Supporting Information

Nitric Oxide Modulates Endonuclease III Redox Activity by a 800 mV Negative Shift upon [Fe₄S₄] Cluster Nitrosylation

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UV–Vis Spectroscopy of Nitrosylation with and without dsDNA

Figure S1. UV–vis spectra of native (black) and nitrosylated EndoIII prepared in the absence (solid red) or presence (dashed red) of dsDNA (Endo/dsDNA ratio of 1:10).

CW EPR Spectroscopy

Figure S2. Continuous-wave EPR spectrum of native EndoIII (20 µM by cluster) prior to nitrosylation, demonstrating a diamagnetic spectrum consistent with the presence of [Fe₄S₄]²⁺.
Figure S3. (A) Continuous-wave EPR spectra and (B) single-integration EPR spectra of EndoIII nitrosylation before (red) and after (blue) incubation with dithionite in 20 mM phosphates, 150 mM NaCl, 0.5 mM EDTA, 10% glycerol, pH 7.5. Acquisition parameters included a temperature of 60 K and microwave power of 204 µW.

Figure S4. (A) Continuous-wave EPR spectra and of EndoIII nitrosylation before (black, top trace) and after (black, bottom trace) incubation with dithionite in 20 mM phosphates, 150 mM NaCl, 0.5 mM EDTA, 10% glycerol, pH 7.5 with simulations of [(Cys)$_2$Fe(NO)$_2$]- (red, top trace) and [(µ-Cys)$_2$Fe$_2$(NO)$_4$] (red, bottom trace) species using parameters in Table 1 of the main text. Arrows indicate the field positions at which X-band HYSCORE spectra were acquired. Acquisition parameters included a temperature of 60 K and microwave power of 204 µW. (B) Pseudomodulated Q-band electron-spin echo (ESE) detected EPR spectra of EndoIII nitrosylation before (black, top trace) and after (black, bottom trace) incubation with dithionite in the same buffer as detailed above with simulations in red using parameters in Table 1 of the main text. Arrows indicate the field positions at which Q-band HYSCORE spectra were acquired. Acquisition parameters: temperature = 20 K; microwave frequency = 34.005 GHz; MW pulse lengths π/2, π = 12, 24 ns; τ = 320 ns; shot repetition time (srt) = 1 ms.
Representative UV–Vis Spectra of Deoxymyoglobin Experiments

![Figure S5](image)

**Figure S5.** UV–vis spectra of conversions from (A) equine heart metmyoglobin (dashed) to deoxymyoglobin (solid) by treatment with sodium dithionite and (B) deoxymyoglobin to nitrosomyoglobin by treatment with $^{15}$NO (solid) or a $^{14}$NO standard (dashed).

UV–Vis Spectroscopy of Nitrosylation using $^{14/15}$NO

![Figure S6](image)

**Figure S6.** UV–vis spectra of native EndoIII (black), $^{14}$NO-nitrosylated EndoIII (red), and $^{15}$NO-nitrosylated EndoIII (dashed red).

HYSCORE Simulations

All EPR spectra (CW, HYSCORE) were simulated using the EasySpin simulation toolbox (version 5.2.16) with Matlab 2016b using the following Hamiltonian:

$$
\hat{H} = \mu_B \vec{B}_0 g \vec{S} + \mu_N g_N \vec{B}_0 \vec{I} + \hbar \vec{S} \cdot \vec{A} + \hbar \vec{I} \cdot \vec{P}
$$

(1)

