A Journal of the Gesellschaft Deutscher Chemiker

Angewandte

GDCh

International Edition

Chemie

www.angewandte.org

Accepted Article

Title: Bioorthogonal Catalytic Activation Of Pt And Ru Anticancer Complexes By FAD And Flavoproteins

Authors: Silvia Alonso-de Castro, Aitziber Lopez Cortajarena, Fernando Lopez-Gallego, and Luca Salassa

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201800288
Angew. Chem. 10.1002/ange.201800288

Link to VoR: http://dx.doi.org/10.1002/anie.201800288
http://dx.doi.org/10.1002/ange.201800288

WILEY-VCH

10.1002/anie.201800288

COMMUNICATION

WILEY-VCH

Bioorthogonal Catalytic Activation Of Pt And Ru Anticancer Complexes By FAD And Flavoproteins

Silvia Alonso-de Castro, Aitziber L. Cortajarena, Fernando López-Gallego,* Luca Salassa*

Abstract: *Recent advances in bioorthogonal catalysis promise to deliver new chemical tools for performing chemoselective transformations in complex biological environments. Herein we report how FAD (flavin adenine dinucleotide), FMN (flavin mononucleotide) and four flavoproteins behave as unconventional photocatalysts capable of converting Pt^{IV} and Ru^{II} complexes into potentially toxic Pt^{II} or Ru^{II}–OH_2 species. Using electron donors and low doses of visible light, the flavoproteins mini Singlet Oxygen Generator (miniSOG) and NADH oxidase (NOX) catalytically activate Pt^{IV} prodrugs with bioorthogonal selectivity. In the presence of NADH, NOX catalyzes Pt^{IV} activation in the dark as well, indicating for the first time that flavoenzymes may contribute to initiate the activity of Pt^{IV} chemotherapeutic agents.*

The latest advancements in bioorthogonal chemistry[1] demonstrate how organometallic compounds and inorganic materials are capable of catalyzing the activation of profluorescent substrates and prodrugs with remarkable efficiency in biological environments.[2-10] These selective catalysts carry out non-natural reactions dodging the interference of biological molecules, using in some cases endogenous cellular components as co-reactants.[3, 9]

In this context, we recently reported a new bioorthogonal reaction in which riboflavin photoactivates a Pt^{IV} prodrug with extremely low doses of blue light through a catalytic mechanism in the presence of zwitterionic electron donors. Light activation of the riboflavin-prodrug pair triggers cisplatin-related antiproliferative activity in PC3 cancer cells.[11] Unlike classic organometallic catalysis, where metals act as catalysts, in this reaction the metal complex is an unconventional substrate[12] and the biocompatible riboflavin is the catalyst.

Herein, we report fundamental discoveries in this new type of bioorthogonal chemistry by (i) investigating the catalytic behavior of various flavin catalysts, including four flavoproteins with diverse biological functions and flavin binding pockets, (ii) increasing the pool of inorganic reactions to different Pt^{IV} and Ru^{II} prodrug complexes and (iii) evaluating the efficiency of (bio)organic electron donors (Figure 1). Furthermore, our work shows for the first time that certain flavoproteins may be directly implicated in the activation of metallodrugs under biologically relevant conditions in the absence of light.

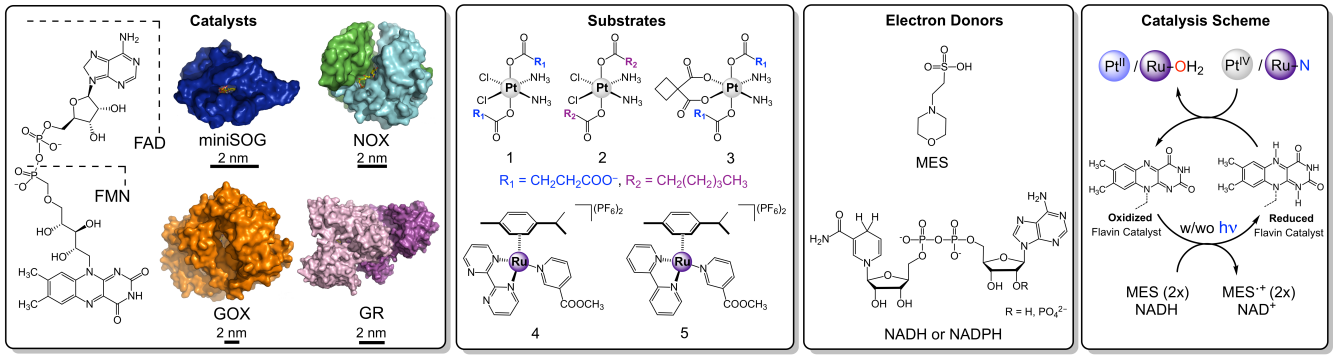


Figure 1. Structures of catalysts, substrates and electron donors employed in this study and proposed catalysis mechanism.

