SUPPORTING INFORMATION FOR

A Substrate-triggered μ-Peroxodiiron(III) Intermediate in the 4-Chloro-L-Lysine-Fragmenting Heme-Oxygenase-like Diiron Oxidase (HDO) BesC: Substrate Dissociation from, and C4 Targeting by, the Intermediate

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SUPPLEMENTARY PROCEDURES

Reactions of the Fe(II)•BesC substrate complex with O_2 (Figure 2). Reactant solutions contained 0.3 mM BesC, 0.6 mM (2 molar equiv) Fe(II), and 1 mM of substrate/analog in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, and 5% glycerol (*v/v*). In control samples that lacked substrate, the compound was omitted from the initial reaction mixture. The anoxic protein solution was mixed at 5 °C with an equal volume of the same buffer at equilibrium (at 5 °C) with ~ 1.05 atm O_2 (~1.8 mM). Absorbance was monitored with the photodiode array detector.

Freeze-Quench Mössbauer (FQ-Möss) Spectroscopy (Figure 3). Freeze-quench Mössbauer samples were prepared according to previously published procedures.[1] The BesC•$Fe(II)_2$•L-Lys reactant complex was assembled from anoxic solutions of 1.7 mM BesC, 3.4 mM $^{57}Fe(II)$, and 5.1 mM L-Lys. An O_2-stable, acidic stock solution of $^{57}Fe(II)$ was prepared from commercial $^{57}Fe^0$, as previously described.[2] In the anoxic chamber, this solution was diluted to 50 mM Fe(II) by mixing with 1 M sodium HEPES, pH 7.5. An appropriate volume of this dilute stock solution was added to an anoxic solution of apo BesC in 100 mM sodium HEPES buffer (pH 7.5), 100 mM NaCl, 5% (*v*/*v*) glycerol to yield the reactant solution described above. This sample was mixed at 5 °C with an equal volume of the same buffer that had been saturated with O_2 (~ 1.8 mM). This solution was allowed to incubate for the varying reaction times indicated in Fig. 2 and subsequently frozen by injection into cold (-150 °C) 2-methylbutane (for reaction times from milliseconds to tens of seconds) or by pipetting into a Mössbauer cell cooled on a metal block that was in contact with liquid N_2 (for reaction times of minutes to hours).

BesC Single-Turnover Assays with 4,4,5,5-[$^2H_4$]-L-lysine or [$^{13}C_6$,$^{15}N_2$]-L-lysine (Figure 4B). Reactions were carried out in the MBraun anoxic chamber in sealed LC-MS vials with pierceable septa. A 25 µL aliquot of a solution containing BesC (0.60 or 1.8 mM) in 100 mM sodium HEPES, pH 7.5, 100 mM NaCl, 5% glycerol, $(NH_4)_2FeSO_4$ (1.2 or 3.6 mM, 2 molar equiv), and substrate (20 mM) was mixed with an equal volume O_2-saturated buffer. After 2 h at 5 °C, reactions were quenched with 1.5 volumes (75 µL) of methanol and 1% formic acid containing 83

μM L-allylglycine. Control reactions omitted one of the reaction components. Samples were centrifuged at 10,000*g* for 10 min to remove precipitated protein. The supernatant was filtered by centrifugation in a 10 kDa Nanosep filter (PALL Corporation) at 10,000*g* for 10 min. Samples were analyzed by LC-MS as described in the main text.

BesC Single-Turnover Assays with 4-thia-L-Lys (Figure 5). Reactions were carried out in a MBraun anoxic chamber in sealed LC-MS vials with pierceable septa. A 25 μL aliquot of a solution containing BesC (0.50 mM) in 100 mM sodium HEPES, pH 7.5, 100 mM NaCl, 5% glycerol, $(NH_4)_2FeSO_4$ (1 mM or 2 molar equiv), and 4-thia-L-Lys (0.50 mM, 1 molar equiv) was mixed with equal volume of O_2-saturated buffer containing either natural abundance O_2 or $^{18}O_2$. After 30 min, reactions were quenched with 1.5 volumes (75 μL) of methanol and 1% formic acid containing 50 μM L-norleucine. Control reactions omitted one of the reaction components. Samples were centrifuged at 10,000*g* for 10 min to remove precipitated protein. The supernatant was filtered by centrifugation in a 10 kDa Nanosep filter (PALL Corporation) at 10,000*g* for 10 min. Samples were analyzed by LC-MS, as described in the main text.

Titrations of the Fe(II)•BesC substrate complex. Reactant solutions contained 0.2 mM BesC; 0.2, 0.4 or 1.2 mM (1, 2 or 6 molar equiv) Fe(II); and 0, 0.1, 0.2, 0.6, 1.8, 5.8 12, 18, 54, or 180 mM substrate/analog. These samples were prepared in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, and 5% glycerol (*v/v*). The anoxic protein solution was mixed at 5 °C with an equal volume of the same buffer at equilibrium (at 5 °C) with ~ 1.05 atm O_2 (~1.8 mM). Absorbance was monitored with the photodiode array detector.

Titrations of the BesC•L-lysine complex with Fe(II). Reactant solutions contained 0.2 mM BesC; 0.2 mM, 5.4 mM, or 12 mM (1, 27, or 60 molar equiv) L-Lys or 4-thia-L-Lys; and 0.2, 0.4, 0.6, 0.8, or 1.2 mM Fe(II). These samples were prepared in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, and 5% glycerol (*v/v*). The anoxic protein solution was mixed at 5 °C with an equal volume of the same buffer at equilibrium (at 5 °C) with ~ 1.05 atm O_2 (~1.8 mM). Absorbance was monitored with the photodiode array detector

Reaction of the Fe(II)•BesC complex with ferrozine. The SF lines were soaked overnight in a solution of 5 mM sodium dithionite prepared anoxically in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, 5% glycerol (*v/v*). For the experiment, a solution containing 160 μM BesC was incubated with 50 μM sodium dithionite in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, 5% glycerol (*v/v*) to scrub out residual O_2 from the samples. To this solution, Fe(II) was added to 0.13 or 0.32 mM (0.8 or 2 molar equiv). In trials containing excess substrate/analog, 12 mM of either L-Lys or 4-thia-L-Lys was included in the protein solution. This solution was then mixed with an equal volume of an anoxic solution containing 4 mM ferrozine and 50 μM sodium dithionite in the same buffer. In samples containing half the amount of protein used, 80 uM BesC was incubated with 1 mM sodium dithionite in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, 5% glycerol (*v/v*). To this solution Fe(II) was added to a concentration of 40, 80, 120, 160, or 480 uM (0.50, 1.0, 1.5, 2.0 or 6.0 molar equiv). In trials containing excess substrate/analog, 12 mM of either L-Lys or 4-thia-L-Lys was added to the protein mixture. This solution was mixed with an equal volume of an anoxic solution containing 4 mM ferrozine and 1 mM sodium dithionite in the same buffer. In both cases, absorbance at 562 nm (A_{562}) was monitored at 5 °C with the photomultiplier tube using a 0.2 cm pathlength. When appropriate (in experiments with equal Fe(II) concentrations), a very slow loss of ~ 5-10% of the absorption amplitude over the course of all-day experiments was corrected for by normalization of traces to the same final amplitude.

