Supplementary Material (ESI) for *PCCP*
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Electronic supplementary information (ESI)

The reduced [2Fe-2S] clusters in adrenodoxin and *Arthrospira platensis* ferredoxin share spin density with protein nitrogens, probed using 2D ESEEM

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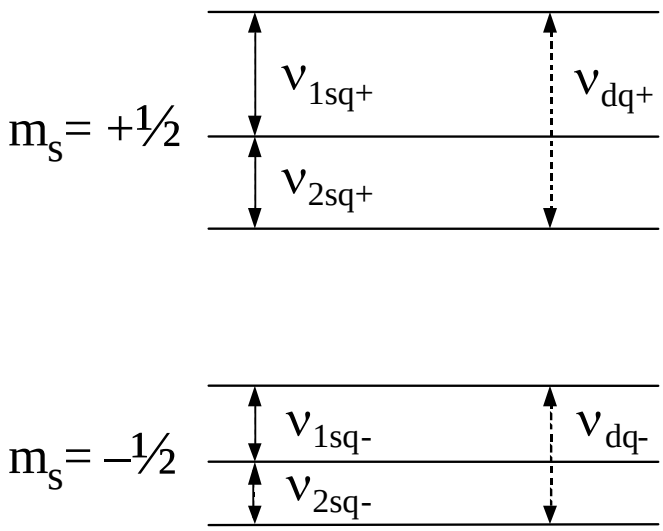


Figure S1. The nuclear frequencies of an *I*=1 nucleus interacting with an electron spin *S*=½**.** There are three nuclear sublevels with three nuclear transitions in both electron spin manifolds with electron spin states $m_S = \pm½$. In each manifold, two of these transitions, between adjacent nuclear sublevels with frequencies $\nu_{(1,2)sq\pm}$ (solid lines), are single-quantum (sq) transitions. The third transition, between extreme sublevels with the frequency $\nu_{dq\pm}$ (dashed lines) is a double-quantum (dq) transition.

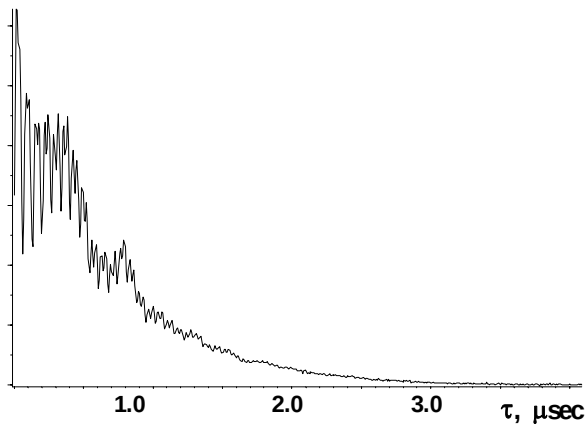


Figure S2. Two pulse ESEEM pattern of the reduced [2Fe-2S] cluster in adrenodoxin recorded in g_1 region at magnetic field 343.8 mT.

Table S1. Direction cosines of the g-tensor principal axes in the coordinate system of the X-ray structure of adrenodoxin (1ayf.pdb)[1] and *Arthrospira platensis* ferredoxin (4fxc.pdb).[2]

Protein	g-value	Direction cosines		
adx	$g_{1(\|\|)\ max}$	0.3266	-0.9308	-0.1642
	$g_{2\ int}$	-0.3732	0.0326	-0.9272
	$g_{3\ min}$	-0.8684	-0.3641	0.3367
fdx	$g_{1\ max}$	0.168	-0.724	-0.669
	$g_{2\ int}$	-0.188	0.642	-0.743
	$g_{3\ min}$	-0.986	-0.251	0.028

Table S2. N-S distances in adrenodoxin calculated from X-ray structure (1ayf.pdb, 1.85 Ǎ resolution, oxidized protein)[1]

	S1	**S2**	**Cys46**	**Cys52**	**Cys55**	**Cys92**
Gly44	7.37	4.47	7.34	5.20	5.91	7.72
Ala45	6.97	4.40	5.76	3.94	6.99	8.17
Cys46	6.03	4.35	**3.49**	**3.47**	7.60	7.74

