Supplemental material for “Experimentally observed phenomena on cardiac energetics in heart failure emerge from simulations of cardiac metabolism”

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S1. List of model components

The computational model applied in the current study integrates computational models of cellular metabolism and oxygen transport in cardiac tissue previously published by Beard et al. (1-3). Please refer to these published papers and the associated supplemental material for detailed descriptions of model parameterization and validation.

This appendix lists the basic components of the current model. Figure S1 illustrates components of the computational model of cardiac energetics and oxygen transport. Table S1 summarizes the metabolite pools in canine hearts at different stages of left ventricular hypotrophy (LVH). Table S2 lists values of myocardiac cellular volumes and water fractions from Reference (4). Tables S3 and S4 list the state variables and reaction and transport fluxes considered in the model. Table S5 lists model parameters.

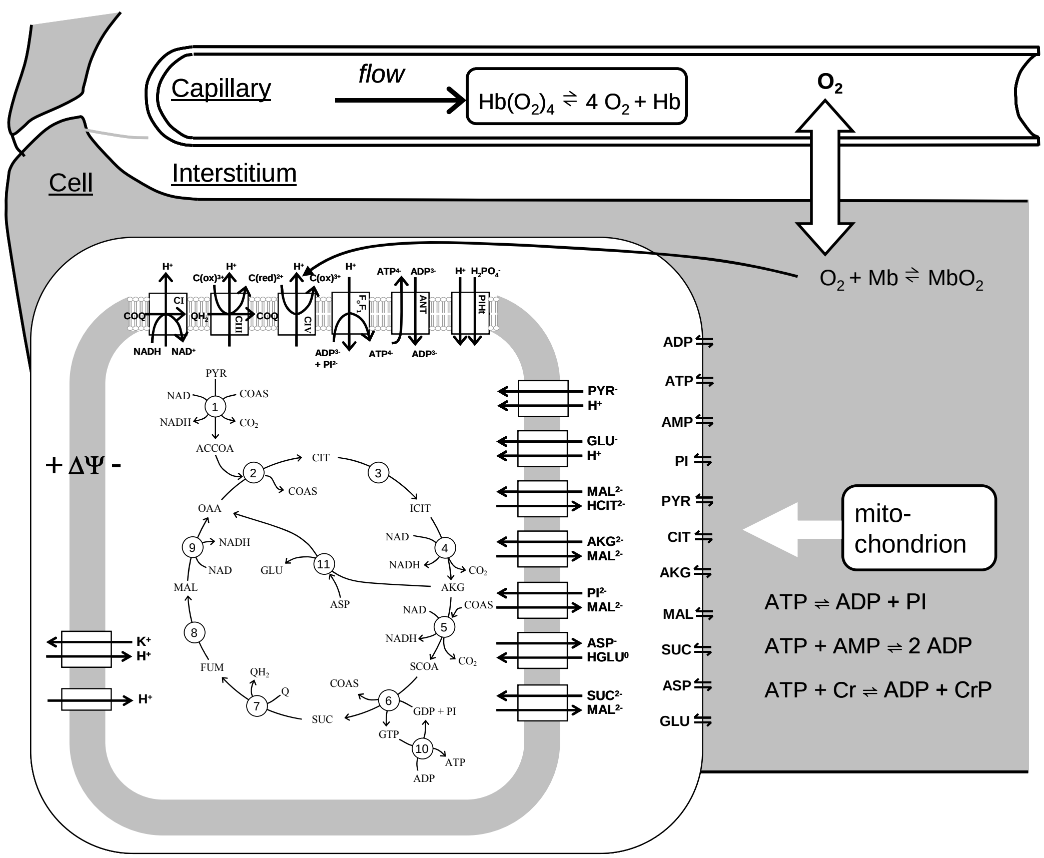

Figure S1: Diagram of model used to simulate cardiac tissue oxygen transport and energy metabolism. Oxygen is transported via advection in capillaries, diffuses into cardiomyocytes from capillaries through interstitium, and is reduced into water via the complex IV reaction in mitochondria. Please see references (1, 5) for detailed procedures of parameterization and validation of models of cellular energy metabolism and oxygen transport.

Table S1: Metabolic pools in canine hearts at different stages of left ventrical hypotrophy (LVH).

Concentrations*† (mmol (l cell)$^{-1}$)	Normal	Early-stage LVH‡	Moderate LVH‡
CR_{tot}	35.04	35.56 (+1.5%)	30.26 (-13.6%)
ATP_{BL}	7.23	6.81 (-5.8%)	5.27 (-27.1%)
CrP_{BL}	15.33	15.05 (-1.8%)	8.16 (-46.8%)
TEP	29.78	28.69 (-3.7%)	18.72 (-37.1%)
TAN	8.62	8.20 (-4.9%)	6.85 (-20.5%)

*Concentrations of total creatine pool (CR_{tot}), basal ATP content (ATP_{BL}), and basal CrP content (CrP_{BL}) are obtained from the biopsy measurement in Zhang et al. (6), Bache et al. (7), and Bache et al. (8), respectively.
†Concentrations of total exchangeable phosphate pool (TEP) and total adenine nucleotide pool (TAN) are estimated based on the experimental measurements of CR_{tot}, ATP_{BL}, and CrP_{BL}.
‡Percentages listed in parentheses denote relative changes of the concentrations from the basal values.

Table S2: Myocardial cellular volumes and water fractions

Variables	Value	Unit
V_{cyto}	0.6801	ml cytoplasm (ml cell)$^{-1}$
V_{mito}	0.2882	ml mitochondria (ml cell)$^{-1}$
W_c	0.8425	ml water (ml cytoplasm)$^{-1}$
W_i	0.0724	ml water (ml mitochondria)$^{-1}$
W_x	0.6514	ml water (ml mitochondria)$^{-1}$

Table S3: Model variables

Variables	Descriptions	Units
Oxygen Concentration:		
$C_{O2,capillary}$	Total oxygen concentration in capillary	mol (l capillary)$^{-1}$
$C_{O2,interstitium}$	Total oxygen concentration in interstitium	mol (l interstitium)$^{-1}$
$C_{O2,cell}$	Total oxygen concentration in myocyte	mol (l cell)$^{-1}$
Mitochondrial membrane potential :		
$\Delta\Psi$	Mitochondrial membrane potential	mV
Mitochondrial Matrix Variables:		
$[H^+]_x$	Concentration of H^+ ion in mito matrix	mol (l matrix water)$^{-1}$
$[K^+]_x$	Concentration of K^+ ion in mito matrix	mol (l matrix water)$^{-1}$
$[Mg^{2+}]_x$	Concentration of Mg^{2+} ion in mito matrix	mol (l matrix water)$^{-1}$
$[NADH]_x$	Concentration of NADH in mito matrix	mol (l matrix water)$^{-1}$

$[NAD]_x$	Concentration of NAD in mito matrix	mol (l matrix water) $^{-1}$
$[QH_2]_x$	Concentration of reduced ubiquinol in mito matrix	mol (l matrix water) $^{-1}$
$[COQ]_x$	Concentration of oxidized ubiquinol in mito matrix	mol (l matrix water) $^{-1}$
$[ATP]_x$	Concentration of total ATP in mito matrix	mol (l matrix water) $^{-1}$
$[ADP]_x$	Concentration of total ADP in mito matrix	mol (l matrix water)$^{-1}$
$[GTP]_x$	Concentration of total GTP in mito matrix	mol (l matrix water) $^{-1}$
$[GDP]_x$	Concentration of total GDP in mito matrix	mol (l matrix water)$^{-1}$
$[PI]_x$	Concentration of inorganic phosphate in mito matrix	mol (l matrix water) $^{-1}$
$[PYR]_x$	Concentration of pyruvate in mito matrix	mol (l matrix water) $^{-1}$
$[COASH]_x$	Concentration of CoA-SH in mito matrix	mol (l matrix water) $^{-1}$
$[ACCOA]_x$	Concentration of acetyl-CoA in mito matrix	mol (l matrix water) $^{-1}$
$[OAA]_x$	Concentration of oxaloacetate in mito matrix	mol (l matrix water) $^{-1}$
$[CIT]_x$	Concentration of citrate in mito matrix	mol (l matrix water) $^{-1}$
$[ICIT]_x$	Concentration of isocitrate in mito matrix	mol (l matrix water) $^{-1}$
$[AKG]_x$	Concentration of α-ketoglutarate in mito matrix	mol (l matrix water) $^{-1}$
$[SCOA]_x$	Concentration of succinyl-CoA in mito matrix	mol (l matrix water) $^{-1}$
$[SUC]_x$	Concentration of pyruvate in mito matrix	mol (l matrix water) $^{-1}$
$[FUM]_x$	Concentration of fumarate in mito matrix	mol (l matrix water) $^{-1}$
$[MAL]_x$	Concentration of malate in mito matrix	mol (l matrix water) $^{-1}$
$[ASP]_x$	Concentration of aspartate in mito matrix	mol (l matrix water) $^{-1}$
$[GLU]_x$	Concentration of glutamate in mito matrix	mol (l matrix water) $^{-1}$
$[O_2]_x$	Concentration of oxygen in mito matrix	mol (l matrix water) $^{-1}$
$[CO_2tot]_x$	Concentration of total CO_2 in mito matrix	mol (l matrix water) $^{-1}$
Mitochondrial Inter-Membrane Space Variables:		
$[H^+]_i$	Concentration of H^+ ion in IM space	mol (l IM water) $^{-1}$
$[K^+]_i$	Concentration of K^+ ion in IM space	mol (l IM water) $^{-1}$
$[Mg^{2+}]_i$	Concentration of Mg^{2+} ion in IM space	mol (l IM water) $^{-1}$
$[Cred]_i$	Concentration of reduced cytochrome C in IM space	mol (l IM water) $^{-1}$
$[Cox]_i$	Concentration of oxidized cytochrome C in IM space	mol (l IM water) $^{-1}$
$[ATP]_i$	Concentration of total ATP in IM space	mol (l IM water) $^{-1}$
$[ADP]_i$	Concentration of total ADP in IM space	mol (l IM water) $^{-1}$
$[AMP]_i$	Concentration of total AMP in IM space	mol (l IM water) $^{-1}$
$[PI]_i$	Concentration of inorganic phosphate in IM space	mol (l IM water) $^{-1}$
$[PYR]_i$	Concentration of pyruvate in IM space	mol (l IM water) $^{-1}$

