Supplementary Information

1. Model Equations

The full mathematical models are described below. Note that the encoding of models in COPASI and SBML format are also included as supplementary data files.

Model I

d ([CuCyt] · V _{vcyto})	ν νCcoA·[CuPP] γ
d <i>t</i>	$= + \nu_{\text{Vpp}} \cdot \frac{1}{(\text{KmCcoA} + [\text{CuPP}])}$
	- / ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·/ ·
	^v _{Vcyto} (KmCopA1 + [CuCyt])
	$-V_{Vcyto} \cdot \left(\frac{VCusSystem \cdot [CuCyt]}{KmCusSystem + [CuCyt]}\right)$
	- V _{Vort} o ·(kasCuCytCP ·[CuCyt] ·[CytCP])
	$+V_{v_{r}}$ (kdsCuCytCP · [CuCytCP])
$\frac{d(t_{\text{out}}, t_{\text{out}}, t_{\text{out}}, t_{\text{out}})}{dt}$	$= + V_{\text{vpp}} \cdot \left(\frac{\text{vOprC} \cdot \text{cuExt0}}{\text{KmOprC} + \text{cuExt0}} \right)$
	- <i>V</i> _{vpp} ·(kasCuPPCP·[CuPP]·[PPCP])
	vCcoA·[CuPP]
	^V Vpp (KmCcoA + [CuPP])
	+ $V_{v_{cyto}} \cdot \left(\frac{v_{CopA1} \cdot [CuCyt]}{KmCopA1 + [CuCyt]} \right)$
	vPcoB·[CuPP]
	(KmPcoB + [CuPP])
	+ V _{vpp} · (kdsCuPPCP · [CuPPCP])
d ([CuCytCP] · V _{Vcyto})	
d <i>t</i>	$= + V_{V_{cyto}} \cdot (kasCuCytCP \cdot [CuCyt] \cdot [CytCP])$
	- V _{Vcvto} · (kdsCuCytCP · [CuCytCP])
d([CuPPCP]·V _{Vpp})	
d <i>t</i>	$= + V_{Vpp} \cdot (kasCuPPCP \cdot [CuPP] \cdot [PPCP])$
	- V _{Vpp} · (kdsCuPPCP · [CuPPCP])
d([PPCP]·V _{Vpp})	
d <i>t</i>	
	+ V _{Vpp} · (kdsCuPPCP · [CuPPCP])
d([CytCP] · V _{Vcyto})	
d <i>t</i>	- V _{Vcyto} (Nascucyter [cucyt] [cyter])
	+ V _{vcyto} · (kdsCuCytCP · [CuCytCP])
"Total_Cu (attomol/cell)	" = "TotalCu_PP (attomol/cell)" + "TotalCu_Cyt (attomol/cell)"
"TotalCu_Cyt (attomol/cell)	<pre>" = ([CuCyt] + [CuCytCP]) · Compartments[Vcyto].InitialVolume · 1e18</pre>
"TotalCu_PP (attomol/cell)	<pre>" = ([CuPP]+[CuPPCP]).Compartments[Vpp].InitialVolume.1e18</pre>
TotalCu con	$c = ([CuCyt] + [CuCytCP]) \cdot Compartments[Vcyto].InitialVolume + ([CuPP] + [CuPPCP]) \cdot Compartments[Vpp].InitialVolume + ([CuPP] + [CuPPCP]) \cdot Compartments[Vpp] + ([CuPPCP]) \cdot$
	Compartments[Vcyto].InitialVolume + Compartments[Vpp].InitialVolume

