Online Supplement to
Andrienko T., Kuznetsov A.V., Kaambre T., Usson Y., Orosco A., Appaix F., Tiivel T., Sikk P., Vendelin M. , Margreiter R. and Saks V.A.: Metabolic consequences of functional complexes of mitochondria, myofibrils and sarcoplasmic reticulum in muscle cells, *J. Exp. Biol.*, 206(Pt 12):2059-2072, 2003.

Mathematical model of heterogeneous diffusion of ATP and ADP in permeabilized cardiac cells and fibers

Geometry. The skinned cardiac muscle fiber was considered as cylinder with 20μm in diameter, i.e. the diameter of skinned cardiac muscle fibers was close to that of cardiomyocytes. This assumption is in concord with an observation that the apparent Km for exogenous ADP is equally very high both for isolated cardiomyocytes and skinned cardiac fibers (Kümmel, 1988; Saks et al., 1991, 1993; Kay et al., 1997). We assumed that the concentrations of the metabolites within the solution were uniform due to the stirring during the experiments. Using this assumption and taking into account small ratio of diameter to the length of the fiber, we simulated the diffusion between the fiber and solution only in one cross-section. The cross-section was populated with mitochondria (diameter 1μm), which were distributed randomly in the cross-section to fill 1/4 of the fiber volume (Aliev and Saks, 1997). Within the fiber, the concentration of the metabolites in cytoplasm and myofibrils was approximated using the finite elements. The concentrations of metabolites within mitochondria depend on concentrations of metabolites surrounding every mitochondrion in myofibrillar and cytoplasmic compartments. However, inside every mitochondrion, the concentrations were assumed to be independent from the spatial coordinate.

Solution volume was taken equal to 3 ml. The volume of the skinned fiber (0.00625 ml) was computed from its dry weight (2.5 mg), wet weight to dry weight ratio (5), and volume to wet weight ratio (0.5 ml/g ww). The relative volumes occupied by the compartments were as follows: 3/4, 1/16, and 3/16 for cytoplasmic, mitochondrial intermembrane space (IMS) and mitochondrial matrix compartments, respectively (Aliev and Saks, 1997). On the basis of the relative volumes and volume of the fiber, the volume of cytoplasmic (V_f), IMS (V_i) and matrix (V_x) compartments were computed.

Model solution. The model solution was obtained in the form of concentration distributions within the fiber. Each metabolite was described by its concentration distribution within the cytoplasmic and myofibrillar compartment (Met_f), concentrations in intermembrane space (IMS) of different mitochondria (Met_i), and concentrations in solution surrounding the fiber (Met_s). From the computed distributions of the metabolites in the fiber, the average reaction rates were found and compared with the corresponding experimental measurements. The equations used to compute the model solution are presented below.

Cytoplasmic and myofibrillar compartment. Concentrations of metabolites were gov-

erned by reaction-diffusion type equations

$$\frac{\partial ATP_f}{\partial t} = D^{ATP} \cdot \nabla^2 ATP_f - v_{ATPase}/V_f + v_{PK}/V_f, \quad (1)$$

$$\frac{\partial ADP_f}{\partial t} = D^{ADP} \cdot \nabla^2 ADP_f + v_{ATPase}/V_f - v_{PK}/V_f, \quad (2)$$

$$\frac{\partial Pi_f}{\partial t} = D^{Pi} \cdot \nabla^2 Pi_f + v_{ATPase}/V_f, \quad (3)$$

where D^{Met} is the diffusion coefficient of metabolite (Met, i.e., ATP, ADP, Pi), v_{ATPase} is ATPase reaction rate, v_{PK} is the rate of ATP synthesis by pyruvate kinase (PK) and phosphoenolpyruvate (PEP) system, the symbol ∇^2 denotes the Laplace operator.

ATPase rate v_{ATPase} is given by simple Michaelis–Menten equation

$$v_{ATPase} = \frac{V_{ATPase} \cdot ATP}{ATP + K_{ATP}\left[1 + (ADP/K_{ADP}) \cdot (1 + Pi/K_{Pi})\right]}, \quad (4)$$

where V_{ATPase} was determined from the maximal respiration rate recorded after stimulation of respiration by ATP. Since inorganic phosphate (Pi) concentration was not changed in this work but kept constant at 3 mM, in calculations we used an apparent dissociation constant for ADP, $K'_{ADP} = K_{ADP}/(1+Pi/K_{Pi})$. Here, K_{ATP} and K'_{ADP} were taken equal to 100μM.

