# 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

$$\frac{d([CuCyt]\cdot V_{Vcyto})}{dt} = +V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) - V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vcyto}\cdot\left(\frac{vCusSystem\cdot[CuCyt]}{KmCusSystem+[CuCyt]}\right) - V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\frac{d([CuPP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot\left(\frac{vOprC\cdot CuExt0}{KmOprC+CuExt0}\right) - V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) + V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vpp}\cdot\left(\frac{vPcoB\cdot[CuPP]}{KmPcoB+[CuPP]}\right) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([CuCytCP]\cdot V_{Vcyto})}{dt} = +V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) - V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\frac{d([CuPPCP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([PPCP]\cdot V_{Vpp})}{dt} = -V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([CytCP]\cdot V_{Vcyto})}{dt} = -V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\text{"Total\_Cu (attomol/cell)"} = \text{"TotalCu\_PP (attomol/cell)"} + \text{"TotalCu\_Cyt (attomol/cell)"}$$

$$\text{"TotalCu\_Cyt (attomol/cell)"} = ([CuCyt]+[CuCytCP])\cdot Compartments[Vcyto].InitialVolume\cdot 1e18$$

$$\text{"TotalCu\_PP (attomol/cell)"} = ([CuPP]+[CuPPCP])\cdot Compartments[Vpp].InitialVolume\cdot 1e18$$

$$TotalCu\_conc = \frac{([CuCyt]+[CuCytCP])\cdot Compartments[Vcyto].InitialVolume + ([CuPP]+[CuPPCP])\cdot Compartments[Vpp].InitialVolume}{Compartments[Vcyto].InitialVolume + Compartments[Vpp].InitialVolume}$$

## Model II

$$\frac{d([CuCyt]\cdot V_{Vcyto})}{dt} = +V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) - V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vcyto}\cdot\left(\frac{vCusSystem\cdot[CuCyt]}{KmCusSystem+[CuCyt]}\right) - V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP]) - V_{Vcyto}\cdot\left(\frac{vCopA2\cdot[CuCyt]}{KmCopA2+[CuCyt]}\right)$$

$$\frac{d([CuPP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot\left(\frac{vOprC\cdot CuExt0}{KmOprC+CuExt0}\right) - V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) + V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vpp}\cdot\left(\frac{vPcoB\cdot[CuPP]}{KmPcoB+[CuPP]}\right) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP]) + V_{Vcyto}\cdot\left(\frac{vCopA2\cdot[CuCyt]}{KmCopA2+[CuCyt]}\right)$$

$$\frac{d([CuCytCP]\cdot V_{Vcyto})}{dt} = +V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) - V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\frac{d([CuPPCP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([PPCP]\cdot V_{Vpp})}{dt} = -V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([CytCP]\cdot V_{Vcyto})}{dt} = -V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\text{"Total\_Cu (attomol/cell)"} = \text{"TotalCu\_PP (attomol/cell)"} + \text{"TotalCu\_Cyt (attomol/cell)"}$$

$$\text{"TotalCu\_Cyt (attomol/cell)"} = ([CuCyt]+[CuCytCP])\cdot Compartments[Vcyto].InitialVolume\cdot 1e18$$

$$\text{"TotalCu\_PP (attomol/cell)"} = ([CuPP]+[CuPPCP])\cdot Compartments[Vpp].InitialVolume\cdot 1e18$$

$$TotalCu\_conc = \frac{([CuCyt]+[CuCytCP])\cdot Compartments[Vcyto].InitialVolume + ([CuPP]+[CuPPCP])\cdot Compartments[Vpp].InitialVolume}{Compartments[Vcyto].InitialVolume + Compartments[Vpp].InitialVolume}$$

## Model III

$$\frac{d([CuCyt]\cdot V_{Vcyto})}{dt} = +V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) - V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vcyto}\cdot\left(\frac{vCusSystem\cdot[CuCyt]}{KmCusSystem+[CuCyt]}\right) - V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\frac{d([CuPP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot\left(\frac{vOprC\cdot CuExt0}{KmOprC+CuExt0}\right) - V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) + V_{Vpp}\cdot(kdPPCP\cdot[CuPPCP]) - V_{Vpp}\cdot\left(\frac{vCcoA\cdot[CuPP]}{KmCcoA+[CuPP]}\right) + V_{Vcyto}\cdot\left(\frac{vCopA1\cdot[CuCyt]}{KmCopA1+[CuCyt]}\right) - V_{Vpp}\cdot\left(\frac{vPcoB\cdot[CuPP]}{KmPcoB+[CuPP]}\right) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([CuCytCP]\cdot V_{Vcyto})}{dt} = +V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) - V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