Reaction of the peroxodiiron(III) complex triggered by L-lysine with more reactive substrate analogs. In a sequential mixing configuration, a reactant solution containing 0.4 mM BesC, 0.8 mM (2 molar equiv) Fe(II), and 2.7 mM L-Lys in 100 mM sodium HEPES (pH 7.5), 100 mM NaCl, and 5% glycerol (*v/v*). This anoxic protein solution was mixed at 5 °C with an equal volume of the same buffer at equilibrium (at 5 °C) with ~ 1.05 atm O_2 (~1.8 mM). After being allowed to react to accumulate the peroxodiiron(III) intermediate, this reaction solution was then mixed with an equal volume of either 10 mM L-Lys (control) or 0.1, 1.35, of 6.75 mM of either 4-thia-L-Lys or 4-Cl -Lys. Absorbance was monitored with the photodiode array detector.

Over-expression of BesC for X-ray crystallography. E. coli BL21 Star (DE3) was transformed with a plasmid encoding N-terminally- His_{10}-tagged BesC with a Prescission protease-cleavable linker. An overnight TB culture of freshly transformed cells was used to inoculate TB (1 L) containing the appropriate antibiotics (50 µg/ml carbenicillin) in a 2.8 L baffled shake flask to OD_{600}=0.05. The cultures were grown at 37 °C at 200 rpm to OD_{600} = 0.6 to 0.8 at which point cultures were cooled on ice for 20 min. Protein expression was induced with IPTG (0.2 mM) and cells were grown overnight at 16 °C. Cell pellets were harvested by centrifugation at 9,800 *g* for 7 min at 4 °C and stored at -80 °C.

Purification of BesC for X-ray crystallography. Frozen cell pellets were thawed and resuspended at 5 mL/g of cell paste in lysis buffer (50 mM HEPES, 300 mM NaCl, 20 mM βME, 10% (v/v) glycerol, pH 7.5) supplemented with protease inhibitor cocktail (Roche). The cell paste was homogenized and then lysed by passage through a French Pressure cell (Thermo Scientific; Waltham, MA) at 9,000 psi. The lysate was then centrifuged at 13,500 *g* for 20 min at 4 °C to separate the soluble and insoluble fractions. DNA was precipitated with 0.15% (w/v) polyethyleneimine and stirring at 4 °C for 30 min. The precipitated DNA was then removed by centrifugation at 13,500 *g* for 20 min at 4 °C. The soluble lysate was incubated with Ni-NTA (0.5 mL resin/g of cell paste) for 45 min at 4 °C, then resuspended and loaded onto a column by gravity flow. The column was washed with wash buffer (50 mM HEPES, 300 mM NaCl, 20 mM imidazole, 20 mM βME, 10% (v/v) glycerol, pH 7.5) for 15-20 column volumes. The column was then eluted with elution buffer (50 mM HEPES, 300 mM NaCl, 300 mM imidazole, 20 mM βME, 10% (v/v) glycerol, pH 7.5). The protein was dialyzed following elution from Ni-NTA column 3 x 1:50 against dialysis buffer (50 mM HEPES, 100 mM NaCl, 1 mM DTT, pH 7.5) for 1.5 h to remove imidazole. After the third round of dialysis, protein was incubated with Prescission protease (1 mg protease/50 mg protein) and dialyzed overnight into dialysis buffer (1:50). Cleaved and dialyzed protein was passed through Ni-NTA (2 mL) to remove Prescission and the His_{10} tag. The eluent was then diluted to a final salt concentration of 20 mM NaCl using

Buffer A (50 mM HEPES, 20% (v/v) glycerol, 1 mM DTT, 1 mM EDTA, pH 7.5) and loaded onto a 5 mL HiTrap-Q column for ion exchange with the AKTA Purifier FPLC system (GE Healthcare). The protein was eluted using a gradient from 0-100% buffer A to buffer B (50 mM HEPES, 1 M NaCl, 20% (v/v) glycerol, 1 mM DTT, 1 mM EDTA, pH 7.5) over 40 min. The protein sample was concentrated to 2 mL and loaded onto a Superdex 75 16/60 pg (GE Healthcare) column equilibrated with SEC Buffer (25 mM HEPES, 100 mM NaCl, 1 mM DTT, pH 7.5). The protein eluent was concentrated to 15 mg/mL and glycerol was added to a final concentration of 5% (v/v) before flash freezing in liquid nitrogen and storage at -80 °C.

Over-expression and Purification of Selenomethione-substituted BesC. Expression of selenomethionine (SeMet)- substituted BesC was based on a previously reported protocol.[3] Cells were grown at 37 °C in 2xYT media containing the appropriate antibiotics (50 µg/ml carbenicillin) to an OD_{600} of 0.7-0.8. Cells were pelleted by centrifugation at 1500 *g* for 15 min and then washed twice in M9 minimal media by gentle resuspension and centrifugation. Washed cells were resuspended in M9 media with the appropriate antibiotics and supplemented with all the amino acids except methionine at concentrations of 40 mg/L (SAWYRHDEGPQNC), 50 mg/L (ILV), or 100 mg/L (KFT). Cells were grown for 20 min at 25 °C to exhaust residual methionine. Selenomethionine was added at 80 mg/L and protein expression was induced by addition of IPTG to a final concentration of 0.4 mM. Protein was expressed overnight at 25 °C and then harvested and purified as described above, with one exception. For Semet-substituted BesC, the SEC buffer contained 1 mM TCEP in place of 1 mM DTT.

