊂ S112A Sav and [Cp*Ir(biot-*p*-L)Cl] ⊂ S112K Sav yield the opposite enantiomers of salsolidine in 96 and 78% ee under optimized conditions using purified Sav.[6b,8]

Herein, we report our efforts to address *ex vivo* the GSH poisoning of precious-metal organometallic catalysts. For this purpose, we set out to screen a variety of Michael acceptors as well as oxidizing agents known to react with glutathione.

RESULTS AND DISCUSSION

Inspired by the reports of Meggers[3] Sadler[8] and Teply,[2b] suggesting that d^6-pianostool complexes are particularly robust scaffolds for reactions within cells or in the presence of cell lysates, we selected the IrCp*-catalyzed asymmetric transfer hydrogenation as a benchmark reaction.

For screening purposes, we selected *Escherichia coli* cell-free extracts and cell lysates (see experimental details in the Supporting Information (SI)). Following overexpression of a Sav isoform in the *E. coli* strain BL21(DE3) pLysS, the cell pellet was lysed by freeze−thaw cycles followed by addition of Tris-HCl buffer containing DNase I and phenylmethylsulfonyl-

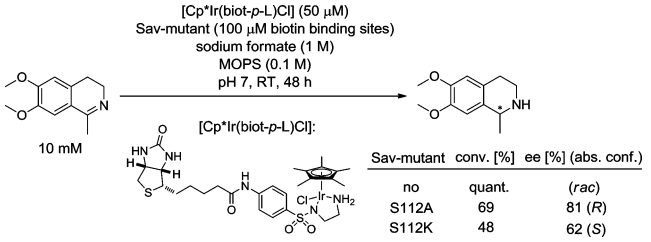
Received: January 20, 2014
Published: June 11, 2014

   dx.doi.org/10.1021/ja500613n | *J. Am. Chem. Soc.* 2014, 136, 8928−8932

fluoride as protease inhibitor. Catalysis was either performed in the resulting cell lysate or with dried cell free extract (cfe) obtained by centrifugation and lyophilization of the supernatant to yield a brown powder. The cfe was stored at 4 °C until use. The biotin-binding site concentration of the cell lysates and cfe was determined using the biotin-4-fluorescein assay (see Figure SI1).[9] The total thiol concentration of the cfe was estimated to approximatively 500 μM using Ellman's reagent.[10] As Sav, which is devoid of cysteines, represents >50% of the proteins present in the cfe, we conclude that most of the cysteines quantified by the Ellman test are contained in the glutathione present in cfe.

For comparison purposes, catalytic transfer hydrogenase experiments using [Cp*Ir(biot-*p*-L)Cl] were carried out with (i) purified Sav samples, (ii) purified Sav samples spiked with GSH, (iii) cfe containing Sav isoforms, (iv) cfe containing no Sav (resulting from an *E. coli* culture using an plasmid devoid of the Sav gene), and (v) cell lysates containing Sav isoforms. Prior to this study, typical catalysis experiments using purified Sav samples were performed using 250 μM [Cp*Ir(biot-*p*-L)Cl], 500 μM biotin binding sites (i.e., 125 μM tetrameric Sav). With *in vivo* catalysis in mind, we set out to decrease the catalyst and substrate concentrations. Initial experiments were thus performed using 50 μM [Cp*Ir(biot-*p*-L)Cl], 100 μM biotin binding sites (i.e. 25 μM tetrameric Sav), 1 M HCO_2Na in MOPS buffer (100 mM, pH 7) (see Scheme 1). For comparison purposes, the imine reduction was performed with [Cp*Ir(biot-*p*-L)Cl]; [Cp*Ir(biot-*p*-L)Cl] ⊂ S112A Sav and [Cp*Ir(biot-*p*-L)Cl] ⊂ S112K Sav, Scheme 1. The results are summarized in Table 1.

