Supplemental Material for "Mitochondrial Iron-Sulfur-Cluster Activity and Cytosolic Iron Regulate Iron Traffic in *Saccharomyces cerevisiae*" by Wofford and Lindahl

Table S1 Comparison between simulated values and literature data relevant to the model. Unless otherwise indicated, [N] = 40 µM and $\alpha = 0.2\ hr^{-1}$. Residuals were calculated using the function $rmsd_{S1} = \frac{2\left|[S]_m - [D]_m\right|}{[S]_m + [D]_m}$. Grey shading indicates experiments that are poorly simulated ($rmsd_{S1} > 1.0$).

Experimental Condition (Ref)	Experimental Measurement	Experimental Result	Simulation Details	Simulation vs. Experiment
WT at Fe-deficient, Fe-sufficient (14)	FET3 expression	4-5↑at Fe-deficient vs. Fe sufficient	$[Fe]_{med} = 4, 40$	$S_1 = \frac{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{N=4}}{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{N=40}} = 1.6$ $D_1 = 4.5$ $rmsd_{S1} = 0.95$
ΔYfh1				
ΔYfh1 (9, 20)	Fermenting $[Fe]_{mit}$	5-15 time higher than WT	$R_{isu} = 15$ µM/hr $\alpha = 0.07\ hr^{-1}$	$S_2 = \left(\frac{[Fe_{mit}]_{\Delta Yfh1}}{[Fe_{mit}]_{WT}}\right)_{N=40} = 16$ $D_2 = 10$ $rmsd_{S1} = 0.46$
ΔYfh1 (9)	mRNA levels of Mrs4	Levels were ~ 2.5 times increased relative to WT	$R_{isu} = 15$ µM/hr $\alpha = 0.07\ hr^{-1}$	$S_3 = \frac{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{mit}\right)_{\Delta\text{Yfh1}}}{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{mit}\right)_{\text{WT}}} = 1.8$ $D_3 = 2.5$ $rmsd_{S1} = 0.33$
ΔYfh1 (9)	$[^{55}Fe_{mit}], [^{55}Fe_{super}]$	Mitochondria from ΔYfh1 contained 40%-45% of cellular ^{55}Fe; mitochondria from WT cells contained 5 – 10% of cellular ^{55}Fe. (Ratio is ca. 42/7.5 = 5.7)	$R_{isu} = 15$ µM/hr $\alpha = 0.07\ hr^{-1}$	$S_4 = \frac{\left([Fe_{mit}]/[Fe_{cell}]\right)_{\Delta Yfh1}}{\left([Fe_{mit}]/[Fe_{cell}]\right)_{WT}} = 8.2$ $D_4 = 5.7$ $rmsd_{S1} = 0.36$
ΔYfh1 (9)	Aconitase and COX activities	Aconitase activity was 41% of WT COX activity was 74% of WT Ave = 58%	$R_{isu} = 15$ µM/hr $\alpha = 0.07\ hr^{-1}$	$S_5 = \frac{[FS]_{\Delta Yfh1}}{[FS]_{WT}} = 0.52$ $D_5 = 0.58$ $rmsd_{S1} = 0.11$
Cu treatment to mimic ISC mutant (46)	Mrs4 expression	2.1↑ vs. WT	$R_{isu} = 15$ µM/hr $\alpha = 0.07\ hr^{-1}$	$S_6 = \frac{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{mit}\right)_{Cu}}{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{mit}\right)_{WT}} = 1.8$ $D_6 = 2.1$ $rmsd_{S1} = 0.15$
ΔMrs3/4				

