Supporting Information

Cells on Pores: A Simulation-Driven Analysis of Transcellular Small Molecule Transport

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Running title: Cells on Pores

- Designed research: GR
- Performed research: XZ, NZ, PZ, HZ
- Contributed analytic tools: JH
- Analyzed data: GR, XZ, JH
- Wrote the paper: GR, XZ

Supporting Table and Figure Legends

Table S1. Parameter ranges used as input for Monte Carlo simulations

Table S2. Simulation and quantitative experimental data of CQ transport across MDCK cells on polyester membranes of varying porosity, at donor compartment pH 6.5 and 7.4. The prefix 'sim.' indicates simulation data corresponding to 10%, 50%, and 90% quantiles of simulated dM/dt (10^{-6} pmol/sec/cell), P_{cell} (10^{-6}cm/sec), P_{app} (10^{-6}cm/sec) and intracellular mass accumulation (10^{-3} pmol/cell) at the end of the 5th minute, using the parameters in Table S1 (non-lysosomal swelling cells). The prefix 'exp.' indicates the experimental data.

Figure S1: CQ mass accumulation in the receiver compartment with time for different experimental conditions. Initial concentration was 1mM except for AP→BL transport, pH=6.5 on 0.4μm membrane which with a initial concentration of 2.5mM. Three lines indicate 10%, 50%, and 90% quantiles of Monte Carlo simulations, respectively. Symbols indicate experimental measured mass change in the receiver compartment with time. Experiments performed on different days were pooled together.

Figure S2. Cell images stained with Hoechst 33342 after transport experiments. (A) Images were taken for AP→BL transport. (B) Images were taken for BL→AP transport. Images in the same row were taken for the transport experiments with the same concentration in the donor compartment. Images in the same column were taken for the transport experiments with the same type of membrane and pH value in the donor compartment.

Figure S3. Binding of mono- and di-cationic species of CQ to resident, anionic macromolecules and phospholipids, can account for observed accumulation during 4 hour incubation period. Monte Carlo simulations were performed the same as before except that $\log K_d = \log K_{dd} = 2.3$ (calculation was described in Method) were used instead of $\log P_{d1} = [-0.07,\ 0.93]$ and $\log P_{d1} = [-1.41,\ -0.41]$ (Table S1) in equation 17 of reference 1 in cytosol, lysosomes, and mitochondria, i.e. the adsorption coefficient $K_d = L \times 1.22 \times 10^{2.3}$ for mono- and di-cationic species of CQ, where L is the lipid fraction in each compartment. The lipid fraction was fixed as 5% in these simulations. Thus the adsorption coefficient for mono- and di-cationic species of CQ was also fixed as above. X-axis indicates log10 (intracellular mass, pmol/cell) and y-axis indicates density. Red solid lines indicate mean values of measured intracellular accumulation of CQ (pmol/cell) after 4 hours incubation with initial concentration of 1mM. Red dashed lines indicate standard deviation. Simulations were also performed with initial concentration of 1mM. The first and third columns indicate simulations without lysosomal swelling or intra-lysosomal pH increment. The second and fourth columns indicate simulation with lysosomal swelling and intra-lysosomal pH increment.

Figure S4. Parametric sensitivity analysis. For each individual parametric analysis, one parameter was changed and other parameters were fixed at mean values in Table S1. Unit

for concentration is mM, for intracellular mass (Intra. Mass) is pmol/cell, for permeability (P_{cell} and P_{app}) is 10^{-6}cm/sec, and for transport rate (dM/dt) is 10^{-6} pmol/sec/cell.

Table S1.

	$logP_n$	[3.43, 4.43]
	pK_{a1}	[9.46, 10.46]
	pK_{a2}	[6.97, 7.97]
	$logP_{d1}$	[-0.07, 0.93]
	$logP_{d2}$	[-1.41, -0.41]
	cell number / insert	[2×10^5, 4×10^5]
	pore density	[3.2×10^6, 4.8×10^6] for membranes with 0.4μm pores
	(pore number / cm^2)	[1.6×10^6, 2.4×10^6] for membranes with 3μm pores
	A_a (μm^2)	[100, 1000]
	A_{insert} (cm^2)	1.12
	A_{pore} (μm^2)	$\textbf{average pore area/cell} = \frac{\textbf{cell number/insert}}{\textbf{pore density} \times \mathbf{A_{insert}}} \times \textbf{area of single pore}$
	A_b (μm^2)	[A_{pore}, 100]
	V_c (μm^3)	[500, 3000]
b	V_l (μm^3)	[9.24, 23.8] / [196.5, 906.3]
	V_m (μm^3)	[10.48, 262]
a	A_l (μm^2)	314
a	A_m (μm^2)	314
a	V_b (μm^3)	1.5mL for AP->BL transport, volume of donor compartment 0.5mL for BL->AP transport, volume of donor compartment
	E_a (mV)	[-14.3, -4.3]
	E_l (mV)	[5, 15]
	E_b (mV)	[6.9, 16.9]
a	E_m (mV)	-160mV
	pH_c	[7.0, 7.4]
c	pH_l	[4.8, 5.2] / [4.63, 6.37]
	pH_m	[7.8, 8.2]
	pH_a	[7.0, 7.4] for pH=7.4 in the donor compartment [6.4, 6.6] for pH=6.5 in the donor compartment
	L_c	[0.05, 0.15]
	L_m	[0.05, 0.15]
	L_l	[0.05, 0.15]
a	$pH_{a/b}$	7.4; pH value in the receiver compartment

[a] indicates parameters that do not influence permeability or intracellular accumulation calculations shown by performing parametric studies

[b] Uniform distribution upper and lower boundaries for lysosomal volume were calculated based on experimental measurement and calculated as described below. The measured lysosomal volume was calculated by equation (s)E1 using measured number and diameter of lysosomes.

$$V_l = n \times (\frac{1}{6}\pi(d)^3), \text{ (s)E1}$$

where *n* is the number of lysosomes / cell, and *d* is the diameter of a lysosome. The average number of lysosomes per cell was 200 ± 35 (n = 6) and 253 ± 45 (n = 5) for treated (50μM CQ for 4hours) and untreated cells, respectively. The diameter of lysosomes was 1.74 ± 0.19 μm (n = 6) and 0.50 ± 0.03 μm (n = 5) for treated (50μM CQ for 4hours) and untreated cells, respectively. Thus the measured lysosomal volume was 551.4 ± 204.9 and 16.5 ± 4.19 μm^3 (mean ± SD) for treated and untreated cells, respectively. The standard deviation of lysosomal volume was estimated by equation (s)E2 (partial derivative method for error propagation estimation)[2] assuming there is no correlation between *n* and *d*.

$$SD_{V_l} = \sqrt{(\frac{\partial V_l}{\partial n})^2 SD_n{}^2 + (\frac{\partial V_l}{\partial d})^2 SD_d{}^2}, \text{ (s)E2}$$

The equations (s)E3 and (s)E4 were applied to calculate the upper (b) and lower (a) boundaries of the uniform distribution of V_l.

$$mean = \frac{1}{2}(a+b), \text{ (s)E3}$$

$$\text{variance} = \frac{1}{12}(b-a)^2, \text{ (s)E4}$$

By plugging in the above measurement, uniform distribution [9.24, 23.8] and [196.5, 906.3] μm^3 were used for V_l for untreated and treated cells, respectively.

[C] Uniform distribution upper and lower boundaries of lysosomal pH for Monte Carlo Simulations with CQ-expanded lysosomal volume (Figure 6) were calculated as the following. The measured mean value and maximum standard deviation are 5.5 and 0.5, respectively. Thus the variance is 0.25. The upper (b) and lower (a) boundaries of the distributions were calculated from equations (s)E3 and (s)E4, which are derived for uniform distribution probability function. Thus uniform distribution [4.63, 6.37] was set for pH in lysosomes of cells under 50 μM CQ treatment.

