Differential equation system for the two-compartment model with two SNARE pairs (X, U and Y, V) and one cargo (C)

# The programm calculates the time dependent changes of
# compartment sizes, amounts of SNAREs, and amounts of cargo, starting with
# initial
# values for these variables until a steady state is reached.

# The program can be directly used for integration by the
# Gear's method, implemented in xpp by B. Ermentrout
# (http://www.pitt.edu/~phase)

# The program can be used to reproduce the results
# depicted in Fig.2a-c, and Fig. 4c.
# The results depicted in Fig. 3a are obtained by using
# the following initial conditions: xx1(0)=0.5, xx2(0)=0, uu1(0)=0.5,
# uu2(0)=0, yy1(0)=0.495,
# yy2(0)=0.005, vv1(0)=0.495, vv2(0)=0.005, nxa1(0)=0, nxb1(0)=0, nya1(0)=0,
# nya2(0)=0, nxa2(0)=0, nxb2(0)=0, nya2(0)=0, nyb2(0)=0,
# s1(0)=0.94, s2(0)=0.02, na1(0)=0.01,
# nb1(0)=0.01, na2(0)=0.01, nb2(0)=0.01

# Basic notations

# xx1, xx2, uu1, uu2: amounts of X and U SNAREs in compartments 1 and 2
# yy1, yy2, vv1, vv2: amounts of Y and V SNAREs in compartments 1 and 2
# xx, uu: total amounts of X and U SNAREs
# yy, vv: total amounts of Y and V SNAREs
# x1, x2, u1, u2: concentrations of X and U SNAREs in compartments 1 and 2
# y1, y2, v1, v2: concentrations of Y and V SNAREs in compartments 1 and 2
# cc: total amount of cargo
# cc1, cc2: total amount of cargo in compartments 1 and 2
# c1, c2: concentrations of cargo in compartments 1 and 2
# ca1, ca2, cb1, cb2: amounts of cargo in vesicles originating
# from compartments 1 and 2 with coats A and B, respectively

# s1, s2: normalized sizes of compartments 1 and 2
# ss: normalized total size of compartments
# na1, nb1, na2, nb2: normalized number of vesicles originating
# from compartments 1 and 2 with coats A and B, respectively

# nxa1, nxa2, nxb1, nxb2: amounts of X SNAREs in vesicles originating
# from compartments 1 and 2 with coats A and B, respectively
# (similar notations for U, Y, and V SNAREs)

# nxa1, nxa2, nxb1, nxb2: amounts of X SNAREs in vesicles originating
# from compartments 1 and 2 with coats A and B, respectively
# (similar notations for U, Y, and V SNAREs)

# nca1, nca2, ncb1, ncb2: amounts of cargo in vesicles originating
# from compartments 1 and 2 with coats A and B, respectively
# $k_{xa}$, $k_{xb}$, $k_{ua}$, $k_{ub}$, $k_{ya}$, $k_{yb}$, $k_{va}$, $k_{vb}$: dissociation constants for SNARE binding to coats
# $k_{ca}$, $k_{cb}$: dissociation constants for cargo binding to coats
# $\kappa$: fusion rate constant

# concentrations of SNAREs and cargo in compartments 1 and 2

\begin{align*}
x_1 &= \frac{xx_1}{s_1} \\
x_2 &= \frac{xx_2}{s_2} \\
u_1 &= \frac{uu_1}{s_1} \\
u_2 &= \frac{uu_2}{s_2} \\
y_1 &= \frac{yy_1}{s_1} \\
y_2 &= \frac{yy_2}{s_2} \\
v_1 &= \frac{vv_1}{s_1} \\
v_2 &= \frac{vv_2}{s_2} \\
c_1 &= \frac{cc_1}{s_1} \\
c_2 &= \frac{cc_2}{s_2}
\end{align*}

