# Photo-induced H2 production by [NiFe]-hydrogenase from *T. roseopersicina* covalently linked to a Ru(II) photosensitizer

Authors: Oleg A. Zadvornyy, Janice E. Lucon, Robin Gerlach, Nikolay A. Zorin, Trevor Douglas, Timothy E. Elgren, & John W. Petersa

# Photo-induced $H_2$ production by [NiFe]-hydrogenase from *T. roseopersicina* covalently linked to a Ru(II) photosensitizer

Oleg A. Zadvornyy [a,c], Janice E. Lucon [a], Robin Gerlach [b], Nikolay A. Zorin [c], Trevor Douglas [a], Timothy E. Elgren [d], John W. Peters [a]

[a] Department of Chemistry and Biochemistry, Montana State University, 103 Chemistry and Biochemistry Building, Bozeman, MT 59717, USA
[b] Department of Chemical and Biological Engineering, Montana State University, 366 EPS Building, Bozeman, MT 59717, USA
[c] Laboratory of Biochemistry and Biotechnology of Phototrophic Microorganisms, Institute of Basic Biological Problems of Russian Academy of Sciences, Institutskaya 2, Pushchino, Russia [d] Department of Chemistry, Hamilton College, Clinton, NY 13323, USA

A B S T R A C T

The potential of hydrogen as a clean renewable fuel source and the finite reserves of platinum metal to be utilized in hydrogen production catalysts have provided the motivation for the development of non-noble metal-based solutions for catalytic hydrogen production. There are a number of microorganisms that possess highly efficient hydrogen production catalysts termed hydrogenases that generate hydrogen under certain metabolic conditions. Although hydrogenases occur in photosynthetic microorganisms, the oxygen sensitivity of these enzymes repre-sents a significant barrier in directly coupling hydrogen production to oxygenic photosynthesis. To overcome this barrier, there has been considerable interest in identifying or engineering oxygen tolerant hydrogenases or gen-erating mimetic systems that do not rely on oxygen producing photocatalysts. In this work, we demonstrate photo-induced hydrogen production from a stable [NiFe]-hydrogenase coupled to a [Ru(2,2'-bipyridine)$_2$(5-amino-1,10-phenanthroline)]$^{2+}$ photocatalyst. When the Ru(II) complex is covalently attached to the hydroge-nase, photocatalytic hydrogen production occurs more efficiently in the presence of a redox mediator than if the Ru(II) complex is simply present in solution. Furthermore, sustained hydrogen production occurs even in the presence of oxygen by presumably creating a local anoxic environment through the reduction of oxygen similar to what is proposed for oxygen tolerant hydrogenases. These results provide a strong proof of concept for engi-neering photocatalytic hydrogen production in the presence of oxygen using biohybrid mimetic systems.

## 1. Introduction

Hydrogen gas has tremendous potential to serve as a clean, efficient energy source. Harnessing this potential has been the focus of hydrogen fuel technology research in recent years [1]. The three primary areas of development are in hydrogen production, efficient storage, and catalyt-ic oxidation for use in hydrogen fuel cells. With respect to hydrogen production, much attention has focused on utilizing hydrogenase ($H_2$ase) [EC 1.12.2.1] for the catalytic production of hydrogen from aqueous protons and electrons. $H_2$ases, found in a variety of microor-ganisms including several lower eukaryotes, catalyze hydrogen oxida-tion and proton reduction according to the following reaction [2].

$$2H^+(aq) + 2e^- \underset{H_2ase}{\rightleftharpoons} H_2(g).$$

$H_2$ases that formally catalyzed this reaction are classified as [FeFe]-$H_2$ases or [NiFe]-$H_2$ases based on the composition of their metal-containing active sites. Both classes have unique active site metal centers with Fe ions ligated by carbon monoxide and cyanide. This set of ligands serves to stabilize lower oxidation states of Fe and thus a key feature in catalytic function.

The high catalytic activities of $H_2$ases make them exciting potential non-noble metal alternatives for hydrogen oxidation and hydrogen production for energy applications. However, as is the case for biologically derived materials, most of these enzymes lack the inherent dura-bility for practical use in materials applications. Nevertheless, a number of strategies have been reported to deliver reducing equiva-lents to hydrogenase in order to drive hydrogen production, including coupling the enzyme to an electrode [1], carbon nano-tubes [3–9] and photosensitizers [10–15].