In this expression, the first term corresponds to the electron Zeeman interaction term where $\mu_B$ is the Bohr magneton, $g$ is the electron spin $g$-value matrix with principle components
$g = [g_{xx} g_{yy} g_{zz}]$, and $\hat{S}$ is the electron spin operator; the second term corresponds to the nuclear Zeeman interaction term where $\mu_N$ is the nuclear magneton, $g_N$ is the characteristic nuclear $g$-value for each nucleus (e.g. $^1\text{H},^1\text{H},^3\text{P}$) and $I$ is the nuclear spin operator; the third term corresponds to the electron-nuclear hyperfine term, where $A$ is the hyperfine coupling tensor with principle components $A = [A_{xx} A_{yy} A_{zz}]$; and for nuclei with $I \geq 1$, the final term corresponds to the nuclear quadrupole (NQI) term which arises from the interaction of the nuclear quadrupole moment with the local electric field gradient (efg) at the nucleus, where $P$ is the quadrupole coupling tensor. In the principle axis system (PAS), $P$ is traceless and parametrized by the quadrupole coupling constant $e^2Qq/h$ and the electric field gradient (efg) asymmetry parameter $\eta$ such that:

$$
P = \begin{pmatrix}
    p_{xx} & 0 & 0 \\
    0 & p_{yy} & 0 \\
    0 & 0 & p_{zz}
\end{pmatrix} = \frac{e^2Qq/h}{4I(2I-1)} \begin{pmatrix}
    -(1- \eta) & 0 & 0 \\
    0 & -(1+ \eta) & 0 \\
    0 & 0 & 2
\end{pmatrix} \tag{2}
$$

where $\frac{e^2Qq}{h} = 2I(2I-1)p_{zz}$ and $\eta = \frac{p_{xx} - p_{yy}}{p_{zz}}$. The asymmetry parameter may have values between 0 and 1, with 0 corresponding to an electric field gradient with axial symmetry and 1 corresponding to a fully rhombic efg.

The orientations between the hyperfine and NQI tensor principle axis systems and the g-matrix reference frame are defined by the Euler angles ($\alpha, \beta, \gamma$). In general, the HYSCORE spectrum for a given nucleus with spin $I = \frac{1}{2}$ ($^1\text{H}$) coupled to the $S = \frac{1}{2}$ electron spin exhibits a pair of peaks at frequencies

$$
\nu_{\pm} = \left| \frac{A}{2} \pm \nu_N \right| \tag{4}
$$

Where $\nu_N$ is the nuclear Larmor frequency and $A$ is the hyperfine coupling. For nuclei with $I \geq 1$ ($^{14}\text{N}, ^2\text{H}$), an additional splitting of the $\nu_{\pm}$ manifolds is produced by the nuclear quadrupole interaction (P)

$$
\nu_{\pm,m_l} = \left| \nu_N \pm \frac{3P(2m_l-1)}{2} \right| \tag{5}
$$

In HYSCORE spectra, these signals manifest as cross-peaks or ridges in the 2-D frequency spectrum which are generally symmetric about the diagonal of a given quadrant. This technique allows hyperfine levels corresponding to the same electron-nuclear submanifold to be differentiated, as well as separating features from hyperfine couplings in the weak-coupling regime ($|A| < 2|\nu_I|$ ) in the (+,-) quadrant from those in the strong coupling regime ($|A| > 2|\nu_I|$ ) in the (-,-) quadrant. The (-,-) and (+,-) quadrants of these frequency spectra are symmetric to the (+,+), and (-,+) quadrants, thus typically only two of the quadrants are typically displayed in literature. For systems with appreciable hyperfine anisotropy in frozen solutions or solids, HYSCORE spectra typically do not exhibit sharp cross peaks, but show ridges that represent the sum of cross peaks from selected orientations at the magnetic field position at
which the spectrum is collected. The length and curvature of these correlation ridges allow for
the separation and estimation of the magnitude of the isotropic and dipolar components of the
hyperfine tensor.