[*] Prof. Luca Salassa
Donostia International Physics Center
Paseo Manuel de Lardizabal 4, Donostia, 20018 (Spain)
E-mail: lsalassa@dipc.org

S. Alonso-de Castro, Prof. A. L. Cortajarena, Prof. F. López-Gallego
CIC biomaGUNE
Paseo de Miramón 182, Donostia, 20014 (Spain)
E-mail: flopezgallego@unizar.es

Prof. Luca Salassa, Prof. A. L. Cortajarena
Ikerbasque, Basque Foundation for Science
Bilbao, 48011 (Spain)

Dr. F. López-Gallego
ARAID Foundation
Zaragoza, Spain

Supporting information for this article is given via a link at the end of the document.

Initially we investigated the capacity of FAD (flavin adenine dinucleotide) as catalyst for the photoactivation of two classes of anticancer metal complexes, namely Pt^{IV} octahedral and Ru^{II}-arene piano-stool complexes. Complexes **1**–**3** are prodrugs of cisplatin and carboplatin,[13] and complexes **4** and **5** are photoactivatable scaffolds capable of generating reactive Ru–OH_2 species, which can bind to biomacromolecules (Figure 2a).[14-17] Importantly, these Pt^{IV} and Ru^{II} complexes have poor absorption properties in the visible (Figure 2b) compared to other photoactivatable complexes, e.g. Ru polypyridyls. Therefore, novel strategies to prompt their photochemistry at longer wavelengths are pivotal for use in photochemotherapy. Complexes **1**–**5** are stable towards hydrolysis in the dark, and have either no or poor photoreactivity under blue light excitation.[11, 17-18]

FAD photocatalysis towards **1**–**5** was performed employing 10 μM of catalyst and 200 μM of metal substrate (5% catalyst load). In all irradiation experiments, we used an LED light source (6 mW·cm^{-2}) with an emission maximum at 460 nm and ^{1}H NMR to monitor and quantify the evolution of the photoreactions. Description of experimental methods and a complete set of dark and light-irradiation experiments are provided in the Supporting Information (Figure S1–S76).

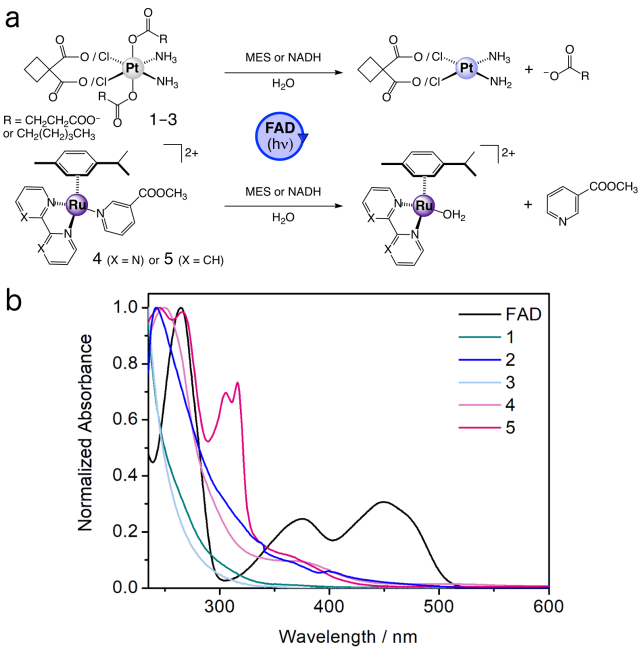


Figure 2. (a) Flavin-mediated photoactivation reactions of complexes **1**–**5;** (b) absorption spectra of FAD and **1**–**5.**