Our goal is to utilize the reducing power of a photo-activated ruthe-nium photocatalyst to drive hydrogen production from hydrogenase. Photoexcitation of Ru(II)(bpy)$_3^{2+}$ (bpy=2,2′-bipyridine) results from excitation into a metal-to-ligand charge transfer transition creating an excited state of the complex, Ru(III)(bpy)$_2$(bpy$^{\bullet-}$)$^{2+}$, in which a ground state electron from a metal based d-orbital has been excited

into a low lying $\pi^*$ orbital of the bpy ligand [16]. Electron transfer occurs when the excited state undergoes quenching by a redox mediator such as methyl viologen, which can subsequently deliver the reducing equivalents to the resting state of the enzyme (Fig. 1). Oxidation of a sacrificial or terminal reductant such as EDTA, completes the catalytic cycle by regenerating the Ru(II) state. There are several examples of systems involving a variety of photocatalysts, sacrificial reductants, and redox mediators in solution with hydrogenases from different sources that are capable of light dependent hydrogen production [10–13].

Covalently linking the Ru-complex to the surface of the enzyme has the potential to greatly enhance the efficiency of photoreduction over the aforementioned solution studies by increasing the proximity and local concentration of photocatalyst and reduced redox mediator relative to the proton reduction site on the hydrogenase [14, 15]. Carbodiimide mediated formation of amide bonds allows for the selective addition of primary amines to carboxylates located on the surface of a protein [17, 18]. Incorporation of 5-amino-1,10-phenanthroline ($NH_2$phen) into the ruthenium complex, $[Ru(bpy)_2(NH_2phen)]^{2+}$, allows for direct coupling to carboxylate residues on the surface of the hydrogenase.

In this study we report the covalent linkage of $[Ru(bpy)_2(NH_2phen)](PF_6)_2$ (Ru(II) photocatalyst) to *Thiocapsa roseopersicina* $H_2$ase to create an efficient photoreduction system to drive hydrogenase catalyzed hydrogen production. Because of its stability and low oxygen sensitivity, the [NiFe]-$H_2$ase from *T. roseopersicina* might present an ideal system for applications in catalysis. Ruthenium based photoreduction used to drive hydrogen production from $H_2$ase requires the presence of a redox mediator and sacrificial reductant, such as methyl viologen and EDTA respectively. Highest efficiencies of light-activated hydrogen production were observed when the Ru(II) photocatalyst was covalently linked to the $H_2$ase ($H_2$ase–Ru(II)) and sustained hydrogen production was observed in the presence of oxygen.

## 2. Experimental

### 2.1. Protein purification

*T. roseopersicina*, strain BBS, was grown under anoxic conditions on modified Pfennig medium [19]. $H_2$ase (2.5–3 μM protein/1 g cells) was purified from *T. roseopersicina* cells, strain BBS according to previously published protocols [9]. Cells were collected in the logarithmic growth phase and were acetone treated before DEAE-cellulose $DE_{52}$ (Whatman, Great Britain), phenyl-sepharose CL-4B, and Sephacryl S-300 (Pharmacia, Sweden) chromatography described previously [20]. The purity was confirmed by SDS-PAGE. The concentration of protein in these samples was determined using the Bradford assay [21] with bovine serum albumin as a protein standard.

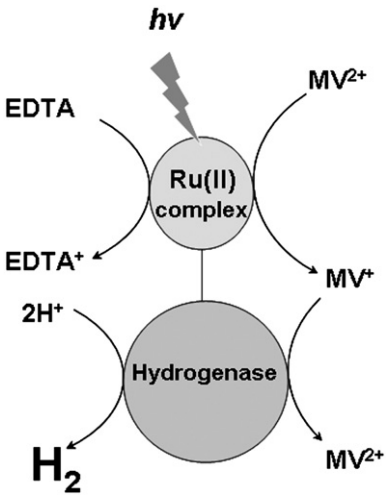


**Fig. 1.** Scheme of photo-activated hydrogen production by hydrogenase covalently linked to a Ru(II) complex with EDTA as a sacrificial reductant and MV as a redox mediator.

### 2.2. Synthesis of Ru(II) photocatalyst

$[Ru(bpy)_2(NH_2phen)](PF_6)_2$ was synthesized according to previously published procedures [22]. In brief, 0.350 g (0.725 mmol) $[Ru(bpy)_2Cl_2]$ and 0.142 g (0.725 mmol) of 5-amino-1,10-phenanthroline were added to 25 ml of methanol and allowed to reflux under argon for 3 h. The red solution was gravity filtered and an aqueous saturated solution of ammonium hexafluorophosphate was added to the warm mixture. The precipitate was collected and washed with cold water and ether to obtain the desired product with a 68% yield.

