Structural Basis of Regiospecificity of a Mononuclear Iron Enzyme in Antibiotic Fosfomycin Biosynthesis

Supplementary Information

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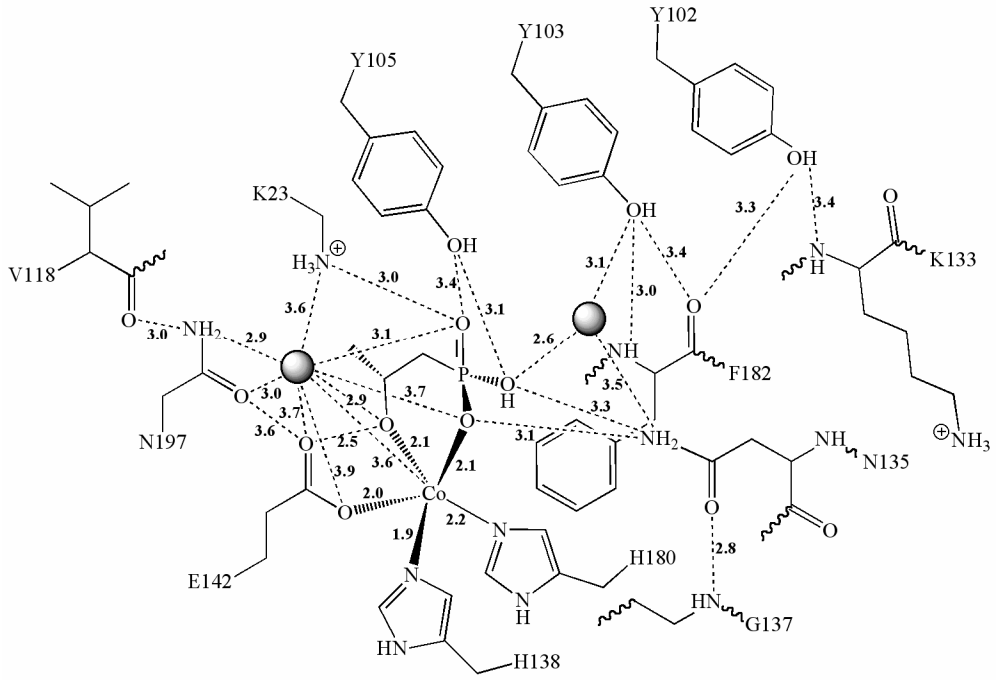
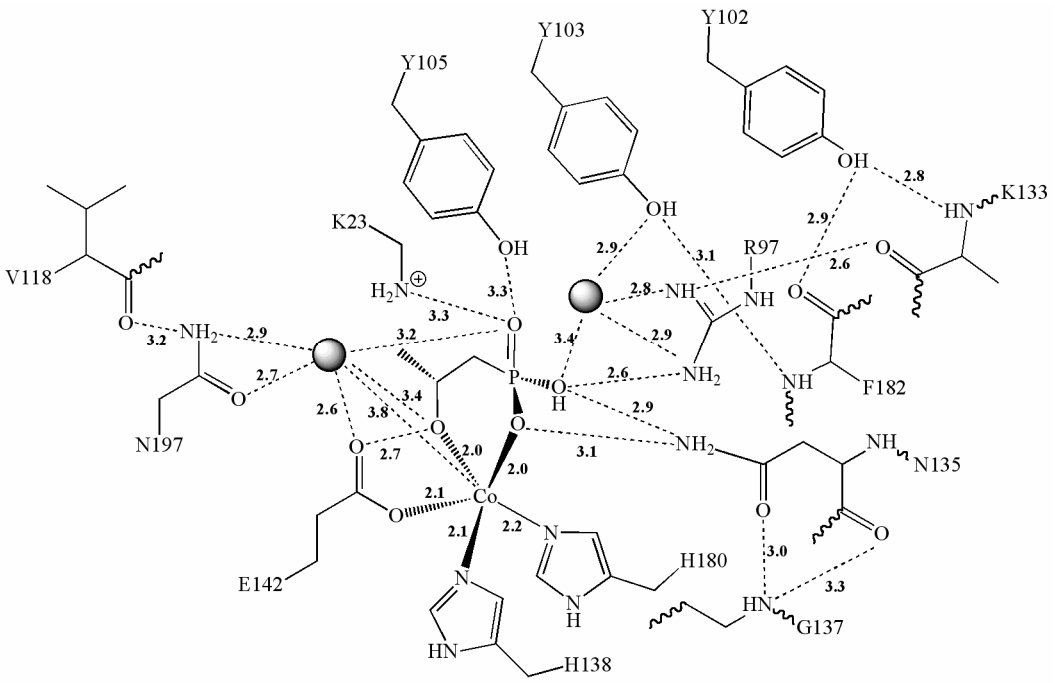
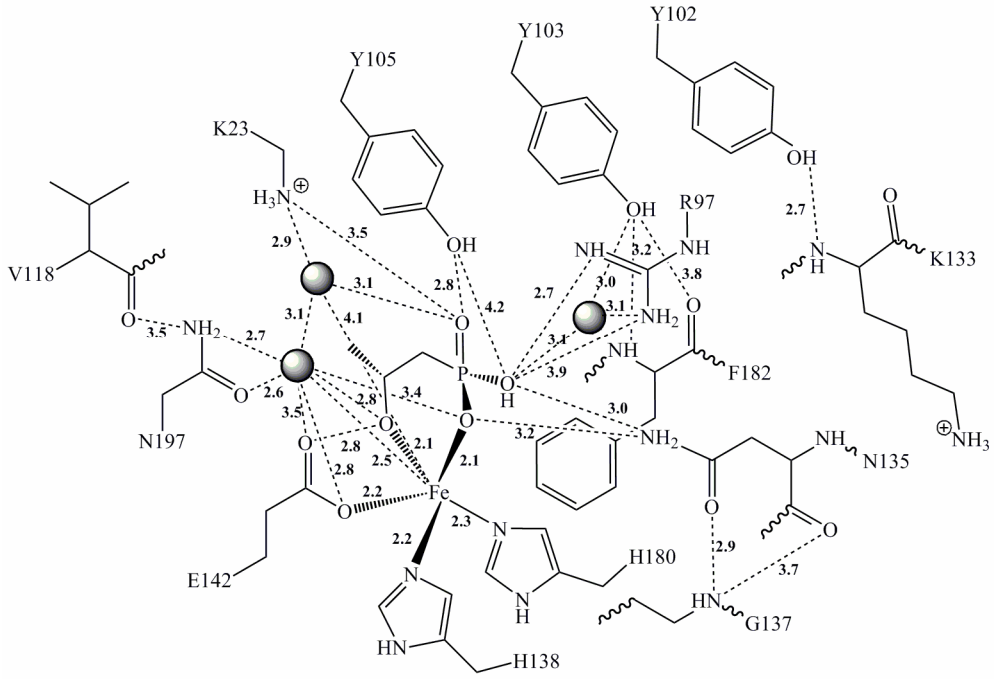
Supplementary Figure 1. Two dimensional active site contact maps. Distances (Å) shown with dotted lines and grey spheres are water molecules (a) *R*-HPP, Co(II), monomer A, bidentate binding mode; (b) *R*-HPP, Co(II), monomer B, bidentate; (c) *R*-HPP, Fe(II), monomer A, bidentate; (d) *R*-HPP, Fe(II), monomer B, bidentate; (e) *R*-HPP, Fe(II), monomer C, bidentate; (f) NO-soak, *S*-HPP, Fe(II), monomer A, bidentate, NO; (g) NO-soak, *S*-HPP, Fe(II), monomer B, bidentate, NO; (h) NO-soak, *S*-HPP, Fe(II), monomer C, bidentate, H_2O; and (i) Fe-*S*-HPP, monomer C, bidentate (1zz8).