ΔMrs3/4 (9)	$[^{55}Fe_{mit}],[^{55}Fe_{super}]$	Total cellular Fe was about 1.7 times that of WT cells.	R_{mit} = 180 μM/hr	$S_7 = \frac{[Fe_{cell}]_{\Delta Mrs3/4}}{[Fe_{cell}]_{WT}} = .88$ $D_7 = 1.7$ $rmsd_{S1} = 0.64$
ΔMrs3/4 (9)	$[Fe]_{mit}$	Percentage of cellular iron in mito was 2.5% for ΔMrs3/4 and 4.0% for WT; the ratio was 0.62	R_{mit} = 180 μM/hr	$S_8 = \frac{([Fe_{mit}]/[Fe_{cell}])_{\Delta Mrs3/4}}{([Fe_{mit}]/[Fe_{cell}])_{WT}} = 1.0$ $D_8 = 0.62$ $rmsd_{S1} = 0.47$
ΔMrs3/4 (9)	Aconitase and COX activities	Aconitase activity was 60% of WT COX activity was 59% of WT Ave = 60%	R_{mit} = 180 μM/hr	$S_9 = \frac{[FS]_{\Delta Mrs3/4}}{[FS]_{WT}} = 0.82$ $D_9 = 0.60$ $rmsd_{S1} = 0.31$
ΔMrs3/4 (10)	FET3 expression FTR1 expression	FET3 = 2.2↑ vs. WT FTR1 = 1.9 ↑vs. WT (microarray)	R_{mit} = 180 μM/hr	$S_{10} = \frac{(\text{Reg}_{-C} \cdot \text{Reg}_{-FS} \cdot \text{R}_{cyt})_{\Delta Mrs3/4}}{(\text{Reg}_{-C} \cdot \text{Reg}_{-FS} \cdot \text{R}_{cyt})_{WT}} = .89$ $D_{10} = 2.0$ $rmsd_{S1} = 0.77$
ΔMrs3/4 (10)	[hemes], [Bio2] aconitase, SDH	Hemes: 1.4↓ vs. WT (was 2-fold down in Fe-starved cells) Bio2: 2.5↓ vs. WT (was 1.7 fold lower in Fe-depleted mitos) Aconitase unchanged SDH unchanged (Ave ca. 1.5 fold down)	R_{mit} = 180 μM/hr $[Fe]_{med}$ = 4	$S_{11} = \left(\frac{[FS]_{\Delta Mrs3/4}}{[FS]_{WT}}\right)_{[Femed]=4} = 0.82$ $D_{11} = 0.67$ $rmsd_{S1} = 0.20$
ΔMrs3/4 (10)	Aconitase and SDH activities	In mito preparations, no significant differences in the activities of aconitase and SDH.	R_{mit} = 180 μM/hr	$S_{12} = \left(\frac{[FS]_{\Delta Mrs3/4}}{[FS]_{WT}}\right)_{[Femed]=40} = 0.82$ $D_{12} = 1.0$ $rmsd_{S1} = 0.19$
ΔMrs3/4 (10)	$[Fe]_{mit}$	Mitochondria had 2.1 fold less Fe than WT mitochondria	R_{mit} = 180 μM/hr $[Fe]_{med}$ = 4	$S_{13} = \left(\frac{[Fe_{mit}]_{\Delta Mrs3/4}}{[Fe_{mit}]_{WT}}\right)_{[Femed]=4} = 0.89$ $D_{13} = 0.48$ $rmsd_{S1} = 0.59$
ΔMrs3/4 (10)	$[Fe]_{mit}$	In mitochondria, no substantial changes in iron accumulation relative to WT; heme formation was similar to WT	R_{mit} = 180 μM/hr	$S_{14} = \left(\frac{[Fe_{mit}]_{\Delta Mrs3/4}}{[Fe_{mit}]_{WT}}\right)_{[Femed]=40} = 0.89$ $D_{14} = 1.0$ $rmsd_{S1} = 0.12$
ΔMrs3/4 (36)	FET3, FRE1, FTR1 expression	FET3 = 4.7↑ vs. WT FRE1 = 4.1↑ vs. WT FTR1 =3.5↑ vs. WT Ave = 4.1	R_{mit} = 180 μM/hr	$S_{15} = \frac{(\text{Reg}_{-C} \cdot \text{Reg}_{-FS} \cdot R_{cyt})_{\Delta Mrs3/4}}{(\text{Reg}_{-C} \cdot \text{Reg}_{-FS} \cdot R_{cyt})_{WT}} = 0.89$ $D_{15} = 4.1$ $rmsd_{S1} = 1.3$