Scheme 1. Asymmetric Transfer Hydrogenation Catalyzed by an Artificial Imine Reductase As a Benchmark Reaction to Test the Neutralization of the Detrimental Effect of GSH



Catalysis results from experiments performed at 125 μM Sav and 25 μM Sav are similar, suggesting that the ATHase is well suited to operate at low concentrations (Table 1, compare entries 1−3 to 4−6). Upon addition of ≥0.1 mM GSH (i.e., two equivalents vs Ir), all three catalytic systems were completely inhibited (Table 1, entries 7−18). This emphasizes the severe poisoning effect of GSH toward the catalyst precursor. Substitution of the chloride ligand by a pyridine (i.e., [Cp*Ir(biot-*p*-L)pyridine]), as suggested by Sadler,[8b] did not improve significantly the catalytic performance in the presence of GSH toward the reduction of the salsolidine precursor. In contrast, addition of GSSG had a less negative impact on the reaction, although in the case of the bare Ir-complex and the S112K mutant, the conversions were found to be modest (Table 1, entries 19−21). We thus speculated that oxidizing GSH or derivatizing its thiol function could—at least partially—prevent inhibition of the transfer hydrogenation catalyst.

We selected Michael acceptors and oxidizing agents known to react with GSH to yield the corresponding thioether and disulfide, respectively. The following Michael acceptors were tested: maleinimide **MalIn**, 2-bromo-1-phenylethanone **BrPheOne**, phenyl-vinylsulfone **PheViSul**, and 3-phenyl-2-propynenitrile **PhePropNit**. The following oxidizing agents were selected: oxone **Ox**, 1,4-benzoquinone **BQ**,[11] $K_3[Fe(CN)_6]$ $\mathbf{Fe^{3+}}$,[12] and diamide **DiAm**,[13] Figure 1. For this purpose, solutions either with or without a Sav isoform were spiked with 2.5 mM GSH and incubated overnight in the presence of different concentrations of a particular GSH neutralizing agent before adding the Ir-catalyst. The reactions were initiated by addition of the imine substrate and run for 2 days (see Table 1 in the SI). The main findings of this screening include: (1) $\mathbf{Fe^{3+}}$ and **BQ** are not compatible with any of the three catalytic systems; even in the absence of GSH, no or low conversions are observed. (2) **MalIn** exhibits limited compatibility with the Ir-catalyst, especially when the latter is embedded within Sav. However, apart from this, **MalIn** proved to be a poor GSH neutralizing agent under the selected experimental conditions. (3) **PhePropNit** is most effective when applied at a ratio **PhePropNit**/GSH 2:1. An equimolar amount has no benefit on the reaction, whereas four equivalents led to a decrease in conversion and enantioselectivity in the case of the S112K mutant. (4) Despite the fact that H_2O_2 is commonly used to oxidize GSH, we favored **Ox** for this purpose. Indeed, it was found to be more compatible with the experimental setup as the presence of catalases in the cfe lead to significant gushing upon addition of H_2O_2. Strikingly, although **Ox** is a two electron oxidant, its efficacy is most pronounced with four equivalents vs GSH. This behavior may be traced back to the fact that, in the presence of formate, GSH is oxidized to the corresponding sulfonic acid rather than the disulfide GSSG.[14] (5) The most effective GSH neutralizing agents are **BrPheOne**, **PheViSul**, and **DiAm.** Compared to reactions where GSH is absent, all these agents led to comparable conversions and enantioselectivities in most cases when incubated for either 2 or 15 h to GSH-spiked solutions prior to catalysis. Increasing the concentration of the GSH neutralizing agents leads to less consistent results; the outcome of the reaction depends on the Sav mutant (if any) is used. For example, **DiAm** is fully compatible with the artificial metalloenzymes but not with free [Cp*Ir(biot-*p*-L)Cl] as increasing concentrations led to a decrease in conversion. A similar behavior is observed with **BrPheOne** when no Sav or the S112K mutant was present. On the other hand, **PheViSul** does not affect the free Ir-complex but limited compatibility was observed with the hybrid catalysts especially in absence of GSH.

With regard to these results, the following properties of the GSH neutralizing agents have to be considered: (i) efficiency of GSH neutralization; (ii) (mutual) inhibition between the catalyst and the GSH neutralizing agent (compatibility), (iii) inhibition of the catalyst and the product of the GSH neutralization (disulfide and thioether respectively), (iv) reduction of GSSG to GSH by the Ir-catalyst, (v) competing catalytic reduction of the GSH neutralizing agent by the Ir-catalyst when used in excess and vi) derivatization of Sav amino acid side chains by the Michael acceptors.