Table S2.

	pH = 6.5, 0.4µm			pH = 6.5, 3µm			pH = 7.4, 0.4µm			pH = 7.4, 3µm		
	10%	**50%**	**90%**	**10%**	**50%**	**90%**	**10%**	**50%**	**90%**	**10%**	**50%**	**90%**
	A. overall effects of parameters (AP→BL)											
sim.dM/dt	1.78	7.75	23.7	5.00	12.0	31.3	11.7	54.7	229	29.2	91.8	321
exp.dM/dt	2.20 ± 0.718			5.19 ± 1.01			22.8 ± 0.741			46.6 ± 6.28		
sim.P_{cell}	91.0	407	1264	9.26	22.6	59.7	602	2904	12401	54.1	172	612
exp. P_{cell}	218 ± 34.4			14.0 ± 3.33			1560 ± 161			85.9 ± 15.6		
sim.P_{app}	0.455	2.04	6.24	1.31	3.17	8.35	3.04	14.5	62.3	7.64	23.9	84.4
exp.P_{app}	1.35 ± 0.442			1.98 ± 0.471			7.85 ± 0.810			12.1 ± 2.21		
sim.mass	0.490	1.04	2.16	0.451	0.971	2.03	3.14	7.61	18.0	2.99	7.23	17.8
exp.mass	3.73 ± 0.14			1.88 ± 0.54			8.72 ± 0.94			8.90 ± 0.26		
	B. overall effects of parameters (BL→AP)											
sim.dM/dt	1.70	7.42	22.9	4.88	12.0	30.9	10.7	52.9	214	27.2	84.6	309
exp.dM/dt	5.25 ± 1.24			7.12 ± 0.473			29.4 ± 1.54			63.8 ± 15.9		
sim.P_{cell}	85.4	390	1228	9.10	22.3	58.9	548	2767	11616	50.8	159	585
exp. P_{cell}	382 ± 81.7			15.8 ± 2.45			2000 ± 353			114 ± 19.0		
sim.P_{app}	0.439	1.96	6.19	1.28	3.13	8.18	2.77	13.8	57.4	7.11	22.4	82.3
exp.P_{app}	1.92 ± 0.411			2.24 ± 0.346			10.0 ± 1.77			16.2 ± 2.69		
sim.mass	0.020	0.091	0.309	0.060	0.151	0.425	0.137	0.679	2.56	0.378	1.11	3.56
exp.mass	3.52 ± 0.93			4.94 ± 1.06			8.28 ± 0.75			11.8 ± 1.9		

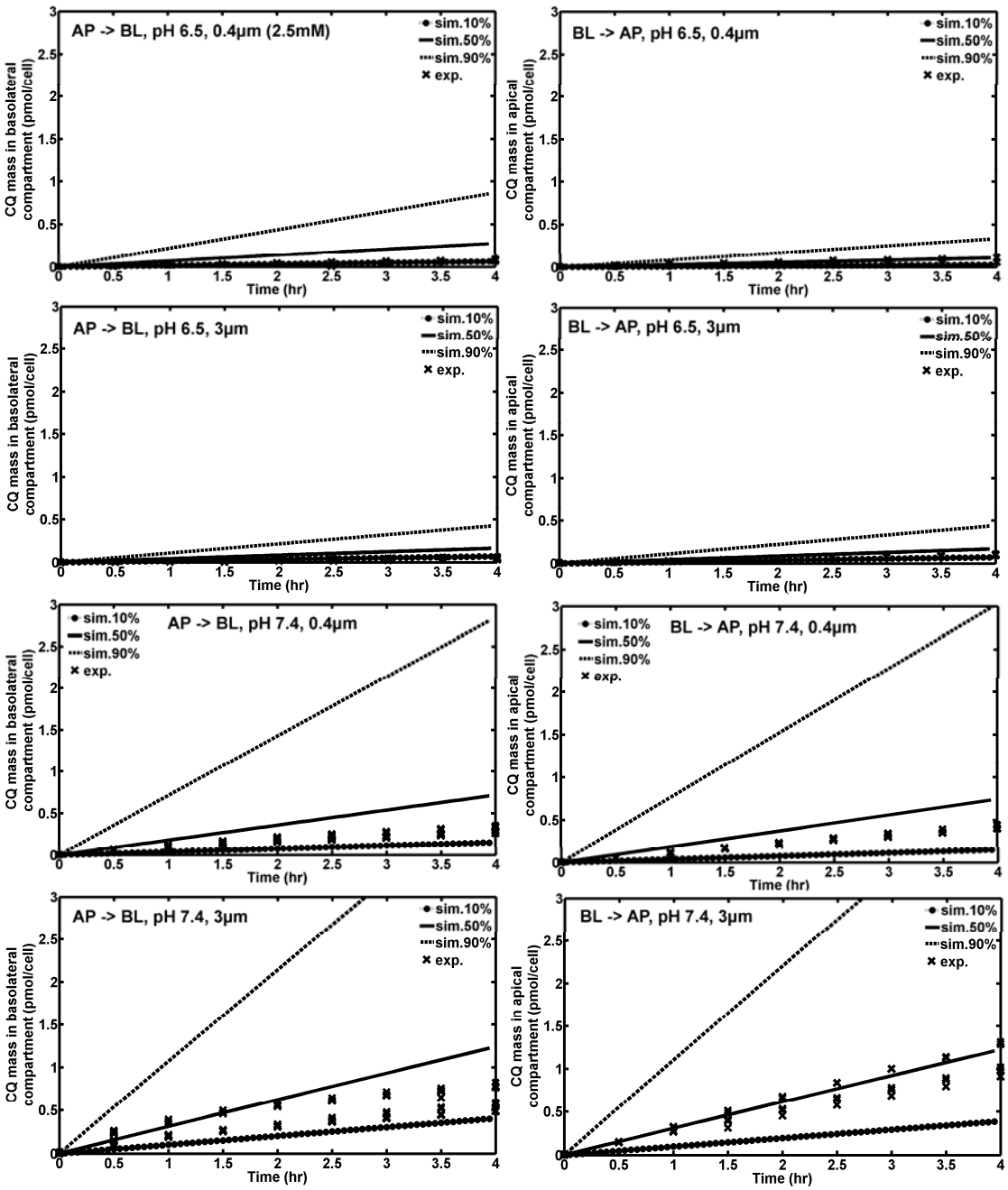

AP -> BL, pH 6.5, 0.4µm (2.5mM)
BL -> AP, pH 6.5, 0.4µm
AP -> BL, pH 6.5, 3µm
BL -> AP, pH 6.5, 3µm
AP -> BL, pH 7.4, 0.4µm
BL -> AP, pH 7.4, 0.4µm
AP -> BL, pH 7.4, 3µm
BL -> AP, pH 7.4, 3µm
sim.10%
sim.50%
sim.90%
exp.
CQ mass in basolateral compartment (pmol/cell)
CQ mass in apical compartment (pmol/cell)
Time (hr)

Figure S1.

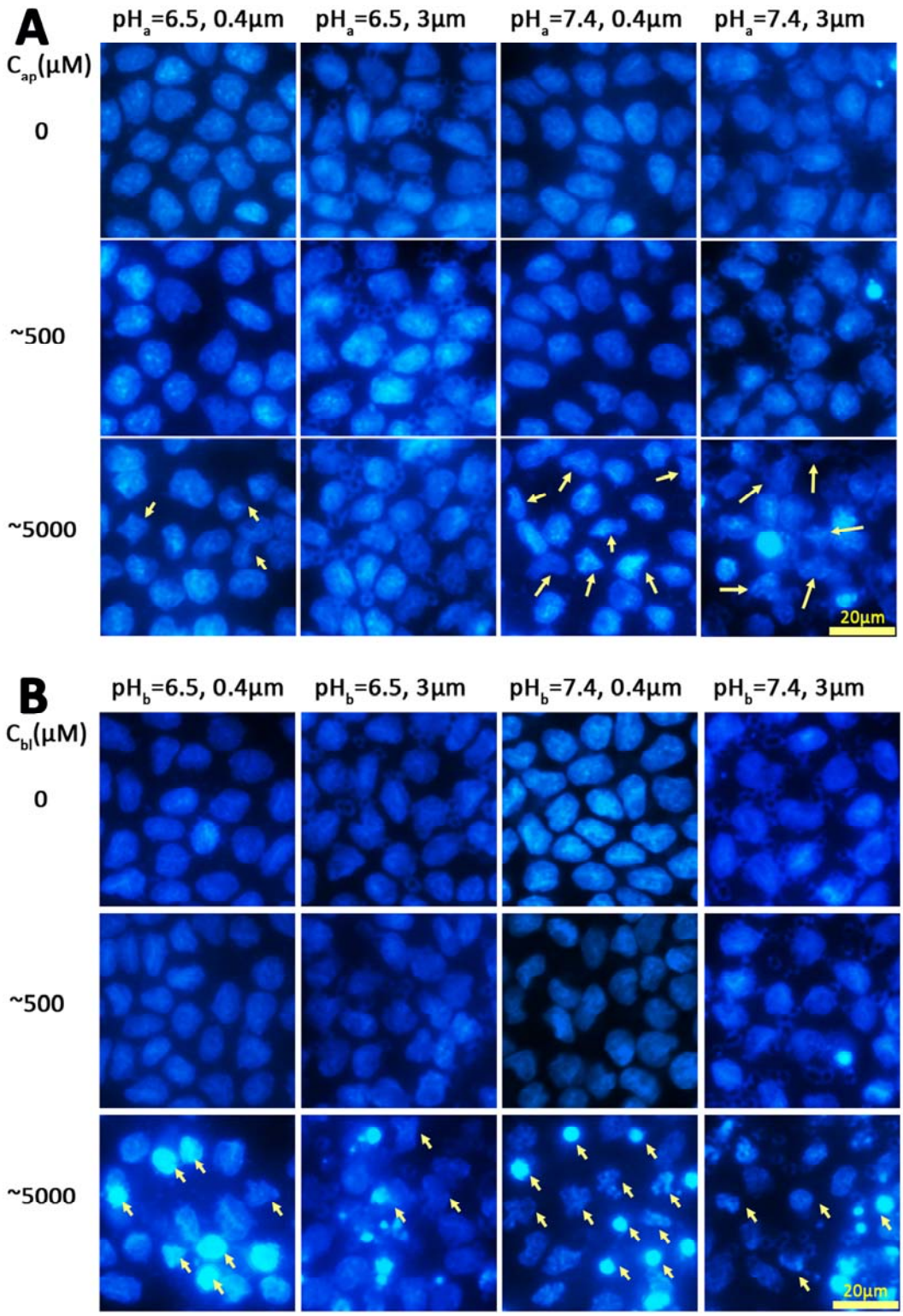

A
pH$_a$=6.5, 0.4μm
pH$_a$=6.5, 3μm
pH$_a$=7.4, 0.4μm
pH$_a$=7.4, 3μm
C$_{ap}$(μM)
0
~500
~5000
20μm
B
pH$_b$=6.5, 0.4μm
pH$_b$=6.5, 3μm
pH$_b$=7.4, 0.4μm
pH$_b$=7.4, 3μm
C$_{bl}$(μM)
0
~500
~5000
20μm

Figure S2.

