Electronic Supplementary Information for

Cluster-Selective ^{57}Fe Labeling of a Twitch-Domain-Containing Radical SAM Enzyme

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Table of Contents:

A. Experimental Methods ……………………………… S2

B. Experimental Procedures ……………………………… S3

C. Calculating the Label Incorporation ……………………………… S5

D. Simultaneous Fitting of the Mössbauer Spectra ……………………………… S7

E. Supplementary Figures ……………………………… S10

F. Supplementary References ……………………………… S15

A. Experimental Methods

UV/vis spectroscopy

UV/vis spectra were collected at room temperature with a Cary 50 UV/vis spectrophotometer using a 1 cm path length quartz cuvette. For unlabeled BtrN and BtrN(^{57}Fe$_8$) samples, the reconstituted, oxidized protein was diluted appropriately prior to acquiring the spectrum. For BtrN-RS(^{57}Fe$_4$) and BtrN-Aux(^{57}Fe$_4$) samples, a 20 µL aliquot was taken from the final sample, oxidized with 20 µL of 20 mM indigodisulfonic acid (IDS), and gel-filtered through a P-30 spin column (Bio-Rad) equilibrated with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl. The resulting flowthrough was diluted appropriately prior to acquiring the spectrum.

EPR spectroscopy

Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker EMX spectrometer at 9.37 GHz as frozen solutions. Samples were transferred to a quartz tube (made from clear fused quartz tubing of 3 mm I.D. & 4 mm O.D. from Wale Apparatus) in an anaerobic glovebox (<5 ppm O_2), capped with a rubber septum, and frozen in liquid N_2 outside the glovebox. Each sample contained 100 µL of approximately 0.1 mM BtrN with 5 mM sodium dithionite (DTH).

B. Experimental Procedures

Construction of *E. coli* strain BL21(DE3)-pDB1282-pBtrNWt

The p1282 plasmid (encoding the ISC system from *Azotobacter vinelandii*; originally generated by Prof. Dennis R. Dean's laboratory (Virginia Tech.)) and the pET-26b vector encoding *C*-terminal His$_6$-tagged BtrN (pBtrNWt) were gifts from Prof. Squire J. Booker's laboratory (Penn State University). *E. coli* strain BL21(DE3) was transformed with pDB1282 using a standard protocol. Then, competent cells were prepared from the strain BL21(DE3)-pDB1282, and the resulting cell was transformed with pBtrNWt. A 200 mL LB media containing 100 µg/mL ampicillin and 50 µg/mL kanamycin was inoculated with a single colony and shaken at 37 °C, 180 rpm for 18 h. Glycerol stocks of the *E. coli* strain BL21(DE3)-pDB1282-pBtrNWt were prepared by mixing 0.5 mL of the cultured media with 0.5 mL of 50% glycerol in cryotubes. The glycerol stocks were frozen in liquid nitrogen and stored at −80 °C until further use.

Overproduction, purification, and chemical reconstitution of BtrN

All procedures were adapted from reported protocols[1,2] with minor modifications.

Overproduction. A glycerol stock of *E. coli* strain BL21(DE3)-pDB1282-pBtrNWt was streaked on an LB-agar plate containing 100 µg/mL ampicillin and 50 µg/mL kanamycin. A 200 mL LB starter culture containing 100 µg/mL ampicillin and 50 µg/mL kanamycin was inoculated with a single colony and shaken at 37 °C, 180 rpm for 18 h. 4 × 1 L M9 minimal media containing 100 µg/mL ampicillin and 50 µg/mL kanamycin were inoculated with 10 mL starter culture, supplemented with $FeCl_3$ (or $^{57}FeCl_3$) to a final concentration of 25 µM, and shaken at 37 °C, 180 rpm. At OD_{600} = 0.3, the cultures were supplemented with L-cysteine and $FeCl_3$ (or $^{57}FeCl_3$) to final concentrations of 300 µM and 25 µM, respectively, before the overexpression of *isc* operon (encoded in pDB1282 plasmid) was induced by adding L-arabinose to a final concentration of 0.2% w/v. At OD_{600} = 0.6, the cultures were cooled in an ice bath to 18 °C before the overexpression of *btrN* was induced by adding IPTG to a final concentration of 100 µM. The cultures were shaken at 18 °C, 180 rpm for 18 h before the cells were harvested by centrifugation at 8,000 × *g*, 4 °C for 10 min. The cell paste was frozen in liquid N_2 and stored at −80 °C until further use.

