Supplementary Materials

The Effect of Phospholipidosis on the Cellular Pharmacokinetics of Chloroquine

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Table S1. Parameter ranges for Monte Carlo simulations[1]. pH, E, A, V, L and Is indicate *pH*, *membrane potential relative to the cytosol*, *surface area*, *volume*, *lipid fraction* and *ionic strength* in each compartment, while the subscripts a, c, l, and m indicate the *apical/extracellular*, *cytosolic*, *lysosomal* and *mitochondrial compartment*. pH_a, pH_l, V_l, V_c, A_l, rate of change in surface area or volume and cellular partition coefficient ($\log P_{n,d1,d2\_cell}$) were measurement as described in the manuscript. pK_{a1}, pK_{a2}, $\log P_n$, $\log P_{d1}$ and $\log P_{d2}$ were calculated by ChemAxon® based on weighted method prediction with 0.5 log units variant. Temperature (T) is set to 310.15 K during uptake experiment. Ionic strength and membrane potential values were based on literature report (Barry and Eggenton, 1972; Trapp and Horobin, 2005; Zhang et al., 2006).

		CQ				CQ/Suc.				CQ/Baf.			
		25	50	100	200	25	50	100	200	25	50	100	200
pH_c [2, 4]	**a**	7.29	7.34	7.28	7.27	7.34	7.34	7.34	7.32	7.33	7.32	7.29	7.10
	b	7.43	7.38	7.42	7.33	7.40	7.38	7.40	7.38	7.43	7.38	7.39	7.24
pH_l [2]	**a**	4.88	5.22	5.45	5.20	5.51	6.03	6.09	6.25	5.26	5.05	5.64	5.48
	b	5.84	5.80	6.17	6.76	6.31	6.73	6.81	7.21	5.84	6.33	6.12	7.04
V_c (μm^3) [2]	**a**	1452	1752	1616	1314	2761	2572	2391	2630	1491	1437	1512	1608
	b	1874	1922	2042	1916	3250	3535	3516	3904	1989	1961	2022	1945
V_l_initial (μm^3) [2]	**a**	8.8	8.8	8.8	8.8	54.9	54.9	54.9	54.9	8.8	8.8	8.8	8.8
	b	32.4	32.4	32.4	32.4	128.0	128.0	128.0	128.0	32.4	32.4	32.4	32.4
A_l_initial [2, 4]	**a**	111.5	111.5	111.5	111.5	517.5	517.5	517.5	517.5	111.5	111.5	111.5	111.5
	b	335.0	335.0	335.0	335.0	899.7	899.7	899.7	899.7	335.0	335.0	335.0	335.0
rate of change A: μm^2/hr [3, 4]	**A**	239.3	466.3	247.9	163.0	339.0	549.9	624.2	206.2	0.0	0.0	0.0	0.0
V: μm^3/hr [3]	**V**	68.7	106.9	66.0	45.1	121.4	227.2	266.3	127.3	0.0	0.0	0.0	0.0
T (K)		310.15											
$\log P_n$		(3.68, 4.18)											
$\log P_{d1}$		(0.18, 0.68)											
$\log P_{d2}$		(-1.16, -0.66)											
$\log P_{n,d1,d2\ cell}$		(1.70, 1.83)											
pK_{a1}		(9.71, 10.21)											
pK_{a2}		(7.22, 7.72)											
E_a (mV) [4]		-10											
E_l (mV)		(5, 15)											
E_m (mV)		-160											
pH_a		(7.4, 7.5)											
pH_m [4]		8											
cellNo (/well)		(50, 70) × 10^4											
V_a (μm^3)		0.5×10^{12}/cellNo											
V_m (μm^3)		16.35											
A_a (μm^2) [4]		100											
A_m (μm^2) [4]		196.35											
L_c [4]		0.05											
L_l		(0.025, 0.075)											
L_m [4]		0.05											
Is_c [4]		0.3											
Is_l		(0.2, 0.4)											
Is_m [4]		0.3											

[1] The center points for each range were used to simulate the typical kinetic curves under specific each treatment, as shown in Figure 6.

[2] The upper (b) and lower (a) boundaries of uniform distribution were calculated from the following equations based on measurements:

$$mean = \frac{1}{2}(a+b), \text{ (S1)}$$

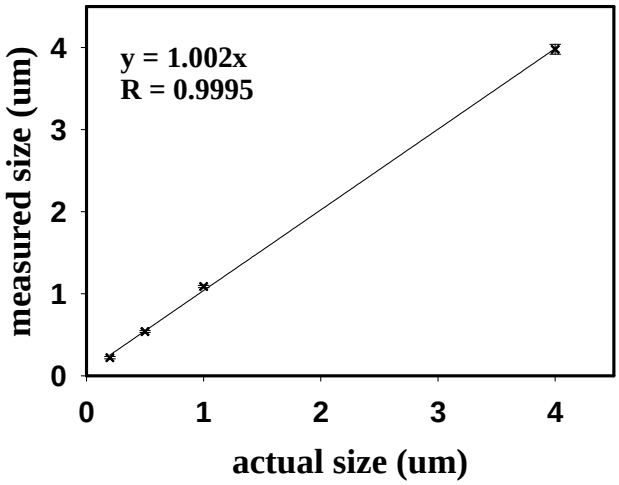
$$\text{variance} = \frac{1}{12}(b-a)^2, \text{ (S2)}$$

where the mean values were reported in Table 1, and the variance was calculated as the squared s.d..