In first instance, we evaluated the effect of electron donors on the catalytic process using complex **1**, with the aim of optimizing reaction conditions. Three concentrations (0.2, 2 and 20 mM) of MES (as buffer, pH 6) or NADH (pH 7, 100 mM PB, i.e. phosphate buffer) were employed for this purpose. MES was selected as electron donor to follow up our previous work on riboflavin,[11] while NADH for its relevance as biological cofactor in numerous reactions catalyzed by flavoenzymes.[19] Moreover, metal-based catalytic drugs have been recently shown to kill cancer cells by interfering with the cellular NAD^{+}/NADH homeostasis.[20-22]

Upon 460-nm light excitation, FAD photoconverted the Pt^{IV} substrate and showed a catalytic efficiency which increased linearly alongside the MES concentration. FAD was fully inactive in the dark at any tested MES concentration. In the absence of light, 0.2 and 2 mM NADH did not induce any reaction for **1**, whereas light irradiation switched on the generation of photoproducts at 2 mM when FAD was present. At 2 mM NADH, FAD photocatalyzed the full conversion of **1** in only 2.5 min against the 5–10 min required by 20 mM MES. At the lowest concentration (0.2 mM), NADH was instantaneously photooxidized to NAD^{+} by molecular oxygen (O_2), precluding any catalytic reaction between FAD and the complex. Conversely, reduction of **1** at 20 mM NADH took place readily in the dark when FAD was present, or under light irradiation when the flavin was absent (Figure S1–8).

On this basis, we used 20 mM MES and 2 mM NADH to determine FAD photocatalytic activity towards **1**–**5** (Figure 2).[23] All complexes underwent photochemical activation in the presence of catalytic quantities of FAD (Figure S1–38). Consistently with their redox chemistry in the biological context,[24] FAD photoactivation of **1**–**4** with NADH was approximately twice as fast as MES. The only exception was **5**, towards which FAD displayed 3.4 times lower turnover frequency (TOF) using NADH than using MES (Table 1).

The kinetics of these catalytic reactions showed clear dependency on the substrate nature. Complexes **1**, **2** and **4** were the best substrates, having the highest TOFs and total turnover numbers (TTNs). Remarkably, FAD was able to complete the conversion of **1** and **2** into its corresponding photoproducts regardless of the electron donor used.

Table 1. Turnover frequency (TOF, min^{-1}) and total turnover number (TTN) for the FAD- and flavoprotein-catalyzed photoactivation of complexes **1**–**5** in the presence of MES and NADH.

Complex	MES (20 mM)			NADH (2 mM)[a]		
	TOF	TTN	% Conv.	TOF	TTN	% Conv.
	FAD					
1	2.3 ± 0.2	20	100	5.0 ± 1.7	20	100
2	4.0 ± 0.5	20	100	7.1 ± 1.8	20	100
3	0.6 ± 0.1	11	55	2.3 ± 0.6	14	70
4	4.5 ± 0.6	16	80	9.0 ± 2.3	20	100
5	2.2 ± 0.5	16	80	0.6 ± 0.1	14	70
	miniSOG					
1	1.0 ± 0.2	20	100	7.1 ± 0.4	20	100
2	1.2 ± 0.1	20	100	8.6 ± 2.2	20	100
	NOX					
1	0.62 ± 0.01	20	100	4.3 ± 1.6[b]	20	100
2	4.7 ± 1.2	20	100	8.3 ± 1.6[b]	20	100
	GOX					
1	Not active					
2	<0.1	5	20	<0.2	7.4	37
	GR[a]					
1	<0.1	10	50	0.42 ± 0.07	20	100
2	Not active			1.2 ± 0.3	20	100

[a] = experiments for GR were run using NADPH; [b] = in the dark

FAD allowed employing a convenient excitation wavelength (460 nm) and an extremely low light dose for the photoactivation of **1**–**5**. In the case of **1**, **2** and **4**, a light dose of ca. 1 J·cm^{-2} was sufficient to fully convert the complexes in their activated photoproducts. Ru^{II}-arene derivatives such as **4** and **5** typically require irradiation times exceeding 1 hour to reach ca. 50% conversion (Figure S24 and S32).[14-17] Herein, we show that less than 15 min are sufficient to achieve comparable effects in **4** and **5** when FAD was used as photocatalyst.