PK reaction rate v_{PK} was simulated using equations from (Saks et al., 1994). Homogeneous distribution of PK was assumed in solution and myofibrillar and cytoplasmic compartments.

The following boundary conditions were applied:

$$D^{ATP} \cdot \frac{\partial ATP_f}{\partial \vec{n}} = R^{ATP} \cdot (ATP_i - ATP_f), \quad (5)$$

$$D^{ADP} \cdot \frac{\partial ADP_f}{\partial \vec{n}} = R^{ADP} \cdot (ADP_i - ADP_f), \quad (6)$$

$$D^{Pi} \cdot \frac{\partial Pi_f}{\partial \vec{n}} = R^{Pi} \cdot (Pi_i - Pi_f), \quad (7)$$

on the boundary of cytoplasmic and myofibrillar compartment against each mitochondria in the cross-section and

$$D^{ATP} \cdot \frac{\partial ATP_f}{\partial \vec{n}} = R_S^{ATP} \cdot (ATP_s - ATP_f), \quad (8)$$

$$D^{ADP} \cdot \frac{\partial ADP_f}{\partial \vec{n}} = R_S^{ADP} \cdot (ADP_s - ADP_f), \quad (9)$$

$$D^{Pi} \cdot \frac{\partial Pi_f}{\partial \vec{n}} = R_S^{Pi} \cdot (Pi_s - Pi_f), \quad (10)$$

on the boundary of the fiber. Here, R^{Met} and R_S^{Met} are the permeabilities of metabolite Met through mitochondrial outer membrane and permeabilized sarcolemma, respectively; $\vec{n}$ is outward normal to the domain boundary. To ensure a fast diffusion through permeabilized sarcolemma, R_S^{Met} was set to a large value.

Solution. In the solution surrounding the fiber, we assume that Pi concentration is constant. Since, in all simulated experiments, Pi initial concentration in solution was

relatively large (3mM) and all the measurements were performed within 30-45 minutes, influence of Pi concentration changes to mitochondrial respiration rate can be safely neglected due to the high affinity of mitochondrion to Pi. Concentrations of ATP and ADP in the solution were determined from PK reaction rate and fluxes through the boundary between the fiber and solution:

$$\frac{dATP_s}{dt} = v_{PK}/V_s - \frac{V_f}{S_f \cdot V_s} \oint_{\mathcal{L}_S} R_S^{ATP} \cdot (ATP_s - ATP_f) \cdot dl, \tag{11}$$

$$\frac{dADP_s}{dt} = -v_{PK}/V_s - \frac{V_f}{S_f \cdot V_s} \oint_{\mathcal{L}_S} R_S^{ADP} \cdot (ADP_s - ADP_f) \cdot dl, \tag{12}$$

where S_f is the area occupied by cytoplasmic and myofibrillar compartment in the cross-section and $\mathcal{L}_S$ is the boundary of the fiber.

Intermembrane space. The metabolite concentrations in IMS are determined by

$$\frac{dATP_i}{dt} = +v_{ANT}/V_i - \frac{V_f \cdot N_{mito}}{S_f \cdot V_i} \oint_{\mathcal{L}_V} R^{ATP} \cdot (ATP_i - ATP_f) \cdot dl, \tag{13}$$

$$\frac{dADP_i}{dt} = -v_{ANT}/V_i - \frac{V_f \cdot N_{mito}}{S_f \cdot V_i} \oint_{\mathcal{L}_V} R^{ADP} \cdot (ADP_i - ADP_f) \cdot dl, \tag{14}$$

$$\frac{dPi_i}{dt} = -v_{PI}/V_i - \frac{V_f \cdot N_{mito}}{S_f \cdot V_i} \oint_{\mathcal{L}_V} R^{Pi} \cdot (Pi_i - Pi_f) \cdot dl, \tag{15}$$

where $\mathcal{L}_V$ is the boundary of mitochondrion against the cytoplasmic and myofibrillar compartment, L_v is the length of this boundary, and N_{mito} is the number of mitochondria in the cross-section.