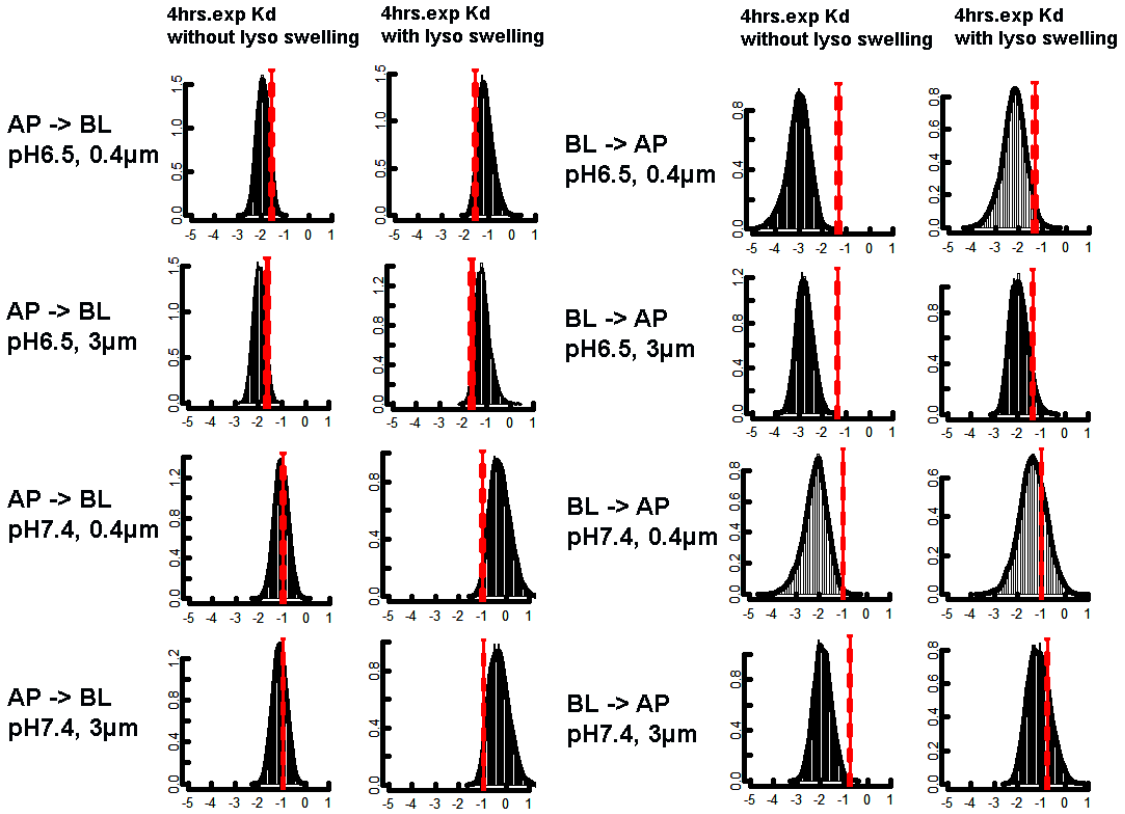
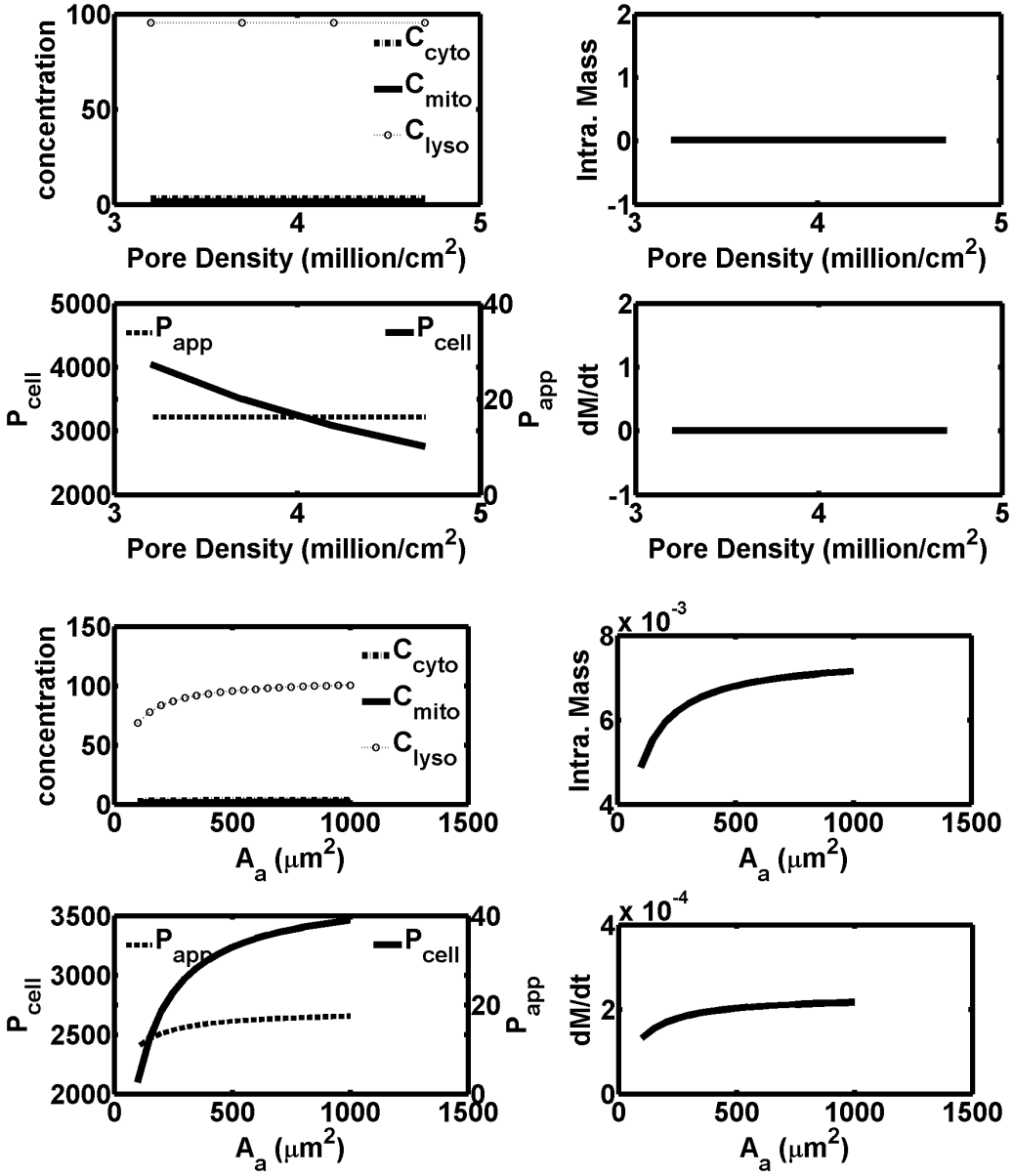
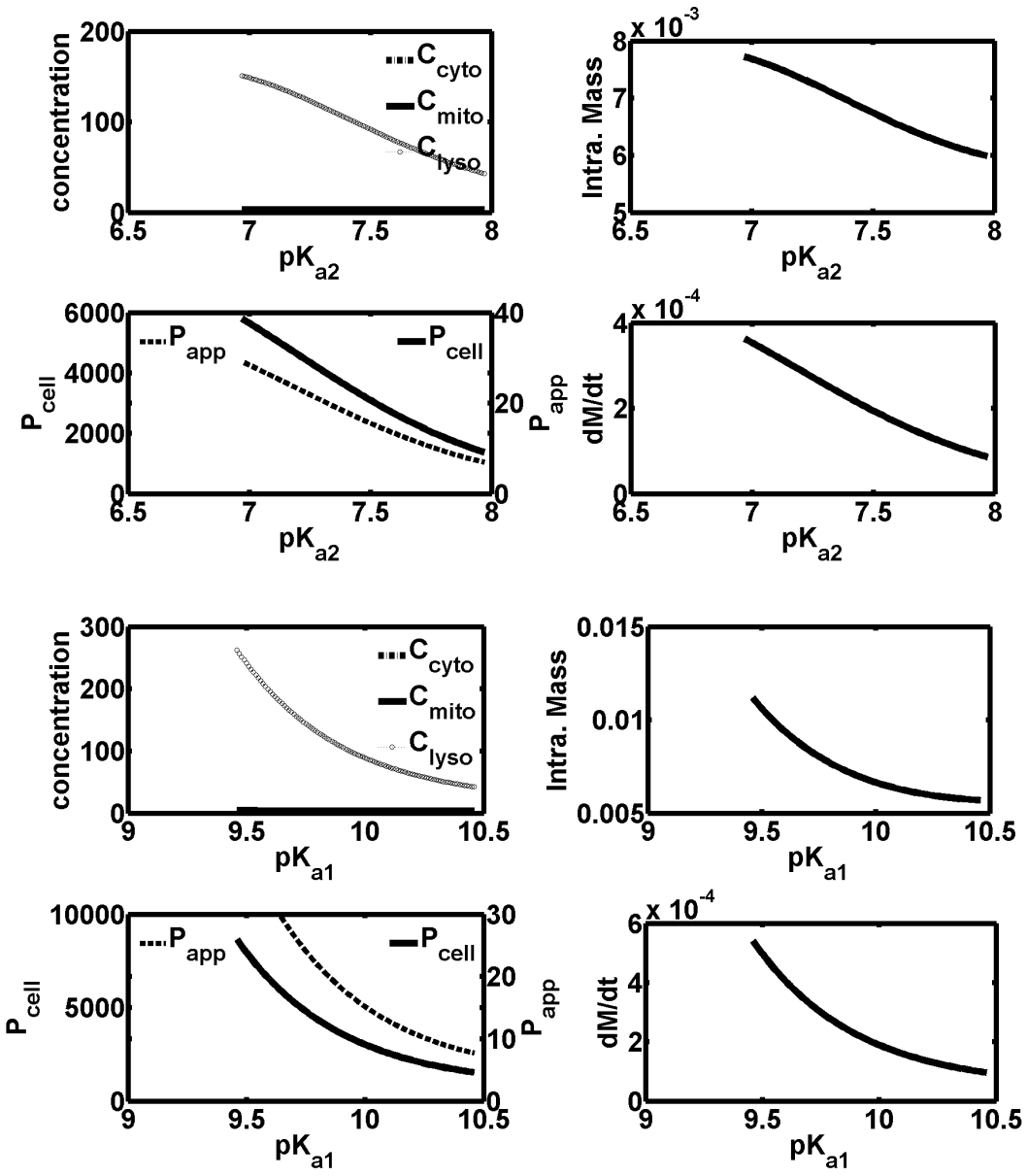
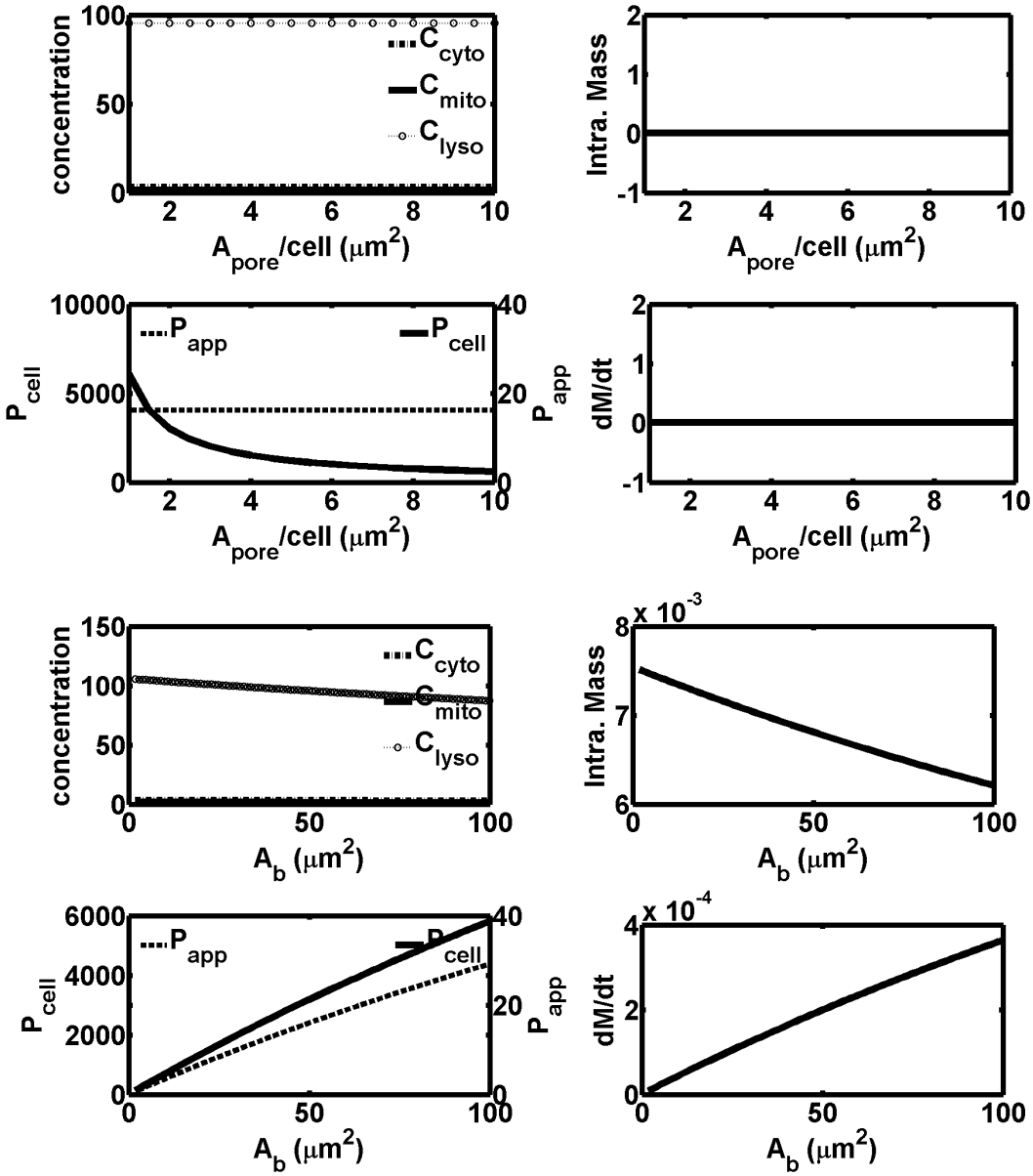