In the cell milieu, flavins are bound to proteins through covalent and non-covalent interactions,[19] which control their (photo)redox properties.[24] Exploiting flavoproteins as selective catalysts is therefore an exciting prospect for the design of bioorthogonal activation strategies for metal-based prodrugs. Accordingly, we selected four flavoproteins for their diverse flavin-binding pockets and explored their capacity to catalyze the photoreduction of **1** and **2** as substrates. This part of the study was limited to these derivatives for their relevance as anticancer compounds[13, 25] with respect to the Ru^{II} complexes **4** and **5**. Furthermore, FAD had superior activity towards these Pt^{IV} substrates with respect to **3**. The use of **2** was also aimed at gauging the role played by the charge of the substrates on the catalysis, having this complex a neutral alkyl chain at the axial position compared to the negatively charged succinate of **1**.

The flavoprotein catalysts tested were miniSOG (mini Singlet Oxygen Generator),[26] NOX (NADH oxidase from *Thermus thermophilus*),[27] GOX (glucose oxidase from *Aspergillus niger*)[28] and GR (glutathione reductase from *S. cerevisiae*).[29] MiniSOG is an FMN-containing (flavin mononucleotide) small protein investigated as a genetically encodable photosensitizer for the selective generation of singlet O_2.[30-32] The bacterial NOX enzyme generates hydrogen peroxide (H_2O_2) from O_2 oxidizing NADH, while the eukaryotic GOX naturally oxidizes glucose to H_2O_2 and D-glucono-δ-lactone. NOX and GOX have been both widely exploited in biocatalysis.[27, 33] GR is a NADPH-dependent oxidoreductase exerting a central role in glutathione metabolism for most aerobic organisms.[34] Conversely from the other flavoproteins, GR was selected because does not generate reactive oxygen metabolites.

Different chemical environments surround the flavin binding-pocket in these four flavoproteins, controlling solvent and substrate accessibility to the active site. As shown in Figure 3, miniSOG[35] and NOX, have more exposed flavins than GOX and GR, in which FAD is deeply buried into the protein scaffold. Solvent accessible surface areas of the flavins are 45.50 Å^2 for miniSOG, 67.92 Å^2 for NOX, while only 2.39 Å^2 for GOX and 4.01 Å^2 for GR. Moreover, they display different electrostatic surfaces in the proximity of the flavin binding-pocket (Figure S39–43). At pH 6–7, the NOX and GR active sites are neutral, whereas miniSOG and GOX display positive and negative electrostatic charges respectively.

Unless otherwise stated, photocatalysis experiments (Figure S44–76) were performed employing 10 μM flavoprotein catalysts, 200 μM **1** or **2** and either 20 mM MES or 2 mM NADH, in order to directly compare activities with the corresponding free flavin. Concentrations of flavins bound to proteins were calibrated by optical methods using FAD and FMN (for miniSOG) absorbance at 460 nm. Catalysis results for flavoproteins are summarized in Table 1.

As anticipated from inspecting their flavin active site, GOX and GR showed the lowest catalytic activity towards the Pt^{IV} substrates. GOX presented no catalytic activity towards **1** under none of the experimental conditions tested. Lack of activity was also found when glucose (20 mM), a natural substrate for the enzyme, was employed as source of electrons instead of MES or NADH. Conversion of **2** by GOX occurred in the presence of both electron donors, however reactions were slow and did not reach completion after 1 h of light irradiation (conversion < 40%). In MES, GR was poorly or no active towards **1** and **2**. On the contrary, NADPH prompted significantly higher TOF values and complete substrate conversion within few minutes of light exposure.

In MES, miniSOG and NOX converted **1** and **2** into their photoproducts exclusively upon blue light excitation. Whereas miniSOG showed no preference between the two substrates, NOX was ca. 7 times more efficient towards **2** than **1**. Light irradiation also switched on the catalytic activity of miniSOG in PB/NADH. The flavoprotein achieved full conversion of 200 μM **1** and **2** in ca. 4 min. In the case of **1**, this is approximately 5 times less efficient than free FMN (TOF 35.6 ± 4.3 min^{-1}, Figure S50).

To our surprise, NOX behaved differently, activating **1** and **2** in the dark when co-incubated with 2 mM NADH. Under such conditions, 10 μM NOX completely converted **1** in less than 7.5 min, while free FAD did not give any reaction with **1** over 3 h (*vide supra*). The TOF values of **1** and **2** for NOX were estimated to be 4.3 ± 1.6 min^{-1} and 8.3 ± 1.6 min^{-1} respectively, using less than 2 μM of flavoprotein to allow monitoring of the reaction by NMR.