Matrix. For the oxidative phosphorylation, we use the model by Korzeniewski (1998) as in (Vendelin et al., 2000).

$$\frac{dUQ}{dt} = (v_{C3} - v_{C1})/V_x \tag{16}$$

$$\frac{dc^{3+}}{dt} = 2(2v_{O_2} - v_{C3})/V_x \tag{17}$$

$$\frac{dNAD^+}{dt} = (v_{C1} - v_{dh})/V_x \tag{18}$$

$$\frac{dH_x}{dt} = -(2(2+2u)v_{O_2} + 4v_{C1} + (4-2u)v_{C3} - n_a v_{sn} - v_{ANT}u - (1-u)v_{PI} - v_{leak})/(V_x \cdot r_{buff}) \tag{19}$$

$$\frac{dATP_x}{dt} = (v_{sn} - v_{ANT})/V_x \tag{20}$$

$$\frac{dPi_x}{dt} = (v_{PI} - v_{sn})/V_x. \tag{21}$$

Here are given the concentrations of the following metabolites: UQ — oxidized form of coenzyme Q; c^{3+} — oxidized form of cytochrome c; NAD^+ — NAD^+ in the matrix; H_x, ATP_x and Pi_x — H^+, ATP and Pi in the matrix, respectively. V_x is the fractional volume of the mitochondrial matrix with respect to the cell volume. The following reaction rates are used in the above equations (Korzeniewski, 1998):

Substrate dehydrogenation

$$v_{dh} = \frac{k_{dh}}{\left(1 + \frac{K_{mN}}{NAD^+/NADH}\right)^{p_D}}. \tag{22}$$

Complex I

$$v_{C1} = k_{C1}(E_u - E_n - 2\Delta p). \tag{23}$$

Complex III

$$v_{C3} = k_{C3}(E_c - E_u - (2-u)\Delta p). \tag{24}$$

Complex IV

$$v_{O_2} = \frac{1}{2} k_{C4} \cdot a^{2+} \cdot c^{2+} \frac{O_2}{O_2 + k_{mO}}. \tag{25}$$

ATP synthase

$$v_{sn} = k_{sn} \frac{10^{\Delta G_{sn}/Z} - 1}{10^{\Delta G_{sn}/Z} + 1}. \tag{26}$$

Phosphate carrier

$$v_{PI} = v_{fPI} H_{ext} \frac{Pi_i}{1 + 10^{pH_{ext} - pK_a}} - v_{bPI} H_x \frac{Pi_x}{1 + 10^{pH_x - pK_a}}. \tag{27}$$

Proton leak

$$v_{leak} = k_{L1}(10^{k_{L2}\Delta p} - 1). \tag{28}$$

In eqs. (22)–(28), we used the total metabolite pools for substrate, coenzyme Q, cytochrome c, cytochrome a_3, and adenine nucleotides (in matrix) as follows.

$$\begin{aligned} NAD_{tot} &= NAD^+ + NADH, & (29)\\ UQ_{tot} &= UQ + UQH_2, & (30)\\ c_{tot} &= c^{3+} + c^{2+}, & (31)\\ a_{tot} &= a^{3+} + a^{2+}, & (32)\\ A_x &= ATP_x + ADP_x. & (33) \end{aligned}$$

For computing pH in matrix pH_x, pH difference across the inner membrane ΔpH, protonmotive force Δp, electrical potential $\Delta\Psi$, and buffering rate r_{buff}, the following equations were used

$$\begin{aligned} pH_x &= -\log(H_x/\eta), & (34)\\ \Delta pH &= Z \cdot (pH_x - pH_{ext}), & (35)\\ \Delta p &= \frac{1}{1-u}\Delta pH, & (36)\\ \Delta\Psi &= -(\Delta p - \Delta pH), & (37)\\ H_{ext} &= \eta \cdot 10^{-pH_{ext}}, & (38)\\ r_{buff} &= r_{buff0} \cdot \eta \cdot \frac{dpH_x}{dH_x}. & (39) \end{aligned}$$

Here coefficient $u = \Delta\Psi/\Delta p$ is kept constant. Because of the high permeability of the mitochondrial outer membrane for protons, pH in intermembrane space pH_{ext} is considered being equal to that in cytoplasm and constant. The coefficient η is used to convert between molar concentrations and the concentrations used in the model (micromolars). The coefficient Z is expressed as

$$Z = \ln(10)\frac{RT}{F}, \tag{40}$$

where R is the gas constant, T is the absolute temperature and F is the Faraday's number. Throughout this text, the notations ln and log stand for logarithms on bases $e = 2.718\ldots$ and 10, respectively.