ΔMrs3/4 (36)	$[Fe]_{cell}$	2.8↑ vs. WT	R_{mit} = 180 μM/hr	$S_{16} = \frac{[Fe_{cell}]_{\Delta Mrs3/4}}{[Fe_{cell}]_{WT}} = 0.89$ $D_{16} = 2.8$ $rmsd_{S1} = 1.0$
ΔMrs3/4 (36)	$[Fe]_{vac}$	4.0↑ vs. WT	R_{mit} = 180 μM/hr	$S_{17} = \frac{[Fe_{vac}]_{\Delta Mrs3/4}}{[Fe_{vac}]_{WT}} = 0.84$ $D_{17} = 4.0$ $rmsd_{S1} = 1.3$
ΔMrs3/4 (36)	FET3 expression Iron uptake	FET3: 8.0↑ vs. WT Iron uptake: 14.8 times WT Ave: 11.4	R_{mit} = 180 μM/hr	$S_{18} = \frac{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{\Delta Mrs3/4}}{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{WT}} = 0.89$ $D_{18} = 11.4$ $rmsd_{S1} = 1.7$
ΔMrs3/4 (36)	$[Fe]_{cell}$	2.0↑ vs. WT	R_{mit} = 180 μM/hr	$S_{19} = \frac{[Fe_{cell}]_{\Delta Mrs3/4}}{[Fe_{cell}]_{WT}} = 0.89$ $D_{19} = 2.0$ $rmsd_{S1} = 0.77$
ΔMrs3/4 (36)	Aconitase activity	0.25 times WT	R_{mit} = 180 μM/hr	$S_{20} = \frac{[FS]_{\Delta Mrs3/4}}{[FS]_{WT}} = 0.82$ $D_{20} = 0.25$ $rmsd_{S1} = 1.1$
MRS3/4 **Overexpression**				
MRS3/4-over (35)	FET3 expression FTR1 expression	FET3 = 2.8↑ vs. WT FTR1 = 2.5↑ vs. WT (microarray)	R_{mit} = 2300 μM/hr $[Fe]_{med}$ = 4	$S_{21} = \frac{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{Mrs3/4over}}{\left(\text{Reg}_{\text{-C}} \cdot \text{Reg}_{\text{-FS}} \cdot R_{cyt}\right)_{WT}} = 0.61$ $D_{21} = 2.6$ $rmsd_{S1} = 1.2$
MRS3/4-over (35)	[hemes], [ISC] aconitase, SDH	Hemes: 1.7↑ vs. WT ISCs: 2↑ vs. WT Aconitase unchanged SDH unchanged	R_{mit} = 2300 μM/hr	$S_{22} = \left(\frac{[FS]_{Mrs3/4over}}{[FS]_{WT}}\right)_{[Femed]=4} = 1.1$ $D_{22} = 1.8$ $rmsd_{S1} = 0.48$
MRS3/4-over (35)	$[Fe]_{mit}$	2.5↑ vs. WT	R_{mit} = 2300 μM/hr $[Fe]_{med}$ = 4	$S_{23} = \left(\frac{[Fe_{mit}]_{Mrs3/4over}}{[Fe_{mit}]_{WT}}\right)_{[Femed]=4} = 1.2$ $D_{23} = 2.5$ $rmsd_{S1} = 0.70$
MRS3/4-up (36)	$[Fe]_{cell}$	$[Fe]_{cell}$ = 40% of WT	R_{mit} = 2300 μM/hr	$S_{24} = \frac{[Fe_{cell}]_{Mrs3/4UP}}{[Fe_{cell}]_{WT}} = 0.55$ $D_{24} = 0.40$ $rmsd_{S1} = 0.32$
ΔCcc1				

ΔCcc1 (36)	FET3 expression Iron uptake	FET3: 0.67 times WT Iron uptake: 0.75 times WT Ave: 0.7	R_{vac} = 510 μM/hr	$S_{25}=\frac{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{cell})_{\Delta CCC1}}{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{cell})_{WT}}=0.60$ $D_{25}=0.70$ $rmsd_{S1}=0.15$
ΔCcc1 (36)	$[Fe]_{cell}$	0.59 times WT	R_{vac} = 510 μM/hr	$S_{26}=\frac{[Fe_{cell}]_{\Delta CCC1}}{[Fe_{cell}]_{WT}}=0.60$ $D_{26}=0.59$ $rmsd_{S1}=0.02$
ΔCcc1 (36)	Aconitase activity	1.5 times WT	R_{vac} = 510 μM/hr	$S_{27}=\frac{[FS]_{\Delta CCC1}}{[FS]_{WT}}=1.1$ $D_{27}=1.5$ $rmsd_{S1}=0.31$
ΔCcc1 (12)	$[Fe_{vac}]$	27% of WT value	R_{vac} = 510 μM/hr	$S_{28}=\frac{[Fe_{vac}]_{\Delta CCC1}}{[Fe_{vac}]_{WT}}=0.54$ $D_{28}=0.27$ $rmsd_{S1}=0.67$
Ccc1 overexpression				
Ccc1 over (12)	$[Fe_{vac}]$	2.2 times greater than in WT vacuoles	R_{vac} = 20,000 μM/hr	$S_{29}=\frac{[Fe_{vac}]_{CCC1over}}{[Fe_{vac}]_{WT}}=2.2$ $D_{29}=2.2$ $rmsd_{S1}=0.0$
Ccc1 overexpression (12)	Aconitase activity	Reduced relative to WT	R_{vac} = 20,000 μM/hr	$S_{30}=\frac{[FS]_{CCC1over}}{[FS]_{WT}}=0.79$ $D_{30}=0.8$ $rmsd_{S1}=0.01$
Aft1/2-1^{UP}				
Aft1-1^{up} (16) Aft2-1^{up} ΔAft1ΔAft2	FET3 expression FTR1 expression	FET3: (25.2+3.7) ↑ Up vs. Δ FTR1: (5+2.6) ↑ Up vs. Δ FRE1: (4+3.5) ↑ Up vs. Δ Ave = 7.3	$[Fe]_{med}$ = 0.4 and 10,000	$S_{31}=\frac{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{cyt})_{N=0.4}}{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{cyt})_{N=10,000}}=2.7$ $D_{31}=7.3$ $rmsd_{S1}=0.92$
Aft1-1^{up} (15) Aft2-1^{up} ΔAft1ΔAft2	Mrs4 expression	3.5 ↑ Up (ave) vs. Δ	$[Fe]_{med}$ = 0.4 and 10,000	$S_{32}=\frac{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{mit})_{N=0.4}}{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{mit})_{N=10,000}}=2.0$ $D_{32}=3.5$ $rmsd_{S1}=0.55$
Aft1-1^{up} (15) Aft2-1^{up} ΔAft1ΔAft2	FET5 expression SMF3expression	~ 2.6↑ Up (ave) vs. Δ (Fet5) ~ 2↑ Up (ave) vs. Δ (Smf3)	$[Fe]_{med}$ = 0.4 and 10,000	$S_{33}=\frac{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{vac})_{N=10,000}}{(\text{Reg}_{\text{-C}}\cdot\text{Reg}_{\text{-FS}}\cdot R_{vac})_{N=0.4}}=574$ $D_{33}=2.3$ $rmsd_{S1}=1.9$
ΔYfh1ΔMrs3/4				