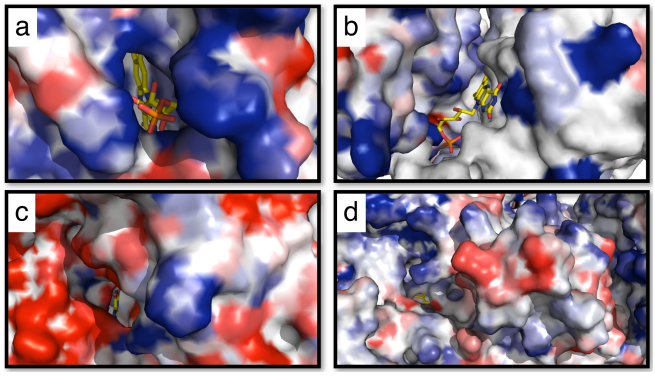


Figure 3. Electrostatic surface potential of the binding sites of (a) miniSOG, (b) NOX, (c) GOX and (d) GR (calculated using Bluues server). Red and blue colors represent anionic and cationic residues, respectively.[36]

The discovery of NOX catalytic activity in the dark has broad relevance for understanding the mechanism of action of Pt^{IV} anticancer agents. It is common assumption that Pt^{IV} complexes

are converted into active species by biological molecular reductants, such as glutathione or ascorbic acid, under physiological conditions.[37] Nevertheless, NOX-catalyzed activation of **1** and **2** in the presence of 2 mM NADH is significantly rapid and suggests that flavoproteins can provide alternative and highly efficient activation pathways for metallodrugs.[38]

Considering that the cellular concentration of NADH is in the 0.1–0.2 mM range,[39] we evaluated the capacity of NOX to convert 200 μM **1** with an equimolar quantity of NADH in PB (Figure S64). NOX naturally uses O_2 as electron acceptor to generate H_2O_2 in the presence of NADH.[27] At such low concentration, the enzyme consumed NADH too rapidly, precluding any catalytic conversion of **1**. For this reason, the reaction was studied under N_2 atmosphere. Accordingly, we determined that NOX could activate approximately a third of **1** in the absence of O_2, revealing that indeed flavoenzymes may turn on Pt drug activity under certain cellular conditions, i.e. hypoxia. Although the Pt^{IV} conversion did not reach completion, the concentration of activated **1** should reasonably be sufficient to induce cell death in cancer tissues.

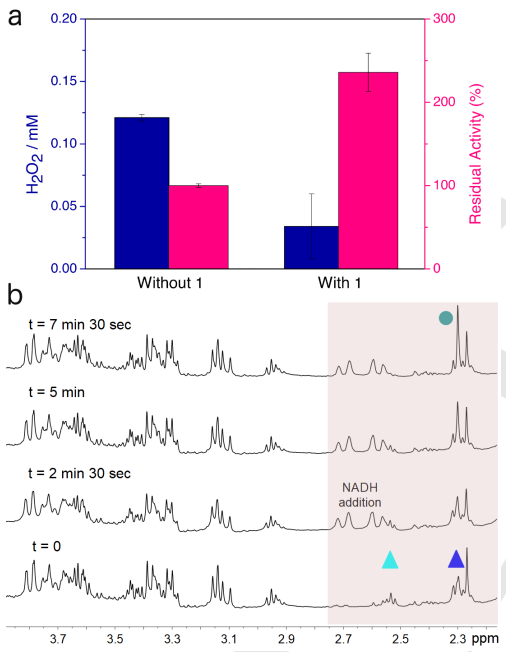


Figure 4. (a) NOX catalytic consumption rate of NADH (magenta) and generation of H_2O_2 (blue) measured employing a 1:1 ration of **1** and NADH at a concentration of 1 mM; (b) Dark catalytic activity of NOX in cell culture medium (pH 7) in the presence of NADH. ^{1}H NMR spectra were recorded for solutions of 200 μM **1** and 10 μM NOX and 2 mM NADH. ^{1}H NMR signal labelling: ▲ Pt–OCO**CH$_2$**CH$_2$CO$_2^-$, ▲ Pt–OCOCH$_2$**CH$_2$**CO$_2^-$, ● free $^-$O$_2$C**CH$_2$CH$_2$**CO$_2^-$.