Description of ANT kinetics is based on the phenomenological model by Korzeniewski (1998). The net rate of ATP export by ANT from matrix to intermembrane space is

$$v_{ANT} = \left(\frac{fADP_i}{fATP_i 10^{+0.35\Delta\Psi/Z} + fADP_i} - \frac{fADP_x}{fATP_x 10^{-0.65\Delta\Psi/Z} + fADP_x} \right) \quad (41)$$

where $fADP_i$ and $fATP_x$ are concentrations magnesium-free forms of ADP and ATP in matrix, respectively. The total concentrations of ATP and ADP and their magnesium-bound forms are related as follows

$$fATP = \frac{ATP}{1 + Mg/K_{DT}}, \quad (42)$$

$$mATP = ATP - fATP, \quad (43)$$

$$fADP = \frac{ADP}{1 + Mg/K_{DD}}, \quad (44)$$

$$mADP = ADP - fADP. \quad (45)$$

Here Mg is the level of free magnesium, and K_{DT} and K_{DD} are magnesium dissociation constants.

The thermodynamic span of ATP synthase is calculated as follows

$$\Delta G_{sn} = n_a \Delta p - \frac{\Delta G_0}{F} - Z \log \frac{\eta ATP_x}{ADP_x Pi_x}, \quad (46)$$

where n_a and ΔG_0 are the H^+/ATP stoichiometry and standard free energy for ATP synthase, respectively.

In eqs. (23) and (24) are used the NAD, coenzyme Q, and cytochrome c redox potentials

$$E_n = E_{n0} + \frac{Z}{2} \log \frac{NAD^+}{NADH}, \quad (47)$$

$$E_u = E_{u0} + \frac{Z}{2} \log \frac{UQ}{UQH_2}, \quad (48)$$

$$E_c = E_{c0} + \frac{Z}{2} \log \frac{c^{3+}}{c^{2+}}, \quad (49)$$

respectively. For computing the concentration of reduced cytochrome a_3 used in eq. (25), the redox potential of cytochrome a_3

$$E_a = E_c + (1 + u)\Delta p \quad (50)$$

is substituted into relation

$$a^{3+} = 10^{(E_a - E_{a0})/Z}, \quad (51)$$

which gives the result (together with eq. (32)).

Numerical methods. The model equations were numerically solved by a finite-element method in conjunction with Galerkin's method. The resulting system of ordinary differential equations was solved by the backward differentiation formula that is able to treat

stiff equations. The accuracy of the solution was tested by comparing different spatial discretizations and varying the tolerance of the ordinary differential equation solver. The finite-element discretization was performed using the software package Diffpack (Bruaset and Langtagen, 1997) and the system was integrated using the DVODE package (Brown et al., 1989). The finite-element mesh was generated using software package GEOMPACK (Joe, 1991). The required optimization was performed using the Levenberg-Marquardt algorithm (Moré et al., 1984).

Table 1

Event	Parameter	Dimension	Value
Diffusion			
	D_{ATP}	$\mu m^2/s$	$1.45 \cdot 10^2$
	D_{ADP}	$\mu m^2/s$	$1.45 \cdot 10^2$
	D_{PCr}	$\mu m^2/s$	$2.6 \cdot 10^2$
	D_{Cr}	$\mu m^2/s$	$2.6 \cdot 10^2$
	D_{Pi}	$\mu m^2/s$	$3.27 \cdot 10^2$
Mg^{2+}, buffering			
	Mg_{ext}	μM	$1.0 \cdot 10^3$
	K_{DText}	μM	24.0
	K_{DDext}	μM	347.0
	Mg_x	μM	380.0
	k_{DTx}	μM	17.0
	k_{DDx}	μM	282.0
H^+, buffering			
	pH_{ext}		6.9
	r_{buff0}	M	0.022
Diffusion through mitochondrial outer membrane			
	R_{ATP}	$\mu m/s$	160
	R_{ADP}	$\mu m/s$	160
	R_{PCr}	$\mu m/s$	260
	R_{Cr}	$\mu m/s$	260
	R_{Pi}	$\mu m/s$	327
Diffusion through permeabilized sarcolemma			
	R_{ATP}	$\mu m/s$	1600
	R_{ADP}	$\mu m/s$	1600
	R_{PCr}	$\mu m/s$	2600
	R_{Cr}	$\mu m/s$	2600
	R_{Pi}	$\mu m/s$	3270
ATP/ADP translocation by ANT			
	V_{ANT}	$\mu M/s$	$5.70 \cdot 10^3$

Table 1 (continued)