$[CIT]_i$	Concentration of citrate in IM space	mol (l IM water) $^{-1}$
$[AKG]_i$	Concentration of α-ketoglutarate in IM space	mol (l IM water) $^{-1}$
$[SUC]_i$	Concentration of pyruvate in IM space	mol (l IM water) $^{-1}$
$[FUM]_i$	Concentration of fumarate in IM space	mol (l IM water) $^{-1}$
$[MAL]_i$	Concentration of malate in IM space	mol (l IM water) $^{-1}$
$[ASP]_i$	Concentration of ssparatate in IM space	mol (l IM water) $^{-1}$
$[GLU]_i$	Concentration of glutamate in IM space	mol (l IM water) $^{-1}$
Cytoplasm Variables:		
$[H^+]_c$	Concentration of H^+ ion in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[K^+]_c$	Concentration of K^+ ion in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[Mg^{2+}]_c$	Concentration of Mg^{2+} ion in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[ATP]_c$	Concentration of total ATP in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[ADP]_c$	Concentration of total ADP in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[AMP]_c$	Concentration of total ADP in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[PI]_c$	Concentration of inorganic phosphate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[PYR]_c$	Concentration of pyruvate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[CIT]_c$	Concentration of citrate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[AKG]_c$	Concentration of α-ketoglutarate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[SUC]_c$	Concentration of pyruvate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[FUM]_c$	Concentration of fumarate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[MAL]_c$	Concentration of malate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[ASP]_c$	Concentration of aspartate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[GLU]_c$	Concentration of glutamate in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[CrP]_c$	Concentration of phosphate creatine in cytoplasm	mol (l cytoplasm water) $^{-1}$
$[Cr]_c$	Concentration of creatine in cytoplasm	mol (l cytoplasm water) $^{-1}$

Table S4: Reaction and transport fluxes

Flux	**Description**	**Units**
Mitochondrial Reactions:		
J_{C1}	Complex I	mol s^{-1} (l mito)$^{-1}$
J_{C3}	Complex III	mol s^{-1} (l mito)$^{-1}$
J_{C4}	Complex IV	mol s^{-1} (l mito)$^{-1}$
J_{F1}	F_1F_0 ATPase reaction	mol s^{-1} (l mito)$^{-1}$
J_{ANT}	Adenine nucleotide translocase	mol s^{-1} (l mito)$^{-1}$

J_{PIHt}	Phosphate-hydrogen co-transporter	mol s^{-1} (l mito)$^{-1}$
J_{Hle}	Proton leak	mol s^{-1} (l mito)$^{-1}$
J_{KH}	Mitochondrial K^+ / H^+ exchanger	mol s^{-1} (l mito)$^{-1}$
J_{pdh}	Pyruvate dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{cits}	Citrate synthetase	mol s^{-1} (l mito)$^{-1}$
J_{acon}	Aconitase	mol s^{-1} (l mito)$^{-1}$
J_{isod}	Isocitrate dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{akgd}	α-Ketoglutarate dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{scoas}	Succinyl-CoA synthetase	mol s^{-1} (l mito)$^{-1}$
J_{sdh}	Succinate dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{fum}	Fumarase	mol s^{-1} (l mito)$^{-1}$
J_{mdh}	Malate dehydrogenase	mol s^{-1} (l mito)$^{-1}$
J_{ndk}	Nucleoside diphosphokinase	mol s^{-1} (l mito)$^{-1}$
J_{got}	Glutamate oxaloacetate transaminase (Aspartate Transaminase)	mol s^{-1} (l mito)$^{-1}$
J_{AKi}	Mitochondrial adenylate kinase	mol s^{-1} (l mito)$^{-1}$
Mitochondrial Transport Fluxes:		
J_{PYRH}	Pyruvate-H^+ co-transporter	mol s^{-1} (l mito)$^{-1}$
J_{GLUH}	Glutamate-H^+ co-transporter	mol s^{-1} (l mito)$^{-1}$
J_{CITMAL}	Citrate/malate antiporter	mol s^{-1} (l mito)$^{-1}$
J_{AKGMAL}	α-Ketoglutarate/malate antiporter	mol s^{-1} (l mito)$^{-1}$
J_{SUCMAL}	Succinate/malate antiporter	mol s^{-1} (l mito)$^{-1}$
J_{MALPI}	Malate/phosphate antiporter	mol s^{-1} (l mito)$^{-1}$
J_{ASPGLU}	Aspartate/glutamate antiporter	mol s^{-1} (l mito)$^{-1}$
J_{PIt}	Phosphate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{ATPt}	ATP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{ADPt}	ADP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{AMPt}	AMP transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{PYRt}	Pyruvate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{CITt}	Citrate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{MALt}	Malate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{AKGt}	α-Ketoglutarate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{SUCt}	Succinate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
J_{GLUt}	Glutamate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$

J_{ASPt}	Aspartate transport across outer membrane	mol s^{-1} (l mito)$^{-1}$
Cytoplasm Reactions:		
J_{ATPase}	Cytoplasmic ATP consumption rate	mol s^{-1} (l cyto)$^{-1}$
J_{AKc}	Cytoplasmic adenylate kinase	mol s^{-1} (l cyto)$^{-1}$
J_{CK}	Creatine kinase	mol s^{-1} (l cyto)$^{-1}$

Table S5: Model parameter

Parameter	**Description**	**Value**	**Units**	**Reference**
Enzyme activity and kinetic constant				
X_{pdh}	Pyruvate dehydrogenase activity	1.22×10^{-1}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{cits}	Citrate synthase activity	1.16	mol s^{-1} (l mito)$^{-1}$	(5)
X_{acon}	Aconitase activity	3.21×10^{-2}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{isod}	Isocitrate dehydrogenase activity	4.25×10^{-1}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{akgd}	α-Ketoglutarate dehydrogenase activity	7.70×10^{-2}	mol s^{-1} (l mito)$^{-1}$	(5)
$K_{ir,akgd}$	Inhibition constant of NADH in α-Ketoglutarate dehydrogenase reaction	6.04×10^{-7}	M	(5)
X_{scoas}	Succinyl-CoA synthetase activity	5.82×10^{-1}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{sdh}	Succinate dehydrogenase activity	6.23×10^{-2}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{fuma}	Fumarase activity	7.12×10^{-3}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{mdh}	Malate dehydrogenase activity	6.94×10^{-2}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{ndk}	Nucleoside diphosphokinase activity	2.65×10^{-2}	mol s^{-1} (l mito volume)$^{-1}$	(5)
X_{got}	Glutamate oxaloacetate transaminase	7.96	mol s^{-1} (l mito)$^{-1}$	(5)
X_{PYRH}	PYR^{-}/H^{+} co-transporter activity	4.12×10^{8}	mol s^{-1} M^{-2} (l mito)$^{-1}$	(5)
X_{GLUH}	GLU^{-}/H^{+} co-transporter activity	3.26×10^{8}	mol s^{-1} M^{-2} (l mito volume)$^{-1}$	(5)
X_{CITMAL}	$HCIT^{2-}/MAL^{2-}$ antiporter activity	7.31×10^{1}	mol s^{-1} M^{-2} (l mito volume)$^{-1}$	(5)
X_{AKGMAL}	AKG^{2-}/MAL^{2-} antiporter activity	3.46×10^{-1}	mol s^{-1} (l mito volume)$^{-1}$	(5)
X_{SUCMAL}	SUC^{2-}/MAL^{2-} antiporter activity	9.54×10^{1}	mol s^{-1} M^{-2} (l mito)$^{-1}$	(5)
X_{MALPI}	MAL^{2-}/PI^{2-} antiporter activity	1.58×10^{1}	mol s^{-1} M^{-2} (l mito)$^{-1}$	(5)
X_{ASPGLU}	$ASP^{-}/HGLU^{0}$ antiporter activity	7.48×10^{-5}	mol s^{-1} (l mito)$^{-1}$	(5)
X_{C1}	Complex I activity	2.47×10^{4}	mol s^{-1} M^{-2} (l mito)$^{-1}$	(5)
X_{C3}	Complex III activity	6.65×10^{-1}	mol s^{-1} $M^{-3/2}$ (l mito)$^{-1}$	(5)

$k_{PI,1}$	Complex III/PI parameter	2.81×10^{-5}	M	(5)
$k_{PI,2}$	Complex III/PI parameter	3.14×10^{-3}	M	(5)
X_{C4}	Complex IV activity	9.93×10^{-5}	mol s^{-1} M^{-1} (l mito)$^{-1}$	(5)
X_{F1}	F_oF_1-ATPase activity	5.95×10^{3}	mol s^{-1} M^{-1} (l mito)$^{-1}$	(5)
X_{ANT}	ANT activity	1.52×10^{-1}	unitless	(1)
X_{PIHt}	$H_2PO_4^-/H^+$ co-transporter activity	2.01×10^{7}	mol s^{-1} M^{-1} (l mito)$^{-1}$	(5)
k_{PIHt}	$H_2PO_4^-/H^+$ co-transporter parameter	1.01×10^{-3}	M	(5)
X_{KH}	K^+/H^+ antiporter activity	5.65×10^{6}	mol s^{-1} M^{-2} (l mito)$^{-1}$	(5)
X_{Hle}	Proton leak activity	3.05×10^{2}	mol s^{-1} mV^{-1} M^{-1} (l mito)$^{-1}$	(5)
X_{AKi}	IM space adenylate kinase activity	1×10^{10}	mol s^{-1} M^{-2} (l mito)$^{-1}$	-
X_{AKc}	Cytoplasmic adenylate kinase activity	1×10^{10}	mol s^{-1} M^{-2} (l cyto)$^{-1}$	-
X_{CKc}	Cytoplasmic creatine kinase	1×10^{10}	mol s^{-1} M^{-2} (l cyto)$^{-1}$	-
Oxygen Transport Parameters				
α_1	Plasma O_2 solubility	1.30×10^{-6}	M mm Hg^{-1}	(9)
α_2	Interstitial fluid O_2 solubility	1.25×10^{-6}	M mm Hg^{-1}	(10)
α_3	Myocyte O_2 solubility	1.74×10^{-6}	M mm Hg^{-1}	(11)
PS_{12}	Capillary wall PS product	50	ml s^{-1} (ml tissue)$^{-1}$	(12, 13)
PS_{23}	Myocyte fiber PS product	10	ml s^{-1} (ml tissue)$^{-1}$	(14)
Hct	Hematocrit	0.45	unitless	(2)
C_{Hb}	Oxyhemoglobin binding site concentration	0.0213	mol (l RBC)$^{-1}$	(9)
$P_{50,Hb}$	Hemoglobin half-saturation P_{O2}	30.0	mm Hg	(15)
n_H	Hemoglobin Hill coefficient	2.55	unitless	(15)
C_{Mb}	Myoglobin saturation	200×10^{-6}	mol (l cell)$^{-1}$	(2)
$P_{50,Mb}$	Myoglobin half-saturation P_{O2}	2.39	mm Hg	(16)
P_{input}	Arterial oxygen tension	100	mm Hg	(2)
Physicochemical Parameters:				
RT	Gas constant times temperature	2.5775	kJ mol^{-1}	–[a]
F	Faraday's constant	0.096484	kJ mol^{-1} mV^{-1}	–[a]
Structure/Volume Parameters:				
ρ	Tissue density	1.053	g (ml tissue)$^{-1}$	(4)
L	Capillary length	550	μm	(17)