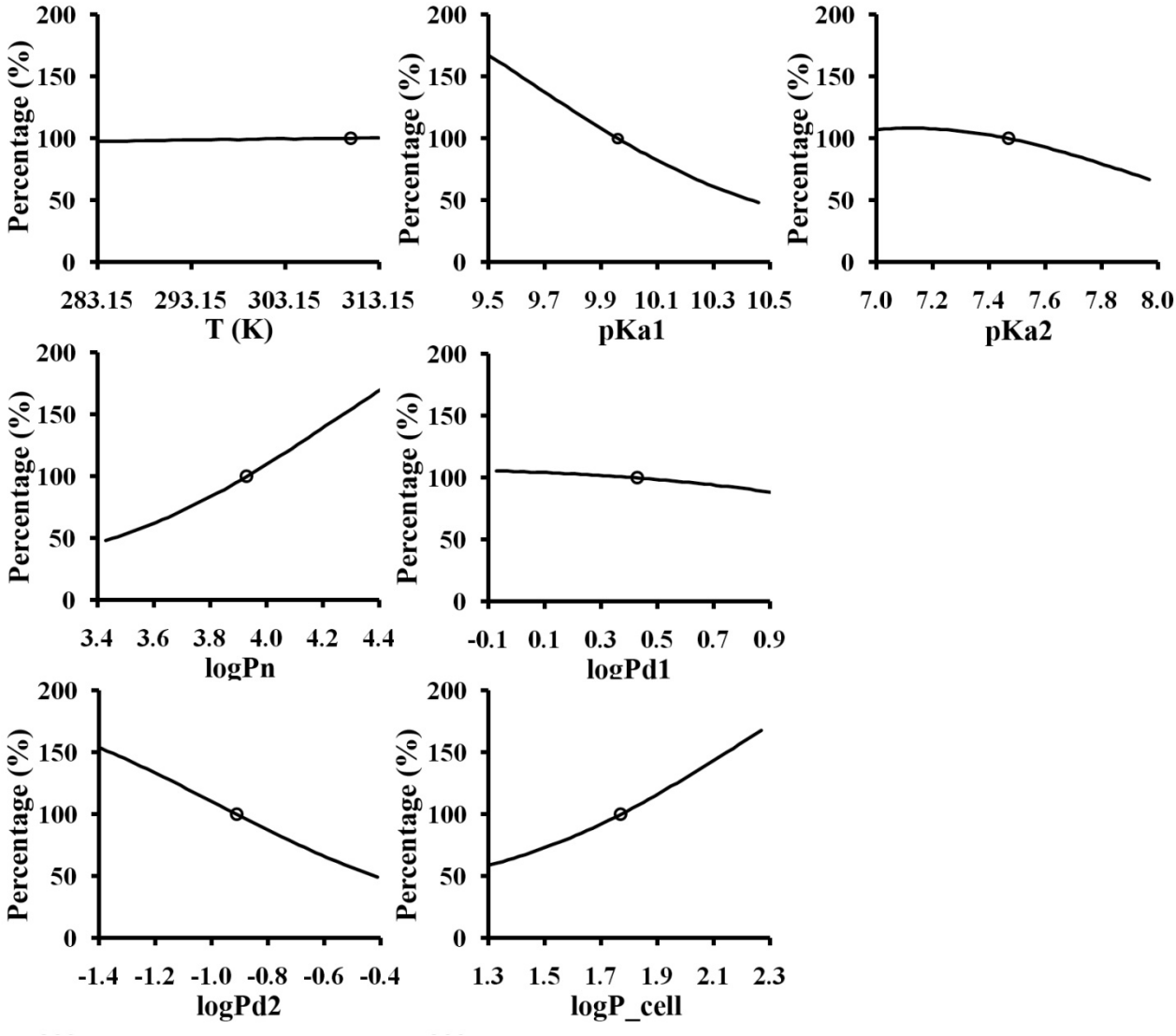
[3] Rate of changes of vesicular volume and surface area were obtained by fitting measurements at 1-4 hour time points with a linear model using the initial values as intercepts. The slope of vesicular volume and surface area under CQ treatments with bafilomycin A1 were essentially 0 after statistical analysis.

[4] These parameters do not significantly affect intracellular mass of CQ as suggested by sensitivity studies. (Figure S2).

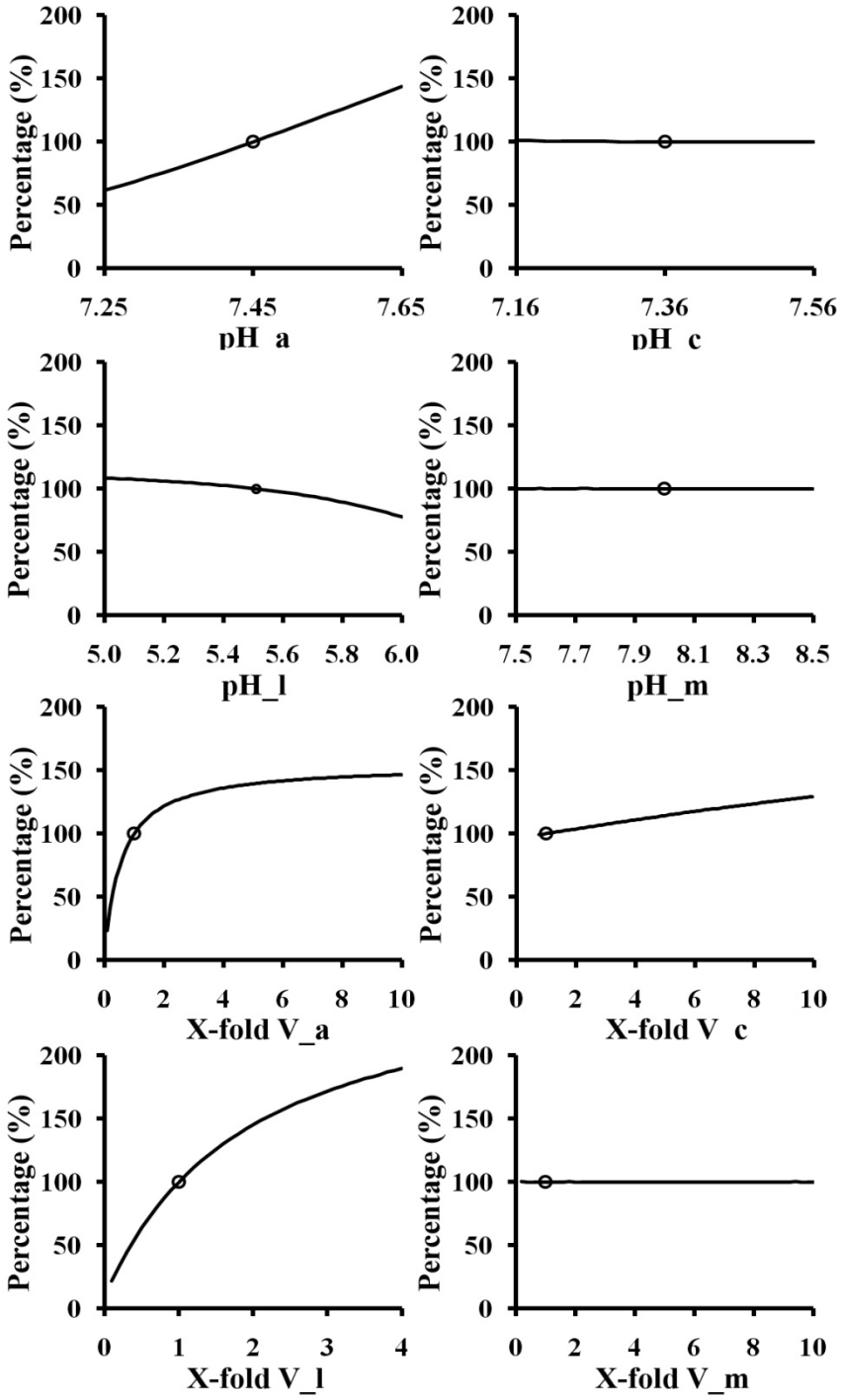
Movie S1. Movement of LTG-rich MVB/MLB within the xenosomes. MDCK cells were treated with 50 μM CQ for 4 hours and stained with LTG for 30 min. A series of 20 images were acquired at 2 sec intervals with a Nikon TE2000S *e*pifluorescence microscope coupled with a standard mercury bulb illumination, a CCD camera *(*Roper Scientific, Tucson, AZ), a 100X oil immersion objective (Nikon CFI Plan Fluor 100xH oil), and a triple-pass DAPI/FITC/TRITC filter set (Chroma Technology Corp. 86013v2). Images were compiled as a movie and played at 10X speed with the Stack Tool box in MetaMorph®.



