Supporting information

for

The O_2-stable [FeFe]-hydrogenase CbA5H reveals high resilience against organic solvents

By Martin Gerbaulet, Anja Hemschemeier and Thomas Happe*

Ruhr University Bochum, Faculty of Biology and Biotechnology, Photobiotechnology, Universitätsstr. 150, 44801 Bochum, Germany

Content:

Additional results

Residual H_2 production activity of [FeFe]-hydrogenases CbA5H from *Clostridium beijerinckii*, HydA1 from *Chlamydomonas reinhardtii* and CpI from *Clostridium pasteurianum* after incubation in 30 % (v/v) of acetone, dimethylsulfoxide (DMSO) and acetonitrile at 23 °C over the course of 4 h (HydA1) and 24 h (CbA5H, CpI).

Figure SI1: H_2 production activity of *C. beijerinckii* CbA5H after incubation in 30 % (v/v) acetone, DMSO or acetonitrile over the course of 24 h

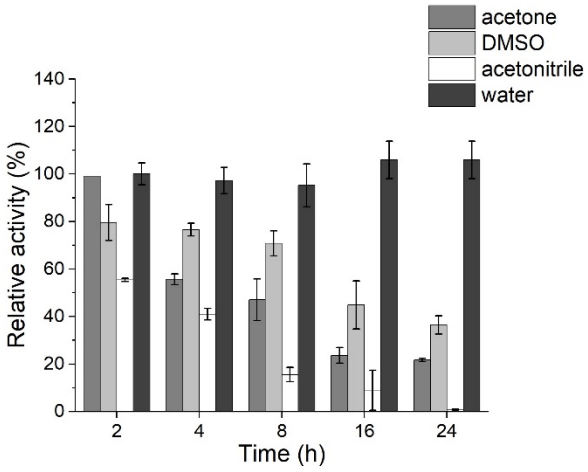

Figure SI1*:* H_2 production activity of *C. beijerinckii* **CbA5H** after incubation in 30 % (v/v) of the indicated solvents over the course of 24 h. The enzyme was incubated in acetone (grey), DMSO (light grey), acetonitrile (white) and water (dark grey) for the indicated time-points at 23 °C. Afterwards, 10 µl of these solutions were added to a hydrogenase activity assay as described in the caption of Fig. 1 as well as the Experimental section. The columns show the averages of two independent experiments employing biological duplicates. The standard deviation is indicated by the error bars. The activities were always calculated to the initial activities of the CbA5H enzymes incubated in water, which were set to 100 % and equalled 2400 U $\times$ mg^{-1}.

Figure SI2: H_2 production activity of *C. reinhardtii* HydA1 after incubation in 30 % (v/v) acetone, DMSO or acetonitrile over the course of 4 h

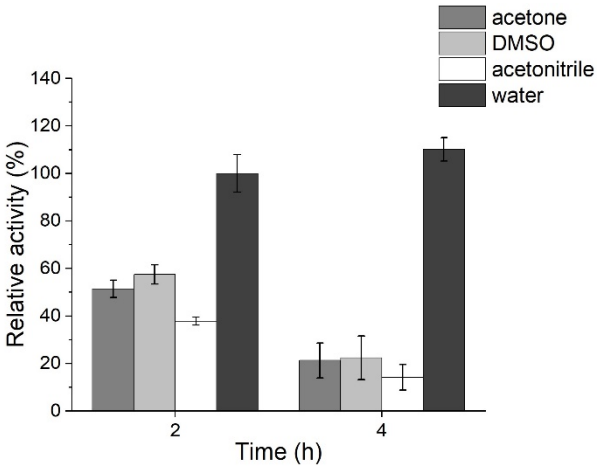

Figure SI2*:* H_2 production activity of *C. reinhardtii* **HydA1** after incubation in 30 % (v/v) of the indicated solvents for 2 and 4 h. The hydrogenase was incubated in acetone (grey), DMSO (light grey), acetonitrile (white) and water (dark grey) for 2 and 4 h at 23 °C. Afterwards, 10 µl of these solutions were added to our standard hydrogenase activity assay. The columns show the averages of two independent experiments employing biological duplicates. The standard deviation is indicated by the error bars. The activities were always calculated to the initial activities of the HydA1 enzyme incubated in water, which were set to 100 % and equalled 905 U $\times$ mg^{-1}.