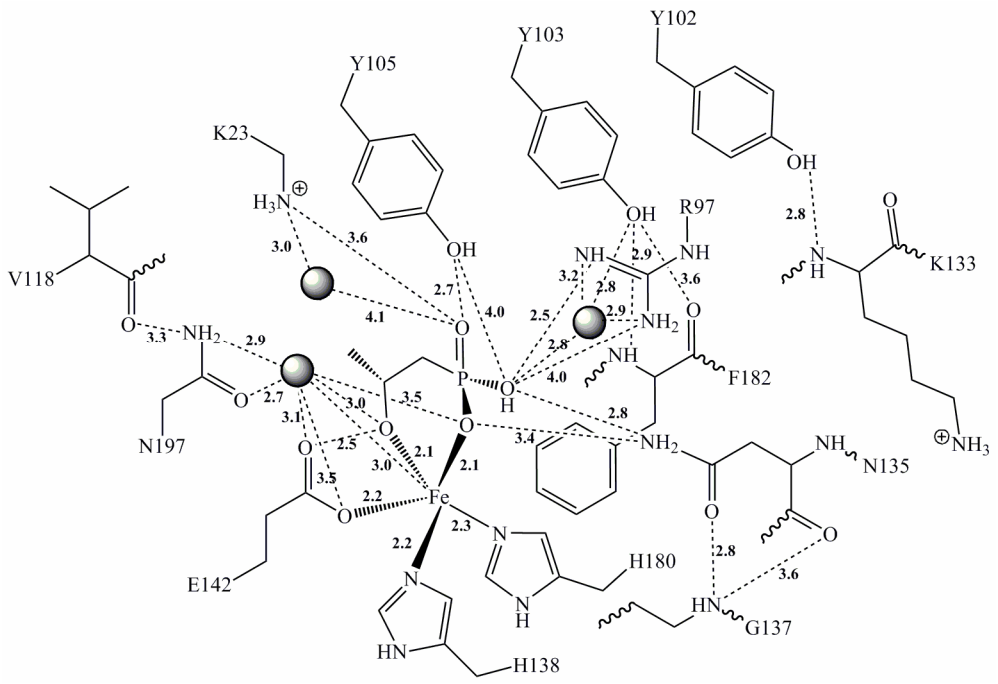
Supplementary Figure 1a. *R*-HPP, Co(II), monomer A, bidentate.



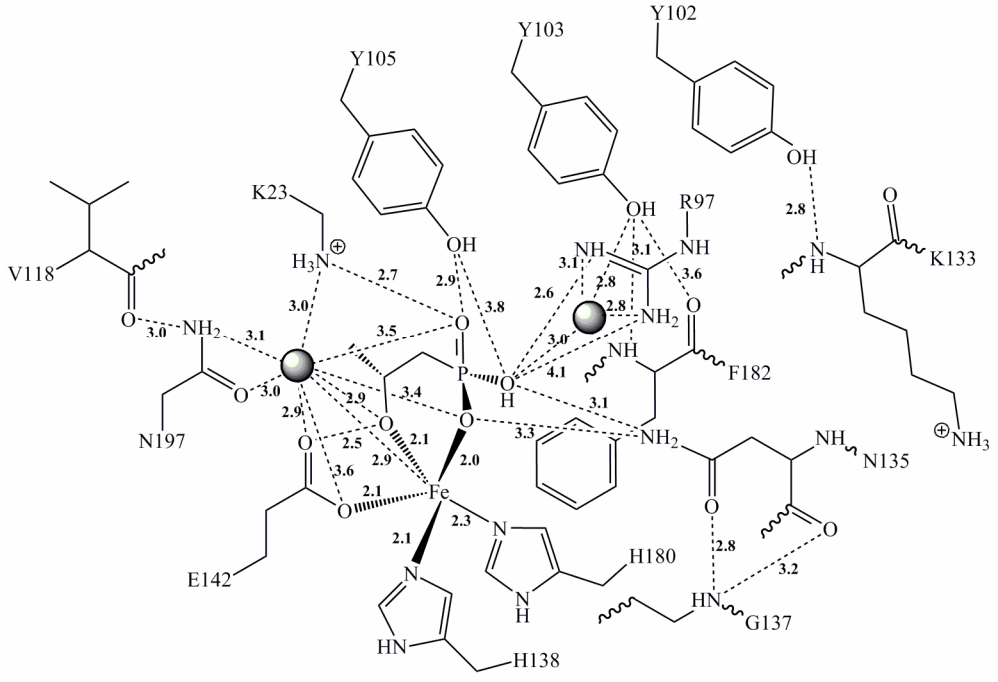
Supplementary Figure 1b. *R*-HPP, Co(II), monomer B, bidentate.