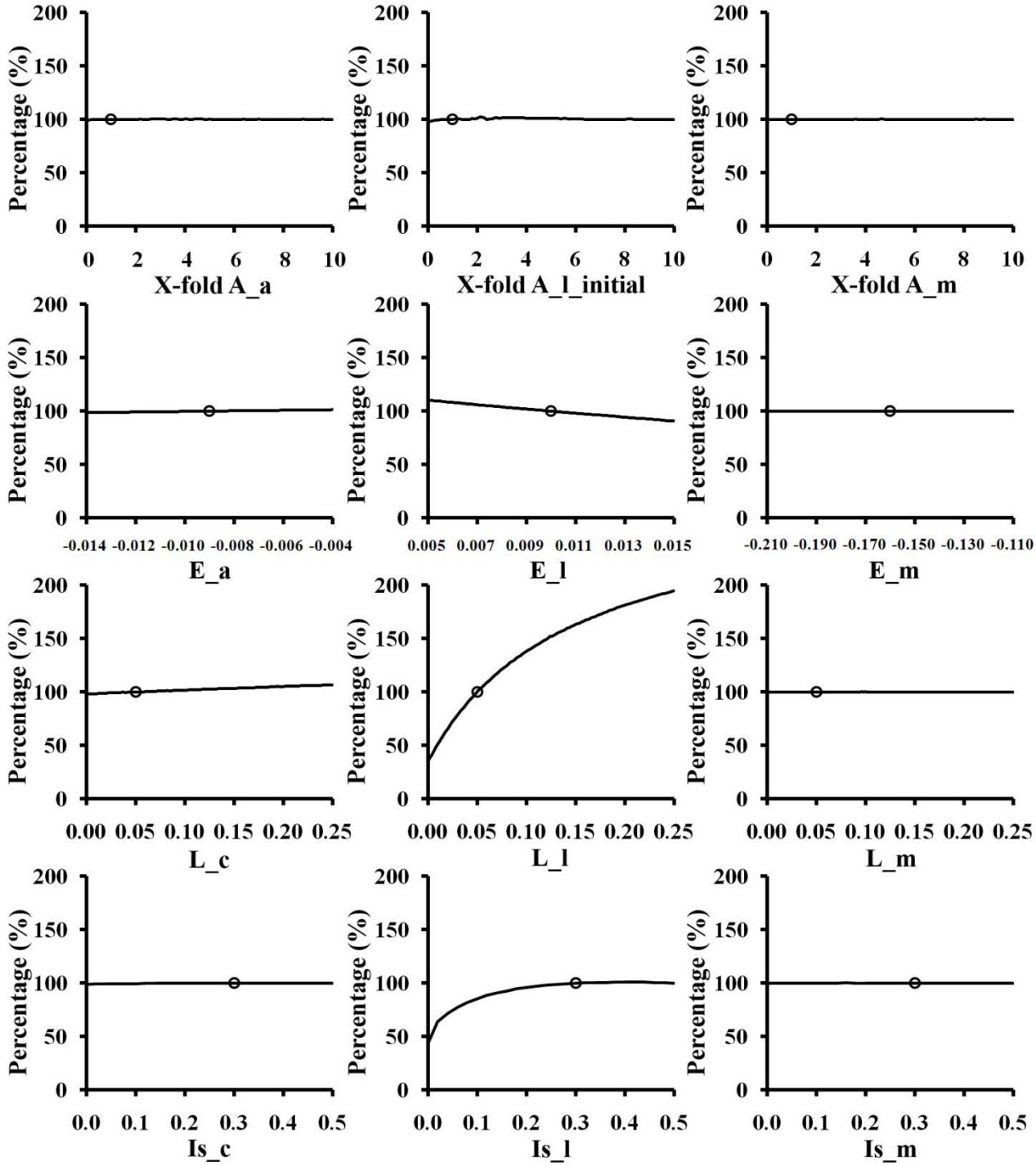
Supplemental Figure 1. Validation of vesicular volume measurement. Fluorescent microspheres of known sizes (TetraSpeck^{TM} T14792) were imaged and the diameter of individual bead was measured as mentioned in text. Six images were used to determine the diameter of each kind of microsphores. Size measurement was plotted against know diameter. For 0.2, 0.5, 1 and 4 μm microsphere, the slope was essentially 1 after statistical analysis, indicating that this method is able to capture spatial information of particles from 0.2 μm and above. To determine the thickness of the focal plane, stack images of the 0.5 μm fluorescent beads (which can be considered as point objects) were acquired at step size 1 μm. In each stack, the size of microsphores can be accurately determined in 3 or 4 images, indicating that one image captures signals from 3-4 μm distance space (images not shown). Considering the thickness of cell monolayer is 10 μm, the total vesicular volume/surface area was calculated as the product of 2.5 and the sum of vesicular volume/surface area associated with one image.



