H_2 Activation

DOI: 10.1002/anie.201006255

Iron-Chromophore Circular Dichroism of [Fe]-Hydrogenase: The Conformational Change Required for H_2 Activation**

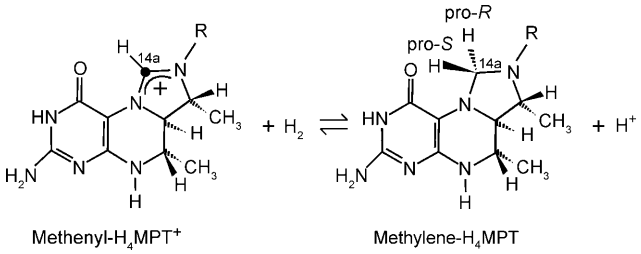
Seigo Shima, Sonja Vogt, Andreas Göbels, and Eckhard Bill*

Circular dichroism (CD) spectroscopy is a very sensitive method used to detect changes in inherently chiral chromophores and in achiral chromophores embedded in chiral surroundings.[1] Information on the secondary structures of proteins can be obtained by CD spectroscopy in the far-UV spectral region (190–250 nm), and CD in the near-UV region (250–350 nm) can be sensitive to certain aspects of tertiary structure.[1b] Near-UV and visible CD spectroscopy is also used to analyze metal complexes[1a,c] and the active site of metal-containing enzymes, such as P450 (300–500 nm), galactose oxidase (300–700 nm), and biotin sulfoxide reductase (300–650 nm).[2]

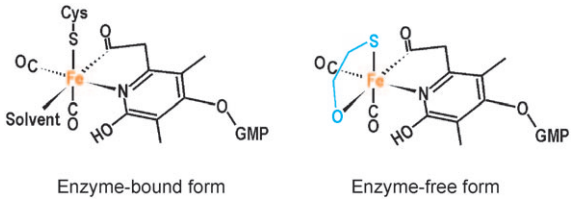
The [Fe]-hydrogenase found in many methanogenic archaea catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$) with H_2 to methylenetetrahydromethanopterin (methylene-H_4MPT) in the methanogenic pathway from CO_2 and H_2 (Scheme 1).[3] This hydrogenase contains a unique iron guanylyl pyridinol (FeGP) cofactor and lacks iron–sulfur clusters.[4] A model of the iron-complex structure of the FeGP cofactor bound to the enzyme has been proposed on the basis of crystallographic, spectroscopic, and chemical analyses. In the model, the iron ion is ligated with an sp^2-hybridized nitrogen atom, an acyl carbon atom, the Cys176 sulfur atom, two CO molecules, and one "solvent" molecule to form a chiral iron complex (Scheme 2).[4] Several model compounds of the FeGP cofactor have been synthesized.[5]

The crystal structure of the [Fe]-hydrogenase from *Methanocaldococcus jannaschii* reveals that the homodimer is composed of three domains: two peripheral N-terminal domains to which the FeGP cofactor binds, and one central domain composed of two intertwined C-terminal domains.[4a,b,6] The peripheral domains and the central domain form the two active-site clefts. In the cocrystal structure of the [Fe]-hydrogenase C176A mutant and the substrate methylene-H_4MPT, the substrate is bound to the central domain in the open form of the enzyme (see Figure S1 A in the Supporting Information). The distance between the iron center of the FeGP cofactor and the substrate is too far for the iron center and the substrate to interact.

Hiromoto et al.[6a] predicted that the active-site cleft is closed upon binding of the substrates, and that the iron site moves close to the carbocation of the substrate, which is the hydride acceptor in the reaction. It was also proposed that H_2 is supplied to the active site through a narrow channel formed between the peripheral and central domains after closing of the cleft, and that H_2 then interacts with methenyl-H_4MPT$^+$ and/or the iron site. Thus, the proposed open/closed conformational change has a crucial role in the catalytic mechanism. However, since the crystal structure of [Fe]-hydrogenase has only been solved in its open conformation in a complex with its substrate, such structural changes



Scheme 1. Reaction catalyzed by [Fe]-hydrogenase. The $\Delta G^{\circ\prime}$ value for the reversible reduction is $-5{,}5$ kJ mol^{-1}.



