Mathematics for Mitochondrial Membrane Potential

$c_{\mathrm{int,free}}$	free concentration of probe ion inside mitochondria
$c_{\mathrm{ext,free}}$	free concentration of probe ion outside mitochondria
K_{i}'	apparent partition coefficient describing internal binding
K_{o}'	apparent partition coefficient describing external binding
n_{add}	total amount of probe ions added to the system
$n_{\mathrm{int,free}}$	free (in solution) amount of probe ions inside mitochondria
$n_{\mathrm{int,bound}}$	bound (in the membrane) amount of probe ions inside mitochondria
$n_{\mathrm{ext,free}}$	free (in solution) amount of probe ions outside mitochondria
$n_{\mathrm{ext,bound}}$	bound (in the membrane) amount of probe ions outside mitochondria
$n_{\mathrm{total,bound}}$	total bound (in the membrane) amount of probe ions
P_{mt}	total mitochondrial protein content (as a marker for mitochondrial membrane content)
P_{C}	total cellular protein content (as a marker for cellular membrane and other material content); note that the mitochondrial protein content is accounted twice: in P_{mt} and in P_{C}, this may be partially justified: while the outside of the mitochondrial membrane contributes to the external binding, the inside contributes to internal binding. However, also the outside of the cell membrane contributes twice. There is probably no numerical significance in this question, considering the low impact of external binding at reasonable high membrane potentials
V_{mt}(abs)	total mitochondrial volume in the system (chamber)
V_{mt}(spec)	mass specific mitochondrial volume (per mass of mitochondrial protein)
V_{ext}	external volume: total solution volume outside mitos
$x_{\mathrm{int,bound}}$	internal „binding ratio“ (M. Brand), ratio of internally bound probe molecule to total amount of probe molecules taken up by the mitos, see definition B1; 1- binding correction factor (M. Brand)
$x_{\mathrm{ext,bound}}$	external „binding ratio“, ratio of externally bound probe molecule to total amount of probe molecules outside of mitos, see definition D1.

only required for the Rottenberg 1984 line of reasoning:

Rottenberg H (1984) Membrane potential and surface potential in mitochondria: uptake and binding of lipophilic cations. J Membr Biol 81: 127-138.

K_{mi}	partition coefficient between liquid phase inside mitos and membrane phase inside mitos, taking into account only different chemical affinity
K_{mo}	partition coefficient between liquid phase outside mitos and membrane phase outside mitos, taking into account only different chemical affinity
α_{i}	ratio describing probe ion distribution between liquid phase inside mitos and membrane phase inside mitos taking into account only different electrical potentials; assumed to be 1 for high enough salt concentrations
α_{o}	ratio describing probe ion distribution between liquid phase outside mitos and membrane phase outside mitos taking into account only different electrical potentials; assumed to be 1 for high enough salt concentrations
$V_{\mathrm{memb,in}}$	Volume of membranes taking part in binding of probe molecules from inside mitos
$V_{\mathrm{memb,out}}$	Volume of membranes taking part in binding of probe molecules from outside mitos

A.) Calculation of $\Delta\Psi$:

(A1) $\Delta\Psi = \frac{RT}{zF} \cdot \ln\left(\frac{c_{\text{int,free}}}{c_{\text{ext,free}}}\right)$

(A2)

$$\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} = \frac{\frac{n_{\text{int,free}}}{V_{\text{mt}}(\text{abs})}}{c_{\text{ext,free}}}$$

(A3) $\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} = \frac{n_{\text{int,free}}}{c_{\text{ext,free}} \cdot V_{\text{mt}}(\text{abs})}$

(A4) $n_{\text{int,free}} = n_{\text{add}} - n_{\text{ext,free}} - n_{\text{int,bound}} - n_{\text{ext,bound}}$

A4 in A3

(A5) $\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} = \frac{n_{\text{add}} - n_{\text{ext,free}} - n_{\text{int,bound}} - n_{\text{ext,bound}}}{c_{\text{ext,free}} \cdot V_{\text{mt}}(\text{abs})}$

Following the Rottenberg 1984 approach, or go directly to A8:

(A6a) $n_{\text{int,bound}} = K_{\text{mi}} \cdot \alpha_{\text{i}} \cdot V_{\text{memb,in}} \cdot c_{\text{int}}$

(A6b) $n_{\text{ext,bound}} = K_{\text{mo}} \cdot \alpha_{\text{o}} \cdot V_{\text{memb,out}} \cdot c_{\text{out}}$