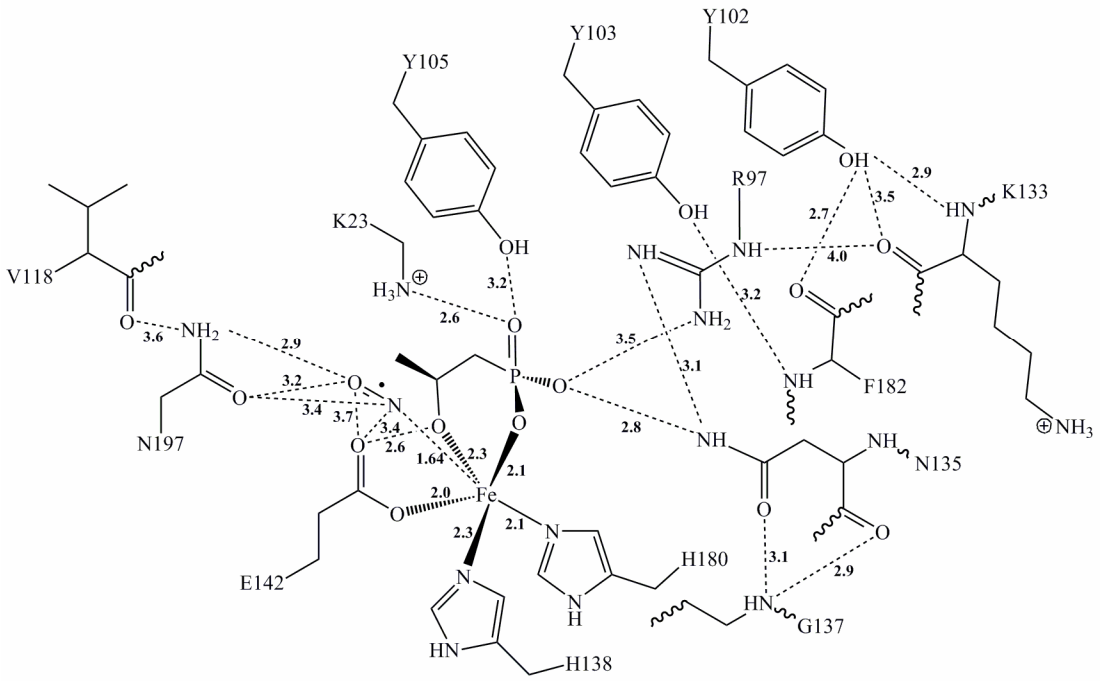
Supplementary Figure 1c. *R*-HPP, Fe(II), monomer A, bidentate.