Model II

d ([CuCyt] · V _{vcvto})	v vCcoA·[CuPP] v
d <i>t</i>	$= + V_{\text{vpp}} \cdot \left(\frac{1}{(\text{KmCcoA} + [\text{CuPP}])} \right)$
	$-V_{v_{cyto}} \cdot \left(\frac{v_{cyto}}{KmCopA1 + [CuCyt]} \right)$
	- V _{vcyto} · (<u>vCusSystem · [CuCyt]</u>) (<u>KmCusSystem + [CuCyt]</u>)
	- V _{vcyto} ·(kasCuCytCP · [CuCyt] · [CytCP])
	+V _{Vcyto} ·(kdsCuCytCP·[CuCytCP])
	$-V_{V_{cyto}} \cdot \left(\frac{V_{CopA2} \cdot [CuCyt]}{KmCopA2 + [CuCyt]} \right)$
$\frac{d([CuPP] \cdot V_{Vpp})}{d t}$	$= + V_{\text{Vpp}} \cdot \left(\frac{\text{vOprC} \cdot \text{CuExt0}}{\text{KmOprC} + \text{CuExt0}} \right)$
	- V _{Vpp} ·(kasCuPPCP ·[CuPP] ·[PPCP])
	$-V_{Vpp} \cdot \left(\frac{VCcoA \cdot [CuPP]}{KmCcoA + [CuPP]}\right)$
	$+ V_{Vcyto} \cdot \left(\frac{VCopA1 \cdot [CuCyt]}{KmCopA1 + [CuCyt]} \right)$
	$-V_{\text{Vpp}} \cdot \left(\frac{\text{VPcoB} \cdot [\text{CuPP}]}{(\text{KmPcoB} + [\text{CuPP}])}\right)$
	+V _{vpp} ·(kdsCuPPCP ·[CuPPCP])
	+ V_{Vcyto} · $\left(\frac{vCopA2 \cdot [CuCyt]}{KmCopA2 + [CuCyt]}\right)$
$\frac{d([CuCytCP] \cdot V_{Vcyto})}{d t}$	= + <i>V</i> _{Vcyto} ·(kasCuCytCP·[CuCyt]·[CytCP])
	- V _{Vcvto} ·(kdsCuCytCP ·[CuCytCP])
$\frac{d([CuPPCP] \cdot V_{Vpp})}{dt}$	= $+V_{\text{vpp}}$ (kasCuPPCP · [CuPP] · [PPCP])
	- V _{von} ·(kdsCuPPCP ·[CuPPCP])
$\frac{d([PPCP] \cdot V_{Vpp})}{dt}$	= - V _{vpp} · (kasCuPPCP · [CuPP] · [PPCP])
	+ <i>V</i> _{Vnn} ·(kdsCuPPCP·[CuPPCP])
$\frac{d([CytCP] \cdot V_{V_{cyto}})}{d t}$	= -V _{Vcyto} ·(kasCuCytCP · [CuCyt] · [CytCP])
	+V _{Vcyto} · (kdsCuCytCP · [CuCytCP])
"Total_Cu (attomol/cell)	" = "TotalCu_PP (attomol/cell)" + "TotalCu_Cyt (attomol/cell)"
"TotalCu_Cyt (attomol/cell)	" = ([CuCyt]+[CuCytCP])·Compartments[Vcyto].InitialVolume·1e18
"TotalCu_PP (attomol/cell)	" = ([CuPP]+[CuPPCP])·Compartments[Vpp].InitialVolume·1e18
TotalCu_con	$c = ([CuCyt] + [CuCytCP]) \cdot Compartments[Vcyto].InitialVolume + ([CuPP] + [CuPPCP]) \cdot Compartments[Vpp].InitialVolume + ([CuPP] + [CuPPCP]) \cdot Compartments[Vpp] + ([CuPP] + [CuPPCP]) \cdot Compartmen$
	Compartments[vcyto].Initialvolume + Compartments[Vpp].InitialVolume

