

Electronic Supplementary Information for:

Characterization of the [3Fe-4S]^{0/1+} cluster from the D14C variant of *Pyrococcus furiosus* ferredoxin via combined NRVS and DFT analyses

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Experimental Materials and Methods

Preparation. The D14C ferredoxin from *Pyrococcus furiosus* was produced, purified, and uniformly ^{57}Fe labelled as previously described.¹ D14C (70 μM in 100 mM Tris pH 8.0) was treated and stirred aerobically with a 40 fold molar excess of $\text{K}_3[\text{Fe}(\text{CN})_6]$ and EDTA overnight at 25°C in order to obtain the [3Fe-4S] form. The protein samples were then concentrated (Amicon Ultra-4 3 kDa MWCO, Millipore) and washed in 100 mM Tris pH 8.0 buffer. The oxidized sample was loaded into the NRVS cell directly. For reduction, the sample was treated with 100 mM Tris pH 8.0 containing 20 mM sodium dithionite in an anaerobic box by repeated washing and concentrating with Amicon Ultras. This sample was loaded into a Lucite NRVS cuvette inside the chamber and sealed with vacuum grease. All samples were immediately flash frozen in liquid nitrogen.

Mössbauer Spectroscopy. Mössbauer spectra were recorded on a SEE Co. spectrometer working in a constant acceleration mode in transmission geometry. The measurements were performed at 80 K using a Janis cryostat. A 100 mCi ^{57}Co in a Rh matrix held at room temperature (RT) was used as source. The spectra were least square fitted using Recoil software.² All centroid shifts, δ , are given with respect to metallic α -iron at RT. The parameters from the fittings, which are centroid shift Centroid shift (δ), quadrupole splitting (ΔE_Q), linewidth (Γ), and intensity (I) are summarized in Table 1.

NRVS Measurements. Nuclear resonance vibrational spectroscopy measurements were performed at SPring-8 BL09XU with a 0.8 meV energy resolution at 14.4125 keV as described previously.³ A 4-element avalanche photo diode detector array was used to detect delayed nuclear fluorescence and K fluorescence by ^{57}Fe atoms. All measurements were performed at a 10 K reading of the cryostat sensor. The real sample temperature, as obtained from the spectral analysis, was 30–80 K. The raw data were analyzed with the PHOENIX software⁴ to gain the iron partial vibrational density of states (^{57}Fe -PVDOS).

DFT Calculations

Initial coordinates for the DFT calculations were extracted from PDB 3PNI, based on the 2.8 Å resolution X-ray diffraction (XRD) analysis of the chemically oxidized *Pf* Fd D14C crystals.⁵ The three Cys11/17/56 ligands to the [3Fe-4S] cluster core were modeled using CH₃CH₂S⁻ ethylthiolates, and Cys14 of the D14C protein variant was modeled using CH₃CH₂SH ethylthiol as shown in Fig. S2. The cluster core has not been protonated, ensured by the experimental pH 8.0 conditions (protonation of the [3Fe-4S]⁰ reduced core has been indicated for *Azotobacter vinelandii* ferredoxin I (Av FdI) at acidic pH < 7.0).⁶ The resulting 40-atoms model complex can then be denoted as [[Fe₃S₄](CH₃CH₂S)₃(CH₃CH₂SH)]^{3-/2-}, respectively for the [3Fe-4S]^{0/1+} cluster oxidation levels.

The broken-symmetry (BS)⁷ electronic structures were constructed using an option to assign a number of unpaired α/β electrons to Fe atomic fragments, as implemented in JAGUAR 7.9.⁸ These BS densities were used as starting points for geometry optimizations and analytic Hessian calculations employing harmonic approximation using GAUSSIAN 09, Revision D.01.⁹ The four cysteine C α positions, represented by methyl carbons of the [Fe₃S₄](CH₃CH₂S)₃(CH₃CH₂SH) model, were fixed to their original XRD positions during geometry optimization (Fig. S2). This level of protein restraint has been found optimal in our earlier studies on the [4Fe-4S]^{1+/2+} and [4Fe-4S]^{0/1+/2+} cubanes from *Pf* Fd D14C and *Azotobacter vinelandii* nitrogenase Fe protein (Av2), respectively.^{1, 10} Further protein environment of the [3Fe-4S] cluster was considered via self-consistent reaction field (SCRF) polarizable continuum model using the integral equation formalism (IEF-PCM)¹¹ as implemented in GAUSSIAN, with the static dielectric constant set to $\epsilon = 4.0$ as often used for proteins, and the remaining IEF-PCM parameters at their default values for water. The SCRF molecular cavity was built using UFF atomic radii scaled by 1.1. Using dielectric screening with $\epsilon = 4.0$ or water-like $\epsilon = 80.0$ environment has been earlier shown to improve optimized structures accuracy in [[Fe₃S₄](CH₃S)₃]³⁻, [[Fe₄S₄](CH₃S)₄]²⁻, and [[Fe₄S₄](CH₃CH₂S)₄]^{4-/3-/2-} DFT models of protein-bound clusters [3Fe-4S]⁰, [4Fe-4S]²⁺, and [4Fe-4S]^{0/1+/2+}, respectively;^{10, 12, 13} in absence of dielectric screening (optimization in vacuum), significant negative charge of these models leads to unrealistic expansion of the Fe-S core.

In consistency with the previous study on the *Pf* Fd D14C [4Fe-4S] cluster,¹ the generalized gradient approximation (GGA) PW91¹⁴ density functional was used for all the calculations if not otherwise mentioned. A customized B(5HF)P86^{15, 16} hybrid functional based on the GGA BP86^{17, 18} has been additionally used for tests on the relative energies of the BS states and electronic structures. While non-hybrid PW91 and BP86 (0% of Hartree-Fock exchange) are known to perform well on Fe-S cluster geometries and normal modes,^{12, 16, 19-21} a calibrated level of 5% Hartree-Fock exchange in the customized B(5HF)P86 functional has been indicated to give optimal description of covalency in Fe-S clusters.^{15, 16}

The LACV3P** basis set as implemented in JAGUAR was used for all the calculations, implying a 6-311G** triple- ζ basis set including polarization function for the 1st- and 2nd-row elements. For the Fe atom, LACV3P** uses the Los Alamos effective core potential (ECP), and the valence part is essentially of triple- ζ quality. Relevant to NRVs, the ⁵⁷Fe isotope mass was used for the normal mode analysis following Hessian calculations. Based on the normal mode outputs from GAUSSIAN, ⁵⁷Fe partial vibrational density of states (⁵⁷Fe-PVDOS) and kinetic energy distribution (KED) spectral profiles were generated using Q-SPECTOR, an in-house Python tool successively applied as well earlier.^{1, 10, 22-25} To empirically account for the resolution of the present NRVs experiment, the simulated spectra were broadened by convolution with a FWHM = 14 cm⁻¹ Lorentzian.

