

# Supplementary Information for Protonation of a subsite analogue of [FeFe]-hydrogenase: mechanism of a deceptively simple reaction revealed by time-resolved IR spectroscopy

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## General experimental

HPLC grade acetonitrile (Fisher) was degassed by bubbling for 15 minutes with nitrogen and dried using a Braun solvent purifier.  $\text{HBF}_4 \cdot \text{Et}_2\text{O}$  (Aldrich) was used as received. Compound **1** was prepared according to ref. 7 (main text).

## Stop-flow instrumentation

All stop-flow measurements were carried out using Tgk Scientific stop-flow drive systems mounted inside Belle Technology glove boxes. The reaction solutions were prepared inside the glove box before loading into syringes in the drive unit. This was thermostated at  $21.0^\circ\text{C} \pm 0.2^\circ\text{C}$  by means of a Thermo Scientific recirculating bath.

For UV-visible measurements, the syringes were driven by compressed air into a mixing chamber attached to the drive unit inside the glove box and equipped with a stop syringe. The light source was mounted outside of the glove box and UV-visible light was coupled to the cell by means of a fibre optic cable. Initial measurements were carried out using a Tgk diode array mounted outside of the box and coupled to the cell by means of a second fibre optic cable. This allowed measurement in the range 310 nm to 700 nm. For time-resolved measurements, the diode array was replaced by a photomultiplier tube and a monochromator was introduced between the lamp and the cell, supplying UV radiation at a 348 nm.

Lowering the acid concentration below 20 mM led to a linear decay of **1** with time, implying a zero-order dependence on both acid and substrate. This occurred even though the ratio of acid to substrate remained high (at least 50 : 1). One possible explanation for this is that there are two possible proton sources in the reaction,  $[\text{HCNMe}]^+$  and  $[\text{HOEt}_2]^+$ . The protonated solvent will predominate, but it is possible that only the protonated ether is sufficiently acidic to react with **1**. The amount of  $[\text{HOEt}_2]^+$  will be very much lower than the overall acid concentration.

For IR measurements, the syringes were driven by a computer-controlled stepper motor. The reaction solutions were fed through a thermostated "umbilical" to the mixing chamber, which was part of a custom IR flow cell fitted with  $\text{CaF}_2$  windows, and mounted inside a small inert atmosphere chamber (also fitted with  $\text{CaF}_2$  windows) in a Bruker Vertex 80 spectrometer. A Northumbria Optical Coatings band pass filter was used between the cell. Initial experiments used a filter with pass range  $1650\text{ cm}^{-1}$  to  $2200\text{ cm}^{-1}$ ; no signals were seen below  $1800\text{ cm}^{-1}$  and so time-resolved data were acquired using a filter with pass band  $1790\text{ cm}^{-1}$  to  $2200\text{ cm}^{-1}$ . Data analysis was carried out in the software package *fit\_3D* (S. J. George, University of California, 2009).

## Variable temperature NMR

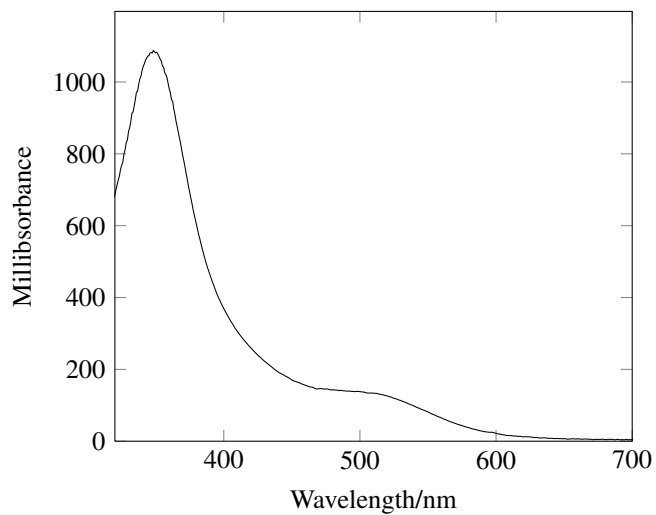
NMR spectra were recorded on a Bruker Avance 300 spectrometer. A solution of **1** (4.5 mg,  $9.3\ \mu\text{mol}$ ) in  $d_3$ -MeCN ( $0.5\text{ cm}^3$ ) was cooled to  $10^\circ\text{C}$  in the spectrometer. The sample was ejected and neat  $\text{HBF}_4 \cdot \text{Et}_2\text{O}$  ( $10\ \mu\text{l}$ ,