V_1	Capillary blood volume	0.05	ml (ml tissue)$^{-1}$	(18, 19)
V_2	Interstitial volume	0.17585	ml (ml tissue)$^{-1}$	(4)
V_3	Myocyte volume	0.73078	ml (ml tissue)$^{-1}$	(4)
V_{cyto}	Cytoplasm Volume	0.894	(l cytoplasm) (l cell)$^{-1}$	(20)
V_{mito}	Mitochondrial Volume	0.056	(l mito) (l cell)$^{-1}$	(21)
W_x	Matrix water space fraction	0.6514	(l water) (l mito)$^{-1}$	(4, 22)
W_i	IM space water fraction	0.0724	(l water) (l mito)$^{-1}$	(4, 22)
W_c	Cytoplasm water fraction	0.8425	(l water) (l cyto)$^{-1}$	(4)
γ	Outer membrane area per mito volume	5.99	μm^{-1}	(23)
ρ_m	Protein density of mitochondria	2.725×10^5	(mg Protein) (l mito)$^{-1}$	(4)
Mitochondrial Model Parameters:				
n_A	H^+ stoich. coef. for F_1F_0-ATPase	3	unitless	(24)
p_{PI}	Mitochondrial membrane permeability to inorganic phosphate	327	$\mu m\ sec^{-1}$	(25)
p_A	Mitochondrial outer membrane permeability to nucleotides	85.0	$\mu m\ sec^{-1}$	(26)
k_{O2}	Kinetic constant for complex IV	1.2×10^{-4}	M	(25)[d]
C_{IM}	Capacitance of inner membrane	6.75×10^{-6}	mol (l mito)$^{-1}$ mV^{-1}	(22, 27)
B_x	Matrix buffering parameter	0.02	M	(28, 29)[b]
K_{Bx}	Matrix buffering parameter	1×10^{-7}	M	(28, 29)[b]
Fixed Concentrations and Concentration Pools:				
NAD_{tot}	Total matrix NAD(H) concentration	2.97	mol (l matrix water)$^{-1}$	(25)[c]
Q_{tot}	Total matrix ubiquinol concentration	1.35	mol (l matrix water)$^{-1}$	(25)[c]
$cytC_{tot}$	Total IM cytochrome c concentration	2.70	mol (l IM water)$^{-1}$	(25)[c]
A_{tot}	Total matrix ATP+ADP concentration	10	mol (l matrix water)$^{-1}$	(25)[c]
CR_{tot}	Total Cr+CrP concentration	42.7	mol (l cytoplasm water)$^{-1}$	(30)
$[CO_2tot]_x$	Total CO_2 concentration in the matrix	21.4×10^{-3}	Molar	(31)

Standard Gibbs Free Energy or Equilibrium Constants of Reference Reactions:[d]				
$\Delta_r G^0_{\mathrm{C1}}$	Complex I	-109.7	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{C3}}$	Complex III	46.69	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{C4}}$	Complex IV	-202.2	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{F1}}$	F_oF_1-ATPase	-4.51	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{pdh}}$	Pyruvate dehydrogenase	19.59	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{cits}}$	Citrate synthase	42.36	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{acon}}$	Aconitase	12.82	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{isod}}$	Isocitrate dehydrogenase	91.75	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{akgd}}$	α-Ketoglutarate dehydrogenase	12.82	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{scoas}}$	Succinyl-CoA synthetase	47.61	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{sdh}}$	Succinate dehydrogenase	-1.35	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{fum}}$	Fumarase	-3.60	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{mdh}}$	Malate dehydrogenase	69.12	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{ndk}}$	Nucleoside diphosphokinase	0	kJ mol^{-1}	(5)
$\Delta_r G^0_{\mathrm{got}}$	Glutamate Oxaloacetate Transaminase	-1.47	kJ mol^{-1}	(5)
$K^o_{eq,\mathrm{AK}}$	Adenylate kinase	3.97×10^{-1}	unitless	(1)
$K^o_{eq,\mathrm{CK}}$	Creatine kinase	3.57×10^{8}	M^{-1}	(1)

[a]Standard physicochemical constants
[b]Values are adjusted to match experimental data.
[c]Value used is taken from previous modeling studies, not direct experimental measure.
[d]Values used are calculated for reference reactions at physiological temperature (310.15 K) and ionic strength (0.17 M) based on standard thermodynamic data collected from the Alberty's book (32) and NIST database (33). Please refer to Appendices of (5) and (1) for detailed computation procedures.

S2. Computational model

The model is mathematically described by using the differential equations listed below. The oxidative phosphorylation component of the model is derived from previously published work (22, 34, 35). The details behind the TCA cycle enzyme kinetic schemes are provided in Appendix C of our previously published work (5). Here the subscripts "x", "i", and "c" on variable names denote matrix, intermembrane, and cytoplasmic (extra-mitochondrial) spaces, respectively. For example $[\text{ATP}]_x$ denotes matrix ATP concentration while $[\text{ATP}]_c$ denotes ATP concentration in the cytoplasm or buffer space for an isolated mitochondria experiment.

S2.1. Differential equations

The differential equations are grouped into equations for oxygen concentration, membrane potential, mitochondrial matrix variables, intermembrane space variables, and cytoplasm variables. The time derivatives of free $[\text{H}^+]$, $[\text{Mg}^{2+}]$, and $[\text{K}^+]$ are treated separately.

Oxygen concentration:

$$\partial C_{\text{O}_2,\text{capillary}} / \partial t = -\frac{\rho GL}{V_1} \partial C_{\text{O}_2,\text{capillary}} / \partial x - \frac{\alpha_1 PS_{12}}{V_1}(P_{\text{O}_2,\text{capillary}} - P_{\text{O}_2,\text{interstitium}}) \quad \text{(S1)}$$

$$\partial C_{\text{O}_2,\text{interstitium}} / \partial t = -\frac{\alpha_1 PS_{12}}{V_2}(P_{\text{O}_2,\text{capillary}} - P_{\text{O}_2,\text{interstitium}}) - \frac{\alpha_1 PS_{23}}{V_2}(P_{\text{O}_2,\text{interstitium}} - P_{\text{O}_2,\text{cell}}) \quad \text{(S2)}$$

$$\partial C_{\text{O}_2,\text{cell}} / \partial t = -\frac{\alpha_1 PS_{23}}{V_2}(P_{\text{O}_2,\text{capillary}} - P_{\text{O}_2,\text{cell}}) - V_{\text{mito}}\left(\frac{J_{C4}}{2}\right) \quad \text{(S3)}$$

where

$$C_{\text{O}_2,\text{capillary}} = \alpha_1 P_{\text{O}_2,\text{capillary}} + Hct C_{\text{Hb}} S_{\text{Hb}} \quad \text{(S4)}$$

$$S_{\text{Hb}} = \frac{P^{n_H}_{\text{O}_2,\text{capillary}}}{P^{n_H}_{\text{O}_2,\text{capillary}} + P^{n_H}_{50,\text{Hb}}} \quad \text{(S5)}$$

$$C_{\text{O}_2,\text{cell}} = \alpha_3 P_{\text{O}_2,\text{cell}} + C_{\text{Mb}} S_{\text{Mb}} \quad \text{(S6)}$$

$$S_{\text{Mb}} = \frac{P_{\text{O}_2,\text{cell}}}{P_{\text{O}_2,\text{cell}} + P_{50,\text{Mb}}}. \quad \text{(S7)}$$

Mitochondrial Inner Membrane Electrical Potential:

$$\partial \Delta\Psi / \partial t = (+4J_{\text{C1}} + 2J_{\text{C3}} + 4J_{\text{C4}} - n_{\text{A}} J_{\text{F1}} - J_{\text{ANT}} - J_{\text{Hle}} + J_{\text{ASPGLU}}) / C_{\text{IM}} \quad \text{(S8)}$$

Mitochondrial Matrix:

$$\partial[\text{ATP}]_{\text{x}} / \partial t = (+J_{\text{ndk}} + J_{\text{F1}} - J_{\text{ANT}}) / W_{\text{x}} \quad \text{(S9)}$$

$$\partial[\text{ADP}]_{\text{x}} / \partial t = (-J_{\text{ndk}} - J_{\text{F1}} + J_{\text{ANT}}) / W_{\text{x}} \quad \text{(S10)}$$

$$\partial[\text{AMP}]_{\text{x}} / \partial t = 0 / W_{\text{x}} \quad \text{(S11)}$$

$$\partial[\text{GTP}]_{\text{x}} / \partial t = (+J_{\text{scoas}} - J_{\text{ndk}}) / W_{\text{x}} \quad \text{(S12)}$$

$$d[\mathrm{GDP}]_\mathrm{x} / dt = (-J_\mathrm{scoas} + J_\mathrm{ndk}) / W_\mathrm{x} \tag{S13}$$

$$\partial[\mathrm{PI}]_\mathrm{x} / \partial t = (-J_\mathrm{scoas} - J_\mathrm{F1} + J_\mathrm{PIHt} - J_\mathrm{SUCPI} - J_\mathrm{MALPI}) / W_\mathrm{x} \tag{S14}$$

$$\partial[\mathrm{NADH}]_\mathrm{x} / \partial t = (+J_\mathrm{pdh} + J_\mathrm{isod} + J_\mathrm{akgd} + J_\mathrm{mdh} - J_\mathrm{C1}) / W_\mathrm{x} \tag{S15}$$

$$\partial[\mathrm{QH_2}]_\mathrm{x} / \partial t = (+J_\mathrm{sdh} + J_\mathrm{C1} - J_\mathrm{C3}) / W_\mathrm{x} \tag{S16}$$

$$\partial[\mathrm{PYR}]_\mathrm{x} / \partial t = (-J_\mathrm{pdh} + J_\mathrm{PYRH}) / W_\mathrm{x} \tag{S17}$$

$$\partial[\mathrm{ACCOA}]_\mathrm{x} / \partial t = (-J_\mathrm{cits} + J_\mathrm{pdh}) / W_\mathrm{x} \tag{S18}$$

$$\partial[\mathrm{CIT}]_\mathrm{x} / \partial t = (+J_\mathrm{cits} - J_\mathrm{acon} + J_\mathrm{CITMAL}) / W_\mathrm{x} \tag{S19}$$

$$\partial[\mathrm{ICIT}]_\mathrm{x} / \partial t = (+J_\mathrm{acon} - J_\mathrm{isod}) / W_\mathrm{x} \tag{S20}$$

$$\partial[\mathrm{AKG}]_\mathrm{x} / \partial t = (+J_\mathrm{isod} - J_\mathrm{akgd} - J_\mathrm{got} + J_\mathrm{AKGMAL}) / W_\mathrm{x} \tag{S21}$$

$$\partial[\mathrm{SCOA}]_\mathrm{x} / \partial t = (+J_\mathrm{akgd} - J_\mathrm{scoas}) / W_\mathrm{x} \tag{S22}$$