Mössbauer Spectroscopy Results

The [3Fe-4S] clusters show two physiological redox states. The oxidized form of the cluster [3Fe-4S]¹⁺ has the electronic spin of $S = 1/2$, which results from spin-frustrated coupling of three high-spin ferric ($S = 5/2$) ions. The reduced [3Fe-4S]⁰ form has a $S = 2$ ground state due to antiferromagnetic coupling of the ferric ion to two mixed-valent ($\text{Fe}^{2.5+}$) ions ($S = 9/2$).^{26, 27}

Mössbauer spectra were measured on oxidized and reduced samples of D14C within the same sample cuvette as for NRVS. The spectrum from the oxidized sample exhibits one doublet (see Fig. S1). Due to the asymmetry of the lines, the spectrum is fitted with two doublets with close lying hyperfine parameters. The 1st component has $\delta = 0.257$ mm/s and $\Delta E_Q = 0.534$ mm/s. The 2nd component has $\delta = 0.328$ mm/s and $\Delta E_Q = 0.638$ mm/s. The widths are 0.30 mm/s and 0.41 mm/s, respectively. These parameters are typical for high-spin Fe^{3+} with thiolate ligands in tetrahedral geometry. It is worth-mentioning that the reason for having two subspectra is due to the different environments for Fe atoms. Although the electronic structure for all atoms is the same, i.e. being in the high-spin Fe^{3+} , the slightly different ligands and/or small differences of the charge distributions at different Fe atoms give rise to different quadrupole splitting, resulting in the asymmetry in the spectrum. This shows all three irons of the [3Fe-4S] cluster have the same redox state in its oxidized state. Reduction by one electron by dithionite results in a $S = 2$ state. The Mössbauer spectrum for the reduced cluster consists of three doublets. The 1st doublet has $\delta = 0.307$ mm/s and $\Delta E_Q = 0.475$ mm/s, which are typical values for high-spin Fe^{3+} . The 2nd doublet has $\delta = 0.446$ mm/s and $\Delta E_Q = 1.396$ mm/s, representing a valence-delocalized $\text{Fe}^{2.5+}$ pair with a shared electron between two irons.^{27, 28} The ratio of these components is 1:2. The 3rd doublet, X, has $\delta = 0.403$ mm/s and $\Delta E_Q = 0.406$ mm/s. The values are close to the values for the weighted average between Fe^{3+} and $\text{Fe}^{2.5+}$. Previous Mössbauer studies on Fe-S cluster suggest that this species represent a fully delocalized state with one electron equally spread over all three irons,²⁷ with a temperature-dependent contribution. The intensity of this component, 24%, is in the range of the previous study.²⁷ The comparison of our results with Mössbauer spectra of the [4Fe-4S] form of *Pf* Fd D14C from an earlier study¹ reveals that the oxidized and the reduced sample are in a homogenous state and the conversion of the [4Fe-4S] to [3Fe-4S] form was complete.

[3Fe-4S] Cluster: Spin Coupling

As disclosed previously,^{26, 27, 29} a $[2\text{Fe}\uparrow:1\text{Fe}\downarrow]$ spin-coupling pattern applies to both oxidation levels of the [3Fe-4S] cluster considered here, where ' $\uparrow$ ' and ' $\downarrow$ ' respectively denote $M_{Si} > 0$ and $M_{Si} < 0$ for the spin-projection values of the Fe_i iron site spins S_i , $i = 1, 2, 3$. Three combinations are available for the $[2\text{Fe}\uparrow:1\text{Fe}\downarrow]$ broken-symmetry (BS) pattern distribution among the three Fe ions. These BS states can be named as BS12, BS13, and BS23 after the Fe_i numbers of the two spin-up sites ($[2\text{Fe}\uparrow]$); here, the Fe_i numbering scheme follows Figs. 1 and S2. At the $[3\text{Fe-4S}]^{0/1+}$ oxidation levels, the relative energies between the three structurally optimized BS states are respectively within 1.0/0.2 kcal/mol using PW91 and within 0.5/0.2 kcal/mol using B(5HF)P86 DFT functionals (Table S2). Because these energy differences are within the inherent accuracy limits of DFT, they cannot provide a conclusive preference to any of the three BS states. A comparison of the observed and BS-dependent calculated ^{57}Fe -PVDOS spectra in Fig. S4 does not favor particular BS state either. BS12, which has the lowest energy in most of the cases (except the PW91 calculation on $[3\text{Fe-4S}]^{1+}$), has been regarded as the representative spin configuration reported in this study; see further support for BS12 below.

A strong inhomogeneity of Fe_i spin densities (which approximate the M_{Si} values) reported in Table S2 for the $[3\text{Fe-4S}]^{1+}$ ($S = 1/2$) oxidized cluster is indicative of spin-frustration (which implies S_i vector orientations different from parallel or antiparallel) between the three high-spin ferric (Fe^{3+} , d^5 , $S = 5/2$) iron sites; for the computed $[3\text{Fe-4S}]^{1+}$ $\text{Fe}_{1/2/3}$ spin densities of BS12, we note a qualitative correspondence to the $A_i = 5/18/-41$ MHz isotropic parts of the Fe_i magnetic hyperfine interaction tensors observed for $[3\text{Fe-4S}]^{1+}$ from Av FdI.²⁶ In contrast, Fe_i spin densities obtained for the $[3\text{Fe-4S}]^0$ ($S = 2$) reduced cluster are largely homogeneous in their magnitudes; accepting for moderate spin density deviations from their intrinsic values within the cluster, the BS12 solution here corresponds to a (ferromagnetic) mixed-valence pair formed by Fe_1 and Fe_2 ($\text{Fe}^{2.5+}$, $S = 9/4$), coupled antiferromagnetically to ferric Fe_3 .²⁷ Provided the Fe sites permutation, solutions received for BS13 and BS23 bear their qualities similar to BS12 at both $[3\text{Fe-4S}]^{0/1+}$ oxidation levels (Table S2). Finally, PW91 and B(5HF)P86 provide qualitatively similar electronic structures, with magnitudes of Fe_i spin densities somewhat higher from the hybrid B(5HF)P86 functional.

Via combination of EPR, variable-temperature magnetic circular dichroism (VTMCD), and sequence specific ^1H NMR spectroscopies, the identity of the noncoordinating residue equivalent to position 14 in *Pf* Fd has been indicated as a determinant in the location of the reducible pair of irons in [3Fe-4S] clusters of wild-type and mutant ferredoxins.^{30, 31} This residue is at position X(II) of the conserved C(I)-X-X-X(II)-X-X-C(III) polypeptide cluster binding motif; for *Pf* Fd D14C, C(I) = Cys11, X(II) = Cys14, and C(III) = Cys17. For the X(II) = C(II) = Cys case, it has been concluded that the two Fe coordinated by C(I) and C(III) cysteines form the reducible pair, and the remaining Fe coordinated by cysteine outside the conserved motif (here, Cys56) is a 'unique' site.^{30, 31} Our selection of BS12 to underlie the representative DFT results (see above) is then consistent with the outlined spectroscopic indication: Fe_1 and Fe_2 form the reducible pair, and Fe_3 remains 'unique' high-spin ferric in $[3\text{Fe-4S}]^{0/1+}$ of *Pf* Fd D14C.

[3Fe-4S] Cluster: Structural Aspects

Trends in redox-dependent structural shifts in the *Pf* Fd D14C [3Fe-4S]/[4Fe-4S] clusters, well mapped by observed and DFT-simulated NRVS (^{57}Fe -PVDOS) vibrational properties, are discussed in the main text employing averaged Fe–Fe/ $S_{\text{I}}/S_{\text{b}}$ internuclear distances from DFT optimization. This section extends to a comparison of the optimized *Pf* Fd D14C [3Fe-4S] $^{0/1+}$ structures to relevant experimental data collected in Table S3; for the [4Fe-4S] cluster from the very same protein, this type of comparison has been carried out earlier.¹

The only available X-ray diffraction (XRD) structure PDB 3PNI⁵ of specifically *Pf* Fd D14C variant is for the oxidized protein and has been presently used as a starting point for the corresponding [3Fe-4S] $^{1+}$ DFT modeling. A critical assessment of the DFT structure quality is however problematic here due to a relatively low 2.8 Å resolution of its experimental source, PDB 3PNI. We note that the DFT optimization leads to a consistent mean Fe–Fe/ $S_{\text{I}}/S_{\text{b}}$ distances expansion by respectively 0.05/0.05/0.04 Å (Table S3). Interestingly, higher 1.5 Å resolution data on the oxidized wild-type (WT) *Pf* Fd variant from PDB 1SJ1³² shows notable expansion of the cluster, as compared to PDB 3PNI. A critical assessment is impeded here as well due to residual electron density in PDB 1SJ1, observed at location of the missing 4th Fe site; notably, the averaged Fe–Fe/ $S_{\text{I}}/S_{\text{b}}$ distances from PDB 1SJ1 better match those from our DFT modeling on *Pf* Fd D14C [4Fe-4S] $^{2+/1+}$.