At higher concentrations of electron donor in aerated solution, the enzyme reaction pathway is altered by **1**, which effectively competes with O_2 and intercepts electrons from the reduced flavoenzyme. In fact, the activity of NOX was increased by 2.3 fold while the production of H_2O_2 simultaneously lowered (Figure 4a) when 1 mM **1** was incubated with equimolar NADH. Under these conditions, the enzyme worked faster because it had access to higher concentrations of electron acceptors (**1** and O_2), and produced lower amounts of H_2O_2 because the hydride of NADH ought to be shared between O_2 and the metal complex reduction reactions. Conversely, miniSOG production of H_2O_2 is independent of the presence of **1** (Table S1), as it is likely occurring via photosensitization.[40]

The capacity of miniSOG and NOX to act as bioorthogonal catalysts towards Pt^{IV} prodrugs was investigated using **1** in cell culture medium, where components such as proteins, vitamins and salts can interfere with the activation process. Reactions in the presence of NADH (2 mM) showed that miniSOG converted **1** in the biological environment only under light irradiation, while the same reaction occurred already in the dark with NOX (Figure 4b and Figure S75). The flavoproteins retained practically the same selectivity and efficiency of free FAD under the same conditions (Figure S76).

A molecular description of the catalytic mechanism through which free flavins and flavoproteins activate Pt^{IV} and Ru^{II} complexes requires further investigations and is out of the scope of this manuscript. Our previous study suggests the catalysis is linked to the generation of the reduced forms of FAD and FMN (e.g. $FADH_2$ and $FMNH_2$), either through photoinduced electron transfer (MES) or by hydride transfer (NADH/NADPH). High levels of electron donors and light help increasing the catalytic efficiency of the flavins by stabilizing their active species and consequently enhancing reaction rates. Metal complexes may form transient adducts with the reduced flavins and undergo chemical and photochemical transformations.[11]

Nevertheless, it is clear from the results of this study that protein scaffolds play a crucial role in governing the accessibility of the metal substrate to the flavin catalytic site. So, the negative electrostatic surface of GOX and the shielded channel in which its FAD is bound prevent any interaction with the negatively charged **1**. Consistently, we observed that the consumption of glucose by GOX was not affected by the presence of equimolar **1** (Table S2). Although with poor efficiency, GOX activated **2** in agreement with the absence of charged chemical groups in the complex and its lower reduction potential compared to **1**.[41]

In the case of miniSOG and NOX, however, the protein scaffold enables the artificial catalysis by facilitating the formation of reduced FMN/FAD and favoring its stabilization for the subsequent electron transfer interaction with the Pt^{IV} substrates. Actually, miniSOG and NOX have more solvent exposed flavins and suitable electrostatic surfaces to allow metal substrates to access the active site. The role played by the protein scaffold is dramatic for NOX, which is an enzyme optimized by nature to transfer hydrides from NADH to electron acceptors. Consistently, NOX activity towards **1** and **2** is observed almost instantaneously in the dark with NADH. On the contrary, miniSOG, a protein derived from phototropin 2, which naturally does not use NADH as cofactor, requires light activation. Similarly to NOX and in contrast to GOX, GR has a mostly neutral FAD binding pocket and slightly positive surface charge, which allow catalytic activation of the **1** and **2**.

Nonetheless, GR requires light triggering for the catalysis likely due to the limited accessibility of its FAD with respect to NOX.

In conclusion, we show that free flavins and flavoproteins can catalyze artificial reactions of Pt^{IV} and Ru^{II} complexes, operating either in the dark or upon light excitation. Some of these unconventional reactions have promising catalytic efficiency and bioorthogonal selectivity. These findings open new opportunities for the design of chemically- and light-activated metal-based prodrugs, whose biological effects could be triggered endogenously by bioorthogonal flavoprotein catalysts.

Acknowledgements

We thank the Spanish MINECO for grant CTQ2016-80844-R, BIO2016-77367, BIO2015-69887-R and BES-2013-065642. COST action CM1303 is also acknowledged for support (FLG). We thank the European Research Council ERC-CoG-648071-ProNANO (ALC). Dr. D. Padró is acknowledged for his kind support with NMR experiments.

