PS 3 Solution

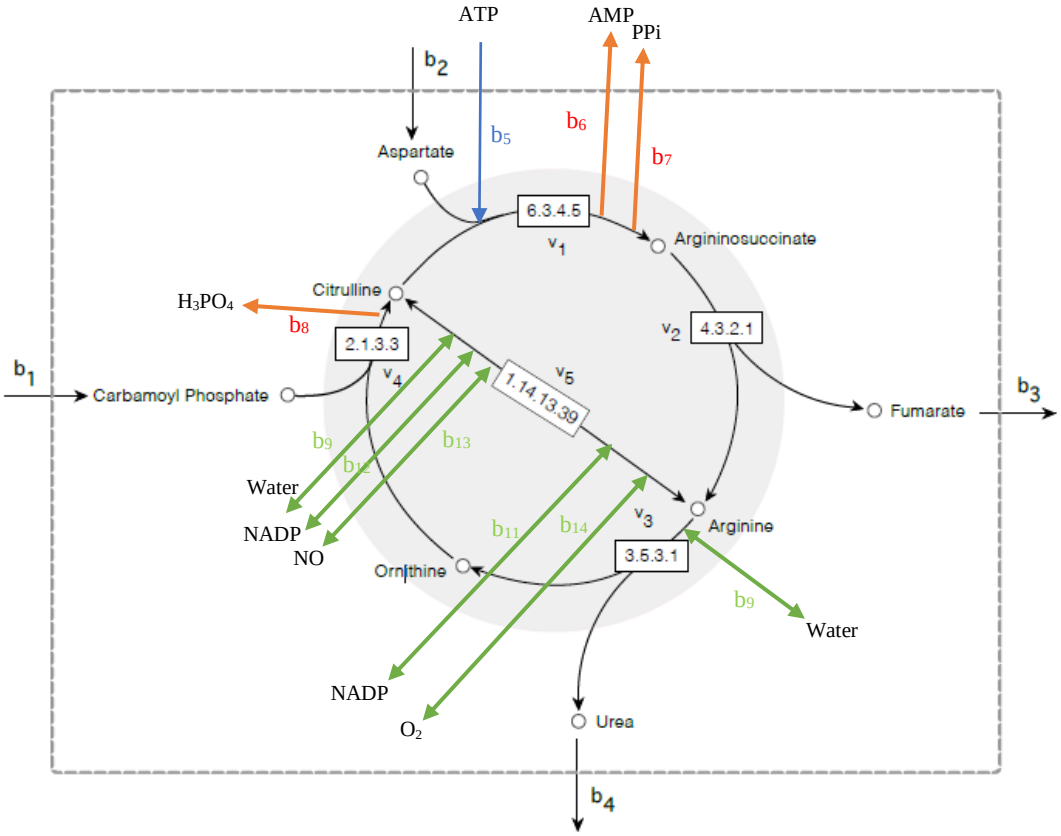

Figure 1: Schematic of the Urea cycle.

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

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

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

E.C. 4.3.2.1

$C_{10}H_{18}N_4O_6 ===> C_6H_{14}N_4O_2 + C_4H_4O_4$

arg_s ===> arg + fum

E.C. 3.5.3.1

$C_6H_{14}N_4O_2 + H_2O ===> C_5H_{12}N_2O_2 + CH_4N_2O$

Arg + water ==> orn + urea

E.C. 2.1.3.3

$C_5H_{12}N_2O_2 + CH_4NO_5P ===> C_6H_{13}N_3O_3 + H_3PO_4$

orn + camp ===> cit + orthophosphate

E.C. 1.14.13.39

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

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 v_5 reversibility has been assumed to be: citrulline ===> arginine)

Solution 1 (b)

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.

- 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”)

Solution 1 (c)

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

- Upper bounds for the fluxes were calculated using:

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

- 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 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 25, 26 and activate lines 29 and 30 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