Supplemental Materials for "Catalytic Coupling of Oxidative Phosphorylation, ATP Demand, Oxygen Availability, and Mitochondrial Reactive Oxygen Species Generation"

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The Supplemental Materials presents a detailed description of the integrated model used to simulate the results in the main paper. For convenience, the model code is included as supplemental material.

Integrated Model of Mitochondrial Bioenergetics and ROS Dynamics

The present model is extended from the previous model of mitochondrial bioenergetics [1]. Several flux expressions and model parameters were updated based on the recent works of Bazil et al. [2, 3], as laid out in this model supplemental material section. This supplement consists of four parts. Part 1 lists all the state variables comprising the model, dissociation constants, and physiochemical and general model parameters. Part 2 introduces the set of nine non-linear ODEs, four algebraic conservation expressions (for mitochondrial ATP, NADH and UQH_2 and inter-membrane space c^{2+}), and six non-linear cation ODEs (mitochondrial and extra-mitochondrial H^+, K^+, and Mg^{2+}). Part 3 presents the model rate equations and the associated parameter definitions and values. Part 4 introduces the code used to simulate the model and generate the plots given in the paper.

The outer-mitochondrial membrane (OMM) is highly permeable to ions, metabolites and substrates of low molecular weight under the given conditions. As such, all permeable inter-membrane space (IMS) state variables were replaced with their respective extra-mitochondrial counterparts, and thus not included in the model. This has the benefit of making the system of non-linear ODEs less stiff and shortens simulation time without significantly altering the simulation results. The only IMS state variables explicitly simulated in the model are oxidized and reduced forms of cytochrome c (c^{3+} and c^{2+}).

S1 - Model State Variables, Dissociation Constants, and Physiochemical and General Parameter Values

The state variables used in the model, their definitions, and their units are provided in Table S1.1. All the dissociation constants used in the model are presented in Table S1.2. Most of the

dissociation constants are taken from Li et al. [4] and corrected for appropriate temperature (T = 37 ^{o}C) and ionic strength (I = 0.17 M), corresponding to the present experiments. The physiochemical and general model parameters are presented in Table S1.3.

Table S1.1. Model State Variables

State Variable	Definition	Units
$\Delta\Psi$	Mitochondrial membrane potential	mV
Mitochondrial State Variables		
$[H^+]_x$	Mitochondrial free proton concentration	M
$[K^+]_x$	Mitochondrial free potassium concentration	M
$[Mg^{2+}]_x$	Mitochondrial free magnesium concentration	M
$[ATP]_x$	Total mitochondrial ATP concentration	M
$[ADP]_x$	Total mitochondrial ADP concentration	M
$[Pi]_x$	Total mitochondrial Pi concentration	M
$[NADH]_x$	Total mitochondrial NADH concentration	M
$[NAD]_x$	Total mitochondrial NAD concentration	M
$[UQH_2]_x$	Total mitochondrial ubiquinol concentration	M
$[UQ]_x$	Total mitochondrial ubiquinone concentration	M
$[O_2^{\cdot -}]_x$	Total mitochondrial superoxide concentration	M
$[H_2O_2]_x$	Total mitochondrial hydrogen peroxide concentration	M
Intermembrane Space (IMS) State Variables		
$[c^{2+}]_i$	Total IMS cytochrome c^{2+} (reduced) concentration	M
$[c^{3+}]_i$	Total IMS cytochrome c^{3+} (oxidized) concentration	M
Extra-Mitochondrial State Variables		
$[H^+]_e$	Extra-mitochondrial free proton concentration	M
$[K^+]_e$	Extra-mitochondrial free potassium concentration	M
$[Mg^{2+}]_e$	Extra-mitochondrial free magnesium concentration	M
$[ATP]_e$	Total extra-mitochondrial ATP concentration	M
$[ADP]_e$	Total extra-mitochondrial ADP concentration	M
$[AMP]_e$	Total extra-mitochondrial AMP concentration	M
$[Pi]_e$	Total extra-mitochondrial Pi concentration	M
$[O_2^{\cdot -}]_e$	Total extra-mitochondrial superoxide concentration	M
$[H_2O_2]_e$	Total extra-mitochondrial hydrogen peroxide concentration	M
$[CrP]_e$	Total extra-mitochondrial creatine phosphate concentration	M
$[Cr]_e$	Total extra-mitochondrial creatine concentration	M

Table S1.2. Dissociation Constants at 37 ºC, I = 0.17 M

Parameter	Definition	Value	Reference
K_{ATP}^{H}	Proton ATP binding constant	$10^{-6.33}$ M	[4]
K_{ATP}^{K}	Potassium ATP binding constant	$10^{-1.02}$ M	[4]
K_{ATP}^{Mg}	Magnesium ATP binding constant	$10^{-3.88}$ M	[4]
K_{ADP}^{H}	Proton ADP binding constant	$10^{-6.26}$ M	[4]
K_{ADP}^{K}	Potassium ADP binding constant	$10^{-0.89}$ M	[4]
K_{ADP}^{Mg}	Magnesium ADP binding constant	$10^{-3.00}$ M	[4]
K_{Pi}^{H}	Proton Pi binding constant	$10^{-6.62}$ M	[4]
K_{Pi}^{K}	Potassium Pi binding constant	$10^{-0.42}$ M	[4]
K_{Pi}^{Mg}	Magnesium Pi binding constant	$10^{-1.66}$ M	[4]
K_{MOPS}^{H}	Proton MOPS binding constant	$10^{-7.14}$ M	[a]

[a] NIST Standard Reference Database 46.

Table S1.3. Physiochemical and General Model Parameters[a]