$$\partial[\mathrm{COASH}]_\mathrm{x} / \partial t = (-J_\mathrm{pdh} - J_\mathrm{akgd} + J_\mathrm{scoas} + J_\mathrm{cits}) / W_\mathrm{x} \tag{S23}$$

$$\partial[\mathrm{SUC}]_\mathrm{x} / \partial t = (+J_\mathrm{scoas} - J_\mathrm{sdh} + J_\mathrm{SUCPI} - J_\mathrm{FUMSUC}) / W_\mathrm{x} \tag{S24}$$

$$\partial[\mathrm{FUM}]_\mathrm{x} / \partial t = (+J_\mathrm{sdh} - J_\mathrm{fum} + J_\mathrm{FUMSUC}) / W_\mathrm{x} \tag{S25}$$

$$\partial[\mathrm{MAL}]_\mathrm{x} / \partial t = (+J_\mathrm{fum} - J_\mathrm{mdh} + J_\mathrm{MALPI} - J_\mathrm{AKGMAL} - J_\mathrm{CITMAL}) / W_\mathrm{x} \tag{S26}$$

$$\partial[\mathrm{OAA}]_\mathrm{x} / \partial t = (-J_\mathrm{cits} + J_\mathrm{mdh} + J_\mathrm{got}) / W_\mathrm{x} \tag{S27}$$

$$\partial[\mathrm{GLU}]_\mathrm{x} / \partial t = (+J_\mathrm{got} + J_\mathrm{GLUH} - J_\mathrm{ASPGLU}) / W_\mathrm{x} \tag{S28}$$

$$\partial[\mathrm{ASP}]_\mathrm{x} / \partial t = (-J_\mathrm{got} + J_\mathrm{ASPGLU}) / W_\mathrm{x} \tag{S29}$$

$$\partial[\mathrm{CO_2tot}]_\mathrm{x} / \partial t = 0 / W_\mathrm{x}\,. \tag{S30}$$

Mitochondrial Inter-Membrane Space:

$$\partial[\mathrm{Cred}]_\mathrm{i} / \partial t = (+2J_\mathrm{C3} - 2J_\mathrm{C4}) / W_\mathrm{i} \tag{S31}$$

$$\partial[\mathrm{ATP}]_\mathrm{i} / \partial t = (+J_\mathrm{ATP} + J_\mathrm{ANT} + J_\mathrm{AKi}) / W_\mathrm{i} \tag{S32}$$

$$\partial[\mathrm{ADP}]_\mathrm{i} / \partial t = (+J_\mathrm{ADP} - J_\mathrm{ANT} - 2J_\mathrm{AKi}) / W_\mathrm{i} \tag{S33}$$

$$\partial[\mathrm{AMP}]_\mathrm{i} / \partial t = (J_\mathrm{AMPt} + J_\mathrm{AKi}) / W_\mathrm{i} \tag{S34}$$

$$\partial[\mathrm{PI}]_\mathrm{i} / \partial t = (-J_\mathrm{PIHt} + J_\mathrm{PIt} + J_\mathrm{MALPI} + J_\mathrm{SUCPI}) / W_\mathrm{i} \tag{S35}$$

$$\partial[\mathrm{PYR}]_\mathrm{i} / \partial t = (-J_\mathrm{PYRH} + J_\mathrm{PYRt}) / W_\mathrm{i} \tag{S36}$$

$$\partial[\mathrm{CIT}]_\mathrm{i} / \partial t = (-J_\mathrm{CITMAL} + J_\mathrm{CITt}) / W_\mathrm{i} \tag{S37}$$

$$\partial[\mathrm{ICIT}]_\mathrm{i} / \partial t = (-J_\mathrm{ICITMAL} + J_\mathrm{ICITt}) / W_\mathrm{i} \tag{S38}$$

$$\partial[\mathrm{AKG}]_\mathrm{i} / \partial t = (-J_\mathrm{AKGMAL} + J_\mathrm{AKGt}) / W_\mathrm{i} \tag{S39}$$

$$\partial[\mathrm{SUC}]_\mathrm{i} / \partial t = (-J_\mathrm{SUCPI} + J_\mathrm{SUCt} + J_\mathrm{FUMSUC}) / W_\mathrm{i} \tag{S40}$$

$$\partial[\mathrm{FUM}]_\mathrm{i} / \partial t = (-J_\mathrm{FUMPI} + J_\mathrm{FUMt}) / W_\mathrm{i} \tag{S41}$$

$$\partial[\mathrm{MAL}]_\mathrm{i} / \partial t = (-J_\mathrm{MALPI} + J_\mathrm{MALt} + J_\mathrm{AKGMAL} + J_\mathrm{CITMAL}) / W_\mathrm{i} \quad \text{(S42)}$$

$$\partial[\mathrm{GLU}]_\mathrm{i} / \partial t = (-J_\mathrm{GLUH} + J_\mathrm{ASPGLU} + J_\mathrm{GLUt}) / W_\mathrm{i} \quad \text{(S43)}$$

$$\partial[\mathrm{ASP}]_\mathrm{i} / \partial t = (-J_\mathrm{ASPGLU} + J_\mathrm{ASPt}) / W_\mathrm{i}\,. \quad \text{(S44)}$$

Cytoplasm:

$$\partial[\mathrm{ATP}]_\mathrm{c} / \partial t = (-(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{ATPt} - J_\mathrm{ATPase} + J_\mathrm{CKc} + J_\mathrm{AKc}) / W_\mathrm{c} \quad \text{(S45)}$$

$$\partial[\mathrm{ADP}]_\mathrm{c} / \partial t = (-(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{ADPt} + J_\mathrm{ATPase} - J_\mathrm{CKc} - 2 J_\mathrm{AKc}) / W_\mathrm{c} \quad \text{(S46)}$$

$$\partial[\mathrm{AMP}]_\mathrm{c} / \partial t = (-(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{AMPt} + J_\mathrm{AKc}) / W_\mathrm{c} \quad \text{(S47)}$$

$$\partial[\mathrm{PI}]_\mathrm{c} / \partial t = (-(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{PIt} + J_\mathrm{AKc}) / W_\mathrm{c} \quad \text{(S48)}$$

$$\partial[\mathrm{PYR}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{PYRt} / W_\mathrm{c} \quad \text{(S49)}$$

$$\partial[\mathrm{CIT}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{CITt} / W_\mathrm{c} \quad \text{(S50)}$$

$$\partial[\mathrm{AKG}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{AKGt} / W_\mathrm{c} \quad \text{(S51)}$$

$$\partial[\mathrm{SUC}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{SUCt} / W_\mathrm{c} \quad \text{(S52)}$$

$$\partial[\mathrm{FUM}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{FUMt} / W_\mathrm{c} \quad \text{(S53)}$$

$$\partial[\mathrm{MAL}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{MALt} / W_\mathrm{c} \quad \text{(S54)}$$

$$\partial[\mathrm{GLU}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{GLUt} / W_\mathrm{c} \quad \text{(S55)}$$

$$\partial[\mathrm{ASP}]_\mathrm{c} / \partial t = -(V_\mathrm{cyto} / V_\mathrm{mito}) J_\mathrm{ASPt} / W_\mathrm{c} \quad \text{(S56)}$$

$$\partial[\mathrm{PCr}]_\mathrm{c} / \partial t = -J_\mathrm{CKc} / W_\mathrm{c}\,. \quad \text{(S57)}$$

Assuming constant total concentrations $\mathrm{NAD_{tot}}$, $\mathrm{Q_{tot}}$, $\mathrm{cytC_{tot}}$, and $\mathrm{A_{tot}}$ for nicotinamide nucleotides, ubiquinol, and cytochrome c, we compute concentrations of the following reactants as:

$$[\mathrm{NAD}]_\mathrm{x} = \mathrm{NAD_{tot}} - [\mathrm{NADH}]_\mathrm{x} \quad \text{(S58)}$$

$$[\mathrm{COQ}]_\mathrm{x} = \mathrm{Q_{tot}} - [\mathrm{QH_2}]_\mathrm{x} \quad \text{(S59)}$$

$$[\mathrm{Cox}]_\mathrm{i} = \mathrm{cytC_{tot}} - [\mathrm{Cred}]_\mathrm{i}\,. \quad \text{(S60)}$$

Concentrations of cytoplasmic H^+, Mg^{2+}, and K^+ are assumed to be fixed at buffer conditions or physiological in vivo values. Since the outer membrane is highly permeable to hydrogen ions and cations, we assume here $[\mathrm{H^+}]_\mathrm{i} = [\mathrm{H^+}]_\mathrm{c}$, $[\mathrm{Mg^{2+}}]_\mathrm{i} = [\mathrm{Mg^{2+}}]_\mathrm{c}$, and $[\mathrm{K^+}]_\mathrm{i} = [\mathrm{K^+}]_\mathrm{c}$.

The rate of change of free $[H^+]$, $[Mg^{2+}]$ and $[K^+]$ in the mitochondrial matrix can be calculated based on mass conservation. Mathematical expressions for time derivatives of $[H^+]$, $[Mg^{2+}]$, and $[K^+]$ are listed below. For detailed description and derivation of these expressions, please refer to Chapter 6 of Beard and Qian (36).

$$\frac{\partial[\mathrm{H}^+]}{\partial t} = \Bigg[\left(\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]} - \alpha_{Mg}\alpha_K\right)\Phi^H + \left(\alpha_K\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} - \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]}\cdot\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\right)\Phi^{Mg} + \left(\alpha_{Mg}\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]} - \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]}\right)\Phi^K\Bigg]/D \tag{S61}$$

$$\frac{\partial[\mathrm{Mg}^{2+}]}{\partial t} = \Bigg[\left(\alpha_K\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]} - \frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]}\right)\Phi^H + \left(\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]}\cdot\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]} - \alpha_H\alpha_K\right)\Phi^{Mg} + \left(\alpha_H\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]} - \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]}\right)\Phi^K\Bigg]/D \tag{S62}$$

$$\frac{\partial[\mathrm{K}^+]}{\partial t} = \Bigg[\left(\alpha_{Mg}\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]} - \frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]}\right)\Phi^H + \left(\alpha_H\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} - \frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]}\cdot\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\right)\Phi^{Mg} + \left(\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]}\cdot\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} - \alpha_H\alpha_{Mg}\right)\Phi^K\Bigg]/D \;. \tag{S63}$$

The binding polynomials are calculated as

$$P_i\left([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+]\right) = 1 + [\mathrm{H}^+]/K_i^H + [\mathrm{Mg}^{2+}]/K_i^{Mg} + [\mathrm{K}^+]/K_i^K \,. \tag{S64}$$

