Supplementary Material (ESI) for Chemical Communications
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Supporting Information for

Delocalized Electronic Structure of Hydrogenase H-cluster

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Pages 2-3: Details of XAS data collection

Pages 4-8: Details of DFT calculations and coordinates of initial and optimized structures

Optimized Atomic Coordinates of $^{1}[(LS_3)Fe_4S_4\{Fe_2(CH_3C(\mu\text{-}SCH_2)_3(CO)_5]^{2-}$

Optimized Structure of $^{1}[(LS_3)Fe_4S_4\{Fe_2(CH_3C(\mu\text{-}SCH_2)_3(CO)_5]^{2-}$ with selected internal coordinates

Figure S1: Frontier molecular orbitals for the biomimetic H-cluster compound

(orbital plots were generated in GOpenMol: http://www.csc.fi/gopenmol/)

Figure S2: S K-edge spectra of the wild-type native and CO-bound resting forms of *Cp*I hydrogenase

Figure S3: Difference plot (yellow/green at ±0.003 contour levels) between the electron densities of the [6Fe] and the separate [4Fe]- and [2Fe]-subclusters for the computational model of the hydrogenase active site.

Figure S4: Frontier molecular orbitals for the [6Fe] cluster model of the hydrogenase active site

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Details of XAS data collection

Model complexes $[Fe_2S_2(CO)_6]$,[1] $[Fe_2(SEt)_2(CO)_6]$,[2] [Fe_2(1,3-propanadithiolate)$(CO)_6$],[3] [Fe_2(di(2-methylthiolate)ammine)]$(CO)_6$],[4] $[Fe_2(CH_3C(CH_2S)_2(CH_2SCH_3)(CO)_5]$,[5] $(Bu_4N)_2$[(1,3,5-tris(4,6-dimethyl-3-mercaptophenylthio)-2,4,6-tris(p-tolyl-thio)benzene)Fe_4S_4(SEt)],[6] and $(Bu_4N)_2$[(1,3,5-tris(4,6-dimethyl-3-mercaptophenylthio)-2,4,6-tris(p-tolyl-thio)benzene)$Fe_4S_4\{Fe_2(CH_3C(CH_2S)_3)(CO)_5\}$][6] were synthesized by established protocols in CJP's laboratory. The wild-type and CO-bound native *Cp*I hydrogenase samples were prepared anaerobically in JWP's laboratory in about 1 mM protein concentration. The sulphur K-edge XAS measurements were carried out at BL6-2 of Stanford Synchrotron Radiation Laboratory (beamtime proposal 2893MP). Details of beamline setup and optimization have been published elsewhere.[7] The solid samples were ground and pasted onto a sulphur-free Mylar tape from Shercon in a glovebox with sub ppm oxygen and moisture levels. Protein samples with 20% glycerol were kept in a Teflon cell under liquid nitrogen and directly mounted to a Cu block purged with dry nitrogen gas that was cooled in a liquid nitrogen filled cryostat. A constant -30±5 ^oC temperature were maintained during the measurement. Samples were protected by a thin polypropylene window (Specs CertiPrep) during sample mounting and change at the beamline from exposure to air. Fluorescence signal was collected using Lyttle detector positioned at 45° solid angle relative to the sample cell. The incident photon energy was scanned in 0.5 eV steps outside the rising edge region where the stepsize was 0.1 eV. At least five scans were averaged to obtain a good signal-to-noise ratio. The incident photon energy was calibrated to the first transition (2472.02 eV) of the sodium thiolsulphate pentahydrate spectrum. A smooth background (second order polynomial) was subtracted from the spectra and normalized at 2490 eV of the spline (second order polynomial). Background subtractions, and spline were performed using PeakFit 4.12 (SeaSolve). S K-edge spectra of [2Fe] complexes in the full energy range after background subtraction and spline (along the dotted line) are shown on page S2.