Parameter	Definition	Value	Reference
R	Ideal gas constant	8.314×10^{-3} kJ/K/mol	Physical constant
T	Temperature	310.15 K	Physical constant
F	Faraday's constant	96.487×10^{-3} kJ/mV/mol	Physical constant
C_{mito}	IMM capacitance	6.76×10^{-6} mol/s/l mito/mV	[1]
N_{tot}	Total NAD concentration	3 mM	[1]
Q_{tot}	Total UQ concentration	20 mM	[3]
C_{tot}	Total CytC concentration	2.7 mM	[1]
A_{tot}	Total AdN concentration	10 mM	[1]
Cr_{tot}	Total creatine concentration	20 mM	[5]
ρ_m	Protein density of mitochodnria	2.725×10^{5} mg/l mito	[6]
CS_m	Citrate synthase activity per mg mitochondria	5.62 U CS / mg	-
Vol_m	Matrix H_2O volume fraction	0.65 l mtx/l mito	[b]
Vol_i	IMS H_2O volume fraction	0.072 l ims/l mito	[b]
Vol_e	buffer H_2O volume fraction	4.54×10^{3} l buffer/l mito	[c]
[$MOPS$]	Extra-mitochondrial MOPS concentration	20 mM	-
$d_f G^0_{H_2O_2}$	H_2O_2 formation energy	-134.03 kJ/mol	[7]
$d_f H^0_{H_2O_2}$	H_2O_2 formation enthalpy	-194.17 kJ/mol	[7]
z_{H2O2}	H_2O_2 valence	0	[7]
nH_{H2O2}	Number of protons	2	[7]
$d_f G^0_{O_2^{\cdot-}}$	$O_2^{\cdot-}$ formation energy	13.18 kJ/mol	[8]
$d_f H^0_{O_2^{\cdot-}}$	$O_2^{\cdot-}$ formation enthalpy	0 kJ/mol	[8]
$z_{O2\cdot-}$	$O_2^{\cdot-}$ valence	-1	[8]
$nH_{O2\cdot-}$	Number of protons	0	[8]

Abbreviations: IMM, inner mitochondrial membrane; NAD, nicotinamide adenine dinucleotide; UQ, ubiquinone; CytC, cytochrome c; AdN, adenine nucleotide.

[a] All thermodynamic data obtained from Li et al. [4]. Additional data is included in this table.

[b] Approximate mitochondrial matrix and IMS volumes.

[c] Based on a mitochondrial load of 0.67 U CS in a 2 ml chamber.

S2 - Model Differential-Algebraic Equations

S2.2A - Model Differential Equations (Bioenergetics)

Mitochondrial Membrane Potential:

$$\frac{d\Delta\Psi}{dt} = \left(4J_{C1} + 2J_{C3} + 4J_{C4} - nH_{F_1F_O}J_{F_1F_O} - J_{ANT} - J_{Hleak} - 4J_{CU} - J_{RaM} - J_{NCE}\right)/C_{mito} \quad (2.1)$$

Mitochondrial State Variables:

$$\frac{d[ADP]_x}{dt} = \left(J_{ANT} - J_{F_1F_O}\right)/Vol_m \quad (2.2)$$

$$\frac{d[Pi]_x}{dt} = \left(J_{PIC} - J_{F_1F_O}\right)/Vol_m \quad (2.3)$$

$$\frac{d[NAD]_x}{dt} = \left(J_{C1} - J_{DH}\right)/Vol_m \quad (2.4)$$

$$\frac{d[UQ]_x}{dt} = \left((2-1)J_{C3} + \alpha_{C2}J_{DH} - J_{C1}\right)/Vol_m \quad (2.5)$$

$$\frac{d[\mathrm{O_2^{\cdot -}}]_x}{dt} = \left(\frac{1}{2}J_{C3}^{SO} + J_{C1}^{SO}\right)/Vol_m - 2J_{MnSOD} \quad (2.6)$$

$$\frac{d[\mathrm{H_2O_2}]_x}{dt} = J_{C1}^{H2O2}/Vol_m + J_{MnSOD} - J_{H2O2} - J_{H2O2perm} \quad (2.7)$$

Inter-Membrane Space (IMS) State Variables:

$$\frac{d[\mathrm{c}^{3+}]_i}{dt} = \left(2J_{C4} - 2J_{C3}\right)/Vol_i \quad (2.8)$$

Extra-Mitochondrial State Variables:

$$\frac{d[ATP]_e}{dt} = -J_{ATP}/Vol_e - J_{ATPase} \quad (2.9)$$

$$\frac{d[ADP]_e}{dt} = -J_{ADP}/Vol_e + J_{ATPase} \quad (2.10)$$

$$\frac{d[Pi]_e}{dt} = -J_{Pi}/Vol_e + J_{ATPase} \quad (2.11)$$

$$\frac{d[\mathrm{O_2^{\cdot -}}]_e}{dt} = \frac{1}{2}J_{C3}^{SO}/Vol_e - 2J_{SOD} \quad (2.12)$$

$$\frac{d[\mathrm{H_2O_2}]_e}{dt} = J_{SOD} + J_{H2O2perm}Vol_m/Vol_e \quad (2.13)$$

S2.2B - Mitochondrial Conservation Algebraic Equations

The mitochondrial species for adenine nucleotides (AdNs), nicotinamide adenine dinucleotides (NADH), ubiquinone (UQH_2), and reduced cytochrome c ($CytC^{2+}$) are conserved in the model. The conservation is implemented by using an algebraic expression to govern the conservation.

This is done using a mass matrix with the integration algorithm and setting the appropriate rows equal to a zero row vector.

$$[ATP]_m = A_{tot} - [ADP]_m \tag{2.14}$$

$$[NADH]_m = N_{tot} - [NAD]_m \tag{2.15}$$

$$[UQH_2]_m = Q_{tot} - [UQ]_m \tag{2.16}$$

$$[CytC^{2+}]_i = C_{tot} - [CytC^{3+}]_i \tag{2.17}$$

$$[\mathrm{PCr}]_e = CR_{tot} - [Cr]_e \tag{2.18}$$

S2.2C - Model Differential Equations (Cations)

The cation differential equations for the mitochondrial and extra-mitochondrial compartments are derived using the method outlined in Vinnakota et al. [9]. The general method will be presented with the understanding that compartment specific concentrations and transport rates are to be used where appropriate. In some cases, these compartment specific equations will be given. Due to the large expressions resulting from the derivation, the method used to obtain them is presented versus explicitly showing all the terms that enter the differential equations. The equations used in the model are obtained by solving the linear system of equations given in Equation 2.19.

The generalized system of equations relating the cation differential equations is

$$\begin{bmatrix} \frac{d[H^+]}{dt} \\ \frac{d[K^+]}{dt} \\ \frac{d[Mg^{2+}]}{dt} \end{bmatrix} = \begin{bmatrix} 1+H_{\partial H} & H_{\partial K} & H_{\partial Ca} \\ K_{\partial H} & 1+K_{\partial K} & K_{\partial Mg} \\ Mg_{\partial H} & Mg_{\partial K} & 1+Mg_{\partial Mg} \end{bmatrix}^{-1} \begin{bmatrix} \Phi_H \\ \Phi_K \\ \Phi_{Mg} \end{bmatrix}, \tag{2.19}$$

where $X_{\partial Y}$ is the partial derivative of the concentration of *bound* X with respect to Y (Y can equal X) and Φ_X is the flux of X into or out of the compartment.

