Ca^{2+} Uniporter

Transporter

(Version 2)
In Mitochondria

Author(s): Ranjan K. Dash, Feng Qi and Daniel A. Beard
Biotechnology and Bioengineering Center, Department of Physiology
Medical College of Wisconsin
Contact: dbeard@mcw.edu
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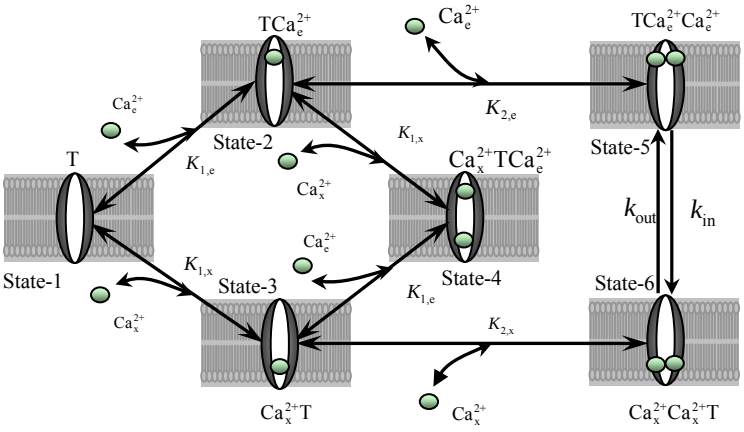
Function

Ca^{2+} is taken up in energized mitochondria via the Ca^{2+} uniporter (CU). It is driven by the electrochemical gradient of Ca^{2+} across the inner mitochondrial membrane (IMM). It is also influenced by the kinetics of Ca^{2+} binding to the CU. CU plays a major role in mitochondrial function or dysfunction by regulating the dynamics of mitochondrial [Ca^{2+}].



Mechanism

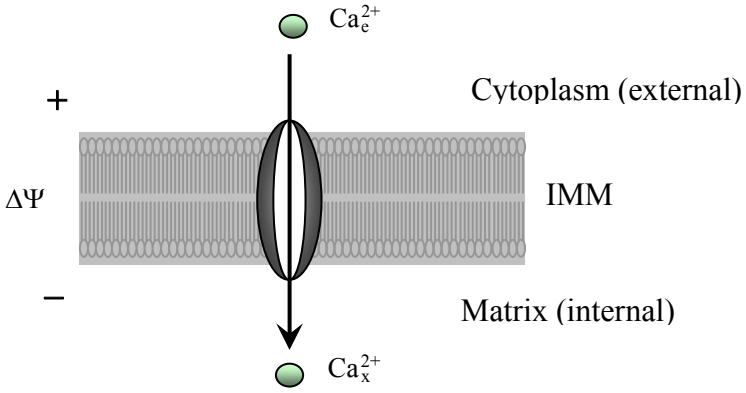


Figure 1a: General schematic of the Ca^{2+} transport via the Ca^{2+} uniporter. The arrow shows the direction of net flux. The scheme also shows the sign convention for membrane potential.

Figure 1b: The proposed 6-states kinetic mechanism of Ca^{2+} transport into mitochondria through the Ca^{2+} uniporter. The uniporter is assumed to have two binding sites for Ca^{2+} and the Ca^{2+} is assumed to bind to the uniporter from either side of the inner mitochondrial membrane (IMM). The ionized free Ca^{2+} from the cytoplasmic side of the IMM cooperatively binds to the unbound uniporter (T) (state-1) in two steps to form the complex $TCa_e^{2+}Ca_e^{2+}$ (state-5) which then undergo conformal changes (or flips upside down) to form the complex $Ca_x^{2+}Ca_x^{2+}T$ (state-6). The complex $Ca_x^{2+}Ca_x^{2+}T$ in the matrix side of the IMM

goes through the reverse process where it dissociates in two steps to form the unbound uniporter (T) and ionized free Ca^{2+}. The model also assumes the cross-interactions between the uniporter, external Ca^{2+}, and internal Ca^{2+} to form the intermediate complex $Ca_x^{2+}TCa_e^{2+}$ (dead end, state-4). The other two states of the uniporter are the bound uniporter TCa_e^{2+} (state-2) and TCa_x^{2+} (state-3). ($K_{1,e}$, $K_{1,x}$) and ($K_{2,e}$, $K_{2,x}$) are the two pairs of dissociation (binding) constants for the two step uniporter binding reactions with the external and internal Ca^{2+}. The transport of Ca^{2+} via the Ca^{2+} uniporter is limited by the rate constants k_{in} and k_{out} which are dependent on the membrane potential $\Delta\Psi$.