# saturation functions for binding of SNAREs to coats

\begin{align*}
s_{xa1} &= \frac{x_1}{k_{xa}} \left( \frac{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}}{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}} \right) \\
s_{xb1} &= \frac{x_1}{k_{xb}} \left( \frac{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}}{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}} \right) \\
s_{ua1} &= \frac{u_1}{k_{ua}} \left( \frac{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}}{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}} \right) \\
s_{ub1} &= \frac{u_1}{k_{ub}} \left( \frac{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}}{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}} \right) \\
s_{ya1} &= \frac{y_1}{k_{ya}} \left( \frac{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}}{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}} \right) \\
s_{yb1} &= \frac{y_1}{k_{yb}} \left( \frac{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}}{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}} \right) \\
s_{va1} &= \frac{v_1}{k_{va}} \left( \frac{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}}{1 + \frac{x_1}{k_{xa}} + \frac{u_1}{k_{ua}} + \frac{y_1}{k_{ya}} + \frac{v_1}{k_{va}}} \right) \\
s_{vb1} &= \frac{v_1}{k_{vb}} \left( \frac{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}}{1 + \frac{x_1}{k_{xb}} + \frac{u_1}{k_{ub}} + \frac{y_1}{k_{yb}} + \frac{v_1}{k_{vb}}} \right)
\end{align*}

# saturation functions for binding of cargo to coats

\begin{align*}
s_{ca1} &= \frac{c_1}{k_{ca}} \left( \frac{1 + \frac{c_1}{k_{ca}}}{1 + \frac{c_1}{k_{ca}}} \right) \\
s_{cb1} &= \frac{c_1}{k_{cb}} \left( \frac{1 + \frac{c_1}{k_{cb}}}{1 + \frac{c_1}{k_{cb}}} \right)
\end{align*}

# concentrations of SNAREs in vesicles

\begin{align*}
x_{a1} &= \frac{nx_{a1}}{na_{a1}}
\end{align*}
\[ x_{b1} = n_{xb1}/n_{b1} \]
\[ u_{a1} = n_{ua1}/n_{a1} \]
\[ u_{b1} = n_{ub1}/n_{b1} \]
\[ y_{a1} = n_{ya1}/n_{a1} \]
\[ y_{b1} = n_{yb1}/n_{b1} \]
\[ v_{a1} = n_{va1}/n_{a1} \]
\[ v_{b1} = n_{vb1}/n_{b1} \]
\[ x_{a2} = n_{xa2}/n_{a2} \]
\[ x_{b2} = n_{xb2}/n_{b2} \]
\[ u_{a2} = n_{ua2}/n_{a2} \]
\[ u_{b2} = n_{ub2}/n_{b2} \]
\[ y_{a2} = n_{ya2}/n_{a2} \]
\[ y_{b2} = n_{yb2}/n_{b2} \]
\[ v_{a2} = n_{va2}/n_{a2} \]
\[ v_{b2} = n_{vb2}/n_{b2} \]

# concentrations of cargo in vesicles

\[ c_{a1} = n_{ca1}/n_{a1} \]
\[ c_{b1} = n_{cb1}/n_{b1} \]
\[ c_{a2} = n_{ca2}/n_{a2} \]
\[ c_{b2} = n_{cb2}/n_{b2} \]

# fusion frequencies

\[ r_{a1} = \kappa (x_{a1}u_{1} + u_{a1}x_{1} + y_{a1}v_{1} + v_{a1}y_{1}) \]
\[ r_{b1} = \kappa (x_{b1}u_{1} + u_{b1}x_{1} + y_{b1}v_{1} + v_{b1}y_{1}) \]
\[ r_{a2} = \kappa (x_{a2}u_{2} + u_{a2}x_{2} + y_{a2}v_{2} + v_{a2}y_{2}) \]
\[ r_{b2} = \kappa (x_{b2}u_{2} + u_{b2}x_{2} + y_{b2}v_{2} + v_{b2}y_{2}) \]

\[ f_{a1} = \kappa (x_{a1}u_{2} + u_{a1}x_{2} + y_{a1}v_{2} + v_{a1}y_{2}) \]
\[ f_{b1} = \kappa (x_{b1}u_{2} + u_{b1}x_{2} + y_{b1}v_{2} + v_{b1}y_{2}) \]
\[ f_{a2} = \kappa (x_{a2}u_{1} + u_{a2}x_{1} + y_{a2}v_{1} + v_{a2}y_{1}) \]
\[ f_{b2} = \kappa (x_{b2}u_{1} + u_{b2}x_{1} + y_{b2}v_{1} + v_{b2}y_{1}) \]