Keywords: photocatalysis, flavoproteins, metal-based prodrugs, bioorthogonal, photochemotherapy

[1] J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* 2005, **1**, 13-21.
[2] P. K. Sasmal, C. N. Streu, E. Meggers, *Chem. Commun.* 2013, **49**, 1581-1587.
[3] T. Völker, F. Dempwolff, P. L. Graumann, E. Meggers, *Angew. Chem. Int. Ed.* 2014, **53**, 10536-10540.
[4] A. M. Pérez-López, B. Rubio-Ruiz, V. Sebastián, L. Hamilton, C. Adam, T. L. Bray, S. Irusta, P. M. Brennan, G. C. Lloyd-Jones, D. Sieger, J. Santamaría, A. Unciti-Broceta, *Angew. Chem. Int. Ed.* 2017, **56**, 12548-12552.
[5] J. T. Weiss, J. C. Dawson, K. G. Macleod, W. Rybski, C. Fraser, C. Torres-Sánchez, E. E. Patton, M. Bradley, N. O. Carragher, A. Unciti-Broceta, *Nat. Commun.* 2014, **5**, 3277.
[6] R. M. Yusop, A. Unciti-Broceta, E. M. V. Johansson, R. M. Sánchez-Martín, M. Bradley, *Nat. Chem.* 2011, **3**, 239-243.
[7] J. Clavadetscher, S. Hoffmann, A. Lilienkampf, L. Mackay, R. M. Yusop, S. A. Rider, J. J. Mullins, M. Bradley, *Angew. Chem. Int. Ed.* 2016, **55**, 15662-15666.
[8] M. I. Sanchez, C. Penas, M. E. Vazquez, J. L. Mascareñas, *Chem. Sci.* 2014, **5**, 1901-1907.
[9] M. Tomás-Gamasa, M. Martínez-Calvo, J. R. Couceiro, J. L. Mascareñas, *Nat. Commun.* 2016, **7**, 12538.
[10] G. Y. Tonga, Y. Jeong, B. Duncan, T. Mizuhara, R. Mout, R. Das, S. T. Kim, Y.-C. Yeh, B. Yan, S. Hou, V. M. Rotello, *Nat. Chem.* 2015, **7**, 597-603.
[11] S. Alonso-de Castro, E. Ruggiero, A. Ruiz-de-Angulo, E. Rezabal, J. C. Mareque-Rivas, X. Lopez, F. Lopez-Gallego, L. Salassa, *Chem. Sci.* 2017, **8**, 4619-4625.
[12] L. Gong, Z. Lin, K. Harms, E. Meggers, *Angew. Chem. Int. Ed.* 2010, **49**, 7955-7957.
[13] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* 2016, **116**, 3436-3486.
[14] F. Barragán, P. López-Senín, L. Salassa, S. Betanzos-Lara, A. Habtemariam, V. Moreno, P. J. Sadler, V. Marchán, *J. Am. Chem. Soc.* 2011, **133**, 14098-14108.
[15] S. Betanzos-Lara, L. Salassa, A. Habtemariam, O. Novakova, A. M. Pizarro, G. J. Clarkson, B. Liskova, V. Brabec, P. J. Sadler, *Organometallics* 2012, **31**, 3466-3479.
[16] S. Betanzos-Lara, L. Salassa, A. Habtemariam, P. J. Sadler, *Chem. Commun.* 2009, 6622-6624.
[17] A. Habtemariam, C. Garino, E. Ruggiero, S. Alonso-de Castro, C. J. Mareque-Rivas, L. Salassa, *Molecules* 2015, **20**, 7276-7291.
[18] I. Infante, J. M. Azpiroz, N. G. Blanco, E. Ruggiero, J. M. Ugalde, J. C. Mareque-Rivas, L. Salassa, *J. Phys. Chem. C* 2014, **118**, 8712-8721.
[19] D. P. H. M. Heuts, N. S. Scrutton, W. S. McIntire, M. W. Fraaije, *FEBS J.* 2009, **276**, 3405-3427.
[20] R. J. Needham, C. Sanchez-Cano, X. Zhang, I. Romero-Canelón, A. Habtemariam, M. S. Cooper, L. Meszaros, G. J. Clarkson, P. J. Blower, P. J. Sadler, *Angew. Chem. Int. Ed.* 2017, **56**, 1017-1020.
[21] J. J. Soldevila-Barreda, I. Romero-Canelón, A. Habtemariam, P. J. Sadler, *Nat. Commun.* 2015, **6**, 6582.