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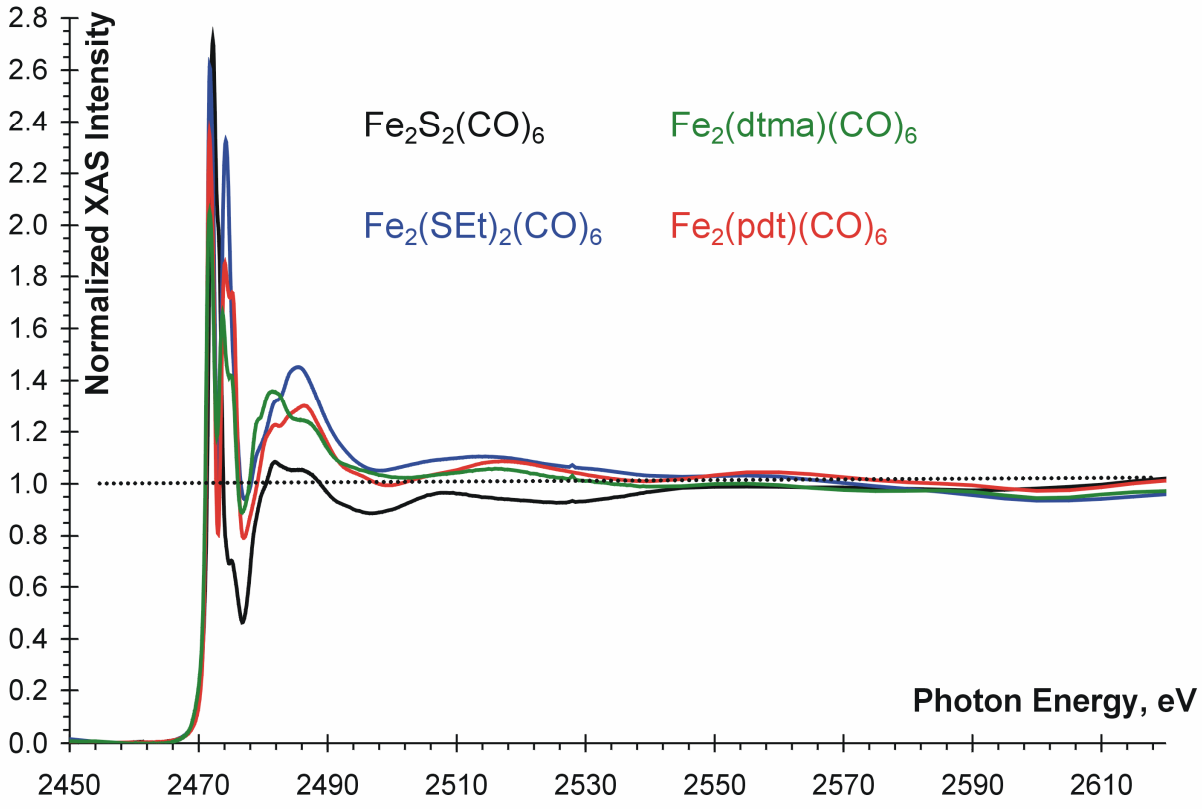

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Details of DFT Calculations

Density functional calculations were carried out using the Gaussian03 suite[8]. The electronic structures of the computational models $^{1}[(LS_3)Fe_4S_4\{Fe_2(CH_3C(\mu\text{-}SCH_2)_3(CO)_5\}]^{2-}$, $^{2}[(EtS)_3Fe_4S_4(\mu\text{-}SEt)Fe_2(pdt)(\mu\text{-}CO)(CN)_2(CO)_2(OH_2)]^{3-}$ and their ionic fragments were calculated employing a gradient corrected density functional composed Becke non-local and Slater local density functional exchange[9] and Perdew non-local and Vosko-Wilk-Nussair local density functional correlation[10] functions (BP). Due to the size of a sufficiently large all-electron basis set, calculations were carried out using Stuttgart-Dresden effective core potential and corresponding valence triple-ζ basis set (ECP2),[11] which approximates the basis set convergence limit reasonably well.[12] We have utilized an ionic fragment based approach[12] to evaluate all possible spin coupling schemes for the [6Fe] models and results obtained for only the lowest energy structures are discussed in the text. The initial structure of the H-cluster framework was generated by merging the 4Fe-cluster from $[\{(LS_3)Fe_4S_4\}_2(\mu\text{-}SEt)]$[13] and the 2Fe-cluster from $[Fe_2(CH_3C(\mu\text{-}SCH_2)_2(CH_2SCH_3))(CO)_5]$[5]. The composite structure of the H-cluster was generated by merging the active site structures for *Cp*I[14] and *Dd*[15] hydrogenases to contain a bridging CO ligand, terminal water at the distal position, and a propanadithiolate ligand.

