PS 3 Solution

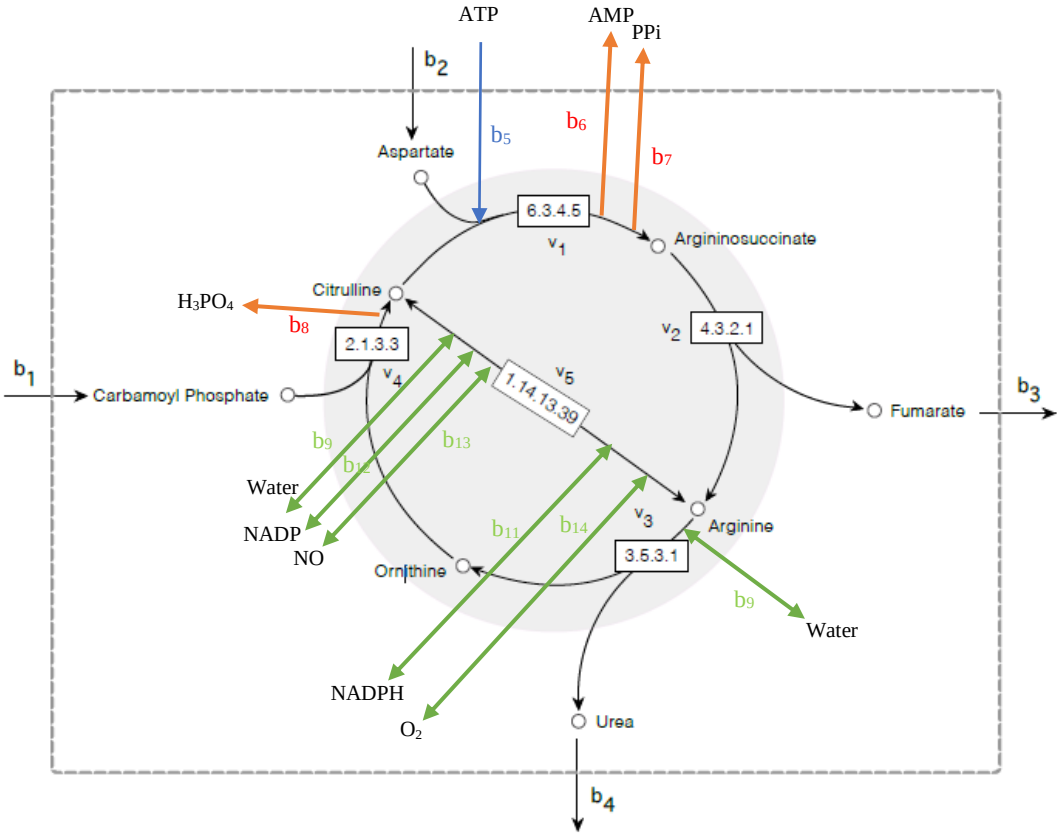

Figure 1: Schematic of the Urea cycle.

Solution 1 (a)

- Table 1 Metabolite ID

#	Metabolite	Symbol	#	Metabolite	Symbol
M.1	Arginine (arg)	$C_6H_{14}N_4O_2$	M.10	ATP	$C_{10}H_{16}N_5O_{13}P_3$
M.2	Ornithine (orn)	$C_5H_{12}N_2O_2$	M.11	AMP	$C_{10}H_{14}N_5O_7P$
M.3	Urea (u)	CH_4N_2O	M.12	Diphosphate (PPi)	H_4PO_7
M.4	Carbamoyl Phosphate (camp)	CH_4NO_5P	M.13	Orthophosphate	H_3PO_4
M.5	L-aspartate (asp)	$C_4H_7NO_4$	M.14	NADPH	$C_{21}H_{30}N_7O_{17}P_3$
M.6	Citrulline (cit)	$C_6H_{13}N_3O_3$	M.15	NADP	$C_{21}H_{29}N_7O_{17}P_3$
M.7	Arginine succinate (arg_s)	$C_{10}H_{18}N_4O_6$	M.16	Oxygen	O_2
M.8	Fumarate (fum)	$C_4H_4O_4$	M.17	Nitric Oxide	NO
M.9	Water	H_2O	M.18	H^+	H