As already discussed in the main text, high 1.4 Å resolution determinations PDB 6FD1/6FDR of respectively [3Fe-4S] $^{0/1+}$ forms of *Azotobacter vinelandii* ferredoxin (Av FdI) displayed structural deviations within experimental error only.^{33, 34} Collectively, these two Av FdI structures are in better agreement with our DFT modeling of the reduced *Pf* Fd D14C [3Fe-4S] 0 cluster. For the [3Fe-4S] 0 case, the averaged Fe–Fe/ $S_{\text{I}}/S_{\text{b}}$ distances are different by respectively 0.0/0.06/0.04 Å when comparing the Av FdI PDB 6FD1 to our DFT modeling (Table S3).

High-resolution XRD data on a synthetic compound hosting the reduced [Fe₃S₄] 0 cluster³⁵ provides the most consistent match to our [3Fe-4S] 0 DFT structure: here, experiment-to-DFT shift in averaged Fe–Fe/ $S_{\text{I}}/S_{\text{b}}$ distances is respectively -0.02/0.02/0.02 Å only (Table S3). A comparable level of structural quality has been achieved in an earlier DFT study on the [3Fe-4S] 0 cluster,¹² where the best computational protocol delivered mean absolute error of 0.016 Å in Fe–S bonding distances, as compared to the synthetic [Fe₃S₄] 0 species.

A separate comment goes to the Fe–Fe distances distribution, well recognized to be dependent on spin configuration (among other factors, such as protein environment) in Fe-S clusters. While in the all-ferric [3Fe-4S] $^{1+}$ cluster the optimized Fe–Fe distances are essentially homogeneous and distribute within 0.03 Å only, for the reduced [3Fe-4S] 0 cluster spin-dependent delocalization in the mixed-valence [Fe $^{2.5+}\uparrow$ -Fe $^{2.5+}\uparrow$] pair leads to the corresponding short Fe1–Fe2 distance, as compared to 0.07-0.08 Å longer Fe1/2–Fe3 distances in the two [Fe $^{2.5+}\uparrow$ -Fe $^{3+}\downarrow$] pairs (Table S3). This is consistent with the BS12 spin configuration clarified in the previous section. The outlined 'one short / two long' Fe–Fe distances pattern for [3Fe-4S] 0 has been resolved in the synthetic [Fe₃S₄] 0 species XRD structure as well.³⁵

[3Fe-4S] Cluster: Reorganization Energies

The present analysis focuses on λ_i self-exchange inner-sphere contribution to the reorganization energy λ of the *Pf* Fd D14C [3Fe-4S]^{0/1+} cluster. Here, the inner-sphere implies the cluster inorganic core and its first coordination sphere, represented by the DFT model. Calculation of λ_i was done in a manner following Ryde et al^{36, 37} and others^{1, 12, 19, 21}:

$$\lambda_i = \lambda_{ox} + \lambda_{red}$$

$$\lambda_{ox} = E(\text{Ox}^{\text{Ox}}) - E(\text{Red}^{\text{Ox}})$$

$$\lambda_{red} = E(\text{Red}^{\text{Red}}) - E(\text{Ox}^{\text{Red}})$$

where λ_{ox} , the reorganization energy during oxidation, is the difference between the energies of the oxidized complex in its equilibrium geometry (Ox^{Ox}) and oxidized complex at the equilibrium structure of the reduced complex (Red^{Ox}). Similarly, the λ_{red} reorganization energy during reduction is the difference between the energies of the reduced complex in its equilibrium geometry (Red^{Red}) and reduced complex at the equilibrium structure of the oxidized complex (Ox^{Red}).

The representative spin configuration BS12 (as detailed above) has been selected for the *Pf* Fd D14C [3Fe-4S]^{0/1+} cluster reorganization energy assessment, and the values computed are collected as BS12 _{λ} (kcal/mol) in Table S2. These values translate to $\lambda_{ox} = 52.7$, $\lambda_{red} = 58.6$, and $\lambda_i = 111.3$ kJ/mol using the PW91 functional; alternative application of the B(5HF)P86 functional leads to a very similar result of $\lambda_{ox} = 51.9$, $\lambda_{red} = 60.7$, and $\lambda_i = 112.5$ kJ/mol. A previous study on the *Pf* Fd D14C [4Fe-4S]^{1+/2+} cluster employing the equivalent DFT protocol provided $\lambda_i = 18.8$ kJ/mol.¹ Within the very same *Pf* Fd D14C protein framework, our results therefore indicate $113.3/18.8 \approx 6$ higher reorganization energy of the [3Fe-4S]^{0/1+} cluster as compared to the [4Fe-4S]^{1+/2+} variant.

We note that reorganization energies calculated for Fe-S clusters vary significantly depending on the computational protocol and environment treatment, as discussed earlier.^{1, 12, 21} $\lambda_i = 67$ kJ/mol has been reported for a generic [3Fe-4S]^{0/1+} cluster,¹² and broad range of $\lambda_i \approx 18$ to 50 kJ/mol is available from the literature on [4Fe-4S]^{1+/2+}.^{21, 37, 38} Notably, our modeling of *both* [3Fe-4S] and [4Fe-4S] clusters from *Pf* Fd D14C included (i) all four Cys ligands (as shown in Fig. S2) coordinating the [4Fe-4S] variant prior to its oxidative damage, (ii) C α carbon fixations to their original XRD positions, and (iii) implicit protein environment; the outer-sphere λ_o contribution to the reorganization energy is then partially accounted as well. This adds extra confidence in relative assessment of the *Pf* Fd D14C [3Fe-4S]^{0/1+} and [4Fe-4S]^{1+/2+} clusters reorganization energies as given above.

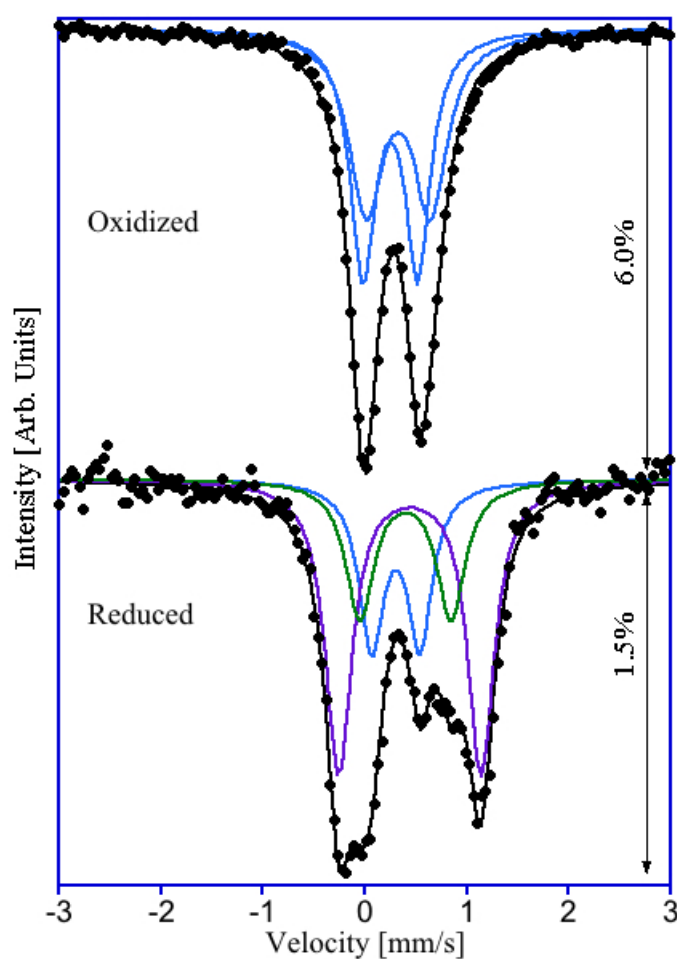


Fig. S1 Mössbauer spectra of [3Fe-4S]-containing *Pf* Fd D14C recorded at 80 K. Black circles represent oxidized and reduced D14C experimental Mössbauer data. The subspectra are Lorentzian doublets of Fe^{3+} (blue), $\text{Fe}^{2.5+}$ (violet) and species X (green, see text and Table S1 for details). The sum of subspectra is shown in black lines.