### 2.3. Covalent attachment of Ru(II) photocatalyst to hydrogenase

For covalent attachment of the Ru(II) photocatalyst to the *T. roseopersicina* $H_2$ase, the enzyme (5–7 μM) in 0.1 M MES (2-[N-morpholino]ethane sulfonic acid) buffer, pH 5.0, was mixed with 20 mM Ru(II) photocatalyst and a 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) solution in a molar ratio of 1:100:8 (protein:Ru(II)-complex:EDC). After 2 h incubation under constant agitation, unreacted Ru(II) photocatalyst was removed by washing the hydrogenase sample 3 times with 0.1 MES buffer (pH 5.0) on YM-50 Microcon filters (Millipore Corporation, Bedford, MA). Ruthenium was quantified using an Agilent 7500ce inductively coupled plasma-mass spectrometer (ICP-MS) [23].

### 2.4. Hydrogen production activity

Ru(II)-labeled *T. roseopersicina* $H_2$ase was examined for the ability to produce hydrogen via photoreduction of water to hydrogen. Reaction mixtures containing 20 μl (20 nM) $H_2$ase or $H_2$ase–Ru(II), 0.02–5.0 μM Ru(II) photocatalyst, 4 mM methyl viologen (MV), 45 mM EDTA and 50 mM phosphate buffer, pH 5.5, were placed in a sealed quartz cuvette with an atmosphere of 100% nitrogen. The reaction mixture was stirred continuously (magnet stir bar) and initiated by adding sodium dithionite from a concentrated stock solution to a final concentration of 5 mM (control reaction) or by irradiating of the reaction mixture with 150,000 lx Xenon lamp (light reaction). The gas headspace of the cuvette was sampled using a gas tight syringe and analyzed by gas chromatography (GC-8A, Shimadzu Scientific Instruments Inc). In control assays containing just the Ru(II) photocatalyst, without added MV, a small amount of oxygen could be detected and is attributed to a small amount of decomposition of the Ru(II) photocatalyst. The $H_2$ase activity is presented in units, where one unit is equal to 1 nmol $H_2$/min per mg protein. Three or four replicates of each experiment were conducted and replicate measurements were never found to vary by greater than 5%.

## 3. Results and discussion

### 3.1. Ru(II) photocatalyst as an electron source for T. roseopersicina $H_2$ase

Ru(II) photocatalyst and EDTA has been used as a donor of electrons to study photo-activated hydrogen production by the $H_2$ase from *T. roseopersicina*. In a control experiment an anoxic solution containing 5 μM Ru(II) photocatalyst, 45 mM EDTA, and 20 nM $H_2$ase was placed under constant illumination and monitored continuously for hydrogen production for 4 h. Hydrogen production was not detected, indicating that direct electron transfer from the light activated Ru(II) photocatalyst does not occur or is too slow to yield detectable hydrogen production over the course of the assay. These assays were repeated with the addition of a redox mediator (4 mM MV) and hydrogen was produced in response to illumination in the range of 200 units of $H_2$ase activity. $H_2$ production activity for the stable *T. roseopersicina*

$H_2$ase is not detected in assays with sodium dithionite as a reductant so viologen dyes (benzyl and methyl viologen) are used as redox mediators to enhance the rate of reduction of $H_2$ase. In control experiments with 4 mM MV and 5 mM sodium dithionite used as a reductant instead of the EDTA and Ru(II) photocatalyst, the activity of $H_2$ase was 510 nmol $H_2$/min per mg protein which remained constant after continued irradiation of this reaction mixture with 150,000 lx Xenon lamp for 10–12 h.

To investigate the dependence of hydrogen production on the concentration of Ru(II) photocatalyst, assays were conducted as described above but over a range of concentrations of Ru(II) photocatalyst (0.02–5.0 μM). The results of these experiments indicated that 2 μM Ru(II) complex is the concentration for maximal $H_2$ production in these assays (Fig. S1 in the Supporting information). Photoactivation of $H_2$ase in the presence of 2 μM free Ru(II) complex, 4 mM MV and an excess of the sacrificial reductant, EDTA (45 mM), results in 224 nmol of hydrogen per min per mg of protein which is 44% of the activity of $H_2$ase using sodium dithionite as a terminal electron donor in the absence of light (Table 1). This specific activity is over 180 times the activities reported previously for hydrogen production by *Desulfovibrio vulgaris* $H_2$ase driven by photo-activated $Ru(bpy)_3^{2+}$/MV (1.22 nmol/min per mg protein) [12].