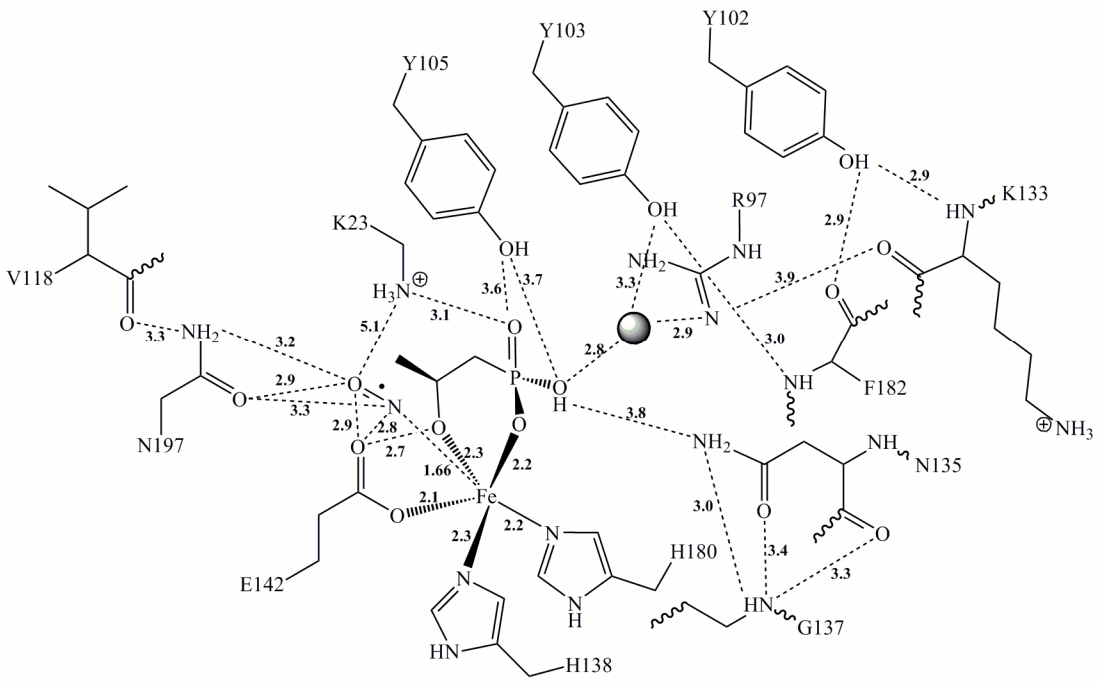
Supplementary Figure 1d. *R*-HPP, Fe(II), monomer B, bidentate.



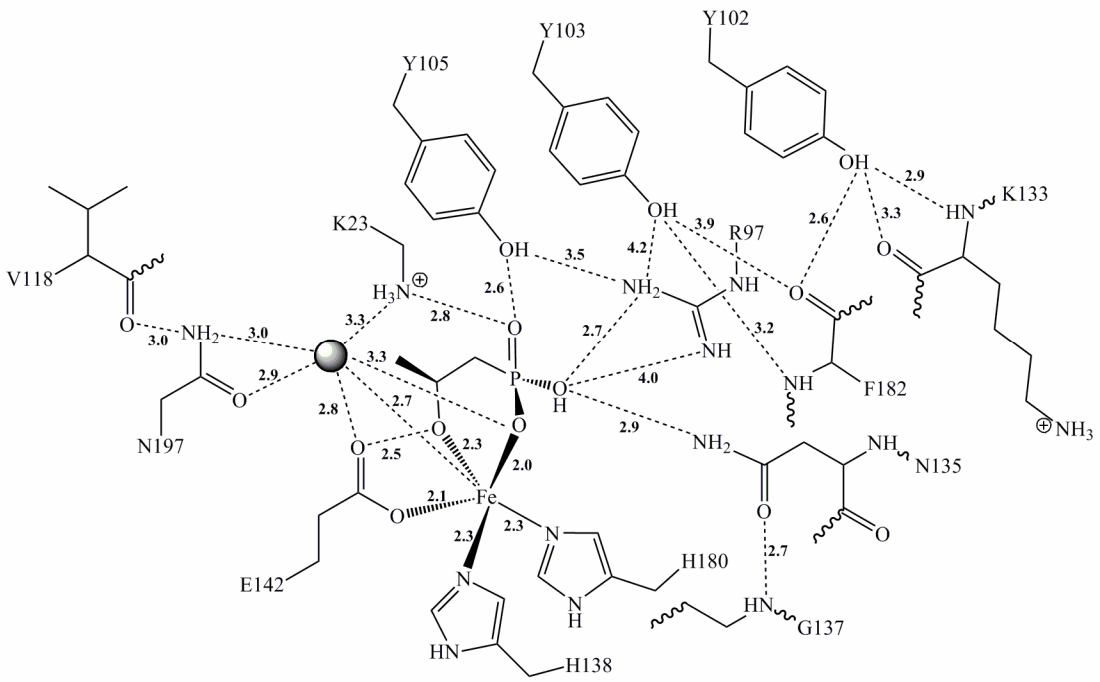
Supplementary Figure 1e. *R*-HPP, Fe(II), monomer C, bidentate.