Supplemental Figure 2 Sensitivity analysis on variations induced by model parameters. Model was run with one parameter continuously sampled from a given range but all the other parameter fixed to simulated intracellular uptake for cells treated with 50 μM CQ. The predicted intracellular mass was divided by that under the typical condition, and the ratio was plotted again parameter value to show the effect of uncertainty of model parameter on cellular drug accumulation. Parameters that lead to less than 5% variance were considered as not significant factors for CQ uptake, thus they were fixed at specific values in Monte Carlos Simulation.



Supplemental Figure 2 (cont.) Sensitivity analysis on model parameters.



Supplemental Figure 2 (cont.) Sensitivity analysis on model parameters.

MATLAB® code and R code

To simulate CQ uptake in MDCK cells with the assumption of lysosomal swelling and CQ binding to cellular lipid fractions, save Code_S1 and Code_S2 as .m files. To simulate CQ uptake in without binding, in Code_S1, use $\log P_{n,d1,d2}$ instead of $\log P_{n,d1,d2\_cell}$ in calculated sorption coefficients ($K_{n,d1,d2}$) in each compartment. To simulate CQ uptake without volume expansion, in Code_S2, substitute A_slope_Gr and V_slope_Gr (rate of change in lysosomal area or volume) to an array of 0s. MATLAB® R2009b was used to code the programs; higher versions MATLAB® should be able to run the files. Code_S3.txt was copied into R 2.8.1 program to plot Monte Carlos simulation of CQ uptake. To generate green or blue histograms when simulating uptake without binding or swelling, substitute code "`col="black"`" or "`col="white"`" to "`col="green"`" or "`col="blue"`" in the "hist" and "lines" command.