ΔYfh1ΔMrs3/4 (9)	$[^{55}Fe_{mit}],[^{55}Fe_{super}]$	Mitochondrial iron concentration is decreased to almost WT levels (Ratio is ca. 1.0)	R_{isu} = 15 μM/hr R_{mit} = 180 μM/hr α = 0.07 hr^{-1}	$S_{34}=\frac{[Fe_{mit}]/[Fe_{cell}]_{\Delta Yfh1\Delta Mrs3/4}}{[Fe_{mit}]/[Fe_{cell}]_{WT}}=7.1$ $D_{34}=1.2$ $rmsd_{S1}=1.4$
ΔYfh1ΔMrs3/4 (9)	$[^{55}Fe_{mit}],[^{55}Fe_{super}]$	Mitochondrial iron concentration is 13% of cellular Fe.	R_{isu} = 15 μM/hr R_{mit} = 180 μM/hr $[Fe_{med}]$ = 10000 μM α = 0.07 hr^{-1}	$S_{35}=\frac{[Fe_{mit}]_{\Delta Yfh1\Delta Mrs3/4}}{[Fe_{cell}]_{\Delta Yfh1\Delta Mrs3/4}}=0.83$ $D_{35}=0.13$ $rmsd_{S1}=1.5$
ΔYfh1ΔMrs3/4 (9)		Cellular Fe is about 3.5 times WT cellular iron level	R_{isu} = 15 μM/hr R_{mit} = 180 μM/hr $[Fe_{med}]$ = 10000 μM α = 0.07 hr^{-1}	$S_{36}=\frac{[Fe_{cell}]_{\Delta Yfh1\Delta Mrs3/4}}{[Fe_{cell}]_{\Delta Yfh1\Delta Mrs3/4}}=0.49$ $D_{36}=3.5$ $rmsd_{S1}=1.5$
ΔYfh1ΔMrs3/4 (9)	Aconitase and COX activities	Aconitase activity was 16% of WT COX activity was 45% of WT Ave = 30%	R_{isu} = 15 μM/hr R_{mit} = 180 μM/hr α = 0.07 hr^{-1}	$S_{37}=\frac{[FS]_{\Delta Yfh1\Delta Mrs3/4}}{[FS]_{WT}}=0.06$ $D_{37}=0.3$ $rmsd_{S1}=1.3$
ΔYfh1; MRS3/4over				
ΔYfh1;MRS3/4over (36)	$[Fe]_{mit}$	2.5 ↑ vs. ΔYfh1	R_{isu} = 15 μM/hr R_{mit} = 4000 μM/hr α = 0.07 hr^{-1}	$S_{38}=\frac{[Fe_{mit}]_{\Delta Yfh1-Mrs3/4UP}}{[Fe_{mit}]_{\Delta Yfh1}}=2.3$ $D_{38}=2.5$ $rmsd_{S1}=0.08$
ΔCcc1;ΔMrs3/4				
ΔCcc1;ΔMrs3/4 (36)	FET3, FRE1, FTR1 expression	FET3: 0.978 vs. WT FRE1: 1.374 vs WT FTR1: 0.834 vs. WT Ave: 1.1	R_{vac} = 510 μM/hr R_{mit} = 180 μM/hr	$S_{39}=\frac{(Reg_{-C}\cdot Reg_{-FS}\cdot R_{cell})_{\Delta CCC1\Delta Mrs3/4}}{(Reg_{-C}\cdot Reg_{-FS}\cdot R_{cell})_{WT}}=0.24$ $D_{39}=1.1$ $rmsd_{S1}=1.3$
ΔCcc1;ΔMrs3/4 (36)	FET3 expression Iron uptake	FET3: 4 times vs. WT Iron uptake: 3.4 times vs. WT Ave: 3.7 times vs. WT	R_{vac} = 510 μM/hr R_{mit} = 180 μM/hr	$S_{40}=\frac{(Reg_{-C}\cdot Reg_{-FS}\cdot R_{cell})_{\Delta CCC1\Delta Mrs3/4}}{(Reg_{-C}\cdot Reg_{-FS}\cdot R_{cell})_{WT}}=0.24$ $D_{40}=3.7$ $rmsd_{S1}=1.8$
ΔCcc1;ΔMrs3/4 (36)	Whole cell iron	1.6 times vs. WT	R_{vac} = 510 μM/hr R_{mit} = 180 μM/hr	$S_{41}=\frac{[Fe_{cell}]_{\Delta Yfh1\Delta Ccc1}}{[Fe_{cell}]_{WT}}=0.46$ $D_{41}=3.7$ $rmsd_{S1}=1.6$
ΔCcc1;ΔMrs3/4 (36)	$[Fe_{vac}]$	2 times vs. WT vacuoles	R_{vac} = 510 μM/hr R_{mit} = 180 μM/hr	$S_{42}=\frac{[Fe_{vac}]_{\Delta Yfh1\Delta Ccc1}}{[Fe_{vac}]_{WT}}=0.35$ $D_{42}=2.0$ $rmsd_{S1}=1.4$