# budding rates of vesicles

\[ w_{a1} = \omega_{1}a_{1} \]
\[ w_{b1} = \omega_{1}b_{1} \]
\[ w_{a2} = \omega_{2}a_{2} \]
\[ w_{b2} = \omega_{2}b_{2} \]

# backward fusion rates of vesicles

\[ r_{ra1} = r_{a1}s_{1}n_{a1} \]
\[ r_{rb1} = r_{b1}s_{1}n_{b1} \]
\[ r_{ra2} = r_{a2}s_{2}n_{a2} \]
\[ r_{rb2} = r_{b2}s_{2}n_{b2} \]

# forward fusion rates of vesicles

\[ f_{fa1} = f_{a1}s_{2}n_{a1} \]
\[ f_{fb1} = f_{b1}s_{2}n_{b1} \]
ffa2=fa2*s1*na2
ffb2=fb2*s1*nb2

# SNARE fluxes for budding
ixa1=wa1*sxa1
ixb1=wb1*sxb1
iual=wa1*sual
iub1=wb1*sub1
iya1=wa1*syal
iyb1=wb1*syb1
ival=wa1+sval
ivb1=wb1*svb1
ixa2=wa2*sxa2
ixb2=wb2*sxb2
iual=wa2*sual
iub2=wb2*sub2
iya2=wa2*syal
iyb2=wb2*syb2
ival=wa2+sval
ivb2=wb2*svb2

# SNARE fluxes for backward fusion
jxal=rra1*xa1
jxbl=rrb1*xb1
jual=rra1*uual
jub1=rrb1*ub1
jyal=rra1*yal
jybl=rrb1*yb1
jval=rra1*val
jvbl=rrb1*vbl
jxa2=rra2*xa2
jxb2=rrb2*xb2
jual=rra2*uual
jub2=rrb2*ub2
jyal=rra2*yal
jybl=rrb2*yb2
jval=rra2*val
jvbl=rrb2*vbl

# SNARE fluxes for forward fusion
mxa1=ffa1*xa1
mxbl=ffb1*xb1
mual=ffa1*uual
mub1=ffb1*ub1
\[\text{mya}_1 = f_{fa1} \times y_1 \]
\[\text{myb}_1 = f_{fb1} \times y_1 \]
\[\text{mva}_1 = f_{fa1} \times v_1 \]
\[\text{mvb}_1 = f_{fb1} \times v_1 \]
\[\text{mxa}_2 = f_{fa2} \times x_2 \]
\[\text{mxb}_2 = f_{fb2} \times x_2 \]
\[\text{mua}_2 = f_{fa2} \times u_2 \]
\[\text{mub}_2 = f_{fb2} \times u_2 \]
\[\text{mya}_2 = f_{fa2} \times y_2 \]
\[\text{myb}_2 = f_{fb2} \times y_2 \]
\[\text{mva}_2 = f_{fa2} \times v_2 \]
\[\text{mvb}_2 = f_{fb2} \times v_2 \]

# cargo fluxes for budding

\[\text{ica}_1 = w_{a1} \times s_{c1} \]
\[\text{icb}_1 = w_{a1} \times s_{cb1} \]
\[\text{ica}_2 = w_{a2} \times s_{c2} \]
\[\text{icb}_2 = w_{a2} \times s_{cb2} \]

# cargo fluxes for backward fusion

\[\text{jca}_1 = r_{ra1} \times c_1 \]
\[\text{jcb}_1 = r_{rb1} \times c_1 \]
\[\text{jca}_2 = r_{ra2} \times c_2 \]
\[\text{jcb}_2 = r_{rb2} \times c_2 \]

# cargo fluxes for forward fusion

\[\text{mca}_1 = f_{fa1} \times c_1 \]
\[\text{mcb}_1 = f_{fb1} \times c_1 \]
\[\text{mca}_2 = f_{fa2} \times c_2 \]
\[\text{mcb}_2 = f_{fb2} \times c_2 \]