Assuming higher order cation binding is negligible, the partial derivative expressions are defined below where N_r is the number of reactants, L_i is the i^{th} ligand, K_i^j is the dissociation constant for the i^{th} ligand and j^{th} cation couple and P_i is the binding polynomial for the ith ligand as originally defined by Alberty [7].

$$H_{\partial H} = \frac{\partial [H_{bound}]}{\partial [H^+]} = \sum_{i=1}^{Nr} \frac{[L_i]\left(1+[Na^+]/K_i^{Na}+[K^+]/K_i^{K}+[Mg^{2+}]/K_i^{Mg}+[Ca^{2+}]/K_i^{Ca}\right)}{K_i^H P_i^2} \tag{2.20}$$

$$H_{\partial K} = \frac{\partial [H_{bound}]}{\partial [K^+]} = -[H^+] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^H}{K_i^K P_i^2} \tag{2.21}$$

$$H_{\partial Mg} = \frac{\partial [H_{bound}]}{\partial [Mg^{2+}]} = -[H^+] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^H}{K_i^{Mg} P_i^2} \tag{2.22}$$

$$K_{\partial H} = \frac{\partial [K_{bound}]}{\partial [H^+]} = -[K^+] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^K}{K_i^H P_i^2} \tag{2.23}$$

$$K_{\partial K} = \frac{\partial [K_{bound}]}{\partial [K^+]} = \sum_{i=1}^{Nr} \frac{[L_i] \left(1 + [H^+] / K_i^H + [Na^+] / K_i^{Na} + [Mg^{2+}] / K_i^{Mg} + [Ca^{2+}] / K_i^{Ca}\right)}{K_i^K P_i^2} \tag{2.24}$$

$$K_{\partial Mg} = \frac{\partial [K_{bound}]}{\partial [Mg^{2+}]} = -[K^+] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^K}{K_i^{Mg} P_i^2} \tag{2.25}$$

$$Mg_{\partial H} = \frac{\partial [Mg_{bound}]}{\partial [H^+]} = -[Mg^{2+}] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^{Mg}}{K_i^H P_i^2} \tag{2.26}$$

$$Mg_{\partial K} = \frac{\partial [Mg_{bound}]}{\partial [K^+]} = -[Mg^{2+}] \sum_{i=1}^{Nr} \frac{[L_i] / K_i^{Mg}}{K_i^K P_i^2} \tag{2.27}$$

$$Mg_{\partial Mg} = \frac{\partial [Mg_{bound}]}{\partial [Mg^{2+}]} = \sum_{i=1}^{Nr} \frac{[L_i] \left(1 + [H^+] / K_i^H + [Na^+] / K_i^{Na} + [K^+] / K_i^K + [Ca^{2+}] / K_i^{Ca}\right)}{K_i^{Mg} P_i^2} \tag{2.28}$$

and

$$P_i = 1 + [H^+] / K_i^H + [K^+] / K_i^K + [Mg^{2+}] / K_i^{Mg} . \tag{2.29}$$

Additional Buffering in the Mitochondrial Compartment

To account for additional buffering not attributed to metabolites and substrates in the mitochondrial compartment, Eqs. 2.20 is modified by adding the following terms:

$$H_{\partial H} = H_{\partial H} + \frac{[B_H]}{K_H\left(1+[H^+]/K_H\right)^2} \tag{2.30}$$

$$Mg_{\partial Mg} = Mg_{\partial Mg} + \frac{[B_{Mg}]}{K_{Mg}\left(1+[Mg^{2+}]/K_{Mg}\right)^2} \tag{2.31}$$

Table S2.1. Additional Mitochondrial Buffering Parameters

Parameter	Definition	Value	Reference
$[B_H]$	Total H^+ binding sites	20 mM	[10]
K_H	H^+ binding constant	$10^{-7.5}$ M	[10]
$[B_{Mg}]$	Total Mg^{2+} binding sites	8.7 mM	[11][a]
K_{Mg}	Mg^{2+} binding constant	340 µM	[11]

[a] based on 32 nmol/mg

Flux Terms

The terms representing flux into a given compartment are defined as

$$\Phi_H = -\sum_{i=1}^{Nr}\frac{\partial[H_{bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} + \sum_{k=1}^{Nk} n_k J_k + J_t^H \tag{2.32}$$

$$\Phi_K = -\sum_{i=1}^{Nr}\frac{\partial[K_{bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} + J_t^K \tag{2.33}$$

and

$$\Phi_{Mg} = -\sum_{i=1}^{Nr}\frac{\partial[Mg_{bound}]}{\partial[L_i]}\frac{d[L_i]}{dt} + J_t^{Mg} \tag{2.34}$$

where N_k is the number of reactions, n_k is the stoichiometric coefficient of k^{th} reaction, J_k is the k^{th} reaction rate and J_t^i is the i^{th} cation transport rate into the compartment.

The ligand dependent partial derivative expressions are defined as

$$\frac{\partial[H_{bound}]}{\partial[L_i]} = [H^+]\sum_{i=1}^{Nr}\frac{1}{K_i^H P_i} \tag{2.35}$$

$$\frac{\partial[K_{bound}]}{\partial[L_i]} = [K^+]\sum_{i=1}^{Nr}\frac{1}{K_i^K P_i} \tag{2.36}$$

and

$$\frac{\partial[Mg_{bound}]}{\partial[L_i]} = [Mg^{2+}]\sum_{i=1}^{Nr}\frac{1}{K_i^{Mg} P_i} \tag{2.37}$$

Compartment Reactions and Fluxes

The generation of protons by biochemical reactions in the mitochondria is defined as

$$\sum_{k=1}^{Nk} n_k J_k = J_{DH} \tag{2.38}$$

.

This term is zero in the extra-mitochondrial compartment.

The transport of cations into the mitochondrial compartment are defined as

$$J_t^H = \left(\left(nH_{F_1F_O} - 1\right)J_{F_1F_O} + 2J_{PIC} + J_{KHE} + J_{Hleak} - 5J_{C1} - 2J_{C3} - 4J_{C4}\right) \tag{2.39}$$

$$J_t^K = -J_{KHE} \tag{2.40}$$

and

$$J_t^{Mg} = 0 \tag{2.41}$$

The transport terms for the extra-mitochondrial compartment are the negative of their mitochondrial counterparts.

S3: Model Rate Equations

Each reaction rate and transport mechanism in the model is presented below. First, the electron transport related reaction rates are shown. Next, the oxidative phosphorylation related reaction rates and transport mechanisms are discussed. Finally, the cation transport and ROS scavenging mechanisms are presented.