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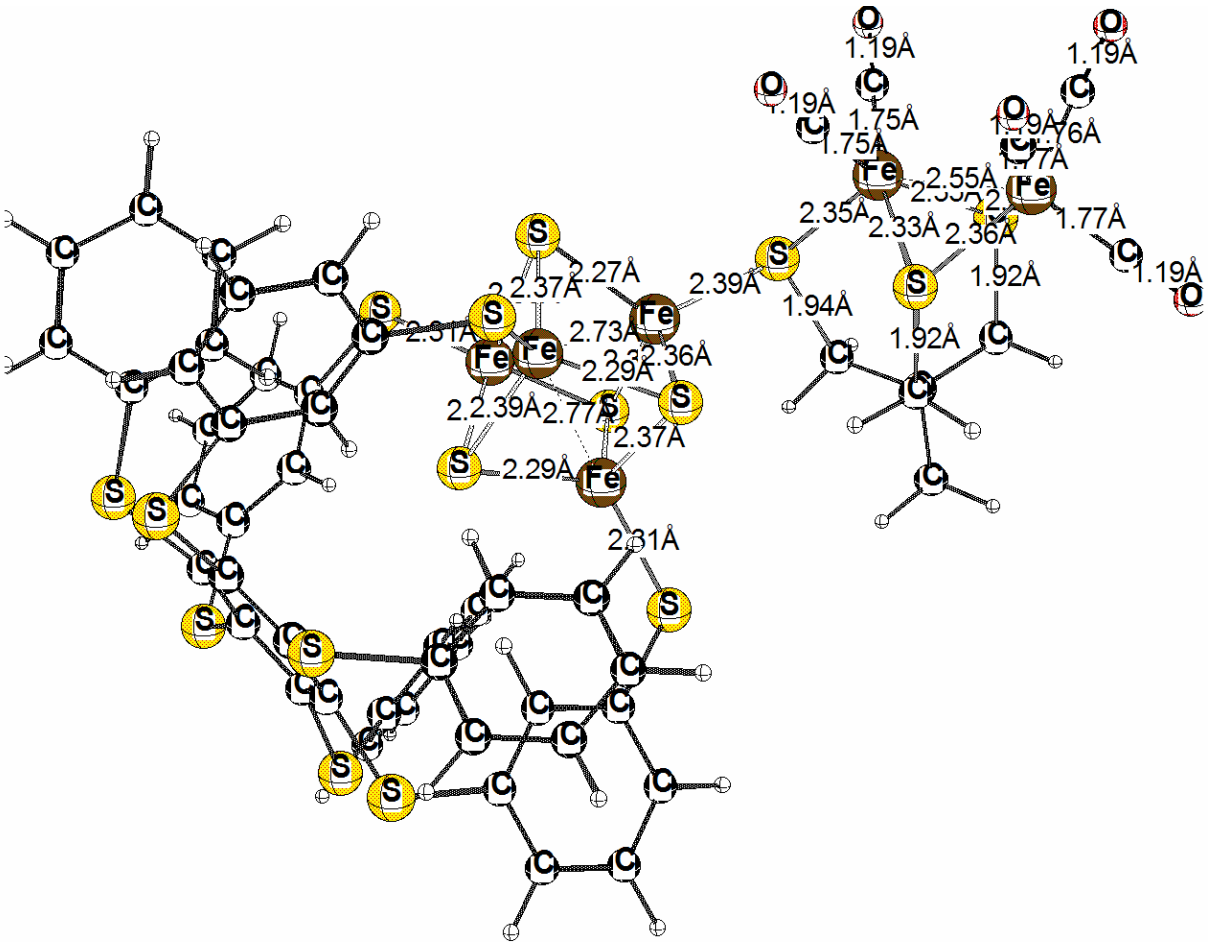
Optimized Atomic Coordinates of $^{1}[(LS_3)Fe_4S_4\{Fe_2(CH_3C(\mu\text{-}SCH_2)_3\}(CO)_5]^{2-}$

S	15.416073	23.403032	8.367902	C	12.911452	26.627520	8.293752
Fe	16.602191	21.656657	7.254397	H	13.215817	25.581388	8.352844
Fe	16.817718	24.344944	6.696926	S	12.150735	23.360785	5.914890
Fe	14.406141	23.072640	6.338407	C	11.071948	23.300398	7.409347
S	18.319708	22.652975	5.964837	C	9.676224	23.379918	7.189625
S	15.096582	20.892514	5.701244	H	9.301458	23.430685	6.164172
S	15.420535	24.669417	4.901640	C	8.789696	23.414253	8.277404
Fe	16.451912	22.534115	4.674551	H	7.712362	23.485520	8.096927
S	17.268013	19.946985	8.628401	C	9.273224	23.386721	9.597727
C	17.665284	20.351414	10.385765	H	8.589412	23.457127	10.447620
C	18.614556	19.526396	11.031198	C	10.662026	23.289471	9.805316
H	19.130547	18.756060	10.453153	C	11.564006	23.229439	8.732899
C	18.876106	19.692896	12.401148	H	12.636781	23.133658	8.911784
H	19.615653	19.054156	12.894393	S	11.193656	23.180335	11.593162
C	18.194792	20.669075	13.148323	C	12.920873	23.880706	11.649550
H	18.392958	20.794282	14.216354	C	13.950712	23.074354	12.198606
C	17.257943	21.491818	12.492549	S	13.695329	21.296690	12.737404
C	16.995246	21.355371	11.122580	C	12.886087	20.413715	11.298531
H	16.285692	22.014928	10.620385	C	13.482531	20.376536	10.028465
S	16.400954	22.722991	13.608250	H	14.385200	20.950020	9.813722
C	15.206603	23.658622	12.528391	C	12.916051	19.572161	9.021592
C	15.453638	25.026590	12.247426	H	13.381995	19.558258	8.032854
S	16.981689	25.932894	12.835437	C	11.777576	18.793240	9.293564
C	18.444384	24.901220	12.300907	H	11.346217	18.165529	8.508618
C	19.458670	24.691435	13.255204	C	11.194679	18.826106	10.575017
H	19.303844	25.004173	14.290933	H	10.301812	18.230913	10.789810
C	20.651223	24.054806	12.866863	C	11.740988	19.644639	11.580102