Purification. All purification procedures were carried out in a Coy Labs glovebox (<5 ppm O_2). All aqueous solutions used were sparged with N_2 overnight. Cells were lysed using the osmotic shock method. BL21(DE3)-pDB1282-pBtrNWt cell paste was resuspended in 50 mM HEPES, pH 7.5, 40% glycerol, 2 mM sodium dithionite (DTH) and stirred at room temperature for 15 min before the cells were pelleted by centrifugation at 40,000 × *g*, 4 °C for 30 min. The supernatant was discarded, the cell pellet was resuspended in 50 mM HEPES, pH 7.5, 2 mM 2 mM DTH, 1 mg/mL lysozyme, 0.1 mg/mL DNase I, 0.1 mg/mL phenylmethylsulfonyl fluoride (PMSF) and stirred at room temperature for 15 min before the lysate was pelleted by centrifugation at 40,000 × *g*, 4 °C for 1 h. The cleared lysate was loaded onto a Co-NTA column (G-Bioscience), equilibrated with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl, 2 mM imidazole, 2 mM DTH. The resulting column was washed with 5 column volumes of the equilibration buffer before the protein was eluted with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl, 300 mM imidazole, 2 mM DTH. The brown eluate was collected and concentrated to 2 mL using an AMICON stirred cell equipped with a 10 kDa filter. The concentrated protein and the excess DTH were oxidized with 0.5 mL of 20 mM IDS, and the IDS was removed by centrifugation at 14,000 × *g* for 3 min and gel-filtration through a PD-10 column (GE Healthcare), equilibrated with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl. The purity of BtrN was determined to be >95% based on SDS-PAGE. The concentration of BtrN was estimated by the Bradford assay[3] using a correction factor of 1.[1] The Fe content of BtrN was determined by ferene assay.[4] The protein was aliquoted, frozen in liquid N_2, and stored in liquid N_2 until further use.

Chemical reconstitution. All reconstitution procedures were carried out in a Coy Labs glovebox (<5 ppm O_2). To a solution of BtrN (100 μM) in 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl in an ice bath was added a solution of dithiothreitol (DTT; 100 mM) in the same buffer to a final concentration of 10 mM. The resulting mixture was stirred for 30 min before 10 equiv $FeCl_3$ (or $^{57}FeCl_3$) was added dropwise as a solution (50 mM) in the buffer in 5 equal portions with 5 min intervals, and subsequently, 10 equiv Na_2S was added dropwise as a solution (50 mM) in the buffer in 5 equal portions with 30 min intervals. The resulting mixture was incubated on ice overnight without stirring before the precipitates were removed by centrifugation at 14,000 × *g* for 5 min. The supernatant was concentrated <5 mL using an AMICON stirred cell equipped with a 10 kDa filter and loaded onto a Superdex-200 column (Amersham Pharmacia Biotech), equilibrated with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl, 2 mM DTH, upon which a black aggregate band was removed and a brown protein band was collected. The brown eluate was concentrated to 2 mL using an AMICON stirred cell equipped with a 10 kDa filter. The concentrated protein and the excess DTH were oxidized with 0.5 mL of 20 mM IDS, and the IDS was removed by centrifugation at 14,000 × *g* for 3 min and gel-filtration through PD-10, equilibrated with 100 mM HEPES, pH 7.5, 10% glycerol, 500 mM NaCl. The purity, concentration, and Fe content of the reconstituted BtrN was determined as described above.

C. Calculating the Label Incorporation

Label incorporation (I) of a sample obtained from an isotope exchange reaction is defined as the proportion of Fe ions in the protein substituted by exogenously added Fe. This value informs on the extent of the Fe exchange reaction and is required for Mössbauer spectroscopic analysis of the samples generated in this study (see SI Section D).