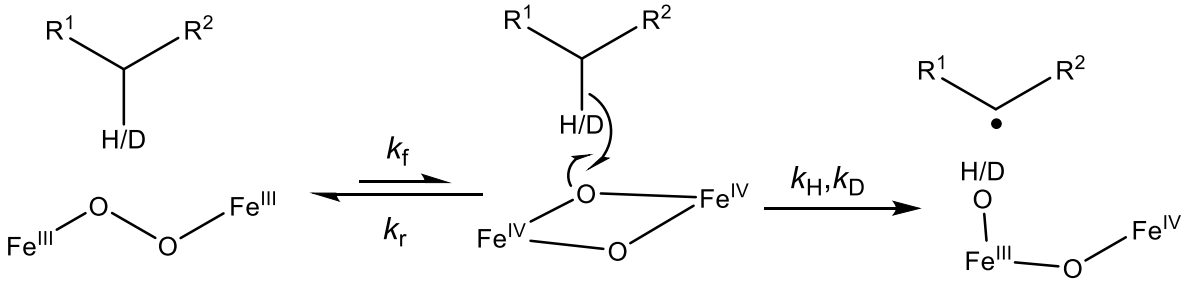
X-ray crystallographic characterization of apo BesC. Apo crystals of native BesC from *S. cattleya* were obtained by the hanging drop vapor diffusion method by combining equal volumes of protein solution (10 mg/mL BesC in 25 mM HEPES pH 7.5 and 1 mM DTT) and reservoir solution (calcium acetate (0.6 mM) and PEG 3350 (25% (v/v))). Small hexagonal plate crystals grew in 1 day. Crystals were transferred to an Eppendorf tube containing 100 µL of seed buffer (calcium acetate (0.6 mM) and PEG 3350 (25% (v/v))) at 4 °C and vortexed for 30 seconds with

10 x 1 mM diameter zirconia/silica beads to produce a micro-seed solution. The seed solution was stored at -80 °C for future use. SeMet crystals were prepared by micro-seeding equal volumes of protein solution (10 mg/mL BesC in 25 mM HEPES pH 7.5 and 1 mM TCEP) and reservoir solution (calcium acetate (0.6 mM), PEG 3350 (25% (v/v)) and 1,3 butane-diol (4%)). Small hexagonal plate crystals grew in 1 day.

Crystals were flash-frozen in liquid nitrogen and data were collected at Beamline 8.3.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory). For phasing purposes, an energy scan was performed at the Se edge and then data were collected at the following two wavelengths with inverse beam: 0.979620. (12656.631 eV, halfway between the peak and the inflection point) and 0.953680. (13000.613 eV, high energy remote). Data were processed with XDS[4-5] and scaled and merged with Aimless[6] within the CCP4 suite.[6] The structure was solved using the experimental phasing module within Phenix, yielding a figure of merif (FOM) of 0.4.[7] Bucaneer[8] was used to build an initial model of the structure that contained 922 amino acids (out of 1028 possible among the four protein chains in the asymmetric unit). This model was extended and refined iteratively with COOT[9] and Phenix.[7] Because analysis of the BesC crystals with Xtriage in Phenix revealed extensive twinning, the dataset was refined with a 50% twin fraction. Coordinates were analyzed with the Molprobity server[10] prior to structure deposition. The Pymol[11] molecular graphics suite was used to prepare figures. Crystals of apo-BesC belong to the $P2_1$ space group with four monomers in the asymmetric unit. The final model of apo-BesC consists of residues 26-203, 212-255 in chain A; 26-202, 209-254 in chain B; 26-201, 210-254 in chain C; 26-202, 210-255 in chain D, and 948 water molecules.

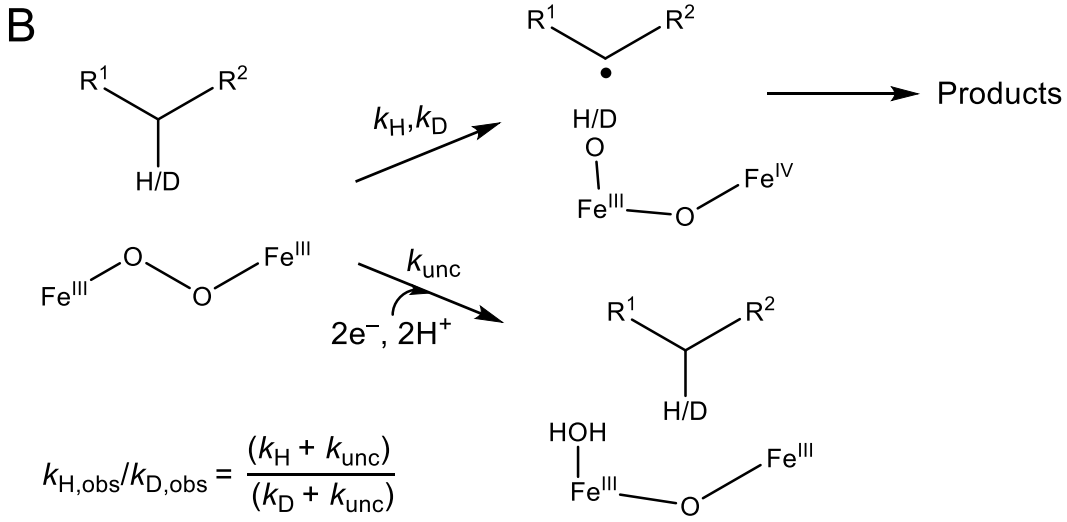
Scheme S1. Potential kinetic masking [in direct monitoring of μ-peroxodiiron(III) intermediate decay] of a large intrinsic D-KIE on C4 HAT by reversible conversion of the μ-peroxodiiron(III) complex to a more reactive intermediate or decay of the absorbing intermediate complex through isotope-insensitive pathway(s).

A



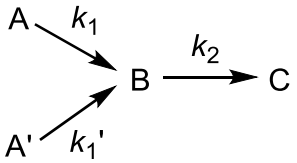
$$k_{H,obs}/k_{D,obs} = \frac{k_f \bullet k_H/(k_H + k_r)}{k_f \bullet k_D/(k_D + k_r)} = \frac{k_H/(k_H + k_r)}{k_D/(k_D + k_r)}$$

B



$$k_{H,obs}/k_{D,obs} = \frac{(k_H + k_{unc})}{(k_D + k_{unc})}$$

Scheme S2. Kinetic model used in regression analysis of the SF-Abs traces.