Scheme 2. Structure of the enzyme-bound and the enzyme-free FeGP cofactor. The ligand geometry of the enzyme-bound form has been determined by X-ray crystallography; the second intrinsic CO-binding site *trans* to the pyridinol nitrogen atom has not yet been unambiguously identified.[4a,b] The 2-mercaptoethanol bridging ligand shown in light blue is predicted from the crystal structure of the C176A mutant enzyme, in which dithiothreitol is the bridging ligand (see Figure S1 A in the Supporting Information).

[*] Dr. S. Shima,[+] Dr. S. Vogt
Max-Planck-Institut für terrestrische Mikrobiologie
Karl-von-Frisch-Strasse 10, 35043 Marburg (Germany)
Fax: (+49) 6421-178-109
E-mail: shima@mpi-marburg.mpg.de
Homepage: http://www.mpi-marburg.mpg.de/
A. Göbels, Dr. E. Bill
Max-Planck-Institut für Bioanorganische Chemie
Stiftstrasse 34–36, 45470 Mülheim an der Ruhr (Germany)
[+] Additional affiliation:
PRESTO Japan Science and Technology Agency (JST)
Honcho, Kawaguchi, Saitama 332-0012 (Japan)

[**] This research was supported by the Max Planck Society, the Fonds der Chemischen Industrie, the BMBF (BioH_2 project), and the PRESTO program of the Japan Science and Technology Agency (JST). S.S. and S.V. were financed by a grant from the Max Planck Society to R. K. Thauer, whom we also thank for helpful discussions and critical reading of the manuscript.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201006255.

induced by the substrates are still hypothetical. In this study, we analyzed the changes induced by inhibitors and substrates in the near-UV and visible CD spectra of the iron chromophore. Our results support conformational changes of the [Fe]-hydrogenase induced by binding of the substrate.

CD spectra of [Fe]-hydrogenase revealed signals of the iron chromophore (250–500 nm; Figure 1 b,c). Because the apoenzyme did not show any CD signals at wavelengths above 300 nm (Figure 1 c), the CD spectrum of the holoenzyme above 300 nm can be attributed to that of the FeGP cofactor bound to the enzyme. In the CD spectrum of the FeGP cofactor isolated from this enzyme, the major peaks are shifted to shorter wavelengths, and the signals are much weaker than those observed for the FeGP cofactor bound to the enzyme (Figure 1). The differences in the CD spectra of the [Fe]-hydrogenase holoenzyme and the protein-free FeGP cofactor could result from the distinct ligation structures of the iron site in the two forms of the cofactor (Scheme 2). It is also possible that the CD signals of the cofactor are shifted and amplified by its binding to the asymmetric protein environment.[1b,7]

Upon the addition of the inhibitor CO (100% in the gas phase), the CD spectrum of [Fe]-hydrogenase changed (Figure 2 b,c). When we exchanged the gas phase of the CO-inhibited sample for N_2, the CD spectrum changed back to that of the noninhibited enzyme (data not shown). This result indicated that CO binding is reversible. The inhibitor CN^- also changed the CD spectrum of the enzyme, but in a different manner. The UV/Vis spectrum of the enzyme was also altered by the addition of the inhibitors (Figure 2 a). Binding of the inhibitors to the iron site of [Fe]-hydrogenase has been observed previously by infrared, Mössbauer, and X-ray absorption spectroscopy.[8] The inhibitors probably bind to the "solvent"-binding site at the iron center (Scheme 2). The results of the inhibition experiments indicated that CD spectroscopy is sensitive enough to detect the changes in the electronic structure of the iron chromophore induced by the binding of extrinsic ligands.

