Electronic supplementary information (ESI)

The reduced [2Fe-2S] clusters in adrenodoxin and *Arthospira 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 \( S=\frac{1}{2} \). There are three nuclear sublevels with three nuclear transitions in both electron spin manifolds with electron spin states \( m_S = \pm \frac{1}{2} \). In each manifold, two of these transitions, between adjacent nuclear sublevels with frequencies \( \nu_{1sq} \), are single-quantum (sq) transitions. The third transition, between extreme sublevels with the frequency \( \nu_{dq} \), 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)\textsuperscript{1} and Arthrospira platensis ferredoxin (4fxc.pdb).\textsuperscript{2}

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<th>Protein</th>
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<th>Direction cosines</th>
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Table S2. N-S distances in adrenodoxin calculated from X-ray structure (1ayf.pdb, 1.85 Å resolution, oxidized protein)\textsuperscript{1}

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

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

where

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

and \( T_p = \frac{g_e \beta_x g_N \beta_N}{h r_p^2} \) (\( p=\text{III or II} \)) for point dipoles.

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

\[ r_{II} = [r_{III}^2 + r_{Fe-Fe}^2 - 2r_{III} r_{Fe-Fe} \cos \beta]^{1/2} \]

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

These equations allows us to examine the components of hyperfine tensors from the location of the nucleus, defined by \( r_{III} \) and \( \beta \) or \( r_{II} \) and \( \alpha \). These parameters were calculated using crystallographic coordinates in adrenodoxin (\( \text{adx} \)) (1ayf.pdb\(^1\)) and \( \text{Arthospira platensis} \) ferredoxin (\( \text{fdx} \)) (4fxc.pdb\(^2\)).
Figure S3. Definition of the distances and angles describing the location of the $^{14}\text{N}$ relative to Fe(III) and Fe(II).

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

| Residue  | N-Fe(III), Å | N-Fe(II), Å | Principal values of hfi tensor, MHz | $|T_{\text{max/2}}|$, 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                    |

*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), Å | N- Fe(II), Å | Principal values of hfi tensor, MHz | $|T_{\text{max}/2}|$, 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 $2p$ orbital determines the second contribution to the anisotropic tensor. The computed value of $T_p$ for a $2p$ electron is 55.5 MHz, which is about 32 times less than the value for isotropic coupling obtained for a $2s$ electron. For a spin density $\sim 0.4 \cdot 10^{-3}$ on the $2p$ orbital one can find a value for $T_p$ of $\sim 0.02$ 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, $D_S(Fe_i)$, can be expressed as $D_S(Fe_i)=K(Fe_i) \cdot d_B(Fe_i)$, where $K(Fe_i)=<S_{Fe_i} \cdot S_{total}>/<S_{total}^2>$ 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. The $^{57}$Fe hyperfine couplings in the major classes of iron–sulfur clusters can be obtained by Mössbauer and ENDOR spectroscopies. 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 $\sim 1.50$ for Fe(III) and $\sim 1.00$ for Fe(II). For comparison, simulations of experimental orientation-selected proton ENDOR spectra have provided the spin population coefficients $D_S \sim 1.85$ for
Fe(III) and $\sim -0.9$ for Fe(II) for [2Fe–2S] cluster in ferredoxin from \textit{A. platensis}.\textsuperscript{6} These values were +1.60 and -0.6, respectively, for adrenodoxin.\textsuperscript{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, $a_{spc} = a_{exp} / D_{S(Fe)}$. Therefore, the result would significantly depend on how accurately the $D_{S(Fe)}$ spin populations are determined. The consideration above allows us to suggest that experimental hyperfine couplings are multiplied by a factor $\sim 1.5$ for the nitrogens of the cysteines liganding Fe(III) and nitrogens forming hydrogen bonds with the cysteine sulfurs. The multiplication factor is $\sim -1$ for the nitrogens near Fe(II), and probably has intermediate value for the nitogens interacting with the bridging sulfurs.

\textbf{Protein structure around the cluster in adx and 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.