1) Code _S1. m

```
% The following section is to simulate the intracellular
% concentration of CQ in MDCK cells with volume expansion in acidic
% compartment and binding of CQ to cellular membrane structures.
% Smiles: CCN(CC)CCCC(C)NC1=C2C=CC(=CC2=NC=C1)Cl.OP(=O)(O)O.OP(=O)(O)O
% MDCK cells on 24well plates, 2cm^2 bottom, assuming 60*10^4 cell/well

% Clear the memory
clear
clc

global V_l_initial A_l_initial V_c_initial A_a V_m A_m V_a i
global Pn Pd1 Pd2
global Nd1_a Nd1_m Nd1_l Nd2_a Nd2_m Nd2_l
global fn_a fn_c fn_l fn_m
global fd1_a fd1_c fd1_l fd1_m
global fd2_a fd2_c fd2_l fd2_m

% Constant
T = 310.15 ;                % temperature
R = 8.314 ;                 % Universal gas constant
F = 96484.56 ;              % Faraday constant

% Group conditions: 1-4, CQ treatments (25, 50, 100, 200 uM); 5-8, CQ/Suc.
% treatments (25, 50, 100, 200 uM); and 9-12, CQ/Baf. treatments (25, 50,
% 100, 200 uM).

C_aGr = [0.025, 0.050, 0.100, 0.200, 0.025, 0.050, 0.100, 0.200, 0.025,...
    0.050, 0.100, 0.200] ;          % Apical initial drug concentration (mM)
V_cGr_a = [1452, 1752, 1616, 1314, 2761, 2572, 2391, 2630, 1491, 1437,...
    1512, 1608] ;                   % cell volume, lower bound (um^3)
V_cGr_b = [1874, 1922, 2042, 1916, 3250, 3535, 3516, 3904, 1989, 1961,...
    2022, 1945] ;                   % cell volume, upper bound (um^3)
pH_lGr_a = [4.88, 5.22, 5.45, 5.20, 5.51, 5.73, 6.09, 6.25, 5.26, 5.05,...
    5.64, 5.48] ;                   % pH in lysosome, lower bound
pH_lGr_b = [5.84, 5.80, 6.17, 6.76, 6.31, 6.43, 6.81, 7.21, 5.84, 6.33,...
    6.12, 7.04] ;                   % pH in lysosome, upper bound
pH_cGr_a = [7.29, 7.34, 7.28, 7.27, 7.34, 7.34, 7.34, 7.32, 7.33, 7.32,...
    7.29, 7.10] ;                   % cytosolic pH, lower bound
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```
pH_cGr_b = [7.43, 7.38, 7.42, 7.33, 7.40, 7.38, 7.40, 7.38, 7.43, 7.38,...
    7.39, 7.24] ;                  % cytosolic pH, upper bound
A_l_initial_Gr_a = [111.5, 111.5, 111.5, 111.5, 517.5, 517.5, 517.5,...
    517.5, 111.5, 111.5, 111.5, 111.5] ;
    % initial lysosomal membrane area, lower bound
A_l_initial_Gr_b = [335.0, 335.0, 335.0, 335.0, 899.7, 899.7, 899.7,...
    899.7, 335.0, 335.0, 335.0, 335.0] ;
    % initial lysosomal membrane area, upper bound
V_l_initial_Gr_a = [8.8, 8.8, 8.8, 8.8, 54.9, 54.9, 54.9, 54.9, 8.8,...
    8.8, 8.8, 8.8] ; % initial lysosomal volume, lower bound
V_l_initial_Gr_b = [32.4, 32.4, 32.4, 32.4, 128.0, 128.0, 128.0, 128.0,...
    32.4, 32.4, 32.4, 32.4] ; % initial lysosomal volume, upper bound

rand('seed',2010);

for i = 1:1:12
    for round = 1:1:10000

C_a = C_aGr(i);                              % extracellular concentration
cellNo = (50+20*rand())*10^4;                % cell number per well

% Drug information -- ChemAxon calculation including logPn, pKa1, and pKa2
pKa1 = 9.96+0.25-0.5*rand() ;  % higher pKa
pKa2 = 7.47+0.25-0.5*rand() ;  % lower pKa
z1 = 1 ;                       % electric charge
z2 = 2 ;                       % electric charge
i1 = sign(z1) ;
i2 = sign(z2) ;
logPn = 3.93+0.25-0.5*rand() ;
    % logarithm of octanol/water partition coefficient for neutral species
logPd1 = 0.43+0.25-0.5*rand() ;
    % logarithm of octanol/water partition coefficient for +1 ion species
logPd2 = -0.91+0.25-0.5*rand() ;
    % logarithm of octanol/water partition coefficient for +2 ion species

logPn_cell = 1.70+rand()*(1.83-1.70) ;
logPd1_cell = 1.70+rand()*(1.83-1.70) ;
logPd2_cell = 1.70+rand()*(1.83-1.70) ;
    % logarithm of cellular partition coefficient for all three species

Pn = 10^(logPn-6.7) ;          % membrane permeability for neutral species
Pd1 = 10^(logPd1-6.7) ;        % membrane permeability for +1 ion species
Pd2 = 10^(logPd2-6.7) ;        % membrane permeability for +2 ion species

% pH values
pH_a = 7.4+0.1*rand() ;                          % pH in apical compartment
pH_c = pH_cGr_a(i)+(pH_cGr_b(i)-pH_cGr_a(i))*rand() ; % pH in cytosol
pH_l = pH_lGr_a(i)+(pH_lGr_b(i)-pH_lGr_a(i))*rand() ; % pH in lysosomes
pH_m = 8.0;                                          % pH in mitochondria

% lipid fractions
L_l = 0.025+0.05*rand() ;           % lipid fraction in lysosomes
L_c = 0.05 ;           % lipid fraction in cytosol
L_m = 0.05 ;                        % lipid fraction in mitochondria
```

```
% Areas and volumes (units in m^2 and m^3)
A_a = 100*10^(-10) ;                % apical membrane surface area
A_l_initial = 10^(-12)*(A_l_initial_Gr_a(i)+(A_l_initial_Gr_b(i)...
    -A_l_initial_Gr_a(i))*rand()) ;  % lysosomal membrane surface area
A_m = 250*7.85*10^(-13) ;           % mitochondrial membrane surface area
V_a = 0.5*10^(-6)/cellNo ;          % extracellular drug solution volume
V_c_initial = 10^(-18)*(V_cGr_a(i)+(V_cGr_b(i)-V_cGr_a(i))*rand()) ;
                                    % initial cytosolic volume
V_l_initial = 10^(-18)*(V_l_initial_Gr_a(i)+(V_l_initial_Gr_b(i)...
    -V_l_initial_Gr_a(i))*rand()) ;  % lysosomal volume
V_m = 250*6.55*10^(-20);            % mitochondrial volume

% Membrane potential (units in 'Voltage')
E_a = -0.009 ;                   % membrane potential of apical membrane
E_l = +0.01-0.005+0.01*rand() ;  % membrane potential of lysosomal membrane
E_m = -0.16 ;                % membrane potential of mitochondrial membrane

% Apical Compartment
fn_a = 1/(1+10^(i1*(pKa1-pH_a))+10^(i1*(pKa1-pH_a)+i2*(pKa2-pH_a))) ;
% ratio of the activity of neutral species and
% total molecular concentration in apical compartment
fd2_a = fn_a*10^(i1*(pKa1-pH_a)+i2*(pKa2-pH_a)) ;
% ratio of the activity of +1 ion species and
% total molecular concentration in apical compartment
fd1_a = fn_a*10^(i1*(pKa1-pH_a)) ;
% ratio of the activity of +2 ion species and
% total molecular concentration in apical compartment
Nd2_a = z2*E_a*F/(R*T) ;
Nd1_a = z1*E_a*F/(R*T) ;

% Cytoplasm
W_c = 1-L_c ;        % water fraction in cytosol
Is_c = 0.3 ;         % ionic strength in cytosol (mol)
gamman_c = 10^(0.3*Is_c) ;
                     % activity coefficient of neutral molecules in cytosol