(A7a) $K_{\text{mi}} \cdot \alpha_{\text{i}} \cdot V_{\text{memb,in}} = K_{\text{i}}^{'} \cdot P_{\text{mt}}$ no *P* in Rottenberg 1984

(A7b) $K_{\text{mo}} \cdot \alpha_{\text{o}} \cdot V_{\text{memb,out}} = K_{\text{o}}^{'} \cdot P_{\text{C}}$ no *P* in Rottenberg 1984

A7 in A6 **OR** just directly defining a empirical factor (K') that describes binding

(A8a) $n_{\text{int,bound}} = K_{\text{i}}^{'} \cdot P_{\text{mt}} \cdot c_{\text{int,free}}$

(A8b) $n_{\text{ext,bound}} = K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot c_{\text{ext,free}}$

A8 in A5

(A9) $\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} = \frac{n_{\text{add}} - V_{\text{ext,free}} \cdot c_{\text{ext,free}} - K_{\text{i}}^{'} \cdot P_{\text{mt}} \cdot c_{\text{int,free}} - K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot c_{\text{ext,free}}}{c_{\text{ext,free}} \cdot V_{\text{mt}}(\text{abs})}$

bring all $c_{\text{int,free}}$ terms to left side

(A10) $\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} \cdot \left(1 + \frac{K_{\text{i}}^{'} P_{\text{mt}}}{V_{\text{mt}}\,\text{abs}}\right) = \frac{n_{\text{add}} - V_{\text{ext}} \cdot c_{\text{ext,free}} - K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot c_{\text{ext,free}}}{c_{\text{ext,free}} \cdot V_{\text{mt}}(\text{abs})}$

right side: separate $c_{\text{ext,free}}$ as common term and cancel $c_{\text{ext,free}}$

$$(\text{A11})\ \frac{c_{\text{int,free}}}{c_{\text{ext,free}}}\cdot\left(1+\frac{K_i^{'}P_{\text{mt}}}{V_{\text{mt}}\,\text{abs}}\right)=\frac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}}-V_{\text{ext}}-K_o^{'}\cdot P_C}{V_{\text{mt}}(\text{abs})}$$

because $\dfrac{\dfrac{A}{B}}{1+\dfrac{C}{B}}=\dfrac{A}{B+C}$

$$(\text{A12})\ \frac{c_{\text{int,free}}}{c_{\text{ext,free}}}=\frac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}}-V_{\text{ext}}-K_O^{'}\cdot P_C}{V_{\text{mt}}(\text{abs})+K_i^{'}\cdot P_{\text{mt}}}$$

A12 in A1

$$(\text{A13})\ \Delta\Psi=\frac{RT}{zF}\cdot\ln\left(\frac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}}-V_{\text{ext}}-K_O^{'}\cdot P_C}{V_{\text{mt}}(\text{abs})+K_i^{'}\cdot P_{\text{mt}}}\right)$$

$$(\text{A14})\ V_{\text{mt}}(\text{abs})=V_{\text{mt}}(\text{spec})\cdot P_{\text{mt}}$$

A14 in A13

$$(\text{A15})\ \Delta\Psi=\frac{RT}{zF}\cdot\ln\left(\frac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}}-V_{\text{ext}}-K_O^{'}\cdot P_C}{V_{\text{mt}}(\text{spec})\cdot P_{\text{mt}}+K_i^{'}\cdot P_{\text{mt}}}\right)$$

B.) Conversion internal „Binding Factor (M. Brand)" to K_i' (Rottenberg 1984)

For "binding factor (M. Brand)" see e.g.:

Brand M.D. (1995) Measurement of mitochondrial protonmotive force. In Bioenergetics A Practical Approach (G.C. Brown, C.E. Cooper eds.) p. 39-62. Oxford University Press, Oxford.

Definition:

(B1) $x_{\text{int,bound}} = \text{Fbi} = \text{F(bound,in)} = 1 - \text{binding correction factor (M.Brand)} = \dfrac{n_{\text{int,bound}}}{n_{\text{int,bound}} + n_{\text{int,free}}}$

(A8a) $n_{\text{int,bound}} = K_i' \cdot P_{\text{mt}} \cdot c_{\text{int,free}}$

(B2) $n_{\text{int,free}} = V_{\text{mt}}(\text{abs}) \cdot c_{\text{int,free}}$

A8a und B2 in B1

(B3) $x_{\text{int,bound}} = \dfrac{K_i' \cdot P_{\text{mt}} \cdot c_{\text{int,free}}}{K_i' \cdot P_{\text{mt}} \cdot c_{\text{int,free}} + V_{\text{mt}}(\text{abs}) \cdot c_{\text{int,free}}}$