- Reactions involved in the urea cycle (Source: KEGG)

E.C. 6.3.4.5

$C_4H_7NO_4 + C_6H_{13}N_3O_3 + C_{10}H_{16}N_5O_{13}P_3 \Longrightarrow C_{10}H_{18}N_4O_6 + C_{10}H_{14}N_5O_7P + H_4PO_7$

asp + cit + ATP ===> arg_s + AMP + PPi

E.C. 4.3.2.1

$C_{10}H_{18}N_4O_6 \Longrightarrow C_6H_{14}N_4O_2 + C_4H_4O_4$

arg_s ===> arg + fum

E.C. 3.5.3.1

$C_6H_{14}N_4O_2 + H_2O \Longrightarrow C_5H_{12}N_2O_2 + CH_4N_2O$

arg + water ==> orn + urea

E.C. 2.1.3.3

$C_5H_{12}N_2O_2 + CH_4NO_5P \Longrightarrow C_6H_{13}N_3O_3 + H_3PO_4$

orn + camp ===> cit + orthophosphate

E.C. 1.14.13.39

$2\ C_6H_{14}N_4O_2 + 3\ C_{21}H_{30}N_7O_{17}P_3 + 3\ H^+ + 4\ O_2 \text{ <===> } 2\ C_6H_{13}N_3O_3 + 2\ NO + 3\ C_{21}H_{29}N_7O_{17}P_3 + 4\ H_2O$

2*arg + 3*NADPH + 3*H + 4*oxygen <==> 2* cit + 2*NO + 3*NADP + 4*H_2O

- Table 2 Reaction ID

#	**Reaction**	#	**Reaction**
v_1	*e.c.6.3.4.5* asp-->arg_s	b_5	[ ]-->ATP
v_2	*e.c.4.3.2.1* arg_s-->arg	b_6	AMP-->[ ]
v_3	*e.c.3.5.3.1* arg-->orn	b_7	PPi-->[ ]
v_4	*e.c.2.1.3.3* orn-->cit	b_8	H_3PO_4-->[ ]
v_5	*e.c.1.14.13.39* arg-->cit	b_9	[ ]<-->H_2O
v_6	rev. v_5 *e.c.1.14.13.39* cit-->arg	b_{10}	NADPH<-->[ ]
b_1	[ ]-->camp	b_{11}	H<-->[ ]
b_2	[ ]-->asp	b_{12}	NADP<-->[ ]
b_3	fum-->[ ]	b_{13}	NO<-->[ ]
b_4	u--> [ ]	b_{14}	O_2<-->[ ]

(answer) Table 3 **Stoichiometric Matrix, S**

	v_1	v_2	v_3	v_4	v_5	v_6	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}
M.1	0	1	-1	0	-2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M.2	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M.3	0	0	1	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0
M.4	0	0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
M.5	-1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
M.6	-1	0	0	1	2	-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M.7	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M.8	0	1	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0
M.9	0	0	-1	0	4	-4	0	0	0	0	0	0	0	0	1	0	0	0	0	0
M.10	-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
M.11	1	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0
M.12	1	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0
M.13	0	0	0	1	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0
M.14	0	0	0	0	-3	3	0	0	0	0	0	0	0	0	0	-1	0	0	0	0
M.15	0	0	0	0	3	-3	0	0	0	0	0	0	0	0	0	0	0	1	0	0
M.16	0	0	0	0	-4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	-1
M.17	0	0	0	0	2	-2	0	0	0	0	0	0	0	0	0	0	0	0	1	0
M.18	0	0	0	0	-3	3	0	0	0	0	0	0	0	0	0	0	-1	0	0	0

(Convention for the reversible reaction v_5 has been assumed to be: cit ===> arg)

Solution 1 (b)