gammad1_c = 10^(-0.5*z1*z1*(sqrt(Is_c)/(1+sqrt(Is_c))-0.3*Is_c)) ;
                     % activity coefficient of monovalent base in cytosol
gammad2_c = 10^(-0.5*z2*z2*(sqrt(Is_c)/(1+sqrt(Is_c))-0.3*Is_c)) ;
                     % activity coefficient of bivalent base in cytosol
Kn_c = L_c*1.22*10^(logPn_cell) ;
                     % sorption coefficient for neutral species in cytosol
Kd1_c = L_c*1.22*10^(logPd1_cell) ;
                     % sorption coefficient for +1 ion species in cytosol
Kd2_c = L_c*1.22*10^(logPd2_cell) ;
                     % sorption coefficient for +2 ion species in cytosol
an_c = 1/(1+10^(i1*(pKa1-pH_c))+10^(i1*(pKa1-pH_c)+i2*(pKa2-pH_c))) ;
                     % activity of neutral species in cytosol
ad2_c = an_c*10^(i1*(pKa1-pH_c)+i2*(pKa2-pH_c)) ;
                     % activity of +1 ion species in cytosol
ad1_c = an_c*10^(i1*(pKa1-pH_c)) ;
                     % activity of +2 ion species in cytosol
Dd2_c = ad2_c/an_c ;
Dd1_c = ad1_c/an_c ;
fn_c = 1/(W_c/gamman_c+Kn_c/gamman_c+Dd2_c*W_c/gammad2_c...
    +Dd2_c*Kd2_c/gammad2_c+Dd1_c*W_c/gammad1_c+Dd1_c*Kd1_c/gammad1_c) ;
% ratio of the activity of neutral species and
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% total molecular concentration in cytosol
fd2_c = fn_c*Dd2_c ;
% ratio of the activity of +1 ion species and
% total molecular concentration in cytosol
fd1_c = fn_c*Dd1_c ;
% ratio of the activity of +2 ion species and
% total molecular concentration in cytosol

% Mitochondria
W_m = 1-L_m ;        % water fraction in mitochondria
Is_m = 0.3 ;         % ionic strength in mitochondria (mol)
Nd2_m = z2*E_m*F/(R*T) ;
Nd1_m = z1*E_m*F/(R*T) ;
gamman_m = 10^(0.3*Is_m) ;
                % activity coefficient of neutral molecules in mitochondria
gammad1_m = 10^(-0.5*z1*z1*(sqrt(Is_m)/(1+sqrt(Is_m))-0.3*Is_m)) ;
                % activity coefficient of +1 ion molecules in mitochondria
gammad2_m = 10^(-0.5*z2*z2*(sqrt(Is_m)/(1+sqrt(Is_m))-0.3*Is_m)) ;
                % activity coefficient of +2 ion molecules in mitochondria
Kn_m = L_m*1.22*10^(logPn_cell) ;
                % sorption coefficient for neutral species in mitochondria
Kd1_m = L_m*1.22*10^(logPd1_cell) ;
                % sorption coefficient for +1 ion species in mitochondria
Kd2_m = L_m*1.22*10^(logPd2_cell) ;
                % sorption coefficient for +2 ion species in mitochondria
an_m = 1/(1+10^(i1*(pKa1-pH_m))+10^(i1*(pKa1-pH_m)+i2*(pKa2-pH_m))) ;
                % activity of neutral species in mitochondria
ad2_m = an_m*10^(i1*(pKa1-pH_m)+i2*(pKa2-pH_m)) ;
                % activity of +1 ion species in mitochondria
ad1_m = an_m*10^(i1*(pKa1-pH_m)) ;
                % activity of +2 ion species in mitochondria
Dd2_m = ad2_m/an_m ;
Dd1_m = ad1_m/an_m ;
fn_m = 1/(W_m/gamman_m+Kn_m/gamman_m+Dd2_m*W_m/gammad2_m...
    +Dd2_m*Kd2_m/gammad2_m+Dd1_m*W_m/gammad1_m+Dd1_m*Kd1_m/gammad1_m ) ;
% ratio of the activity of neutral species and
% total molecular concentration in mitochondria
fd2_m = fn_m*Dd2_m ;
% ratio of the activity of +1 ion species and
% total molecular concentration in mitochondria
fd1_m = fn_m*Dd1_m ;
% ratio of the activity of +2 ion species and
% total molecular concentration in mitochondria

% lysosomes
W_l = 1-L_l ;                  % water fraction in lysosomes
Is_l = 0.2 + 0.2 * rand() ;    % ionic strength in lysosomes (mol)
Nd2_l = z2*E_l*F/(R*T) ;
Nd1_l = z1*E_l*F/(R*T) ;
gamman_l = 10^(0.3*Is_l) ;
                  % activity coefficient of neutral molecules in lysosomes
gammad1_l = 10^(-0.5*z1*z1*(sqrt(Is_l)/(1+sqrt(Is_l))-0.3*Is_l));
                  % activity coefficient of +1 ion molecules in lysosomes
gammad2_l = 10^(-0.5*z2*z2*(sqrt(Is_l)/(1+sqrt(Is_l))-0.3*Is_l));
                  % activity coefficient of +2 ion molecules in lysosomes
Kn_l = L_l*1.22*10^(logPn_cell) ;
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```
                    % sorption coefficient for neutral species in lysosomes
Kd1_l = L_l*1.22*10^(logPd1_cell) ;
                    % sorption coefficient for +1 ion species in lysosomes
Kd2_l = L_l*1.22*10^(logPd2_cell) ;
                    % sorption coefficient for +2 ion species in lysosomes
an_l = 1/(1+10^(i1*(pKa1-pH_l))+10^(i1*(pKa1-pH_l)+i2*(pKa2-pH_l))) ;
                    % activity of neutral species in lysosomes
ad2_l = an_l*10^(i1*(pKa1-pH_l)+i2*(pKa2-pH_l)) ;
                    % activity of +1 ion species in lysosomes
ad1_l = an_l*10^(i1*(pKa1-pH_l)) ;
                    % activity of +2 ion species in lysosomes
Dd2_l = ad2_l/an_l ;
Dd1_l = ad1_l/an_l ;
fn_l = 1/(W_l/gamman_l+Kn_l/gamman_l+Dd2_l*W_l/gammad2_l...
    +Dd2_l*Kd2_l/gammad2_l+Dd1_l*W_l/gammad1_l+Dd1_l*Kd1_l/gammad1_l ) ;
% ratio of the activity of neutral species and
% total molecular concentration in lysosomes
fd2_l = fn_l*Dd2_l ;
% ratio of the activity of +1 ion species and
% total molecular concentration in lysosomes
fd1_l = fn_l*Dd1_l ;
% ratio of the activity of +2 ion species and
% total molecular concentration in lysosomes


% Solve the differential equation system:
% Given a system of linear ODE's expressed in matrix form:
% Y' = AY+G with initial conditions Y(0) = [0 0 0 1 1 C_a]'

time = 14400;
Y0 = [0 0 0 1 1 C_a]';
[TI,Y] = ode15s(@Code_S2,[0,time],Y0);
[a,b]=size(Y);
Mass_cell = Y(a,1)*(V_c_initial-V_l_initial*Y(a,4))*10^12+...
    (Y(a,2)*V_m+Y(a,3)*V_l_initial*Y(a,4))*10^12 ;
    % intracellular mass (pmol/cell)
Mass_all = Y(a,1)*(V_c_initial-V_l_initial*Y(a,4))*cellNo*10^9+...
    (Y(a,2)*V_m+Y(a,3)*V_l_initial*Y(a,4)+Y(a,6)*V_a)*cellNo*10^9 ;
    % total mass (nmol)
N = [i, round, C_a, Y(a,1), Y(a,2), Y(a,3), Y(a,4), Y(a,5), Y(a,6),...
    Mass_cell, Mass_all];
if mod(i,4)==1
    fid = fopen('Monte25.dat','a');
elseif mod(i,4)==2
    fid = fopen('Monte50.dat','a');
elseif mod(i,4)==3
    fid = fopen('Monte100.dat','a');
else fid = fopen('Monte200.dat','a');
end
fprintf(fid,'%+12.0f %+12.0f %+12.2f %+12.6f %+12.6f %+12.6f %+12.6f %+12.6f
%+12.6f %+12.6f %+12.6f\n', N);
fclose(fid);
clear Y TI;

    end
end
```