Equations

The reactions for the uniporter-Ca^{2+} binding and the reaction for the conformational change of the ternary uniporter-$2Ca^{2+}$ binding complex can be written as

$$T + Ca_e^{2+} \underset{K_{1,e}}{\longleftrightarrow} T.Ca_e^{2+}, \quad T + Ca_x^{2+} \underset{K_{1,x}}{\longleftrightarrow} Ca_x^{2+}.T,$$

$$TCa_e^{2+} + Ca_x^{2+} \underset{K_{1,x}}{\longleftrightarrow} Ca_x^{2+}TCa_e^{2+}, \quad Ca_x^{2+}T + Ca_e^{2+} \underset{K_{1,e}}{\longleftrightarrow} Ca_x^{2+}TCa_e^{2+},$$

$$TCa_e^{2+} + Ca_e^{2+} \underset{K_{2,e}}{\longleftrightarrow} TCa_e^{2+}Ca_e^{2+}, \quad Ca_x^{2+}T + Ca_x^{2+} \underset{K_{2,x}}{\longleftrightarrow} Ca_x^{2+}Ca_x^{2+}T,$$

$$T.Ca_e^{2+}Ca_e^{2+} \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} Ca_x^{2+}Ca_x^{2+}T,$$

Under the quasi-steady state, rapid equilibrium binding assumptions, we have the following relationships between the various states of the uniporter:

$$[TCa_e^{2+}] = \frac{[Ca^{2+}]_e}{K_{1,e}}[T], \quad [Ca_x^{2+}T] = \frac{[Ca^{2+}]_e}{K_{1,x}}[T], \quad [TCa_e^{2+}Ca_e^{2+}] = \frac{[Ca^{2+}]_e^2}{K_{1,e}K_{2,e}}[T],$$

$$[Ca_x^{2+}Ca_x^{2+}T] = \frac{[Ca^{2+}]_x^2}{K_{1,x}K_{2,x}}[T], \quad [Ca_x^{2+}TCa_e^{2+}] = \frac{[Ca^{2+}]_x[Ca^{2+}]_e}{K_{1,x}K_{1,e}}[T],$$

where the concentrations of free and Ca^{2+}-bound uniporter states are expressed with respect to the mitochondrial matrix volume; $[Ca^{2+}]_e$ and $[Ca^{2+}]_x$ denote the extra-mitochondrial and matrix concentrations of Ca^{2+}; $K_{1,e}$, $K_{1,x}$, $K_{2,e}$, and $K_{2,x}$ are in the units of concentration (molar).

Since the total uniporter concentration $[T]_{tot}$ is constant, we have by mass conservation

$$[T]+[Ca_e^{2+}T]+[TCa_x^{2+}]+[Ca_x^{2+}TCa_e^{2+}]+[Ca_e^{2+}Ca_e^{2+}T]+[TCa_x^{2+}Ca_x^{2+}]=[T]_{tot}.$$

By combining the above equations, we can say

$$[T] = [T]_{tot} / D,$$

where,

$$D = 1 + \frac{[Ca^{2+}]_e}{K_{1,e}} + \frac{[Ca^{2+}]_x}{K_{1,x}} + \frac{[Ca^{2+}]_e[Ca^{2+}]_x}{K_{1,e}K_{1,x}} + \frac{[Ca^{2+}]_e^2}{K_{1,e}K_{2,e}} + \frac{[Ca^{2+}]_x^2}{K_{1,x}K_{2,x}}.$$

The flux through the uniporter can be expressed as

$$J_{Uni} = k_{in}[Ca_e^{2+}Ca_e^{2+}T] - k_{out}[TCa_x^{2+}Ca_x^{2+}] = \frac{[T]_{tot}}{D}\left(k_{in}\frac{[Ca^{2+}]_e^2}{K_{1,e}K_{2,e}} - k_{out}\frac{[Ca^{2+}]_x^2}{K_{1,x}K_{2,x}}\right).$$

which is characterized by six unknown parameters.