### 3.2. Activity of $H_2$ase–Ru photocatalyst adduct

Covalent coupling of Ru(II) photocatalyst to *T. roseopersicina* $H_2$ase was achieved by carbodiimide mediated formation of an amide bond from the primary amine of the $NH_2$phen ligand of the ruthenium complex presumably to carboxylates on the protein surface. SDS-PAGE was used to demonstrate successful coupling of the Ru(II) photocatalyst to the protein (Fig. 2). Coomassie staining of the SDS-PAGE gel shows the two primary bands associated with the $H_2$ase (Fig. 2A, lanes 1–3). The presence of the attached fluorescent Ru(II) photocatalyst was monitored by SDS-PAGE gels under UV light. Two fluorescent $H_2$ase bands appear after the coupling reaction (Fig. 2B, lane 2) and unreacted Ru(II) photocatalyst is removed by filtration (Fig. 2B, lane 3).

The activity of $H_2$ase samples after modification with Ru(II) photocatalyst was measured in the absence of light to investigate levels of $H_2$ase activity recovered after covalent attachment of Ru(II) photocatalyst using sodium dithionite as a terminal reductant and MV as mediator. When assayed with sodium dithionite as a terminal electron donor and MV as a redox mediator the $H_2$ase labeled with the Ru(II) photocatalyst retains greater than 50% of the activity of the unlabeled enzyme (Table 1). Each $H_2$ase was covalently linked to 2–3 molecules of the Ru (II) photocatalyst resulting in an overall concentration of photocatalyst in the assays of 27 ± 1 nM as determined by ICP-MS (data not shown). The activity of the covalently modified $H_2$ase in the light dependent

**Table 1**
Light dependent $H_2$ production by *T. roseopersicina* hydrogenase.

| Sample | Activity (units)[a] | Activity (units/μM Ru) |
|---|---|---|
| $H_2$ase + DT (non illuminated) | ND[b] | NA[c] |
| $H_2$ase + MV + DT (non illuminated) | 510 ± 2 | NA |
| $H_2$ase + MV + 2 μM Ru(II) + EDTA (illuminated) | 224 ± 3 | 112 ± 2 |
| $H_2$ase + 2 μM Ru(II) + EDTA (illuminated) | ND | NA |
| $H_2$ase–Ru(II) + MV + DT (non illuminated) | 286 ± 4 | NA |
| $H_2$ase–Ru(II) + MV + EDTA (illuminated) | 121 ± 1 | 4481 ± 43 |
| $H_2$ase–Ru(II) + EDTA (illuminated) | ND | ND |
| $H_2$ase + MV + 27 nM Ru(II) + EDTA (illuminated) | 16 ± 1 | 592 ± 3 |

[a] Unit is nmol $H_2$/min per mg protein.
[b] Not detected.
[c] Not applicable.

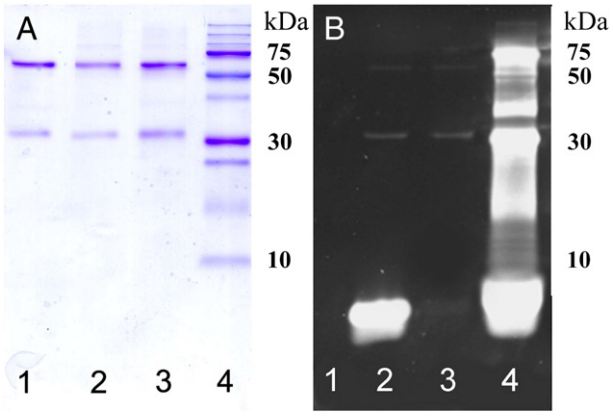


**Fig. 2.** 7.5% SDS-PAGE gel of *T. roseopersicina* hydrogenase samples (5 nM) stained with Coomassie reagent (A) and fluorescent image of the gel (B). Lanes in the gel correspond to hydrogenase (1), hydrogenase labeled with the Ru(II) photocatalyst (2), hydrogenase labeled with the Ru(II) photocatalyst after removing non-reacted Ru(II)-complexes (3) and protein markers (4).