(answer) Table 4 **Atom Matrix, A**

	M.1	M.2	M.3	M.4	M.5	M.6	M.7	M.8	M.9	M.10	M.11	M.12	M.13	M.14	M.15	M.16	M.17	M.18
C	6	5	1	1	4	6	10	4	0	10	10	0	0	21	21	0	0	0
H	14	12	4	4	7	13	18	4	2	16	14	4	3	30	29	0	0	1
N	4	2	2	1	1	3	4	0	0	5	5	0	0	7	7	0	1	0
O	2	2	1	5	4	3	6	4	1	13	7	7	4	17	17	2	1	0
P	0	0	0	1	0	0	0	0	0	3	1	2	1	3	3	0	0	0

I have used the atom matrix for this part and the **epsilon = A*S** matrix can be generated by running the ("**S_and_epsillon_generator.jl**") file. Here, S is the stoichiometric matrix for reactions in the urea cycle only ($v_1 - v_6$)

- in julia console run include("a,b S_and_epsillon_generator.jl")

This will generate “atom_matrix.csv” and “epsilon.txt” files.

If the urea cycle reconstruction is elementally balanced, all components of the epsilon matrix should be zero, which is true in this case. (view “epsilon.txt”)

If all elemental matrix components were not zero, then we would have to check the stoichiometry for the reactions corresponding to the non-zero columns in the matrix.

Solution 1 (c)

The code for solving the FBA problem is given in “c run.jl”

- Upper bounds for the fluxes were calculated using:

$$v_{max,i} = k_{cat,i} \times E \times \prod_{i=1}^{n} \frac{C_i}{K_m + C_i}$$

- K_m values and metabolite concentration , C_i were obtained from Park et al Nat Chem Biol 12:482-9, 2016 and used to calculate:

For these values, the following order of preference was followed:

$$Homo\ sapiens > Mus\ musculus > other\ organism$$

Data for S. cerevisiae was used for the enzyme E.C.2.1.3.3

In the absence of enzyme and substrate specific data, it was assumed $C_i >> K_m$, which effectively gives $\frac{C_i}{K_m + C_i} = 1$

- **in julia console run** include("c run.jl")

Note: I have assumed the metabolites involved in reaction v_5 can be exchanged through the boundary (so there is a net positive flux in arg ==> cit direction [Table5]).

Deactivate lines 26, 27 and activate lines 30 and 31 in the "c run.jl" file to see optimum fluxes with **no boundary exchange of reaction v_5 metabolites** (in this case flux $v_5 = v_6 = 0.0$ mmol/gDW.hr to maximize urea)

(answer) Table 5 Optimum fluxes for maximum urea production

Flux ID	Reaction	LB	UB	Optimum value (mmol/gdW.hr)
v_1	*e.c.6.3.4.5* asp-->arg_s	0	v_{max1}	1.242
v_2	*e.c.4.3.2.1* arg_s-->arg	0	v_{max2}	1.242
v_3	*e.c.3.5.3.1* arg-->orn	0	v_{max3}	1.271095
v_4	*e.c.2.1.3.3* orn-->cit	0	v_{max4}	1.271095
v_5	*e.c.1.14.13.39* arg-->cit	0	v_{max5}	0.478652
v_6	rev. v_5 *e.c.1.14.13.39* cit-->arg	0	v_{max6}	0.4932
b_1	[ ]-->camp	0	10	1.271095
b_2	[ ]-->asp	0	10	1.242
b_3	fum-->[ ]	0	10	1.242
b_4	**u--> [ ]**	**0**	**10**	**1.271095**
b_5	[ ]-->ATP	0	10	1.242
b_6	AMP-->[ ]	0	10	1.242
b_7	PPi-->[ ]	0	10	1.242
b_8	H_3PO_4-->[ ]	0	10	1.271095
b_9	[ ]<-->H_2O	-10	10	1.329286
b_{10}	NADPH<-->[ ]	-10	10	0.043643
b_{11}	H<-->[ ]	-10	10	0.043643
b_{12}	NADP<-->[ ]	-10	10	0.043643
b_{13}	NO<-->[ ]	-10	10	0.029095
b_{14}	O_2<-->[ ]	-10	10	0.05819