Electronic Supplementary Material (ESI) for Dalton Transactions

Supplementary information

Evidence for acyl–iron ligation in the active site of [Fe]-hydrogenase provided by mass spectrometry and infrared spectroscopy

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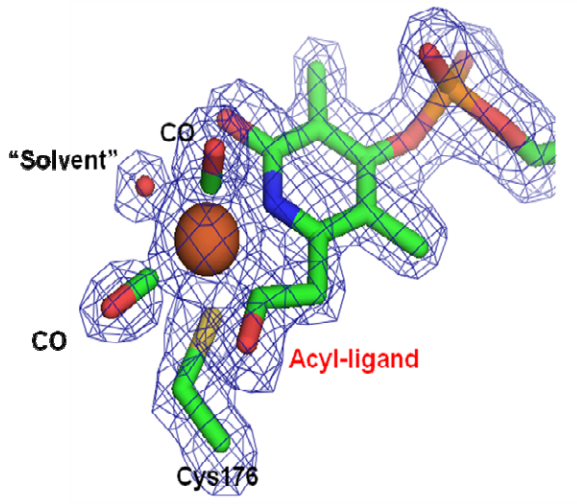
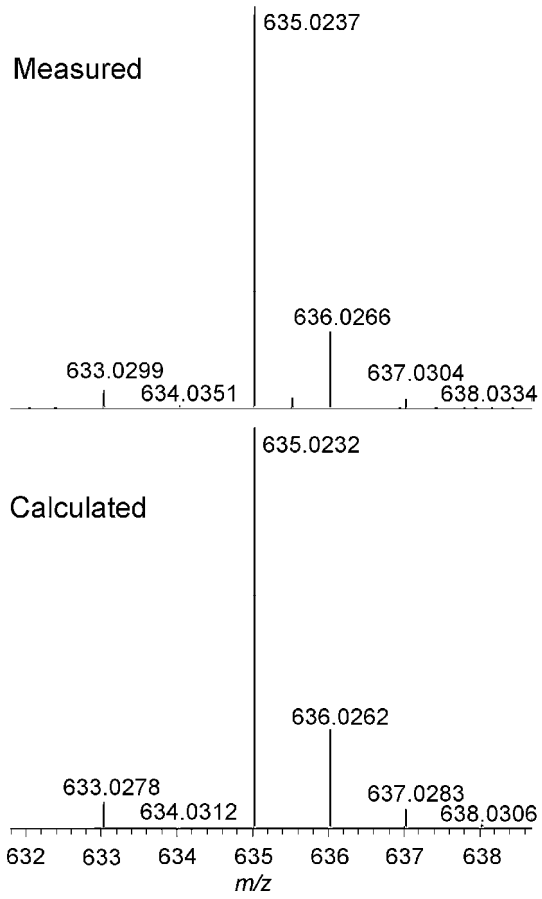
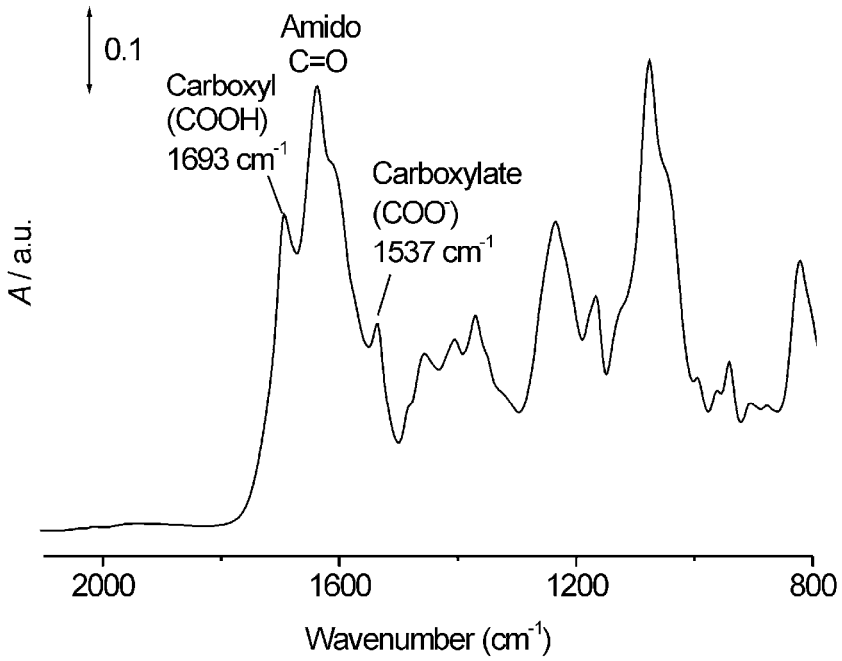


