Oroboros **O2k-Procedures mtMP calculation**

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Calculations for Mitochondrial Membrane Potential

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Content

1. General information

In this document we provide the calculations for mitochondrial membrane potential measured with different fluorescence dyes complying with Oroboros transparency policy.

2. Abbreviations

$c_{int,free}$	free concentration of probe ion inside mitochondria
$c_{ext,free}$	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
$n_{int,free}$	free (in solution) amount of probe ions inside mitochondria
$n_{int,bound}$	bound (in the membrane) amount of probe ions inside

	mitochondria
$n_{ext,free}$	free (in solution) amount of probe ions outside mitochondria
$n_{ext,bound}$	bound (in the membrane) amount of probe ions outside mitochondria
$n_{total,bound}$	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 for 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.
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 mitochondria
$x_{int,bound}$;$F_{bound,int}$	internal „binding ratio‟ (M. Brand), ratio of internally bound probe molecule to total amount of probe molecules taken up by the mitochondria, see definition B1; 1-binding correction factor (M. Brand)
$x_{ext,bound}$;$F_{bound,ext}$	external „binding ratio‟, ratio of externally bound probe molecule to total amount of probe molecules outside of mitochondria, see definition D1.

Only required for calculation according to Rottenberg (1984):

K_{mi}	partition coefficient between liquid phase inside mitochondria and membrane phase inside mitochondria, taking into account only different chemical affinities
K_{mo}	partition coefficient between liquid phase outside mitochondria and membrane phase outside mitochondria, taking into account only different chemical affinities
α_i	ratio describing probe ion distribution between liquid phase inside mitochondria and membrane phase inside mitochondria 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 mitochondria and membrane phase outside mitochondria taking into account only different electrical potentials; assumed to be 1 for high enough salt concentrations

$V_{memb,in}$ Volume of membranes taking part in binding of probe molecules from inside mitochondria

$V_{memb,out}$ Volume of membranes taking part in binding of probe molecules from outside mitochondria

3. Calculation of Δψ

(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 an 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 $c_{\text{ext,free}}$

(A11) $$\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} \cdot \left(1 + \frac{K_{\text{i}}^{'} P_{\text{mt}}}{V_{\text{mt}} \text{abs}}\right) = \frac{\frac{n_{\text{add}}}{c_{\text{ext,free}}} - V_{\text{ext}} - K_{\text{o}}^{'} \cdot P_{\text{C}}}{V_{\text{mt}} (\text{abs})}$$

taking in account that $$\frac{\frac{A}{B}}{1 + \frac{C}{B}} = \frac{A}{B + C}$$

(A12) $$\frac{c_{\text{int,free}}}{c_{\text{ext,free}}} = \frac{\frac{n_{\text{add}}}{c_{\text{ext,free}}} - V_{\text{ext}} - K_{\text{O}}^{'} \cdot P_{\text{C}}}{V_{\text{mt}} (\text{abs}) + K_{\text{i}}^{'} \cdot P_{\text{mt}}}$$

A12 in A1

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

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

A14 in A13

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

4. Relationship between internal "binding factor (M. Brand)" to K_i' (H. Rottenberg)

Definition:

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

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

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

A8a und B2 in B1

(B3) $$x_{\text{int,bound}} = \frac{K_{\text{i}}^{'} \cdot P_{\text{mt}} \cdot c_{\text{int,free}}}{K_{\text{i}}^{'} \cdot P_{\text{mt}} \cdot c_{\text{int,free}} + V_{\text{mt}}(\text{abs}) \cdot c_{\text{int,free}}}$$

(B4) $$x_{\text{int,bound}} = \frac{K_{\text{i}}^{'} \cdot P_{\text{mt}}}{K_{\text{i}}^{'} \cdot P_{\text{mt}} + V_{\text{mt}}(\text{abs})}$$

A14 in B4

(B5) $$x_{\text{int,bound}} = \frac{K_{\text{i}}^{'} \cdot P_{\text{mt}}}{K_{\text{i}}^{'} \cdot P_{\text{mt}} + V_{\text{mt}}(\text{spec}) \cdot P_{\text{mt}}}$$

(B6) $$x_{\text{int,bound}} = \frac{K_{\text{i}}^{'}}{K_{\text{i}}^{'} + V_{\text{mt}}(\text{spec})}$$

or

(B7) $$K_{\text{i}}^{'} = \frac{x_{\text{int,bound}} \cdot V_{\text{mt}}(\text{spec})}{1 - x_{\text{int,bound}}}$$

5. 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 $n_{\text{ext,bound}} = 0$

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

C1 in C2

$$n_{\text{int,free}} = n_{\text{add}} - n_{\text{ext,free}} - \frac{x_{\text{int,bound}} \cdot n_{\text{int,free}}}{1 - x_{\text{int,bound}}}$$ (C3)

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

$$n_{\text{int,free}} = \frac{n_{\text{add}} - n_{\text{ext,free}}}{1 + \frac{x_{\text{int,bound}}}{1 - x_{\text{int,bound}}}}$$ (C4)

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

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

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

(C11) correction term $\Delta\Delta\Psi = \frac{RT}{zF} \cdot \ln(1 - x_{\text{int,bound}})$ =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 ΔΨ values do not depend on K_i' (describing the internal binding correction). Therefore, differences between different measured ΔΨ values (ΔΔΨ) can be considered absolute values within the assumptions stated above.
Note the different use of ΔΔΨ: a difference between two measured ΔΨ values in the conclusion and a difference between corrected and uncorrected ΔΨ in the proof.

6. Relationship between external "binding factor" to *K*o'

Definition:

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

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

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

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: $x_{ext, bound}$ = 0.012

E.) Calulation of K' from zero Psi Experiment

(E1) $K_i' = K_o' = K'$
(E2) $\Delta\psi = 0$
(E3) $c_{ext,free} = c_{int,free}$

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

E1 and E2 in A15

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

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

P_{mt} is still counted twice!

Alternatively, analogous to

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

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

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

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

E7 in E5

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

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

using E4

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

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

P_{mt} now only counted once!

7. References

1. Brand MD, Chien LF and Diolez P (1994) Experimental discrimination between proton leak and redox slip during mitochondrial electron transport Biochem J 297, 27-29.
2. Rottenberg H (1984) Membrane potential and surface potential in mitochondria: uptake and binding of lipophilic cations. J Membr Biol 81:127-38.