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SUPPORTING INFORMATION

# $^{14}$N HYSCORE investigation of the H-cluster of [FeFe] hydrogenase: Evidence for a nitrogen in the dithiol bridge.

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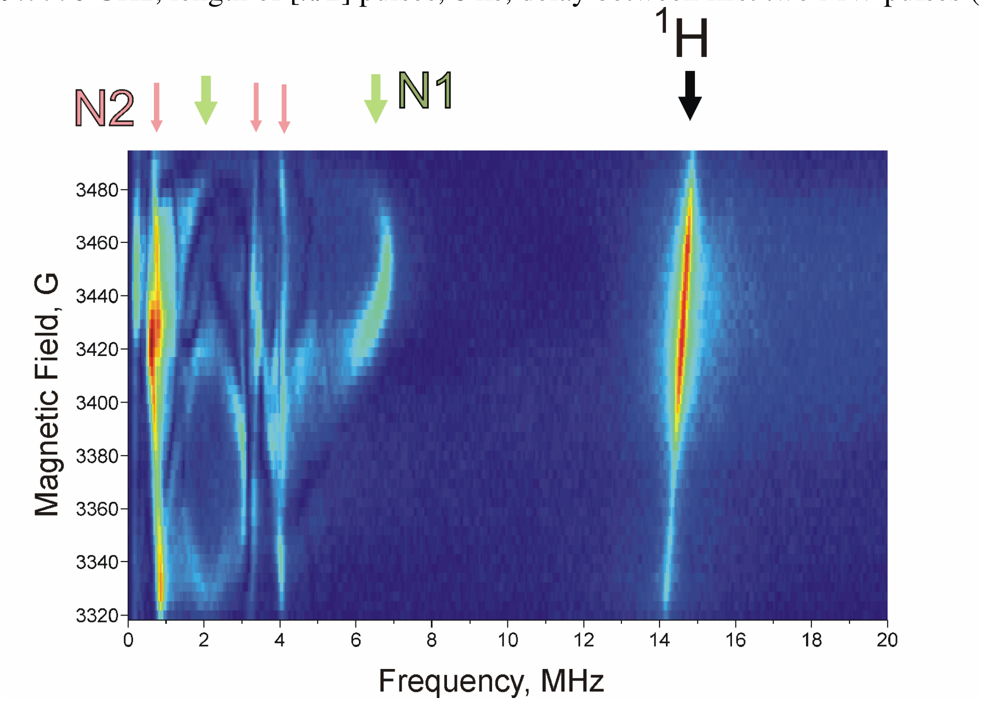
Figure S1. X-band 3pulse ESEEM vs Magnetic field of the Hox state of the H-clustre from *Desulfovibrio desulfuricans*. Experimental conditions: T, 15K; MW frequency, 9.7776 GHz; length of [π/2] pulses, 8 ns; delay between first two MW pulses (τ), 180 ns.

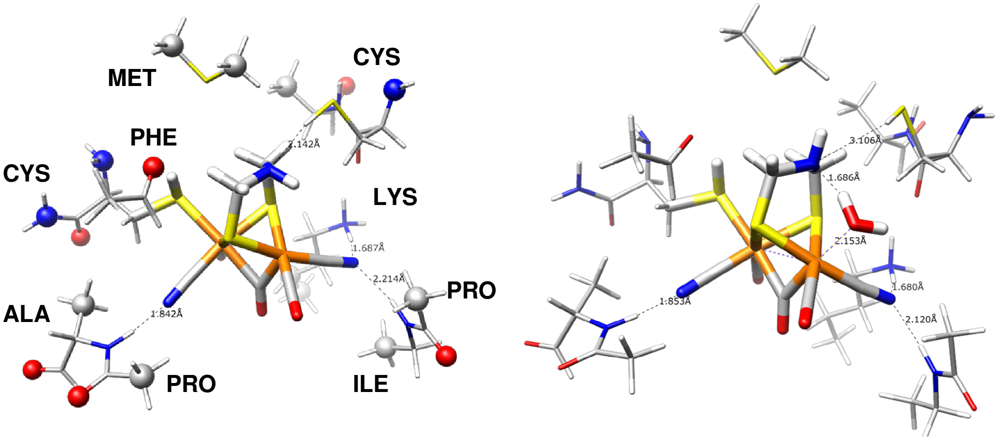


Two types of $^{14}$N signals can be identified in this ESEEM spectrum and assigned to two $^{14}$N nuclei (green and magenta arrows). Magenta arrows indicate signals which show very little field dependence while the ones indicated with green show a strong dependents.

Figure S2. DFT models, used in the study of the quadrupole couplings of the H-cluster in the $H_{ox}$ state. Balls on the structure of **A** show the constraints, made for the geometry optimization. The same constraints were used for **B** and **C**. In **D** no atom was fixed. Distances of some N--H bonds are included.