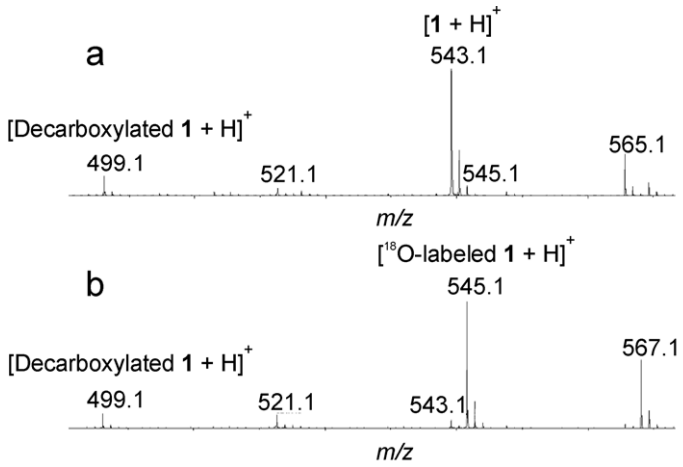
Supplementary Fig. S1 Structure of FeGP cofactor in the reconstituted [Fe]-hydrogenase holoenzyme from *Methanocaldococcus jannaschii*. The revised model containing the acyl-carbon ligand in the wild-type holoenzyme (PDB accession code 3F47) is shown. The electron density maps around the iron complex and the guanylyl pyridinol moiety are shown in blue. The figure was modified from.[1]



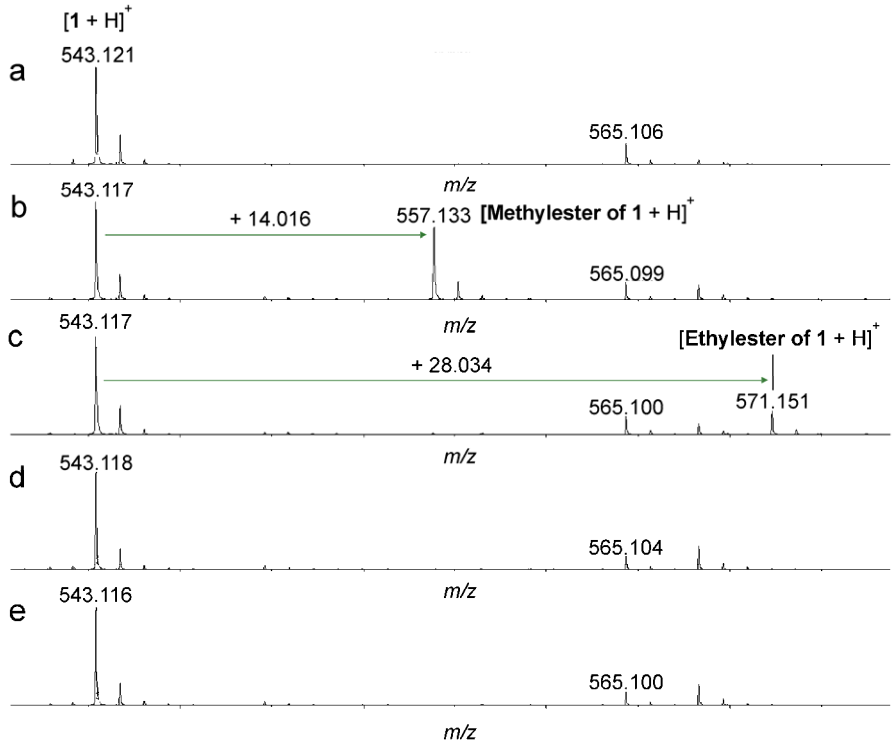
Supplementary Fig. S2 ESI-FT-ICR-MS analysis (negative mode) of isolated FeGP cofactor. The presence of the iron ion in the m/z 635 species is indicated by the characteristic ^{54}Fe isotopic peak (*m/z* 633.0299), which is smaller than the main peak (*m/z* 635.0237) by *m/z* 2.