The analysis was adapted from previous works.[5,6] For all calculations, we take the natural isotopic abundance of Fe to have 91.7% ^{56}Fe and 2.12% ^{57}Fe, and the isotopic abundance of purchased ^{57}Fe to have 95.5% ^{57}Fe and 3.6% ^{56}Fe. Also, we assume that BtrN contains exactly 8 Fe per polypeptide (*i.e.*, two [Fe_4S_4] clusters).

As a generalized form, let I be the label incorporation ($0 \leq I \leq 1$) over n sites of Fe ions among total N Fe sites in the protein ($n \leq N$), and $\chi_0(S, i)$ be the initial mole fraction of isotope i in species S (below, P and M denote protein and exogenous mononuclear Fe, respectively). After the exchange process, the unexchanged sites ($(N-n)$ sites) and $(1-I) \times 100\%$ of the exchanged sites (n sites) remain in the initial isotopic abundance of the protein, while $I \times 100\%$ of the exchanged sites (n sites) is substituted by exogenous Fe (and thus, is in the initial isotopic abundance of the exogenous Fe). Then, the ratio of ^{56}Fe:^{57}Fe, denoted r, in the final protein sample would be:

$$r = \frac{\chi_0(P, {}^{56}Fe) \cdot (N-n) + \chi_0(P, {}^{56}Fe) \cdot (1-I) \cdot n + \chi_0(M, {}^{56}Fe) \cdot I \cdot n}{\chi_0(P, {}^{57}Fe) \cdot (N-n) + \chi_0(P, {}^{57}Fe) \cdot (1-I) \cdot n + \chi_0(M, {}^{57}Fe) \cdot I \cdot n} \qquad \text{(Eq. S1)}$$

This can be rearranged to:

$$I = \frac{\chi_0(P, {}^{56}Fe) - r \cdot \chi_0(P, {}^{57}Fe)}{\left(\chi_0(P, {}^{56}Fe) - \chi_0(M, {}^{56}Fe)\right) - r \cdot \left(\chi_0(P, {}^{57}Fe) - \chi_0(M, {}^{57}Fe)\right)} \cdot \frac{N}{n} \qquad \text{(Eq. S2)}$$

For an Fe exchange reaction between unlabeled BtrN and $^{57}FeCl_2$ with no assumption on cluster-selectivity, we use the following values

$$\chi_0(P, {}^{56}Fe) = 0.917$$

$$\chi_0(P, {}^{57}Fe) = 0.0212$$

$$\chi_0(M, {}^{56}Fe) = 0.036$$

$$\chi_0(M, {}^{57}Fe) = 0.955$$

$$N = n = 8$$

in Eq. S2 to obtain:

$$I = \frac{0.917 - 0.0212r}{0.881 + 0.9338r} \qquad \text{(Eq. S3)}$$

If we cluster-selective exchange is assumed, then,

$$N = 8;\ n = 4$$

and thus:

$$I_{cluster-selective} = \frac{0.917 - 0.0212r}{0.881 + 0.9338r} \cdot 2 \qquad \text{(Eq. S4)}$$

For an Fe exchange reaction between fully labeled BtrN (BtrN($^{57}Fe_8$); its isotopic abundance was determined by ICP-MS and ferene assay; see Supplementary Table 1) and natural abundance $FeCl_2$ with no assumption on cluster-selectivity, we use the following values

$$\chi_0(P, {}^{56}Fe) = 0.059$$

$$\chi_0(P, {}^{57}Fe) = 0.852$$

$$\chi_0(M, {}^{56}Fe) = 0.917$$

$$\chi_0(M, {}^{57}Fe) = 0.0212$$

$$N = n = 8$$

in Eq. S2 to obtain:

$$I = \frac{0.852r - 0.059}{0.8308r + 0.658} \quad \text{(Eq. S5)}$$

If we cluster-selective exchange is assumed, then,

$$N = 8;\ n = 4$$

and thus:

$$I_{cluster-selective} = \frac{0.852r - 0.059}{0.8308r + 0.658} \cdot 2 \quad \text{(Eq. S6)}$$

Note that r is determined by ICP-MS analysis (Supplementary Table 1).