Model 1

We can reduce the number of parameters by two by making the approximation that the first binding constants $K_{1,e}$ and $K_{1,x}$ are to be large compared to the second binding constants $K_{2,e}$ and $K_{2,x}$ with the constraints that $K_{1,e}.K_{2,e} = K_e^2$ and $K_{1,x}.K_{2,x} = K_x^2$ are finite (cooperative binding). These approximations can be valid under the assumptions $K_{1,e} >> 1$, $K_{1,x} >> 1$, $K_{2,e} << 1$ and $K_{2,x} << 1$. In this case, the concentrations of TCa_e^{2+}, $Ca_x^{2+}T$ and $Ca_x^{2+}TCa_e^{2+}$ can be considered negligible compared to the concentrations of the other binding states of the uniporter. The flux expression can be reduced to

$$J_{\text{Uni}} = \frac{[\text{T}]_{\text{tot}}}{D_1}\left(k_{\text{in}}\frac{[\text{Ca}^{2+}]_{\text{e}}^2}{K_{\text{e}}^2} - k_{\text{out}}\frac{[\text{Ca}^{2+}]_{\text{x}}^2}{K_{\text{x}}^2}\right),$$

where

$$D_1 = 1 + \frac{[\text{Ca}^{2+}]_{\text{e}}^2}{K_{\text{e}}^2} + \frac{[\text{Ca}^{2+}]_{\text{x}}^2}{K_{\text{x}}^2}.$$

At equilibrium conditions, the kinetic parameters K_{e}, K_{x}, k_{in} and k_{out} can be further constrained by the following equilibrium relationships:

$$\frac{k_{\text{in}}.K_{\text{x}}^2}{k_{\text{out}}.K_{\text{e}}^2} = \frac{[\text{Ca}^{2+}]_{\text{x,eq}}^2}{[\text{Ca}^{2+}]_{\text{e,eq}}^2} = K_{\text{eq}}.$$

By taking into account the membrane potential $\Delta\Psi$ dependencies (see Figure 2), we can define the kinetic parameters as follows based on Eyring's free-energy barrier theory:

$$\begin{aligned}
&K_{eq} = \exp(2\Delta\Phi), \quad \Delta\Phi = Z_{\text{Ca}}F\Delta\Psi/RT,\\
&K_e = K_e^0\exp(-\alpha_e\Delta\Phi),\\
&K_x = K_x^0\exp(+\alpha_x\Delta\Phi),\\
&k_{\text{in}} = k_{\text{in}}^0\exp(+2\beta_{\text{e}}\Delta\Phi), \text{ and}\\
&k_{\text{out}} = k_{\text{out}}^0\exp(-2\beta_{\text{x}}\Delta\Phi),
\end{aligned}$$

where K_e^0 and K_x^0 are the values of the binding constants in the absence of an electric field ($\Delta\Psi$=0 mV) and

$$k_{\text{in}}^0 = \frac{k_BT}{h}\exp(-\Delta G_{\text{in}}^0/RT) \text{ and } k_{\text{out}}^0 = \frac{k_BT}{h}\exp(-\Delta G_{\text{out}}^0/RT)$$

are the forward and reverse rate constants in the absence of electric field.

By substituting the parameters into the flux expression, we derive the final flux expression

$$J_{\text{Uni}} = X_{\text{Uni}}\frac{\left([\text{Ca}^{2+}]_{\text{e}}^2\exp(+\Delta\Phi) - [\text{Ca}^{2+}]_{\text{x}}^2\exp(-\Delta\Phi)\right)\exp\left((2\alpha_{\text{e}} + 2\beta_{\text{e}} - 1)\Delta\Phi\right)}{\left(K_{\text{x}}^{02} + (K_{\text{x}}^0/K_{\text{e}}^0)^2[\text{Ca}^{2+}]_{\text{e}}^2\exp(2\alpha_{\text{e}}\Delta\Phi) + [\text{Ca}^{2+}]_{\text{x}}^2\exp(-2\alpha_{\text{x}}\Delta\Phi)\right)},$$

where $X_{\text{Uni}} = [\text{T}]_{\text{tot}}k_{\text{out}}^0$.

Model 2

We can also reduce the number of parameters by two by making the assumption that the first binding constants $K_{1,\text{e}}$ and $K_{1,\text{x}}$ are to be equal to the second binding constants $K_{2,\text{e}}$ and $K_{2,\text{x}}$, respectively; in other words, $K_{1,\text{e}} = K_{2,\text{e}} = K_{\text{e}}$ and $K_{1,\text{x}} = K_{2,\text{x}} = K_{\text{x}}$. In this case, the flux expression can be reduced to

$$J_{\text{Uni}} = \frac{[\text{T}]_{\text{tot}}}{D_2}\left(k_{\text{in}}\frac{[\text{Ca}^{2+}]_{\text{e}}^2}{K_{\text{e}}^2} - k_{\text{out}}\frac{[\text{Ca}^{2+}]_{\text{x}}^2}{K_{\text{x}}^2}\right),$$

where

$$D_2 = 1 + \frac{[\text{Ca}^{2+}]_{\text{e}}}{K_{\text{e}}} + \frac{[\text{Ca}^{2+}]_{\text{x}}}{K_{\text{x}}} + \frac{[\text{Ca}^{2+}]_{\text{e}}[\text{Ca}^{2+}]_{\text{x}}}{K_{\text{e}}K_{\text{x}}} + \frac{[\text{Ca}^{2+}]_{\text{e}}^2}{K_{\text{e}}^2} + \frac{[\text{Ca}^{2+}]_{\text{x}}^2}{K_{\text{x}}^2}.$$