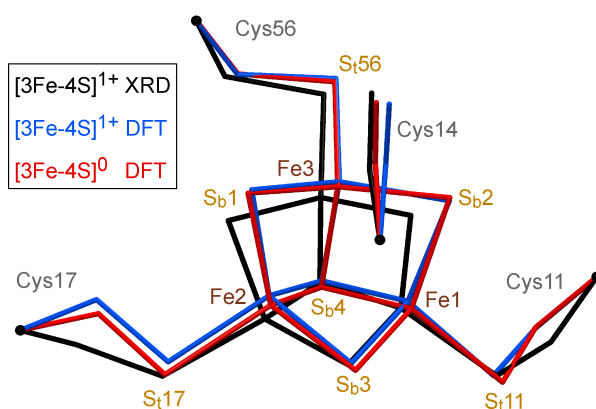


Fig. S2 Overlay of the *Pf* Fd D14C [3Fe-4S] $^{1+}$ cluster reference PDB 3PNI⁵ XRD structure (black) with the DFT model optimized at the [3Fe-4S] $^{1+}$ oxidized (blue) and [3Fe-4S] 0 reduced (red) levels. The Fe/S atom labels are given in brown/yellow, respectively. Hydrogen atoms are omitted for clarity, and Cys14 sulfur is protonated. The four cysteine alpha-carbons fixed to their original crystallographic positions during the optimization are marked with black spheres. All models are shown in 0.05 Å radius tubes.

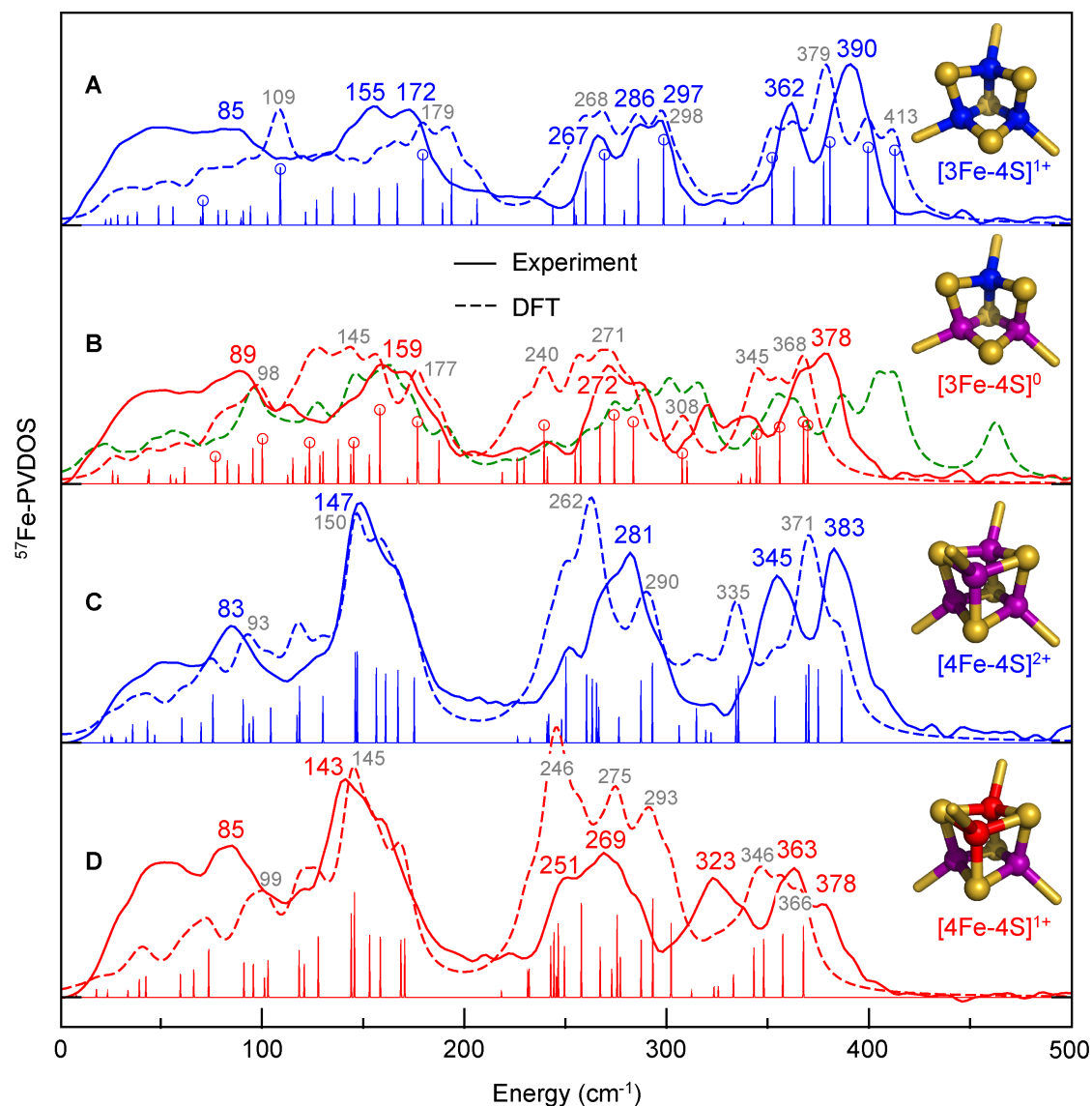


Fig. S3 Overlay of the observed NRVS (solid line) and representative DFT-calculated (broken line) ^{57}Fe -PVDOS spectra for the ^{57}Fe -enriched [3Fe-4S] and [4Fe-4S] variants of *Pf* Fd D14C at the reduced and oxidized levels. The non-broadened intensities (and normal mode positions) from DFT are given in stick-style. On right, electronic structures of the Fe-S clusters are schematically shown, where $\text{Fe}^{3+/2.5+/2+}$ are in blue/purple/red, respectively, and sulfide ligands are in yellow. *Top to bottom*: [3Fe-4S] $^{1+}$ (A); [3Fe-4S] 0 (B); [4Fe-4S] $^{2+}$ (C); [4Fe-4S] $^{1+}$ (D). For the selected [3Fe-4S] $^{0/1+}$ normal modes labeled with circles (A,B), animated GIF files are available as separate part of ESI † . In (B), the green line corresponds to the reduced [3Fe-4S] 0 state DFT modeling using the equilibrium structure of the oxidized [3Fe-4S] $^{1+}$ state. The data on the [4Fe-4S] variant have been in part published previously.¹