Figure SI3: H_2 production activity of *C. pasteurianum* CpI after incubation in 30 % (v/v) acetone, DMSO or acetonitrile over the course of 24 h

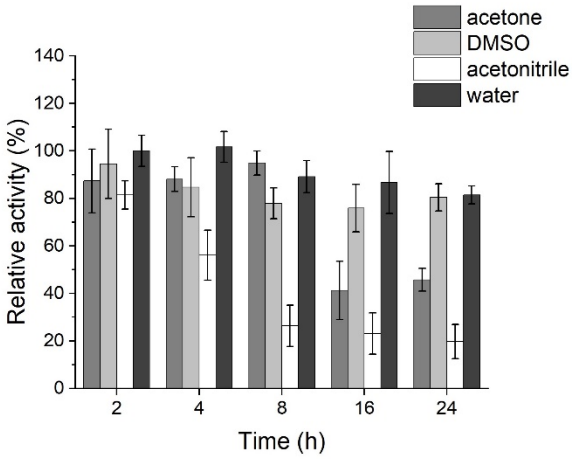

Figure SI3*:* H_2 production activity of *C. pasteurianum* **CpI** after incubation in 30 % (v/v) of the indicated solvents over the course of 24 h. The hydrogenase was incubated in acetone (grey), DMSO (light grey), acetonitrile (white) and water (dark grey) for 2, 4, 8, 16 and 24 h at 23 °C. After this incubation period, 10 µl of the enzyme solutions were added to the hydrogenase activity assay as described in the caption of Fig. 1 and the Experimental section. The columns show the averages of two independent experiments employing biological duplicates. The standard deviation is indicated by the error bars. The activities were calculated to the initial activities of the CpI enzyme incubated in water, which were set to 100 % and equalled 2184 U $\times$ mg^{-1}.

Residual H_2 production activity of [FeFe]-hydrogenases HydA1 from *Chlamydomonas reinhardtii* and CpI from *Clostridium pasteurianum* after incubation in different concentrations of acetone, dimethylsulfoxide (DMSO) and acetonitrile at 23 °C for 30 minutes.

Figure SI4: H_2 production activity of *C. reinhardtii* HydA1 after incubation in acetone, DMSO or acetonitrile

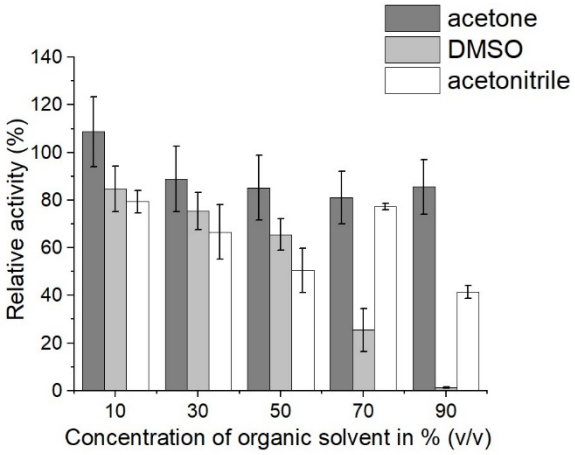

Figure SI4*:* H_2 production activity of *C. reinhardtii* **HydA1** after incubation in the indicated concentrations (all in (v/v) in water) of acetone (dark grey), DMSO (light grey) or acetonitrile (white) for 30 min. After the incubation period, 10 µl of the solutions were added to a hydrogenase activity assay as described in the caption of Fig. 1 and the Experimental section. The columns show the averages of two independent experiments employing biological duplicates. The standard deviation is indicated by the error bars. The activities were always calculated to those of the HydA1 enzyme incubated in water, which were set to 100 % and equalled 1035 U $\times$ mg^{-1}.

Figure SI5: H_2 production activity of *C. pasteurianum* CpI after incubation in acetone, dimethylsulfoxide (DMSO) or acetonitrile