Glu47	6.49	4.13	3.95	5.38	7.83	6.94
Gly48	5.20	**3.26**	**3.79**	5.94	6.51	4.54
Thr49	4.66	4.90	**3.13**	6.51	7.39	4.82
Leu50	**3.55**	5.65	**3.48**	6.30	7.01	4.91
Ala51	3.99	6.34	**3.26**	5.25	7.71	6.88
Cys52	3.94	5.96	**3.78**	**3.49**	6.96	7.44
Ser53	5.10	6.45	6.37	**3.52**	6.03	8.26
Thr54	5.74	5.77	7.14	**3.65**	5.03	8.03
Cys55	5.63	4.80	7.54	4.71	**3.19**	6.62
His56	6.87	6.84	9.54	6.96	**3.80**	7.42
Leu90	7.25	8.77	10.76	9.81	5.26	6.71
Gly91	7.61	7.19	10.37	9.56	4.38	5.48
Cys92	6.05	5.41	8.43	8.32	**3.62**	**3.25**
Gln93	6.80	7.10	9.24	9.95	5.58	**3.65**
Gln93	5.70	6.93	7.47	9.35	6.68	**3.40**
Ile94	9.39	9.52	11.93	12.39	7.58	6.32

Table S3. N-S distances in *Arthrospira platensis* ferredoxin calculated from X-ray structure (4fxc.pdb, 2.5 Ǎ resolution)[2]

	S1	**S2**	**Cys41**	**Cys46**	**Cys49**	**Cys79**
Leu37	10.65	13.42	14.12	13.45	10.16	11.51
Pro38	7.96	10.67	11.53	10.19	7.46	9.52
Tyr39	4.87	8.09	8.23	7.13	5.98	7.46
Ser40	**3.11**	6.45	5.40	4.99	6.26	6.62
Cys41	**3.35**	5.53	**3.13**	4.31	7.09	6.35
Arg42	**3.33**	4.88	**2.82**	5.69	6.54	4.48
Arg42 Ne	7.09	9.37	8.21	10.54	9.16	6.98
Arg42 Nh1	7.73	10.33	9.68	11.36	9.20	7.71
Arg42 Nh2	9.06	11.58	10.34	12.50	11.07	9.15
Ala43	4.93	4.35	**3.02**	6.58	6.98	4.05
Gly44	5.86	**3.30**	**3.78**	6.32	6.69	4.37
Ala45	6.70	3.96	**3.87**	5.61	7.86	6.45
Cys46	6.03	**3.64**	4.12	**3.53**	7.05	6.98
Ser47	5.85	4.69	6.12	**2.79**	5.93	7.69
Thr48	5.52	5.97	7.27	**3.67**	5.40	8.03
Cys49	4.59	5.75	7.49	4.95	**3.37**	6.54
Ala50	7.06	7.28	9.84	7.56	4.06	7.57
Gly51	10.06	9.60	12.56	10.53	6.52	9.60
Val76	11.75	9.99	13.29	11.88	8.16	10.22
Leu77	8.73	6.85	10.12	9.36	5.27	6.75
Thr78	7.58	6.77	9.69	9.27	4.03	5.46
Cys79	6.01	6.13	8.27	8.76	**3.65**	**3.47**
Val80	7.06	6.20	8.09	9.60	5.54	**3.38**
Ala81	9.59	8.51	10.66	11.97	7.47	5.99

^{14}N anisotropic hyperfine tensor in the point-dipole approximation.

The anisotropic hyperfine tensor of the protein nitrogen is the result of two factors: dipole-dipole couplings with Fe(III) and Fe(II) and unpaired spin density transfer onto the nitrogen. The dipole-dipole contribution to the hyperfine tensor is determined chiefly by the relative location of irons and nitrogen. General analytical formulae developed by Randall *et al.*[3] and adapted for the case of the reduced [2Fe-2S] cluster, Fe(III)–Fe(II), interacting with ^{14}N nuclei, were used for the calculation of the principal values of the resulting dipolar tensor