4hrs.exp Kd
without lyso swelling
4hrs.exp Kd
with lyso swelling
4hrs.exp Kd
without lyso swelling
4hrs.exp Kd
with lyso swelling
AP -> BL
pH6.5, 0.4µm
AP -> BL
pH6.5, 3µm
AP -> BL
pH7.4, 0.4µm
AP -> BL
pH7.4, 3µm
BL -> AP
pH6.5, 0.4µm
BL -> AP
pH6.5, 3µm
BL -> AP
pH7.4, 0.4µm
BL -> AP
pH7.4, 3µm

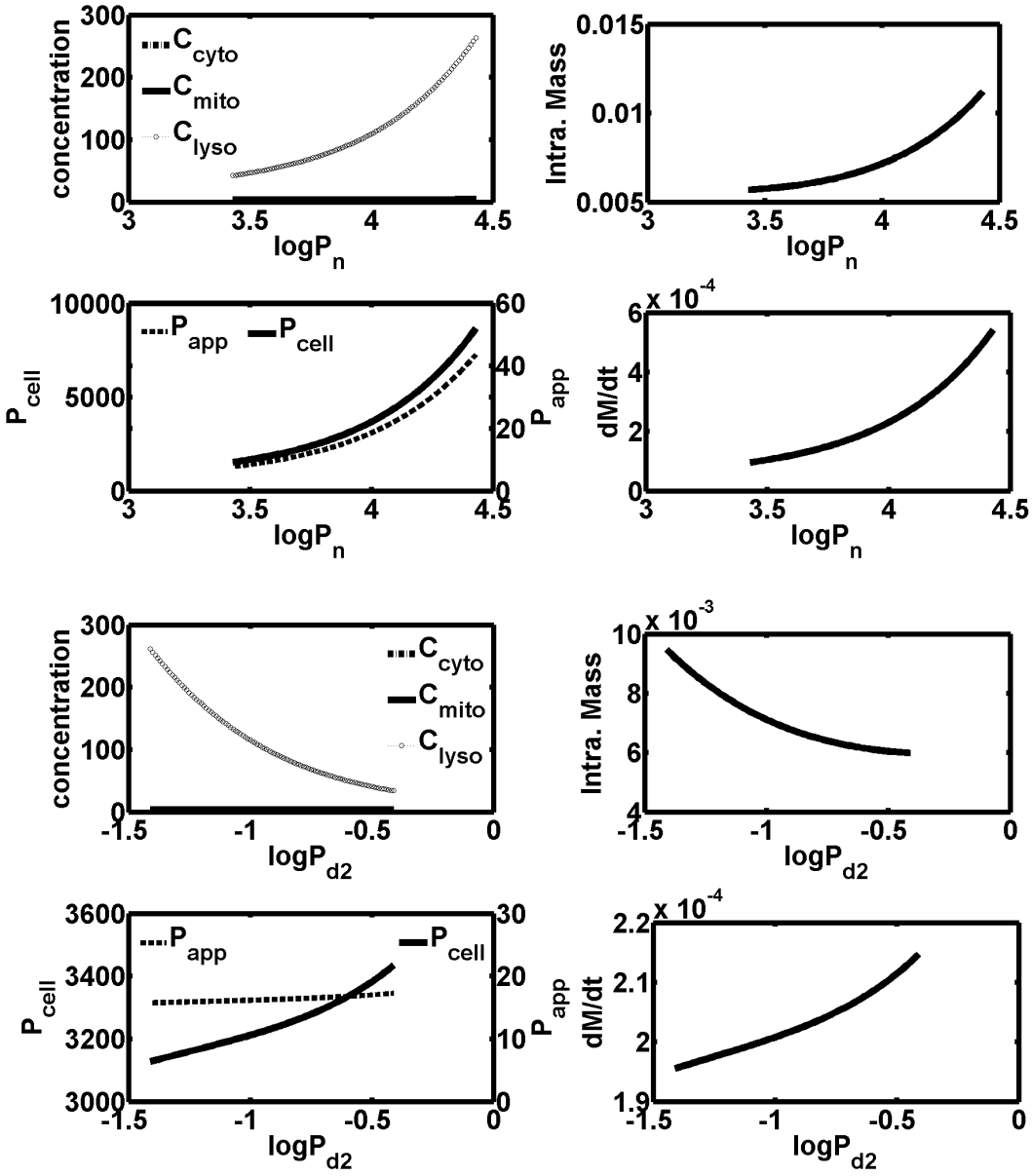
Figure S3.