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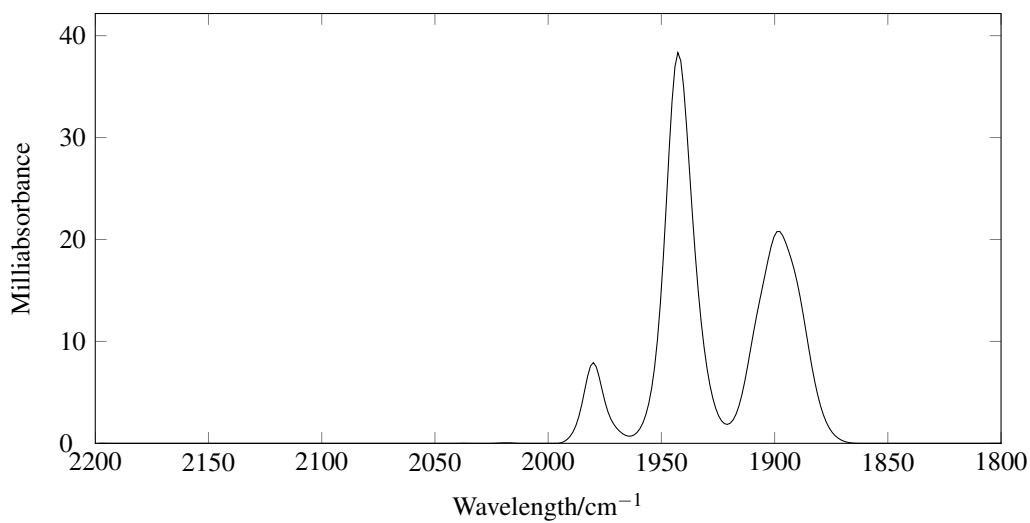
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73  $\mu\text{mol}$ ) was added, the mixture shaken briefly and re-introduced into the probe. Spectra were then acquired over approximately ten minutes, with each acquisition consisting of 4 scans. Separate samples were used for the proton and phosphorus experiments.

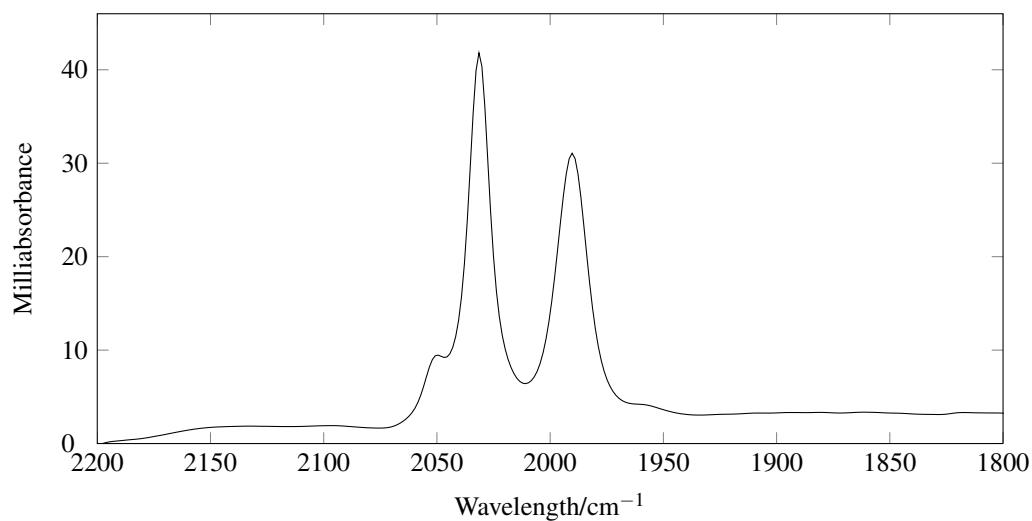
## Spectra



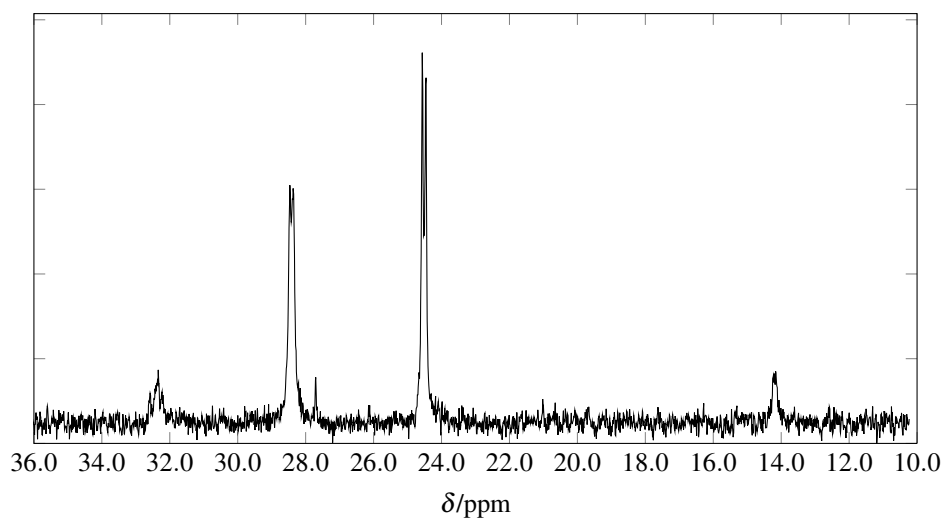
**Fig. 1** UV spectrum of **1** in MeCN.