$$\mathrm{T_{dip}} = \begin{bmatrix} \frac{1}{2}\left(\frac{7}{3}T_{III} - \frac{4}{3}T_{II} - 3Z\right) \\ -\left(\frac{7}{3}T_{III} - \frac{4}{3}T_{II}\right) \\ \frac{1}{2}\left(\frac{7}{3}T_{III} - \frac{4}{3}T_{II} + 3Z\right) \end{bmatrix} \qquad \text{(S1)}$$

where

$$\mathrm{Z} = \left[\frac{49}{9}T_{III}^2 + \frac{16}{9}T_{II}^2 - \frac{56}{9}T_{III}T_{II}\cos(2\alpha + 2\beta)\right]^{1/2}$$

and $\mathrm{T_p} = \frac{g_e\beta_e g_N\beta_N}{hr_p^3}$ (*p=III or II*) for point dipoles.

The geometric relations between the sides and angles of the triangle, Fe(III)-N-Fe(II), allow for definition of the tensor components based on one distance and one angle, for instance $\mathrm{r_{III}}$ and β, using equations (Figure S3)

$$\mathrm{r_{II}} = [r_{iii}^2 + r_{Fe-Fe}^2 - 2r_{III}r_{Fe-Fe}\cos\beta]^{1/2} \qquad (6)$$

$$\alpha = \arcsin\left[\frac{\mathrm{r_{III}}}{\mathrm{r_{II}}}\sin\beta\right]$$

These equations allows us to examine the components of hyperfine tensors from the location of the nucleus, defined by $\mathrm{r_{III}}$ and β or $\mathrm{r_{II}}$ and α. These parameters were calculated using crystallographic coordinates in adrenodoxin (**adx**) (1ayf.pdb[1]) and *Arthrospira platensis* ferredoxin (**fdx**) (4fxc.pdb[2]).

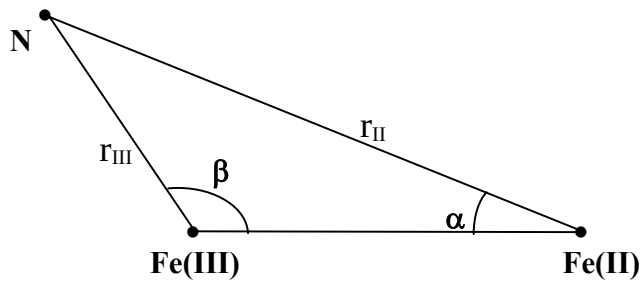


Figure S3. Definition of the distances and angles describing the location of the ^{14}N relative to Fe(III) and Fe(II).

Table S4. Anisotropic hyperfine tensors calculated in the point-dipole model for the ^{14}N nitrogens of selected residues around [2Fe-2S] cluster in adx.

Residue	N-Fe(III),[a] Å	N-Fe(II), Å	Principal values of hfi tensor, MHz	$\lvert T_{max}/2 \rvert$, MHz
Cys46	6.12	3.88	-0.17,0.07, 0.10	0.085
Gly48	4.43	4.17	-0.21,-0.04, 0.22	0.125
Thr49	5.09	4.49	-0.13,-0.02, 0.15	0.075
Leu50	4.97	4.42	-0.15,-0.02, 0.17	0.085
Ala51	4.83	4.53	-0.12,0.03, 0.09	0.06
Cys52	5.78	3.77	-0.19,0.07, 0.12	0.095
Ser53	6.13	4.93	-0.08,0.01, 0.07	0.04
Thr54	5.51	3.54	-0.08,-0.01,0.09	0.045
Cys55	5.97	4.79	-0.13,-0.08, 0.21	0.105
His56	5.97	7.21	-0.05,-0.04,0.09	0.045
Cys92	4.2	6.80	-0.16,-0.15, 0.31	**0.165**
Gln93	5.56	8.07	-0.06,-0.06,0.12	0.06
Gln87(e)	5.46	7.16	-0.07,-0.06,0.13	0.065

[a]Fe(III) is Fe2 coordinated by Cys55 and Cys92

Table S5. Anisotropic hyperfine tensors calculated in the point-dipole model for the ^{14}N nitrogens of selected residues around [2Fe-2S] cluster in fdx.