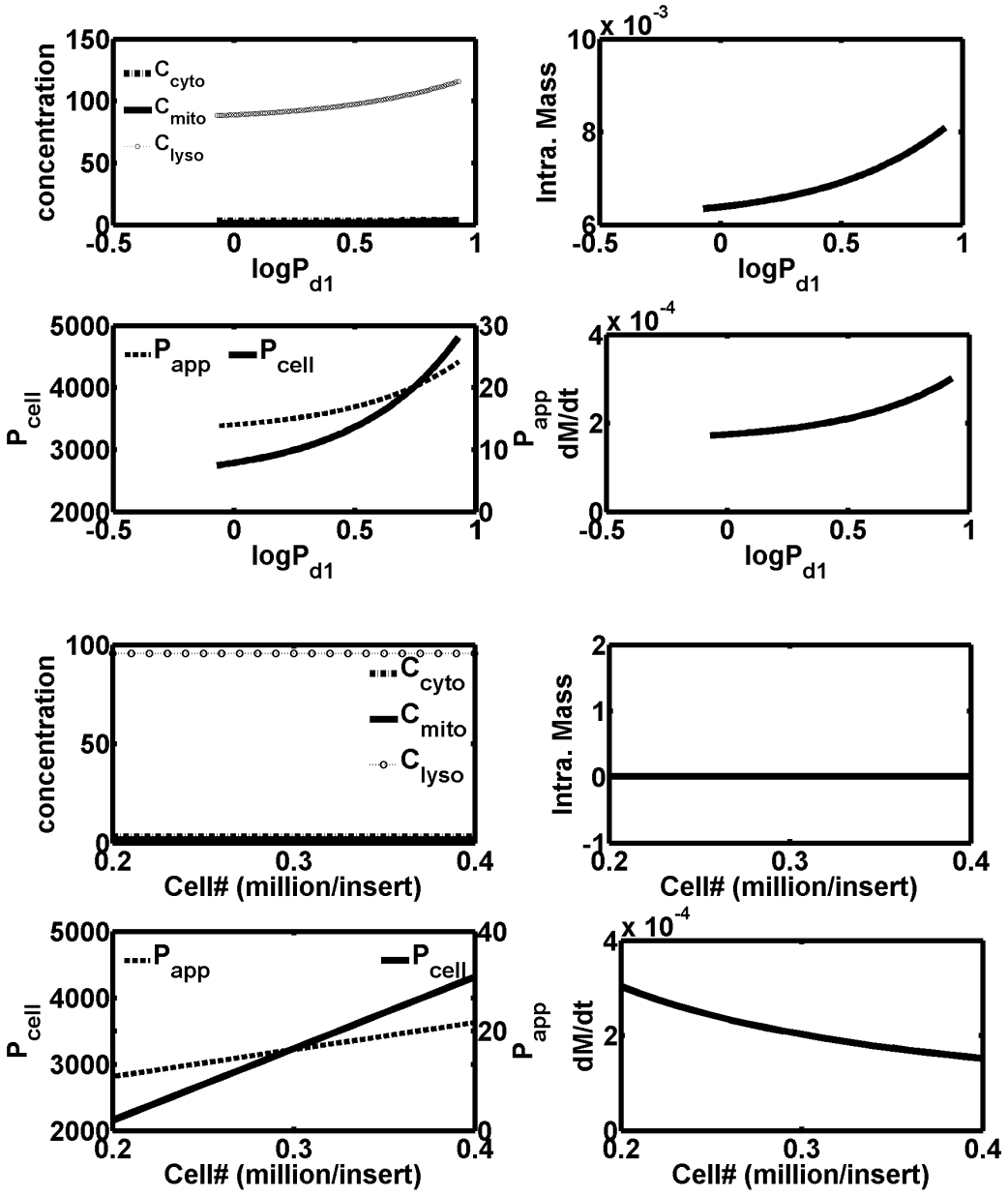
Parameter Sensitivity Analysis Plots

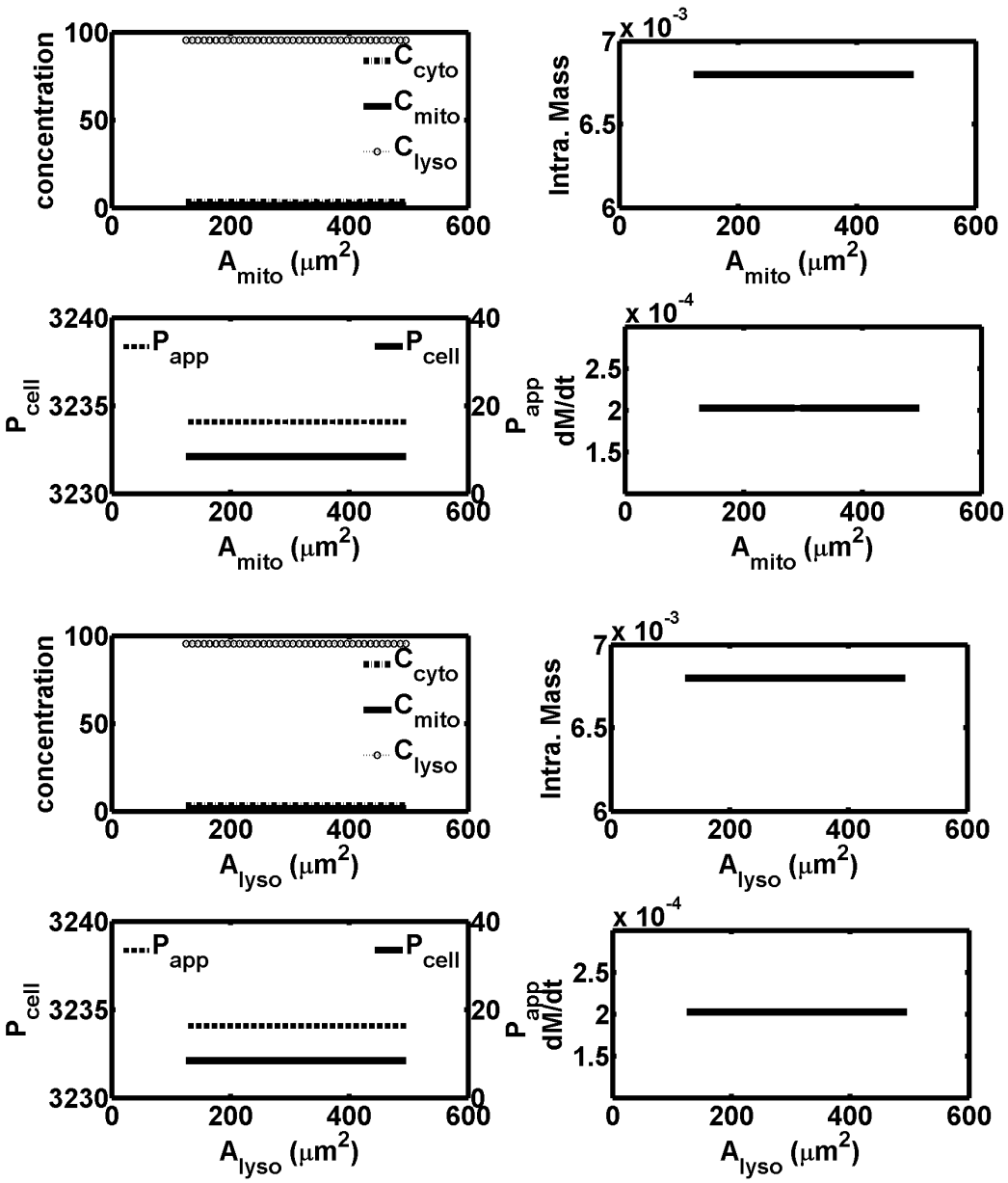


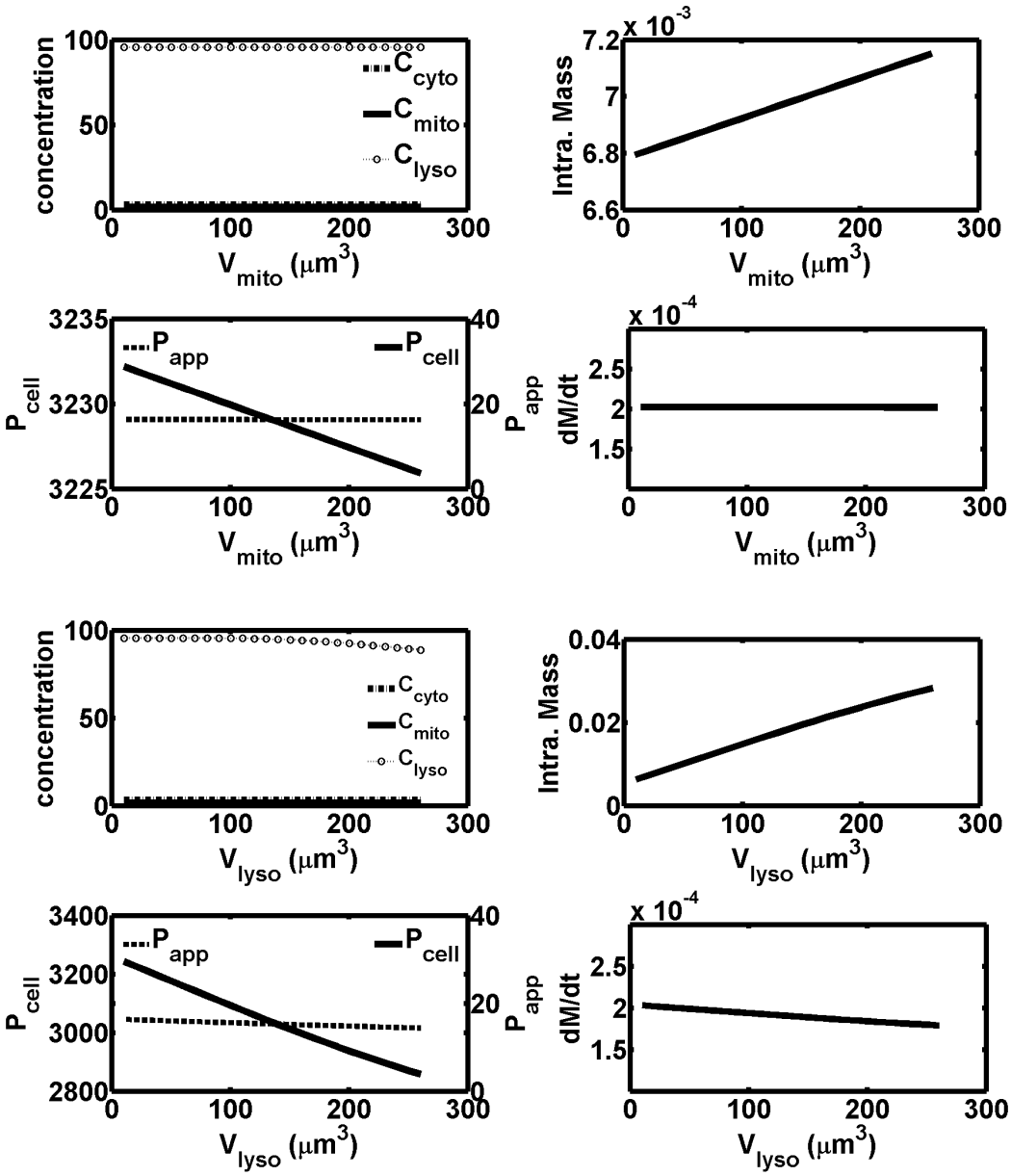

concentration
200
100
0
6.5
7
7.5
8
pK$_{a2}$
C$_{cyto}$
C$_{mito}$
C$_{lyso}$
Intra. Mass
x 10^{-3}
8
7
6
5
P$_{cell}$
6000
4000
2000
0
P$_{app}$
40
20
0
dM/dt
x 10^{-4}
4
2
0
concentration
300
200
100
0
9
9.5