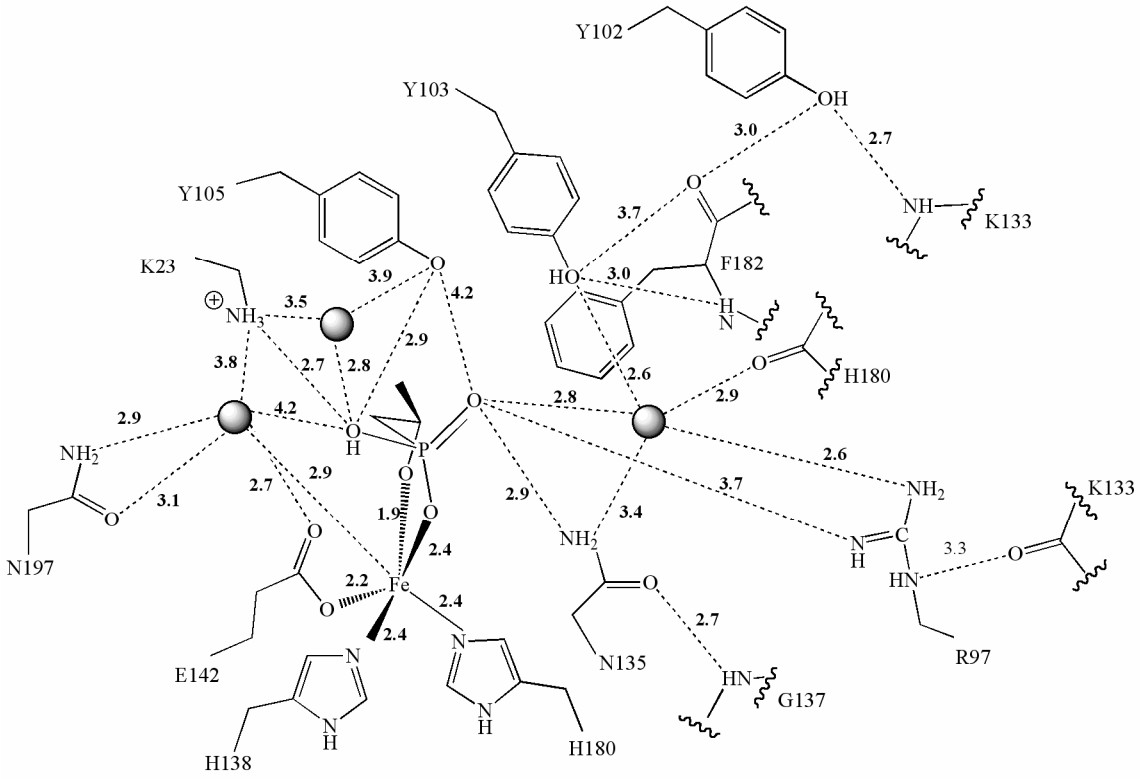
Supplementary Figure 1f. NO-Soak, *S*-HPP, Fe(II), monomer A, bidentate, NO.