By substituting the same parameters as before into the flux expression, we drive the final flux expression

$$J_{\text{Uni}} = X_{\text{Uni}}\frac{\left([\text{Ca}^{2+}]_{\text{e}}^2\exp(+\Delta\Phi) - [\text{Ca}^{2+}]_{\text{x}}^2\exp(-\Delta\Phi)\right)\exp\left((2\alpha_{\text{e}} + 2\beta_{\text{e}} - 1)\Delta\Phi\right)}{\left(\begin{array}{l}K_{\text{x}}^{02} + (K_{\text{x}}^{02}/K_{\text{e}}^0)[\text{Ca}^{2+}]_{\text{e}}\exp(\alpha_{\text{e}}\Delta\Phi) + K_{\text{x}}^0[\text{Ca}^{2+}]_{\text{x}}\exp(-\alpha_{\text{x}}\Delta\Phi)\\ \quad + (K_{\text{x}}^0/K_{\text{e}}^0)[\text{Ca}^{2+}]_{\text{e}}[\text{Ca}^{2+}]_{\text{x}}\exp((\alpha_{\text{e}} - \alpha_{\text{x}})\Delta\Phi)\\ \quad + (K_{\text{x}}^0/K_{\text{e}}^0)^2[\text{Ca}^{2+}]_{\text{e}}^2\exp(2\alpha_{\text{e}}\Delta\Phi) + [\text{Ca}^{2+}]_{\text{x}}^2\exp(-2\alpha_{\text{x}}\Delta\Phi)\end{array}\right)}.$$

where $X_{\text{Uni}} = [\text{T}]_{\text{tot}}k_{\text{out}}^0$.

Parameters

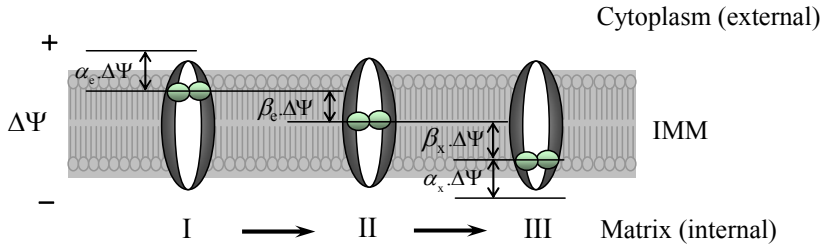
Parameter	Model 1 Case 1 $(K_e^0 = K_x^0)$	Model 1 Case 2 $(K_e^0 \neq K_x^0)$	Model 2 Case 1 $(K_e^0 = K_x^0)$	Model 2 Case 2 $(K_e^0 \neq K_x^0)$	References
k_{in}^0 (nmol/mg/sec)	49.55	0.55	35.0	0.5	[3]
	0.32	3.55×10^{-3}	0.39	5.56×10^{-3}	[1]
	0.354	3.986×10^{-3}	0.4375	6.76×10^{-3}	[4]
k_{out}^0 (nmol/mg/sec)	49.55	0.0085	35.0	0.028	[3]
	0.32	54.84×10^{-6}	0.39	311.1×10^{-6}	[1]
	0.354	61.59×10^{-6}	0.4375	378.4×10^{-6}	[4]
K_e^0 (M)	48×10^{-6}	15×10^{-6}	40×10^{-6}	12×10^{-6}	[3]
	48×10^{-6}	15×10^{-6}	40×10^{-6}	12×10^{-6}	[1]
	90×10^{-6}	78.75×10^{-6}	75×10^{-6}	63×10^{-6}	[4]
K_x^0 (M)	48×10^{-6}	1.865×10^{-6}	40×10^{-6}	2.84×10^{-6}	[3]
	48×10^{-6}	1.865×10^{-6}	40×10^{-6}	2.84×10^{-6}	[1]
	90×10^{-6}	9.79×10^{-6}	75×10^{-6}	14.91×10^{-6}	[4]
α_e (unitless)	0.0	0.0	0.0	0.0	[3],[1],[4]
α_x (unitless)	0.038	-0.214	0.0375	-0.239	[3],[1],[4]
β_e (unitless)	0.112	0.264	0.1125	0.259	[3],[1],[4]
β_x (unitless)	0.85	0.95	0.85	0.98	[3],[1],[4]