H	21.439278	23.887815	13.607798	H	11.274102	19.705902	12.566238
C	20.818589	23.612349	11.540457	- merging point -			
H	21.739236	23.103047	11.242038	S	16.645046	22.365689	2.297607
C	19.794785	23.819374	10.599777	C	14.835787	22.752647	1.725880
H	19.906397	23.483450	9.566126	H	14.384934	23.228500	2.609822
C	18.607332	24.476335	10.973816	H	14.954240	23.517094	0.939360
H	17.835208	24.656915	10.225566	C	13.903861	21.627980	1.201999
S	18.253148	26.030539	7.350975	C	12.457798	22.230943	1.257986
C	17.473937	27.277601	8.469985	H	12.152972	22.407709	2.304142
C	18.049377	28.569797	8.486277	H	12.420045	23.195188	0.720013
H	18.857491	28.801505	7.788150	H	11.730172	21.542625	0.791879
C	17.595468	29.532528	9.402302	C	13.873347	20.360464	2.073408
H	18.052461	30.527158	9.415159	H	13.765236	20.623178	3.138485
C	16.576791	29.225349	10.321330	H	13.028227	19.717841	1.774254
H	16.249533	29.960783	11.061250	S	15.399866	19.190545	2.101512
C	15.995541	27.943201	10.283443	C	14.177816	21.292197	-0.272236
C	16.418043	26.974553	9.361083	H	14.057703	22.197868	-0.892074
H	15.948585	25.988917	9.334043	H	13.484689	20.515714	-0.633371
S	14.620672	27.670892	11.517680	S	15.946660	20.685002	-0.704509
C	14.432349	25.822303	11.657028	Fe	17.223755	20.351740	1.242998
C	13.164959	25.253571	11.371769	Fe	16.111825	18.440744	-0.025126
S	11.690543	26.292538	10.869485	C	18.245491	19.618602	2.463486
C	12.239559	27.244527	9.359972	O	18.975534	19.094914	3.243061
C	11.831212	28.589175	9.274778	C	18.620464	20.920287	0.353033
H	11.337022	29.063932	10.126447	O	19.583325	21.294190	-0.244799
C	12.092637	29.316225	8.099396	C	16.769904	17.039856	0.827890
H	11.780236	30.363019	8.032645	O	17.200421	16.068864	1.361126
C	12.774344	28.709468	7.027335	C	17.258199	18.239926	-1.350110
H	12.987921	29.278167	6.117768	O	18.013416	18.079377	-2.256425
C	13.187798	27.369213	7.129990	C	14.631132	17.758489	-0.710299
H	13.719629	26.882837	6.308112	O	13.657637	17.265494	-1.194367