2) Code_S2.

```
% The following section is the function called by Code_S1.m to simulate
% intracellular concentration of CQ in MDCK cells with volume expansion in
% acidic compartment and binding of CQ to cellular membrane structures.

function [dCR] = Code_S2(t,CR)

global V_l_initial A_l_initial V_c_initial A_a V_m A_m V_a i
global Pn Pd1 Pd2
global Nd1_a Nd1_m Nd1_l Nd2_a Nd2_m Nd2_l
global fn_a fn_c fn_l fn_m
global fd1_a fd1_c fd1_l fd1_m
global fd2_a fd2_c fd2_l fd2_m

% Solve the differential equation system for each drug:
% Given a system of linear ODE's expressed in matrix form:
% Y' = AY+G with initial conditions Y(0) = [0 0 0 1 1 C_a]'

A_slope_Gr = [237.75, 464.69, 246.37, 161.42, 339.03, 549.90, 624.23,...
    206.23, 0.00, 0.00, 0.00, 0.00] ; % rate of change in lyso surface area
V_slope_Gr = [68.77, 106.88, 66.07, 45.08, 121.37, 227.24, 266.29,...
    127.33, 0.00, 0.00, 0.00, 0.00] ; % rate of change in lyso volume
V_slope_base = [20.6, 20.6, 20.6, 20.6, 91.42, 91.42, 91.42, 91.42,...
    20.6, 20.6, 20.6, 20.6] ; % initial lysosomal volume (um^3)
A_slope_base = [223.26, 223.26, 223.26, 223.26, 708.61, 708.61, 708.61,...
    708.61, 223.26, 223.26, 223.26, 223.26] ;
    % initial lysosomal surface area (um^2)

V_l = V_l_initial*CR(4) ;
A_l = A_l_initial*CR(5) ;
V_c = V_c_initial-V_l ;

k11 = -(A_a/V_c)*Pn*fn_c...
       -(A_a/V_c)*Pd1*Nd1_a*fd1_c*exp(Nd1_a)/(exp(Nd1_a)-1)...
       -(A_a/V_c)*Pd2*Nd2_a*fd2_c*exp(Nd2_a)/(exp(Nd2_a)-1)...
       -(A_m/V_c)*Pn*fn_c-(A_m/V_c)*Pd1*Nd1_m*fd1_c/(exp(Nd1_m)-1)...
       -(A_m/V_c)*Pd2*Nd2_m*fd2_c/(exp(Nd2_m)-1)...
       -(A_l/V_c)*Pn*fn_c-(A_l/V_c)*Pd1*Nd1_l*fd1_c/(exp(Nd1_l)-1)...
       -(A_l/V_c)*Pd2*Nd2_l*fd2_c/(exp(Nd2_l)-1) ;
k12 = (A_m/V_c)*Pn*fn_m+(A_m/V_c)*Pd1*Nd1_m*fd1_m*exp(Nd1_m)/...
    (exp(Nd1_m)-1)+(A_m/V_c)*Pd2*Nd2_m*fd2_m*exp(Nd2_m)/(exp(Nd2_m)-1) ;
k13 = (A_l/V_c)*Pn*fn_l+(A_l/V_c)*Pd1*Nd1_l*fd1_l*exp(Nd1_l)/...
    (exp(Nd1_l)-1)+(A_l/V_c)*Pd2*Nd2_l*fd2_l*exp(Nd2_l)/(exp(Nd2_l)-1) ;
k16 = (A_a/V_c)*Pn*fn_a+(A_a/V_c)*Pd1*Nd1_a*fd1_a/...
    (exp(Nd1_a)-1)+(A_a/V_c)*Pd2*Nd2_a*fd2_a/(exp(Nd2_a)-1) ;

k21 = (A_m/V_m)*Pn*fn_c+(A_m/V_m)*Pd1*Nd1_m*fd1_c/(exp(Nd1_m)-1)...
       +(A_m/V_m)*Pd2*Nd2_m*fd2_c/(exp(Nd2_m)-1) ;
k22 = -(A_m/V_m)*Pn*fn_m-(A_m/V_m)*Pd1*Nd1_m*fd1_m*exp(Nd1_m)/...
    (exp(Nd1_m)-1)-(A_m/V_m)*Pd2*Nd2_m*fd2_m*exp(Nd2_m)/(exp(Nd2_m)-1) ;
```

```
S4 = V_slope_Gr(i)/V_slope_base(i)/3600 ;
S5 = A_slope_Gr(i)/A_slope_base(i)/3600 ;

k31 = (A_l/V_l)*Pn*fn_c+(A_l/V_l)*Pd1*Nd1_l*fd1_c/(exp(Nd1_l)-1)...
       +(A_l/V_l)*Pd2*Nd2_l*fd2_c/(exp(Nd2_l)-1) ;
k33 = -(A_l/V_l)*Pn*fn_l-(A_l/V_l)*Pd1*Nd1_l*fd1_l*exp(Nd1_l)/...
    (exp(Nd1_l)-1)-(A_l/V_l)*Pd2*Nd2_l*fd2_l*exp(Nd2_l)/(exp(Nd2_l)-1)...
    -S4/CR(4) ;

k61 = (A_a/V_a)*Pn*fn_c+(A_a/V_a)*Pd1*Nd1_a*fd1_c*exp(Nd1_a)...
    /(exp(Nd1_a)-1)+(A_a/V_a)*Pd2*Nd2_a*fd2_c*exp(Nd2_a)/(exp(Nd2_a)-1) ;
k66 = -(A_a/V_a)*Pn*fn_a-(A_a/V_a)*Pd1*Nd1_a*fd1_a/...
    (exp(Nd1_a)-1)-(A_a/V_a)*Pd2*Nd2_a*fd2_a/(exp(Nd2_a)-1) ;

% CR = [0,0,0,1,1, C_a];
dCR(1) = k11*CR(1)+k12*CR(2)+k13*CR(3)+ k16*CR(6);
dCR(2) = k21*CR(1)+k22*CR(2);
dCR(3) = k31*CR(1)+k33*CR(3);
dCR(4) = S4;
dCR(5) = S5;
dCR(6) = k61*CR(1)+k66*CR(6);

dCR = [dCR(1),dCR(2),dCR(3),dCR(4),dCR(5), dCR(6)]' ;

end
```