Supplementary Table 1. Fe analysis of various BtrN samples.

Samples	[protein] (mM)[a]	[total Fe] (mM)[b]	Fe per polypeptide[c]	^{56}Fe:^{57}Fe ratio[d]
BtrN	0.25 ± 0.01	1.9 ± 0.1	7.5 ± 0.3	-[e]
BtrN-RS(^{57}Fe$_4$)	0.22 ± 0.01	1.6 ± 0.1	7.1 ± 0.3	1.42 : 1[f]
BtrN(^{57}Fe$_8$)	0.41 ± 0.01	3.3 ± 0.1	7.9 ± 0.3	1 : 14.5[g]
BtrN-Aux(^{57}Fe$_4$)	0.36 ± 0.01	2.6 ± 0.2	7.3 ± 0.1	1 : 1.50[h]

[a] Determined by Bradford assay. Mean ± S.D. from triplicate measurements.
[b] Determined by ferene assay. Mean ± S.D. from triplicate measurements.
[c] [total Fe]/[protein] ± S.D.
[d] Determined by ICP-MS.
[e] Natural isotopic abundance.
[f] Individual isotope concentrations determine by ICP-MS: [^{56}Fe] = 0.78 mM, [^{57}Fe] = 0.55 mM
[g] Individual isotope concentrations determine by ICP-MS: [^{56}Fe] = 0.19 mM, [^{57}Fe] = 2.8 mM
[h] Individual isotope concentrations determine by ICP-MS: [^{56}Fe] = 0.90 mM, [^{57}Fe] = 1.4 mM

D. Simultaneous Fitting of the Mössbauer Spectra

General considerations

The simultaneous fitting of BtrN(^{57}Fe$_8$), BtrN-RS(^{57}Fe$_4$), and BtrN-Aux(^{57}Fe$_4$) was performed using the linear least-square fitting function (lsqcurvefit) in MATLAB, adapting the procedure described in previous works.[5,6]

All three spectra were simulated as a combination of an [Fe$_4$S$_4$]$^+$ cluster and an [Fe$_4$S$_4$]$^{2+}$ cluster, each having 1:1:2 and 1:3 pattern[1] for their subcomponents, respectively. Each subcomponent was modeled as a symmetric quadrupole doublet with Lorentzian lineshape using the following function:

$$L(v;\ \delta, |\Delta E_{\mathrm{Q}}|, \Gamma) = -\frac{\Gamma}{2} \times \left(\frac{1}{\left(v-\left(\delta-\frac{|\Delta E_{\mathrm{Q}}|}{2}\right)\right)^2+\left(\frac{\Gamma}{2}\right)^2} + \frac{1}{\left(v-\left(\delta+\frac{|\Delta E_{\mathrm{Q}}|}{2}\right)\right)^2+\left(\frac{\Gamma}{2}\right)^2} \right) \qquad \text{(Eq. S7)}$$

where v is the Mössbauer source velocity relative to the centroid of the spectrum of a metallic foil of α-Fe at room temperature (the *x*-axis), δ is the isomer shift, $|\Delta E_{\mathrm{Q}}|$ is the quadrupole splitting, and Γ is the linewidth.