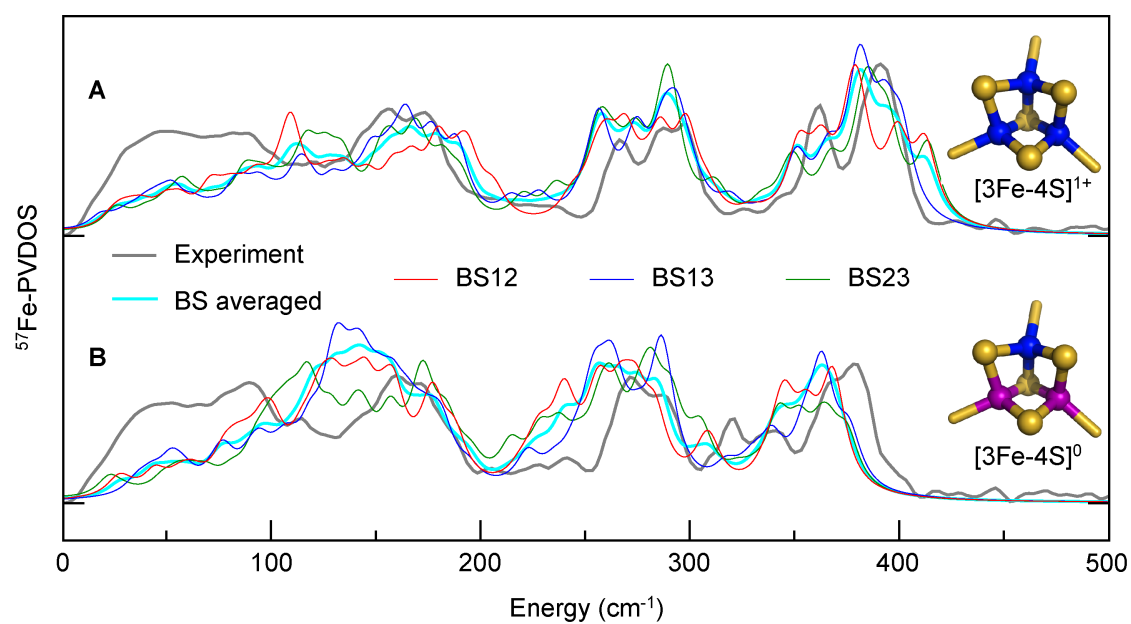


Fig. S4 Overlay of the observed NRVs and broken-symmetry (BS) state variants of the DFT-calculated ^{57}Fe -PVDOS spectra for the ^{57}Fe -enriched $[\text{3Fe-4S}]$ *Pf* Fd D14C at the oxidized $[\text{3Fe-4S}]^{1+}$ (A) and reduced $[\text{3Fe-4S}]^0$ (B) levels. The experimental line is in grey, BS12 in red, BS13 in blue, BS23 in green, and arithmetic mean from the three BS states spectra is in cyan. BS12 is the representative DFT solution used to report $[\text{3Fe-4S}]$ *Pf* Fd D14C spectra elsewhere in this study (Figs. 2 and S3). On right, electronic structures of the $[\text{3Fe-4S}]$ cluster are schematically shown, where $\text{Fe}^{3+/2.5+/2+}$ are in blue/purple/red, respectively, and sulfide ligands are in yellow.

Table S1 Mössbauer parameters for the iron sites of the oxidized and reduced [3Fe-4S] cluster. Centroid shift (δ), quadrupole splitting (ΔE_Q), Lorentzian linewidth (Γ) and intensities (I) are shown.

	Type and S_{tot}	δ (mm/s)	ΔE_Q (mm/s)	Γ (mm/s)	I (%)
ox. D14C	$[3\text{Fe-4S}]^{1+}$				
Fe³⁺	$S = 1/2$	0.257	0.534	0.30	50
Fe³⁺		0.328	0.638	0.41	50
red. D14C	$[3\text{Fe-4S}]^0$				
Fe³⁺	$S = 2$	0.307	0.475	0.29	26
Fe^{2.5+}		0.446	1.396	0.32	49
species X		0.403	0.896	0.33	25

Table S2 Relative energies in three broken-symmetry (BS) states and Mulliken spin populations for the three iron sites of the *Pf* Fd D14C $[3\text{Fe-4S}]^{0/1+}$ cluster from DFT calculations using PW91 (top) and B(5HF)P86 (bottom) functionals.^a

	Relative Energy (kcal/mol) and Spin Population (e)							
	$[3\text{Fe-4S}]^{1+}$				$[3\text{Fe-4S}]^0$			
	BS12	BS12 _{λ} ^d	BS13	BS23	BS12	BS12 _{λ} ^d	BS13	BS23
PW91								
Energy^b	0.0	+12.6	+0.7	+1.0	0.0	+14.0	-0.1	-0.2
Fe1^c	1.50	1.37	2.55	-3.02	3.34	3.06	3.31	-3.28
Fe2^c	2.49	2.98	-3.13	1.20	3.35	3.15	-3.30	3.32
Fe3^c	-3.13	-3.23	1.44	2.83	-3.31	-3.16	3.35	3.34
B(5HF)P86								
Energy^b	0.0	+12.4	+0.5	0.0	0.0	+14.5	+0.1	+0.2
Fe1^c	1.80	1.89	2.87	-3.25	3.43	3.25	3.41	-3.42
Fe2^c	2.68	3.01	-3.33	1.33	3.44	3.22	-3.44	3.42
Fe3^c	-3.34	-3.42	1.52	3.05	-3.44	-3.34	3.44	3.43

^a The DFT results from PW91 are representative and implied elsewhere, if not otherwise mentioned.

^b Energies (kcal/mol) are relatively to BS12 at both oxidation levels.

^c Fe sites labeling for Mulliken spin populations (e) corresponds to Figs. 1 and S2.

^d BS12 _{λ} correspond to states Ox^{Red}/Red^{Ox} of the $[3\text{Fe-4S}]^{0/1+}$ cluster used for the $\lambda_{\text{red/ox}}$ reorganization energies calculation, respectively, as explained in the text.

Table S3 Fe–Fe and bonding Fe–S internuclear distances (Å) in selected ferredoxin [3Fe-4S] and [4Fe-4S] clusters and [Fe₃S₄(LS₃)]³⁻ model compound from X-ray diffraction (XRD) data analysis and DFT geometry optimization.^a

	Internuclear Distance (Å)					
	XRD ^b	XRD ^c	XRD ^d	XRD ^e	DFT ^f	DFT ^g
	[Fe ₃ S ₄ (LS ₃)] ³⁻ [Fe ₃ S ₄] ⁰	Av FdI [3Fe-4S] ^{1+/0}	Pf Fd WT [3Fe-4S] ¹⁺	Pf Fd D14C [3Fe-4S] ¹⁺	Pf Fd D14C [3Fe-4S] ^{1+/0}	Pf Fd D14C [4Fe-4S] ^{2+/1+}
Fe – Fe						
Fe1 – Fe2	2.67	2.64 / 2.65	2.65	2.53	2.61 / 2.63	
Fe1 – Fe3	2.73	2.73 / 2.73	2.71	2.56	2.60 / 2.70	
Fe2 – Fe3	2.71	2.67 / 2.65	2.75	2.54	2.61 / 2.71	
Fe – Fe	2.70	2.68 / 2.68	2.70	2.54	2.61 / 2.68	2.68 / 2.70
Fe – S_t (terminal cysteinates)						
Fe1 – S _t 11	2.33	2.31 / 2.30	2.28	2.18	2.21 / 2.35	
Fe2 – S _t 17	2.32	2.32 / 2.28	2.32	2.18	2.24 / 2.35	
Fe3 – S _t 56	2.31	2.29 / 2.26	2.23	2.21	2.27 / 2.32	
Fe – S _t	2.32	2.31 / 2.28	2.28	2.19	2.24 / 2.34	2.27 / 2.32
Fe – S_b (bridging inorganic)						
Fe1						
– S _b 2	2.25	2.29 / 2.21	2.32	2.24	2.17 / 2.27	
– S _b 3	2.28	2.28 / 2.32	2.17	2.18	2.15 / 2.32	
– S _b 4	2.33	2.32 / 2.32	2.33	2.10	2.23 / 2.38	
Fe1 – S _b	2.29	2.30 / 2.28	2.27	2.17	2.18 / 2.32	
Fe2						
– S _b 1	2.25	2.21 / 2.20	2.26	2.23	2.21 / 2.26	
– S _b 3	2.27	2.28 / 2.29	2.25	2.21	2.20 / 2.33	
– S _b 4	2.31	2.33 / 2.33	2.31	2.10	2.27 / 2.37	
Fe2 – S _b	2.28	2.27 / 2.27	2.27	2.18	2.23 / 2.32	
Fe3						
– S _b 1	2.24	2.21 / 2.23	2.24	2.21	2.23 / 2.26	
– S _b 2	2.27	2.23 / 2.24	2.34	2.19	2.24 / 2.25	
– S _b 4	2.27	2.27 / 2.23	2.38	2.13	2.32 / 2.31	
Fe3 – S _b	2.26	2.24 / 2.23	2.32	2.18	2.26 / 2.27	
Fe – (μ ₂)S _b 1/2/3	2.26	2.25 / 2.25	2.26	2.21	2.20 / 2.28	
Fe – (μ ₃)S _b 4	2.31	2.31 / 2.29	2.34	2.11	2.27 / 2.35	
Fe – S _b	2.28	2.27 / 2.26	2.29	2.18	2.22 / 2.30	2.27 / 2.28
Fe – S_{t/b}						
Fe – S _{t/b}	2.29	2.28 / 2.27	2.29	2.18	2.23 / 2.31	2.27 / 2.29

^a Atom labeling corresponds to Figs. 1 and S2. The arithmetic mean distances are shadowed with grey fields.