ΔCcc1;ΔMrs3/4 (36)	Aconitase activity	0.81 times vs. WT	R_{vac} = 510 μM/hr R_{mit} = 180 μM/hr	$S_{43} = \frac{[FS]_{\Delta Yfh1 \Delta Ccc1}}{[FS]_{WT}} = 0.84$ $D_{43} = 0.81$ $rmsd_{S1} = 0.04$

Table S2 Data from previous Mössbauer-based studies. Data are from ref 6 and references therein, except for data of ISC40 which are from ref 22. Concentrations are given in μM. Residuals were calculated from the relationship $rmsd_{S2} = \frac{1}{6}\left(\sum_{j=FM,FS,MP,F3,VP,Fe^{II}} \frac{\left|[S]_j - [D]_j\right|}{[D]_{cell}}\right)$.

Experiment	[N]	[C]	[F2]	[F3]	[VP]	[FM]	[FS]	[MP]	[O2]	[ROS]	$[Fe^{II}]$	$[Fe_{cell}]$	$rmsd_{S2}$
BPS	4	2.3 S	130 S	450 S 1 D	44 S 1 D	98 S 34 D	170 S 330 D	190 S 48 D	2.2 S	190 S	44 S 56 D	200 S 150 D	0.95
WT1	7	2.6 S	120 S	690 S 400 D	68 S 120 D	100 S 150 D	180 S 550 D	190 S 50 D	2.1 S	190 S	42 S 60 D	270 S 250 D	0.61
WT10	16	3.0 S	120 S	1000 S 1000 D	98 S 1 D	110 S 150 D	290 S 110 D	190 S 310 D	2.0 S	190 S	42 S 65 D	370 S 400 D	0.16
WT40	46	3.3 S	110 S	1300 S 1200 D	120 S 180 D	110 S 150 D	200 S 210 D	190 S 250 D	1.9 S	190 S	42 S 44 D	410 S 440 D	0.10
WT100	110	3.4 S	110 S	1400 S 1400 D	140 S 80 D	120 S 170 D	200 S 220 D	190 S 190 D	1.8 S	190 S	42 S 30 D	460 S 470 D	0.053
WT1000	1000	3.6 S	110 S	1500 S 1400 D	140 S 120 D	120 S 130 D	200 S 220 D	190 S 200 D	1.8 S	190 S	42 S 26 D	490 S 460 D	0.046
WT10000	10000	3.6 S	110 S	1500 S 1500 D	150 S 140 D	120 S 110 D	200 S 140 D	190 S 200 D	1.8 S	190 S	42 S 40 D	490 S 440 D	0.044
ISC40 (20)	46	32 S	57 S	7.2 S 0 D	17 S 0 D	800 S 370 D	100 S 0 D	7000 S 7000 D	3.7 S	7000 S	120 S 20 D	830 S 1000 D	0.11
ΔCcc1-1	7	3.0 S	61 S	110 S 36 D	33 S 36 D	57 S 390 D	220 S 390 D	84 S 240 D	1.6 S	84 S	23 S 27 D	89 S 150 D	0.82
ΔCcc1-10	16	3.3 S	93 S	180 S 210 D	53 S 40 D	58 S 340 D	230 S 340 D	84 S 210 D	1.6 S	84 S	31 S 54 D	120 S 210 D	0.47
ΔCcc1-20	26	3.5 S	110 S	220 S 330 D	64 S 92 D	59 S 310 D	230 S 310 D	84 S 190 D	1.6 S	84 S	35 S 98 D	140 S 280 D	0.37
ΔCcc1-40	46	3.6 S	120 S	250 S 460 D	74 S 240 D	59 S 320 D	230 S 320 D	84 S 200 D	1.6 S	84 S	39 S 110 D	180 S 360 D	0.42
CCC1-UP-1	7	1.7 S	940 S	190 S 180 D	270 S 180 D	83 S 120 D	150 S 120 D	190 S 74 D	2.6 S	190 S	240 S 130 D	400 S 250 D	0.27
CCC1-UP-10	16	1.9 S	1400 S	350 S 370 D	500 S 520 D	88 S 140 D	160 S 140 D	190 S 87 D	2.4 S	190 S	350 S 300 D	600 S 560 D	0.079
CCC1-UP-20	26	2.0 S	1600 S	430 S 610 D	620 S 1400 D	90 S 270 D	160 S 270 D	190 S 170 D	2.4 S	190 S	400 S 410 D	700 S 980 D	0.22
CCC1-UP-40	46	2.0 S	1800 S	520 S 1500 D	740 S 2100 D	92 S 440 D	160 S 440 D	190 S 270 D	2.3 S	190 S	450 S 830 D	800 S 1800 D	0.31
CCC1-UP-1+adenine	7	1.9 S	53 S	600 S 160 D	660 S 170 D	88 S 130 D	160 S 130 D	190 S 80 D	2.4 S	190 S	2 S 270 D	370 S 380 D	0.59
CCC1-UP-	46	2.6 S	16 S	1000 S	1100 S	100 S	180 S	190 S	2.0 S	190 S	16 S	600 S	0.21