Standard physiochemical/thermodynamic parameters used in the model

RT	Gas constant times temperature	2.5775	kJ mol^{-1}
F	Faraday's constant	0.096484	kJ mol^{-1} mV^{-1}
Z_{Ca}	Valence of Ca^{2+}	2	unitless

Table 1: Parameter estimates for both models under the assumptions $K_e^0 = K_x^0$ and $K_e^0 \neq K_x^0$. The constraints $(k_{in}^0 / k_{out}^0).(K_x^0 / K_e^0)^2 = 1$ and $\alpha_e + \alpha_x + \beta_e + \beta_x = 1$ were used to reduce the number of final parameters. In other words, only six parameters were estimated for each case, and the final two were calculated based upon the above constraints.

A



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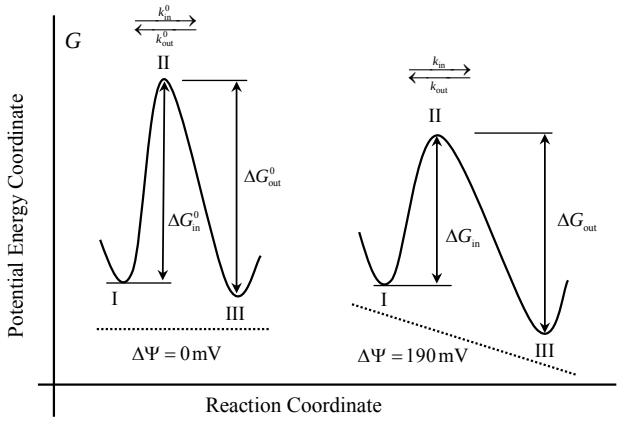
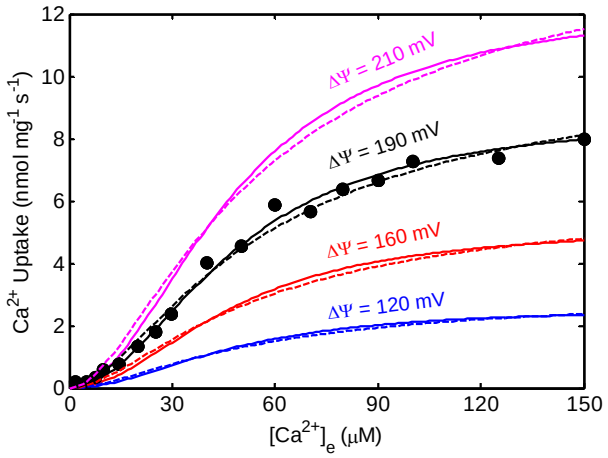


Figure 2: Free-energy barrier formalism for Ca^{2+} transport into mitochondria via the Ca^{2+} uniporter. A(I-III) Consecutive states of the Ca^{2+}-bound uniporter functional unit in the process of Ca^{2+} translocation that is used to derive the dependence of the rate of Ca^{2+} transport on the electrostatic membrane potential $\Delta\Psi$. Here, α_e is the ratio of the potential difference between Ca^{2+} bound at the site of uniporter facing the external side of the IMM and Ca^{2+} in the bulk phase to the total membrane potential $\Delta\Psi$, α_x is the ratio of the potential difference between Ca^{2+} bound at the site of uniporter facing the internal side of the IMM and Ca^{2+} in the bulk phase to the total membrane potential $\Delta\Psi$, β_e is the displacement of external Ca^{2+} from the coordinate of maximum potential barrier, and β_x is the displacement of internal Ca^{2+} from the coordinate of maximum potential barrier. (B) The potential energy barrier profile along the reaction coordinate that is used to derive the dependence of the rate of Ca^{2+} transport on the electrostatic membrane potential $\Delta\Psi$. The dashed line shows the profile of the potential created by the electric field of the charged membrane. The points I, II, and III correspond to the Ca^{2+}-bound uniporter states depicted in the upper panel (A). The rate constants k_{in} and k_{out} are related to the changes in potential energy (Gibbs free-energy) ΔG_{in} and ΔG_{out}. Note that in the absence of electric field ($\Delta\Psi = 0$ mV), the heights of the free-energy barriers in the forward and reverse directions are equal when the dissociation constants for the binding of the uniporter to the external and internal Ca^{2+} are equal: that is, $\Delta G^0_{in} = \Delta G^0_{out} = \Delta G^0$ if and only if $K^0_e = K^0_x = K^0$.