(B4) $x_{\text{int,bound}} = \dfrac{K_i' \cdot P_{\text{mt}}}{K_i' \cdot P_{\text{mt}} + V_{\text{mt}}(\text{abs})}$

A14 in B4

(B5) $x_{\text{int,bound}} = \dfrac{K_i' \cdot P_{\text{mt}}}{K_i' \cdot P_{\text{mt}} + V_{\text{mt}}(\text{spec}) \cdot P_{\text{mt}}}$

(B6) $x_{\text{int,bound}} = \dfrac{K_i'}{K_i' + V_{\text{mt}}(\text{spec})}$

or

(B7) $K_i' = \dfrac{x_{\text{int,bound}} \cdot V_{\text{mt}}(\text{spec})}{1 - x_{\text{int,bound}}}$

C.) Error by neglecting internal binding; no external binding considered

(A1) $\Delta\Psi = \frac{RT}{zF} \cdot \ln\left(\frac{c_{\text{int,free}}}{c_{\text{ext,free}}}\right)$

from A1 and A3

(C6) $\Delta\Psi = \frac{RT}{zF} \cdot \ln\left(\frac{n_{\text{int,free}}}{c_{\text{ext,free}} V_{\text{mt}}(\text{abs})}\right)$

(B1) $x_{\text{int,bound}} = \frac{n_{\text{int,bound}}}{n_{\text{int,bound}} + n_{\text{int,free}}}$

solve B1 for $n_{\text{int,bound}}$

(C1) $n_{\text{int,bound}} = \frac{x_{\text{int,bound}} \cdot n_{\text{int,free}}}{1 - x_{\text{int,bound}}}$

for only internal binding correction:

from A4 for $n_{\text{ext,bound}} = 0$

(C2) $n_{\text{int,free}} = n_{\text{add}} - n_{\text{ext,free}} - n_{\text{int,bound}}$

C1 in C2

(C3)

$$n_{\text{int,free}} = n_{\text{add}} - n_{\text{ext,free}} - \frac{x_{\text{int,bound}} \cdot n_{\text{int,free}}}{1 - x_{\text{int,bound}}}$$

bring all $n_{\text{int,free}}$ to left side

(C4)

$$n_{\text{int,free}} = \frac{n_{\text{add}} - n_{\text{ext,free}}}{1 + \frac{x_{\text{int,bound}}}{1 - x_{\text{int,bound}}}}$$

multiply with (1- $x_{\text{int,bound}}$)

(C5) $n_{\text{int,free}} = (n_{\text{add}} - n_{\text{ext,free}}) \cdot (1 - x_{\text{int,bound}})$

C5 in C6

(C7) $\Delta\Psi(\text{corrected}) = \frac{RT}{zF} \cdot \ln\left(\frac{(n_{\text{add}} - n_{\text{ext,free}}) \cdot (1 - x_{\text{int,bound}})}{c_{\text{ext,free}} V_{\text{mt}}(\text{abs})}\right)$

for no binding correction at all:

(C8) $\Delta\Psi(\text{simple}) = \frac{RT}{zF} \cdot \ln\left(\frac{n_{\text{add}} - n_{\text{ext,free}}}{c_{\text{ext,free}} V_{\text{mt}}(\text{abs})}\right)$

Influence of correction:

(C9) correction term $\Delta\Delta\Psi = \Delta\Psi(\text{corrected}) - \Delta\Psi(\text{simple})$

C7 and C8 in C9

(C10) correction term $\Delta\Delta\Psi = \frac{RT}{zF} \cdot \ln\left(\frac{\left(\frac{(n_{\mathrm{add}} - n_{\mathrm{ext,free}}) \cdot (1 - x_{\mathrm{int,bound}})}{c_{\mathrm{ext,free}} V_{\mathrm{mt}}(\mathrm{abs})}\right)}{\left(\frac{n_{\mathrm{add}} - n_{\mathrm{ext,free}}}{c_{\mathrm{ext,free}} V_{\mathrm{mt}}(\mathrm{abs})}\right)}\right)$

(C11) correction term $\Delta\Delta\Psi = \frac{RT}{zF} \cdot \ln(1 - x_{\mathrm{int,bound}})$ =constant for a given system if V_{mt}(spec) = const, see B6

Because the correction term for internal binding is a constant, differences between different $\Delta\Psi$ values do not depend on K_i' (describing the internal binding correction).
Therefore differences between different measured $\Delta\Psi$ values ($\Delta\Delta\Psi$) can be considered absolute values within the assumptions stated above.
Note the different use of $\Delta\Delta\Psi$: a difference between two measured $\Delta\Psi$ values in the conclusion and a difference between corrected and uncorrected $\Delta\Psi$ in the proof!