[22] S. Bose, A. H. Ngo, L. H. Do, *J. Am. Chem. Soc.* 2017, **139**, 8792-8795.
[23] After conversion to their aqua complexes, **4** and **5** underwent formation of DMSO and NADH adducts in MES and PB buffer (Figure S30 and 38).
[24] *Flavins and Flavoproteins, Vol.* S. Weber, E. Schleicher, Springer, New York, 2014.
[25] S. Dhar, F. X. Gu, R. Langer, O. C. Farokhzad, S. J. Lippard, *Proc. Nat. Acad. Sci. USA* 2008, **105**, 17356-17361.
[26] X. Shu, V. Lev-Ram, T. J. Deerinck, Y. Qi, E. B. Ramko, M. W. Davidson, Y. Jin, M. H. Ellisman, R. Y. Tsien, *PLOS Biol.* 2011, **9**, e1001041.
[27] J. Rocha-Martín, D. Vega, J. M. Bolivar, C. A. Godoy, A. Hidalgo, J. Berenguer, J. M. Guisán, F. López-Gallego, *BMC Biotechnol.* 2011, **11**, 101.
[28] V. Leskovac, S. Trivić, G. Wohlfahrt, J. Kandrač, D. Peričin, *Int. J. Biochem. Cell Biol.* 2005, **37**, 731-750.
[29] J. Yu, C.-Z. Zhou, *Proteins* 2007, **68**, 972-979.
[30] A. Rodriguez-Pulido, A. L. Cortajarena, J. Torra, R. Ruiz-Gonzalez, S. Nonell, C. Flors, *Chem. Commun.* 2016, **52**, 8405-8408.
[31] R. Ruiz-González, A. L. Cortajarena, S. H. Mejias, M. Agut, S. Nonell, C. Flors, *J. Am. Chem. Soc.* 2013, **135**, 9564-9567.
[32] M. Westberg, L. Holmegaard, F. M. Pimenta, M. Etzerodt, P. R. Ogilby, *J. Am. Chem.Soc.* 2015, **137**, 1632-1642.
[33] M. D. Gouda, M. S. Thakur, N. G. Karanth, *Biotechnol. Tech.* 1997, **11**, 653-655.
[34] M. Deponte, *Biochim. Biophys. Acta* 2013, **1830**, 3217-3266.
[35] Information on the miniSOG structural model is provided in the Experimental Section of the Supporting Information.
[36] I. Walsh, G. Minervini, A. Corazza, G. Esposito, S. C. E. Tosatto, F. Fogolari, *Bioinformatics* 2012, **28**, 2189-2190.
[37] E. Wexselblatt, D. Gibson, *J. Inorg. Biochem.* 2012, **117**, 220-229.
[38] A. Nemirovski, Y. Kasherman, Y. Tzaraf, D. Gibson, *J. Med. Chem.* 2007, **50**, 5554-5556.
[39] N. Ma, M. A. Digman, L. Malacrida, E. Gratton, *Biomed. Opt. Express* 2016, **7**, 2441-2452.
[40] F. M. Pimenta, R. L. Jensen, T. Breitenbach, M. Etzerodt, P. R. Ogilby, *Photochem. Photobiol.* 2013, **89**, 1116-1126.
[41] P. Gramatica, E. Papa, M. Luini, E. Monti, M. B. Gariboldi, M. Ravera, E. Gabano, L. Gaviglio, D. Osella, *J. Biol. Inorg. Chem.* 2010, **15**, 1157-1169.

10.1002/anie.201800288

WILEY-VCH

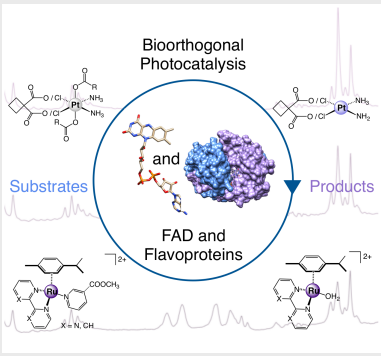
Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Text for Table of Contents

Bioorthogonal inorganic photochemistry: Flavins function as selective photocatalysts for the activation of metal-based prodrugs in the biological environment. Differently from typical catalysis, metal complexes are substrates and not catalysts in these reactions!



((Insert TOC Graphic here))

Silvia Alonso-de Castro, Aitziber L. Cortajarena, Fernando López-Gallego, Luca Salassa,**

Page No. – Page No.

Bioorthogonal Catalytic Activation Of Pt And Ru Anticancer Complexes By FAD and Flavoproteins