40+adenine				1900 D	1200 D	230 D	230 D	140 D			190 D	1000 D	

Table S3: Optimized Parameters and sensitivities. * means nearly optimized.

Parameter	Optimized Value	Unit	Sensitivity
R_{mit}	900	$\mu M \cdot hr^{-1}$	1.15
R_{mit}(ΔMrs3/4)	180	"	1.00
R_{mit}(Mrs3/4-UP)	2300	"	1.00
R_{mit}(ΔYfh1;Mrs3/4-UP)	4000*	"	1.04
k_{O2}	52	hr^{-1}	1.08
k_{res}	150	hr^{-1}	1.07
k_{mp}	0.18	$\mu M^{-1} hr^{-1}$	1.06
K_{mit}	12	µM	1.08
K_{O2}	9	"	1.07
K_{isu}	610	"	1.07
K_{cyt}	14	"	1.00
K_{vac}	5.5	"	1.00
$[FS]_{spcyt}$	190	µM	1.08
$[FS]_{spmit}$	200	"	1.09
$[FS]_{spvac}$	190	"	1.02
$[FS]_{spvac}$(ΔCcc1)	170*	"	1.00
$[FS]_{spvac}$(CCC1-UP)	170	"	1.00
$[FS]_{spvac}$(UP+Ad)	140	"	1.01
$[FS]_{sp23}$	210	"	1.00
$[FS]_{sp23}$(ΔCcc1)	110	"	1.00
$[FS]_{sp23}$(CCC1-UP)	150	"	1.00
$[FS]_{sp23}$(CCC1-UP+Ad)	190	"	1.01
$[C]_{spcyt}$	27	µM	1.05
$[C]_{spmit}$	80*	"	1.00
$[C]_{spvac}$	1.2	"	1.00
$[C]_{spvac}$(ΔCcc1)	4.2	"	1.00
$[C]_{spvac}$(CCC1-UP)	0.2	"	1.00
$[C]_{spvac}$(CCC1-UP+Ad)	0.5*	"	1.00
$[C]_{sp23}$	2	"	1.02
$[C]_{sp23(\Delta Ccc1)}$	2.3	"	1.00
$[C]_{sp23(CCC1\text{-}UP)}$	0.14	"	1.00
$[C]_{sp23(CCC1\text{-}UP+Ad)}$	2	"	1.05
fssenmit	4	none	1.03
fssencyt	9	"	1.00
fssenvac	8	"	1.00
fssenvac(ΔCcc1)	7	"	1.00
fssenvac(CCC1-up)	10	"	1.00
fssenvac(UP+Ad)	10	"	1.00
fssen23	10	"	1.00
fssen23(ΔCCC1)	10	"	1.00
fssens23(CCC1-UP)	10	"	1.00
fsens23(UP+Ad)	7	"	1.01
csenmit	5	none	1.00
csencyt	5	"	1.00
csenvac	3	"	1.00
csenvac(ΔCcc1)	5	"	1.00
csenvac(CCC1-up)	4	"	1.00
csenvac(UP+Ad)	4	"	1.00