# differential equations for sizes of compartments 1 and 2

\[\frac{d s_1}{d t} = -w_{a1} - w_{b1} + r_{ra1} + r_{rb1} + f_{faa} + f_{fb2} \]
\[\frac{d s_2}{d t} = -w_{a2} - w_{b2} + r_{ra2} + r_{rb2} + f_{fa1} + f_{fb1} \]

# differential equations for amounts of SNAREs in compartments 1 and 2

\[\frac{d x_{1}}{d t} = -i_{xa1} - i_{xb1} + j_{xa1} + j_{xb1} + m_{xa2} + m_{xb2} \]
\[\frac{d u_{1}}{d t} = -i_{ua1} - i_{ub1} + j_{ua1} + j_{ub1} + m_{ua2} + m_{ub2} \]
\[\frac{d y_{1}}{d t} = -i_{ya1} - i_{yb1} + j_{ya1} + j_{yb1} + m_{ya2} + m_{yb2} \]
\[\frac{d v_{1}}{d t} = -i_{va1} - i_{vb1} + j_{va1} + j_{vb1} + m_{va2} + m_{vb2} \]

\[\frac{d x_{2}}{d t} = -i_{xa2} - i_{xb2} + j_{xa2} + j_{xb2} + m_{xa2} + m_{xb2} \]
\[\frac{d u_{2}}{d t} = -i_{ua2} - i_{ub2} + j_{ua2} + j_{ub2} + m_{ua2} + m_{ub2} \]
\[\frac{d y_{2}}{d t} = -i_{ya2} - i_{yb2} + j_{ya2} + j_{yb2} + m_{ya2} + m_{yb2} \]
\[\frac{d v_{2}}{d t} = -i_{va2} - i_{vb2} + j_{va2} + j_{vb2} + m_{va2} + m_{vb2} \]

# differential equations for amounts of cargo in compartments 1 and 2
\[
\frac{d c c_1}{d t} = -i c a_1 - i c b_1 + j c a_1 + j c b_1 + m c a_2 + m c b_2 \\
\frac{d c c_2}{d t} = -i c a_2 - i c b_2 + j c a_2 + j c b_2 + m c a_1 + m c b_1 \\
\]

# differential equations for numbers of vesicles

\[
\frac{d n a_1}{d t} = w a_1 - r r a_1 - f f a_1 \\
\frac{d n b_1}{d t} = w b_1 - r r b_1 - f f b_1 \\
\frac{d n a_2}{d t} = w a_2 - r r a_2 - f f a_2 \\
\frac{d n b_2}{d t} = w b_2 - r r b_2 - f f b_2 \\
\]

# differential equations for amounts of SNAREs in vesicles

\[
\frac{d n x a_1}{d t} = i x a_1 - j x a_1 - m x a_1 \\
\frac{d n x b_1}{d t} = i x b_1 - j x b_1 - m x b_1 \\
\frac{d n u a_1}{d t} = i u a_1 - j u a_1 - m u a_1 \\
\frac{d n u b_1}{d t} = i u b_1 - j u b_1 - m u b_1 \\
\frac{d n y a_1}{d t} = i y a_1 - j y a_1 - m y a_1 \\
\frac{d n y b_1}{d t} = i y b_1 - j y b_1 - m y b_1 \\
\frac{d n v a_1}{d t} = i v a_1 - j v a_1 - m v a_1 \\
\frac{d n v b_1}{d t} = i v b_1 - j v b_1 - m v b_1 \\
\frac{d n x a_2}{d t} = i x a_2 - j x a_2 - m x a_2 \\
\frac{d n x b_2}{d t} = i x b_2 - j x b_2 - m x b_2 \\
\frac{d n u a_2}{d t} = i u a_2 - j u a_2 - m u a_2 \\
\frac{d n u b_2}{d t} = i u b_2 - j u b_2 - m u b_2 \\
\frac{d n y a_2}{d t} = i y a_2 - j y a_2 - m y a_2 \\
\frac{d n y b_2}{d t} = i y b_2 - j y b_2 - m y b_2 \\
\frac{d n v a_2}{d t} = i v a_2 - j v a_2 - m v a_2 \\
\frac{d n v b_2}{d t} = i v b_2 - j v b_2 - m v b_2 \\
\]