The partial derivatives of total concentrations of bound [H^+], [Mg^{2+}], and [K^+] are expressed as:

$$\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{H}^+]/K_i^H}{K_i^{Mg}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S65}$$

$$\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{H}^+]/K_i^H}{K_i^{K}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S66}$$

$$\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{H}^+]} = \sum_{i=1}^{N_r}\frac{[\mathrm{L}_i]\left(1+[\mathrm{Mg}^{2+}]/K_i^{Mg}+[\mathrm{K}^+]/K_i^K\right)}{K_i^{H}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S67}$$

$$\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{Mg}^{2+}]/K_i^{Mg}}{K_i^{H}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S68}$$

$$\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{Mg}^{2+}]/K_i^{Mg}}{K_i^K\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S69}$$

$$\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} = \sum_{i=1}^{N_r}\frac{[\mathrm{L}_i]\left(1+[\mathrm{H}^+]/K_i^H+[\mathrm{K}^+]/K_i^K\right)}{K_i^{Mg}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S70}$$

$$\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{K}^+]/K_i^K}{K_i^H\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S71}$$

$$\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} = -\sum_{i=1}^{N_r}\frac{[\mathrm{L}_i][\mathrm{K}^+]/K_i^K}{K_i^{Mg}\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2} \tag{S72}$$

$$\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{K}^+]} = \sum_{i=1}^{N_r}\frac{[\mathrm{L}_i]\left(1+[\mathrm{H}^+]/K_i^H+[\mathrm{Mg}^{2+}]/K_i^{Mg}\right)}{K_i^K\left(P_i([\mathrm{H}^+],[\mathrm{Mg}^{2+}],[\mathrm{K}^+])\right)^2}. \tag{S73}$$

The flux terms for H^+, Mg^{2+}, and K^+ are:

$$\Phi^H = -\sum_{i=1}^{N_r}\frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{L}_i]}\frac{d[\mathrm{L}_i]}{dt} + \sum_{k=1}^{N_f}n_k J_k + J_t^H \tag{S74}$$

$$\Phi^{Mg} = -\sum_{i=1}^{N_r}\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{L}_i]}\frac{d[\mathrm{L}_i]}{dt} + J_t^{Mg} \tag{S75}$$

$$\Phi^K = -\sum_{i=1}^{N_r}\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{L}_i]}\frac{d[\mathrm{L}_i]}{dt} + J_t^K. \tag{S76}$$

where N_r is the number of reactants, N_f is the number of reactions, n_k is the stoichiometric coefficient of k^{th} reaction, J_k is the flux of k^{th} reaction, J_t^H (J_t^{Mg}, J_t^K) is the transport flux of $[H^+]$ ($[Mg^{2+}]$, $[K^+]$) into the system. In the current model, for the mitochondrial matrix, we have

$$\sum_{k=1}^{N_f}n_k J_k = \left(-J_{\mathrm{pdh}}+2J_{\mathrm{cits}}-J_{\mathrm{akgd}}+J_{\mathrm{scoas}}+J_{\mathrm{mdh}}\right)/W_{\mathrm{x}} \tag{S77}$$

$$\begin{aligned} J_t^H = &\left(J_{\mathrm{PYRH}}+J_{\mathrm{GLUH}}+J_{\mathrm{CITMAL}}-J_{\mathrm{ASPGLU}}-5J_{\mathrm{C1}}-2J_{\mathrm{C3}}-4J_{\mathrm{C4}}+(n_A-1)J_{\mathrm{F1}}\right.\\ &\left.+2J_{\mathrm{PIHt}}+J_{\mathrm{Hle}}-J_{\mathrm{KH}}\right)/W_{\mathrm{x}} \end{aligned} \tag{S78}$$

$$J_t^{Mg} = 0 \tag{S79}$$

$$J_t^K = J_{\mathrm{KH}}/W_{\mathrm{x}}. \tag{S80}$$

The buffering terms are:

$$\alpha_H = 1 + \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{H}^+]} + \frac{B_x}{K_{Bx}\left(1+[\mathrm{H}^+]/K_{Bx}\right)^2} \tag{S81}$$

$$\alpha_{Mg} = 1 + \frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{Mg}^{2+}]} \tag{S82}$$

$$\alpha_K = 1 + \frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{K}^+]} \quad \text{(S83)}$$

The denominator term in the time derivatives of $[H^+]$, $[Mg^{2+}]$, and $[K^+]$ is:

$$\begin{aligned} D = \; & \alpha_H \frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]} + \alpha_K \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]} \\ & + \alpha_{Mg} \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]}\cdot\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]} - \alpha_{Mg}\alpha_K\alpha_H \\ & - \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{K}^+]}\cdot\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{H}^+]} \\ & - \frac{\partial[\mathrm{H}_{bound}]}{\partial[\mathrm{Mg}^{2+}]}\cdot\frac{\partial[\mathrm{Mg}_{bound}]}{\partial[\mathrm{K}^+]}\cdot\frac{\partial[\mathrm{K}_{bound}]}{\partial[\mathrm{H}^+]}. \end{aligned} \quad \text{(S84)}$$

S2.2. Flux expressions

Mathematical expressions for oxidative phosphorylation fluxes

Complex I flux:

$$J_{\mathrm{C1}} = X_{\mathrm{C1}}\left(K_{eq,\mathrm{C1}}[\mathrm{NADH}]_{\mathrm{x}}[\mathrm{COQ}]_{\mathrm{x}} - [\mathrm{NAD}]_{\mathrm{x}}[\mathrm{QH}_2]_{\mathrm{x}}\right), \quad \text{(S85)}$$

where $K_{eq,\mathrm{C1}} = K^0_{eq,\mathrm{C1}} \cdot [\mathrm{H}^+]_{\mathrm{x}}^5 / [\mathrm{H}^+]_{\mathrm{i}}^4$, with $K^0_{eq,C1} = \exp\left(-\left(\Delta_r G^0_{\mathrm{C1}} + 4F\Delta\Psi\right)/RT\right)$ and $\Delta_r G^0_{\mathrm{C1}} = \Delta_f G^0_{\mathrm{NAD}} + \Delta_f G^0_{\mathrm{QH_2}} - \Delta_r G^0_{\mathrm{NADH}} - \Delta_r G^0_{\mathrm{COQ}} = -109.7$ kJ/mol.

Complex III flux:

$$J_{\mathrm{C3}} = X_{\mathrm{C3}}\left(\frac{1+[\mathrm{PI}]_{\mathrm{x}}/k_{\mathrm{PI,3}}}{1+[\mathrm{PI}]_{\mathrm{x}}/k_{\mathrm{PI,4}}}\right)\left(K^{1/2}_{eq,\mathrm{C3}}[\mathrm{Cox}]_{\mathrm{i}}[\mathrm{QH}_2]_{\mathrm{x}}^{1/2} - [\mathrm{Cred}]_{\mathrm{i}}[\mathrm{COQ}]_{\mathrm{x}}^{1/2}\right), \quad \text{(S86)}$$

where $K_{eq,\mathrm{C3}} = K^0_{eq,\mathrm{C3}} \cdot [\mathrm{H}^+]_{\mathrm{x}}^2 / [\mathrm{H}^+]_{\mathrm{i}}^4$, with $K^0_{eq,C3} = \exp\left(-\left(\Delta_r G^0_{\mathrm{C3}} + 2F\Delta\Psi\right)/RT\right)$ and $\Delta_r G^0_{\mathrm{C3}} = \Delta_f G^0_{\mathrm{Cred}} + \Delta_f G^0_{\mathrm{COQ}} - \Delta_r G^0_{\mathrm{QH2}} - \Delta_r G^0_{\mathrm{Cox}} = 46.69$ kJ/mol.

Complex IV flux:

$$J_{\mathrm{C4}} = X_{\mathrm{C4}}\left(\frac{1}{1+k_{\mathrm{O_2}}/[\mathrm{O}_2]}\right)\exp\left(\frac{F\Delta\Psi}{RT}\right)\left(\frac{[\mathrm{Cred}]_{\mathrm{i}}}{\mathrm{cytC_{tot}}}\right)\left(K^{1/2}_{eq,\mathrm{C4}}[\mathrm{Cred}]_{\mathrm{i}}[\mathrm{O}_2]_{\mathrm{x}}^{1/4} - [\mathrm{Cox}]_{\mathrm{i}}\right), \quad \text{(S87)}$$

where $[O_2]_x$ is assumed to be equal to $C_{O2,cell}$, $K_{eq,\mathrm{C4}} = K^0_{eq,\mathrm{C4}} \cdot [\mathrm{H}^+]_{\mathrm{x}}^4 / [\mathrm{H}^+]_{\mathrm{i}}^2$, with $K^0_{eq,\mathrm{C4}} = \exp\left(-\left(\Delta_r G^0_{\mathrm{C4}} + 4F\Delta\Psi\right)/RT\right)$ and $\Delta_r G^0_{\mathrm{C4}} = \Delta_f G^0_{\mathrm{Cox}} + \Delta_f G^0_{\mathrm{H_2O}} - \Delta_r G^0_{\mathrm{Cred}} - \Delta_r G^0_{\mathrm{O_2}} = -202.2$ kJ/mol.

F_oF_1-ATPase flux:

$$J_{\mathrm{F1}} = X_{\mathrm{F1}}\left(K_{eq,\mathrm{F1}}[\mathrm{ADP}]_{\mathrm{x}}[\mathrm{PI}]_{\mathrm{x}} - [\mathrm{ATP}]_{\mathrm{x}}\right). \quad \text{(S88)}$$

where $K_{eq,\mathrm{F1}} = K^0_{eq,\mathrm{F1}} \cdot \frac{[\mathrm{H}^+]_{\mathrm{i}}^{n_{\mathrm{A}}}}{[\mathrm{H}^+]_{\mathrm{x}}^{n_{\mathrm{A}}-1}} \cdot \frac{P_{\mathrm{ATP}}}{P_{\mathrm{ADP}}P_{\mathrm{PI}}}$, with $K^0_{eq,\mathrm{F1}} = \exp\left(-\left(\Delta_r G^0_{\mathrm{F1}} - n_{\mathrm{A}} F \Delta\Psi\right)/RT\right)$ and

$\Delta_r G^0_{\mathrm{F1}} = \Delta_f G^0_{\mathrm{ATP}} + \Delta_f G^0_{\mathrm{H_2O}} - \Delta_r G^0_{\mathrm{ADP}} - \Delta_r G^0_{\mathrm{PI}} = -4.51 \text{ kJ/mol}$.

Mitochondrial adenylate kinase flux:

$$J_{\mathrm{AKi}} = X_{\mathrm{AKi}}\left(K_{\mathrm{eq,AK}}[\mathrm{ADP}]_{\mathrm{i}}[\mathrm{ADP}]_{\mathrm{i}} - [\mathrm{AMP}]_{\mathrm{i}}[\mathrm{ATP}]_{\mathrm{i}}\right). \quad \text{(S89)}$$

Mathematical expressions for TCA cycle fluxes

For brevity, values of Michaelis, inhibition, or activation constants are not presented here, but provided in Appendix C of (5). In the following TCA cycle flux expressions, the enzyme activity is represented by V_{mf}, and V_{mr} is related to V_{mf} by obeying the Haledane equation (37).