csen23	10	"	1.00
csen23(ΔCCC1)	2	"	1.00
csens23(CCC1-UP)	5	"	1.00
csens23(UP+Ad)	10	"	1.01
R_{vac}	2200	$\mu M \cdot hr^{-1}$	1.01
$R_{vac(\Delta ccc1)}$	820 (S2) 510 (S1)	"	1.00 1.01
$R_{vac(CCC1\text{-}UP)}$	7900 (S2) 20,000* (S1)	"	1.00 1.00
$R_{vac(CCC1\text{-}UP+Ad)}$	2200	"	1.00
R_{cyt}	410	$\mu M \cdot hr^{-1}$	1.00
R_{isu}	520	$\mu M \cdot hr^{-1}$	1.00
R_{isu}(ΔYfh1)	15	"	1.03
k_{23}	7.3	hr^{-1}	1.00
$k_{23(\Delta Ccc1)}$	0.74	"	1.00
$k_{23(CCC1\text{-}up)}$	0.20	"	1.00
$k_{23(CCC1\text{-}UP+Ad)}$	66	"	1.00
k_{vp}	0.02	hr^{-1}	1.00
$k_{vp(\Delta Ccc1)}$	0.06	"	1.00
$k_{vp(Ccc1\text{-}UP)}$	0.29	"	1.00
$k_{vp(Ccc1\text{-}UP+Ad)}$	0.22	"	1.00
k_{ros}	0.014	$\mu M^{-1}hr^{-1}$	Fixed
RO2 (aerobic)	100	μM	Fixed
RO2 (anaerobic)	1	μM	Fixed
α_{health}	0.20	hr^{-1}	Fixed
$\alpha_{disease}$	0.07	hr^{-1}	Fixed
f_{cyt}	0.65	none	Fixed
f_{mit}	0.10	none	Fixed
f_{vac}	0.25	none	Fixed

A. Derivation of Product Reg Functions

This derivation assumes that X-S and Fe_{cyt} react to generate the Grx:Fe_2S_2:Grx homodimer, and that only the apo-form of Grx is active. Following the nomenclature in the text, X-S = FS and Fe_{cyt} = C. Also, A = the active form of Aft1/2; inactive forms include AC, AFS and AFSC. We assume that either substrate can bind first and that the binding strength does not change when the other substrate is bound. The dissociation equilibrium expressions for these binding events are…

$$AC \rightleftarrows A + C \quad K_C = \frac{A \cdot C}{AC}$$

$$AFS \rightleftarrows A + FS \quad K_{FS} = \frac{A \cdot FS}{AFS}$$

$$AFSC \rightleftarrows AFS + C \quad K_C = \frac{AFS \cdot C}{AFSC}$$

$$AFSC \rightleftarrows AC + FS \quad K_{FS} = \frac{AC \cdot FS}{AFSC}$$

The fraction of A_{tot} that is active depends on these binding constants and the concentrations of FS and C, as follows.

$$A_{tot} = A + AFS + AC + AFSC$$

$$A_{tot} = A + \frac{A \cdot FS}{K_{FS}} + \frac{A \cdot C}{K_C} + \frac{AFS \cdot C}{K_C}$$

$$A_{tot} = A + \frac{A \cdot FS}{K_{FS}} + \frac{A \cdot C}{K_C} + \frac{A \cdot C \cdot FS}{K_C \cdot K_{FS}}$$

$$A_{tot} = A\left(1 + \frac{FS}{K_{FS}} + \frac{C}{K_C} + \frac{C \cdot FS}{K_C \cdot K_{FS}}\right)$$

$$A_{tot} = A\left(1 + \frac{FS}{K_{FS}}\right)\left(1 + \frac{C}{K_C}\right)$$

$$\frac{A}{A_{tot}} = \frac{1}{\left(1 + \frac{FS}{K_{FS}}\right)} \frac{1}{\left(1 + \frac{C}{K_C}\right)}$$

$$\frac{A}{A_{tot}} = \mathrm{Re}\, g_{-FS} \cdot \mathrm{Re}\, g_{-C}$$

B. Derivation of cellular iron concentrations

Multiply the ODEs associated with each of the 7 Fe-containing species in the model ([2] – [8]) by the fractional volume associated with each species whose concentration-change is described by the ODE. For example, both sides of ODE [2] are multiplied by fractional cytosolic volume (V_{cyt}/V_{cell}) because the component described by that ODE, namely C, is found in the cytosol. We have…

$$\frac{V_{cyt}}{V_{cell}}\frac{d[C]}{dt}=\frac{V_{cyt}}{V_{cell}}\frac{R_{cyt}[N]}{K_{cyt}+[N]}\mathrm{Reg}_{-C}\cdot\mathrm{Reg}_{-FS}-\frac{V_{mit}}{V_{cell}}\frac{R_{mit}[C]}{K_{mit}+[C]}\mathrm{Reg}_{-C}\cdot\mathrm{Reg}_{-FS}-\frac{V_{vac}}{V_{cell}}\frac{R_{vac}[C]}{K_{vac}+[C]}\mathrm{Reg}_{+C}\cdot\mathrm{Reg}_{+FS}-\frac{V_{cyt}}{V_{cell}}\alpha[C]$$