# differential equations for amounts of cargo in vesicles

\[
\frac{d n c a_1}{d t} = i c a_1 - j c a_1 - m c a_1 \\
\frac{d n c b_1}{d t} = i c b_1 - j c b_1 - m c b_1 \\
\frac{d n c a_2}{d t} = i c a_2 - j c a_2 - m c a_2 \\
\frac{d n c b_2}{d t} = i c b_2 - j c b_2 - m c b_2 \\
\]

\[
\text{aux } w a_1 = w a_1 \\
\text{aux } w b_1 = w b_1 \\
\text{aux } w a_2 = w a_2 \\
\text{aux } w b_2 = w b_2 \\
\text{aux } r r a_1 = r r a_1 \\
\text{aux } r r b_1 = r r b_1 \\
\text{aux } r r a_2 = r r a_2 \\
\text{aux } r r b_2 = r r b_2 \\
\text{aux } f f a_1 = f f a_1 \\
\text{aux } f f b_1 = f f b_1 \\
\text{aux } f f a_2 = f f a_2 \\
\]
aux affb2=ffb2
aux ax1=xx1/0.5
aux ax2=xx2/0.5
aux au1=uu1/0.5
aux au2=uu2/0.5
aux ay1=yy1/0.5
aux ay2=yy2/0.5
aux av1=vv1/0.5
aux av2=vv2/0.5
aux ass=s1+s2+na1+nb1+na2+nb2
aux axx=xx1+xx2+nxa1+nxb1+nxa2+nxb2
aux auu=uu1+uu2+nua1+nub1+nua2+nub2
aux ayy=yy1+yy2+nya1+nyb1+nya2+nyb2
aux avv=vv1+vv2+nva1+nvb1+nva2+nvb2
aux acc=cc1+cc2+nca1+ncb1+nca2+ncb2

#Initial conditions (can, of course, be changed)

xx1(0)=0.45
xx2(0)=0.05
uu1(0)=0.45
uu2(0)=0.05

yy1(0)=0.05
yy2(0)=0.45
vv1(0)=0.05
vv2(0)=0.45

nxa1(0)=0
nxb1(0)=0
nua1(0)=0
nub1(0)=0

nya1(0)=0
nyb1(0)=0
nva1(0)=0
nvb1(0)=0

nxa2(0)=0
nxb2(0)=0
nua2(0)=0
nub2(0)=0

nya2(0)=0
nyb2(0)=0
nva2(0)=0
nvb2(0)=0

s1(0)=0.48
s2(0)=0.48
na1(0)=0.01
nb1(0)=0.01
na2(0)=0.01
nb2(0)=0.01
cc1(0)=0.5
cc2(0)=0.5
nca1(0)=0
ncb1(0)=0
nca2(0)=0
ncb2(0)=0

#Parameters (again, can be changed)

#dissociation constants of SNAREs

param kxa=1
param kxb=100
param kua=1
param kub=100
param kya=100
param kyb=1
param kva=100
param kvb=1

#dissociation constants of cargo

param kca=1
param kcb=10

#budding rate constants

param wa=1
param wb=1

#fusion rate constant

param kappa=40

@ total=600, dt=0.2
done
Supplement 2:
Effect of inhibitory (i) SNAREs

The model in which two different SNAREs pair during fusion is extended by assuming interactions between the non-cognate SNAREs X and Y and SNAREs U and V, which reduce the fusion-competent concentrations of the SNAREs. The steady state concentrations of the SNAREs in the two compartments were calculated. Note that with increased inhibition by i-SNAREs, the gradients become steeper. The circles refer to no inhibition, the squares to $K_I = 10$, and the triangles to $K_I = 1$. Other parameters were: $w^A = w^B = 1$, $\kappa = 40$, $K_x^A = K_y^A = K_y^B = K_y^V = 1$, $K_x^B = K_y^U = K_x^A = K_y^V = 1$.

Amounts of SNAREs are normalized with respect to their total amounts $X = Y = U = V = 0.5$.