Pyruvate dehydrogenase flux:

$$J_{\mathrm{pdh}} = \frac{V_{mf}\left(1 - \frac{1}{K_{eq,\mathrm{pdh}}}\frac{[\mathrm{P}][\mathrm{Q}][\mathrm{R}]}{[\mathrm{A}][\mathrm{B}][\mathrm{C}]}\right)}{K_{mC}\alpha_{i2}[\mathrm{A}][\mathrm{B}] + K_{mB}\alpha_{i1}[\mathrm{A}][\mathrm{C}] + K_{mA}[\mathrm{B}][\mathrm{C}] + [\mathrm{A}][\mathrm{B}][\mathrm{C}]}, \quad \text{(S90)}$$

where [A] = [PYR], [B] = [COASH], [C] = [NAD], [P] = [CO_2tot], [Q] = [ACCOA], and [R] = [NADH],

$$K_{eq,\mathrm{pdh}} = K^0_{eq,\mathrm{pdh}} \frac{1}{[\mathrm{H}^+]} \frac{P_{\mathrm{CO_2tot}} P_{\mathrm{ACCOA}} P_{\mathrm{NADH}}}{P_{\mathrm{PYR}} P_{\mathrm{COASH}} P_{\mathrm{NAD}}} \text{ with } K^0_{eq,\mathrm{pdh}} = \exp\left(-\frac{\Delta_r G^0_{\mathrm{pdh}}}{RT}\right) = 5.02 \times 10^{-4} \text{ M}^{-1}.$$

Citrate Synthase flux:

$$J_{\mathrm{cits}} = \frac{V_{mf}\left([\mathrm{A}][\mathrm{B}] - \frac{[\mathrm{P}][\mathrm{Q}]}{K_{eq,\mathrm{cits}}}\right)}{K_{ia}K_{mB}\alpha_{i1} + K_{mA}\alpha_{i1}[\mathrm{B}] + K_{mB}\alpha_{i2}[\mathrm{A}] + [\mathrm{A}][\mathrm{B}]}, \quad \text{(S91)}$$

where [A] = [OAA], [B] = [ACCOA], [P] = [COASH], and [Q] = [CIT],

$$K_{eq,\mathrm{cits}} = K^0_{eq,\mathrm{cits}} \frac{1}{[\mathrm{H}^+]^2} \frac{P_{\mathrm{COA}} P_{\mathrm{CIT}}}{P_{\mathrm{OAA}} P_{\mathrm{ACCOA}}} \text{ with } K^0_{eq,\mathrm{cits}} = \exp\left(-\frac{\Delta_r G^0_{\mathrm{cits}}}{RT}\right) = 7.34 \times 10^{-8} \text{ M}^{-1}.$$

Aconitase flux:

$$J_{\mathrm{acon}} = \frac{V_{mf}V_{mr}\left([\mathrm{A}] - \frac{[\mathrm{P}]}{K_{eq,\mathrm{acon}}}\right)}{K_{mA}V_{mr} + V_{mr}[\mathrm{A}] + \frac{V_{mf}}{K_{eq,\mathrm{acon}}}[\mathrm{P}]}, \quad \text{(S92)}$$

where [A] = [CIT] and [P] = [ICIT], $K_{eq,\mathrm{acon}} = K^0_{eq,\mathrm{acon}} \frac{P_{\mathrm{ICIT}}}{P_{\mathrm{CIT}}}$ with $K^0_{eq,\mathrm{acon}} = \exp\left(-\frac{\Delta_r G^0_{\mathrm{acon}}}{RT}\right) = 7.59 \times 10^{-2}$.

Isocitrate dehydrogenase flux:

$$J_{\text{isod}} = \frac{V_{mf}\left(1 - \frac{1}{K_{eq,\text{isod}}}\frac{[\text{P}][\text{Q}][\text{R}]}{[\text{A}][\text{B}]}\right)}{1 + \left(\frac{K_{mB}}{[\text{B}]}\right)^{n_H}\alpha_i + \frac{K_{mA}}{[\text{A}]}\left(1 + \left(\frac{K_{ib}}{[\text{B}]}\right)^{n_H}\alpha_i + \frac{[\text{Q}]}{K_{iq}}\alpha_i\right)}, \quad \text{(S93)}$$

where [A] = [NAD], [B] = [ICIT], [P] = [AKG], [Q] = [NADH], and [R] = [CO_2tot],

$$K_{eq,\text{isod}} = K^0_{eq,\text{isod}}\frac{1}{[\text{H}^+]^2}\frac{P_{\text{AKG}}P_{\text{NADH}}P_{\text{CO}_2\text{tot}}}{P_{\text{NAD}}P_{\text{ICIT}}} \text{ with } K^0_{eq,\text{isod}} = \exp\left(-\frac{\Delta_r G^0_{\text{isod}}}{RT}\right) = 3.50\times10^{-16}.$$

α-Ketoglutarate dehydrogenase flux:

$$J_{\text{akgd}} = \frac{V_{mf}\left(1 - \frac{1}{K_{eq,\text{akgd}}}\frac{[\text{P}][\text{Q}][\text{R}]}{[\text{A}][\text{B}][\text{C}]}\right)}{\left(1 + \frac{K_{mA}}{[\text{A}]}\alpha_i + \frac{K_{mB}}{[\text{B}]}\left(1 + \frac{[\text{Q}]}{K_{iq}}\right) + \frac{K_{mC}}{[\text{C}]}\left(1 + \frac{[\text{R}]}{K_{ir}}\right)\right)}, \quad \text{(S94)}$$

where [A] = [AKG], [B] = [COASH], [C] = [NAD], [P] = [CO_2tot], [Q] = [SCOA], and [R] = [NADH],

$$K_{eq,\text{akgd}} = K^0_{eq,\text{akgd}}\frac{1}{[\text{H}^+]}\frac{P_{\text{CO}_2\text{tot}}P_{\text{SCOA}}P_{\text{NADH}}}{P_{\text{AKG}}P_{\text{COASH}}P_{\text{NAD}}} \text{ with } K^0_{eq,\text{akgd}} = \exp\left(-\frac{\Delta_r G^0_{\text{akgd}}}{RT}\right) = 6.93\times10^{-3}.$$

Succiny-CoA synthetase flux:

$$J_{\text{scoas}} = \frac{V_{mf}V_{mr}\left([\text{A}][\text{B}][\text{C}] - \frac{[\text{P}][\text{Q}][\text{R}]}{K_{eq,\text{scoas}}}\right)}{\begin{aligned} & V_{mr}K_{ia}K_{ib}K_{mC} + V_{mr}K_{ib}K_{mC}[\text{A}] + V_{mr}K_{ia}K_{mB}[\text{C}] \\ & + V_{mr}K_{mC}[\text{A}][\text{B}] + V_{mr}K_{mB}[\text{A}][\text{C}] + V_{mr}K_{mA}[\text{B}][\text{C}] + V_{mr}[\text{A}][\text{B}][\text{C}] \\ & + \frac{V_{mf}K_{ir}K_{mQ}[\text{P}]}{K_{eq,\text{scoas}}} + \frac{V_{mf}K_{iq}K_{mP}[\text{R}]}{K_{eq,\text{scoas}}} + \frac{V_{mf}K_{mR}[\text{P}][\text{Q}]}{K_{eq,\text{scoas}}} + \frac{V_{mf}K_{mQ}[\text{P}][\text{R}]}{K_{eq,\text{scoas}}} \\ & + \frac{V_{mf}K_{mP}[\text{Q}][\text{R}]}{K_{eq,\text{scoas}}} + \frac{V_{mf}[\text{P}][\text{Q}][\text{R}]}{K_{eq,\text{scoas}}} + \frac{V_{mf}K_{mQ}K_{ir}[\text{A}][\text{P}]}{K_{ia}K_{eq,\text{scoas}}} + \frac{V_{mr}K_{ia}K_{mB}[\text{C}][\text{R}]}{K_{ir}} \\ & + \frac{V_{mf}K_{mQ}K_{ir}[\text{A}][\text{B}][\text{P}]}{K_{ia}K_{ib}K_{eq,\text{scoas}}} + \frac{V_{mr}K_{mA}[\text{B}][\text{C}][\text{R}]}{K_{ir}} + \frac{V_{mf}K_{mR}[\text{A}][\text{P}][\text{Q}]}{K_{ia}K_{eq,\text{scoas}}} \\ & + \frac{V_{mr}K_{ia}K_{mB}[\text{C}][\text{Q}][\text{R}]}{K_{iq}K_{ir}} + \frac{V_{mf}K_{ir}K_{mQ}[\text{A}][\text{B}][\text{C}][\text{P}]}{K_{ia}K_{ib}K_{ic}K_{eq,\text{scoas}}} + \frac{V_{mf}K_{ip}K_{mR}[\text{A}][\text{B}][\text{C}][\text{Q}]}{K_{ia}K_{ib}K_{ic}K_{eq,\text{scoas}}} \\ & + \frac{V_{mf}K_{mR}[\text{A}][\text{B}][\text{P}][\text{Q}]}{K_{ia}K_{ib}K_{eq,\text{scoas}}} + \frac{V_{mr}K_{mA}[\text{B}][\text{C}][\text{Q}][\text{R}]}{K_{iq}K_{ir}} + \frac{V_{mr}K_{mA}K_{ic}[\text{B}][\text{P}][\text{Q}][\text{R}]}{K_{ip}K_{iq}K_{ir}} \\ & + \frac{V_{mr}K_{ia}K_{mB}[\text{C}][\text{P}][\text{Q}][\text{R}]}{K_{ip}K_{iq}K_{ir}} + \frac{V_{mf}K_{mR}[\text{A}][\text{B}][\text{C}][\text{P}][\text{Q}]}{K_{ia}K_{ib}K_{ic}K_{eq,\text{scoas}}} + \frac{V_{mr}K_{mA}[\text{B}][\text{C}][\text{P}][\text{Q}][\text{R}]}{K_{ip}K_{iq}K_{ir}} \end{aligned}}, \quad \text{(S95)}$$

where [A] = [GDP], [B] = [SCOA], [C] = [PI], [P] = [COASH], [Q] = [SUC], and [R] = [GTP], $K_{eq,\text{scoas}} = K^0_{eq,\text{scoas}} \frac{1}{[\text{H}^+]} \frac{P_{\text{COASH}} P_{\text{SUC}} P_{\text{GTP}}}{P_{\text{GDP}} P_{\text{SCOA}} P_{\text{PI}}}$ with $K^0_{eq,\text{scoas}} = \exp\left(-\frac{\Delta_r G^0_{\text{scoas}}}{RT}\right) = 9.54\times 10^{-9}\ \text{M}^{-1}$.