Defining the parameters and functions

For the BtrN(^{57}Fe$_8$) spectrum (F_1), because all the Fe sites are uniformly enriched, the contributions from the RS and Aux clusters should be equal. Including a small contribution from adventitious, mononuclear Fe^{2+} (Supplementary Fig. 3), the overall spectrum can be described as the following function:

$$F_1 = A_1 \times \left[\left(0.25L_{\mathrm{RS\_1}} + 0.25L_{\mathrm{RS\_2}} + 0.5L_{\mathrm{RS\_3}}\right) + \left(0.25L_{\mathrm{Aux\_1}} + 0.75L_{\mathrm{Aux\_2}}\right)\right] + A^1_{mono} \times L^1_{mono} + B_1 \qquad \text{(Eq. S8)}$$

where A_1 is the area factor that is proportional to the sample concentration, $L_{\mathrm{RS\_1}}$, $L_{\mathrm{RS\_2}}$, $L_{\mathrm{RS\_3}}$ are the three subcomponents of the RS cluster, $L_{\mathrm{Aux\_1}}$, $L_{\mathrm{Aux\_2}}$ are the two subcomponents of the Aux cluster, A^1_{mono} and L^1_{mono} are the area factor and the quadrupole doublet, respectively, for the adventitious Fe, and B_1 is the baseline-correction factor.

For the BtrN-RS(^{57}Fe$_4$) (F_2) and the BtrN-Aux(^{57}Fe$_4$) (F_3) spectra, the contributions of each cluster are determined by their ^{57}Fe enrichment, which depends on the label incorporation (I) and the cluster-selectivity of the exchange (S).

In the BtrN-RS(^{57}Fe$_4$) sample, $I_2 \times 100\%$ of all the Fe ions in the protein (8 sites) of 2.12% ^{57}Fe enrichment are being substituted with exogenous Fe of 95.5% ^{57}Fe enrichment, where $S_2 \times 100\%$ of those Fe being exchanged are from the RS cluster and $(1 - S_2) \times 100\%$ are from the Aux cluster. Therefore, the contributions of each cluster can be described as the following:

$$C^2_{\mathrm{RS}} = 0.0212 \times 4 - 0.0212 \times 8 \times I_2 \times S_2 + 0.955 \times 8 \times I_2 \times S_2 \qquad \text{(Eq. S9)}$$

$$C^2_{\mathrm{Aux}} = 0.0212 \times 4 - 0.0212 \times 8 \times I_2 \times (1 - S_2) + 0.955 \times 8 \times I_2 \times (1 - S_2) \qquad \text{(Eq. S10)}$$

Including a small contribution from adventitious, mononuclear Fe^{2+} (Supplementary Fig. 4), the overall spectrum can then be described as the following function:

$$F_2 = A_2 \times \left[C^2_{\mathrm{RS}} \times \left(0.25L_{\mathrm{RS_1}} + 0.25L_{\mathrm{RS_2}} + 0.5L_{\mathrm{RS_3}}\right) + C^2_{\mathrm{Aux}} \times \left(0.25L_{\mathrm{Aux_1}} + 0.75L_{\mathrm{Aux_2}}\right)\right] + A^2_{mono} \times L^2_{mono} + B_2$$

(Eq. S11)

where A_2 is the overall area factor proportional to the protein concentration, A^2_{mono} and L^2_{mono} are the area factor and the quadrupole doublet, respectively, for the adventitious Fe, and B_1 is the baseline-correction factor.

In the BtrN-Aux(^{57}Fe$_4$) sample, $I_3 \times 100\%$ of all the Fe ions in the protein (8 sites) of 85.2% ^{57}Fe enrichment are being substituted with exogenous Fe of 2.12% ^{57}Fe enrichment, where $S_3 \times 100\%$ of those Fe being exchanged are from the RS cluster and $(1 - S_3) \times 100\%$ are from the Aux cluster. Therefore, the contribution of each cluster can be described as the following:

$$C^3_{\mathrm{RS}} = 0.852 \times 4 - 0.852 \times 8 \times I_3 \times S_3 + 0.0212 \times 8 \times I_3 \times S_3 \qquad \text{(Eq. S12)}$$

$$C^3_{\mathrm{Aux}} = 0.852 \times 4 - 0.852 \times 8 \times I_3 \times (1 - S_3) + 0.0212 \times 8 \times I_3 \times (1 - S_3) \qquad \text{(Eq. S13)}$$

The overall spectrum can then be described as the following function:

$$F_3 = A_3 \times \left[C^3_{\mathrm{RS}} \times \left(0.25L_{\mathrm{RS\_1}} + 0.25L_{\mathrm{RS\_2}} + 0.5L_{\mathrm{RS\_3}}\right) + C^3_{\mathrm{Aux}} \times \left(0.25L_{\mathrm{Aux\_1}} + 0.75L_{\mathrm{Aux\_2}}\right)\right] + B_3$$

(Eq. S14)

where A_3 is the overall area factor and B_3 is the baseline-correction factor. Note that the contribution from adventitious Fe^{2+} is very small in this spectrum (Supplementary Fig. 5) and is thus not included as a component for the simulation here.