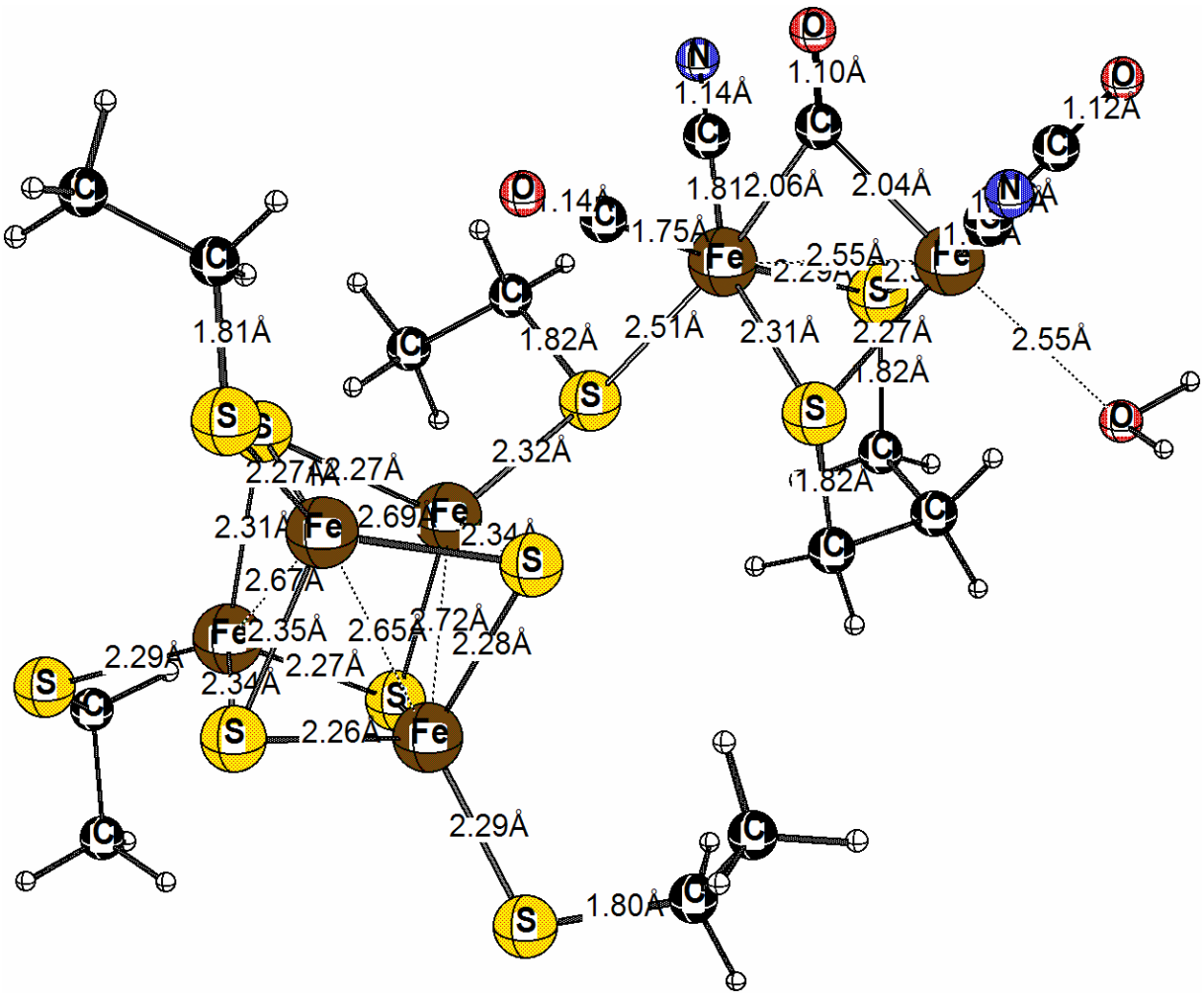
Optimized Structure of $^{1}[(LS_3)Fe_4S_4\{Fe_2(CH_3C(\mu\text{-}SCH_2)_3\}(CO)_5]^{2-}$