Mathematical details:
The existence of i-SNAREs reduces the concentrations of SNAREs in the compartments that are available for fusion. The following formulas are used:

\[
\begin{align*}
X_{i,\text{eff}} &= \frac{X_i}{1 + \frac{Y_i}{K_I}}, \\
Y_{i,\text{eff}} &= \frac{Y_i}{1 + \frac{X_i}{K_I}} \\
U_{i,\text{eff}} &= \frac{U_i}{1 + \frac{V_i}{K_I}}, \\
V_{i,\text{eff}} &= \frac{V_i}{1 + \frac{U_i}{K_I}}
\end{align*}
\]

(S2.1)

where $K_I$ is an inhibition constant.
Compartment 1

\[ Y_1 \]

\[ V_1 \]

amount of SNAREs in compartments

Compartment 2

\[ X_1 \]

\[ U_1 \]

\[ Y_2 \]

\[ V_2 \]

no inhibition

no inhibition

amount of SNAREs in compartments

Compartment 1

Compartment 2
Supplement 3:
Cargo distribution and recycling with net flux through the two compartments

The simple two-compartment system was extended by assuming net flux through the system, such that cargo enters into compartment 1 and exits from compartment 2. The steady state concentration of cargo and the fraction that recycles from compartment 2 back to compartment 1 was calculated for different dissociation constants of cargo for the coats. The following parameters were used: \( J_{\text{input}} = 0.04 \), \( k_{\text{output}} = 0.05 \), \( K^A_X = 1 \), \( K^B_X = 10 \), \( K^A_Y = 10 \), \( K^B_Y = 1 \), \( w^A = w^B = 1.0 \), \( \kappa = 4 \), \( X = U = Y = V = 0.5 \).

**Mathematical details:**
Output flux (from compartment 2) is defined as \( J_{\text{output}} = k_{\text{output}}[\text{cargo}]_2 \) (\([\text{cargo}]_2\) is the concentration of cargo in compartment 2). The cycling rate is defined as follows:

\[
\rho = \frac{F^A_{2,\text{cargo}} + F^B_{2,\text{cargo}}}{J_{\text{input}} + F^A_{2,\text{cargo}} + F^B_{2,\text{cargo}}} \quad (S3.1)
\]

\( F^A_{2,\text{cargo}}, F^B_{2,\text{cargo}} \) are net fluxes of cargo from compartment 2 back into compartment 1 with coat A and B, respectively.

<table>
<thead>
<tr>
<th>Dissociation constants of cargo for vesicle coats</th>
<th>Cargo in Compartment 1</th>
<th>Cargo in Compartment 2</th>
<th>Fraction of cargo cycled ( \rho )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^A_{\text{cargo}} = 10, \ K^B_{\text{cargo}} = 10 )</td>
<td>2.2</td>
<td>0.8</td>
<td>0.51</td>
</tr>
<tr>
<td>( K^A_{\text{cargo}} = 2.5, \ K^B_{\text{cargo}} = 10 )</td>
<td>7.6</td>
<td>0.8</td>
<td>0.70</td>
</tr>
<tr>
<td>( K^A_{\text{cargo}} = 10, \ K^B_{\text{cargo}} = 1.0 )</td>
<td>0.1</td>
<td>0.8</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Supplement 4:
Two-compartment model with inhibition of back-fusion

The two-compartment system containing two pairs of SNAREs (X, U and Y, V) and two cargo proteins (cargo 1 and cargo 2), which stimulate the budding of vesicles (cargo 1 stimulates budding of coat B vesicles, and cargo 2 stimulates budding of coat A vesicles; see Fig. 4c) was extended to include two additional cargo proteins (inh 1 and inh 2). These inhibit the fusion of vesicles (inh 1 inhibits the fusion of coat B vesicles, and inh 2 inhibits the fusion of coat A vesicles). The steady-state concentrations of cargos and the vesicle fluxes (normalized so that the sum of forward fluxes equals unity) were calculated for two different values of the inhibition constant (a, weak inhibition, b, strong inhibition). The distribution of the SNAREs and the compartment sizes are approximately as in Fig. 4c. Note that both back and forward fusion are assumed to be inhibited, consistent with our basic assumption that the compartments are not a priori different. The preferential effect of inh 1 and inh 2 on back fusion comes from their accumulation in different compartments. The following other parameter values were used:

\[ w^A = w^B = 0.2, \quad \kappa = 4C, \quad K^A_X = 1, \quad K^A_U = 1, \quad K^B_X = 10C, \quad K^B_U = 10C, \quad K^A_Y = 10C, \quad K^A_V = 10C, \quad K^B_Y = 1, \quad K^B_V = 1, \quad K^A_{\text{cargo}1} = 1, \quad K^B_{\text{cargo}1} = 1C, \quad K^A_{\text{cargo}2} = 10, \quad K^B_{\text{cargo}2} = 1, \quad K^A_{\text{inh}1} = 1, \quad K^B_{\text{inh}1} = 10C, \quad K^A_{\text{inh}2} = 1, \quad K^B_{\text{inh}2} = 1, \quad X = U = Y = V = 0.5, \]

\[ [\text{cargo}1] = [\text{cargo}2] = [\text{inh}1] = [\text{inh}2] = 1. \]

Mathematical details:
The following equations are used for budding

\[ W^A_1 = w^A[\text{cargo}2]_1 S_1, \quad W^B_1 = w^B[\text{cargo}1]_1 S_1 \quad (S4.1) \]

\[ W^A_2 = w^A[\text{cargo}2]_2 S_2, \quad W^B_2 = w^B[\text{cargo}1]_2 S_2 \quad (S4.2) \]
where \([\text{cargo}d]_1\) and \([\text{cargo}d]_2\) are the concentrations of cargo 1 in compartments 1 and 2, respectively, and \([\text{cargo}2]_1\) and \([\text{cargo}2]_2\) the concentrations of cargo 2 in compartments 1 and 2, respectively.

For fusion, the rate constant \(\kappa\) is replaced by:
\[
\frac{\kappa}{1 + [\text{inh}2]_1/K_{inh}} \text{ for back and forward fusion of coat A vesicles with compartment 1}
\]
\[
\frac{\kappa}{1 + [\text{inh}1]_1/K_{inh}} \text{ for back and forward fusion of coat B vesicles with compartment 1}
\]
\[
\frac{\kappa}{1 + [\text{inh}2]_2/K_{inh}} \text{ for back and forward fusion of coat A vesicles with compartment 2}
\]
\[
\frac{\kappa}{1 + [\text{inh}1]_2/K_{inh}} \text{ for back and forward fusion of coat B vesicles with compartment 2}
\]

where \([\text{inh}1]_1\) and \([\text{inh}1]_2\) are the concentrations of inh 1 in compartments 1 and 2, respectively, and \([\text{inh}2]_1\) and \([\text{inh}2]_2\) the concentrations of inh 2 in compartments 1 and 2, respectively.
$K_{inh} = 5$

$F_2^A = 0.999$

$W_2^A = 1606$

$R_2^A = 0.607$

$F_2^B = 0.001$

$W_2^B = 0.161$

$R_2^B = 0.160$

$W_1^A = 0.161$

$F_1^A = 0.001$

$F_1^B = 0.999$

$R_1^A = 0.160$

$R_1^B = 0.607$

$[inh 1] = 3.06$

$[inh 2] = 0.03$

$[cargo 1] = 2.63$

$[cargo 2] = 0.26$

$K_{inh} = 1$

$F_2^A = 0.9995$

$W_2^A = 12310$

$R_2^A = 0.2315$

$F_2^B = 0.0005$

$W_2^B = 0.1230$

$R_2^B = 0.1225$

$W_1^A = 0.1230$

$F_1^A = 0.0005$

$F_1^B = 0.9995$

$R_1^A = 0.1225$

$R_1^B = 0.2315$

$[inh 1] = 3.44$

$[inh 2] = 0.03$

$[cargo 1] = 2.89$

$[cargo 2] = 0.29$
Supplement 5:
Numerical calculations with a linear three-compartment model