**Q-Band HYSCORE Spectroscopy**

![HYSCORE Spectra](image)

**Figure S7.** Q-band HYSCORE spectra and simulations of $^{14}$N/$^{15}$N hyperfine couplings of
$[\text{Cys}_2\text{Fe(NO)}_2]^-$ generated with (a) $^{14}$NO (b) $^{15}$NO and $[\mu\text{-Cys}_2\text{Fe}_2\text{(NO)}_4]^-$ generated with (c) $^{14}$NO and (d) $^{15}$NO. Top panels show the experimental spectra, with intensities indicated by the
color map ranging from blue to red in order of increasing intensity. The bottom panels reproduce...
the experimental data in grey and overlay simulations from a relatively strongly coupled $^{14/15}\text{N}$ nucleus (blue, denoted $N_1$) and a relatively weakly coupled $^{14/15}\text{N}$ nucleus (red, denoted $N_2$). Specific simulation parameters are detailed in Table 1. Acquisition parameters: temperature = 20 K; microwave frequency = 33.986 GHz (a,b), 34.000 GHz (c,d); magnetic field = 1193.6 mT ($g = 2.034$) (a,b), 1209 mT ($g = 2.009$) (c,d), MW pulse lengths $\pi/2$, $\pi = 12$, 24 ns; $\tau = 100$ ns; $T_1 = T_2 = 100$ ns; $\Delta T_1 = \Delta T_2 = 16$ ns; shot repetition time (srt) = 1 ms.

**Figure S8.** Q-band HYSCORE spectra and simulations of $^{14}\text{N}$ hyperfine couplings of $[(\text{Cys})_2\text{Fe(\text{NO})}_2]^-$ generated with $^{14}\text{NO}$. For each spectrum, the top panels show the experimental spectra, with intensities indicated by the color map ranging from blue to red in order of increasing intensity. The bottom panels reproduce the experimental data in grey and overlay simulations from a relatively strongly coupled $^{14}\text{N}$ nucleus (blue, denoted N1) and a relatively weakly coupled $^{14/15}\text{N}$ nucleus (red, denoted N2). Specific simulation parameters are detailed in Table 1 of the main text. Acquisition parameters: temperature = 20 K; microwave frequency = 33.986 GHz; MW pulse lengths $\pi/2$, $\pi = 12$, 24 ns; $\tau = 100$ ns; $T_1 = T_2 = 100$ ns; $\Delta T_1 = \Delta T_2 = 16$ ns; shot repetition time (srt) = 1 ms.
Figure S9. Q-band HYSCORE spectra and simulations of $^{14}$N/$^{15}$N hyperfine couplings of [(µ-Cys)$_2$Fe$_2$(NO)$_4$]$^-$ generated with $^{14}$NO (top row) and $^{15}$NO (bottom row). For each spectrum, the top panels show the experimental spectra, with intensities indicated by the color map ranging from blue to red in order of increasing intensity. The bottom panels reproduce the experimental data in grey and overlay simulations from a relatively strongly coupled $^{14/15}$N nucleus (blue, denoted N1) and a relatively weakly coupled $^{14/15}$N nucleus (red, denoted N2). Specific simulation parameters are detailed in Table 1 of the main text. Acquisition parameters: temperature = 20 K; microwave frequency = 34.000 GHz; MW pulse lengths $\pi/2$, $\pi$ = 12, 24 ns; $\tau$ = 100 ns; $T_1$ = $T_2$ = 100 ns; $\Delta T_1$ = $\Delta T_2$ = 16 ns; shot repetition time (srt) = 1 ms.
Trypsin-Digested Native and Nitrosylated EndoIII

Figure S10. (A) Logarithm intensity of peptides either non-modified or modified by tyrosine nitration for native (black) and nitrosylated (red) EndoIII. (B) Treated/control ratio demonstrating tyrosine nitration was not observed specific to NO exposure.
Representative Gels from Electrophoretic Mobility Shift Assays

Figure S11. Electrophoretic mobility shift assay gels of (a) native and (b) nitrosylated EndoIII.
Wide-Potential Sweep on DNA-Modified Gold Electrodes

Figure S12. Cyclic voltammograms of buffer (20 mM phosphates, 150 mM NaCl, 0.5 mM EDTA, 10% v/v glycerol, pH 7.5) on DNA-modified gold electrodes using a standard (black) or wide (dotted) potential sweep with a scan rate of 100 mV/s.