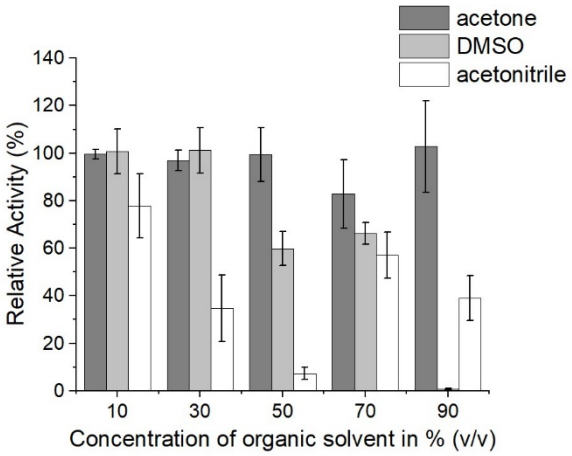

Figure SI5*:* H_2 production activity of *C. pasteurianum* **CpI** after incubation in the indicated concentrations (all in (v/v) in water) of acetone (dark grey), DMSO (light grey) or acetonitrile (white). The experimental procedure was the same as described in the caption of Fig. SI4. The columns show the averages of two independent experiments employing biological duplicates. The standard deviation is indicated by the error bars. The activities were always calculated to those of the CpI enzyme incubated in water, which were set to 100 % and equalled 2000 U $\times$ mg^{-1}.

ATR-FTIR spectra of CbA5H after incubation in alcohols

Figure SI6: ATR-FTIR spectra of *C. beijerinckii* CbA5H after incubation in different concentrations of methanol