Succinate dehydrogenase flux:

$$J_{\text{sdh}} = \frac{V_{mf} V_{mr}\left([\text{A}][\text{B}] - \frac{[\text{P}][\text{Q}]}{K_{eq,\text{sdh}}}\right)}{\begin{array}{l} V_{mr} K_{ia} K_{mB} \alpha_i + V_{mr} K_{mB}[\text{A}] + V_{mr} K_{mA} \alpha_i [\text{B}] + \frac{V_{mf} K_{mQ} \alpha_i}{K_{eq,\text{sdh}}}[\text{P}] + \frac{V_{mf} K_{mP}}{K_{eq,\text{sdh}}}[\text{Q}] \\ + V_{mr}[\text{A}][\text{B}] + \frac{V_{mf} K_{mQ}}{K_{eq,\text{sdh}} K_{ia}}[\text{A}][\text{P}] + \frac{V_{mr} K_{mA}}{K_{iq}}[\text{B}][\text{Q}] + \frac{V_{mf}}{K_{eq,\text{sdh}}}[\text{P}][\text{Q}] \end{array}}, \quad \text{(S96)}$$

where A] = [SUC], [B] = [COQ], [P] = [QH_2], and [Q] = [FUM], $K_{eq,\text{sdh}} = K^0_{eq,\text{sdh}} \frac{P_{\text{QH}_2} P_{\text{FUM}}}{P_{\text{SUC}} P_{\text{COQ}}}$ with

$K^0_{eq,\text{sdh}} = \exp\left(-\frac{\Delta_r G^0_{\text{sdh}}}{RT}\right) = 1.69$.

Fumarase flux:

$$J_{\text{fum}} = \frac{V_{mf} V_{mr}\left([\text{A}] - \frac{[\text{P}]}{K_{eq,\text{fum}}}\right)}{K_{mA} V_{mr} \alpha_i + V_{mr}[\text{A}] + \frac{V_{mf}[\text{P}]}{K_{eq,\text{fum}}}}, \quad \text{(S97)}$$

where [A] = [FUM] and [P] = [MAL], $K_{eq,\text{fum}} = K^0_{eq,\text{fum}} \frac{P_{\text{MAL}}}{P_{\text{FUM}}}$ with $K^0_{eq,\text{fum}} = \exp\left(-\frac{\Delta_r G^0_{\text{fum}}}{RT}\right) = 4.04\ \text{M}^{-1}$.

Malate dehydrogenase flux:

$$J_{\text{mdh}} = \frac{V_{mf} V_{mr}\left([\text{A}][\text{B}] - \frac{[\text{P}][\text{Q}]}{K_{eq,\text{mdh}}}\right)}{\begin{array}{l} V_{mr} K_{ia} K_{mB} \alpha_i + V_{mr} K_{mB}[\text{A}] + V_{mr} K_{mA} \alpha_i [\text{B}] + \frac{V_{mf} K_{mQ} \alpha_i [\text{P}]}{K_{eq,\text{mdh}}} + \frac{V_{mf} K_{mP}[\text{Q}]}{K_{eq,\text{mdh}}} \\ + V_{mr}[\text{A}][\text{B}] + \frac{V_{mf} K_{mQ}[\text{A}][\text{P}]}{K_{eq,\text{mdh}} K_{ia}} + \frac{V_{mf}[\text{P}][\text{Q}]}{K_{eq,\text{mdh}}} + \frac{V_{mr} K_{mA}[\text{B}][\text{Q}]}{K_{iq}} \\ + \frac{V_{mr}[\text{A}][\text{B}][\text{P}]}{K_{ip}} + \frac{V_{mf}[\text{B}][\text{P}][\text{Q}]}{K_{ib} K_{eq,\text{mdh}}} \end{array}}, \quad \text{(S98)}$$

where [A] = [NAD], [B] = [MAL], [P] = [OAA], and [Q] = [NADH], $K_{eq,\text{mdh}} = K^0_{eq,\text{mdh}} \dfrac{1}{[\text{H}^+]} \dfrac{P_{\text{OAA}} P_{\text{NADH}}}{P_{\text{NAD}} P_{\text{MAL}}}$

with $K^0_{eq,\text{mdh}} = \exp\left(-\dfrac{\Delta_r G^0_{\text{mdh}}}{RT}\right) = 2.27\times 10^{-12}$.

Nucleoside diphosphokinase flux:

$$J_{\text{ndk}} = \frac{V_{mf}V_{mr}\left([\text{A}][\text{B}] - \dfrac{[\text{P}][\text{Q}]}{K_{eq,\text{ndk}}}\right)\Big/\alpha_i}{V_{mr}K_{mB}[\text{A}] + V_{mr}K_{mA}[\text{B}] + \dfrac{V_{mf}K_{mQ}[\text{P}]}{K_{eq,\text{ndk}}} + \dfrac{V_{mf}K_{mP}[\text{Q}]}{K_{eq,\text{ndk}}} + V_{mr}[\text{A}][\text{B}] + \dfrac{V_{mf}K_{mQ}[\text{A}][\text{P}]}{K_{eq,\text{ndk}}K_{ia}} + \dfrac{V_{mf}[\text{P}][\text{Q}]}{K_{eq,\text{ndk}}} + \dfrac{V_{mr}K_{mA}[\text{B}][\text{Q}]}{K_{iq}}}, \quad \text{(S99)}$$

where [A] = [GTP], [B] = [ADP], [P] = [GDP], and [Q] = [ATP], $K_{eq,\text{nkd}} = K^0_{eq,\text{nkd}}$ with

$K^0_{eq,\text{ndk}} = \exp\left(-\dfrac{\Delta_r G^0_{\text{ndk}}}{RT}\right) = 1$.

Glutamate oxaloacetate transaminase flux:

$$J_{\text{got}} = \frac{V_{mf}V_{mr}\left([\text{A}][\text{B}] - \dfrac{[\text{P}][\text{Q}]}{K_{eq,\text{got}}}\right)}{V_{mr}K_{mB}[\text{A}] + V_{mr}K_{mA}[\text{B}] + \dfrac{V_{mf}K_{mQ}[\text{P}]}{K_{eq,\text{got}}} + \dfrac{V_{mf}K_{mP}[\text{Q}]}{K_{eq,\text{got}}} + V_{mr}[\text{A}][\text{B}] + \dfrac{V_{mf}K_{mQ}[\text{A}][\text{P}]}{K_{eq,\text{got}}K_{ia}} + \dfrac{V_{mf}[\text{P}][\text{Q}]}{K_{eq,\text{got}}} + \dfrac{V_{mr}K_{mA}[\text{B}][\text{Q}]}{K_{iq}}}, \quad \text{(S100)}$$

where [A] = [ASP], [B] = [AKG], [P] = [OAA], and [Q] = [GLU], $K_{eq,\text{got}} = K^0_{eq,\text{got}} \dfrac{P_{\text{OAA}} P_{\text{GLU}}}{P_{\text{ASP}} P_{\text{AKG}}}$ with

$K^0_{eq,\text{got}} = \exp\left(-\dfrac{\Delta_r G^0_{\text{got}}}{RT}\right) = 1.77$.

Mathematical expressions for substrate and cation transport across the inner mitochondrial membrane

Adenine nucleotide translocase (ANT) flux:

$$J_{\text{ANT}} = x_{\text{ANT}} \frac{k_2^{\text{ANT}} q \dfrac{[\text{ATP}^{4-}]_\text{x} [\text{ADP}^{3-}]_\text{i}}{K_o^\text{D}} - k_3^{\text{ANT}} \dfrac{[\text{ATP}^{4-}]_\text{i} [\text{ADP}^{3-}]_\text{x}}{K_o^\text{T}}}{\left(1 + \dfrac{[\text{ATP}^{4-}]_\text{i}}{K_o^\text{T}} + \dfrac{[\text{ADP}^{3-}]_\text{i}}{K_o^\text{D}}\right)\left(\dfrac{[\text{ATP}^{4-}]_\text{x}}{K_o^\text{T}} + q\dfrac{[\text{ADP}^{3-}]_\text{x}}{K_o^\text{D}}\right)}, \quad \text{(S101)}$$

where

$$k_2^{\mathrm{ANT}} = k_2^{\mathrm{ANT,o}} \exp\left(\left(-3a_1 - 4a_2 + a_3 \right) \frac{F\Delta\Psi}{RT} \right), \tag{S102}$$

$$k_3^{\mathrm{ANT}} = k_3^{\mathrm{ANT,o}} \exp\left(\left(-4a_1 - 3a_2 + a_3 \right) \frac{F\Delta\Psi}{RT} \right), \tag{S103}$$

$$K_o^{\mathrm{D}} = K_o^{\mathrm{D,o}} \exp\left(\frac{3\delta_D RT}{F\Delta\Psi} \right), \tag{S104}$$

$$K_o^{\mathrm{T}} = K_o^{\mathrm{T,o}} \exp\left(\frac{4\delta_T RT}{F\Delta\Psi} \right), \tag{S105}$$

and

$$q = \frac{k_3^{\mathrm{ANT}} K_o^{\mathrm{D}}}{k_2^{\mathrm{ANT}} K_o^{\mathrm{T}}} \exp\left(\frac{F\Delta\Psi}{RT} \right), \tag{S106}$$

with $k_2^{\mathrm{ANT,o}} = 0.159\ \mathrm{sec}^{-1}$, $k_3^{\mathrm{ANT,o}} = 0.501\ \mathrm{sec}^{-1}$, $K_o^{\mathrm{D},o} = 38.89$ mM, $K_o^{\mathrm{T},o} = 56.05$ mM, $a_1 = 0.2829$, $a_2 = -0.2086$, $a_3 = 0.2372$, $\delta_{\mathrm{T}} = 0.0167$, and $\delta_{\mathrm{D}} = 0.0699$ (cited from Supplemental Material of (1)).