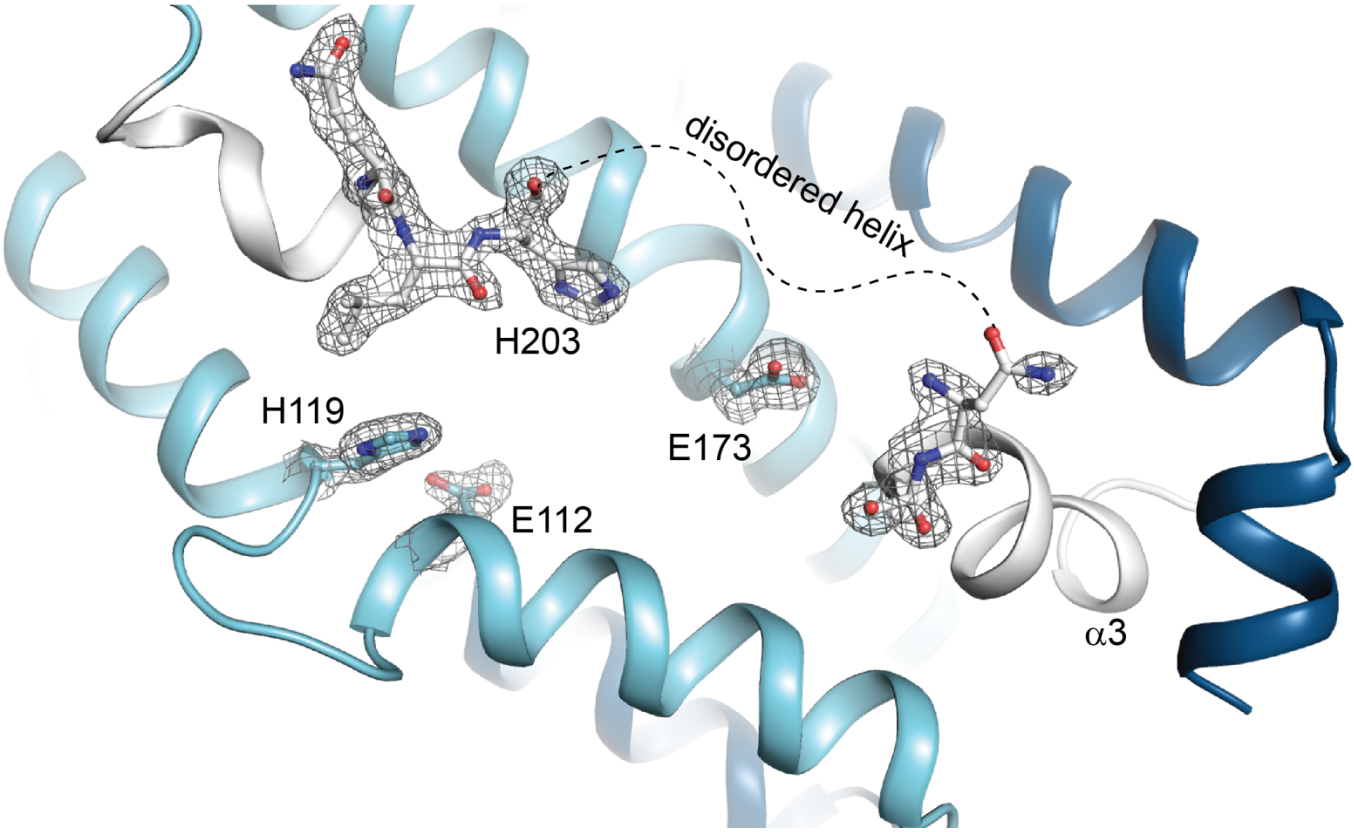


Figure S1. Apo BesC (PDB ID: T7WA) in cartoon representation with α3 shown in *white*, other core helices are shown in *light blue* and auxiliary helices are colored in *dark blue*. Helix α3 is shown in stick format with electron density in *gray* mesh contoured at 1.5σ. Due to the disorder present in helix α3, several residues are missing and there is a complete lack of electron density in this region. Two key metal binding ligands are also missing due to apparent disorder. Similar disorder is observed in apo or partially occupied HDO structures such as SznF (PDB IDs: 6M9S, 6M9R, 6XCV) and UndA (PDB IDs: 6P5Q, 4WWJ).

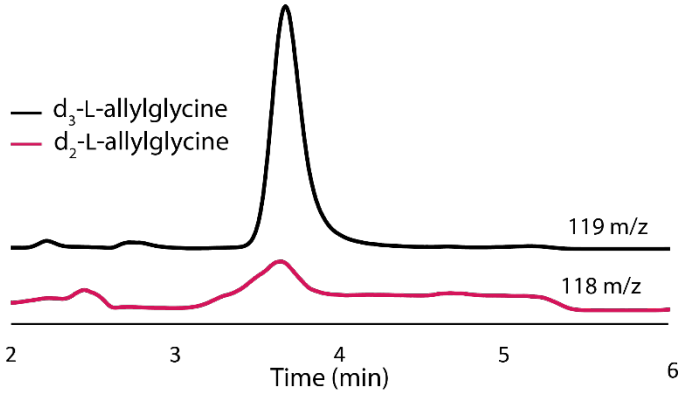


Figure S2. LC-MS detection of products in multiple-turnover assays of BesC (0.15 mM) after incubation with Fe(II) (0.30 mM) and d_4-4,4,5,5-L-Lys (1 mM) in 100 mM sodium HEPES pH 7.5, 100 mM NaCl, 5% glycerol (v/v) at 22 °C. Single ion monitoring (SIM) of the reaction with d_4-L-Lys shows a substantially larger peak for d_3-L-allylglycine at 119 *m/z* relative to the peak at 118 *m/z* that corresponds to d_2-L-allylglycine. The latter peak arises from a small fraction of incompletely deuterium-labeled substrate in the commercial product.

