Supporting Information

Thermodynamics of iron(II) and substrate binding to the ethylene-forming enzyme

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Table S1. The Thermodynamic Cycle for Iron(II)·TES Interaction

Buffer	Reaction	Coeff.	Δ*H* (kcal/mol)
TES	$H_2EDTA^{2-} \leftrightarrows HEDTA^{3-} + H^+$	0.05	4.2[a]
	$HEDTA^{3-} \leftrightarrows EDTA^{4-} + H^+$	1.0	5.4[a]
	$TES + H^+ \leftrightarrows H^+\text{-}TES$	1.05	-7.7[b]
	$Fe^{2+} + EDTA^{4-} \leftrightarrows [Fe{\cdot}EDTA]^{2-}$	1	-3.8[a]
	$Fe^{2+}{\cdot}TES \leftrightarrows Fe^{2+} + TES$	1	-0.5
	$Fe^{2+}{\cdot}TES + H^+\text{-}EDTA \leftrightarrows Fe^{2+}{\cdot}EDTA + H^+\text{-}TES$		-6.8

The ionization enthalpies and enthalpy of the iron(II)·EDTA interaction were taken from the following references:

a) NIST Standard database 46, version 7.0 (2003) Gaithersburg, MD

b) Goldberg, R. N., Kishore, N., Lennon, R.M. (2002) *J. Phys. Chem.* Ref. data 31, 231-370.

Table S2. The enthalpy values of Fe^{2+}·buffer interaction

25 mM buffer, pH 7.4	$\Delta H_{Fe \cdot buffer}$ (kcal/mol)
MOPS	-1.83[a]
ACES	-2.5[a]
TES	0.5[b]

[a]values obtained from Henderson, K. L., Müller, T. A., Hausinger, R. P. & Emerson, J. P. (2015). *Inorg. Chem*. 54, 2278–2283.

[b]Calculated from Table S1.

Table S3. Thermodynamic Cycle for Iron(II) Binding to EFE in ACES Buffer

Reaction	Coefficient	Δ*H* (kcal/mol)
$Fe^{2+}\cdot ACES \leftrightarrows Fe^{2+} + ACES$	1.0	2.5[a]
$ACES + H^{+} \leftrightarrows ACES\text{-}H^{+}$	1.0	-7.27[b]
$Fe^{2+} + EFE\text{-}H^{+} \leftrightarrows Fe^{2+}\cdot EFE + H^{+}$	1.0	0.67
$ACES + Fe\cdot ACES + EFE\text{-}H^{+} \leftrightarrows Fe\cdot EFE + ACES\text{-}H^{+}$		-4.1[d]

Table S4. Thermodynamic Cycle for Iron(II) Binding to EFE in TES Buffer

Reaction	Coefficient	Δ*H* (kcal/mol)
$Fe^{2+}\cdot TES \leftrightarrows Fe^{2+} + TES$	1.0	-0.5[a]
$TES + H^{+} \leftrightarrows TES\text{-}H^{+}$	1.0	-7.7[b]
$Fe^{2+} + EFE\text{-}H^{+} \leftrightarrows Fe^{2+}\cdot EFE + H^{+}$	1.0	1.1
$TES + Fe\cdot TES + EFE\text{-}H^{+} \leftrightarrows Fe\cdot EFE + TES\text{-}H^{+}$		-7.1[d]

Table S5. Deconvolution of the enthalpy of Iron(II) Binding to EFE in ACES Buffer

Reaction			Coefficient	ΔH (kcal/mol)
$EFE{\cdot}H_2O\text{-}H^+$	$\leftrightarrows$	$EFE + H^+$	0.9	13.34[a]
$EFE\text{-}His\text{-}H^+$	$\leftrightarrows$	$EFE + H^+$	0.1	7.17[b]
Fe^{2+} + EFE	$\leftrightarrows$	**$Fe^{2+}{\cdot}EFE$**	**1.0**	**-12.1**
$Fe^{2+} + EFE\text{-}H^+ \leftrightarrows Fe^{2+}{\cdot}EFE + H^+$				0.67[c]

[a]Represents the ionization of water within the $H_2O\text{-}Fe^{2+}{\cdot}EFE$ complex involving 1 deprotonation to the $HO\text{-}Fe^{2+}{\cdot}EFE$ species. Value is taken from NIST Standard Database 69 (2014).