3) Code_S3.txt

```
#---Remove extra top margin:
par(mar=c(3,3,1,1))  # Trim margin around plot [b,l,t,r]
par(tcl=0.35)  # Switch tick marks to insides of axes
par(mgp=c(1.5,0.2,0))  # Set margin lines; default c(3,1,0)
[title,labels,line]
par(xaxs="r",yaxs="r")  # Extend axis limits by 4% ("i" does no extension)
par(lwd=1)
par(mfrow=c(4,3))


## 25uM


IntraMass_exp= 0.0052
IntraMass_exp_std = 0.0015
IntraMass_expS = 0.0062
IntraMass_exp_stdS = 0.001
IntraMass_expB = 0.0020
IntraMass_exp_stdB = 0.0014
```

```
file <- "Monte25.dat"
Data <- read.table(file,header=F)


Data.IntraMass <- log(Data[1:10000,10], base=10)
Data.IntraMassS <- log(Data[10001:20000,10], base=10)
Data.IntraMassB <- log(Data[20001:30000,10], base=10)


Histo <- hist(Data.IntraMass, freq=T, breaks=c(-125:0)/25, axes=TRUE, main="",
xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_exp), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_exp+IntraMass_exp_std), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_exp-IntraMass_exp_std), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassS, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expS), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expS+IntraMass_exp_stdS), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expS-IntraMass_exp_stdS), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassB, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expB), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expB+IntraMass_exp_stdB), col="red", lty=2, lwd=3)
```

```
abline(v=log10(IntraMass_expB-IntraMass_exp_stdB), col="red", lty=2, lwd=3)

## 50uM

IntraMass_exp= 0.0108
IntraMass_exp_std = 0.0004
IntraMass_expS = 0.0170
IntraMass_exp_stdS = 0.0044
IntraMass_expB= 0.0034
IntraMass_exp_stdB = 0.0012

file <- "Monte50.dat"
Data <- read.table(file,header=F)

Data.IntraMass <- log(Data[1:10000,10], base=10)
Data.IntraMassS <- log(Data[10001:20001,10], base=10)
Data.IntraMassB <- log(Data[20001:30000,10], base=10)

Histo <- hist(Data.IntraMass, freq=T, breaks=c(-125:0)/25, axes=TRUE, main="",
xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_exp), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_exp+IntraMass_exp_std), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_exp-IntraMass_exp_std), col="red", lty=2, lwd=3)

Histo <- hist(Data.IntraMassS, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expS), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expS+IntraMass_exp_stdS), col="red", lty=2, lwd=3)
```

```
abline(v=log10(IntraMass_expS-IntraMass_exp_stdS), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassB, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expB), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expB+IntraMass_exp_stdB), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expB-IntraMass_exp_stdB), col="red", lty=2, lwd=3)


## 100uM


IntraMass_exp= 0.0153
IntraMass_exp_std = 0.0019
IntraMass_expS = 0.0216
IntraMass_exp_stdS = 0.0057
IntraMass_expB= 0.0048
IntraMass_exp_stdB = 0.0012


file <- "Monte100.dat"
Data <- read.table(file,header=F)


Data.IntraMass <- log(Data[1:10000,10], base=10)
Data.IntraMassS <- log(Data[10001:20000,10], base=10)
Data.IntraMassB <- log(Data[20001:30000,10], base=10)


Histo <- hist(Data.IntraMass, freq=T, breaks=c(-125:0)/25, axes=TRUE, main="",
xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_exp), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_exp+IntraMass_exp_std), col="red", lty=2, lwd=3)
```

```
abline(v=log10(IntraMass_exp-IntraMass_exp_std), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassS, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expS), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expS+IntraMass_exp_stdS), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expS-IntraMass_exp_stdS), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassB, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expB), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expB+IntraMass_exp_stdB), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expB-IntraMass_exp_stdB), col="red", lty=2, lwd=3)


## 200uM


IntraMass_exp= 0.0110
IntraMass_exp_std = 0.0013
IntraMass_expS = 0.0191
IntraMass_exp_stdS = 0.0047
IntraMass_expB= 0.0056
IntraMass_exp_stdB = 0.0003


file <- "Monte200.dat"
Data <- read.table(file,header=F)


Data.IntraMass <- log(Data[1:10000,10], base=10)
Data.IntraMassS <- log(Data[10001:20000,10], base=10)
```

```
Data.IntraMassB <- log(Data[20001:30000,10], base=10)


Histo <- hist(Data.IntraMass, freq=T, breaks=c(-125:0)/25, axes=TRUE, main="",
xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_exp), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_exp+IntraMass_exp_std), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_exp-IntraMass_exp_std), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassS, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expS), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expS+IntraMass_exp_stdS), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expS-IntraMass_exp_stdS), col="red", lty=2, lwd=3)


Histo <- hist(Data.IntraMassB, freq=T, breaks=c(-125:0)/25, axes=TRUE,
main="", xlim=c(-4,-1), ylim=c(0,1500), col="white")
axTicks(1)
axTicks(2)
axis(1, lwd = 4.5)
axis(2, lwd = 4.5)
lines(Histo$mids, Histo$counts, lwd=4.5, col="black")
abline(v=log10(IntraMass_expB), col="red", lty=1, lwd=3)
abline(v=log10(IntraMass_expB+IntraMass_exp_stdB), col="red", lty=2, lwd=3)
abline(v=log10(IntraMass_expB-IntraMass_exp_stdB), col="red", lty=2, lwd=3)
```

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