S3A - Electron Transport Related Reaction Rates

Substrate oxidation and the electron transport system are based on the rate equations presented in Beard [1]. The mitochondrial dehydrogenase rate (substrate oxidation rate) will be introduced first, followed by the electron transport system rate equations and finally the passive proton leak rate equation is presented.

Mitochondrial Dehydrogenases

The biochemical equation for the Mitochondrial dehydrogenase is defined as

$$NAD_x^+ \rightarrow NADH_x + H_x^+ .$$

The rate expression used in the model is

$$J_{\mathrm{DH}} = \frac{X_{\mathrm{DH}}}{1+\left(\frac{[\mathrm{ATP}]_x K_{DH}}{[\mathrm{ADP}]_x[\mathrm{Pi}]_x}\right)^{n_{DH}}} \left(\mathrm{r}_{\mathrm{DH}}\ [\mathrm{NAD}^+]_x - [\mathrm{NADH}]_x\right).$$

Table S3.1. Mitochondrial Dehydrogenase Parameters

Parameter	Definition	Value	Reference
X_{DH}	Dehydrogenase activity	$2.82\text{x}10^{-2}$ mol/lmito/s	-[a]
r_{DH}	Apparent NADH/NAD^+ equilibrium constant	35.4 unitless	-[a]
K_{DH}	ATPase feedback constant	$2.87\text{x}10^{-2}$ M	-[a]
n_{DH}	ATPase Hill coefficient	1.45 unitless	-[a]

[a] Adjustable parameter.

NADH-ubiquinone oxidoreductase: Complex I

The biochemical equation for Complex I is defined as

$$UQ_x + NADH_x + 5H_x^+ \rightarrow NAD_x^+ + UQH_{2x} + 4H_e^+ .$$

For model details, see [2]. In breif, the model is a multi-state model that includes the minimal components necessary to simulate NADH-quinone oxidoreductase activity as a function of pH and mitochondrial membrane potential (ΔΨ) and also the detailed redox biochemistry required to simulate ROS generation by both the FMN and the SQ sites. Below is an updated list of parameters.

Table S3.2. NADH-Ubiquinone Oxidoreductase Parameters[a]

Parameter	Definition	Value	Reference
Structural Parameters			
$E_m^0(FMN / \mathrm{FMNH_2})$	$FMN/FMNH_2$ midpoint potential	55.14 mV	[12][b]
$E_m^0(FMN / \mathrm{FMNH^{\cdot -}})$	$FMN/FMNH^{\cdot -}$ midpoint potential	23.54 mV	[12][b]
$E_m^0(FMNH^{\cdot -} / \mathrm{FMNH_2})$	$FMNH^{\cdot -}/FMNH_2$ midpoint potential	86.74 mV	[12][b]
pK_{FMNH_2}	$FMNH_2$ pK	7.1	[12][b]
$pK_{FMNH^{\cdot -}}$	$FMNH^{\cdot -}$ pK	7.9	[12][b]
$E_m^0(\mathrm{N2_{ox}} / \mathrm{N2_{red}})$	$N2_{ox}/N2_{red}$ midpoint potential	-90 mV	[13][c]

$pK_{N2^{ox}}$	$N2_{ox}$ pK	6	[13][c]
$pK_{N2^{red}}$	$N2_{red}$ pK	8.5	[13][c]
Kinetic Parameters			
$Etot_{C1}$	Total Complex I content	$1.67x10^{-4}$ mol/lmito	-[d]
K_{NADH}^{ox}	NADH dissociation constant for oxidized FMN	4.61E-05 M	[2]
$K_{NAD^+}^{ox}$	NAD^+ dissociation constant for oxidized FMN	7.05E-04 M	[2]
K_{NADH}^{red}	NADH dissociation constant for reduced FMN	4.99E-04 M	[2]
$K_{NAD^+}^{red}$	NAD^+ dissociation constant for reduced FMN	1.18E-05 M	[2]
K_{NADH}^{rad}	NADH dissociation constant for FMN radical	1.00E+00 M	[2]
$K_{NAD^+}^{rad}$	NAD^+ dissociation constant for FMN radical	1.54E-04 M	[2]
$K_{Q_{10}H_2}$	$Q_{10}H_2$ dissociation constant	1.00E-01 M	[2]
$K_{Q_{10}}$	Q_{10} dissociation constant	1.75E-02 M	[2]
$K_{Q_{10}}^{stability}$	Q_{10} stability constant	1.00E+01	[2]
$k_{f,0\to2}^{NADH}$	NADH oxidation rate for state 0	1.18E+05 s^{-1}	[2]
$k_{f,2\to4}^{NADH}$	NADH oxidation rate for state 2	1.12E+04 s^{-1}	[2]
$k_{f,1\to3}^{NADH}$	NADH oxidation rate for state 1	2.77E+02 s^{-1}	[2]
$k_{f,2\to0}^{Q}$	Q reduction rate for state 2	3.49E+05 s^{-1}	[2]
$k_{f,4\to2}^{Q}$	Q reduction rate for state 4	4.67E+12 s^{-1}	[2]
$k_{f,3\to1}^{Q}$	Q reduction rate for state 3	5.20E+02 s^{-1}	[2]
β	Charge translocation parameter	5.00E-01	[2]
pK_{Nsite}	NADH oxidase site pK	7.39	[2]
pK_{Qsite}	Q reductase site pK	6.41	[2]
ROS Parameters			
$k_{f,1\to0}^{SQ^-/O_2^-}$	Superoxide production rate from semiquinone for state 1	1.67E+09 $M^{-1}s^{-1}$	[2]
$k_{fa,2\to1}^{SQ^-/O_2^-}$	Superoxide production rate from semiquinone for state 2a	2.06E-03 $M^{-1}s^{-1}$	[2]
$k_{fb,2\to1}^{SQ^-/O_2^-}$	Superoxide production rate from semiquinone for state 2b	2.07E-03 $M^{-1}s^{-1}$	[2]
$k_{fa,3\to2}^{SQ^-/O_2^-}$	Superoxide production rate from semiquinone for state 3a	1.14E-05 $M^{-1}s^{-1}$	[2]
$k_{fb,3\to2}^{SQ^-/O_2^-}$	Superoxide production rate from semiquinone for state 3b	2.40E-09 $M^{-1}s^{-1}$	[2]