^b High-resolution data on [Fe₃S₄(LS₃)]³⁻ synthetic compound.³⁵ The atom labeling correspondence (present to ref.³⁵) is Fe1/2/3 to Fe(3/1/2); S_b1/2/3/4 to S(3/4/2/1); S_t11/17/56 to S(31/11/21).

^c 1.4 Å resolution data on Azotobacter vinelandii ferredoxin from PDB 6FD1/6FDR.^{33, 34} The atom labeling correspondence (present to ref.³³) is Fe1/2/3 to Fe2/3/1; S_b1/2/3/4 to S2/3/1/4; S_t11/17/56 to S_y16/49/8.

^d 1.5 Å resolution data on wild-type Pf Fd variant from PDB 1SJ1.³²

^e 2.8 Å resolution data on D14C Pf Fd mutant from PDB 3PNI.⁵

^f This work. Representative BS12 structures are reported.

^g Selected mean distances from earlier Pf Fd D14C [4Fe-4S] DFT modeling.¹

Table S4 Cartesian coordinates (Å) and total absolute energies (Hartree) of the *representative* DFT-optimized models using PW91 functional and BS12 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.161016				40 Absolute Energy: -3874.241079			
Fe	4.459172	9.256593	17.254875	Fe	4.157552	9.170956	17.226948
Fe	3.989838	11.643482	16.317570	Fe	3.696602	11.567092	16.234848
Fe	5.215857	11.345545	18.603257	Fe	5.117496	11.336720	18.524805
S	2.734810	9.849057	16.108846	S	2.197410	9.780901	16.148840
S	4.259182	9.484077	19.403911	S	4.222488	9.507814	19.469802
S	6.030154	10.662907	16.536625	S	5.867291	10.632612	16.460422
S	3.655326	12.897832	18.105363	S	3.584453	12.936578	18.032540
C	6.183984	6.164044	18.836942	C	6.183993	6.164027	18.836950
C	6.348704	6.692845	17.414778	C	6.302294	6.712257	17.417385
S	4.750331	7.165568	16.595934	S	4.666319	6.967574	16.574137
H	5.729712	6.934743	19.476113	H	5.607121	6.862139	19.460201
H	6.999492	7.578032	17.401466	H	6.825143	7.679839	17.426788
H	6.806609	5.931721	16.763523	H	6.885748	6.025704	16.783959
H	5.536318	5.275530	18.856726	H	5.675280	5.188392	18.846039
H	7.161729	5.889487	19.265954	H	7.181085	6.036788	19.293373
C	0.129002	10.771015	19.114998	C	0.129001	10.771028	19.114979
C	0.930405	11.251639	20.315846	C	0.954358	11.415550	20.216120
S	-0.219234	11.851160	21.646332	S	-0.163395	12.070334	21.553987
H	-0.533286	9.932377	19.375915	H	-0.458505	9.918021	19.487425
H	1.544552	10.439960	20.723020	H	1.639847	10.685972	20.661912
H	1.595840	12.073666	20.026522	H	1.551143	12.242377	19.811210
H	0.735137	12.251503	22.523930	H	0.805926	12.570999	22.361595
H	-0.481183	11.579124	18.686735	H	-0.559701	11.493190	18.651340
H	0.831725	10.429493	18.340616	H	0.812209	10.406763	18.329052
C	4.023035	15.420899	13.654091	C	4.022995	15.420904	13.654046
C	4.361019	14.399898	14.739777	C	4.508442	14.226825	14.475811
S	3.508687	12.776975	14.443149	S	3.455377	12.724578	14.205744
H	4.541054	16.373684	13.848294	H	4.672669	16.298923	13.811411
H	5.444603	14.218515	14.778505	H	5.546981	13.973471	14.214224
H	4.051011	14.757512	15.731295	H	4.488846	14.462069	15.549372
H	4.330084	15.063513	12.660270	H	4.019190	15.188736	12.578236
H	2.942215	15.619998	13.621457	H	2.997403	15.698667	13.938453
C	6.703979	14.930041	19.886970	C	6.704012	14.930041	19.887025
C	7.267998	13.716321	19.158103	C	7.279046	13.713694	19.165537
S	6.835971	12.116248	19.998097	S	6.779662	12.106806	19.946037
H	5.606395	14.885826	19.917430	H	5.605436	14.912511	19.853043
H	6.882472	13.670467	18.130584	H	6.945745	13.691873	18.118962
H	8.365897	13.764102	19.103266	H	8.379731	13.747903	19.165580
H	7.073424	14.977642	20.921764	H	7.012882	14.945403	20.943025
H	6.995191	15.862390	19.374870	H	7.047121	15.866414	19.413997

Table S5 Cartesian coordinates (Å) and total absolute energies (Hartree) of the DFT-optimized models using PW91 functional and BS13 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.159877				40 Absolute Energy: -3874.241290			
Fe	4.400217	9.281904	16.967370	Fe	4.184847	9.087575	16.987263
Fe	3.557816	11.612732	16.140693	Fe	3.650308	11.485492	15.897650
Fe	4.985871	11.449915	18.293573	Fe	4.840788	11.298902	18.294117
S	2.398253	9.704501	16.140397	S	2.355222	9.641913	15.803304
S	4.395456	9.551784	19.145964	S	4.045348	9.371756	19.299700
S	5.787002	10.947429	16.282522	S	5.782230	10.677296	16.211911
S	3.313308	12.807986	18.045633	S	3.276883	12.820577	17.697907
C	6.183969	6.163947	18.837083	C	6.184058	6.164091	18.836837
C	6.577231	6.919305	17.574361	C	6.458681	6.770181	17.465465
S	5.137342	7.231026	16.451425	S	4.928408	6.942775	16.433814
H	5.456592	6.747835	19.417888	H	5.485346	6.808907	19.388228
H	7.023258	7.890909	17.822877	H	6.900957	7.769730	17.576727
H	7.312599	6.349642	16.986017	H	7.169533	6.150901	16.895609
H	5.731250	5.191767	18.593729	H	5.737371	5.162401	18.745335
H	7.065500	5.984262	19.474799	H	7.115385	6.076669	19.423855
C	0.128990	10.770886	19.115036	C	0.128875	10.771084	19.115032
C	0.992329	10.921402	20.358975	C	0.957818	11.083317	20.350087
S	-0.052654	11.486011	21.788453	S	-0.156814	11.649847	21.730787
H	-0.661202	10.017936	19.254011	H	-0.599686	9.968185	19.304032
H	1.461602	9.968593	20.629390	H	1.511744	10.199304	20.684344
H	1.781159	11.662303	20.182139	H	1.677727	11.880829	20.132514
H	0.950660	11.606455	22.694120	H	0.812469	11.852632	22.658811
H	-0.341052	11.725236	18.837089	H	-0.412054	11.658781	18.756022
H	0.768666	10.454800	18.276858	H	0.806735	10.443787	18.313856
C	4.023024	15.421169	13.653938	C	4.023043	15.420764	13.654040
C	4.377243	14.210822	14.508658	C	4.530348	14.133344	14.294824
S	3.157899	12.835866	14.271277	S	3.406168	12.701719	13.950529
H	4.766352	16.223763	13.789577	H	4.719999	16.254290	13.845487
H	5.374156	13.826815	14.249907	H	5.532839	13.881481	13.918855
H	4.389232	14.472969	15.575926	H	4.601282	14.242300	15.386343
H	3.997001	15.160531	12.585562	H	3.918355	15.310620	12.563826
H	3.036026	15.820474	13.927118	H	3.039523	15.699076	14.058609
C	6.704018	14.929999	19.886944	C	6.704024	14.930062	19.887091
C	7.113305	13.736918	19.024882	C	6.930285	13.742653	18.953225
S	6.544110	12.112821	19.725267	S	6.493616	12.121028	19.745385
H	5.609575	14.989913	19.969850	H	5.647260	14.984878	20.184849
H	6.688902	13.822552	18.015635	H	6.317784	13.846612	18.045729
H	8.208268	13.684616	18.924147	H	7.983955	13.694940	18.638172
H	7.116407	14.847052	20.903108	H	7.307721	14.835806	20.802277
H	7.068515	15.872734	19.446219	H	6.973611	15.879915	19.393341