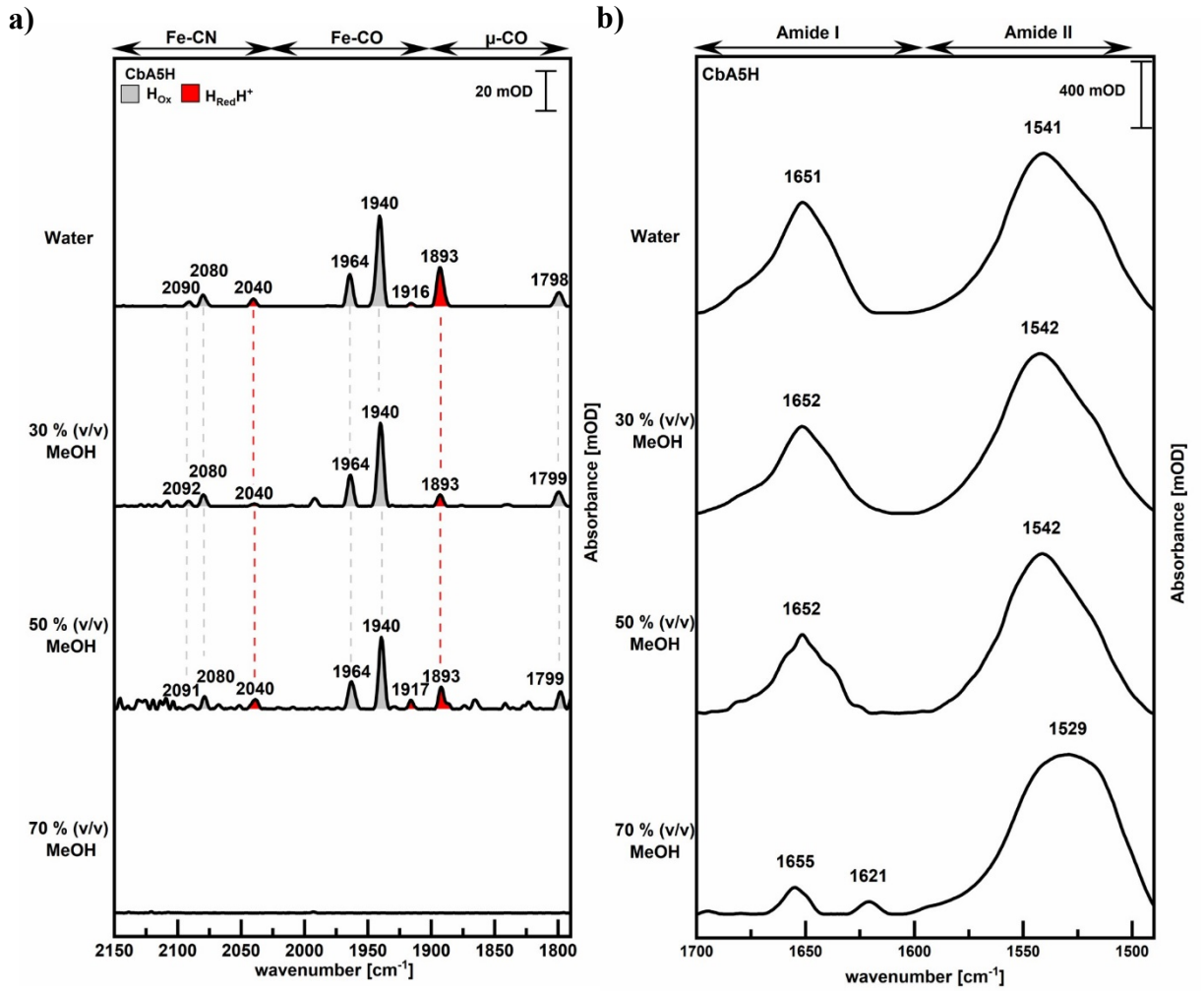

Figure SI6 ATR-FTIR signals of the H-Cluster (**a**) and amide bands (I and II) (**b**) of CbA5H after incubation in different concentrations (30, 50 and 70 % (v/v)) of methanol (MeOH). 3 µg of the purified enzyme were incubated in 10 µl of solvent solution for 30 minutes at 23 C and applied to the ATR crystal afterwards. After drying for 5 – 10 minutes, the spectra were recorded. Blanks were always done with the respective solvent solution. In (**a**), signals corresponding to typical H-cluster redox states are coloured grey (H_{Ox}) and red ($H_{Red}H^+$).

Figure SI7: ATR-FTIR spectra of CbA5H after incubation in different concentrations of ethanol