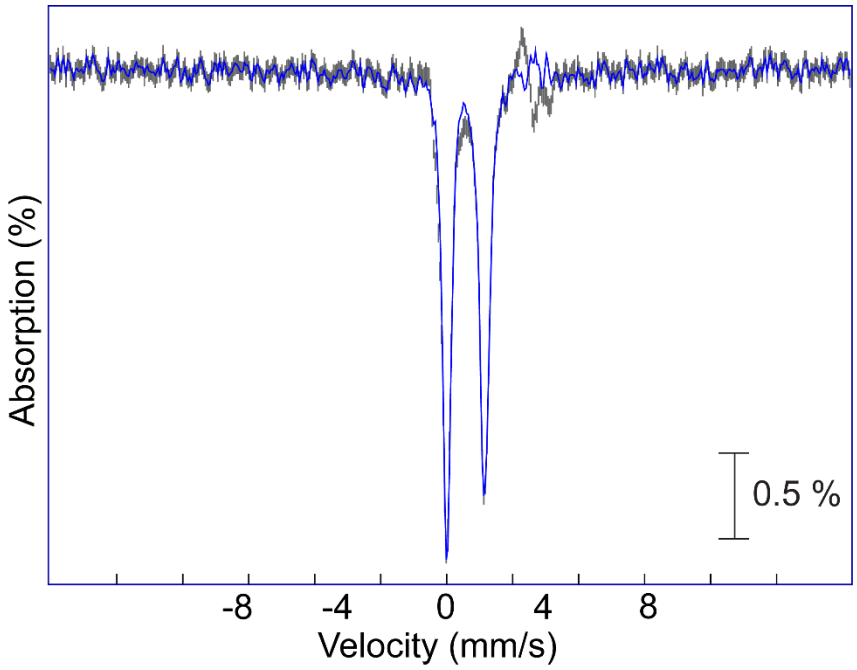


Figure S3. Reference spectrum of the μ-peroxodiiron(III) intermediate generated by subtraction of 57% of the experimental spectrum of Fe(II)•BesC (*grey vertical bars*) or 59% of the theoretical spectrum (*blue line*) from the spectrum of the 1-s freeze-quenched sample shown in Figure 3 of the main text.

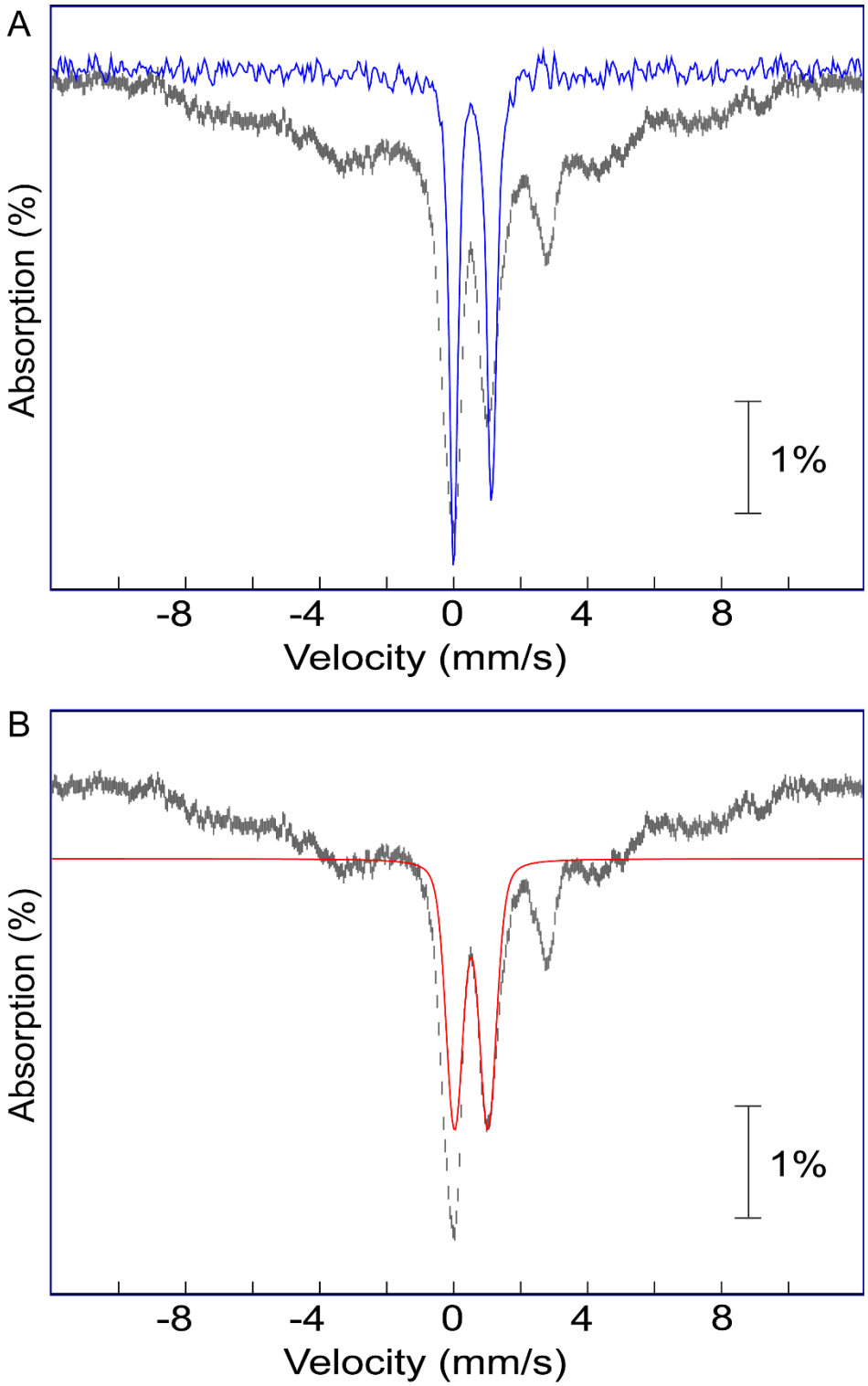


Figure S4. Overlay of the 4.2-K/53-mT Mössbauer spectrum with (***A***) the derived reference spectrum of the μ-peroxodiiron(III) intermediate or (***B***) the theoretical reference spectrum of the diiron(III) product.