10
10.5
pK$_{a1}$
Intra. Mass
0.015
0.01
0.005
P$_{cell}$
10000
5000
0
P$_{app}$
30
20
10
0
dM/dt
x 10^{-4}
6
4
2
0

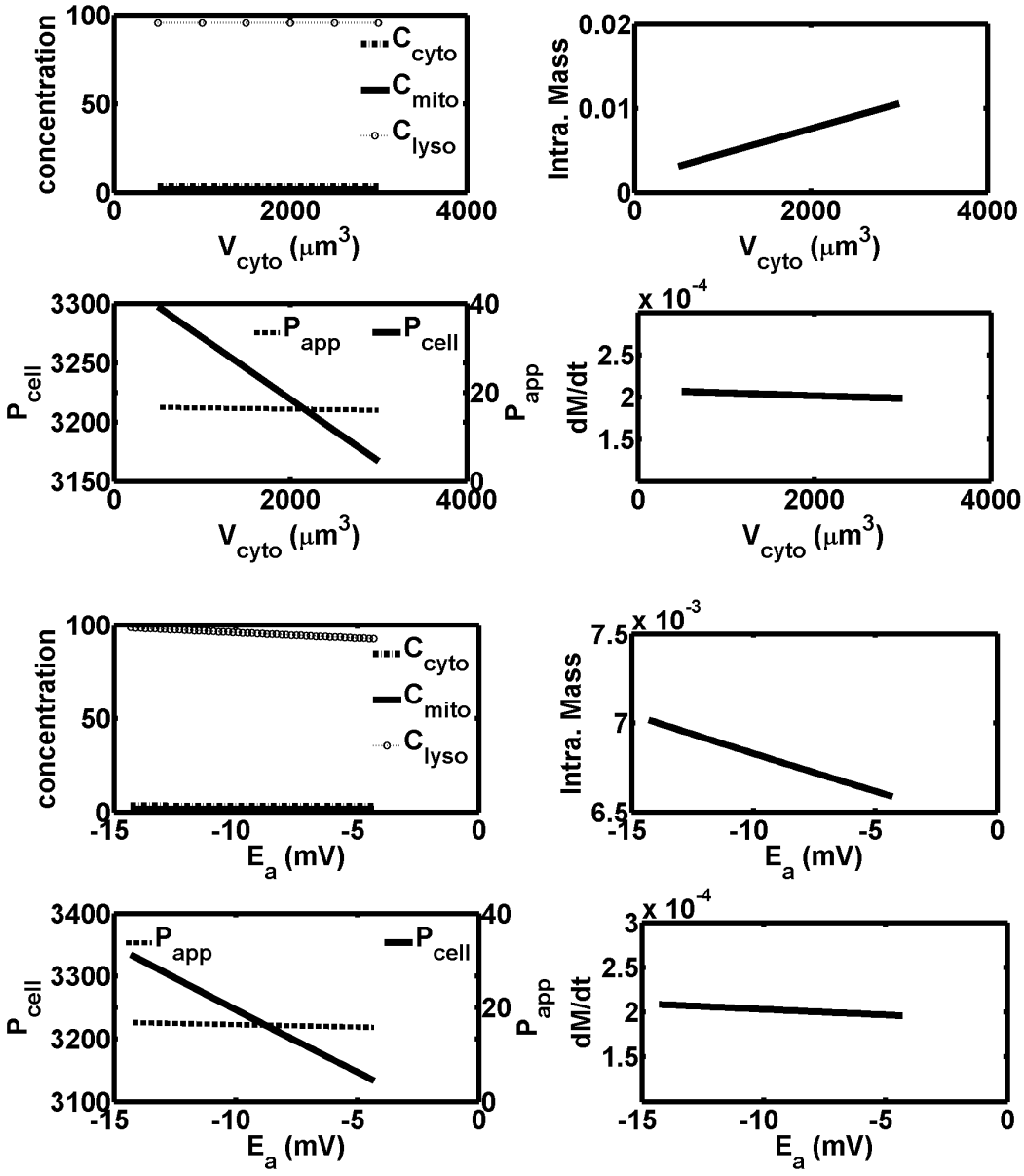

concentration
100
50
0
2
4
6
8
10
A_{pore}/cell (μm^2)
C_{cyto}
C_{mito}
C_{lyso}
Intra. Mass
2
1
0
-1
P_{cell}
P_{app}
10000
5000
0
40
20
0
dM/dt
150
100
50
A_b (μm^2)
x 10^{-3}
8
7
6
x 10^{-4}
4
6000
4000
2000