reactions is 121 ± 1 nmol/min per mg protein, which is approximately 50% of that observed for the maximum activity of hydrogenase in the presence of 2 μM Ru(II) photocatalyst. However the overall concentration of photocatalyst in the labeled samples is ~3 orders of magnitude less than the concentration of Ru(II) photocatalyst needed to achieve maximum activity. For a more valid comparison we determined the activity of the $H_2$ase–Ru(II) based on the concentration of Ru(II) photocatalyst present. These results indicate that the activity of covalently modified $H_2$ase per μmol Ru is 23 times higher (4481 ± 43 units/μM Ru (II)) than the $H_2$ase activity in the presence of 2 μM Ru(II) photocatalyst (112 ± 2 units/μM Ru(II)) demonstrating that covalent attachment facilitates the effective electron delivery to $H_2$ase active sites presumably by increasing the local effective concentration of reducing agent. This is supported by the results of assays in which a concentration of Ru(II) photocatalyst (27 nM) equivalent to that present in the Ru(II) complex coupled to the enzyme is added as free Ru(II) photocatalyst. The activity of non-modified samples of $H_2$ase in the presence of 27 nM Ru(II) photocatalyst is more than 7 times lower (16 ± 1 units) than that of $H_2$ase–Ru (II) (121 ± 1 units).

The catalytic system for photo induced hydrogen production, containing 2 μM Ru(II) photocatalyst, 4 mM MV, 45 mM EDTA, and 20 nM *T. roseopersicina* $H_2$ase reported in our paper is more efficient in comparison to previously published work (Table S1 in Supporting information). For example, the systems containing $Ru(bpy)_3^{2+}$, methyl viologen, and mercaptoethanol as a source of electrons with hydrogenase from *Desulfovibrio desulfuricans* produced 14 nmol $H_2$/min per mg protein [10], which is 16 times less in comparison to the current work. It has been reported that *D. vulgaris* hydrogenase and bisviologen-linked ruthenium (II) complex with different methylene chain lengths between ruthenium complex and viologen produced 0.029 nmol $H_2$/min per mg protein [14]. The highest photoinduced hydrogen production reported so far is 19.8 nmol $H_2$/min per mg protein, published for the system containing 0.1 μmol ($[Ru^{II}(2,2'\text{-bipyridine})_2(4,4'\text{-}(Po_3H_2)_2\text{-}2,2'\text{-bipyridine}]Br_2$) attached to $TiO_2$, EDTA, 5 μM *Desulfomicrobium baculatum* $H_2$ase, which is approximately an order of magnitude lower rate than reported in the present work. The only published work that did not involve the addition of a soluble redox mediator was the aforementioned work in which the photocatalyst was not free in solution and attached to a $TiO_2$ surface [25].

### 3.3. $H_2$ production in the presence of oxygen

Hydrogenase oxygen sensitivity represents a major barrier for many biohydrogen and biomimetic approaches to hydrogen

production. $H_2$ases are known to have varied sensitivities to oxygen [24] and some are irreversibly inactivated by oxygen exposure but there are several examples of $H_2$ases that can support $H_2$ production in the presence of oxygen. It has been shown that the [NiFeSe]-$H_2$ase from *D. baculatum* can support hydrogen production in the presence of 1% of oxygen in $N_2$ using $TiO_2$ as a photocatalyst [25]. The *hyn* encoded [NiFe]-$H_2$ase from *T. roseopersicina* attached to electrode surfaces has been shown to oxidize $H_2$ in reaction mixtures with 20% air [26].

Reducing agents such as sodium dithionite can be added to $H_2$ase reactions and serve as an antioxidant and sacrificial oxidant affording some protection against oxygen inactivation, but under typical assay conditions the consumption of reducing equivalents for these purposes would be impractical. The results presented herein suggest that the ability to generate reducing equivalents in close proximity to the $H_2$ase active site results in a more effective coupling of reducing equivalents to $H_2$ production. To further investigate this relationship, we investigated whether reducing equivalents produced in the coupled complex might also result in a system that is inherently less sensitive to oxygen deactivation.