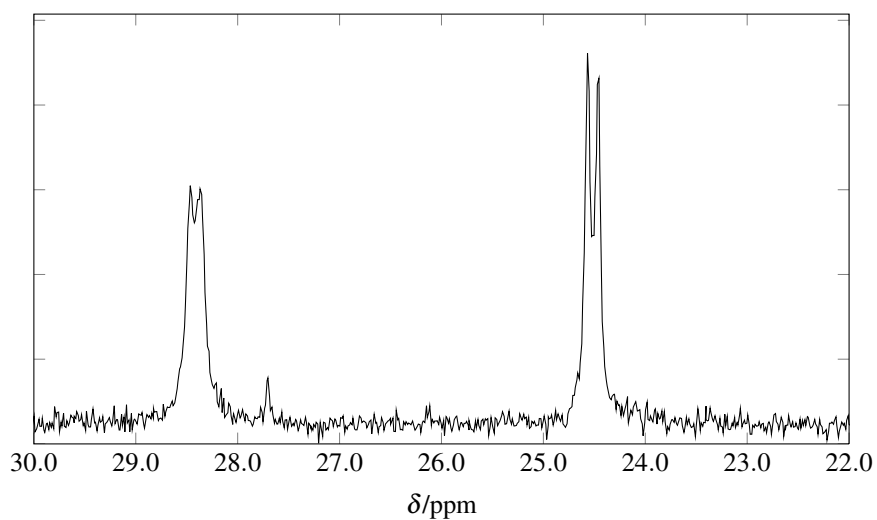
**Fig. 2** IR spectrum of **1** in MeCN.



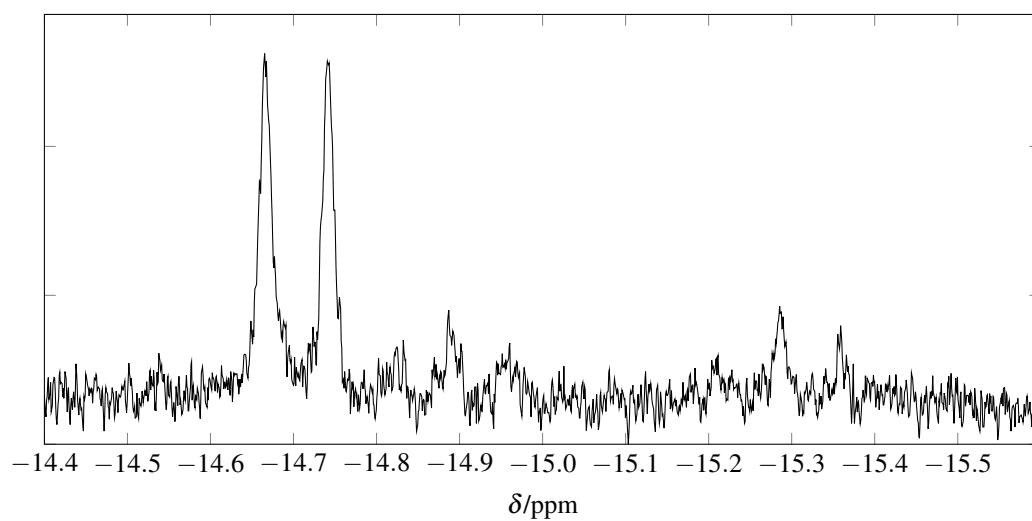
**Fig. 3** IR spectrum of **2** in MeCN.



**Fig. 4**  $^{31}\text{P}\{^1\text{H}\}$  spectrum of **1** in  $d_6$ -acetone at  $-60^\circ\text{C}$ .



**Fig. 5**  $^{31}\text{P}\{^1\text{H}\}$  spectrum of **1** in  $d_6$ -acetone at  $-60^\circ\text{C}$ , enlarged.



**Fig. 6** <sup>1</sup>H spectrum of reaction of **1** with HBF<sub>4</sub>·Et<sub>2</sub>O in d<sub>3</sub>-MeCN at 10°C after approximately one minute.