$k_{f,4\to3}^{SQ^{-}/O_2^{-}}$	Superoxide production rate from semiquinone for state 4	2.16E-07 $M^{-1}s^{-1}$	[2]
$k_{f,2\to1}^{FMNH_2/O_2^{-}}$	Superoxide production rate from $FMNH_2$ for state 2	4.52E+05 $M^{-1}s^{-1}$	[2]
$k_{fa,3\to2}^{FMNH_2/O_2^{-}}$	Superoxide production rate from $FMNH_2$ for state 3a	5.38E-04 $M^{-1}s^{-1}$	[2]
$k_{fb,3\to2}^{FMNH_2/O_2^{-}}$	Superoxide production rate from $FMNH_2$ for state 3b	2.83E-05 $M^{-1}s^{-1}$	[2]
$k_{f,4\to3}^{FMNH_2/O_2^{-}}$	Superoxide production rate from $FMNH_2$ for state 4	1.40E+05 $M^{-1}s^{-1}$	[2]
$k_{f,1\to0}^{FMNH^{\cdot-}/O_2^{-}}$	Superoxide production rate from $FMNH^{\cdot-}$ for state 1	6.81E+07 $M^{-1}s^{-1}$	[2]
$k_{fa,2\to1}^{FMNH^{\cdot-}/O_2^{-}}$	Superoxide production rate from $FMNH^{\cdot-}$ for state 2a	4.04E-11 $M^{-1}s^{-1}$	[2]
$k_{fb,2\to1}^{FMNH^{\cdot-}/O_2^{-}}$	Superoxide production rate from $FMNH^{\cdot-}$ for state 2b	1.19E-07 $M^{-1}s^{-1}$	[2]
$k_{f,3\to2}^{FMNH^{\cdot-}/O_2^{-}}$	Superoxide production rate from $FMNH^{\cdot-}$ for state 3	1.40E-02 $M^{-1}s^{-1}$	[2]
$k_{f,2\to0}^{FMNH_2/H_2O_2}$	Hydrogen peroxide production rate from $FMNH_2$ for state 2	7.82E-07 $M^{-1}s^{-1}$	[2]
$k_{fa,3\to1}^{FMNH_2/H_2O_2}$	Hydrogen peroxide production rate from $FMNH_2$ for state 3a	2.12E+06 $M^{-1}s^{-1}$	[2]
$k_{fb,3\to1}^{FMNH_2/H_2O_2}$	Hydrogen peroxide production rate from $FMNH_2$ for state 3b	1.76E+06 $M^{-1}s^{-1}$	[2]
$k_{f,4\to2}^{FMNH_2/H_2O_2}$	Hydrogen peroxide production rate from $FMNH_2$ for state 4	6.21E+01 $M^{-1}s^{-1}$	[2]

[a] Updated from [2].
[b] Refit from data in Figures 1, 2 and 3 of Sled et al.
[c] Rrefit from data in Figure 1 of Ingledew and Ohnishi.
[d] Adjustable parameter.

Succinate-ubiquinone oxidoreductase: Complex II

The biochemical equation for Complex II is defined as

$$UQ_x + SUC_x \rightarrow FUM_x + UQH_{2x}.$$

The rate expression used in the model is

$$J_{C2} = \alpha_{C2} J_{DH}.$$

Table S3.2. Succinate-Ubiquinone Oxidoreductase Parameters

Parameter	Definition	Value	Reference
α_{C2}	Complex II activity	0.25	[a]

[a]This value is bounded between 0 and 0.25 when pyruvate and malate are the substrates for oxidation. Values less than 0.25 means incomplete oxidation of pyruvate, and there is loss of TCA cycle intermediates before reaching Complex II.

Ubiquinol-cytochrome-c oxidoreductase: Complex III

The biochemical equation for Complex III is defined as

$$UQH_{2x} + 2c_i^{3+} + 2H_x^+ \rightarrow UQ_x + 2c_i^{2+} + 4H_e^+.$$

For model details, see [3]. In breif, the model is a multi-state model that simulates quinol-cytochrome c oxidoreductase activity as a function of pH and ΔΨ, as well as, the redox biochemistry involved in superoxide formation.

Table S3.3. Ubiquinol-Cytochrome-c Oxidoreductase Parameters[a]

Parameter	Definition	Value	Reference
Structural Parameters			
K_{stabo}	Q_o-site semiquinone stability constant	10^{-33} unitless	[14] [b,c]
K_{stabi}	Q_i-site semiquinone stability constant	$10^{-16.11}$ unitless	[15] [c]
pK_{ISPox1}	Oxidized Rieske ISP acidic pK	7.6	[16]
pK_{ISPox2}	Oxidized Rieske ISP alkaline pK	9.2	[16]
pK_{bLox}	Oxidized cytochrome b_L pK	5.9	[17]
pK_{bLred}	Reduced cytochrome b_L pK	7.9	[17]
pK_{bHox}	Oxidized cytochrome b_H pK	5.7	[17]
pK_{bHred}	Reduced cytochrome b_H pK	7.7	[17]
β	Fractional charge transfer coefficient	0.5 unitless	[17-20]
Kinetic and ROS Parameters			
$Etot_{C3}$	Total Complex III content	1.58×10^{-3} mol/lmito	-[d]

k_{120}	State E1 to E2 intrinsic transition-rate constant	3.07×10^3 s^{-1}	[3]
k_{230}	State E2 to E3 intrinsic transition-rate constant	3.31×10^7 s^{-1}	[3]
k_{410}	State E4 to E1 intrinsic transition-rate constant	1.44×10^4 s^{-1}	[3]
k_{340}	State E3 to E4 intrinsic transition-rate constant	3.07×10^3 s^{-1}	-[e]
k_{260}	State E2 to E6 intrinsic transition-rate constant	3.07×10^3 s^{-1}	-[e]
k_{640}	State E6 to E4 intrinsic transition-rate constant	3.31×10^7 s^{-1}	-[f]
k_{450}	State E4 to E5 intrinsic transition-rate constant	3 s^{-1}	-[g]
k_{520}	State E5 to E2 intrinsic transition-rate constant	1.44×10^4 s^{-1}	-[h]
k_{SO}	1st order superoxide production-rate constant	1600 s^{-1}	[14][i]
K_{c3}	Cytochrome c^{3+} binding constant	1.1 µM	[3]
K_{c2}	Cytochrome c^{2+} binding constant	1.2 µM	[3]
$K^o_{QH_2}$	$Q_{10}H_2$ Q_o-site binding constant	0.8 mM	[21][j]
K^o_Q	Q_{10} Q_o-site binding constant	0.5 mM	[21-23][k]
K^i_Q	$Q_{10}H_2$ Q_i-site binding constant	1.0 mM	[24][l]
$K^i_{QH_2}$	Q_{10} Q_i-site binding constant	2.4 mM	-[m]