$H_2$ase–Ru(II) was incubated in air (17 μmol of oxygen or 19% of oxygen in $N_2$) for approximately 2 h in the dark to ensure the complete oxidation of MV. The reactions were initiated by illumination and $H_2$ and $O_2$ were monitored at 10 min intervals (Fig. 3). Sustained $H_2$ production by $H_2$ase–Ru(II) was observed for an hour period 13.5 $\pm$0.2 nmol/min per mg protein (11% of the initial rate). These assays were conducted under the same standard assay conditions described above with an amount of Ru(II)–$H_2$ase that corresponds to 27 nM of Ru(II) catalyst. In control assays in which the same amounts of Ru(II) photocatalyst and $H_2$ase were not coupled covalently, no activity is observed in air. The *T. roseopersicina* hyn encoded $H_2$ase is not irreversibly inactivated by oxygen and greater than 90% of the activity can be restored by removing oxygen and incubating the enzyme under reducing conditions [27]. These results are consistent with sufficient oxygen reduction occurring in the close proximity to the $H_2$ase active sites to maintain activity by protecting the active site from exposure to oxygen. Without the coupling, the available reduced $MV^+$, produced by Ru(II) photocatalyst, is possibly oxidized before it can reduce the $H_2$ase active sites resulting in no subsequent hydrogen production. Oxygen tolerance for the coupled system is presumed to be achieved through creating a local anoxic environment by reducing oxygen in close proximity to the active site in a similar manner to what has been proposed for the uniquely coordinated active site proximal redox FeS cluster of oxygen tolerant hydrogenases commonly found in the Knallgas bacteria [28]. Presumably upon scale up the concentration of Ru(II)–$H_2$ase could be optimized to balance $O_2$ consumption and $H_2$ production to maximize $H_2$ production for a variety of different reactor configurations.

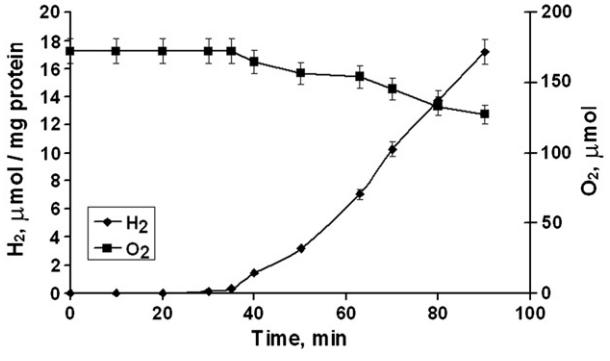


**Fig. 3.** Hydrogen production catalyzed by $H_2$ase–Ru(II) (40 nM) containing 50 mM potassium-phosphate buffer (pH 5.5), 45 mM EDTA, 4 mM MV in air.

## 4. Conclusion

In summary, the coupled photo-induced $H_2$ production catalyst was generated by covalently attaching [NiFe]-$H_2$ase to a Ru(II) based photocatalyst. It has been demonstrated that 1) the $H_2$ase remains active after the covalent modification and attachment of the Ru(II) photocatalyst; 2) the covalent coupling of photocatalyst and $H_2$ase significantly increases the efficiency of photo-induced $H_2$ production and 3) the effect of increasing the local concentration of reducing equivalents in the local environment of the $H_2$ase active sites mimics the properties of oxygen tolerant hydrogenases resulting in oxygen protection that allows for enhanced sustainable $H_2$ production in air.

## Abbreviations

| | |
|---|---|
| bpy | 2,2′-bipyridine |
| $H_2$ase | hydrogenase |
| $H_2$ase–Ru(II) | [Ru(bpy)$_2$($NH_2$phen)] covalently linked to [NiFe]-hydrogenase |
| MV | methyl viologen |
| $NH_2$phen | 5-amino-1,10-phenanthroline |
| ICP-MS | inductively coupled plasma-mass spectrometry |


## Acknowledgment

This work is supported by the Department of Energy (JWP and TD) — (DE-FG02-07ER46477). The Environmental and Biofilm Mass Spectrometry Facility at Montana State University (MSU) was supported by the Defense University Research Instrumentation Program (DURIP, Contract number: W911NF0510255) and the MSU Thermal Biology Institute (NASA Exobiology — Project NAG5-8807).


## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2011.09.012.

