Part A: KEGG was used to construct the stoichiometric matrix **S** for the urea cycle in a growing population of human cells. All reactions involved in the urea cycle were investigated and recorded in order to establish the metabolic network. The internal reactions are shown in Eqns. V1 through V5, with V5 being the only reaction taken to be reversible. The stoichiometric matrix was created using excel, and is show in Figure 1. The external fluxes, b_j, are defined in Table 1.

v1f	v2f	v3f	v4f	v5f	v5r	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12	b13	b14	b15
-1	0	0	0	0	0	0	0	0	0	0	1	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	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	0
1	0	0	0	0	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	0	0	0	0	0	0	0	0	-1	0	0	0
1	-1	0	0	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	0
0	1	-1	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	1	-1	0	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
0	0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	-1	1	0	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	-1	0
0	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	1	-1	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0
0	0	-1	0	1	-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Figure 1: Stoichiometric Matrix, rows are metabolites and columns are reactions/fluxes

External Fluxes			
B1	Carbamoyl phosphate	**B9**	Oxygen
B2	Aspartate	**B10**	Water
B3	Fumarate	**B11**	Orthophosphate
B4	Urea	**B12**	Diphosphate
B5	AMP	**B13**	H+
B6	ATP	**B14**	N-omega-hydroxyarginine
B7	NADPH	**B15**	Arginine
B8	NADP+		

Table 1: Table of external fluxes, b_j

ATP + Citrulline + Aspartate → AMP + diphosphate + Argininiosuccinate **Eqn. V1**

Argininosuccinate → Fumarate + Arginine **Eqn. V2**

Arginine → Ornithine + Urea **Eqn. V3**

Carbamoyl Phosphate + Ornithine → Orthophosphate + Citrulline **Eqn. V4**

Arginine + Oxygen + NADPH + H^+ → N(omega)-hydroxyarginine + $NADP^+$ + water **Eqn. V5**

Part B: It was confirmed that the urea cycle reconstruction is balanced, considering only the internal fluxes inside the cell, using Eqn. 1, where **A** is the atomic matrix and **S** is the stoichiometric matrix. Matlab was used to perform the matrix operations; the result of implementing Eqn. 1 was a matrix of zeros, which confirms that the internal fluxes are balanced. The **A** matrix is shown in Table 2. The Matlab script used to confirm the elemental balances is included in the github repository named Caserto_PS3.

$\mathbf{A}^T\mathbf{S} = [\ ]$ **Eqn. 1**

	C	H	N	O	P
ATP	10	16	5	13	3
Citruline	6	13	3	3	0
Aspartate	4	7	1	4	0
AMP	10	14	5	7	1
diphosphate	0	4	0	7	2
arginino succinate	10	18	4	6	0
fumarate	4	4	0	4	0
arginine	6	14	4	2	0
ornithine	5	12	2	2	0
urea	1	4	2	1	0
carbamoyl phosphate	1	4	1	5	1
orthophosphate	0	3	0	4	1
NADPH	21	30	7	17	3
N-omega-hydroxy-l-arginine	6	14	4	3	0
oxygen	0	0	0	2	0
H+	0	1	0	0	0
NADP+	21	29	7	17	3
water	0	2	0	1	0

Table 2: Atom matrix used for elemental balances.

Part C: Table 3 presents the metabolite concentrations and Km values included in the Julia file named PS3_partc.jl. Metabolites not found in the Park et al. paper include citrulline, argininosuccinate, carbamoyl phosphate, and ornithine. The programming language used to solve this problem is Julia. The Julia script named Flux.jl, provided by Professor Varner via Slack, was used to calculate the optimal flux distribution using flux balance analysis (FBA). The bound constraints for the FBA are shown in Table 4. All Julia script files are included in the github repository name Caserto_PS3.

To execute the code used to obtain the maximum Urea flux, enter the following command in Julia:

> include(PS3_partc.jl)

The maximum rate of production of Urea was found to be 0.83 mmol/gDW/hr.

EC Number	Metabolite	Concentration (M)	K_m (M)	Organism
1.1.1.101	NADPH	6.54E-5	5.25E-6	*Homo sapiens*
2.1.1.5	aspartate	1.49E-2	1.00E-2	*Homo sapiens*
2.3.3.8	ATP	4.67E-3	6.14E-4	*Homo sapiens*
2.1.4.1	arginine	2.55E-4	2.50E-3	*Homo sapiens*

Table 3: Values from Supplementary Data Set 2 of Park et al. that were used to solve for the maximum rate of urea production in PS3_partc.jl.

	Lower Bound	Upper Bound
Intracellular Reactions	0	V_{max}*E * Saturation
Extracellular Fluxes	0	10 mmol/gDW/hr

Table 4: Upper and lower bounds for FBA constraints. Saturation = x / (x+K_m), with x being the metabolite concentration.