Fits to Data

A



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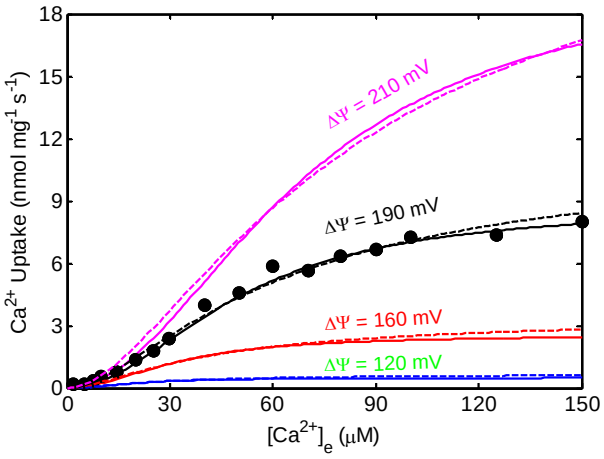

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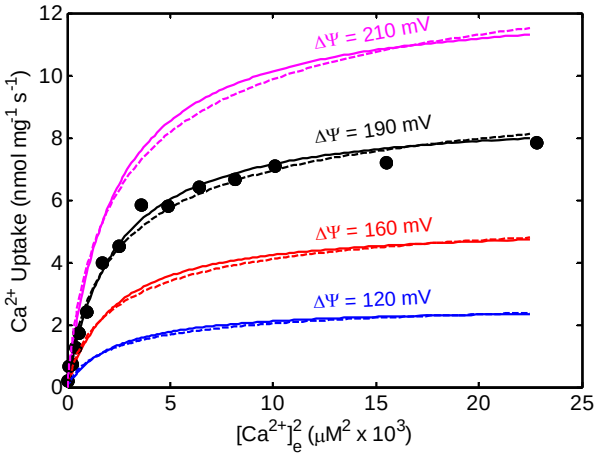

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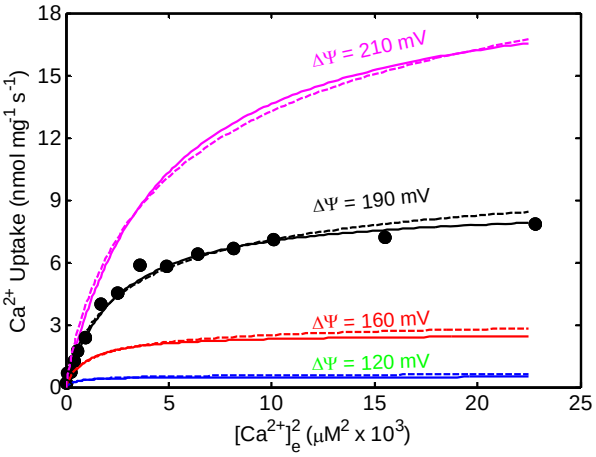

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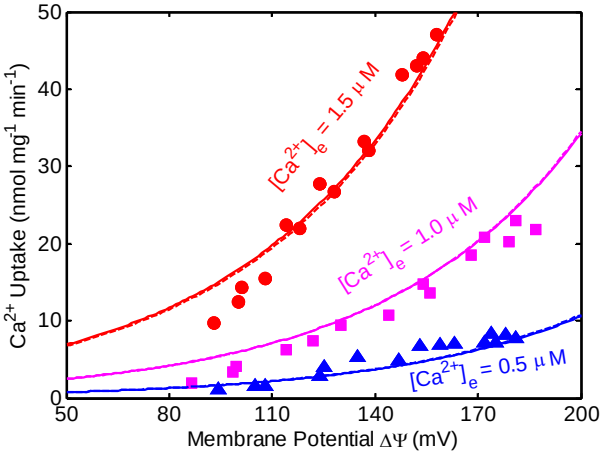