[a] Updated from [3].
[b] Based on approximate K_{eq} for the superoxide reaction.
[c] Extrapolated to pH 0 from given reference. At pH 7, $K_{stabo} = 10^{-9}$ and $K_{stabi} = 10^{-2.11}$.
[d] Adjustable parameter.
[e] Identical state transition as E1 to E2.
[f] Identical state transition as E2 to E3.
[g] This rate must be low to simulate physiological superoxide production rates.
[h] Identical state transition as E4 to E1.
[i] Adjusted to within uncertainty range.
[j] Based on the observation that maximum activity in the absence of a proton-motive force when the Qpool is 50% reduced.
[k] In the range given, and the Q_o-site affinity for QH_2 and Q are very similar.
[l] Based on state-2 respiration rates of 30 nmol O_2/mg/min when $\Delta\Psi = 200$ mV and $\Delta pH = 0$.
[m] Defined via microscopic reversibility where $K^i_{QH_2} = K^o_{QH_2}{}^2 K^i_Q K_{c3}{}^2 / K^o_Q / K_{c2}{}^2$.

Cytochrome-c oxidase: Complex IV

The biochemical equation for Complex IV is defined as

$$2CytC_i^{2+} + \frac{1}{2}O_{2x} + 4H_x^+ \rightarrow 2CytC_i^{3+} + H_2O + 2H_e^+ .$$

The equilibrium constant is defined as

$$K_{eq} = e^{-\left(\Delta_r G_{C4}^0 - 4\Delta\Psi\right)/RT}\left(\left[H^+\right]_x^4 / \left[H^+\right]_i^2\right).$$

The rate expression used in the model is

$$J_{C4} = X_{C4}\left(\frac{1}{1+K_{O_2}/[O_2]_x}\right)\left(\frac{[c^{2+}]_i^n}{[c^{2+}]_i^n + K_M^n\left(1+\beta e^{2\Delta\Psi F/RT}\right)}\right)\left(1-\frac{[c^{3+}]_i^2}{[c^{2+}]_i^2[O_2]_x^{1/2}K_{eq}^{1/2}}\right).$$

Table S3.4. Cytochrome-c Oxidase Parameters

Parameter	Definition	Value	Reference
X_{C4}	Complex IV activity	$8.91\text{x}10^{-2}$ mol/lmito/s	-[a]
$\Delta_r G_{C4}^0$	Gibb's free energy of reaction	-202.16 kJ/mol	[5]
K_{O_2}	O_2 binding constant	$1\text{x}10^{-6}$ M	[1]
K_M	c^{2+} binding constant	162 µM	[25]
n	Hill coefficient for c^{2+}	2	[25]
β	$\Delta\Psi$ constant	$6.6\text{x}10^{-6}$	[25]

[a] Adjustable parameter.

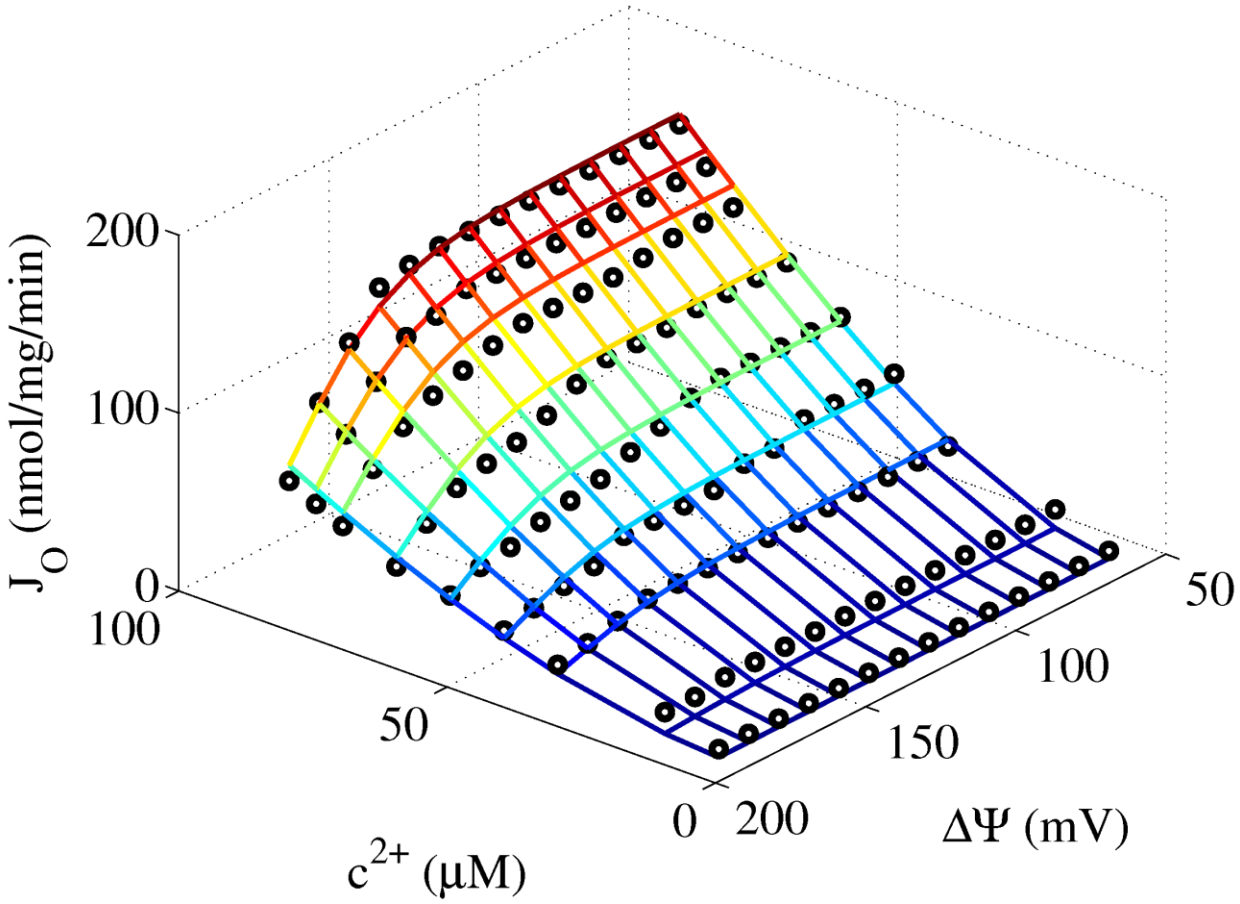

Figure S3.1. Flux through Complex IV as a function of c^{2+} and ΔΨ. The data from Murphy and Brand [25] was used to fit the K_M, n, and β parameters in the flux expression for Complex IV. The reported fraction of c^{2+} was converted to concentration using the measurements given in Estabrook and Holowinsky [26].