[b]Value is taken from ref.[24]

[c]Data from Table S3. .

Table S6. Deconvolution of the enthalpy of Iron(II) Binding to EFE in TES Buffer

Reaction			Coefficient	ΔH (kcal/mol)
$EFE\cdot H_2O\text{-}H^+$	$\leftrightarrows$	$EFE + H^+$	0.9	13.34[a]
$EFE\text{-}His\text{-}H^+$	$\leftrightarrows$	$EFE + H^+$	0.1	7.17[b]
Fe^{2+} + EFE	$\leftrightarrows$	**$Fe^{2+}\cdot EFE$**	**1.0**	**-11.6**
$Fe^{2+} + EFE\text{-}H^+ \leftrightarrows Fe^{2+}\cdot EFE + H^+$				1.1[c]

[a]Represents the ionization of water within the $H_2O\text{-}Fe^{2+}\cdot EFE$ complex involving 1 deprotonation to the $HO\text{-}Fe^{2+}\cdot EFE$ species. Value is taken from NIST Standard Database 69 (2014).

[b]Value is taken from ref.[24]

[c]Data from Table S4.

Table S7. Equilibrium Constant Involved in Iron(II) binding to EFE

Reaction	
Fe^{2+}·EFE ⇆ Fe^{2+} + EFE	**K_d(Fe·EFE)**
Fe^{2+} + HEDTA ⇆ Fe^{2+}·HEDTA	K_a(Fe·EDTA)[a]
Fe^{2+}·EFE + HEDTA ⇆ Fe^{2+}·HEDTA + EFE	K^*_{obs}

[a]The K_a value of M·EDTA were taken from Schwarzenbach, G., Cut, R. A. G. (1954) Komplexone XXV. *Helv. Chim. Acta* 37:937–957.

Table S8. Thermodynamic Properties of 2OG Binding to Fe·EFE Obtained by ITC

ΔG (kcal/mol)	Δ*H* (kcal/mol)	Δ*S* (cal/mol)
-7.21 (± 0.6)	-13.16 (± 2.6)	-19.5

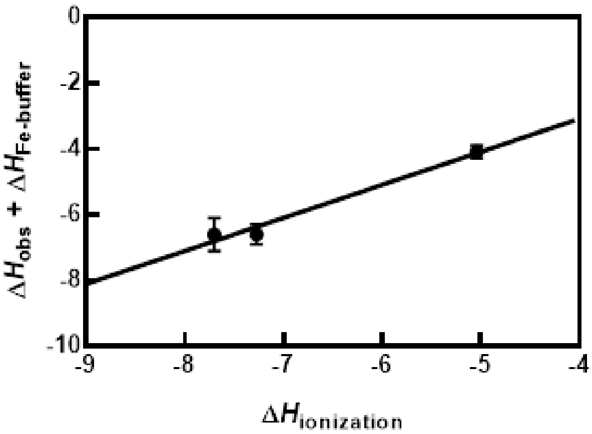

Figure S1. Observed enthalpies corrected for iron(II) buffer interaction were plotted against buffer ionization enthalpies. ITC experiments examined iron(II) binding to EFE using three buffers with each buffer study repeated two to four times. The data points represent the averages collected from these trials with error bars indicating one standard deviation of the mean. The slope of this plot represents the number of protons released (n_p), where n_p = 1.0 (±0.1) with r^2 = 0.9523.