Next, the most promising glutathione neutralizing agents **BrPheOne**, **PheViSul**, and **DiAm** were tested in the presence of cell free extracts, Figure 2. While the bare catalyst was inactive, up to 22 turnovers (i.e., 11% conversion at 0.5 mol %

dx.doi.org/10.1021/ja500613n | J. Am. Chem. Soc. 2014, 136, 8928−8932

Table 1. Catalysis with Purified Sav at Low Catalyst Concentration and in the Presence of GSH and GSSG at RT (for further details see SI)

entry	Sav mutant	[Sav] (μM)	[Ir] (μM)	[formate] (M)	[GSH] (mM)	[GSSG] (mM)	[substrate] (mM)	time (h)	conversion[b] (%)	ee[a,b] (%)
1	no	125	250	3	0	0	50	4.5	quant.	0
2	S112A	125	250	3	0	0	50	4.5	89 ± 1	90 ± 1
3	S112K	125	250	3	0	0	50	4.5	70 ± 3	−75 ± 1
4	no	25	50	1	0	0	10	48	quant.	0
5	S112A	25	50	1	0	0	10	48	69 ± 5	81 ± 1
6	S112K	25	50	1	0	0	10	48	48 ± 1	−62 ± 2
7	no	25	50	1	0.025	0	10	48	92 ± 2	0
8	no	25	50	1	0.05	0	10	48	59 ± 4	0
9	no	25	50	1	0.1	0	10	48	0	–
10	no	25	50	1	2.5	0	10	48	0	0
11	S112A	25	50	1	0.025	0	10	48	56 ± 2	83 ± 1
12	S112A	25	50	1	0.05	0	10	48	37 ± 3	83 ± 2
13	S112A	25	50	1	0.1	0	10	48	0	–
14	S112A	25	50	1	2.5	0	10	48	0	0
15	S112K	25	50	1	0.025	0	10	48	23 ± 2	−60 ± 2
16	S112K	25	50	1	0.05	0	10	48	9 ± 5	−55 ± 3
17	S112K	25	50	1	0.1	0	10	48	0	–
18	S112K	25	50	1	2.5	0	10	48	0	0
19	no	25	50	1	0	2.5	10	48	19 ± 1	0
20	S112A	25	50	1	0	2.5	10	48	50 ± 1	84 ± 2
21	S112K	25	50	1	0	2.5	10	48	15 ± 1	−47 ± 3

[a]Positive ee values correspond to (*R*)-salsolidine, and negative ee values correspond to (*S*)-salsolidine. [b]The ± values represent standard deviations resulting from triplicate measurements. GSH: reduced form of glutathione; GSSG: oxidized form of glutathione (glutathione disulfide).

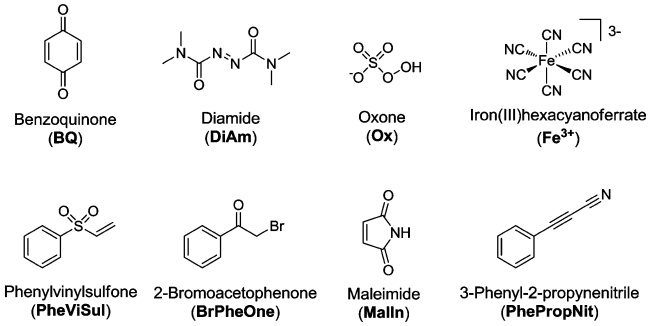


Figure 1. Stoichiometric reagents tested to neutralize glutathione (top oxidizing agents, bottom Michael acceptors).

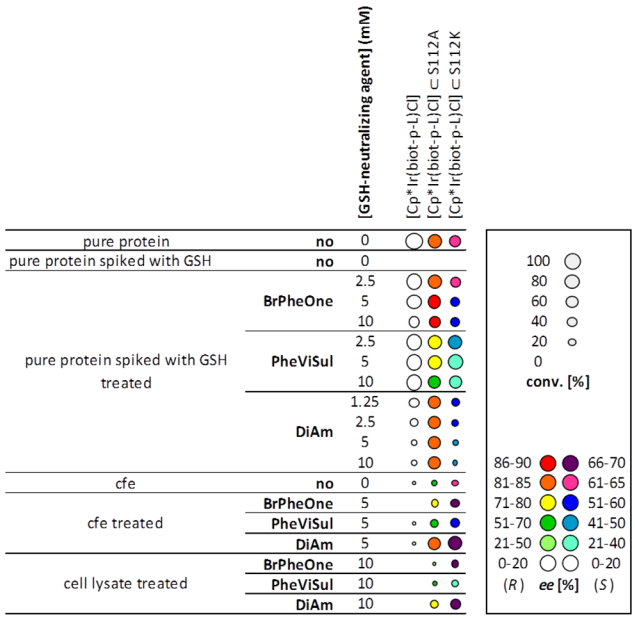


Figure 2. Fingerprint representation of ATHase activity in the presence of GSH neutralizing agents.