Boundary conditions

The Mössbauer parameters of individual quadrupole doublets had the following boundary conditions (*l.b.* = lower boundary; *u.b.* = upper boundary) to limit the simulation space to chemically reasonable values based on the literature:[1,7]

Supplementary Table 2. Boundary conditions of the Mössbauer parameters used in the simultaneous fitting of BtrN(^{57}Fe$_8$), BtrN-RS(^{57}Fe$_4$), and BtrN-Aux(^{57}Fe$_4$) spectra.

Cluster	Component	δ		$\lvert\Delta E_{\mathrm{Q}}\rvert$		Γ	
		l.b.	*u.b.*	*l.b.*	*u.b.*	*l.b.*	*u.b.*
RS	Fe^{2+} (1)	0.5	0.65	0.9	5	0.3	0.5
	Fe^{2+} (1)	0.5	0.65	0.9	5	0.3	0.5
	Fe$^{2.5+}$ (2)	0.4	0.5	0	5	0.3	0.5
Aux	Fe$^{2.5+}$ (1)	0.4	0.45	0	5	0.3	0.5
	Fe$^{2.5+}$ (3)	0.4	0.45	0	5	0.3	0.5
Adventitious Fe^{2+}		1.2	1.5	2.9	5	0.3	0.5

As described above, the label incorporation can be calculated from the ICP-MS data (Supplementary Table 1). The values for BtrN-RS(^{57}Fe$_4$) and BtrN-Aux(^{57}Fe$_4$) are determined and fixed during the simulation of the Mössbauer spectra to be the following:

$$I_2 = 0.40 \text{ (from Eq. S3)}$$

$$I_3 = 0.42 \text{ (from Eq. S5)}$$

The cluster-selectivity, S, is defined such that the sum of the selectivity for the RS cluster and the selectivity for the Aux cluster equals 1; *i.e.*, $S = 0.5$ corresponds to no cluster-selectivity, $S = 1$ corresponds to completely selective exchange at the RS cluster, and $S = 0$ corresponds to completely selective exchange at the Aux cluster. Given that we qualitatively observe preferential

Fe exchange at the RS cluster, we set the boundary condition for S as the following:

$$0.5 \leq S \leq 1$$

Results from the simultaneous fitting

The converged parameters are shown in the main text and Supplementary Table 3. We also obtained $S_2 = 0.99$ and $S_3 = 0.97$, suggesting that Fe exchange occurs essentially exclusively at the RS cluster.

Supplementary Table 3. Converged parameters from the simultaneous fitting of BtrN($^{57}Fe_8$), BtrN-RS($^{57}Fe_4$), and BtrN-Aux($^{57}Fe_4$) spectra.[a]

Components[b]		δ (mm s^{-1})	$\lvert\Delta E_Q\rvert$ (mm s^{-1})	Γ (mm s^{-1})
RS $[Fe_4S_4]^+$	Fe^{2+} (1)	0.61	1.68	0.35
	Fe^{2+} (1)	0.63	1.02	0.30[e]
	$Fe^{2.5+}$ (2)	0.45	1.00	0.31
Aux $[Fe_4S_4]^{2+}$	$Fe^{2.5+}$ (1)	0.45	0.79	0.30[e]
	$Fe^{2.5+}$ (3)	0.43	1.22	0.30[e]
Adventitious Fe^{2+} in BtrN($^{57}Fe_8$)[c]		1.25	2.99	0.32
Adventitious Fe^{2+} in BtrN-RS($^{57}Fe_4$)[d]		1.23	3.01	0.30