We tested the effects of the substrates H_2 and methenyl-H_4MPT, alone and together, on the CD spectrum of [Fe]-hydrogenase. The CD spectrum of the enzyme (0.1 mM) under a gas phase of 100% H_2 was identical to that under 100% N_2 (see Figure S2 in the Supporting Information). If H_2 or hydride anion is bound to the iron site instead of the "solvent" molecule (Scheme 2), definite differences in CD should be observed. However, H_2 did not induce any changes in the CD signal in the absence of methenyl-H_4MPT$^+$. This result strongly supports the hypothesis that H_2 can be activated by [Fe]-hydrogenase only in the presence of methenyl-H_4MPT$^+$.[9]

The addition of methenyl-H_4MPT$^+$ (final concentration: 0.1 mM) to the solution of the enzyme (0.1 mM) under 100% N_2 induced little change in the CD spectrum (Figure 3 b,c). Equilibrium dialysis experiments indicated that under these conditions, only about 10% of the [Fe]-hydrogenase contained bound methenyl-H_4MPT$^+$; probably for this reason, the CD signal changed only slightly (see the Supporting information). However, when both H_2 and methenyl-H_4MPT$^+$ were added simultaneously to the enzyme solution, the CD spectrum of the enzyme solution changed substantially. The large positive peak at 280 nm disappeared, the negative peak at 310 nm and the positive peaks at 340 and 420 nm shifted and changed significantly in size, and a small positive peak appeared at 525 nm (Figure 3 b,c). The UV/Vis absorption peak at 335 nm, which is in the fingerprint region of methenyl-H_4MPT$^+$,[10] decreased in intensity (Figure 3 a).

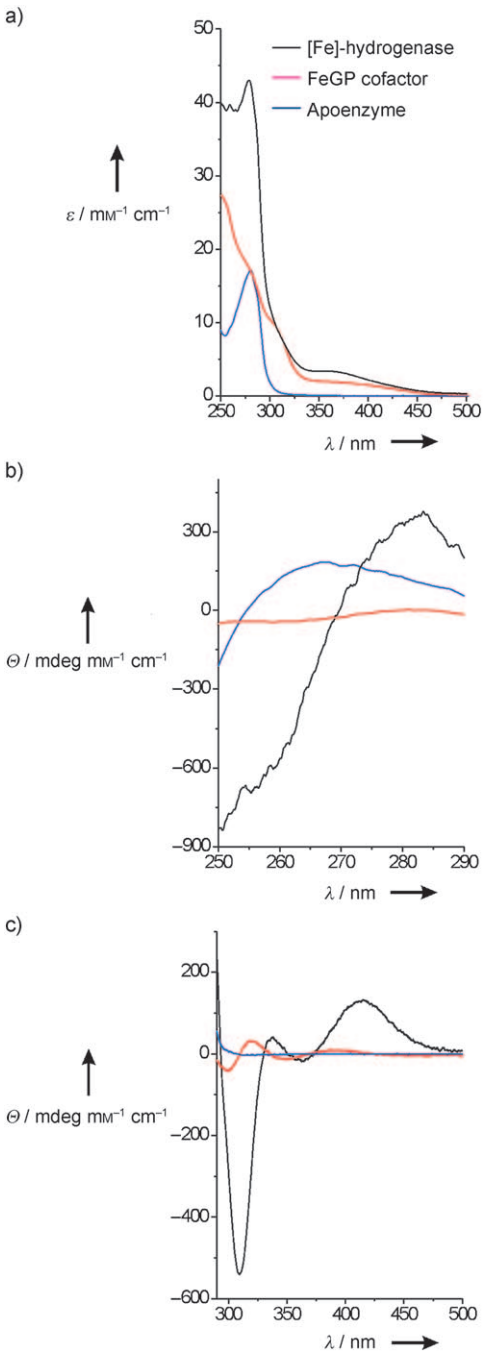


Figure 1. a) UV/Vis and b,c) CD spectra of [Fe]-hydrogenase (black), FeGP cofactor (1.0 mM; red), and the apoenzyme from *M. jannaschii* (2.0 mM; blue) as solutions in 50 mM Mes/NaOH buffer (pH 6.0). The concentration of [Fe]-hydrogenase for CD at 250–290 and 290–500 nm was 0.1 and 1 mM, respectively. For CD spectroscopy, 0.1 cm quartz cuvettes were used.