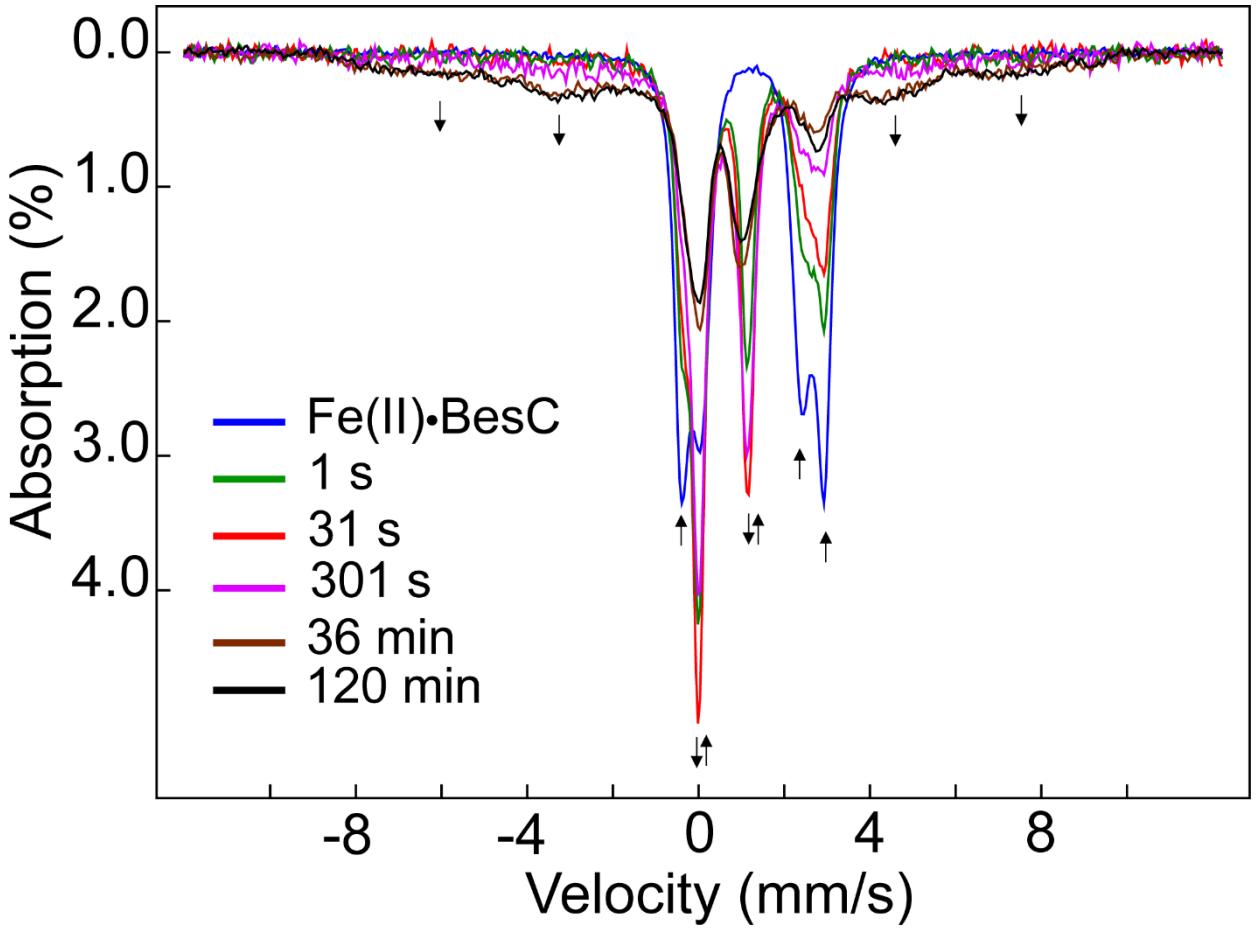


Figure S5. Overlay of all 4.2-K/53-mT Mössbauer spectra from the experiment depicted in Figure 3 of the main text to illustrate the development of the broad features characteristic of uncoupled high-spin Fe(III) species at long reaction times.