[a] Cluster-selectivity values converged to S_2=0.99 and S_3=97 for BtrN-RS($^{57}Fe_4$) and BtrN-Aux($^{57}Fe_4$), respectively

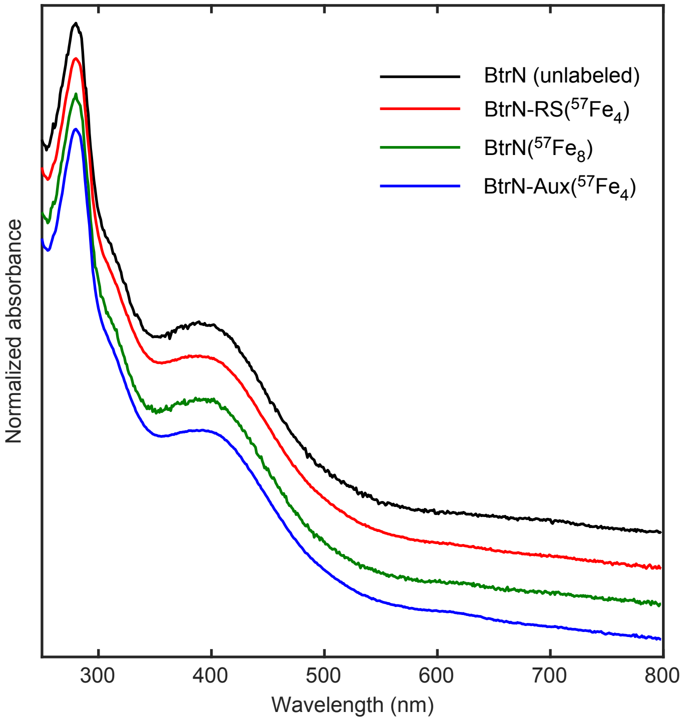
[b] Area factors for the clusters converged to A_1=0.88, A_2=0.12, A_3=0.20 for BtrN($^{57}Fe_8$), BtrN-RS($^{57}Fe_4$), BtrN-Aux($^{57}Fe_4$), respectively.

[c] The area factor converged to A^1_{mono}=0.10.

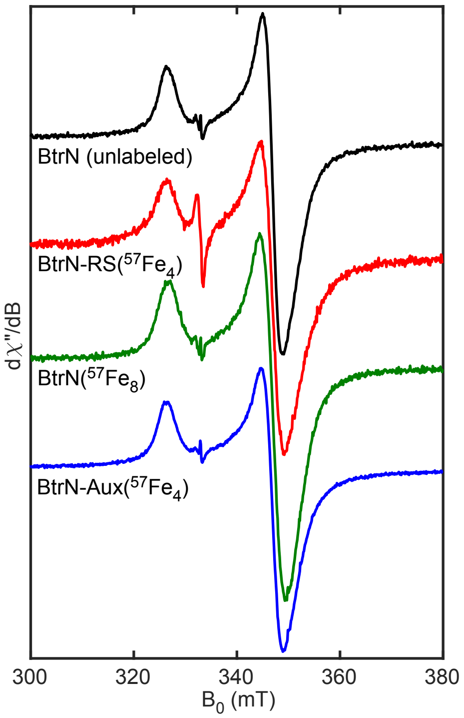
[d] The area factor converged to A^2_{mono}=0.026.

[e] Converged to the lower boundary.