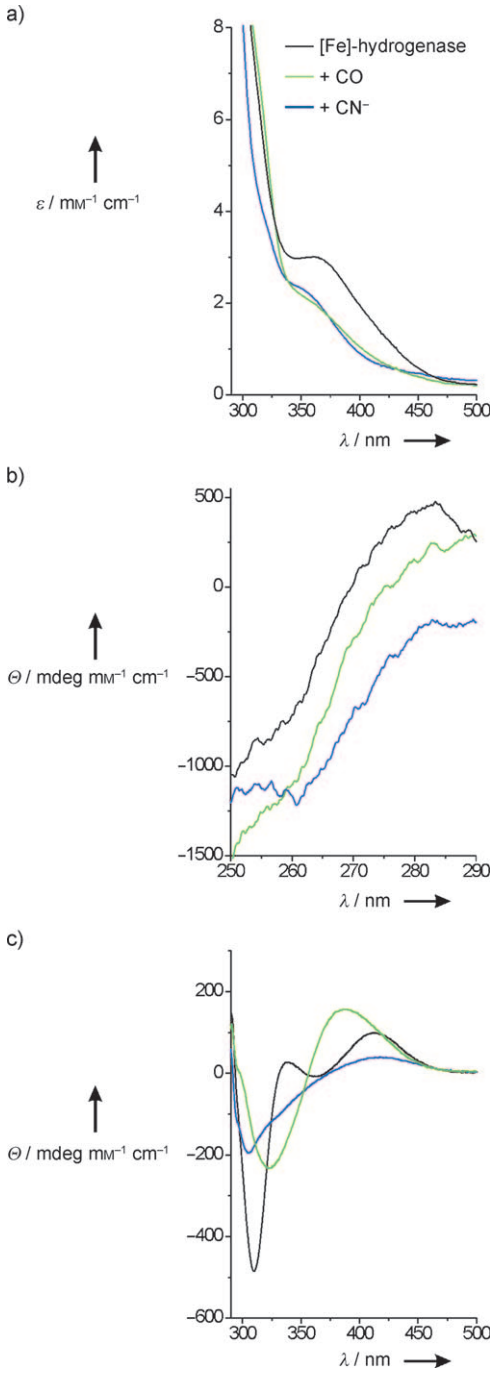


Figure 2. a) UV/Vis and b,c) CD spectra of [Fe]-hydrogenase in the absence (black) and presence of CO (100%; green) or CN^- (4 mM; blue). The spectra were recorded in 50 mM tricine/NaOH buffer (pH 8.0) for stabilization of the CN^- anion. The concentration of [Fe]-hydrogenase for the CD measurements at 250–290 and 290–500 nm was 0.1 and 2.0 mM, respectively; 0.1 cm quartz cuvettes were used. For the CD measurements at 290–500 nm with the inhibitor CO, the concentration of [Fe]-hydrogenase was 0.2 mM, and 1 cm cuvettes were used so that the gas phase could be efficiently exchanged.