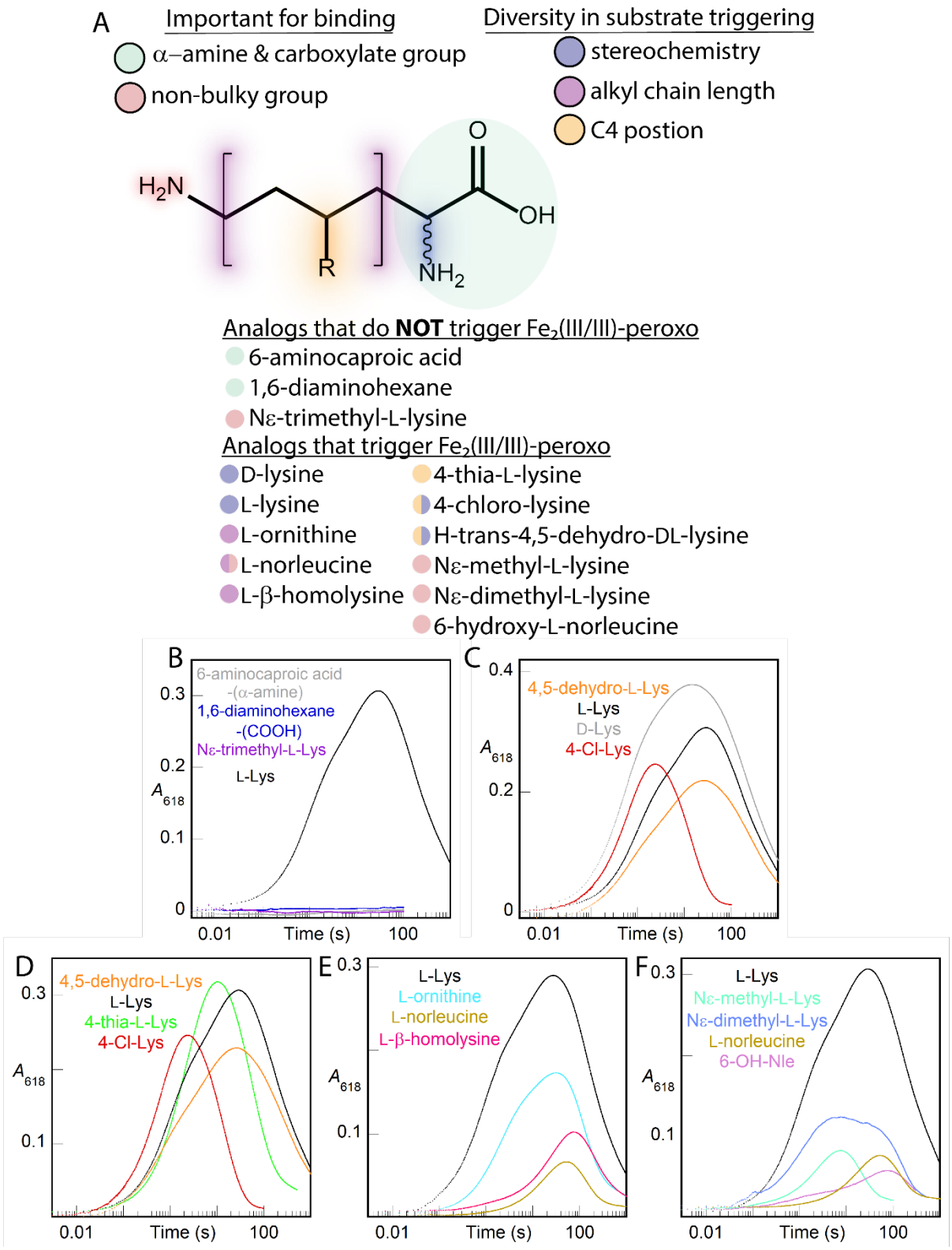

A
Important for binding
α–amine & carboxylate group
non-bulky group
Diversity in substrate triggering
stereochemistry
alkyl chain length
C4 postion
H2N
O
OH
R
NH2
Analogs that do NOT trigger Fe2(III/III)-peroxo
6-aminocaproic acid
1,6-diaminohexane
Nε-trimethyl-L-lysine
Analogs that trigger Fe2(III/III)-peroxo
D-lysine
L-lysine
L-ornithine
L-norleucine
L-β-homolysine
4-thia-L-lysine
4-chloro-lysine
H-trans-4,5-dehydro-DL-lysine
Nε-methyl-L-lysine
Nε-dimethyl-L-lysine
6-hydroxy-L-norleucine
B
6-aminocaproic acid
-(α-amine)
1,6-diaminohexane
-(COOH)
Nε-trimethyl-L-Lys
L-Lys
A618
Time (s)
0.01
100
C
4,5-dehydro-L-Lys
L-Lys
D-Lys
4-Cl-Lys
D
4,5-dehydro-L-Lys
L-Lys
4-thia-L-Lys
4-Cl-Lys
E
L-Lys
L-ornithine
L-norleucine
L-β-homolysine
F
L-Lys
Nε-methyl-L-Lys
Nε-dimethyl-L-Lys
L-norleucine
6-OH-Nle

Figure S6. Analysis of L-Lys analogs reveals the determinants for substrate triggering in formation of the μ-peroxodiiron(III) intermediate. (***A***) Schematic depiction of components of lysine modified in these experiments. These include (*i*) loss of the α-amine or carboxylate functional groups, (*ii*) mono- or di-methylation of the ε-amine, (*iii*) stereoinversion of the C2 position, (*iv*) change in length of the alkyl chain, and (v) subsitution at the C4 position. A_{618}-versus-time traces are shown for each of these five groups. All reactions were carried out by mixing an anoxic solution of BesC (0.30 mM), Fe(II) (0.60 mM) and the substrate analog (1 mM) with O_2-saturated buffer [100 mM sodium HEPES pH 7.5, 100 mM NaCl, 5% (v/v) glycerol] at 5°C. All plots show the kinetic trace for L-Lys for comparison. (***B***) Substrate analogs lacking the α-amine/carboxylate groups or trimethylated at the ε-amine do not trigger intermediate formation in BesC. We posit that these analogs either fail to bind in the active site (6-aminocaproic acid, 1,6-diaminohexane) or contain altered charge that prevents reactivity with O_2 (N^{ε}-trimethyl-L-Lys). (***C***) Substrate analogs with altered stereochemistry at C2 are tolerated by BesC. In the case of D-Lys, the BesC intermediate forms faster and accumulates to a greater extent. (***D***) BesC also tolerates substitutions at the C4 position. In particular, with 4-thia-L-Lys, the μ-peroxodiiron(III) intermediate decays more quickly (by a factor of 5) than with L-Lys. (***E***) The BesC intermediate is triggered by amino acids with shorter alkyl chains; however, the kinetics are markedly perturbed. (***F***) Lastly, substitutions at the ε-amine are permitted by BesC, with the exception of the trimethylated side chain. Although these substitutions are tolerated, the kinetics are similar to substrates that vary in alkyl chain length.

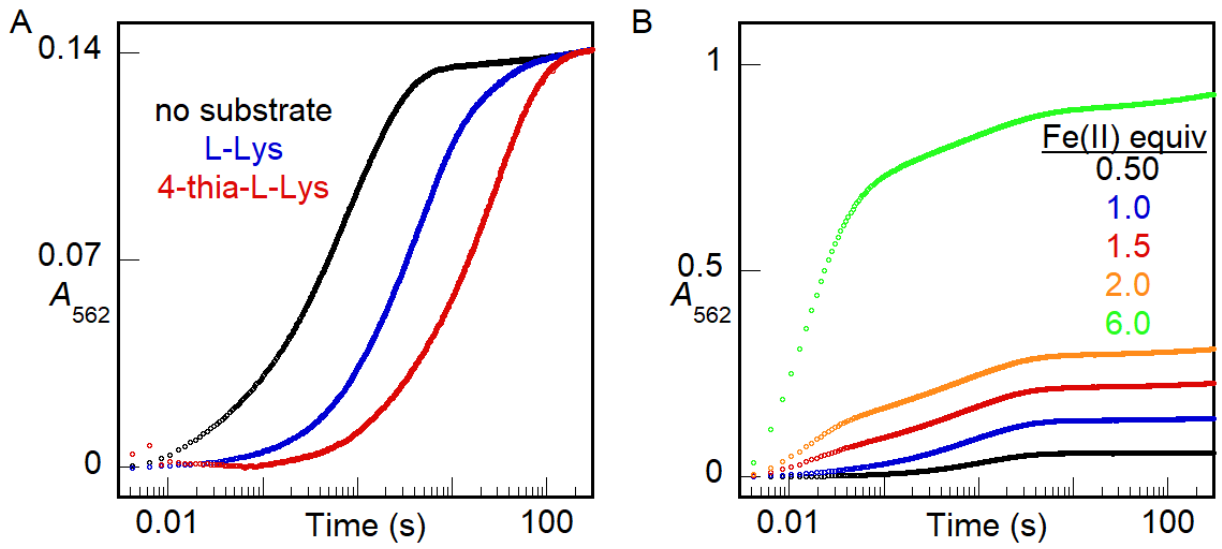


Figure S7. A_{562}-versus-time traces for the detection of Fe(II) by ferrozine following its dissociation from BesC. An anoxic solution of 80 μM apo-BesC was loaded with (***A***) 80 μM (1 molar equiv) Fe(II) in the absence of any substrate (*black*) or in the presence of 12 mM L-Lys (*blue*) or 4-thia-L-Lys (*red*); or (**B**) 40-480 μM (0.5-6 molar equiv) Fe(II) in the absence of any substrate. This solution was then mixed with an equal volume of an anoxic solution of 4 mM ferrozine, and the absorbance of the purple Fe(II)•ferrozine$_3$ complex at 562 nm (A_{562}) was monitored as a function of reaction time. The traces shown here are representative of at least 2 trials for each condition.