Table S6 Cartesian coordinates (Å) and total absolute energies (Hartree) of the DFT-optimized models using PW91 functional and BS23 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.159390				40 Absolute Energy: -3874.241419			
Fe	4.404766	9.290284	17.110698	Fe	4.091362	9.118855	17.189488
Fe	3.758708	11.717254	16.458522	Fe	3.575420	11.578558	16.227992
Fe	5.178110	11.354562	18.524929	Fe	4.793563	11.327353	18.573029
S	2.516547	9.910663	16.118947	S	2.219493	9.784032	16.138103
S	4.285195	9.512021	19.333602	S	3.859536	9.466251	19.418807
S	5.856383	10.932556	16.367140	S	5.713146	10.586156	16.511339
S	3.699590	13.004916	18.222535	S	3.368167	13.085775	18.005075
C	6.183977	6.164070	18.836895	C	6.183988	6.164018	18.837014
C	6.480111	6.963440	17.572895	C	6.236129	6.654876	17.393731
S	4.983658	7.192131	16.492362	S	4.565423	6.926777	16.632219
H	5.445615	6.692330	19.456009	H	5.685404	6.907592	19.473932
H	6.864436	7.960188	17.830652	H	6.786696	7.603504	17.333944
H	7.239305	6.461582	16.954483	H	6.756170	5.924759	16.753873
H	5.785113	5.169028	18.592061	H	5.629842	5.216636	18.916253
H	7.100717	6.030867	19.435047	H	7.202561	6.001528	19.229604
C	0.128954	10.771018	19.114948	C	0.129017	10.770990	19.114991
C	0.824528	11.296038	20.362346	C	0.799589	11.391007	20.328718
S	-0.433884	11.678783	21.675229	S	-0.482696	11.937471	21.564756
H	-0.422409	9.841401	19.318346	H	-0.449940	9.872723	19.379050
H	1.524709	10.551399	20.758419	H	1.467285	10.669142	20.812457
H	1.385422	12.209995	20.134554	H	1.392389	12.262255	20.025463
H	0.435304	12.100623	22.628228	H	0.369338	12.496242	22.461263
H	-0.572043	11.510596	18.701307	H	-0.539704	11.486487	18.614154
H	0.889422	10.557480	18.347991	H	0.910330	10.479637	18.394919
C	4.023012	15.420792	13.654154	C	4.022993	15.421011	13.654006
C	4.247907	14.500260	14.852207	C	4.506190	14.222283	14.467407
S	3.134928	13.020529	14.785358	S	3.437645	12.735122	14.205119
H	4.694689	16.292455	13.705691	H	4.684236	16.292309	13.801153
H	5.284900	14.139101	14.881577	H	5.539478	13.960325	14.193143
H	4.053426	15.022973	15.798690	H	4.495282	14.442525	15.544255
H	4.218694	14.896168	12.707837	H	4.000698	15.189123	12.578228
H	2.987708	15.790644	13.626288	H	3.005108	15.710287	13.953655
C	6.704057	14.930119	19.887002	C	6.704001	14.929981	19.886991
C	7.304512	13.740832	19.146474	C	7.119026	13.654016	19.157761
S	6.804348	12.112876	19.879751	S	6.492260	12.117481	19.982365
H	5.607080	14.902708	19.826493	H	5.608750	15.016137	19.897422
H	6.995098	13.741315	18.093157	H	6.729266	13.659296	18.131310
H	8.404192	13.772127	19.176669	H	8.216344	13.580709	19.099689
H	6.991122	14.924383	20.948596	H	7.057497	14.921058	20.929031
H	7.049268	15.878410	19.442326	H	7.117917	15.824319	19.389001

Table S7 Cartesian coordinates (Å) and total absolute energies (Hartree) of the DFT-optimized models using B(5HF)B86 functional and BS12 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.692326				40 Absolute Energy: -3874.787770			
Fe	4.435423	9.256407	17.253565	Fe	4.155483	9.139226	17.208109
Fe	3.968600	11.666010	16.331863	Fe	3.689263	11.549845	16.212416
Fe	5.221537	11.348179	18.649326	Fe	5.132520	11.329624	18.538052
S	2.752460	9.823525	16.026482	S	2.198224	9.760154	16.129697
S	4.262356	9.460068	19.429236	S	4.224120	9.493042	19.465180
S	6.026320	10.672396	16.560073	S	5.869861	10.616150	16.456876
S	3.655003	12.907543	18.145794	S	3.591709	12.923409	18.028607
C	6.183984	6.164044	18.836942	C	6.183993	6.164027	18.836950
C	6.341252	6.706885	17.417887	C	6.305775	6.667611	17.398911
S	4.738308	7.165090	16.599896	S	4.669845	6.923633	16.553082
H	5.725420	6.925905	19.484646	H	5.626787	6.893476	19.443246
H	6.983743	7.598967	17.411056	H	6.845001	7.626823	17.378324
H	6.807714	5.955923	16.759327	H	6.876534	5.952471	16.784300
H	5.543618	5.268845	18.851089	H	5.653375	5.199734	18.878278
H	7.166162	5.893514	19.261212	H	7.182023	6.027574	19.291427
C	0.129002	10.771015	19.114998	C	0.129001	10.771028	19.114979
C	0.904121	11.265408	20.330032	C	0.940753	11.416860	20.228390
S	-0.277286	11.845168	21.642561	S	-0.196130	12.073826	21.550540
H	-0.520393	9.917949	19.365210	H	-0.464086	9.917867	19.481138
H	1.525342	10.463502	20.747839	H	1.620083	10.687572	20.686055
H	1.561209	12.098392	20.050720	H	1.543088	12.244006	19.830875
H	0.654084	12.248940	22.542655	H	0.760229	12.584506	22.366877
H	-0.492453	11.568252	18.679994	H	-0.554939	11.493960	18.643380
H	0.847999	10.444431	18.347602	H	0.819858	10.405533	18.335962
C	4.023035	15.420899	13.654091	C	4.022995	15.420904	13.654046
C	4.325723	14.405820	14.757653	C	4.513002	14.215763	14.459708
S	3.453356	12.793407	14.454329	S	3.454291	12.718151	14.178516
H	4.549183	16.370080	13.850297	H	4.674903	16.297482	13.817926
H	5.406318	14.210131	14.817692	H	5.549993	13.965089	14.186880
H	4.003047	14.780432	15.739227	H	4.501170	14.440153	15.536353
H	4.346649	15.050280	12.669293	H	4.013285	15.201038	12.574596
H	2.944704	15.633507	13.597260	H	2.998520	15.696867	13.947457
C	6.703979	14.930041	19.886970	C	6.704012	14.930041	19.887025
C	7.310327	13.711244	19.198005	C	7.288777	13.714649	19.168149
S	6.862642	12.114638	20.038863	S	6.803515	12.105439	19.955586
H	5.605863	14.879535	19.865758	H	5.604774	14.904152	19.856094
H	6.973292	13.652656	18.153686	H	6.955697	13.687651	18.121015
H	8.409893	13.768761	19.191908	H	8.389904	13.757673	19.167262
H	7.024344	14.991810	20.938294	H	7.016000	14.952514	20.943089
H	7.014260	15.859242	19.378057	H	7.038666	15.868162	19.409048