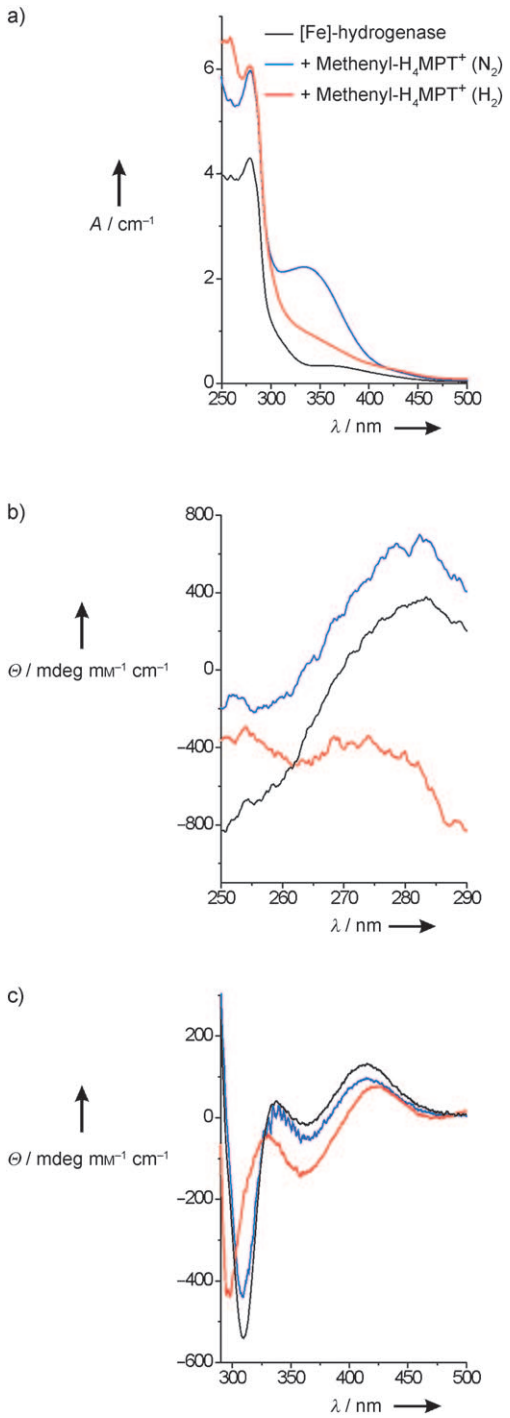


Figure 3. a) UV and b,c) CD spectra of [Fe]-hydrogenase (0.1 mM) in the absence of substrates under N_2 (black), in the presence of methenyl-H_4MPT^+ (0.1 mM) under N_2 (blue), and in the presence of methenyl-H_4MPT^+ (0.1 mM) under H_2 (red). The UV/Vis spectra and the CD spectra at 250–290 and 290–500 nm were measured in 0.3, 0.1, and 1 cm quartz cuvettes, respectively, in 50 mM Mes/NaOH buffer (pH 6.0).

This change in the UV/Vis absorption indicated that in the presence of H_2, about 70% of the methenyl-H_4MPT^+ was converted into methylene-H_4MPT. Equilibrium dialysis indicated that about 50% of the enzyme was complexed with the substrates under these conditions (see the Supporting information). Thus, these results indicated that in the presence of an equimolar amount of the enzyme under a 100% H_2 atmosphere, methenyl-H_4MPT^+ was converted into methyl-

ene-H_4MPT, which bound to the enzyme more tightly than methenyl-H_4MPT$^+$, and that the binding of methylene-H_4MPT caused changes in the CD spectrum of the enzyme. In the presence of a catalytic amount of [Fe]-hydrogenase (1 μM), only 20% of the methenyl-H_4MPT$^+$ was converted into methylene-H_4MPT (see Figure S3 in the Supporting Information). Thus, the equilibrium of the reaction shifted toward methylene-H_4MPT formation in the presence of an equimolar amount of the enzyme. The prerequisite for the observed shift in the equilibrium is that the K_s value of the enzyme for methylene-H_4MPT is higher than that for methenyl-H_4MPT$^+$; a higher K_s value for methylene-H_4MPT fits our observations.

