A Mathematical Model for Copper Homeostasis in *Pseudomonas aeruginosa*

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Abstract

Copper is an ideal biological redox cofactor because it accepts and donates electrons with relative ease. However, free copper can interfere with other important cellular redox reactions. Therefore, bacteria maintain tight control of cellular copper levels. In collaboration with the Argüello group at Worcester Polytechnic Institute, we obtained data measuring copper levels across the periplasmic and cytoplasmic compartments of *P. aeruginosa*. Using this data, we modeled copper homeostasis as a system of chemical reactions transferring copper between protein pools according to mass action kinetics. Mathematically, this is a series of ordinary differential equations describing the fluxes between different copper pools. We used the biochemical modeling software COPASI to simulate and estimate parameters for the model. During the modeling process we determined that knowledge of the protein levels is required to accurately estimate the model parameters. Additionally, it seems necessary to invoke regulation of periplasmic cuproproteins to fit a model to the data. These results will help our collaborators design experiments that produce the most crucial information for developing a more detailed model of the copper homeostasis system in *P. aeruginosa*.

Pseudomonas aeruginosa

Gram-negative bacteria:
- Two membranes
- Two cellular compartments

Opportunistic pathogen: [5]
- Causes ~51,000 cases of healthcare associated infection annually in the U.S.

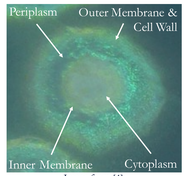


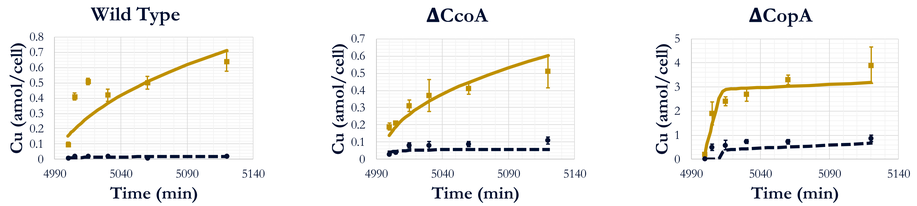
Image from [4]

Copper

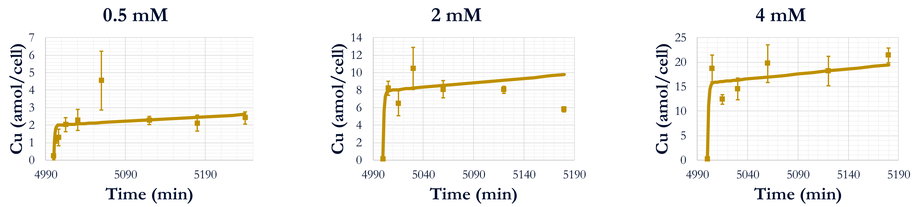
- Bioavailable as Cu^{2+} (oxidized) and Cu^{+} (reduced) [2]
- Easily converts between oxidation states by accepting and donating electrons [2]
- Essential redox cofactor for many cellular processes (ex: cytochrome oxidases in electron transport chain) [2]
- Free copper can be toxic to the cell by participating in harmful redox reactions [3]
- Copper overload can have antimicrobial effects [3]

Results

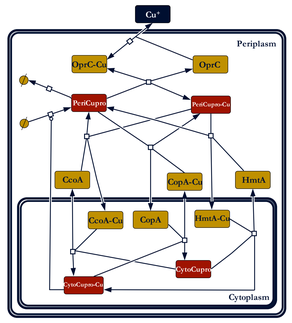
Experiment 1: *P. aeruginosa* mutants under 0.5 mM external copper concentration

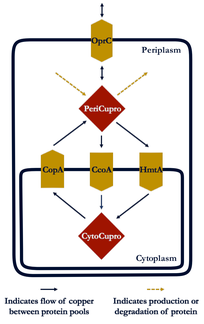


Experiment 2: Wild type *P. aeruginosa* under varying external copper concentrations



Model





Mass action kinetics:
- The rate of a chemical reaction is proportional to the product of the concentrations of the reactants [6]

Model equations:
- The change in the concentration of each protein species is given by an ODE equal to the sum of the rates of the reactions it participates in. For example, for HmtA the ODE is:

$$\frac{d([HmtA]\cdot V_P)}{dt} = k_1\cdot[HmtA-Cu]\cdot[CytoCupro]\cdot V_C - k_2\cdot[PeriCupro-Cu]\cdot[HmtA]\cdot V_P$$

Methods

The biochemical modeling software COPASI [1] was used to implement and analyze our model.

Parameter estimation:
- Protein concentrations and kinetic parameters unknown
- Sequentially generate parameter sets using optimization algorithms and evaluate error between model output and experimental data using the objective function

Algorithms for parameter estimation:
- Global: Particle Swarm, Scatter Search, Simulated Annealing
- Local: Hooke & Jeeves, Levenberg–Marquardt, Nelder–Mead

Objective function:

$$E(P) = \sum_k \sum_{i,j} w_i \cdot (x_{i,j} - y_{i,j}(P))^2$$

$x_{i,j}$ = point in data set — P = parameter set
$y_{i,j}(P)$ = simulated value — w_i = weight for data column

Time course calculations:
- COPASI uses the LSODA algorithm to calculate deterministic time course trajectories

Discussion

- There is evidence in parameter estimation that some parameters are highly interdependent. This makes it difficult to estimate all of them accurately.
- Knowledge of the protein levels is required to accurately estimate the other model parameters.
- It seems necessary to invoke up-regulation of the periplasmic cuproproteins to adequately fit a model to the data.

Future Work

Experimental design:
- Perform sensitivity analysis to determine which protein concentrations should be experimentally measured

Address parameter dependence:
- Analyze the identifiability of parameters
- Obtain and incorporate protein concentration data into model

Possible improvements to model:
- Add specific cuproproteins and chaperones as information regarding their biochemical interactions becomes available
- Change rate equations involving transporters to reflect their confinement to the inner and outer membranes

References

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