catalyst loading) were obtained for the [Cp*Ir(biot-*p*-L)Cl] ⊂ S112A Sav variant without pretreatment of the cfe with a GSH neutralizing agent. This clearly demonstrates the efficacy of the second coordination sphere to protect the precious metal from cell debris, Table 2 entries 5 and 6. In the presence of a GSH neutralizing agent, the catalytic performance could be improved significantly for the artificial metalloenzyme. **DiAm** proved most effective at concentrations of 5−10 mM leading up to 110 turnovers in case of the S112A mutant and 96 turnovers with the S112K mutant (Table 2, entry 14 and 15). This amounts to up to 72% recovery of the original activity of these catalysts, without loss of selectivity. Reducing the preincubation time to 2 h yielded nearly identical results (Table 2, entry 16 and 17). On the other hand, none of the three GSH neutralizing agents had a beneficial effect on the bare Ir-complex in cfe's: conversions do not exceed 5% (Table 2, entry 7, 10 and 13).

Recent saturation kinetic studies demonstrate that the [Cp*Ir(biot-*p*-L)Cl] ⊂ S112A Sav ATHase exhibits higher reaction rates than its [Cp*Ir(biot-*p*-L)Cl] ⊂ S112K Sav counterpart (k_{cat} = 11.4 min^{-1} vs k_{cat} = 2.6 min^{-1}). Although these kinetic profiles are reflected in the initial screening experiments in the presence of purified Sav, the S112K variant generally yields better conversions in the presence of pretreated cfe. Assuming that the neutralization of GSH in the cellular environment is as efficient as in the presence of purified protein, this finding suggests that GSH may be the main but not the sole inhibitor of the precious metal catalyst. Considering the propensity of precious metals to interact with guanine in oligonucleotides,[15] we speculate that the cationic lysine residue at position S121 K interacts with the

dx.doi.org/10.1021/ja500613n | J. Am. Chem. Soc. 2014, 136, 8928−8932

Table 2. Selected Results Obtained from Catalysis in the Presence of Cell Free Extracts (cfe) and Cell Lysates[a]

entry	Sav mutant	origin	GSH neutralizing agent	concentration (mM)	preincubation time (h)	conversion[c] (%)	ee[b,c] (%)
1	no	–	no	–	–	98 ± 1	0
2	S112A	purified	no	–	–	95 ± 3	82 ± 2
3	S112K	purified	no	–	–	67 ± 3	−64 ± 3
4	no	cfe[d]	no	–	–	2 ± 1	o
5	S112A	cfe	no	–	–	11 ± 2	68 ± 2
6	S112K	cfe	no	–	–	8 ± 2	−55 ± 5
7	no	cfe[d]	**BrAcPhe**	5	15	0	–
8	S112A	cfe	**BrAcPhe**	5	15	20 ± 1	74 ± 3
9	S112K	cfe	**BrAcPhe**	5	15	25 ± 3	−62 ± 6
10	no	cfe[d]	**PheViSul**	5	15	2 ± 1	0
11	S112A	cfe	**PheViSul**	5	15	22 ± 5	62 ± 3
12	S112K	cfe	**PheViSul**	5	15	32 ± 1	−44 ± 6
13	no	cfe[d]	**DiAm**	10	15	4 ± 1	0
14	S112A	cfe	**DiAm**	5	15	55 ± 6	83 ± 2
15	S112K	cfe	**DiAm**	5	15	48 ± 5	−60 ± 4
16	S112A	cfe	**DiAm**	10	2	49 ± 10	85 ± 1
17	S112K	cfe	**DiAm**	10	2	42 ± 1	−64 ± 6
18	no	cell lysate	no	–	–	0	–
19	S112A	cell lysate	no	–	–	0	–
20	S112K	cell lysate	no	–	–	0	–
21	S112A	cell lysate	**BrAcPhe**	10	15	6 ± 1	59 ± 17
22	S112K	cell lysate	**BrAcPhe**	10	15	31 ± 12	−66 ± 1
23	S112A	cell lysate	**PheViSul**	10	15	9 ± 1	54 ± 11
24	S112K	cell lysate	**PheViSul**	10	15	20 ± 5	−42 ± 6
25	no	cell lysate[d]	**DiAm**	10	15	<1	n.d.
26	S112A	cell lysate	**DiAm**	10	15	22 ± 3	70 ± 6
27	S112K	cell lysate	**DiAm**	10	15	43 ± 7	−68 ± 1