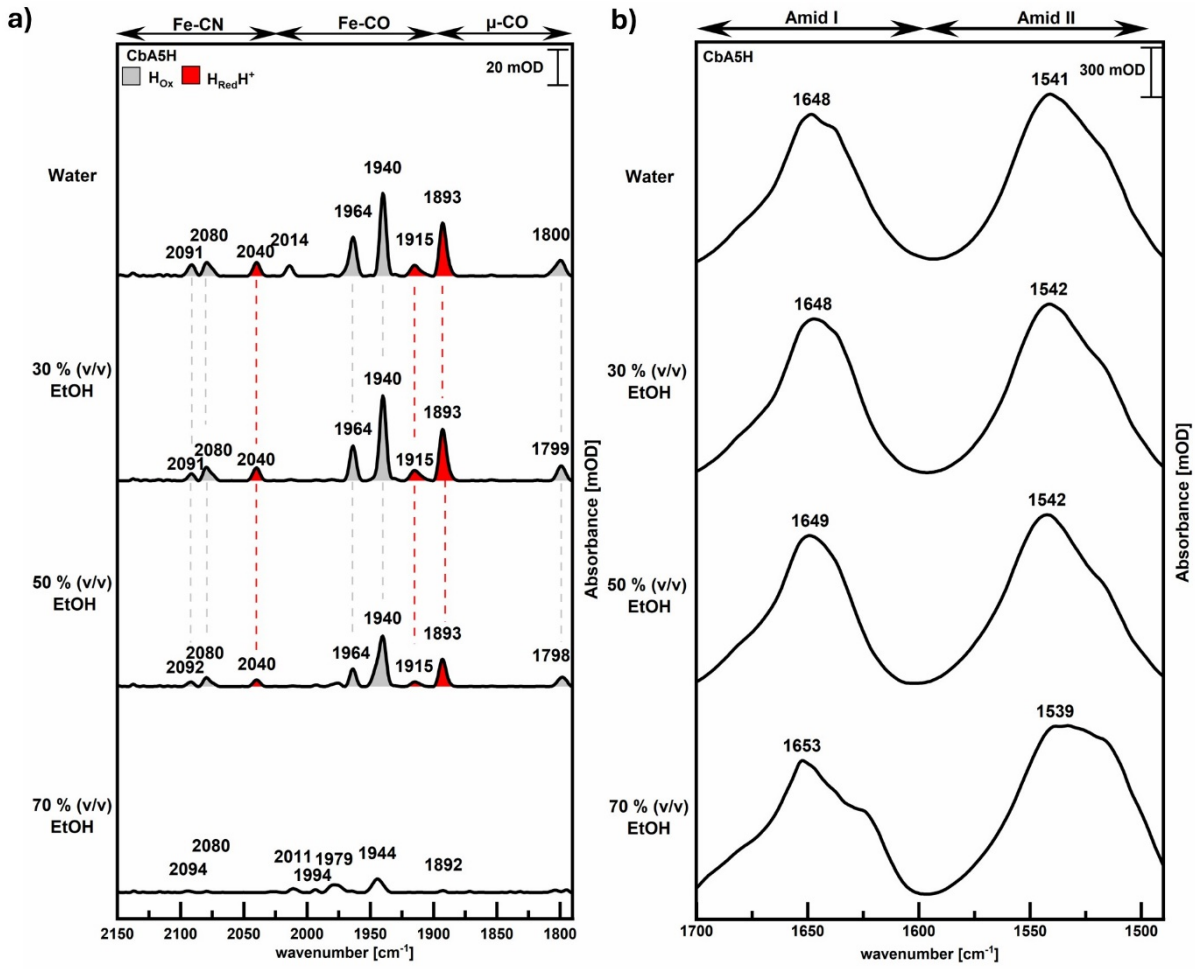

Figure SI7 ATR-FTIR spectroscopy of CbA5H after incubation in different concentrations (30, 50 and 70 % (v/v)) of ethanol (EtOH). The experimental proceeding was the same es described in the caption of Figure SI6. Panel (**a**) shows the H-Cluster signals, and (**b**) the amide bands I and II.

Figure SI8: ATR-FTIR spectra of CbA5H after incubation in different concentrations of 2-propanol