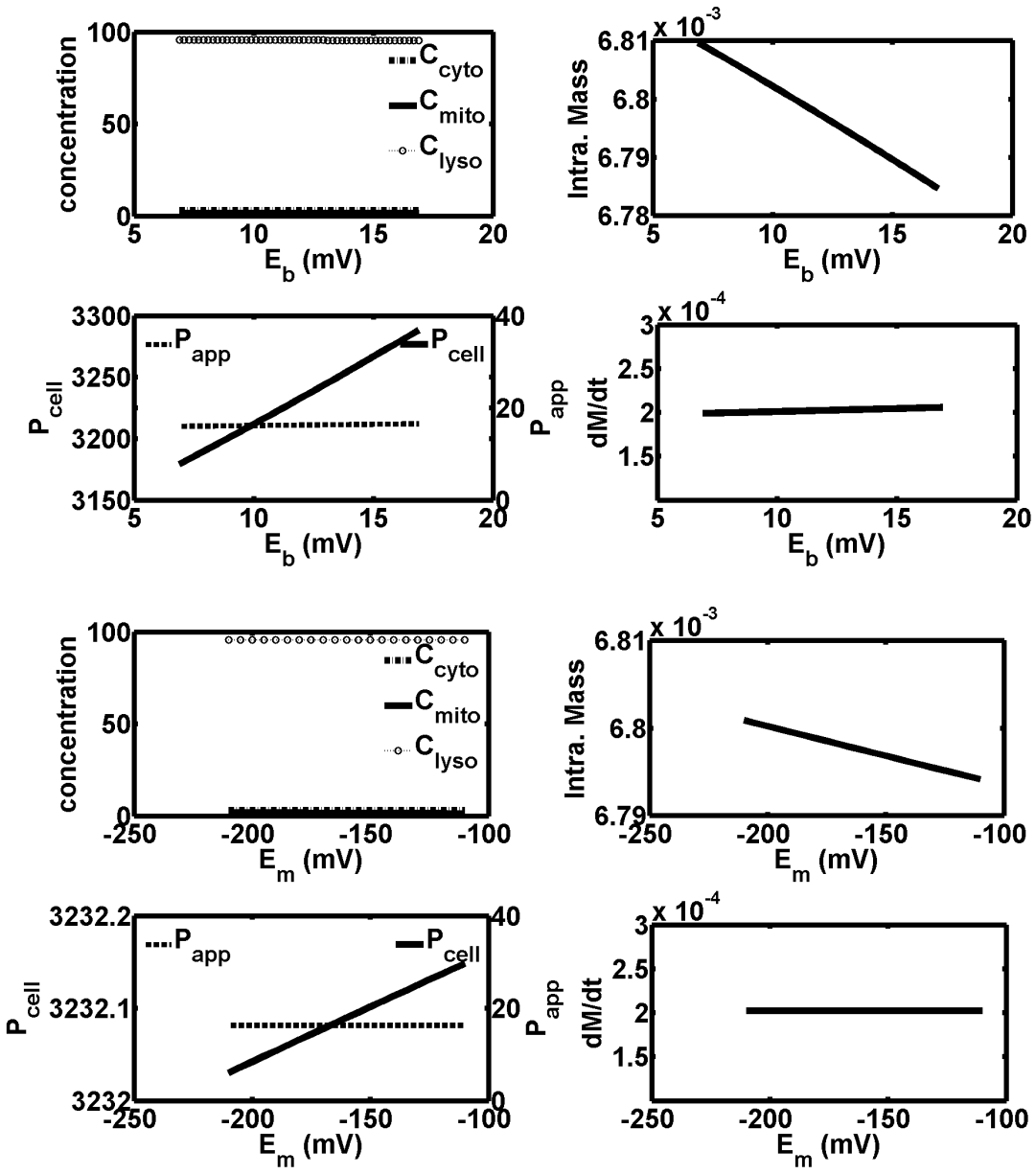

concentration
C cyto
C mito
C lyso
logP n
Intra. Mass
x 10 -4
P cell
P app
dM/dt
x 10 -3
logP d2

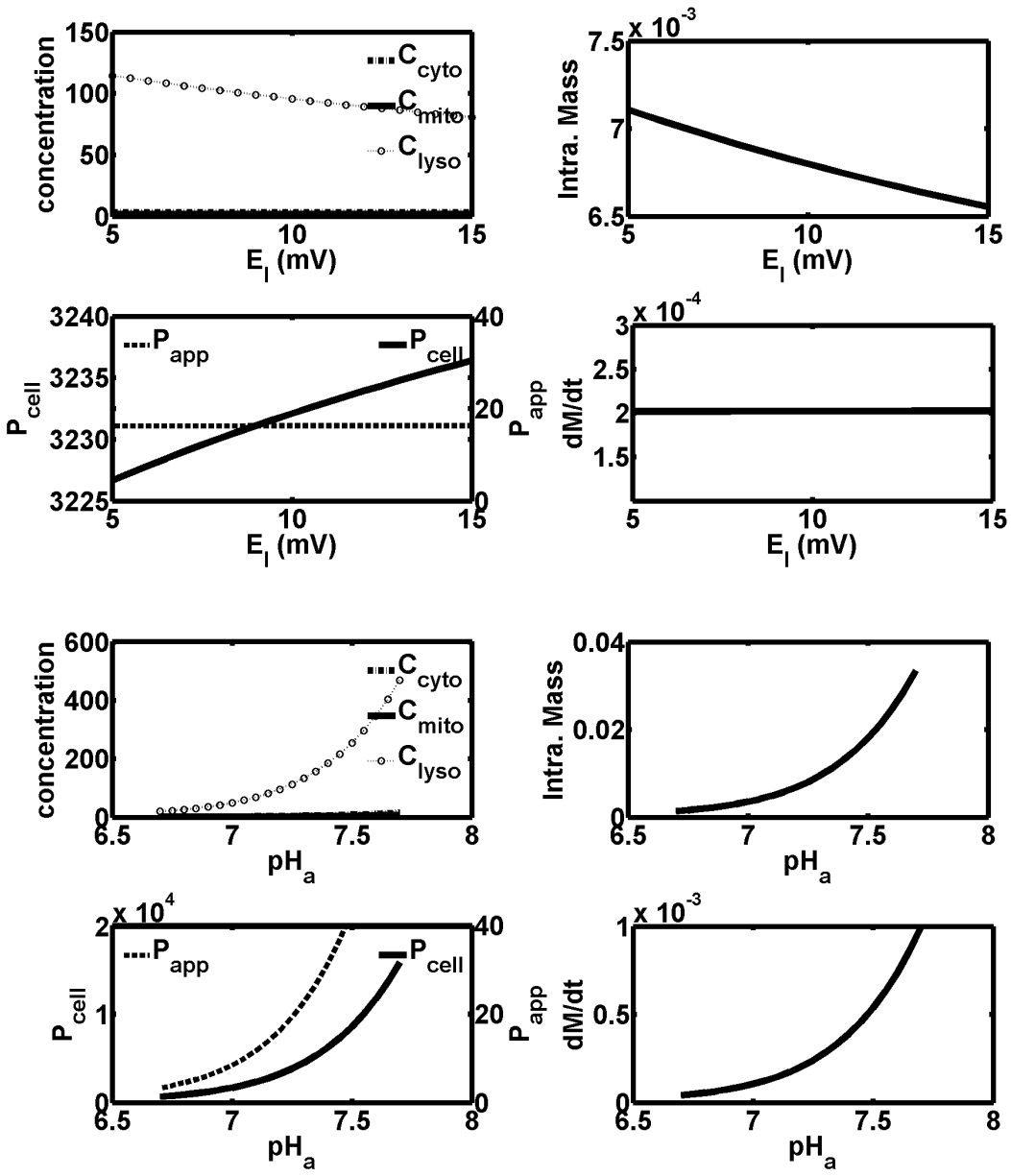

concentration
C_cyto
C_mito
C_lyso
logP_d1
Intra. Mass
x 10^-3
P_cell
P_app
P_app
dM/dt
x 10^-4
concentration
Cell# (million/insert)
Intra. Mass
Cell# (million/insert)
P_cell
P_app
P_app
dM/dt
x 10^-4
Cell# (million/insert)
Cell# (million/insert)