$$\frac{d([CuPPCP]\cdot V_{Vpp})}{dt} = +V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot(kdPPCP\cdot[CuPPCP]) - V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP])$$

$$\frac{d([PPCP]\cdot V_{Vpp})}{dt} = -V_{Vpp}\cdot(kasCuPPCP\cdot[CuPP]\cdot[PPCP]) - V_{Vpp}\cdot(kdPPCP\cdot[PPCP]) + V_{Vpp}\cdot(kdsCuPPCP\cdot[CuPPCP]) + \left(\frac{V_{Vpp}\cdot ksPPCP\cdot[CuCyt]^{hCuCytPPCP}}{KmCuCytPPCP^{hCuCytPPCP}+[CuCyt]^{hCuCytPPCP}}\right)$$

$$\frac{d([CytCP]\cdot V_{Vcyto})}{dt} = -V_{Vcyto}\cdot(kasCuCytCP\cdot[CuCyt]\cdot[CytCP]) + V_{Vcyto}\cdot(kdsCuCytCP\cdot[CuCytCP])$$

"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

$$TotalCu\_conc = \frac{([CuCyt]+[CuCytCP])\cdot Compartments[Vcyto].InitialVolume + ([CuPP]+[CuPPCP])\cdot Compartments[Vpp].InitialVolume}{Compartments[Vcyto].InitialVolume + Compartments[Vpp].InitialVolume}$$

## 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, $\chi_\alpha^2$ 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.**