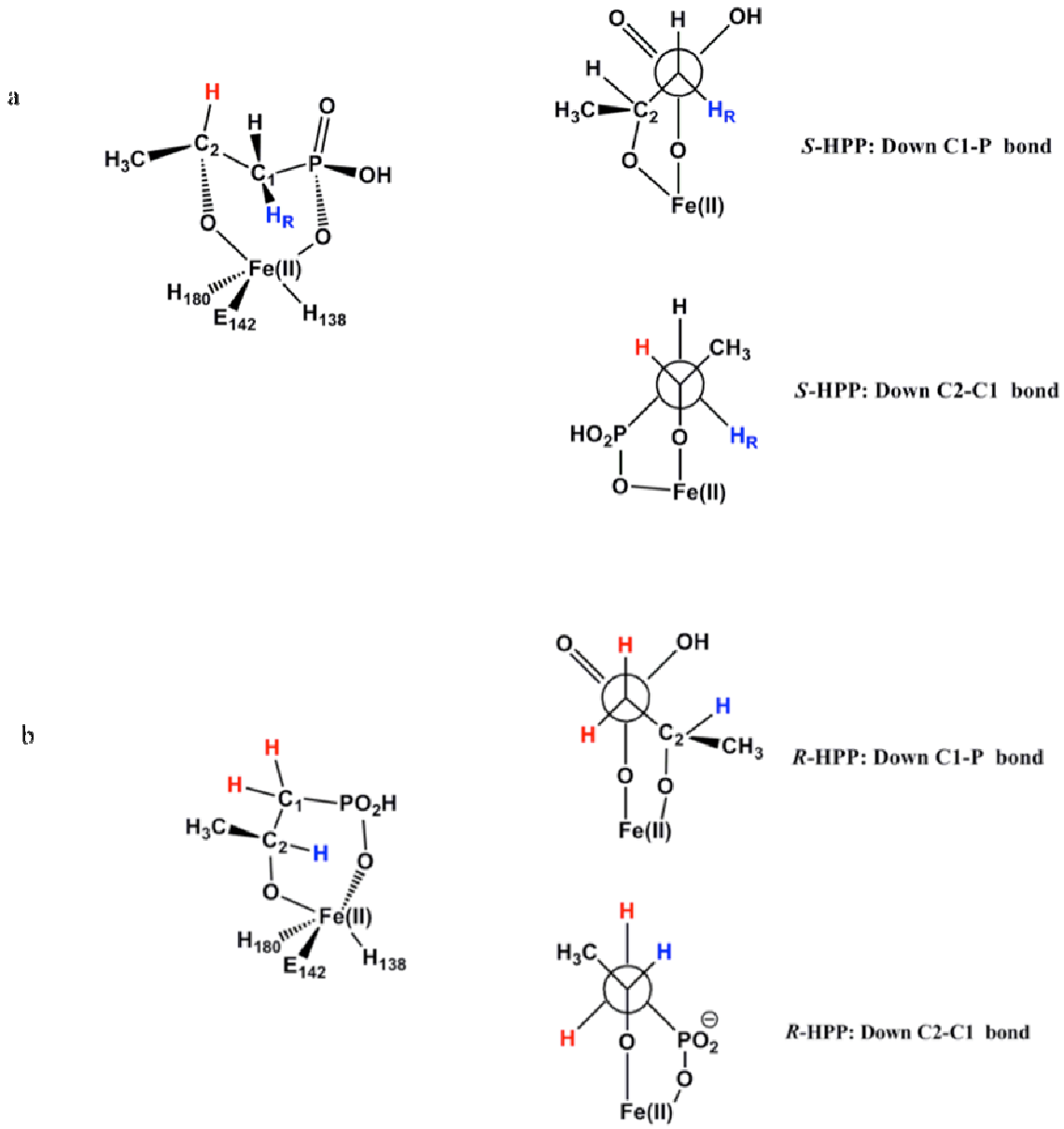
Supplementary Figure 1g. NO-Soak, *S*-HPP, Fe(II), monomer B, bidentate, NO.



Supplementary Figure 1h. NO-Soak, *S*-HPP, Fe(II), monomer C, bidentate, H_2O.



Supplementary Figure 1i. *S*-HPP, Fe(II), monomer C, bidentate (1zz8).



Supplementary Figure 2. Newman projections of *S*- and *R*-HPP anchored in a bidentate fashion to iron. The substrates (a) *S*-HPP and (b) *R*-HPP are shown looking down the C1-P bond (top) and C2-C1 bond (bottom) illustrating bidentate substrate binding in a staggered conformation. Hydrogen atoms accessible for abstraction are shown in blue. Hydrogen atoms inaccessible for abstraction are shown in red (See Figure 6).

