

1 Part A

To construct the stoichiometric array, I first identified 18 nodes or metabolites and 20 reactions or fluxes. Therefore, the stoichiometric array will be 18x20. The tables following define every metabolite and flux needed for the reactions in the urea cycle.

$$\frac{dX_i}{dt} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & -2 & 2 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2 & -2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1.5 & -1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1.5 & -1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & -1.5 & 1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{5f} \\ v_{5r} \\ b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \\ b_6 \\ b_7 \\ b_8 \\ b_9 \\ b_{10} \\ b_{11} \\ b_{12} \\ b_{13} \\ b_{14} \end{pmatrix}$$

Table One: Defined Metabolites in S Consistent with PS3 Diagram

Row	Metabolite	Row	Metabolite
1	Aspartate	10	H_2O
2	Arginosuccinate	11	Pi
3	Fumarate	12	AMP
4	Arginine	13	PPi
5	Urea	14	NO
6	Ornithine	15	O_2
7	Carbamoyl Pi	16	H^+ Sink
8	Citruline	17	$NADPH$ Source
9	ATP	18	$NADP^+$ Source

Table Two: Defined Fluxes Consistent with PS3 Diagram

Variable	Flux	Variable	Flux
v_1	6.3.4.5 Catalyzed Reaction	b_5	ATP Source
v_2	4.3.2.1 Catalyzed Reaction	b_6	Water Source
v_3	3.5.3.1 Catalyzed Reaction	b_7	Phosphate Sink
v_4	2.1.3.3 Catalyzed Reaction	b_8	AMP Sink
v_{5f}	1.14.13.39 Forward Reaction (Cit to R)	b_9	Pyrophosphate Sink
v_{5r}	1.14.13.39 Reverse Reaction (R to Cit)	b_{10}	Nitric Oxide Sink
b_1	Carbamoyl Pi Source	b_{11}	O_2 Sink
b_2	Aspartate Source	b_{12}	H^+ Source
b_3	Fumarate Sink	b_{13}	$NADPH$ Source
b_4	Urea Sink	b_{14}	$NADP^+$ Sink

2 Part B

The code B_balanced.jl shows the balanced nature of the first 6 fluxes (the actual chemical reactions). An atom matrix with dimensions of # of elements by # of metabolites (A) is multiplied by the stoichiometric array (S). The rows are thus C,H,N,O,P, and S respectively. The product E has zero entries for the elementally balanced reactions or the first 6 fluxes v_{1-5r} . The reaction and stoichiometric coefficients were taken from the reactions and conditions on the KEGG database for the urea cycle. When values are not zero in the v reactions, the exact element (row) and reaction (column) entry is shown to not be zero in **E**. We can thus edit the stoichiometric array until balanced.

$$\mathbf{A} * \mathbf{S} = \mathbf{E}$$

$$A = \begin{bmatrix} 4 & 10 & 4 & 6 & 1 & 5 & 1 & 6 & 10 & 0 & 0 & 10 & 0 & 0 & 0 & 0 & 21 & 21 \\ 7 & 18 & 4 & 14 & 4 & 12 & 4 & 13 & 16 & 2 & 3 & 14 & 4 & 0 & 0 & 1 & 30 & 29 \\ 1 & 4 & 0 & 4 & 2 & 2 & 1 & 3 & 5 & 0 & 0 & 5 & 0 & 1 & 0 & 0 & 7 & 7 \\ 4 & 6 & 4 & 2 & 1 & 2 & 5 & 3 & 13 & 1 & 4 & 7 & 7 & 1 & 2 & 0 & 17 & 17 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 3 & 0 & 1 & 1 & 2 & 0 & 0 & 0 & 3 & 3 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 3 & 3 \end{bmatrix}$$

3 Part C

CHEME5440HW3.C.jl calls the Flux.jl function to complete the linear programming problem of FBA. We need bounds and an objective. the objective is to maximize urea flux:

$$Objective = Maximize b_4$$

Subject To:

$$0 \leq v_j \leq k_{cat,j} * E * \theta * \prod_{i=1}^M \frac{X_i}{K_{Mij} + X_i}$$

$$0 \leq b_j \leq 10mmol/gDW - hr$$

Where M is the number of metabolites acted on by that enzyme in v_j . E, $k_{cat,j}$, and θ are given in the problem statement. X_i and K_{Mij} are the metabolite concentration and K_M value for each enzyme and substrate. These are all from the BRENDA enzyme database. A value for human cells was chosen first, then *Mus musculus* if needed, and finally a yeast value if none of those existed. When no metabolite value or K_M value could be found, the reaction had to be assumed to act at saturation. The saturation term was set to be 1. This was especially true for metabolites like water or H^+ whose concentrations are not really measurable. b_j refers to fluxes in and out of the system and the bound is given. There are no metabolite bounds on this problem. All values were converted to $umol/gDW - hr$ via the conversion in Appendix A. The solution or returned flux vector in $umol/gDW - hr$:

$$\mathbf{v} = \begin{bmatrix} 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 0.0 \\ 0.0 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 1033.4 \\ 0.0 \\ 0.0 \\ 0.0 \\ 0.0 \\ 0.0 \\ 0.0 \end{bmatrix}$$

Therefore the maximum urea flux b_4 is:

$$UreaFlux = 1033.4 \mu mol/gDW - hr$$

This answer makes sense because the maximum rate of aspartate to arginosuccinate is governing the entire process and how much flux can continue from there to every other node (since units are in moles). All zero fluxes are reactions or transports that are not used. For example, each NADPH used in the conversion of arginine to citruline is just used up in the reverse reaction (futile cycle). However, this flux is just zero anyway to maximize urea transport out. It is also worth noting that $\mathbf{S}^*\mathbf{v}=\mathbf{0}$ so the system checks itself.

4 Appendix A: Conversion

Molar values were converted to $\mu mol/gDW$ with the following conversion factors. The mass of a mammalian cell ($2.3e - 9g$ from Park[1]) was converted to dry mass via the water fraction (0.798 from Savitz[2]). The Molar value is divided by this and multiplied by the volume of a mammalian cell ($1e - 12L$ from Sims[3]). This is done via the internal function "convertfactor" in CHEME5440HW3_C.jl.

References

- [1] K. Park, J. Jang, D. Irimia, J. Sturgis, J. Lee, J. P. Robinson, M. Toner, and R. Bashir, "living cantilever arrays for characterization of mass of single live cells in fluids," *Lab on a Chip*, vol. 8, no. 7, pp. 1034–1041, 2008.
- [2] D. Savitz, V. W. Sidel, and A. Solomon, "Osmotic properties of human red cells," *The Journal of general physiology*, vol. 48, no. 1, pp. 79–94, 1964.
- [3] C. E. Sims and N. L. Allbritton, "Analysis of single mammalian cells on-chip," *Lab on a Chip*, vol. 7, no. 4, pp. 423–440, 2007.