Table S8 Cartesian coordinates (Å) and total absolute energies (Hartree) of the DFT-optimized models using B(5HF)B86 functional and BS13 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.691582				40 Absolute Energy: -3874.787662			
Fe	4.231580	9.165947	16.891679	Fe	4.180530	9.073751	16.948592
Fe	3.473468	11.512716	15.888978	Fe	3.572684	11.485139	15.825285
Fe	4.732790	11.363807	18.190505	Fe	4.817133	11.303666	18.249535
S	2.295143	9.605661	15.879208	S	2.308495	9.608381	15.789057
S	4.237996	9.472770	19.114899	S	4.047711	9.373535	19.262981
S	5.683753	10.830629	16.262722	S	5.727086	10.704739	16.128966
S	3.102770	12.749801	17.762765	S	3.236672	12.822655	17.643623
C	6.183959	6.163954	18.837070	C	6.184058	6.164091	18.836837
C	6.510280	6.903303	17.544314	C	6.491812	6.774974	17.472747
S	5.032081	7.104931	16.445918	S	4.989038	6.937760	16.399835
H	5.443282	6.727677	19.421982	H	5.481096	6.811154	19.381707
H	6.909971	7.903402	17.758374	H	6.929112	7.775543	17.598542
H	7.264087	6.356509	16.956074	H	7.221469	6.158937	16.921785
H	5.773621	5.164311	18.629144	H	5.730994	5.165754	18.730005
H	7.089306	6.043117	19.457212	H	7.104247	6.064186	19.441484
C	0.129016	10.770900	19.115029	C	0.128875	10.771084	19.115032
C	0.869656	10.815661	20.445380	C	0.969618	11.097276	20.340623
S	-0.324279	11.207017	21.816670	S	-0.135287	11.666413	21.729396
H	-0.646718	9.989701	19.107039	H	-0.592147	9.963176	19.317008
H	1.342870	9.850899	20.663805	H	1.532126	10.217818	20.674873
H	1.646871	11.588931	20.424761	H	1.682994	11.897956	20.109176
H	0.574153	11.212716	22.833415	H	0.840466	11.887465	22.645938
H	-0.343980	11.738373	18.887678	H	-0.423927	11.653391	18.757598
H	0.847505	10.554907	18.309941	H	0.798959	10.444091	18.305494
C	4.023018	15.421142	13.653947	C	4.023043	15.420764	13.654040
C	4.436880	14.138490	14.367667	C	4.525295	14.092777	14.216331
S	3.295284	12.730912	13.968486	S	3.341784	12.708538	13.869941
H	4.727225	16.238321	13.886021	H	4.751757	16.228452	13.844199
H	5.454715	13.840922	14.076129	H	5.499252	13.828068	13.777933
H	4.430394	14.276671	15.458480	H	4.654318	14.154023	15.307208
H	4.011168	15.284534	12.561479	H	3.861787	15.358794	12.565876
H	3.016840	15.739586	13.964863	H	3.067572	15.704704	14.120638
C	6.704007	14.930004	19.886954	C	6.704024	14.930062	19.887091
C	6.728548	13.772909	18.886669	C	6.906778	13.758523	18.924662
S	6.173078	12.175784	19.654469	S	6.482181	12.126051	19.701334
H	5.682938	15.104611	20.256882	H	5.655374	14.980835	20.214902
H	6.060712	13.976936	18.038772	H	6.276552	13.882578	18.031623
H	7.743203	13.620428	18.487416	H	7.954358	13.715334	18.586573
H	7.347908	14.722038	20.755435	H	7.332779	14.819126	20.784660
H	7.062858	15.859423	19.412999	H	6.961879	15.889894	19.403990

Table S9 Cartesian coordinates (Å) and total absolute energies (Hartree) of the DFT-optimized models using B(5HF)B86 functional and BS23 broken-symmetry state.

Oxidized <i>Pf</i> Fd D14C [3Fe-4S] ¹⁺ model				Reduced <i>Pf</i> Fd D14C [3Fe-4S] ⁰ model			
[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ²⁻				[[Fe ₃ S ₄](CH ₃ CH ₂ S) ₃ (CH ₃ CH ₂ SH)] ³⁻			
40 Absolute Energy: -3874.692308				40 Absolute Energy: -3874.787481			
Fe	4.374489	9.249848	17.094312	Fe	4.089323	9.066785	17.218979
Fe	3.719352	11.713823	16.469746	Fe	3.614501	11.572076	16.247546
Fe	5.164126	11.359056	18.523795	Fe	4.810317	11.312498	18.625777
S	2.485606	9.900417	16.093402	S	2.239897	9.776257	16.140224
S	4.287395	9.493936	19.329593	S	3.852530	9.437573	19.453432
S	5.815941	10.923972	16.343526	S	5.738431	10.534807	16.569417
S	3.678847	13.020545	18.217138	S	3.396566	13.067792	18.026116
C	6.183977	6.164070	18.836895	C	6.183988	6.164018	18.837014
C	6.493268	6.956614	17.570029	C	6.191433	6.516259	17.350313
S	5.013309	7.158978	16.460349	S	4.507478	6.854340	16.647051
H	5.426393	6.687002	19.438214	H	5.789137	7.005368	19.424511
H	6.864635	7.959087	17.826605	H	6.816751	7.402237	17.168509
H	7.268699	6.457344	16.968522	H	6.611138	5.686586	16.758446
H	5.804149	5.160030	18.593719	H	5.553721	5.282160	19.032810
H	7.092146	6.049510	19.453729	H	7.207388	5.944016	19.190356
C	0.128954	10.771018	19.114948	C	0.129017	10.770990	19.114991
C	0.819362	11.292861	20.369092	C	0.788090	11.384127	20.341473
S	-0.446376	11.679904	21.675005	S	-0.510239	11.934090	21.560433
H	-0.431745	9.845180	19.315572	H	-0.458019	9.874182	19.369933
H	1.514558	10.544322	20.768872	H	1.444415	10.655768	20.832690
H	1.385434	12.205382	20.144614	H	1.391629	12.252785	20.049215
H	0.417290	12.094709	22.635729	H	0.328174	12.488701	22.471797
H	-0.563658	11.515964	18.694496	H	-0.531626	11.492141	18.609527
H	0.893865	10.549873	18.353585	H	0.916547	10.477669	18.401803
C	4.023012	15.420792	13.654154	C	4.022993	15.421011	13.654006
C	4.229408	14.515961	14.869399	C	4.543630	14.227608	14.456041
S	3.103899	13.046087	14.804512	S	3.500177	12.719570	14.200287
H	4.700500	16.289690	13.700295	H	4.663114	16.309231	13.801685
H	5.264450	14.148964	14.913380	H	5.578644	13.989606	14.163809
H	4.028894	15.055978	15.805439	H	4.545129	14.446660	15.533422
H	4.225505	14.880967	12.716782	H	3.998994	15.194479	12.576067
H	2.989694	15.797892	13.609936	H	2.999309	15.681763	13.962554
C	6.704057	14.930119	19.887002	C	6.704001	14.929981	19.886991
C	7.303581	13.745371	19.135326	C	7.151365	13.642246	19.193897
S	6.816455	12.112142	19.867584	S	6.529823	12.109384	20.033029
H	5.605806	14.897750	19.837563	H	5.606724	15.000663	19.881165
H	6.985733	13.749679	18.083931	H	6.788682	13.622089	18.156974
H	8.404185	13.781516	19.155623	H	8.251517	13.583901	19.163304
H	7.001971	14.921673	20.946667	H	7.042379	14.952321	20.934928
H	7.040426	15.883031	19.442664	H	7.112228	15.819087	19.372961

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