Coordinates of the Protein-Bound H-cluster Model $^{2}[(EtS)_3Fe_4S_4(\mu\text{-}SEt)Fe_2(pdt)(\mu\text{-}CO)(CN)_2(CO)_2(OH_2)]^{3-}$

S	-4.239154	0.730883	-1.638912	S	0.795514	-0.478529	1.911187
Fe	-2.556242	1.791292	-0.555904	C	0.636988	-2.010142	2.874137
Fe	-2.326609	-0.629105	-1.620458	H	1.373371	-2.036119	3.550198
Fe	-3.945823	-0.259052	0.465414	H	0.728790	-2.786403	2.250433
S	-0.632746	0.794923	-1.280133	C	-0.690274	-2.134987	3.604442
S	-2.803018	1.271644	1.691227	H	-1.453026	-2.053154	2.962867
S	-2.436635	-1.990788	0.247169	H	-0.844129	-1.297635	4.285155
Fe	-1.204708	-0.157492	0.780382	H	-0.804094	-3.134985	4.022845
S	-2.677087	4.017968	-1.080656	Fe	2.809966	-0.968883	0.495936
C	-1.011990	4.693721	-0.977835	Fe	4.875012	0.324917	-0.271192
H	-1.083576	5.687916	-0.897119	S	4.166857	0.178949	1.935780
H	-0.574997	4.322750	-0.158479	C	3.296182	1.703160	2.420072
C	-0.119409	4.375685	-2.180395	H	3.746883	2.133998	3.201873
H	0.134625	3.408663	-2.161604	H	2.343067	1.507190	2.650648
H	0.779311	4.989633	-2.122231	C	3.378705	2.596967	1.193467
H	-0.651060	4.556574	-3.114605	H	4.220694	2.397859	0.692083
S	-2.462136	-2.022699	-3.405503	H	3.378976	3.555404	1.478957
C	-1.757549	-3.529034	-2.687977	C	2.177679	2.335745	0.293133
H	-1.610631	-3.348215	-1.715475	H	1.954090	3.143990	-0.251646
H	-0.876509	-3.681475	-3.135950	H	1.378861	2.063057	0.829258
C	-2.538529	-4.834823	-2.785204	S	2.752907	0.969730	-0.766827
H	-3.404420	-4.864642	-2.285758	C	3.427351	-2.297918	1.564933
H	-1.871062	-5.576237	-2.346127	N	3.821752	-3.133934	2.224269
H	-2.865145	-4.995841	-3.812600	C	1.916140	-1.976329	-0.622632
S	-6.014781	-0.879517	1.212478	O	1.316564	-2.589532	-1.373721
C	-5.924187	-0.457122	2.993956	C	4.388054	-1.621127	-0.656440
H	-4.961401	-0.377421	3.252157	O	4.822871	-2.485577	-1.179371
H	-6.349926	-1.195449	3.516824	C	5.270930	0.227533	-2.047863
C	-6.629074	0.852221	3.343164	N	5.492496	0.119472	-3.168268
H	-7.599703	0.817866	3.105524	C	6.510082	-0.118547	0.188815
H	-6.187267	1.664867	2.766417	O	7.559554	-0.402103	0.473515
H	-6.553284	1.010241	4.418956	O	5.568376	2.754673	0.069890
	- merging point -			H	6.487429	2.789945	0.617020
				H	5.701847	3.224565	-0.882304

Structure of the Protein-Bound H-cluster Model $^{2}[(EtS)_3Fe_4S_4(\mu\text{-}SEt)Fe_2(pdt)(\mu\text{-}CO)(CN)_2(CO)_2(OH_2)]^{3-}$