Model III

d([CuCyt]·V _{Vcyto})	$= + V_{\text{Vers}} \cdot \left(\frac{\text{vCcoA} \cdot [\text{CuPP}]}{\frac{1}{2}} \right)$
d <i>t</i>	(KmCcoA + [CuPP])
	$-V_{\text{Vcyto}} \cdot \left(\frac{V \text{CopA1} \cdot [\text{CuCyt}]}{\text{KmCopA1} + [\text{CuCyt}]} \right)$
	$-V_{V_{Cyto}} \cdot \left(\frac{V_{CusSystem} \cdot [CuCyt]}{K_{m}CusSystem + [CuCyt]} \right)$
	- V _{vovo} · (kasCuCytCP · [CuCyt] · [CytCP])
	+ V _{Vcyto} · (kdsCuCytCP · [CuCytCP])
$\frac{d([CuPP] \cdot V_{Vpp})}{d t}$	$= + V_{\text{Vpp}} \cdot \left(\frac{\text{vOprC} \cdot \text{CuExt0}}{\text{KmOprC} + \text{CuExt0}} \right)$
	- V _{VDD} · (kasCuPPCP · [CuPP] · [PPCP])
	+ V _{Vpp} · (kdPPCP · [CuPPCP])
	$-V_{\text{Vpp}} \cdot \left(\frac{\text{vCcoA} \cdot [\text{CuPP}]}{\text{KmCcoA} + [\text{CuPP}]}\right)$
	$+ V_{v_{cyto}} \cdot \left(\frac{v_{CopA1} \cdot [CuCyt]}{KmCopA1 + [CuCyt]} \right)$
	$-V_{Vpp} \cdot \left(\frac{VPcoB \cdot [CuPP]}{KmPcoB + [CuPP]} \right)$
	+ V _{Vvn} · (kdsCuPPCP · [CuPPCP])
$\frac{d([CuCytCP] \cdot V_{Vcyto})}{d t}$	= +V _{Vcyto} ·(kasCuCytCP · [CuCyt] · [CytCP])
	- <i>V</i> _{Vcyto} ·(kdsCuCytCP·[CuCytCP])
$\frac{d([CuPPCP] \cdot V_{Vpp})}{d t}$	= $+ V_{Vpp} \cdot (kasCuPPCP \cdot [CuPP] \cdot [PPCP])$
	- V _{Vpp} ·(kdPPCP·[CuPPCP])
	-V _{Vnn} ·(kdsCuPPCP ·[CuPPCP])
$\frac{d([PPCP] \cdot V_{Vpp})}{dt}$	= -V _{vpp} · (kasCuPPCP · [CuPP] · [PPCP])
	- V _{Von} •(kdPPCP•[PPCP])
	+ V _{Vpp} · (kdsCuPPCP · [CuPPCP])
	/ V _{vpp} ·ksPPCP·[CuCyt] ^{hCuCytPPCP}
	+ $\left(\frac{1}{KmCuCytPPCP^{hCuCytPPCP} + [CuCyt]^{hCucytPPCP}}\right)$
$\frac{d([CytCP] \cdot V_{V_{Cyto}})}{d t}$	= -V _{Vcyto} ·(kasCuCytCP · [CuCyt] · [CytCP])
	+ V _{Vcyto} ·(kdsCuCytCP ·[CuCytCP])
"Total_Cu (attomol/cell)"	= "TotalCu_PP (attomol/cell)" + "TotalCu_Cyt (attomol/cell)"
"TotalCu_Cyt (attomol/cell)"	= ([CuCyt]+[CuCytCP]) Compartments[Vcyto].InitialVolume 1e18
"TotalCu_PP (attomol/cell)"	= ([CuPP] + [CuPPCP]) · Compartments[Vpp].InitialVolume · 1e18
TotalCu_conc	$= ([CuCyt] + [CuCytCP]) \cdot Compartments[Vcyto].InitialVolume + ([CuPP] + [CuPPCP]) \cdot Compartments[Vpp].InitialVolume + ([CuPPCP]) \cdot Compartments[Vpp].InitialVolume$
	Compartments) veyto j. initiarvolume + Compartments) vpp j. initiarvolume

2. Indentifiability analysis

Identifiability analysis was carried out for each of the models using profile likelihood analysis (Raue et al., 2009) using a previously described procedure (Schaber, 2012).

Briefly, hold each estimated parameter at a constant value around the best estimate (here we used ± 1000 fold) and estimate the rest of parameters by parameter estimation. The likelihood ratio is calculated using the following formula:

$$C_{LR} = SSR(p) \cdot \exp\left(\frac{\chi_{\alpha}^2}{n}\right)$$

Where, χ_{α}^2 is the Chi-squared distribution and *n* is the number of data points. For Models I and II, *n* is 32 and for Model III it is 48.

Horizontal lines represent the level for 90% confidence intervals (solid line for identifiability of all parameters simultaneously, dashed line for identifiability of that parameter alone).

Figure S1. Profile likelihood for Model I.



Copper Model-I profile likelihood

Figure S2. Profile likelihood for Model II.



Copper Model-II profile likelihood

Figure S3. Profile likelihood for Model III.



Copper Model-III profile likelihood