F

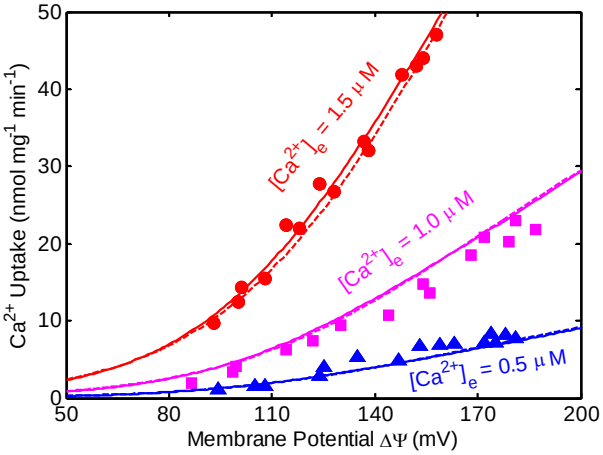

Figure 3: Fitting of Ca^{2+} uniporter model (lines) to data (points) on Ca^{2+} uptake in purified rat liver mitochondria for two different models under two different assumptions. (Upper and middle panels: A-D) The fittings of four different kinetic models of the Ca^{2+} uniporter to the kinetic data of Vinogradov and Scarpa [1] in which the initial rates of Ca^{2+} uptake (points) were measured in respiring mitochondria purified from rat liver with varying levels of extra-mitochondrial buffer Ca^{2+}. Also shown are the model-simulated mitochondrial Ca^{2+} uptake at four different levels of membrane potential $\Delta\Psi$ (lines) in which the models were fitted to the data with $\Delta\Psi$ = 190 mV (states 2 and 4 membrane potential). The plots in the upper panels (A,B) differ from the plots in the middle panels (C,D) through the labeling of the x-axis. (Lower panels: E,F) The fittings of the same four kinetic models to the kinetic data of Gunter and coworkers [2,3] in which the initial rates of Ca^{2+} uptake in respiring mitochondria purified from rat liver were measured with varying membrane potential $\Delta\Psi$ for three different levels of extra-mitochondrial buffer Ca^{2+}. To fit the models to these additional data sets, only the uniporter activity parameter (X_{Uni}) is readjusted, while keeping the other kinetic parameter fixed at values as estimated from the fittings in plots (A-D) (see Table 1). The solid lines are the simulations from Model-1 ($K_{1,e} >> 1$, $K_{1,x} >> 1$, $K_{2,e} << 1$ and $K_{2,x} << 1$, such that $K_{1,e}.K_{2,e} = K_e^2$ and $K_{1,x}.K_{2,x} = K_x^2$ are finite), while the dashed lines are the simulations from Model-2 ($K_{1,e} = K_{2,e} = K_e$ and $K_{1,x} = K_{2,x} = K_x$); the left panels (A,C,E) correspond to the fittings and simulations with the assumption that $K_e^0 = K_x^0 = K^0$, while the right panels (B,D,F) correspond to the fittings and simulations with the assumption that K_e^0 and K_x^0 are distinct.