D.)Relation external „Binding Factor“ to K_o'

works mathematically, but does it make any sense ?
Definition:

(D1) $x_{\text{ext,bound}} = \text{Fbo} = \text{F(bound,ext)} = \dfrac{n_{\text{ext,bound}}}{n_{\text{ext,bound}} + n_{\text{ext,free}}}$

(A8b) $n_{\text{ext,bound}} = K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot c_{\text{ext,free}}$

(D2) $n_{\text{ext,free}} = c_{\text{ext,free}} \cdot V_{\text{ext}}$

A8b and D2 in D1

D3 $x_{\text{ext,bound}} = \dfrac{K_{\text{O}}' \cdot P_{\text{C}} \cdot c_{\text{ext,free}}}{K_{\text{O}}' \cdot P_{\text{C}} \cdot c_{\text{ext,free}} + c_{\text{ext,free}} \cdot V_{\text{ext}}}$

D4 $x_{\text{ext,bound}} = \dfrac{K_{\text{O}}' \cdot P_{\text{C}}}{K_{\text{O}}' \cdot P_{\text{C}} + V_{\text{ext}}}$

e.g. Rottenberg: for P_{C}= 2 (?) mg, V_{ext} = 1500 µl, K_{O}' = 11.1 µl/mg → $x_{\text{ext,bound}}$ = 0.015
for whole cells, P_{C} may be considerable larger, if P_{C} = 10 mg , otherwise equal conditions $x_{\text{ext,bound}}$ = 0.068
for Oroboros: P_{C} = 3.36, Ko' =11, V_{ext} = 3000 : $x_{\text{ext, bound}}$ = 0.012

E.) Calulation of K' from zero Psi Experiment

(E1) $K_{\text{i}}' = K_{\text{o}}' = K'$
(E2) $\Delta\psi = 0$
(E3) $c_{\text{ext,free}} = c_{\text{int,free}}$

(A15) $\Delta\Psi = \dfrac{RT}{zF} \cdot \ln\left(\dfrac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}} - V_{\text{ext}} - K_{\text{O}}^{'} \cdot P_{\text{C}}}{V_{\text{mt}}(\text{spec}) \cdot P_{\text{mt}} + K_{\text{i}}^{'} \cdot P_{\text{mt}}}\right)$

E1 and E2 in A15

(E4) $\dfrac{n_{\text{add}}}{c_{\text{ext,free}}} - V_{\text{ext}} - K^{'} \cdot P_{\text{C}} = V_{\text{mt}}(\text{spec}) \cdot P_{\text{mt}} + K^{'} \cdot P_{\text{mt}}$

(E5) $K^{'} = \dfrac{\dfrac{n_{\text{add}}}{c_{\text{ext,free}}} - V_{\text{ext}} - V_{\text{mt}}(\text{spec}) \cdot P_{\text{mt}}}{P_{\text{mt}} + P_{\text{C}}}$

P_{mt} is still counted twice !

alternatively
analogous to

(A8a) $n_{\mathrm{int,bound}} = K_i' \cdot P_{\mathrm{mt}} \cdot c_{\mathrm{int,free}}$

(E5) $n_{\mathrm{total,bound}} = K' \cdot P_C \cdot c_{\mathrm{ext,free}}$

(E6) $n_{\mathrm{total,bound}} = n_{\mathrm{add}} - n_{\mathrm{ext,free}} - n_{\mathrm{int,free}}$

(E7) $n_{\mathrm{total,bound}} = n_{\mathrm{add}} - V_{\mathrm{ext}} \cdot c_{\mathrm{ext,free}} - V_{\mathrm{mt}}(\mathrm{abs}) \cdot c_{\mathrm{int,free}}$
E7 in E5

(E8) $K' \cdot P_C \cdot c_{\mathrm{ext,free}} = n_{\mathrm{add}} - V_{\mathrm{ext}} \cdot c_{\mathrm{ext,free}} - V_{\mathrm{mt}}(\mathrm{abs}) c_{\mathrm{int,free}}$