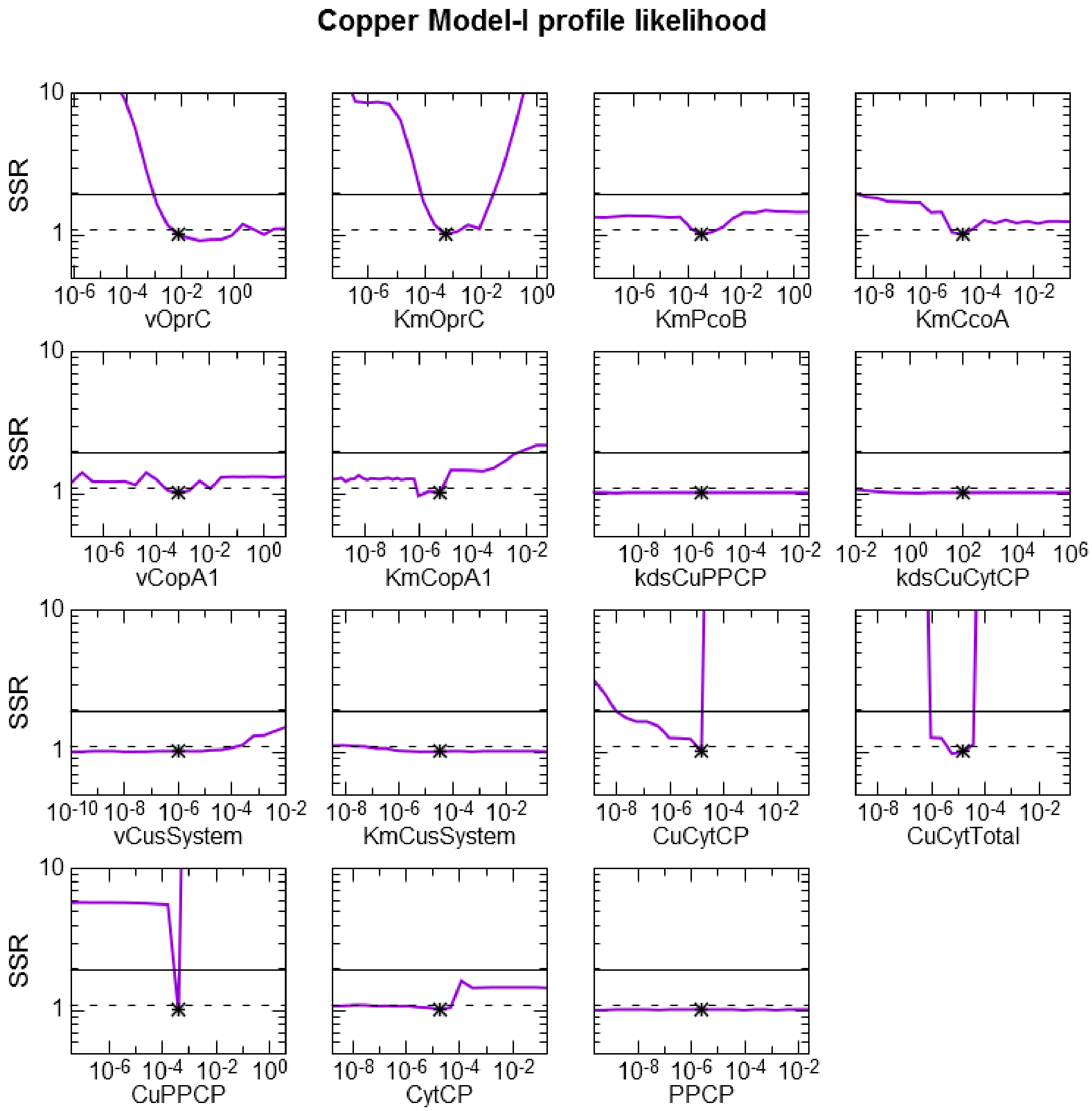

**Figure S2. Profile likelihood for Model II.**

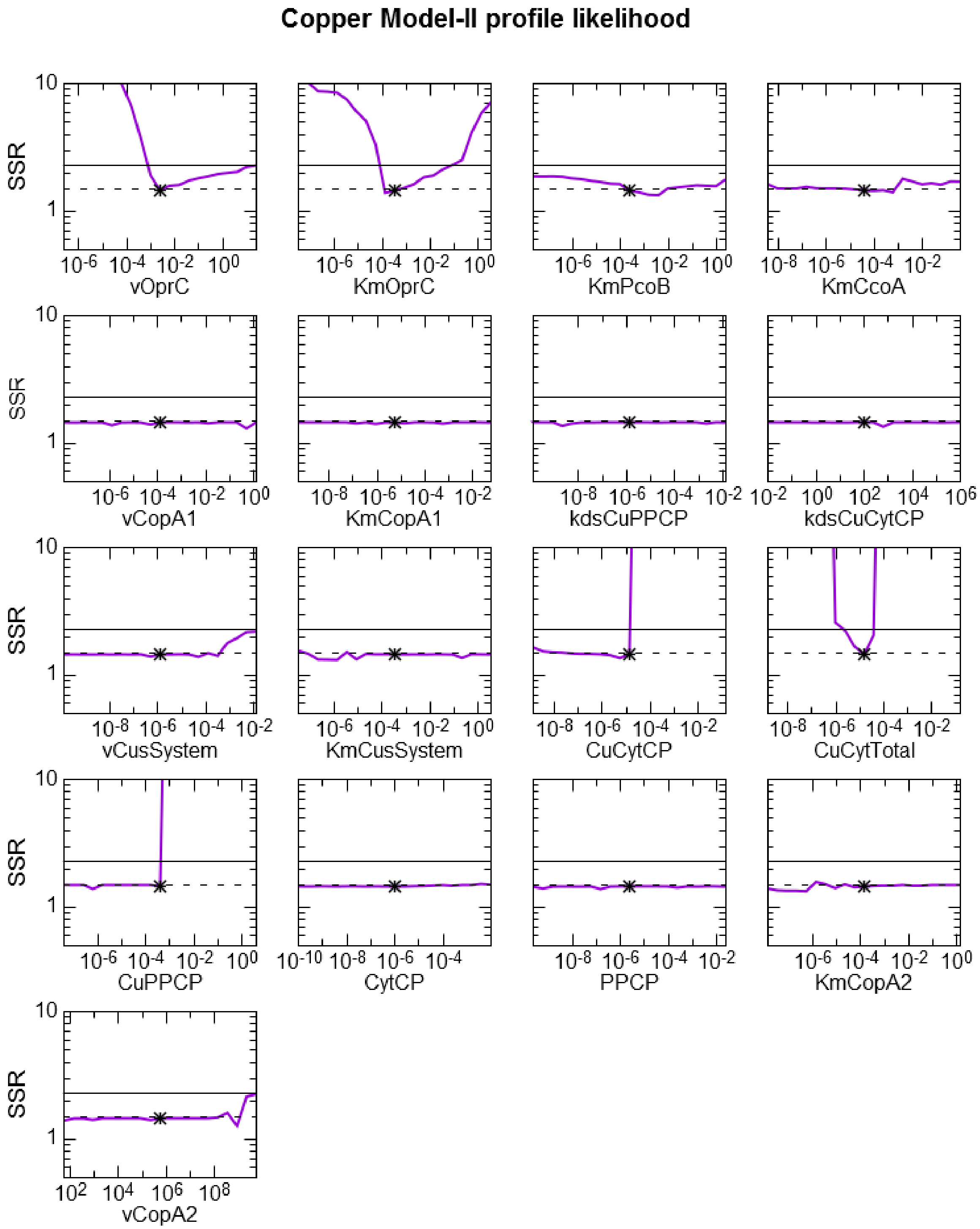

**Figure S3. Profile likelihood for Model III.**