a. The linear three-compartment model shown in Fig. 5 was used to calculate the steady state distribution of SNAREs and cargo proteins. The system is not symmetrical (i.e. compartment 2 differs from compartments 1 and 3) and the steady state depends on the initial conditions. Fig. 5 and Table S5a correspond to a situation in which each compartment accumulates one SNARE and one cargo protein. The calculations were performed with the following dissociation constants for the SNAREs:

\[ K^A_X = 1, \ K^B_U = 10, \ K^C_U = 10 \]
\[ K^A_Y = 10, \ K^B_V = 1, \ K^C_V = 10, \ K^B_C = 10, \ K^A_P = 10 \]
\[ K^B_C = 10, \ K^A_Q = 1, \ K^B_Q = 1, \ K^B_A = 1 \]

And for the cargos:

\[ K^A_{cargo1} = 1, \ K^B_{cargo2} = 10, \ K^C_{cargo3} = 1 \]
\[ K^B_{cargo3} = 10, \ K^C_{cargo3} = 1 \]

b. As in a, but with the initial conditions:

\[ X_1 = U_1 = Y_2 = V_2 = P_3 = Q_3 = 0.4, \]
\[ X_2 = U_2 = X_3 = U_3 = Y_1 = V_1 = Y_3 = V_2 = P_1 = Q_1 = P_2 = Q_2 = 0.05 \]

i.e. each compartment had already a high amount of its preferred SNARE, and [cargo1] = [cargo2] = [cargo3] = 0.5.

The calculated stationary compartment sizes were:

\[ S_1 = 0.217, \ S_2 = 0.227, \ S_3 = 0.217 \]

This stationary state is also attained when essentially all Y and V SNAREs are initially present in compartment 1 and the size \[ S_2 \] of compartment 2 (Golgi) is initially very small, corresponding to the modeling of a BFA wash-out experiment (not shown). b. As in a, but with the initial conditions:

\[ X_1 = U_1 = 0.45, \ X_2 = U_2 = 0.02, \ X_3 = U_3 = 0.02, \ Y_1 = V_1 = 0.4, \]
\[ Y_2 = V_2 = 0.05, \ Y_3 = V_3 = 0.05, \ P_1 = Q_1 = 0.3, \ P_2 = Q_2 = 0.07, \ P_3 = Q_3 = 0.07 \]

(all three SNAREs have high initial levels in compartment 1). The calculated stationary compartment sizes are:

\[ S_1 = 0.49, \ S_2 = 0, \ S_3 = 0.151 \]

i.e. compartment 2 disappears. The SNARE amounts in Tables S5a and S5b were normalized relative to their total amounts.
### a

<table>
<thead>
<tr>
<th>SNAREs/Cargo</th>
<th>Compartment 1</th>
<th>Compartment 2</th>
<th>Compartment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>X, U</td>
<td>0.942</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>Y, V</td>
<td>0.015</td>
<td>0.943</td>
<td>0.015</td>
</tr>
<tr>
<td>P, Q</td>
<td>0.008</td>
<td>0.015</td>
<td>0.942</td>
</tr>
<tr>
<td>Cargo 1</td>
<td>1.057</td>
<td>0.125</td>
<td>0.097</td>
</tr>
<tr>
<td>Cargo 2</td>
<td>0.124</td>
<td>1.089</td>
<td>0.124</td>
</tr>
<tr>
<td>Cargo 3</td>
<td>0.097</td>
<td>0.125</td>
<td>1.057</td>
</tr>
</tbody>
</table>

### b

<table>
<thead>
<tr>
<th>SNAREs/Cargo</th>
<th>Compartment 1</th>
<th>Compartment 2</th>
<th>Compartment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>X, U</td>
<td>0.959</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>Y, V</td>
<td>0.959</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>P, Q</td>
<td>0.020</td>
<td>0</td>
<td>0.939</td>
</tr>
<tr>
<td>Cargo 1</td>
<td>1.268</td>
<td>0</td>
<td>0.054</td>
</tr>
<tr>
<td>Cargo 2</td>
<td>1.268</td>
<td>0</td>
<td>0.054</td>
</tr>
<tr>
<td>Cargo 3</td>
<td>0.326</td>
<td>0</td>
<td>0.840</td>
</tr>
</tbody>
</table>