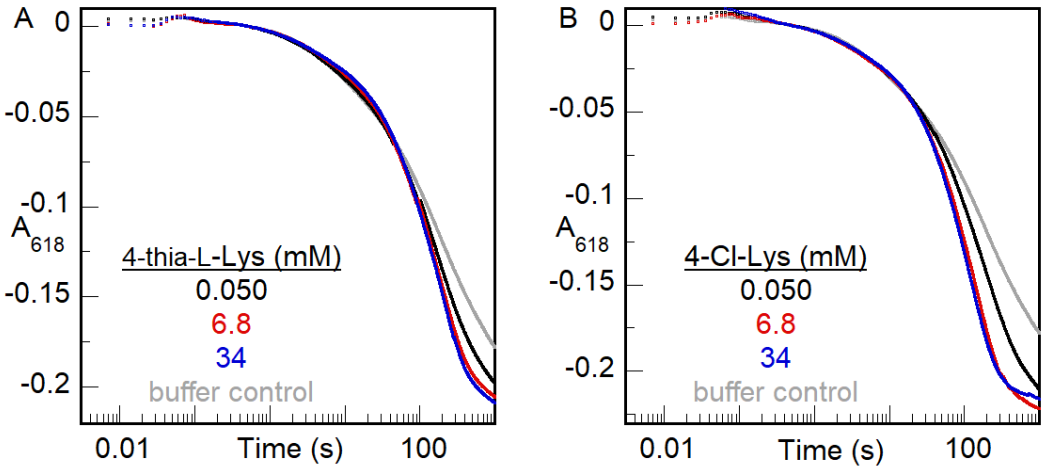


Figure S8. A_{618nm}-versus-time traces from a sequential-mix stopped-flow experiment showing decay of the μ-peroxodiiron(III) intermediate. A reaction solution containing BesC (0.40 mM), Fe(II) (0.8 mM), and L-Lys (2.7 mM) was mixed with O_2-saturated buffer at 5 °C for 30 s. This solution was subsequently mixed with (***A***) 4-thia-L-Lys (0, 0.050, 6.8, 34 mM), or (***B***) 4-Cl-Lys (0, 0.050, 6.8, 34 mM).

Table S1. Data collection and refinement statistics for x-ray structure of apo BesC.

	BesC SeMet (high energy remote)	BesC SeMet (peak-inflection)
Data collection		
Wavelength (Å)	0.953680	0.979620
Space group	$P2_1$	$P2_1$
Cell dimensions		
a, *b*, *c* (Å)	60.9, 68.9, 126.7	60.9, 68.9, 126.7
α, β, γ (°)	90.0, 89.9, 90.0	90.0, 89.9, 90.0
Resolution (Å)	50.00-1.70 (1.73-1.70)	50.00-1.70 (1.76-1.70)
R_{merge}	0.080 (0.635)	0.085 (0.579)
I / σ	14.2 (2.6)	14.6 (2.7)
Completeness (%)	99.7 (94.6)	96.4 (85.6)
Redundancy	8.2 (6.8)	8.0 (6.3)
$CC_{1/2}$	0.999 (0.917)	0.998 (0.887)
Refinement		
Resolution (Å)	50.00-1.70	
No. reflections	115135	
R_{work} / R_{free}	0.20/0.25	
No. atoms		
Protein	7243	
Ligand/ion	39	
Water	948	
B-factors		
Protein	19.7	
Ligand/ion	24.0	
Water	20.3	
r.m.s. deviations		
Bond lengths (Å)	0.004	
Bond angles (°)	0.540	
Molprobity clashscore	6.2	
Rotamer outliers (%)	0	
Ramachandran favored (%)	99	
Ramachandran outliers (%)	0	

*Values in parentheses are for highest-resolution shell.

Table S2. Cloning primers for the preparation of BesC.

Primer	Sequence	Restriction Enzyme Site
Forward	5'- catatgaccgacctgaacaccc-3'	*NdeI*
Reverse	5'- ctcgagtcacttgccgatgc-3'	*XhoI*

Table S3. Recipe for 10,000X trace metals cocktail for minimal (M9) media supplementation.

Reagent	mg/L	Final concentration in medium (nM)
$(NH_4)Mo_7O_{24}$	37.1	3
$CoCl_2 \bullet 6H_2O$	71.4	300
H_3BO_3	247	400
$CuSO_4 \bullet 5H_2O$	25	10
$MnCl_2 \bullet 4H_2O$	198	80
$ZnSO_4 \bullet 7H_2O$	28.8	10

Table S4. Parameters used to simulate Mössbauer spectra.

Fe species	δ (mm/s)	ΔE_Q (mm/s)	Linewidth (mm/s)	Relative area (%)
Peroxo-Fe_2(III/III) intermediate	0.58	1.15	-0.25	Variable
Fe_2(III/III) successor complex	0.51	1.00	-0.80	Variable
Fe_2(II/II) in Figure 4Aa *	1.24	2.35	-0.49	50
	1.27	3.32	-0.373	50
Fe_2(II/II) in Figure 4A, spectra b and c *	1.27	2.67	-0.84	Variable
	1.30	3.38	-0.22	variable

*The contribution of the reactant Fe_2(II/II) complex(es) varies as a function of the reaction time, as is evident by the different shape of the upward-pointing peaks associated with the reactant Fe_2(II/II) at ~+2.7 mm/s (e.g., compare spectra a, b, and c in Figures 4 A & C in main manuscript). This observation suggests heterogeneity of the reactant. We simulated the spectrum of the Fe_2(II/II) complex(es) by assuming two symmetrical quadrupole doublets.

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