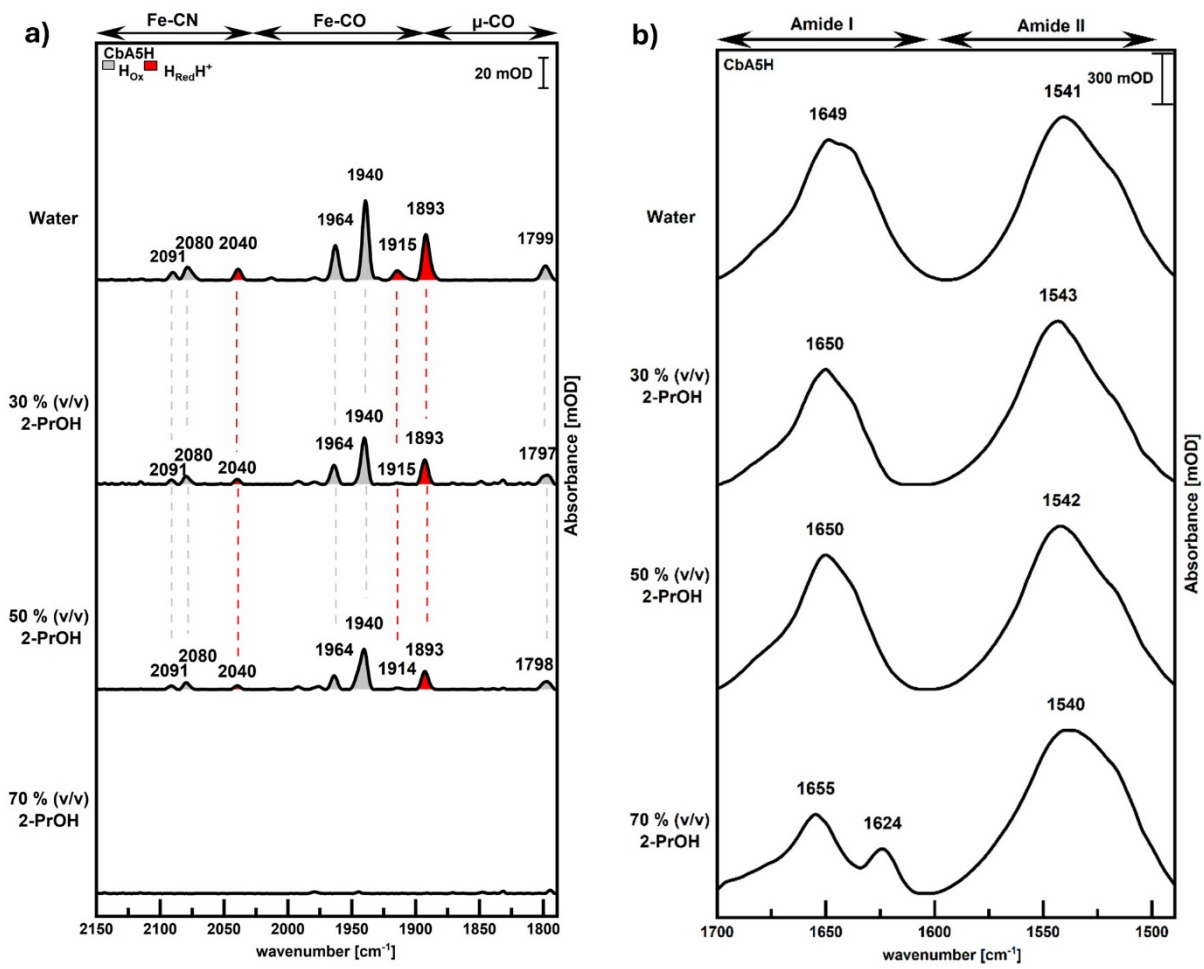

Figure SI8 H-Cluster signals (**a**) and amide bands I and II (**b**) recorded by ATR-FTIR spectroscopy of CbA5H after incubation in different concentrations of 2-propanol (2-PrOH; 30, 50 and 70 % (v/v)). The experiments were done as described in the caption of Fig. SI6.

Figure SI9: ATR-FTIR spectra of CbA5H after incubation in different concentrations of 1-propanol

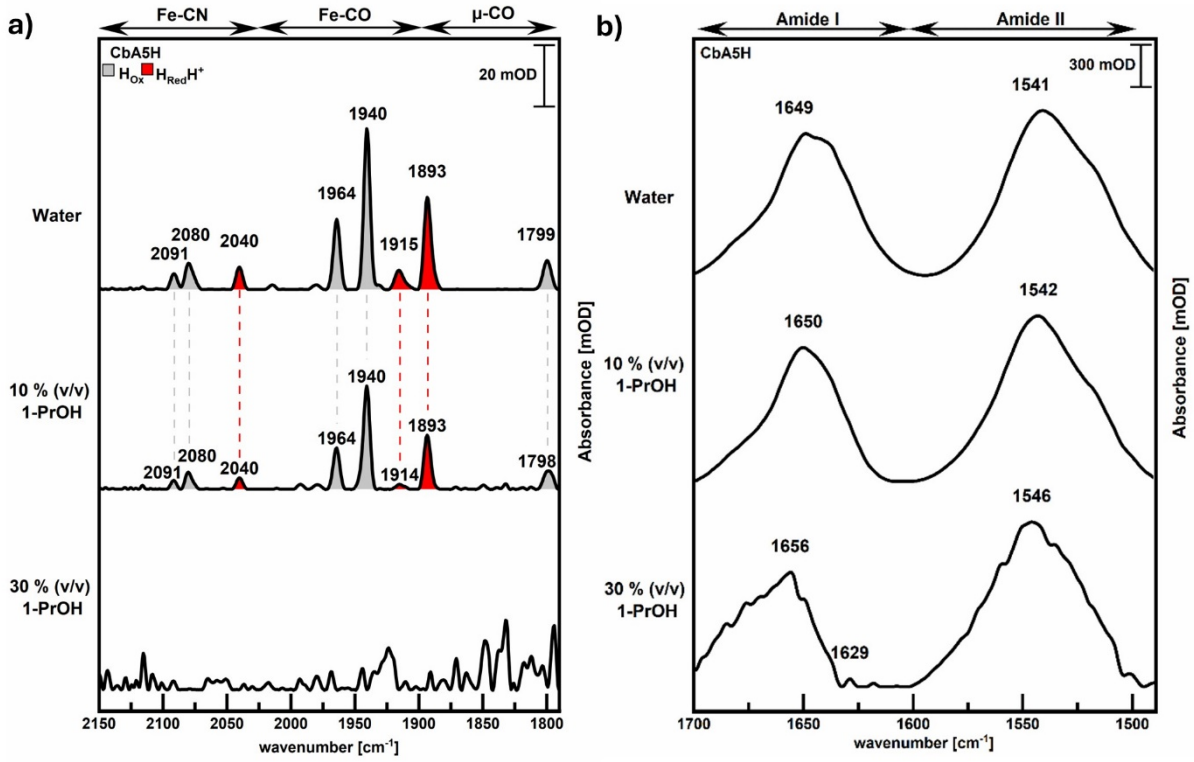

Figure SI9 ATR-FTIR spectroscopy to detect H-Cluster signals (**a**) and amide bands I and II (**b**) of CbA5H after incubation in different concentrations of 1-propanol (1-PrOH). 3 µg of the purified enzyme were employed for each solvent solution, and the experiments were done as described in the caption of Fig. SI6.