$$\frac{V_{mit}}{V_{cell}}\frac{d[FM]}{dt}=\frac{V_{mit}}{V_{cell}}\frac{R_{mit}[C]}{K_{mit}+[C]}\mathrm{Reg}_{-C}\cdot\mathrm{Reg}_{-FS}-\frac{V_{mit}}{V_{cell}}\frac{R_{isu}[FM]}{K_{isu}+[FM]}-\frac{V_{mit}}{V_{cell}}k_{mp}[FM][O2]-\frac{V_{mit}}{V_{cell}}\alpha[FM]$$

$$\frac{V_{vac}}{V_{cell}}\frac{d[F2]}{dt}=\frac{V_{vac}}{V_{cell}}\frac{R_{vac}[C]}{K_{vac}+[C]}\mathrm{Reg}_{+C}\cdot\mathrm{Reg}_{+FS}-\frac{V_{vac}}{V_{cell}}k_{23}[F2]\mathrm{Reg}_{+C}\cdot\mathrm{Reg}_{+FS}-\frac{V_{vac}}{V_{cell}}\alpha[F2]$$

$$\frac{V_{mit}}{V_{cell}}\frac{d[FS]}{dt}=\frac{V_{mit}}{V_{cell}}\frac{R_{isu}[FM]}{K_{isu}+[FM]}-\frac{V_{mit}}{V_{cell}}\alpha[FS]$$

$$\frac{V_{mit}}{V_{cell}}\frac{d[MP]}{dt}=\frac{V_{mit}}{V_{cell}}k_{mp}[FM][O2]-\frac{V_{mit}}{V_{cell}}\alpha[MP]$$

$$\frac{V_{vac}}{V_{cell}}\frac{d[F3]}{dt}=\frac{V_{vac}}{V_{cell}}k_{23}[F2]\mathrm{Reg}_{+C}\cdot\mathrm{Reg}_{+FS}-\frac{V_{vac}}{V_{cell}}k_{vp}[F3]-\frac{V_{vac}}{V_{cell}}\alpha[F3]$$

$$\frac{V_{vac}}{V_{cell}}\frac{d[VP]}{dt}=\frac{V_{vac}}{V_{cell}}k_{vp}[F3]-\frac{V_{vac}}{V_{cell}}\alpha[VP]$$

The sum of these terms on the left-hand-side of the ODEs is

$$\frac{V_{cyt}}{V_{cell}}\frac{d[C]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[FM]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[FS]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[MP]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[F2]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[F3]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[VP]}{dt}$$

This is identical to the derivative of the overall mass balance equation [1].

$$\frac{d[Fe_{cell}]}{dt}=\frac{V_{cyt}}{V_{cell}}\frac{d[C]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[FM]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[FS]}{dt}+\frac{V_{mit}}{V_{cell}}\frac{d[MP]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[F2]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[F3]}{dt}+\frac{V_{vac}}{V_{cell}}\frac{d[VP]}{dt}$$

Indicating that the left-hand-side of the ODEs is equal to $\frac{d[Fe_{cell}]}{dt}$.

The sum of the last terms of the right-hand-side of the ODEs affords

$$-\frac{V_{cyt}}{V_{cell}}\alpha[C]-\frac{V_{mit}}{V_{cell}}\alpha[FM]-\frac{V_{mit}}{V_{cell}}\alpha[FS]-\frac{V_{mit}}{V_{cell}}\alpha[MP]-\frac{V_{vac}}{V_{cell}}\alpha[F2]-\frac{V_{vac}}{V_{cell}}\alpha[F3]-\frac{V_{vac}}{V_{cell}}\alpha[VP]$$

Simplifying yields

$$-\alpha\left(\frac{V_{cyt}}{V_{cell}}[C]+\frac{V_{mit}}{V_{cell}}[FM]+\frac{V_{mit}}{V_{cell}}[FS]+\frac{V_{mit}}{V_{cell}}[MP]+\frac{V_{vac}}{V_{cell}}[F2]+\frac{V_{vac}}{V_{cell}}[F3]+\frac{V_{vac}}{V_{cell}}[VP]\right)$$

The sum of the terms within the parentheses is identical to the right-hand-side of [1], such that we can simply to $-\alpha\cdot[Fe_{cell}]$. Most (but not all) of the remaining terms of the summed ODEs cancel, finally yielding the equation

$$\frac{d[Fe_{cell}]}{dt}=\frac{V_{cyt}}{V_{cell}}\frac{\mathrm{R}_{cyt}\cdot[N]}{K_{cyt}+[N]}\mathrm{Re}\,g_{-C}\cdot\mathrm{Re}\,g_{-FS}-\alpha\cdot[Fe_{cell}]$$