Phosphate-hydrogen co-transporter flux:

$$J_{\mathrm{PIHt}} = X_{\mathrm{PIHt}} \cdot \frac{[\mathrm{H_2PO_4^-}]_{\mathrm{i}}[\mathrm{H^+}]_{\mathrm{i}} - [\mathrm{H_2PO_4^-}]_{\mathrm{x}}[\mathrm{H^+}]_{\mathrm{x}}}{k_{\mathrm{PIHt}} \left(1 + [\mathrm{H_2PO_4^-}]_{\mathrm{i}}/k_{\mathrm{PIHt}} \right) \left(1 + [\mathrm{H_2PO_4^-}]_{\mathrm{x}}/k_{\mathrm{PIHt}} \right)}. \tag{S107}$$

Potassium-hydrogen exchange flux:

$$J_{\mathrm{KH}} = X_{\mathrm{KH}} \left([\mathrm{K^+}]_{\mathrm{i}}[\mathrm{H^+}]_{\mathrm{x}} - [\mathrm{K^+}]_{\mathrm{x}}[\mathrm{H^+}]_{\mathrm{i}} \right). \tag{S108}$$

Pyruvate-hydrogen co-transporter flux:

$$J_{\mathrm{PYRH}} = X_{\mathrm{PYRH}} \left([\mathrm{PYR^-}]_{\mathrm{i}}[\mathrm{H^+}]_{\mathrm{i}} - [\mathrm{PYR^-}]_{\mathrm{x}}[\mathrm{H^+}]_{\mathrm{x}} \right). \tag{S109}$$

Glutamate-hydrogen co-transporter flux:

$$J_{\mathrm{GLUH}} = X_{\mathrm{GLUH}} \left([\mathrm{GLU^-}]_{\mathrm{i}}[\mathrm{H^+}]_{\mathrm{i}} - [\mathrm{GLU^-}]_{\mathrm{x}}[\mathrm{H^+}]_{\mathrm{x}} \right). \tag{S110}$$

Citrate-malate exchange flux:

$$J_{\mathrm{CITMAL}} = X_{\mathrm{CITMAL}} \left([\mathrm{HCIT^{2-}}]_{\mathrm{i}}[\mathrm{MAL^{2-}}]_{\mathrm{x}} - [\mathrm{HCIT^{2-}}]_{\mathrm{x}}[\mathrm{MAL^{2-}}]_{\mathrm{i}} \right). \tag{S111}$$

α-Ketoglutarate-malate exchange flux:

$$J_{\mathrm{AKGMAL}} = \frac{X_{\mathrm{AKGMAL}} \left([\mathrm{AKG^{2-}}]_{\mathrm{i}}[\mathrm{MAL^{2-}}]_{\mathrm{x}} - [\mathrm{AKG^{2-}}]_{\mathrm{x}}[\mathrm{MAL^{2-}}]_{\mathrm{i}} \right)}{\begin{array}{l} r_{\mathrm{AKGMAL}}\alpha_{out1} + (1 - r_{\mathrm{AKGMAL}})\alpha_{in1} + [\mathrm{AKG^{2-}}]_{\mathrm{i}}[\mathrm{MAL^{2-}}]_{\mathrm{x}}\,\alpha_{in2} / K_{d,\mathrm{AKGMAL}} \\ + [\mathrm{AKG^{2-}}]_{\mathrm{x}}[\mathrm{MAL^{2-}}]_{\mathrm{i}}\,\alpha_{out2} / K_{d,\mathrm{AKGMAL}} \\ + [\mathrm{AKG^{2-}}]_{\mathrm{i}}[\mathrm{MAL^{2-}}]_{\mathrm{x}}[\mathrm{AKG^{2-}}]_{\mathrm{x}}[\mathrm{MAL^{2-}}]_{\mathrm{i}} / K_{d,\mathrm{AKGMAL}}^2 \end{array}}, \tag{S112}$$

where r_{AKGMAL} and $K_{d,\text{AKGMAL}}$ are adjustable parameters, and α_{out1}, α_{in1}, α_{out2}, and α_{in2} are inhibition coefficients for the exchanger. The inhibition coefficients are computed as:

$$\alpha_{out1} = 1 + [\text{CIT}]_{\text{i}} / K_{i\text{CIT}} + [\text{GLU}]_{\text{i}} / K_{i\text{GLU}} + [\text{ASP}]_{\text{i}} / K_{i\text{ASP}} + [\text{SUC}]_{\text{i}} / K_{i\text{SUC}},$$

$$\alpha_{in1} = 1 + [\text{CIT}]_{\text{x}} / K_{i\text{CIT}} + [\text{GLU}]_{\text{x}} / K_{i\text{GLU}} + [\text{ASP}]_{\text{x}} / K_{i\text{ASP}} + [\text{SUC}]_{\text{x}} / K_{i\text{SUC}},$$

$$\alpha_{out2} = 1 + r_{\text{AKGMAL}} \left([\text{CIT}]_{\text{i}} / K_{i\text{CIT}} + [\text{GLU}]_{\text{i}} / K_{i\text{GLU}} + [\text{ASP}]_{\text{i}} / K_{i\text{ASP}} + [\text{SUC}]_{\text{i}} / K_{i\text{SUC}} \right),$$

$$\alpha_{in2} = 1 + r_{\text{AKGMAL}} \left([\text{CIT}]_{\text{x}} / K_{i\text{CIT}} + [\text{GLU}]_{\text{x}} / K_{i\text{GLU}} + [\text{ASP}]_{\text{x}} / K_{i\text{ASP}} + [\text{SUC}]_{\text{x}} / K_{i\text{SUC}} \right),$$

with $K_{i\text{CIT}}$ = 3.6 mM, $K_{i\text{GLU}}$ = 2.5 mM, $K_{i\text{ASP}}$ = 2.7 mM, and $K_{i\text{SUC}}$ = 1.6 mM (cited from reference (38)).

Succinate/phosphate exchange flux:

$$J_{\text{SUCPI}} = X_{\text{SUCPI}} \left([\text{SUC}^{2-}]_{\text{i}} [\text{PI}^{2-}]_{\text{x}} - [\text{SUC}^{2-}]_{\text{x}} [\text{PI}^{2-}]_{\text{i}} \right). \quad \text{(S113)}$$

Malate/phosphate exchange flux:

$$J_{\text{MALPI}} = X_{\text{MALPI}} \left([\text{MAL}^{2-}]_{\text{i}} [\text{PI}^{2-}]_{\text{x}} - [\text{MAL}^{2-}]_{\text{x}} [\text{PI}^{2-}]_{\text{i}} \right). \quad \text{(S114)}$$

Fumarate-succinate exchange flux:

$$J_{\text{FUMSUC}} = X_{\text{FUMSUC}} \left([\text{FUM}^{2-}]_{\text{i}} [\text{SUC}^{2-}]_{\text{x}} - [\text{FUM}^{2-}]_{\text{x}} [\text{SUC}^{2-}]_{\text{i}} \right). \quad \text{(S115)}$$

Aspartate-glutamate exchange flux:

$$J_{\text{ASPGLU}} = X_{\text{ASPGLU}} \cdot \frac{e^{-F\Delta\Psi/RT} [\text{ASP}^{-}]_{\text{i}} [\text{HGLU}^{0}]_{\text{x}} - [\text{ASP}^{-}]_{\text{x}} [\text{HGLU}^{0}]_{\text{i}}}{\left(1 + [\text{ASP}^{-}]_{\text{x}} [\text{HGLU}^{0}]_{\text{i}} / K_{d,\text{ASPGLU}}\right)\left(1 + [\text{ASP}^{-}]_{\text{i}} [\text{HGLU}^{0}]_{\text{x}} / K_{d,\text{ASPGLU}}\right)}, \quad \text{(S116)}$$

where $K_{d,\text{ASPGLU}}$ is an adjustable parameter.

Proton leak flux:

$$J_{\text{Hle}} = X_{\text{Hle}} \Delta\Psi \left(\frac{[\text{H}^{+}]_{\text{c}} e^{+F\Delta\Psi/RT} - [\text{H}^{+}]_{\text{x}}}{e^{+F\Delta\Psi/RT} - 1} \right). \quad \text{(S117)}$$

Mathematical expressions for passive permeation across the outer mitochondrial membrane

Adenine nucleoside permeation fluxes:

$$J_{\text{ATPt}} = \gamma p_{A} \left([\text{ATP}]_{\text{c}} - [\text{ATP}]_{\text{i}} \right) \quad \text{(S118)}$$

$$J_{\text{ADPt}} = \gamma p_{A} \left([\text{ADP}]_{\text{c}} - [\text{ADP}]_{\text{i}} \right) \quad \text{(S119)}$$

$$J_{\text{AMPt}} = \gamma p_{A} \left([\text{AMP}]_{\text{c}} - [\text{AMP}]_{\text{i}} \right). \quad \text{(S120)}$$

Inorganic phosphate permeation flux:

$$J_{\text{PIt}} = \gamma p_{\text{PI}} \left([\text{PI}]_{\text{c}} - [\text{PI}]_{\text{i}} \right). \quad \text{(S121)}$$

TCA cycle intermediate permeation fluxes:

$$J_{\text{PYRt}} = \gamma p_{\text{TI}} \left([\text{PYR}]_{\text{c}} - [\text{PYR}]_{\text{i}} \right) \quad \text{(S122)}$$

$$J_{\text{CITt}} = \gamma\, p_{\text{TI}} \left([\text{CIT}]_{\text{c}} - [\text{CIT}]_{\text{i}}\right) \quad \text{(S123)}$$

$$J_{\text{MALt}} = \gamma\, p_{\text{TI}} \left([\text{MAL}]_{\text{c}} - [\text{MAL}]_{\text{i}}\right) \quad \text{(S124)}$$

$$J_{\text{AKGt}} = \gamma\, p_{\text{TI}} \left([\text{AKG}]_{\text{c}} - [\text{AKG}]_{\text{i}}\right) \quad \text{(S125)}$$

$$J_{\text{SUCt}} = \gamma\, p_{\text{TI}} \left([\text{SUC}]_{\text{c}} - [\text{SUC}]_{\text{i}}\right) \quad \text{(S126)}$$

$$J_{\text{FUMt}} = \gamma\, p_{\text{TI}} \left([\text{FUM}]_{\text{c}} - [\text{FUM}]_{\text{i}}\right) \quad \text{(S127)}$$

$$J_{\text{GLUt}} = \gamma\, p_{\text{TI}} \left([\text{GLU}]_{\text{c}} - [\text{GLU}]_{\text{i}}\right) \quad \text{(S128)}$$

$$J_{\text{ASPt}} = \gamma\, p_{\text{TI}} \left([\text{ASP}]_{\text{c}} - [\text{ASP}]_{\text{i}}\right). \quad \text{(S129)}$$

Mathematical expressions for cytoplasmic reaction fluxes

Mitochondrial adenylate kinase flux:

$$J_{\text{AKi}} = X_{\text{AKi}} \left(K_{\text{eq,AK}} [\text{ADP}^{3-}]_{\text{i}}^{2} - [\text{AMP}^{2-}]_{\text{i}} [\text{ATP}^{4-}]_{\text{i}}\right), \quad \text{(S130)}$$

where X_{AKi} is an large arbitrary value to maintain the reaction around equilibrium.

Cytoplasmic adenylate kinase flux:

$$J_{\text{AKc}} = X_{\text{AKc}} \left(K_{\text{eq,AK}} [\text{ADP}^{3-}]_{\text{c}}^{2} - [\text{AMP}^{2-}]_{\text{c}} [\text{ATP}^{4-}]_{\text{c}}\right), \quad \text{(S131)}$$

where X_{AKc} is an large arbitrary value to maintain the reaction around equilibrium.

Creatine kinase flux:

$$J_{\text{CKc}} = X_{\text{CKc}} \left(K_{\text{eq,CK}} [\text{ADP}^{3-}]_{\text{c}} [\text{PCr}^{2-}]_{\text{c}} [\text{H}^{+}]_{\text{c}} - [\text{ATP}^{4-}]_{\text{c}} [\text{Cr}^{0}]_{\text{c}}\right), \quad \text{(S132)}$$

where X_{CKc} is an large arbitrary value to maintain the reaction around equilibrium.

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