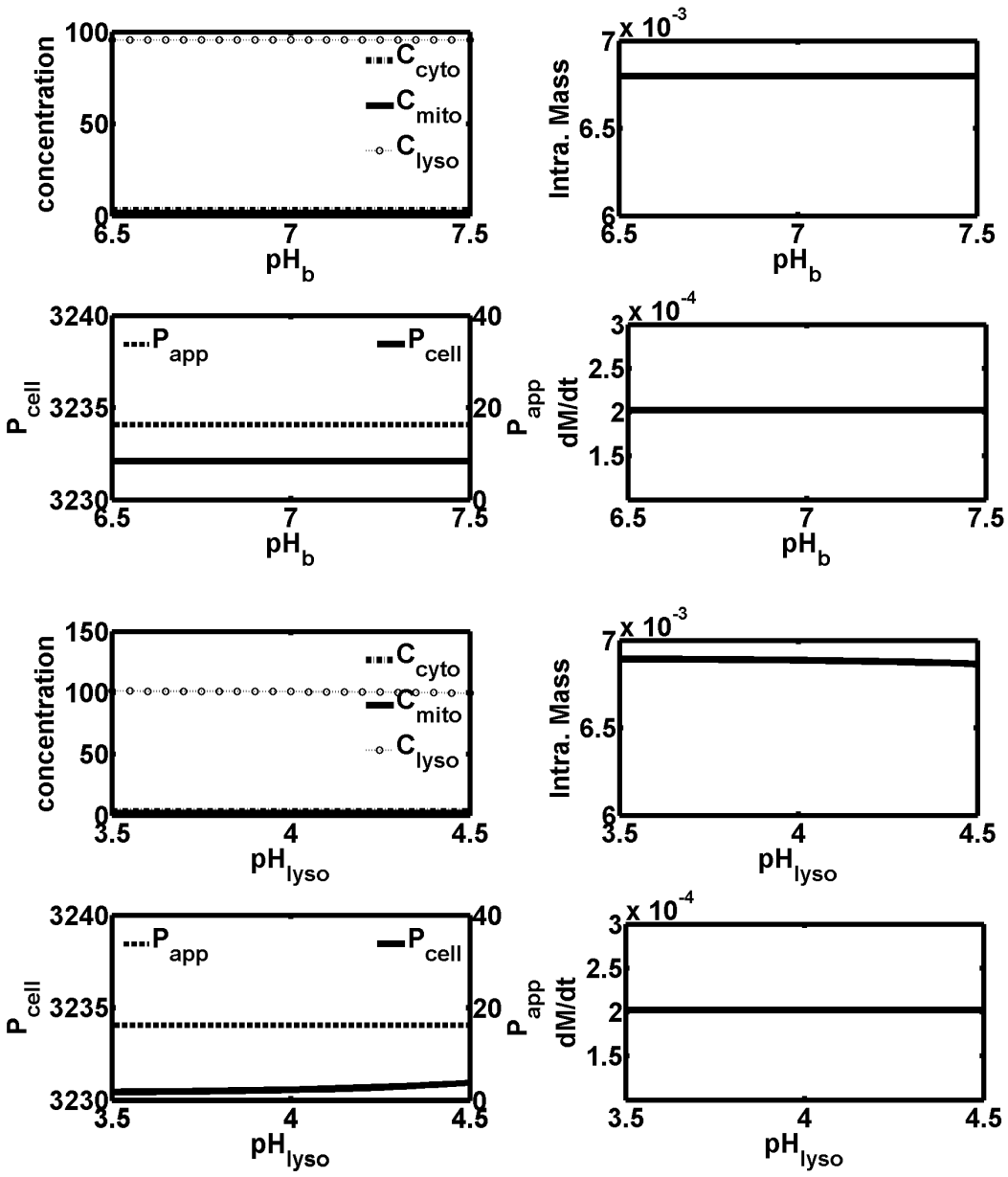

concentration
100
50
0
0
200
400
600
A_{mito} (μm^2)
C_{cyto}
C_{mito}
C_{lyso}
Intra. Mass
x 10^{-3}
7
6.5
6
P_{cell}
3240
3235
3230
40
20
0
P_{app}
P_{cell}
P_{app} dM/dt
x 10^{-4}
2.5
2
1.5
A_{lyso} (μm^2)

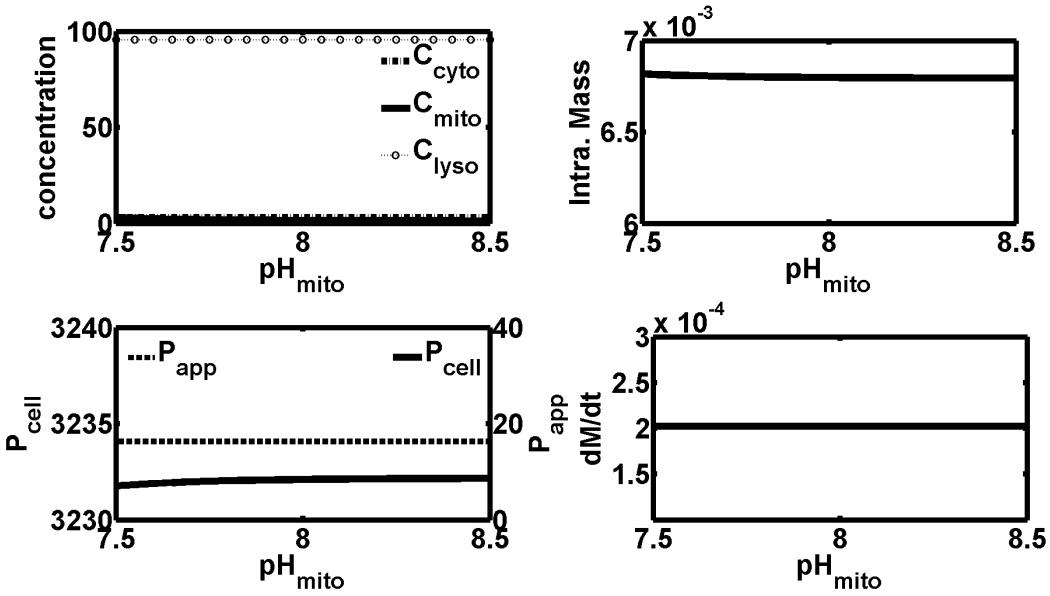

concentration
100
50
0
0
100
200
300
V_{mito} (μm^3)
C_{cyto}
C_{mito}
C_{lyso}
Intra. Mass
x 10^{-3}
7.2
7
6.8
6.6
P_{cell}
3235
3230
3225
P_{app}
40
20
0
x 10^{-4}
dM/dt
2.5
2
1.5
V_{lyso} (μm^3)
3400
3200
3000
2800
0.04
0.02


concentration
100
50
0
0
2000
4000
C_{cyto}
C_{mito}
C_{lyso}
V_{cyto} (μm^3)
Intra. Mass
0.02
0.01
0
V_{cyto} (μm^3)
P_{cell}
3300
3250
3200
3150
P_{app}
P_{cell}
40
20
0
V_{cyto} (μm^3)
dM/dt
x 10^{-4}
2.5
2
1.5
V_{cyto} (μm^3)
concentration
C_{cyto}
C_{mito}
C_{lyso}
-15
-10
-5
0
E_a (mV)
Intra. Mass
x 10^{-3}
7.5
7
6.5
E_a (mV)
P_{cell}
3400
3300
3200
3100
P_{app}
P_{cell}
40
20
0
E_a (mV)
dM/dt
x 10^{-4}
3
2.5
2
1.5
E_a (mV)


concentration
100
50
0
5
10
15
20
E_b (mV)
C_{cyto}
C_{mito}
C_{lyso}
Intra. Mass
x 10^{-3}
6.81
6.8
6.79
6.78
P_{cell}
3300
3250
3200
3150
40
20
0
P_{app}
dM/dt
x 10^{-4}
3
2.5
2
1.5
-250
-200
-150
-100
E_m (mV)
3232.2
3232.1
3232


concentration
150
100
50
0
5
10
15
E_l (mV)
C_cyto
C_mito
C_lyso
Intra. Mass
x 10^-3
7.5
7
6.5
P_cell
3240
3235
3230
3225
P_app
40
20
0
x 10^-4
dM/dt
3
2.5
2
1.5
600
400
200
6.5
7
7.5
8
pH_a
0.04
0.02
x 10^4
2
1
x 10^-3
0.5


concentration
100
50
0
6.5
7
7.5
pH_b
C_cyto
C_mito
C_lyso
Intra. Mass
x 10^-3
7
6.5
6
P_cell
3240
3235
3230
40
20
0
P_app
P_cell
P_app
dM/dt
x 10^-4
3
2.5
2
1.5
150
3.5
4
4.5
pH_lyso


concentration
100
50
0
7.5
8
8.5
pH mito
C cyto
C mito
C lyso
Intra. Mass
x 10^-3
7
6.5
6
P cell
3240
3235
3230
40
20
0
P app
dM/dt
x 10^-4
3
2.5
2
1.5

Reference:

1. Zhang, X.; Shedden, K.; Rosania, G. R. A cell-based molecular transport simulator for pharmacokinetic prediction and cheminformatic exploration. *Mol Pharm* **2006,** 3, (6), 704-16.
2. Ku, H. Notes on the Use of Propagation of Error Formulas, J Research of National Bureau of Standards-C. Engineering and Instrumentation, Vol. 70C, No.4, pp. 263-273. **1966**.