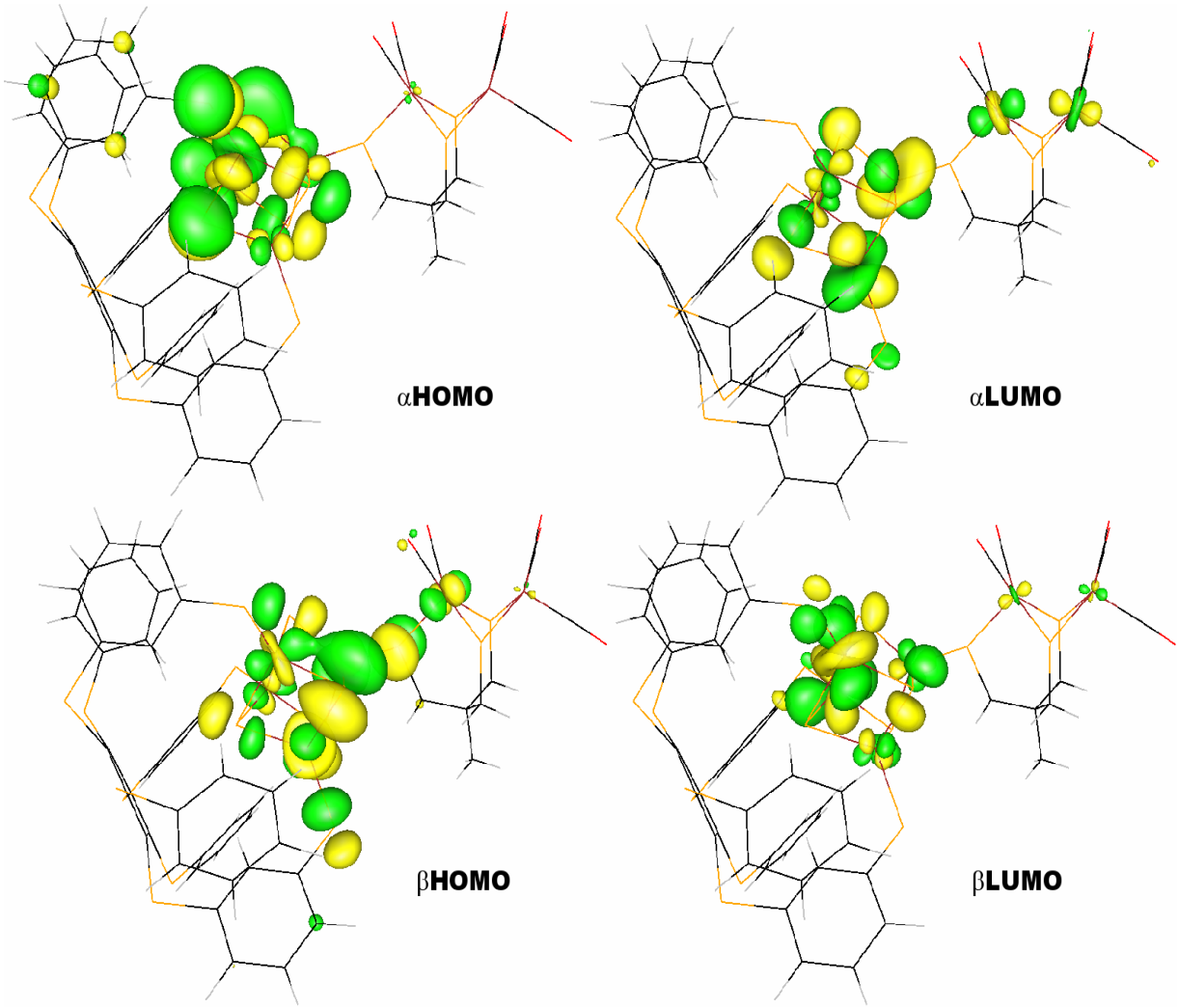


Figure S1

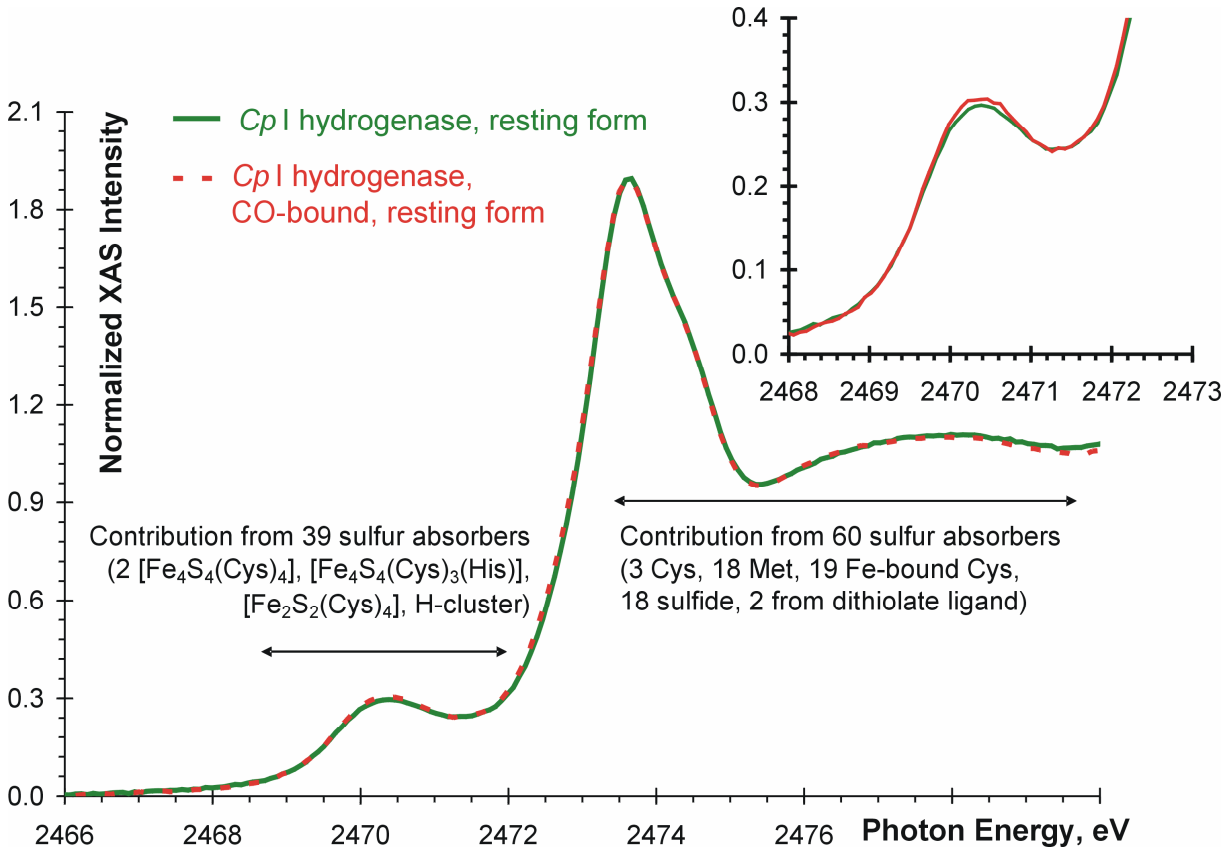

Cp I hydrogenase, resting form
Cp I hydrogenase,
CO-bound, resting form
Normalized XAS Intensity
Photon Energy, eV
Contribution from 39 sulfur absorbers
(2 [Fe4S4(Cys)4], [Fe4S4(Cys)3(His)],
[Fe2S2(Cys)4], H-cluster)
Contribution from 60 sulfur absorbers
(3 Cys, 18 Met, 19 Fe-bound Cys,
18 sulfide, 2 from dithiolate ligand)