Proton leak

The electrophoretically driven proton uptake via leak pathways was updated to account for the highly non-linear nature of the leak across energy-transducing membranes [27]. The proton leak permeability, P_{Hleak}, is an adjustable parameter and set to 1.78×10^3 mol/s/lmito/M:

$$J_{Hleak} = P_{Hleak}\left([H^+]_e e^{\left(\frac{F\Delta\Psi}{2RT}\right)} - [H^+]_m e^{-\left(\frac{F\Delta\Psi}{2RT}\right)}\right).$$

S3B - Oxidative Phosphorylation Related Reactions Rates

Adenine nucleotide translocase (ANT)

The biochemical equation for ANT is defined as

$$ATP_x + ADP_e \quad ATP_e + ADP_x .$$

The rate expression used in the model is

$$J_{\mathrm{ANT}} = E_{\mathrm{ANT}} \frac{k_2^{ANT} q \frac{[\mathrm{ATP}^{4-}]_x [\mathrm{ADP}^{3-}]_e}{K_o^D} - k_3^{ANT} \frac{[\mathrm{ATP}^{4-}]_e [\mathrm{ADP}^{3-}]_x}{K_o^T}}{\left(1 + \frac{[\mathrm{ATP}^{4-}]_e}{K_o^T} + \frac{[\mathrm{ADP}^{3-}]_e}{K_o^D}\right)\left([\mathrm{ATP}^{4-}]_x + q[\mathrm{ADP}^{3-}]_x\right)},$$

where

$$k_2^{ANT} = k_2^{ANT,o} e^{(-3\alpha_1 - 4\alpha_2 + \alpha_3) F\Delta\Psi / RT},$$

$$k_3^{ANT} = k_3^{ANT,o} e^{(-4\alpha_1 - 3\alpha_2 + \alpha_3) F\Delta\Psi / RT},$$

$$K_o^D = K_o^{D,o} e^{3\delta_D RT / F\Delta\Psi},$$

$$K_o^T = K_o^{\mathrm{T},o} e^{4\delta_T RT / F\Delta\Psi},$$

and

$$q = \frac{k_3^{ANT} K_o^D}{k_2^{ANT} K_o^T} e^{F\Delta\Psi / RT}.$$

Table S3.5. Adenine Nucleotide Translocase Parameters[a]

Parameter	Definition	Value	Reference
E_{ANT}	Total ANT content	0.141 mol/lmito	-[b]
$k_2^{ANT,o}$	Forward translocation rate	0.159 s^{-1}	[5]
$k_3^{ANT,o}$	Reverse translocation rate	0.501 s^{-1}	[5]
$K_o^{D,o}$	ADP binding constant	38.89 μM	[5]
$K_o^{T,o}$	ATP binding constant	56.05 μM	[5]
α_1	Translocation displacement constant	0.2829 unitless	[5]
α_2	Translocation displacement constant	-0.2086 unitless	[5]
α_3	Translocation displacement constant	0.2372 unitless	[5]

δ_T	ATP displacement binding constant	0.0167 unitless	[5]
δ_D	ADP displacement binding constant	0.0699 unitless	[5]

[a] Updated from Metelkin et al. [28].
[b] Adjustable parameter.

F_1F_O ATP synthase

The biochemical equation for F_1F_O ATP synthase is defined as

$$ADP_m + Pi_m \rightleftharpoons ATP_m .$$

The equilibrium constant is defined as

$$K_{eq} = e^{-\left(\Delta_r G^0_{F_1F_O} + 3F\Delta\Psi\right)/RT}\left(\frac{[H^+]_e^{nH}}{[H^+]_x^{nH-1}}\right)\left(\frac{P_{ATP}}{P_{ADP}P_{Pi}}\right).$$

The rate expression used in the model is

$$J_{F_1F_O} = X_{F_1F_O}\left([ADP]_x[Pi]_x K_{eq} - [ATP]_x\right).$$

Table S3.6. F_1F_O ATP synthase Parameters

Parameter	Definition	Value	Reference
X_{F1FO}	F_1F_O activity	812 mol/s/M/lmito	[1][a]
$\Delta_r G^0_{F_1F_O}$	Gibb's free energy of reaction	-4.51 kJ/mol	[5]
nH	H^+:ATP ratio	8/3	[29]

[a] The activity was lowered from the cited value to reduced the stiffness of the system of DAEs. This resulted in negligible differences in state variable dynamics for the simulations that were used to identify this parameter value.

Inorganic phosphate carrier (PiC)

The biochemical equation for the PiC is defined as

$$Pi_e + H_e^+ \quad Pi_x + H_x^+ .$$

The rate expression used in the model is

$$J_{\mathrm{PiC}} = X_{\mathrm{PiC}} \left(\frac{[Pi^-]_x [\mathrm{H}^+]_x - [Pi^-]_e [\mathrm{H}^+]_e}{[Pi^-]_e + k_{\mathrm{PiC}}} \right).$$

Table S3.7 Inorganic Phosphate Parameters

Parameter	Definition	Value	Reference
X_{PiC}	PiC activity	3.34×10^7 mol/s/M/lmito	[1]
k_{PiC}	Pi binding constant	1.61 mM	[1]

ATPase

The biochemical equation for an ATPase is defined as

$$ATP_e \rightarrow ADP_e + Pi_e .$$

The equilibrium constant is defined as

$$K_{eq} = e^{-\left(\Delta_r G^0_{ATPase}/RT\right)}\left([H^+]_e^{-1}\right)\left(\frac{P_{ADP}P_{Pi}}{P_{ATP}}\right).$$

The rate expression used in the model is

$$J_{ATPase} = X_{ATPase} / \left(1+[ADP]_e / K_{i,ADP}\right)\left(1-[ADP]_e[Pi]_e / [ATP]_e / K_{eq}\right).$$

Table S3.8. ATPase Parameters

Parameter	Definition	Value	Reference
X_{ATPase}	ATPase activity	0 - 1 U/ml	[a]
$\Delta_r G^0_{ATPase}$	Gibb's free energy of reaction	4.51 kJ/mol	[5]
$K_{i,ADP}$	ADP inhibition constant	$2.41\text{x}10^{-4}$ M	[b]

[a] The value was based on the amount of ATPase added as given in the experimental companion paper. 1 U is defiend as 1 µmol/min of ATP hydrolyzed in the buffer.
[b] Adjustable parameter.