Event	Parameter	Dimension	Value
Respiratory chain			
	k_{dh}	μM/s	$3.2{\cdot}10^3$
	K_{mN}		100.0
	p_D		0.8
	k_{C1}	μM/s	11
	k_{C3}	μM/s	2.8
	k_{C4}	$1/(\mu\text{M}\cdot\text{ s})$	0.65
	k_{mO}	μM	150.0
	E_{n0}	mV	-320.0
	E_{u0}	mV	85.0
	E_{c0}	mV	250.0
	E_{a0}	mV	540.0
	NAD_{tot}	μM	$2.97{\cdot}10^3$
	UQ_{tot}	μM	$1.35{\cdot}10^3$
	c_{tot}	μM	$2.7{\cdot}10^2$
	a_{tot}	μM	$1.35{\cdot}10^2$
	O_2	μM	$2.4{\cdot}10^2$
ATP synthesis			
	k_{sn}	μM/s	$1.6{\cdot}10^3$
	ΔG_0	kJ/mol	31.9
	n_a		2.5
	A_x	μM	$1.0{\cdot}10^4$
Phosphate carrier			
	v_{fPI}	$1/(\mu\text{M}\cdot\text{ s})$	7.6
	v_{bPI}	$1/(\mu\text{M}\cdot\text{ s})$	7.6
	pK_a		6.8
Proton leak			
	k_{L1}	μM/s	438
	k_{L2}	1/mV	0.003
General constants			
	T	K	298
	R	$\text{kJ}/(\text{mol}\cdot\text{ K})$	0.0083
	F	$\text{kJ}/(\text{mol}\cdot\text{ mV})$	0.0965
	Z	mV	$\ln(10)RT/F$
	u		0.861
	η		10^6

References

M. K. Aliev and V. A. Saks. Compartmentalized energy transfer in cardiomyocytes: use of mathematical modeling for analysis of in vivo regulation of respiration. *Biophys J*, 73(1):428–445, 1997.

P. N. Brown, G. D. Byrne, and A. C. Hindmarch. VODE: a variable coefficient ODE Solver. *SIAM J. Sci. Stat. Comput.*, 10:1038–1051, 1989.

A. M. Bruaset and H. P. Langtagen. A comprehensive set of tools for solving partial differential equations: Diffpack. In M. Daehlen and A. Tveito, editors, *Numerical Methods and Software Tools in Industrial Mathematics*, pages 61–90. Birkhauser, Boston, MA, 1997.

B. Joe. GEOMPACK – a software package for the generation of meshes using geometric algorithms. *Adv. Eng. Software*, 13:325–331, 1991.

L. Kay, Z. Li, M. Mericskay, J. Olivares, L. Tranqui, E. Fontaine, T. Tiivel, P. Sikk, T. Kaambre, J. L. Samuel, L. Rappaport, Y. Usson, X. Leverve, D. Paulin, and V. A. Saks. Study of regulation of mitochondrial respiration in vivo. an analysis of influence of adp diffusion and possible role of cytoskeleton. *Biochim Biophys Acta*, 1322(1):41–59, 1997.

B. Korzeniewski. Regulation of atp supply during muscle contraction: theoretical studies. *Biochem J*, 330 (Pt 3):1189–1195, 1998.

L. Kümmel. Ca,Mg-ATPase activity of permeabilized rat heart cells and its functional coupling to oxidative phosphorylation of the cells. *Cardiovasc. Res.*, 22:359–367, 1988.

J. J. Moré, D. C. Sorensen, K. E. Hillstrom, and B. S. Garbow. The MINPACK project. In W. J. Cowell, editor, *Sources and Development of Mathematical Software*. Prentice-Hall, 1984.

V. A. Saks, Y. O. Belikova, and A. V. Kuznetsov. In vivo regulation of mitochondrial respiration in cardiomyocytes: specific restrictions for intracellular diffusion of adp. *Biochim Biophys Acta*, 1074(2):302–311, 1991.

V. A. Saks, Z. A. Khuchua, E. V. Vasilyeva, O. Y. u. Belikova, and A. V. Kuznetsov. Metabolic compartmentation and substrate channelling in muscle cells. role of coupled creatine kinases in in vivo regulation of cellular respiration–a synthesis. *Mol Cell Biochem*, 133-134:155–192, 1994.

V. A. Saks, E. Vasil'eva, Y. u. O. Belikova, A. V. Kuznetsov, S. Lyapina, L. Petrova, and N. A. Perov. Retarded diffusion of adp in cardiomyocytes: possible role of mitochondrial outer membrane and creatine kinase in cellular regulation of oxidative phosphorylation. *Biochim Biophys Acta*, 1144(2):134–148, 1993.

M. Vendelin, O. Kongas, and V. Saks. Regulation of mitochondrial respiration in heart cells analyzed by reaction-diffusion model of energy transfer. *Am J Physiol Cell Physiol*, 278(4):C747–C764, 2000.