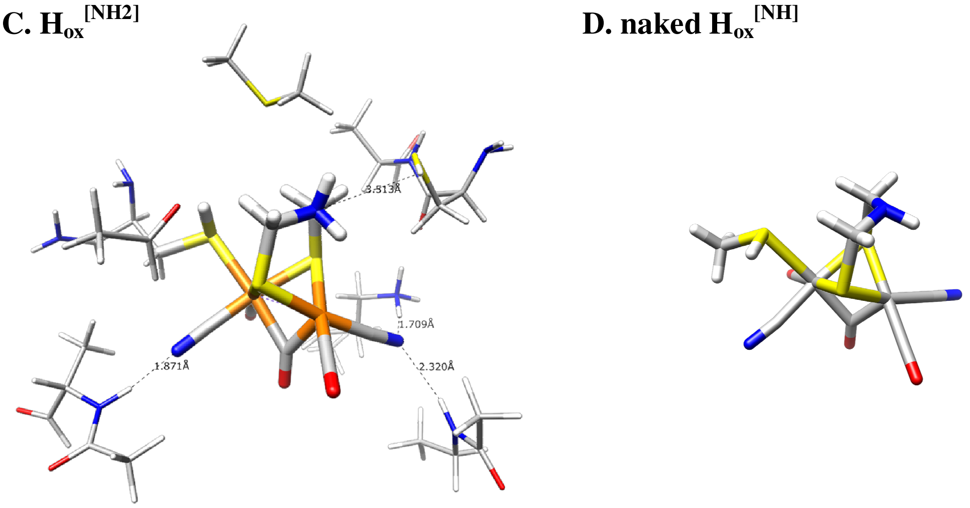
**`A. $H_{ox}^{[NH]}$**

**B. $H_{ox}^{[NH]}$-$H_2O$**



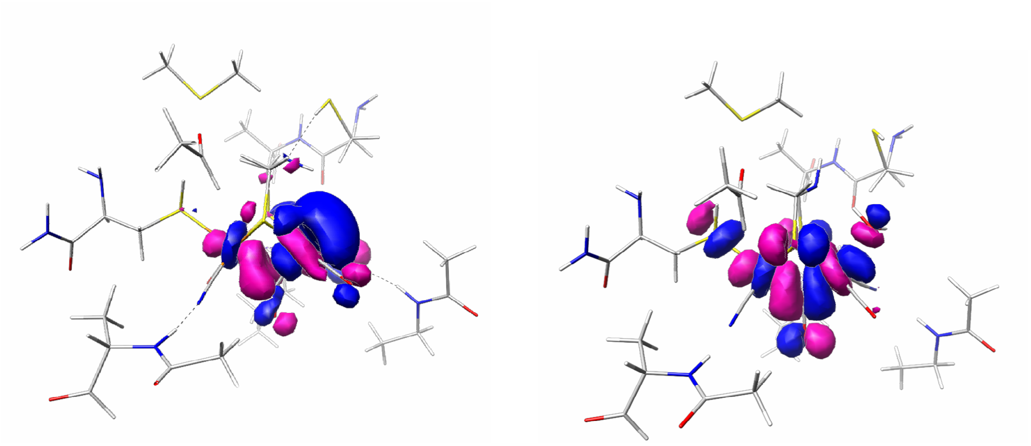
**C. $H_{ox}^{[NH2]}$**

**D. naked $H_{ox}^{[NH]}$**



We note that in the case of the $H_{ox}^{[NH]}$-$H_2O$ model the unpaired spin density was found to be equally distributed over both irons (see figure S3 and table S1) which is rather different from the $H_{ox}^{[NH]}$ model where most of the spin density was found at the distal iron. An equal distribution of the spin density over both sites of the $[2Fe]_H$ subcluster in the $H_{ox}$ state was, however, suggested by our $^{57}$Fe study [`1_Silakov2007`]. Hence it is tempting to suggest that there is a water molecule present at the distal iron in the $H_{ox}$ state of DdH, similar to CpI. More advanced DFT calculations on the complete H-cluster need to be performed in order to clarify this question. A possible way to resolve this experimentally is to investigate DdH in $D_2O$ and/or $H_2{}^{17}O$ by means of advanced EPR methods.

Figure S3. Effect of $H_2O$ on SOMOs of $H_{ox}^{[NH]}$ model. Isosurfaces were obtained at 0.02 level.



**$H_{ox}^{[NH]}$**

**$H_{ox}^{[NH]}$ -$H_2O$**

Table S1. Mulliken spin population analysis on selected atoms:

| | naked $H_{ox}^{[NH]}$ | $H_{ox}^{[NH]}$ | $H_{ox}^{[NH]}$-$H_2O$ | $H_{ox}^{[NH2]}$ |
|---|---|---|---|---|
| Charge | -1 | 0 | 0 | +1 |
| | | Spin Populations | | |
| N (Ala) | - | -0.000007 | -0.000050 | -0.000007 |
| N (Ile) | - | 0.000099 | 0.000046 | 0.000083 |
| N (Lys) | - | 0.000185 | 0.000093 | 0.000077 |
| N ($CN_d$) | 0.074328 | 0.042323 | 0.001382 | 0.044353 |
| N ($CN_p$) | 0.001601 | 0.003034 | 0.000492 | 0.002529 |
| N (DTN) | -0.003330 | -0.004001 | -0.001795 | -0.006738 |
| **Fe ($Fe_p$)** | **0.064978** | **0.126337** | **0.495016** | **0.100359** |
| **Fe ($Fe_d$)** | **1.088289** | **0.970039** | **0.476411** | **1.000737** |
| S (Cys) | 0.005002 | 0.002121 | 0.018734 | 0.000600 |
| S (DTN) | -0.001564 | -0.024939 | -0.001174 | -0.026818 |
| S (DTN) | -0.022621 | -0.022854 | -0.008200 | -0.022520 |
| S (Cys---DTN) | - | 0.000016 | 0.000068 | 0.000103 |
| O ($H_2O$) | - | - | 0.009426 | - |

According to the Mulliken population analysis, the unpaired spin density is mainly localized at the distal iron. The only exception is $H_{ox}^{[NH]}$-$H_2O$ model, in which spin population on both irons is roughly equal.

## References

1. Silakov, A, Reijerse, E. J., Albracht, S. P. J., Hatchikian, E. C., and Lubitz, W.; The electronic structure of the H-cluster in the [FeFe]-hydrogenase from Desulfovibrio desulfuricans: A Q-band Fe-57-ENDOR and HYSCORE study(2007) Journal of the American Chemical Society 129: 11447-11458