[a]All reactions were performed with 50 μM [Cp*Ir(biot-*p*-L)Cl], 25 μM tetrameric Sav, 0.6 M MOPS pH 7, and 3 M sodium formate at RT for 48 h (see SI for details). [b]Positive ee values correspond to (*R*)-salsolidine and negative ee values correspond to (*S*)-salsolidine. [c]The ± values represent standard deviations resulting from triplicate measurements. [d]cfe containing no Sav resulting from an *E. coli* culture using an plasmid devoid of the Sav gene.

phosphate of the (oligo)nucleotide, hampering N^7 guanine coordination to the iridium moiety with this mutant.

Encouraged by these findings, we tested the ATHase with cell lysates (see SI for a detailed procedure). Again here, the best results were achieved with **DiAm** yielding up to 44 turnovers (S112A) and 86 (S112K) turnovers, respectively (Table 2, entry 26 and 27). Besides the lower conversions obtained compared to experiments performed with cfe, in the case of the S112A-ATHase also a slight decrease of enantioselectivity was observed. The S112K mutant shows no degradation in agreement with the assumption that the latter better protects the Ir-center from inhibitors.

OUTLOOK

From the data presented in this study, we conclude that diamide **DiAm** is a promising GSH oxidizing agent which is shown to be compatible with organometallic catalysis on cell free extracts as well as cell lysates. This important finding will allow us to apply directed evolution protocols to optimize the performance of artificial transfer hydrogenases with crude cellular extracts.

With the ultimate goal of performing precious metal catalysis *in vivo*, it is noteworthy that Kosower has shown that diamide **DiAm** is compatible with living cells, including *E. coli*, neither causing lysis nor death.[13,16] The next challenge is to engineer a transport of the abiotic metal cofactor within *E. coli* expressing Sav to ultimately perform catalysis *in vivo*.

ASSOCIATED CONTENT

Supporting Information

General procedure for the preparation of cell free extracts and cell lysates, experimental details, and additional data. This material is available free of charge via the Internet at http://pubs.acs.org

AUTHOR INFORMATION

Corresponding Author

thomas.ward@unibas.ch

Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing financial interest.

†Deceased on January 22, 2014.

ACKNOWLEDGMENTS

T.R.W. thanks the Swiss National Science Foundation (grant 200020_144354) and the EU (ITN Biotrains FP7-ITN-238531 for a Marie Curie fellowship to ESN) for financial support. Prof. C. R. Cantor is thanked for the streptavidin gene and Umicore precious metal chemistry for a loan of iridium. T.R.W. thanks Prof. F. Seebeck for insightful comments. M.D. thanks Fabian Schwizer for help with Figure 2.

REFERENCES

(1) (a) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13. (b) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974. (c) Stephanopoulos, N.; Francis, M. B. *Nat. Chem. Biol.* **2011**, *7*, 876.