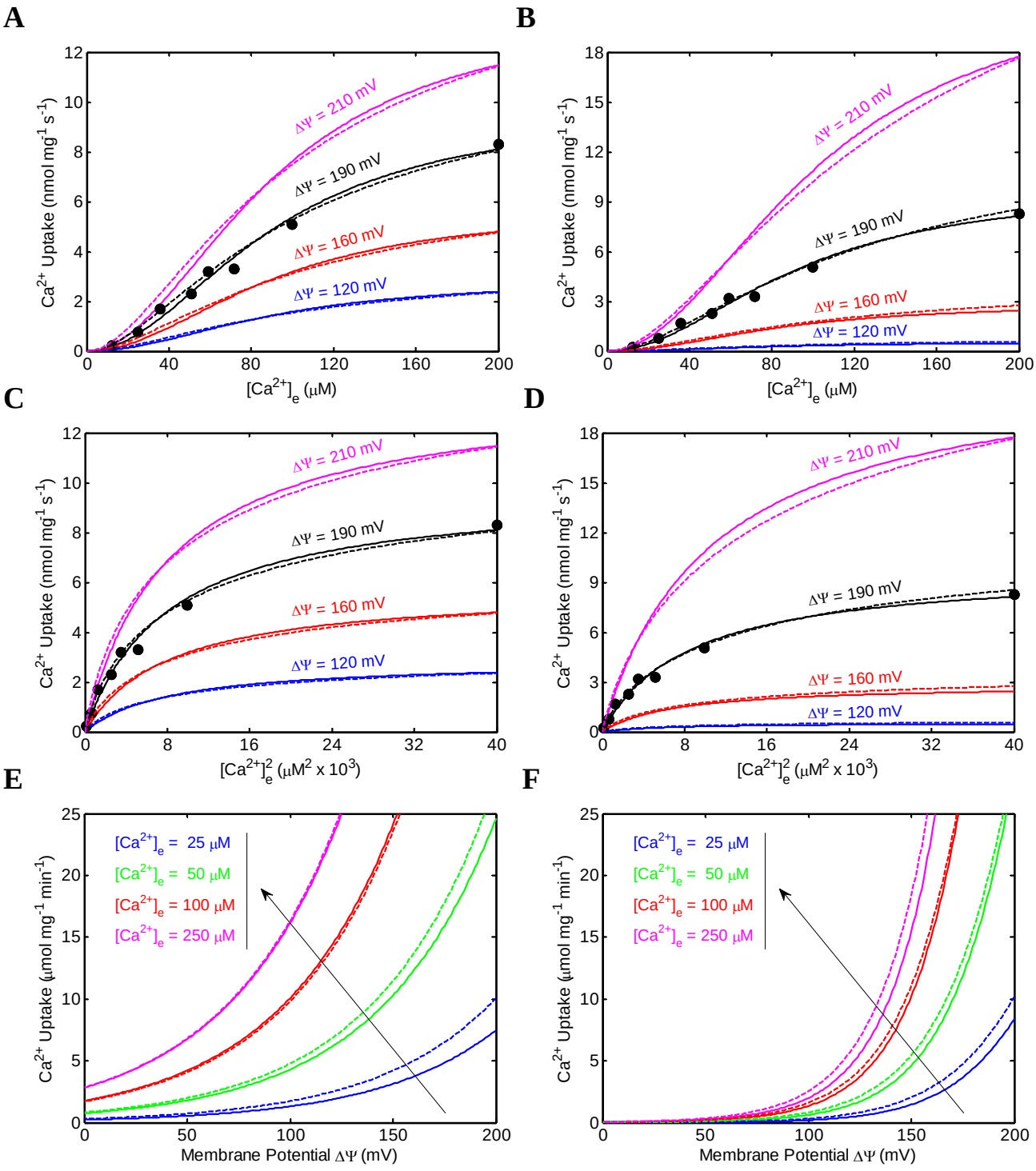
A
B
C
D
E
F
ΔΨ = 210 mV
ΔΨ = 190 mV
ΔΨ = 160 mV
ΔΨ = 120 mV
Ca2+ Uptake (nmol mg-1 s-1)
[Ca2+]e (μM)
[Ca2+]e2 (μM2 x 103)
[Ca2+]e = 25 μM
[Ca2+]e = 50 μM
[Ca2+]e = 100 μM
[Ca2+]e = 250 μM
Ca2+ Uptake (μmol mg-1 min-1)
Membrane Potential ΔΨ (mV)

Figure 4: Fitting of Ca^{2+} uniporter model (lines) to data (points) on Ca^{2+} uptake in isolated rat heart mitochondria for two different models under two different assumptions. (Upper and middle panels: A-D) The fittings of four different kinetic models of the Ca^{2+} uniporter to the kinetic data of Scarpa and Graziotti [4] in which the initial rates of Ca^{2+} uptake (points) were measured in respiring mitochondria isolated from rat heart with varying levels of extra-mitochondrial buffer Ca^{2+}. Also shown are the model-simulated mitochondrial Ca^{2+} uptake at four different levels of membrane potential $\Delta\Psi$ (lines) in which the models were fitted to the data with $\Delta\Psi$ = 190 mV (states 2 and 4 membrane potential). The plots in the upper panels (A,B) differ from the plots in the middle panels (C,D) through the labeling of the x-axis. To fit the models to these additional data sets from rat heart mitochondria, only the kinetic parameters X_{Uni}, K_{e}^{0} and K_{x}^{0} were readjusted, while keeping the other kinetic parameter α_{e}, α_{x}, β_{e} and β_{x} fixed at values as estimated from the fittings in Figure 1 for rat liver mitochondria (see Table 1). (Lower panels: E,F) The model-simulated Ca^{2+} uptake in respiring mitochondria isolated from rat heart as a function of membrane potential $\Delta\Psi$ for four different levels of extra-mitochondrial buffer Ca^{2+} (relatively higher levels of buffer Ca^{2+} than those shown in Figure 3 (E,F) for rat liver mitochondria; corresponding to the experimental protocol of Kirichok et al. [5]). For these simulations, the uniporter activity parameter (X_{Uni}) is increased by 150 times, while keeping the other kinetic parameters fixed at values as estimated from the fittings in plots (A-D) (see Table 1). The solid lines are the simulations from Model-1, while the dashed lines are the simulations from Model-2; the left panels (A,C,E) correspond to the fittings and simulations with the assumption that $K_{\text{e}}^{0} = K_{\text{x}}^{0} = K^{0}$, while the right panels (B,D,F) correspond to the fittings and simulations with the assumption that K_{e}^{0} and K_{x}^{0} are distinct; model specifications are as mentioned in Figure 3.

References

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