Residue	N-Fe(III),[a] Å	N- Fe(II), Å	Principal values of hfi tensor, MHz	$\lvert T_{max}/2 \rvert$, MHz
Ser40	5.29	4.53	-0.12,-0.01,0.13	0.065
Cys41	5.30	3.52	-0.25,0.08,0.17	0.125
Arg42	4.48	3.80	-0.25,-0.01,0.26	0.13
Ala43	4.82	4.51	-0.15,-0.04,0.19	0.095
Gly44	4.82	4.62	-0.15,0.04,0.19	0.095
Ala45	5.78	3.77	-0.09,.0.01,0.08	0.04
Cys46	5.64	3.88	-0.18,0.06,0.12	0.09
Ser47	5.61	4.42	-0.12,0.01,0.11	0.06
Thr48	5.77	5.16	-0.08,-0.01,0.09	0.045
Cys49	4.55	5.24	-0.14,-0.09,0.23	0.105
Cys79	4.48	7.10	-0.125, -0.125, 0.25	0.125
Val80	5.36	7.62	-0.07,-0.07,0.14	0.07

[a]Fe(III) is Fe2 coordinated by Cys49 and Cys79

Unpaired spin density transferred onto the 2*p* orbital determines the second contribution to the anisotropic tensor. The computed value of T_p for a 2*p* electron is 55.5 MHz, which is about 32 times less than the value for isotropic coupling obtained for a 2s electron.[4] For a spin density ~0.4 $\cdot 10^{-3}$ on the 2*p* orbital one can find a value for T_p of ~0.02 MHz. Thus, the dipole-dipole interaction provides the major contribution to the anisotropic hyperfine tensor of the ^{14}N.

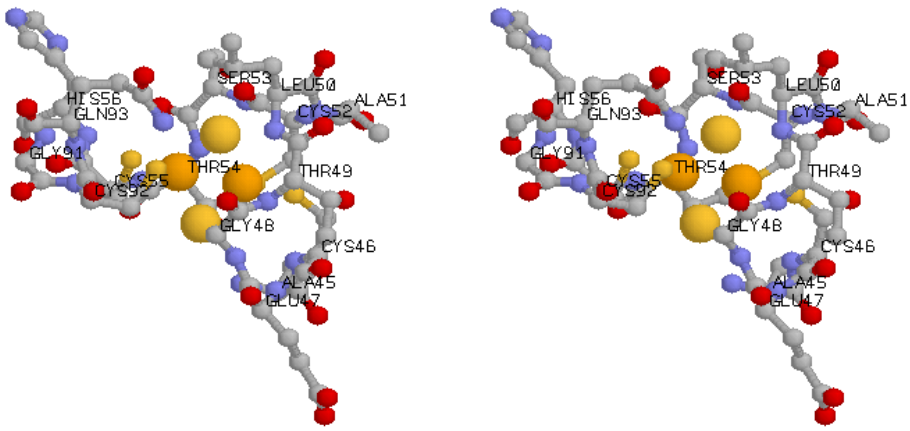
Rescaling of isotropic hyperfine couplings

Although the coupled spins of two irons collectively have the net moment of a single electron spin, each individual iron possesses the larger moment of several electrons, and many nitrogens interact primarily with the moment of the single nearest iron. The spin-population coefficient for each iron, $D_S(\mathrm{Fe_i})$, can be expressed as $D_S(\mathrm{Fe_i})=K(\mathrm{Fe_i})\cdot d_B(\mathrm{Fe_i})$, where $K(\mathrm{Fe_i})=\langle \boldsymbol{S}(\mathrm{Fe_i})\cdot \boldsymbol{S}_{total}\rangle/\langle \boldsymbol{S}^2_{total}\rangle$ is the theoretical vector-coupling coefficient and d_B is a covalency factor, which is the ratio of the spin population of an iron site and the maximum population expected in the valence-bond limit.[5] The ^{57}Fe hyperfine couplings in the major classes of iron–sulfur clusters can be obtained by Mössbauer and ENDOR spectroscopies[5]. Analysis of these values has found that a reliable covalency factor d_B equal to 0.75 for Fe(II) and 0.65 for Fe(III) in reduced [2Fe–2S] clusters with cysteine ligands. This means that instead of a maximal population, which is 4 for Fe(II) and 5 for Fe(III), only about 3 and 3.25 unpaired electrons, respectively, are localized on the irons. Multiplication of these d_B values with vector coupling coefficients (7/3) for Fe(III) and (-4/3) for Fe(II) leads to spin population coefficients D_S of ~1.50 for Fe(III) and ~-1.00 for Fe(II). For comparison, simulations of experimental orientation-selected proton ENDOR spectra have provided the spin population coefficients D_S ~1.85 for