Supplementary Table 1. Data Collection and Refinement Statistics

	***R*-HPP-Co(II)**	***R*-HPP-Fe(II)**	***S*-HPP-Fe(II)-NO**
Data Collection			
Space Group	$P6_522$	$P4_22_12$	$P4_22_12$
Unit Cell Dimensions			
a, b (Å)	86.3	111.6	111.7
c (Å)	219.1	151.3	152.2
Beamline	ALS 5.0.2	SSRL 9-1	APS 24ID-C
Wavelength (Å)	1.1271	1.00	1.00
Resolution Range (Å)	30-2.10 (2.18-2.10)	50-3.00 (3.22-3.00)	50-2.85 (2.95-2.85)
Observations	392445	548792	244234
Unique Reflections	28960	19839	22766
Redundancy	13.6	27.7	10.7
Completeness (%)[a]	99.9 (100.0)	99.9 (100.0)	99.1 (92.8)
$I/\sigma(I)$[a]	40.2 (5.1)	54.5 (11.8)	21.9 (2.3)
R_{sym} (%)[a,b]	6.9 (32.8)	8.2 (31.4)	10.1 (43.8)
Wilson *B*-Factor (Å^2)	35.4	61.9	68.8
Refinement			
R_{work}[c] (R_{free}[d]) (%)	21.7 (24.5)	21.7 (25.5)	24.3 (26.9)
Resolution Range (Å)	30.0-2.10	50-3.00	50-2.85
No. Reflections	26908	19766	22379
No. Molecules per asu[e]	2	3	3
No. of non-hydrogen atoms:			
Protein	2897	4389	4389
Cobalt	2	-	-
Iron		3	3
R-HPP	16	24	
S-HPP	-	-	24
NO	-	-	4
Water	154	49	39
RMS[f] Deviations:			
Bond Lengths (Å)	0.009	0.008	0.009
Bond Angles (°)	1.7	1.6	1.7
Average B-Factors (Å^2)			
Protein atoms	40.4	56.3	69.1
Cobalt	40.5	-	-
Iron		55.0	68.8
R-HPP	49.3	58.4	
S-HPP			73.5
NO			72.7
Water	41.7	42.3	53.6

[a]Values in parentheses are the highest resolution shell. [b]$R_{sym}=\sum_{hkl}\sum_{i}|I_i(hkl)-\langle I(hkl)\rangle|/\sum_{hkl}\sum_{i} I_i(hkl)$, where $I_i(hkl)$ is the i[th] measured diffraction intensity and $\langle I(hkl)\rangle$ is the mean intensity for the reflection with the miller index (hkl). [c]$R_{work}=\sum_{hkl}||F_o(hkl)|-|F_c(hkl)||/\sum_{hkl}|F_o(hkl)|$. [d]$R_{free}=R_{work}$ for 5% of reflections omitted from refinement. [e]asu, asymmetric unit. [f]RMS, root mean square.

Supplementary Table 2. Structures in paper

Data set	Substrate	Metal	Monomer ID	Substrate Conformation	Other ligand	Figure*
	R-HPP	Co(II)	Monomer A	Bidentate		S1a
	R-HPP	Co(II)	Monomer B	Bidentate		2a, S1b
	R-HPP	Fe(II)	Monomer A	Bidentate		S1c
	R-HPP	Fe(II)	Monomer B	Bidentate		S1d
	R-HPP	Fe(II)	Monomer C	Bidentate		2b, S1e
"NO"	*S*-HPP	Fe(II)	Monomer A	Bidentate	NO	S1f
"NO"	*S*-HPP	Fe(II)	Monomer B	Bidentate	NO	S1g
"NO"	*S*-HPP	Fe(II)	Monomer C	Bidentate	--	S1h
1ZZ8	*S*-HPP	Fe(II)	Monomer C	Bidentate		2d, S1i

*-Figure 2c 1ZZB monomer B, Figure 2d 1ZZ8 monomer C.