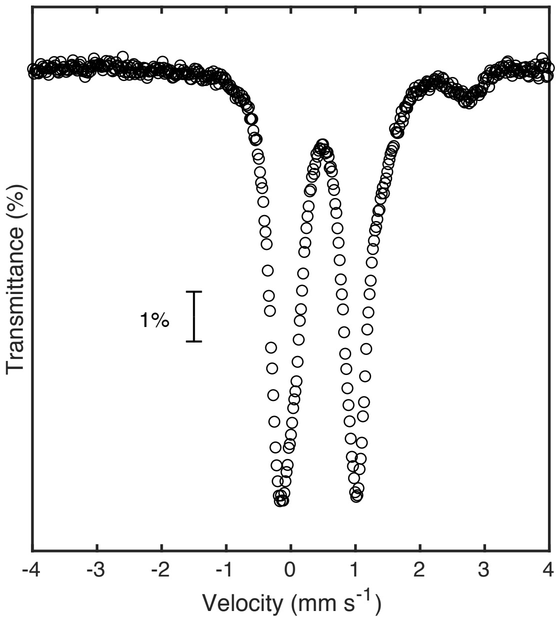
E. Supplementary Figures



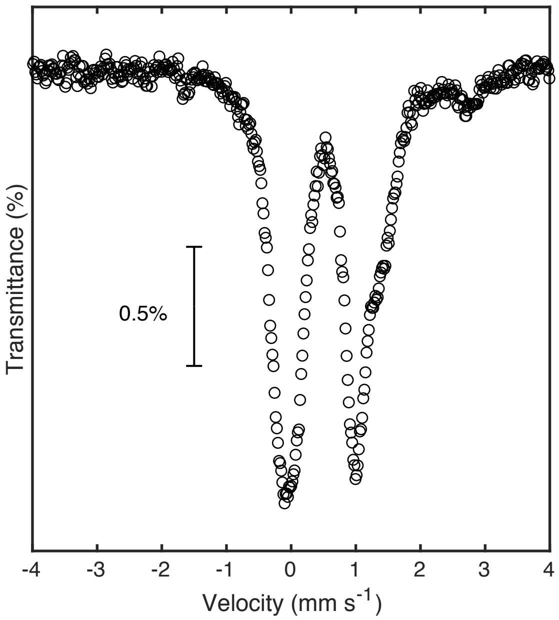
Supplementary Figure 1. UV/vis spectra of various BtrN samples with offsets. Black: unlabeled BtrN. Red: BtrN-RS($^{57}Fe_4$). Green: BtrN($^{57}Fe_8$). Blue: BtrN-Aux($^{57}Fe_4$).



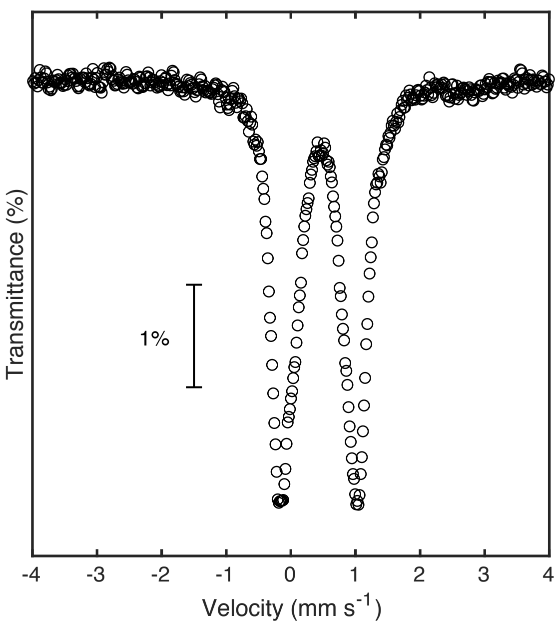
Supplementary Figure 2. 9.37 GHz EPR spectra of DTH-reduced BtrN in various ^{57}Fe-enrichments. Black: unlabeled BtrN. Red: BtrN-RS($^{57}Fe_4$). Green: BtrN($^{57}Fe_8$). Blue: BtrN-Aux($^{57}Fe_4$). All spectra were recorded at 15 K with microwave power = 0.25 mW, and normalized to the protein concentrations determined by Bradford assay.



Supplementary Figure 3. Zero-field, 80 K Mössbauer spectrum of BtrN($^{57}Fe_8$).



Supplementary Figure 4. Zero-field, 80 K Mössbauer spectrum of BtrN-RS($^{57}Fe_4$).



Supplementary Figure 5. Zero-field, 80 K Mössbauer spectrum of BtrN-Aux($^{57}Fe_4$).

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