## References


[1] R. Cammack, M. Frey, R. Robson, Hydrogen as a Fuel: Learning from Nature, Taylor and Francis, London, 2001.
[2] P.M. Vignais, B. Billoud, Chemical Reviews 107 (2007) 4206–4272.
[3] T.E. Elgren, O.A. Zadvorny, E. Brecht, T. Douglas, N.A. Zorin, M.J. Maroney, J.W. Peters, Nano Letters 5 (2005) 2085–2087.
[4] T.J. McDonald, D. Svedruzic, Y.H. Kim, J.L. Blackburn, S.B. Zhang, P.W. King, M.J. Heben, Nano Letters 7 (2007) 3528–3534.
[5] M.A. Alonso-Lomillo, O. Rudiger, A. Maroto-Valiente, M. Velez, I. Rodriguez-Ramos, F.J. Munoz, V.M. Fernandez, A.L. De Lacey, Nano Letters 7 (2007) 1603–1608.
[6] O. Rudiger, J.M. Abad, E.C. Hatchikian, V.M. Fernandez, A.L. De Lacey, Journal of the American Chemical Society 127 (2005) 16008–16009.
[7] H.R. Pershad, J.L. Duff, H.A. Heering, E.C. Duin, S.P. Albracht, F.A. Armstrong, Biochemistry 38 (1999) 8992–8999.
[8] C. Leger, A.K. Jones, W. Roseboom, S.P. Albracht, F.A. Armstrong, Biochemistry 41 (2002) 15736–15746.
[9] O.A. Zadvornyy, M. Allen, S.K. Brumfield, Z. Varpness, E.S. Boyd, N.A. Zorin, L. Serebriakova, T. Douglas, J.W. Peters, Environmental Science and Technology 44 (2010) 834–840.
[10] H.S. Lee, Korean Biochemistry Journal 16 (1983) 228–239.
[11] A.I. Krasna, Photochemistry and Photobiology 29 (1979) 267–276.
[12] I. Okura, N. Kim-Thuan, Chemistry Letters (1980) 1511–1512.
[13] I. Okura, M. Takeuchi, N. Kim-Thuan, Chemistry Letters (1980) 765–766.
[14] T. Hiraishi, T. Kamachi, I. Okura, Journal of Molecular Catalysis A: Chemical 151 (2000) 7–15.
[15] R. Hilhorst, C. Laane, C. Veeger, Proceedings of the National Academy of Sciences of the United States of America 79 (1982) 3927–3930.
[16] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, A. Vonzelewsky, Coordination Chemistry Reviews 84 (1988) 85–277.
[17] D.G. Haore, D.E. Koshland, Am. Chem. Assoc. 88 (1966) 2057–2058.

[18] E.M. Ryan, R. O'Kennedy, M.M. Feeney, J.M. Kelly, J.G. Vos, Bioconjugate Chemistry 3 (1992) 285–290.
[19] L.A. Bogorov, Microbiologiya 43 (1974) 326–332.
[20] O.A. Zadvorny, N.A. Zorin, I.N. Gogotov, Biochemistry (Mosc) 65 (2000) 1287–1291.
[21] M.M. Bradford, Analytical Biochemistry 72 (1976) 248–254.
[22] C.D. Ellis, L.D. Margerum, R.W. Murray, T.J. Meyer, Inorganic Chemistry 22 (1983) 1283–1291.
[23] J.E. Longbottom, T.D. Martin, K.W. Edgell, S.E. Long, M.R. Plantz, B.E. Warden, R. Baraona, D. Bencivengo, D. Cardenas, L. Faires, D. Gerlach, W. King, G. Laing, C. Lord, M. Plantz, T. Rettberg, S. Tan, D. Tye, G. Wallace, Journal of AOAC International 77 (1994) 1004–1023.
[24] A.L. De Lacey, V.M. Fernandez, M. Rousset, R. Cammack, Chemical Reviews 107 (2007) 4304–4330.
[25] E. Reisner, J.C. Fontecilla-Camps, F.A. Armstrong, Chemical Communications (Cambridge, England) (2009) 550–552.
[26] S.V. Morozov, O.G. Voronin, E.E. Karyakina, N.A. Zorin, S. Cosnier, A.A. Karyakin, Electrochemistry Communications (2006) 851–854.
[27] I.N. Gogotov, N.A. Zorin, L.T. Serebriakova, E.N. Kondratieva, Biochimica et Biophysica Acta 523 (1978) 335–343.
[28] T. Goris, A.F. Wait, M. Saggu, J. Fritsch, N. Heidary, M. Stein, I. Zebger, F. Lendzian, F.A. Armstrong, B. Friedrich, O. Lenz, Nature Chemical Biology (2011) 310–318.