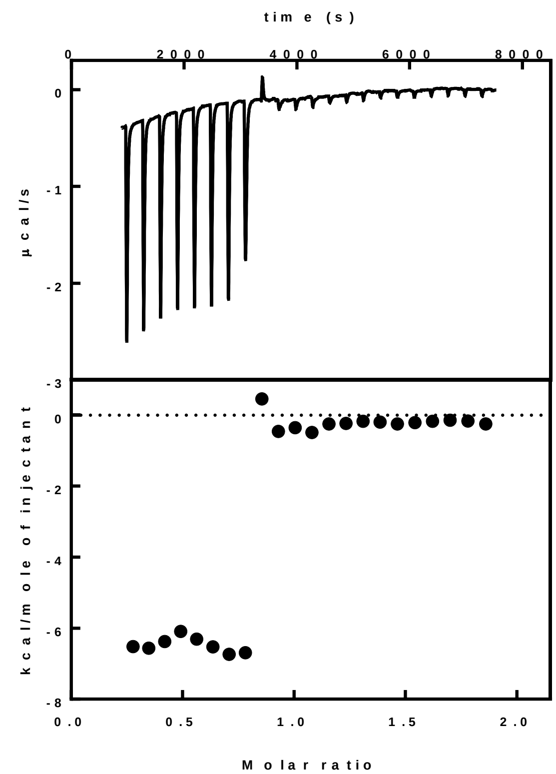

Figure S2. A representative control experiment involving titration of iron(II)-buffer into a solution containing EDTA in TES buffer, pH 7.4. These experiments were performed to elucidate the enthalpy of the iron(II)·TES interaction, which was used to deconvolute the iron(II)·EFE experiments.

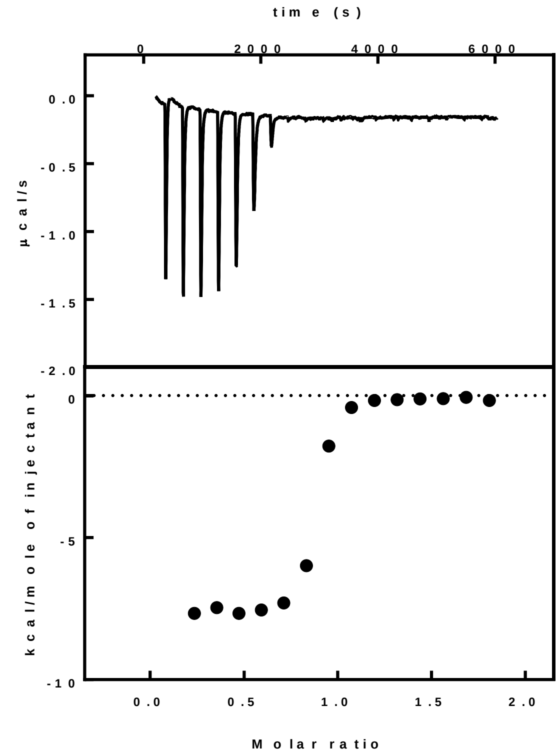

Figure S3. Representative ITC raw data for titration of EDTA into a solution containing Fe·EFE in 25 mM TES buffer at pH 7.4 and 25 °C.

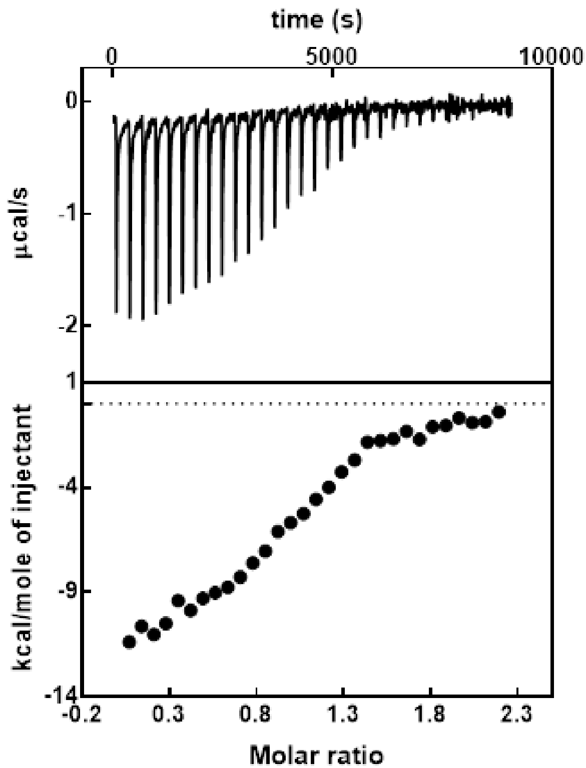

Figure S4. Representative ITC raw data for titration of 2OG into a solution containing Fe·EFE in 25 mM HEPES buffer at pH 7.5 and 25 °C.