Note, the Complex II, adenylate kinase, creatine kinase, and external ATPase reactions shown below were only used for the ischemia/reperfusion simulations.

Succinate-ubiquinone oxidoreductase: Complex II

The biochemical equation for Complex II is defined as

$$UQ_x + SUC_x \rightarrow FUM_x + UQH_{2x} .$$

The rate expression used in the model is

$$J_{C2} = X_{C2}\left(1-[\text{UQH}_2]_x / [\text{UQ}]_x / \text{K}_{eq}^{C2}\right).$$

Table S3.9. Succinate-Ubiquinone Oxidoreductase Parameters

Parameter	Definition	Value	Reference
X_{C2}	Complex II activity	$6.0\text{x}10^{-4}$ M/s/lmito	[a]
K_{eq}^{C2}	Complex II equilibrium constant	1	-

[a]This value is set to give an approximate J_{CII}/J_{CI} ratio of 0.25.

Adenylate Kinase (AK)

The biochemical equation for AK is defined as

$$2ADP_e \rightleftharpoons ATP_e + AMP_e .$$

The equilibrium constant is defined as

$$K_{eq} = e^{-\left(\Delta_r G^0_{AK}/RT\right)}\left(\frac{P_{ATP}P_{AMP}}{P^2_{ADP}}\right).$$

The rate expression used in the model is

$$J_{AK} = X_{AK}\left(1-[ATP]_e[\mathrm{AMP}]_e/[ADP]^2_e/K_{eq}\right).$$

Table S3.10. AK Parameters

Parameter	Definition	Value	Reference
X_{AK}	AK activity	10^6 M/s	[a]
$\Delta_r G^0_{AK}$	Gibb's free energy of reaction	2.38 kJ/mol	[5]

[a]Set arbitrarily high to ensure equilibrium.

Creatine Kinase (CK)

The biochemical equation for CK is defined as

$$CrP_e + ADP_e \rightleftharpoons Cr_e + ATP_{ee} .$$

The equilibrium constant is defined as

$$K_{eq} = e^{-\left(\Delta_r G^0_{CK}/RT\right)}[\mathrm{H}^+]_e\left(\frac{P_{ATP}}{P_{ADP}}\right).$$

The rate expression used in the model is

$$J_{CK} = X_{CK}\left(1-[ATP]_e[\mathrm{AMP}]_e/[ADP]^2_e/K_{eq}\right).$$

Table S3.11. AK Parameters

Parameter	Definition	Value	Reference
X_{CK}	CK activity	10^6 M/s	[a]
$\Delta_r G^0_{CK}$	Gibb's free energy of reaction	-7.91 kJ/mol	[5]

[a]Set arbitrarily high to ensure equilibrium.

ATPase for I/R Simulations

The biochemical equation for an ATPase is defined as

$$ATP_e \rightarrow ADP_e + Pi_e .$$

The rate expression used in the model is

$$J_{ATPase} = \frac{X_{ATPase}}{1 + R\dfrac{[ADP]_e[Pi]_e}{[ATP]_e}} .$$

Table S3.12. ATPase Parameters

Parameter	Definition	Value	Reference
X_{ATPase}	ATPase activity	2.0×10^{-3} M/s	[a]
R	Critical value of mass-action ratio of ATP hydrolysis potential	6.58×10^{4} M^{-1}	[5]

[a]Set to achieve an intermedial level of respiration.

S2.3C - Cation Related Reaction Rates

Potassium-hydrogen exchanger

The biochemical equation for KHE is defined as

$$K_m^+ + H_e^+ \rightarrow K_e^+ + H_m^+ .$$

The rate expression used in the model is

$$J_{KHE} = X_{KHE}\left([K^+]_m[H^+]_e - [K^+]_e[H^+]_m\right)$$

where X_{KHE} is set to 4.76×10^{6} mol/s/M/lmito as in [1].

S4B – ROS Scavenging Related Reactions Rates

Mangenese Superoxide Dismutase (MnSOD)

The biochemical equation for MnSOD is defined as

$$2O_2^{-} + 2H^{+} \rightarrow O_2 + H_2O_2 .$$

The rate expression used in the model is

$$J_{SODx} = X_{SOD}[O_2^{-}]_x$$

and

$$J_{SODc} = X_{SOD}[O_2^{-}]_c$$

where the subscripts *x* and *c* denote matrix and cytosolic, respectively, X_{SOD} is set to 2.0×10^4 s^{-1} based on 10 µM [MnSOD] and a second order rate constant of 2.0×10^9 $M^{-1}s^{-1}$ as given in [30, 31]. For simplicity, the cytosolic rate was set equal to the mitochondrial rate.

H_2O_2 Reduction via GSH and Trx Pathways

The biochemical equation for H_2O_2 scavening is defined as

$$H_2O_2 + 2H^{+} \rightarrow 2H_2O .$$

The rate expression used in the model is

$$J_{H2O2} = X_{H2O2}[H_2O_2]_x$$

where X_{H2O2} is set to 1.32×10^4 s^{-1} based on 60 µM [Prx3] and a second order rate constant of 2.0×10^7 $M^{-1}s^{-1}$ and 2 µM [Gpx1] and a second order rate constant of 6.0×10^7 $M^{-1}s^{-1}$ as given in [30, 31].

The expression for H_2O_2 permeation through the mitochondrial membrane is

$$J_{H2O2perm} = k_{perm}\left([\mathrm{H_2O_2}]_x - [\mathrm{H_2O_2}]_e\right)$$

where k_{perm} is set to 333 s^{-1} based on a membrane permeability coefficient of 2 µm/s [32] and a membrane thickness of 6 nm.

S4: Model Code

Simulate_Vinnakota2015.m – script used to call the necessary functions to simulate the model and reproduce the plots for the Vinnakota data set as given in the main article.

Simulate_FCCP_Titration.m – script used to call the necessary functions to simulate the model and reproduce the plots for the FCCP titration as given in the main article.

Simulate_IR.m – script used to call the necessary functions to simulate the model and reproduce the plots for the ischemia/reperfusion conditions as given in the main article.

DAEs.m – a function that defines the right hand side of the system of differential algebraic equations that comprise the model.

Model_IC.m – a function that defines the model initial conditions.

dGpKcorr.m – a function that corrects the Gibb's free energies of formation and pK for ionic strength and temperature.

herrorbar.m – a function by Jos van der Geest that plots horizontal error bars and downloaded from the Matlab File Exchange server.

data.mat – cell array of experimental data.

params.mat – structure containing model parameters.

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