(E9) $K' = \dfrac{n_{\mathrm{add}} - V_{\mathrm{ext}} \cdot c_{\mathrm{ext,free}} - V_{\mathrm{mt}}(\mathrm{abs}) \cdot c_{\mathrm{int,free}}}{P_C \cdot c_{\mathrm{ext,free}}}$

using E4

(E10) $K' = \dfrac{\dfrac{n_{\mathrm{add}}}{c_{\mathrm{ext,free}}} - V_{\mathrm{ext}} - V_{\mathrm{mt}}(\mathrm{abs})}{P_C}$

(E11) $K' = \dfrac{\dfrac{n_{\mathrm{add}}}{c_{\mathrm{ext,free}}} - V_{\mathrm{ext}} - V_{\mathrm{mt}}(\mathrm{spec}) \cdot P_{\mathrm{mt}}}{P_C}$

P_{mt} now only counted once !

by the way:
x = a/(a+b) → a = (x*b)/(1-x)

F Conversion Kamo / Rottenberg

A simple conversion of the binding correction factors published by Kamo in

Demura M, Kamo N, Kobatake Y (1987) Binding of lipophilic cations to the liposomal membrane: thermodynamic analysis. Biochim. Biophys. A. 903 303-308

to the factors published in Rottenberg (1984) and used here.

U_b	bound probe ion per mass membrane
A	Langmuir parameter (maximal binding capacity)
K	Langmuir parameter (dissociation constant)

(F1) $U_b = \dfrac{n_{\mathrm{int,bound}}}{P_{mt}}$

Langmuir isotherm

(F2) $U_b = \dfrac{Ac}{K+c}$

A8a rearranged:

(F3) $K_i' = \dfrac{n_{\mathrm{int,bound}}}{c_{\mathrm{int,free}} P_{\mathrm{mt}}}$

F1 in F3

(F4) $K_i' = \dfrac{U_b}{c_{\mathrm{int,free}}} =$

(F5) $K_i' = \dfrac{\dfrac{Ac_{\mathrm{int,free}}}{K + c_{\mathrm{int,free}}}}{c_{\mathrm{int,free}}}$

(F5) $K_i' = \dfrac{A}{K + c_{\mathrm{int,free}}}$

For $c_{\mathrm{int,free}}$ below saturation effects (linear part of Langmuir plot):

(F6) $K_i' = \lim_{c_{\mathrm{int,free}} \to 0} \dfrac{A}{K + c_{\mathrm{int,free}}} = \dfrac{A}{K}$

G Beyond Mitochondria: Measuring Cell Membrane Potential

The derivation given here follows the reasoning published by Saito 1992 but applying the nomenclature used above whenever possible.

Saito S, Murakami Y, Miyauchi S, Kamo N (1992) Measurement of plasma membrane potential in isolated rat hepatocytes using the lipophilic cation, tetraphenylphosphonium: correction of probe intracellular binding and mitochondrial accumulation. Biochim. Biophys. A. 1111 221-230.

Conditions:

1.) Two measurements: one under physiological conditions, one with abolished cell membrane potential but unchanged mitochondrial membrane potential. Therefore the method to abolish the cell membrane potential has to be carefully chosen, if the cell membrane is permeabilized the used medium has to be compatible with undisturbed mitochondrial function – the later point is not observed in Saito 1992.
2.) No binding outside of cell (to the outside of the membrane) assumed

Additional terms and terms with changed meaning

$c_{\mathrm{cyt,free}}$	free concentration of probe ion outside mitochondria, inside cytosol
$c_{\mathrm{ext,free}}$	free concentration of probe ion outside cells
$\Delta\Psi_{\mathrm{mito}}$	mitochondrial membrane potential
$\Delta\Psi_{\mathrm{cell}}$	cellular membrane potential
$n_{\mathrm{cyt,free}}$	free (in solution) amount of probe ions outside mitochondria, inside cells
$n_{\mathrm{cyt,bound}}$	bound (in the membrane) amount of probe ions outside mitochondria, inside cell

(G1) $$\Delta\Psi_{\mathrm{mito}} = \frac{RT}{zF} \cdot \ln\left(\frac{c_{\mathrm{int,free}}}{c_{\mathrm{cyt,free}}}\right)$$

(G2) $$\Delta\Psi_{\mathrm{cell}} = \frac{RT}{zF} \cdot \ln\left(\frac{c_{\mathrm{cyt,free}}}{c_{\mathrm{ext,free}}}\right)$$

(G2a) $$c_{\mathrm{cyt,free}} = c_{\mathrm{ext,free}} \cdot e^{-\frac{zF}{RT}(\Delta\Psi_{\mathrm{cell}})}$$