Supplementary Fig. S3 Attenuated total reflection infrared (ATR-IR) spectra of the light decomposition product **1**. The samples (5 µl of 10 mM) were dried on the sample window under an argon stream. This sample was purified as described previously.[2]



Supplementary Fig. S4 MALDI-TOF-MS spectra (positive mode) of the light-decomposition products of the FeGP cofactor. Acetic-acid-extracted FeGP cofactor **3** (0.05 ml of 0.1 mM) was dried by evaporation at 4 °C and dissolved (a) in 0.05 ml of 100 mM Na_2CO_3/$NaHCO_3$ at pH 10 in $H_2{}^{16}O$, (b) in 0.5 ml of 100 mM Na_2CO_3/$NaHCO_3$ at pH 10 in $H_2{}^{18}O$ (97 atom%). After illumination, each sample was mixed with the matrix, and mass spectra were recorded. Illumination was provided by a Schott KL2500LCD with a 250 W halogen lamp (maximum light flux) via flexible light guides. The *m/z* 543.1peak is a monocationic species (intact **1** plus proton) and the *m/z* 499.1 peak is most probably a protonated form of the decarboxylated product of **1.**[2] The additional peaks at *m/z* 521, 565, and 567 are adducts with a sodium cation of the *m/z* 499, 543, and 545 species.[2] This experiment "B" yielded *m/z* 545 species instead of the non-labeled monocationic *m/z* 543 species.



Supplementary Fig. S5 Formation of ester compounds by light decomposition of FeGP cofactor in alcohols. The acetic-acid-extracted FeGP cofactor **3** (0.05 ml of 0.1 mM) was dried by evaporation at 4 °C and dissolved (a) in 0.1 ml of volume in 100 mM Na_2CO_3/$NaHCO_3$ pH 10, (b) in 0.1 ml of 99.9% methanol, and (c) in 0.1 ml of 99.8% ethanol. In the alcohol samples, some particles of molecular sieve were added to remove water. After removal of molecular sieve, the samples were illuminated for 10 min at 0 °C. The samples were then dried and dissolved in 0.05 ml of water. In order to show the non-reactivity of the alcohols with the carboxy group of the pyridinol derivative **1**, aliquots of the light-decomposed sample "A", which contained **1**, were dried again and then dissolved in the same volume of methanol (d) and ethanol (e), and illuminated for 10 min at 0 °C, and then the solvent was exchanged with the same volume of water. Finally, each illuminated sample was mixed with the matrix for MALDI-TOF-MS (positive mode). Illumination was provided by a Schott KL2500LCD with a 250 W halogen lamp (maximum light flux) via flexible light guides.

Supplementary Table S1 ESI-FT-ICR-MS (negative mode) data of the light decomposition products of the acetic-acid extracted FeGP cofactor. The samples were analyzed with ESI-FT-ICR-MS in combination with HPLC.

Species	Formula of the detected anion	Molecular masses (Da)		
		Calculated	Measured	Error
[**1** – H^+]$^-$	$C_{19}H_{22}N_6O_{11}P_1$	541.1090	541.1090	0.0000
[**1** – H^+]$^-$ [a]	$C_{19}H_{22}N_6O_{10}$**$^{18}O_1$**P_1	543.1132	543.1123	- 0.0009
[**Methylester of 1** – H^+]$^-$	$C_{20}H_{24}N_6O_{11}P_1$	555.1246	555.1249	+0.0003
[**Ethylester of 1** – H^+]$^-$	$C_{21}H_{26}N_6O_{11}P_1$	569.1403	569.1413	+0.0010

[a] Decomposed in $H_2{}^{18}O$. [b] Decomposed in ^2H_2O.

1. T. Hiromoto, K. Ataka, O. Pilak, S. Vogt, M. S. Stagni, W. Meyer-Klaucke, E. Warkentin, R. K. Thauer, S. Shima and U. Ermler, *FEBS Lett.*, 2009, **583**, 585-590.
2. S. Shima, E. J. Lyon, M. S. Sordel-Klippert, M. Kauss, J. Kahnt, R. K. Thauer, K. Steinbach, X. L. Xie, L. Verdier and C. Griesinger, *Angew. Chem. Int. Ed.*, 2004, **43**, 2547-2551.