(2) (a) Streu, C.; Meggers, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 5645. (b) driaenssens, L.; Severa, L.; Vávra, J.; Šálová, T.; Hývl, J.; Čížková, M.; Pohl, R.; Šaman, D.; Teplý, F. *Collect. Czech. Chem. Commun.* **2009**, *74*, 1023. (c) Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sánchez-Martín, R. M.; Bradley, M. *Nat. Chem.* **2011**, *3*, 239. (d) Sasmal, P. K.; Carregal-Romero, S.; Han, A. A.; Streu, C. N.; Lin, Z.; Namikawa, K.; Elliott, S. L.; Köster, R. W.; Parak, W. J.; Meggers, E. *ChemBioChem* **2012**, *13*, 1116. (e) Do, J. H.; Kim, H. N.; Yoon, J.; Kim, J. S.; Kim, H.-J. *Org. Lett.* **2010**, *12*, 932. (f) Chalker, J.; Wood, C.; Davis, B. *J. Am. Chem. Soc.* **2009**, *131*, 16346. (g) Lin, Y. A.; Chalker, J. M.; Davis, B. J. *ChemBioChem* **2009**, *10*, 959. (h) Giannini, F.; Süss-Fink, G.; Furrer, J. *Inorg. Chem.* **2011**, *50*, 10552. (i) Soriano del Amo, D.; Wang, W.; Jiang, H. *J. Am. Chem. Soc.* **2010**, *132*, 16894. (j) Lee, Y.; Umeano, A.; Balskus, E. P. *Angew. Chem., Int. Ed.* **2013**, *52*, 11800. (k) Ball, Z. T. *Acc. Chem. Res.* **2013**, *46*, 560. (l) Li, N.; Lim, R. K. V.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, *133*, 15316. (m) Li, J.; Yu, J.; Zhao, J.; Wang, J.; Zheng, S.; Lin, S.; Chen, L.; Yang, M.; Jia, S.; Zhang, X.; Chen, P. R. *Nat. Chem.* **2014**, *6*, 352.

(3) Sasmal, P. K.; Streu, C. N.; Meggers, E. *Chem. Commun.* **2013**, *49*, 1581.

(4) Poizat, M.; Arends, I. W. C. E.; Hollmann, F. *J. Mol. Catal. B: Enzym.* **2010**, *63*, 149.

(5) (a) Rosati, F.; Roelfes, G. *ChemCatChem.* **2010**, *2*, 916. (b) Deuss, P. J.; den Heeten, R.; Laan, W.; Kamer, P. C. *Chem.—Eur. J.* **2011**, *17*, 4680. (c) Ringenberg, M. R.; Ward, T. R. *Chem. Commun.* **2011**, *47*, 8470. (d) Ward, T. R. *Acc. Chem. Res.* **2011**, *44*, 47.

(6) (a) Wilson, M. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1978**, *100*, 306. (b) Koehler, V.; Wilson, Y. M.; Dürrenberger, M.; Ghislieri, D.; Churakova, E.; Quinto, T.; Knörr, L.; Häussinger, D.; Hollmann, F.; Turner, N. J.; Ward, T. R. *Nat. Chem.* **2013**, *5*, 93.

(7) Wang, Z. J.; Clary, K. N.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *Nat. Chem.* **2013**, *5*, 100.

(8) (a) Dougan, S. J.; Habtemariam, A.; McHale, S. E.; Parsons, S.; Sadler, P. J. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 11628. (b) Liu, Z.; Romero-Canelón, I.; Qamar, B.; Hearn, J. M.; Habtemariam, A.; Barry, N. P. E.; Pizarro, A. M.; Clarkson, G. J.; Sadler, P. J. *Angew. Chem., Int. Ed.* **2014**, *53*, 3941.

(9) (a) Kada, G.; Kaiser, K.; Falk, H.; Gruber, H. J. *Biochim. Biophys. Acta* **1999**, *1427*, 44. (b) Humbert, N.; Zocchi, A.; Ward, T. R. *Electrophoresis* **2005**, *26*, 47.

(10) Riddles, P. W. *Anal. Biochem.* **1979**, *94*, 75.

(11) Lau, S. S.; Hill, B. A.; Highet, R. J.; Monks, T. J. *Mol. Pharmacol.* **1988**, *34*, 829.

(12) Stochel, G.; Martinez, P.; Van Eldik, R. *J. Inorg. Biochem.* **1994**, *54*, 131.

(13) Wax, R.; Rosenberg, E.; Kosower, N. S.; Kosower, E. M. *J. Bacteriol.* **1970**, *101*, 1092.

(14) Calam, D. H.; Waley, S. G. *Biochem. J.* **1962**, *85*, 417.

(15) Wang, F.; Xu, J.; Wu, K.; Weidt, S. K.; Mackay, C. L.; Langridge-Smith, P. R. R.; Sadler, P. J. *Dalton Trans.* **2013**, *42*, 3188.

(16) Kosower, E. M.; Kosower, N. S. *Nature* **1969**, *224*, 117.