G2 in G1

(G3) $$c_{\mathrm{int,free}} = c_{\mathrm{ext,free}} \cdot e^{-\frac{zF}{RT}(\Delta\Psi_{\mathrm{mito}} + \Delta\Psi_{\mathrm{cell}})}$$

Definition:

(G4) $$c_{\mathrm{int,app}} = \frac{n_{\mathrm{total,bound}} + n_{\mathrm{int,free}} + n_{cyt,\,free}}{V_{\mathrm{cell}}}$$ („apparent internal concentration“)

(G5) $$\frac{c_{\mathrm{int,app}}}{c_{\mathrm{ext,free}}} = \frac{n_{\mathrm{total,bound}} + n_{\mathrm{int,free}} + n_{cyt,\,free}}{V_{\mathrm{cell}} \cdot c_{\mathrm{ext,free}}}$$

(G6) $$\frac{c_{\mathrm{int,app}}}{c_{\mathrm{ext,free}}} = \frac{n_{\mathrm{int,bound}} + n_{\mathrm{int,free}} + n_{cyt,bound} + n_{cyt,\,free}}{V_{\mathrm{cell}} \cdot c_{\mathrm{ext,free}}}$$

A8a and A8b in G6

$$\text{(G7)}\quad \frac{c_{\text{int,app}}}{c_{\text{ext,free}}} = \frac{c_{\text{int,free}} K_{\text{i}}^{'} P_{\text{mt}} + c_{\text{int,free}} V_{\text{mt}}(\text{abs}) + K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot c_{cyt,\,free} + c_{cyt,\,free} V_{cell}}{V_{\text{cell}} \cdot c_{\text{ext,free}}}$$

G3 in G7

$$\text{(G8)}\quad \frac{c_{\text{int,app}}}{c_{\text{ext,free}}} = \frac{c_{\text{ext,free}} \cdot e^{-\frac{zF}{RT}(\Delta\Psi_{\text{mito}} + \Delta\Psi_{\text{cell}})} (K_{\text{i}}^{'} P_{\text{mt}} + V_{\text{mt}}(\text{abs})) + c_{\text{ext,free}} \cdot e^{-\frac{zF}{RT}(\Delta\Psi_{\text{cell}})} (K_{\text{o}}^{'} \cdot P_{\text{C}} \cdot + V_{cell})}{V_{\text{cell}} \cdot c_{\text{ext,free}}}$$

Cancel $c_{\text{ext,free}}$, isolate $\Delta\Psi_{\text{cell}}$

$$\text{(G9)}\quad \frac{c_{\text{int,app}}}{c_{\text{ext,free}}} = \frac{e^{-\frac{zF}{RT}(\Delta\Psi_{\text{cell}})} (e^{-\frac{zF}{RT}(\Delta\Psi_{\text{mito}})} K_{\text{i}}^{'} P_{\text{mt}} + V_{\text{mt}}(\text{abs})) + (K_{\text{o}}^{'} \cdot P_{\text{C}} + V_{cell}))}{V_{\text{cell}}}$$

For $\Delta\Psi_{\text{cell}} = 0$:

$$\text{(G10)}\quad \frac{c_{\text{int,app}}}{c_{\text{ext,free}}}(0) = \frac{1 \cdot (e^{-\frac{zF}{RT}(\Delta\Psi_{\text{mito}})} K_{\text{i}}^{'} P_{\text{mt}} + V_{\text{mt}}(\text{abs})) + (K_{\text{o}}^{'} \cdot P_{\text{C}} + V_{cell}))}{V_{\text{cell}}}$$

G10 in G9

$$\text{(G11)}\quad \frac{c_{\text{int,app}}}{c_{\text{ext,free}}} = e^{-\frac{zF}{RT}(\Delta\Psi_{\text{cell}})} \cdot \frac{c_{\text{int,app}}}{c_{\text{ext,free}}}(0)$$

$$\text{(G12)}\quad e^{-\frac{zF}{RT}(\Delta\Psi_{\text{cell}})} = \frac{\frac{c_{\text{int,app}}}{c_{\text{ext,free}}}}{\frac{c_{\text{int,app}}}{c_{\text{ext,free}}}(0)}$$

$$\text{(G13)}\quad \Delta\Psi_{\text{cell}} = -\frac{RT}{zF} \ln \frac{\frac{c_{\text{int,app}}}{c_{\text{ext,free}}}}{\frac{c_{\text{int,app}}}{c_{\text{ext,free}}}(0)}$$