As described above, the near-UV and visible CD signals of [Fe]-hydrogenase were changed substantially when both H_2 and methenyl-H_4MPT$^+$ were added simultaneously to the enzyme solution. However, the CD changes observed at 250–300 nm are difficult to interpret, because the protein, the FeGP cofactor, and methylene-H_4MPT exhibit definitive CD signals in this region. On the other hand, the CD signals at wavelengths above 350 nm can be considered as only coming from the FeGP cofactor, since neither the enzyme nor methylene-H_4MPT exhibit a CD signal in this region, and their absorption is much lower than that of the iron chromophore of the enzyme (see Figure S4 in the Supporting Information). Thus, the significant changes in the CD signal observed in the region 350–500 nm indicate that the environment of the FeGP cofactor in the enzyme is altered by binding of the substrate.

The structure of the enzyme–substrate complex revealed that the FeGP cofactor and methylene-H_4MPT each bind to a distinct domain of [Fe]-hydrogenase; thus, the iron chromophore and the substrate molecule do not directly interact in the open conformation (see Figure S1 in the Supporting Information). The CD spectrum of the iron chromophore can therefore only be changed if the ligand structure and/or the environment of the iron chromophore is changed by binding of the substrate. In the predicted closed conformation triggered by the binding of methenyl-H_4MPT$^+$ and/or methylene-H_4MPT, the pro-*R* hydrogen atom of methylene-H_4MPT sits near the iron center located in the hydrophobic cavity formed between the two domains (see Figure S1b in the Supporting Information).[6a] Such a conformational change of the protein should cause changes in the environment of the iron chromophore, and these changes in the environment of the iron chromophore should be detected as changes in the near-UV and visible CD spectra of the enzyme.

Lyon et al.[8b] reported the small but real effects of substrates on the infrared spectrum of the two intrinsic CO ligands of [Fe]-hydrogenase. They interpreted the infrared data as indicating that the substrates bind near to or at the active site and thereby decrease the flexibility of the active-site pocket. The infrared results are in agreement with our CD data.

We have revealed herein that iron-chromophore CD analysis is suitable for detecting changes induced in the iron chromophore of [Fe]-hydrogenase by the binding of substrates and inhibitors. Our CD data and previously reported infrared data support the hypothesis that the binding of methylene-H_4MPT induces a conformational change that closes the active-site cleft of [Fe]-hydrogenase to form the intact active site, as predicted from crystal structures of the protein. Interestingly, the crystal structure of [FeFe]-hydrogenase lacking the dinuclear iron center was recently solved in an open conformation, which is known to close after incorporation of the dinuclear center to thus generate the active enzyme.[11]

Experimental Section

CD spectra were recorded on a Jasco J-715 spectropolarimeter. The signals of the spectra were averaged at least twice with a bandwidth of 1.0 nm at a scan speed of 100 nm min^{-1}; the response was set to 1 s. Each CD curve was corrected by subtracting the corresponding spectrum of the buffer solution. For the methods for the preparation of the enzyme, the preparation of the FeGP cofactor, and equilibrium dialysis, see the Supporting Information.