Figure S2

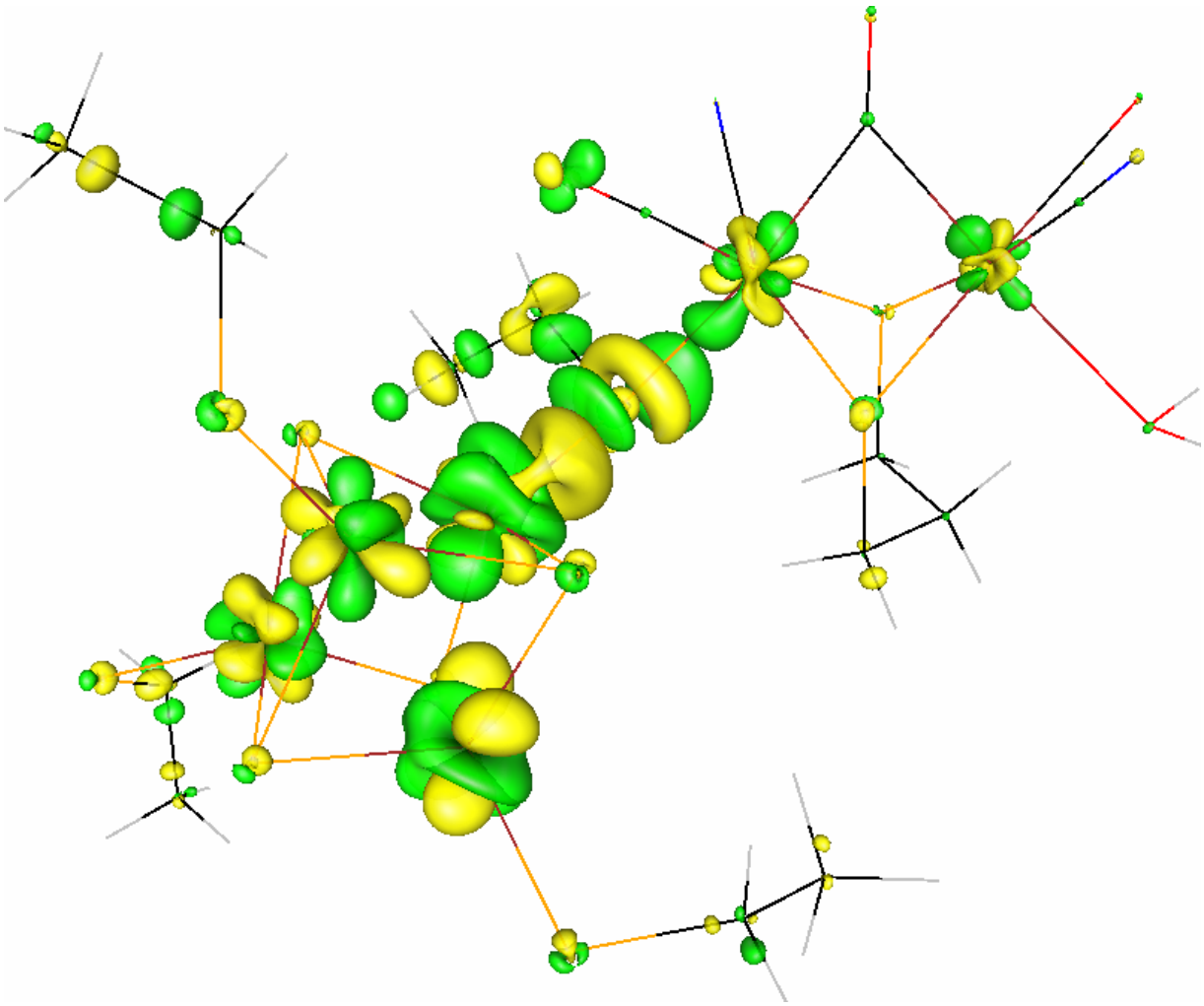


Figure S3

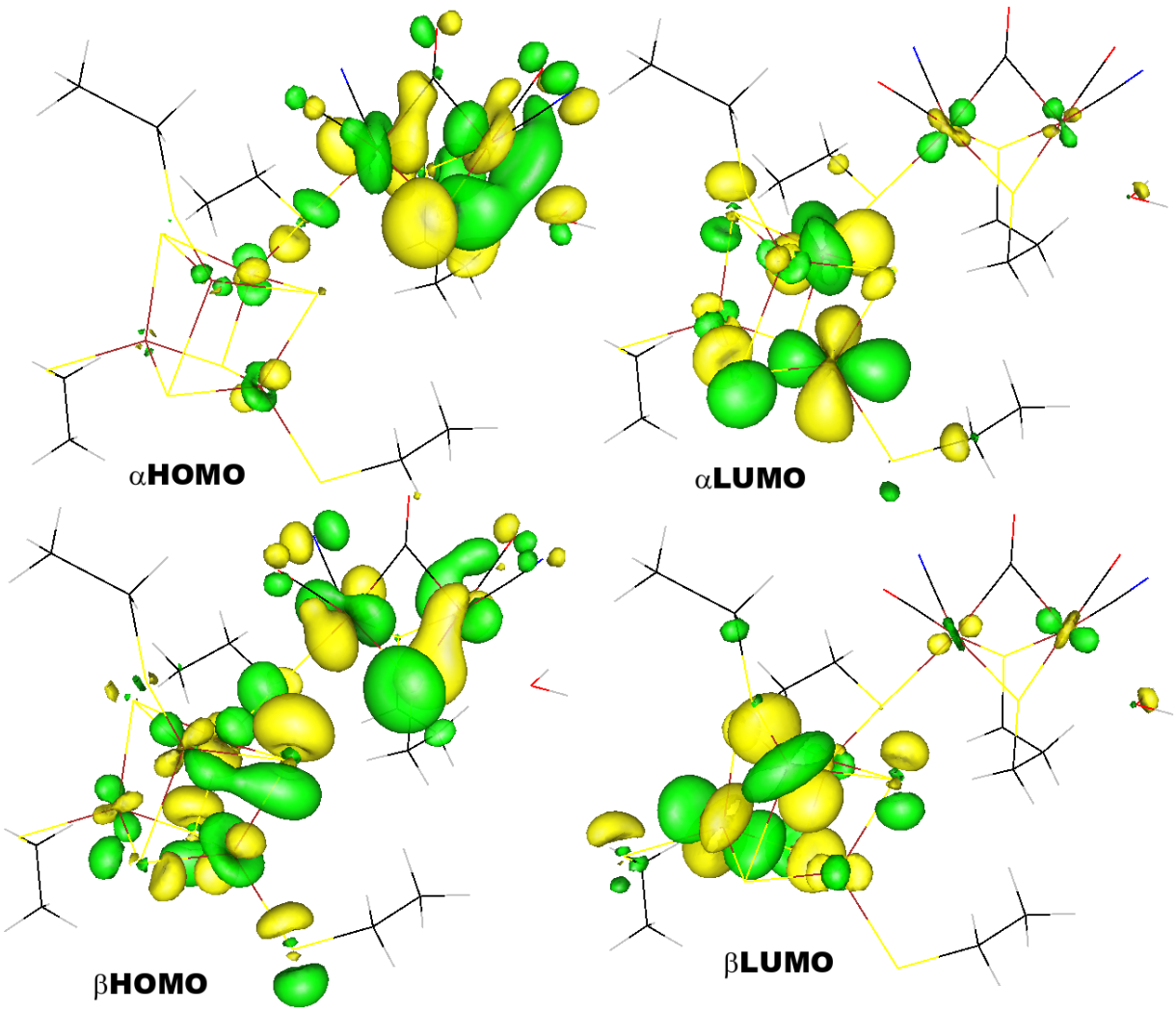


Figure S4