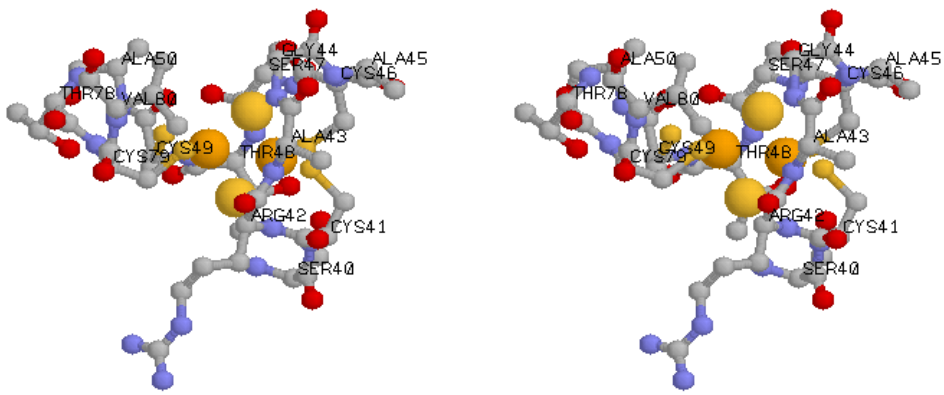
Fe(III) and ~–0.9 for Fe(II) for [2Fe–2S] cluster in ferredoxin from *A. platensis*.[6] These values were +1.60 and -0.6, respectively, for adrenodoxin.[7]

From these considerations we can conclude that real spin density on the nitrogens is proportional to the hyperfine couplings a_{spc}, obtained after normalization of the experimental nitrogen isotropic constants by the spin population coefficients for each individual Fe ion, $a_{spc}=a_{exp}/D_S(Fe_i)$. Therefore, the result would significantly depend on how accurately the $D_S(Fe_i)$ spin populations are determined. The consideration above allows us to suggest that experimental hyperfine couplings are multiplied by a factor ~1.5 for the nitrogens of the cysteines liganding Fe(III) and nitrogens forming hydrogen bonds with the cysteine sulfurs. The multiplication factor is ~-1 for the nitrogens near Fe(II), and probably has intermediate value for the nitogens interacting with the bridging sulfurs.

Protein structure around the cluster in adx and fdx.



adx



fdx

Figure S4. Structure around the cluster in **adx** and **fdx** (the orientation is similar in both proteins) The structures are shown as ball and stick models, colored in CPK, with the cluster atoms at 0.6 van der Waals radii. (Stereo pairs are for crossed eye viewing.)

References.

1. A. Müller, J.J. Müller, Y.A. Muller, H. Uhlmann, R. Bernhardt and U. Heinemann, *Structure,* 1998, **6**, 269.
2. K. Fukuyama, N. Ueki, M. Nakamura, T. Tsukihara and H. Matsubara, *J. Biochem. (Tokyo)*, 1995, **117**, 1017.
3. D.W. Randall, A. Gelasco, M.T. Caudle, V.L. Pecoraro, R. D. Britt, *J. Am. Chem. Soc.* 1997, **119**, 4481.
4. J.R. Morton and K.F. Preston, *J. Magn. Reson.* 1978, **30**, 577.
5. J.-M. Mouesca, L. Noodleman, D.A. Case, B. Lamotte, *Inorg. Chem.* 1995, **34**, 4347.
6. C. Canne, M. Ebelshäuser, E. Gay, J.K. Shergill, R. Cammack, R. Kappl, J. Hüttermann, *J. Biol. Inorg. Chem.* 2000**, 5**, 514.
7. R. Kappl, M. Ebelshäuser, F. Hannemann, R. Bernhardt, J. Hüttermann, *Appl. Magn. Reson.* 2006, 30, 427.