Received: October 6, 2010
Published online: November 23, 2010

Keywords: circular dichroism · cofactors · enzymes · hydrogenation · iron

[1] a) R. D. Peacock, B. Stewart, *Coord. Chem. Rev.* **1982**, *46*, 129; b) S. M. Kelly, N. C. Price, *Curr. Protein Pept. Sci.* **2000**, *1*, 349; c) F. A. Cotton, C. A. Murillo, *Eur. J. Inorg. Chem.* **2006**, 4209.
[2] a) L. A. Andersson, J. A. Peterson, *Biochem. Biophys. Res. Commun.* **1995**, *211*, 389; b) M. S. Rogers, P. F. Knowles, A. J. Baron, M. J. McPherson, D. M. Dooley, *Inorg. Chim. Acta* **1998**, *276*, 175; c) V. V. Pollock, R. C. Conover, M. K. Johnson, M. J. Barber, *Arch. Biochem. Biophys.* **2003**, *409*, 315.
[3] a) R. K. Thauer, A. K. Kaster, M. Goenrich, M. Schick, T. Hiromoto, S. Shima, *Annu. Rev. Biochem.* **2010**, *79*, 507; b) J. C. Fontecilla-Camps, P. Amara, C. Cavazza, Y. Nicolet, A. Volbeda, *Nature* **2009**, *460*, 814.
[4] a) S. Shima, O. Pilak, S. Vogt, M. Schick, M. S. Stagni, W. Meyer-Klaucke, E. Warkentin, R. K. Thauer, U. Ermler, *Science* **2008**, *321*, 572; b) T. Hiromoto, K. Ataka, O. Pilak, S. Vogt, M. S. Stagni, W. Meyer-Klaucke, E. Warkentin, R. K. Thauer, S. Shima, U. Ermler, *FEBS Lett.* **2009**, *583*, 585; c) S. Shima, E. J. Lyon, M. S. Sordel-Klippert, M. Kauss, J. Kahnt, R. K. Thauer, K. Steinbach, X. L. Xie, L. Verdier, C. Griesinger, *Angew. Chem.* **2004**, *116*, 2601; *Angew. Chem. Int. Ed.* **2004**, *43*, 2547.
[5] a) D. Chen, R. Scopelliti, X. Hu, *Angew. Chem.* **2010**, *122*, 7674; *Angew. Chem. Int. Ed.* **2010**, *49*, 7512; b) T. B. Liu, B. Li, C. V. Popescu, A. Bilko, L. M. Perez, M. B. Hall, M. Y. Darensbourg, *Chem. Eur. J.* **2010**, *16*, 3083; c) A. M. Royer, T. B. Rauchfuss, D. L. Gray, *Organometallics* **2009**, *28*, 3618; d) P. J. Turrell, J. A. Wright, J. N. T. Peck, V. S. Oganesyan, C. J. Pickett, *Angew. Chem.* **2010**, *122*, 7670; *Angew. Chem. Int. Ed.* **2010**, *49*, 7508.
[6] a) T. Hiromoto, E. Warkentin, J. Moll, U. Ermler, S. Shima, *Angew. Chem.* **2009**, *121*, 6579; *Angew. Chem. Int. Ed.* **2009**, *48*, 6457; b) O. Pilak, B. Mamat, S. Vogt, C. H. Hagemeier, R. K. Thauer, S. Shima, C. Vonrhein, E. Warkentin, U. Ermler, *J. Mol. Biol.* **2006**, *358*, 798.
[7] J. E. Coleman, *J. Biol. Chem.* **1968**, *243*, 4574.
[8] a) M. Korbas, S. Vogt, E. Bill, W. Meyer-Klaucke, E. J. Lyon, R. K. Thauer, S. Shima, *J. Biol. Chem.* **2006**, *281*, 30804; b) E. J. Lyon, S. Shima, R. Boecher, R. K. Thauer, F.-W. Grevels, E. Bill, W. Roseboom, S. P. J. Albracht, *J. Am. Chem. Soc.* **2004**, *126*, 14239; c) S. Shima, E. J. Lyon, R. K. Thauer, B. Mienert, E. Bill, *J. Am. Chem. Soc.* **2005**, *127*, 10430.

[9] a) A. Berkessel, *Curr. Opin. Chem. Biol.* **2001**, *5*, 486; b) A. Berkessel, R. K. Thauer, *Angew. Chem.* **1995**, *107*, 2418; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2247; c) S. Vogt, E. J. Lyon, S. Shima, R. K. Thauer, *J. Biol. Inorg. Chem.* **2008**, *13*, 97; d) B. H. Geierstanger, T. Prasch, C. Griesinger, G. Hartmann, G. Buurman, R. K. Thauer, *Angew. Chem.* **1998**, *110*, 3491; *Angew. Chem. Int. Ed.* **1998**, *37*, 3300.

[10] A. A. Dimarco, T. A. Bobik, R. S. Wolfe, *Annu. Rev. Biochem.* **1990**, *59*, 355.

[11] D. W. Mulder, E. S. Boyd, R. Sarma, R. K. Lange, J. A. Endrizzi, J. B. Broderick, J. W. Peters, *Nature* **2010**, *465*, 248.

