Reconstruction of the Cobalamin (vitamin B12) Biosynthesis Pathway in *A.woodii* GSM

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Background

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- Characterise acetogen metabolism using a genome-scale metabolic model of *Acetobacterium woodii* DSM1030

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- Investigate the potential of using *A.woodii* for production of valuable products from C1 gases
- Direct *in silico* design of an industrially relevant strain

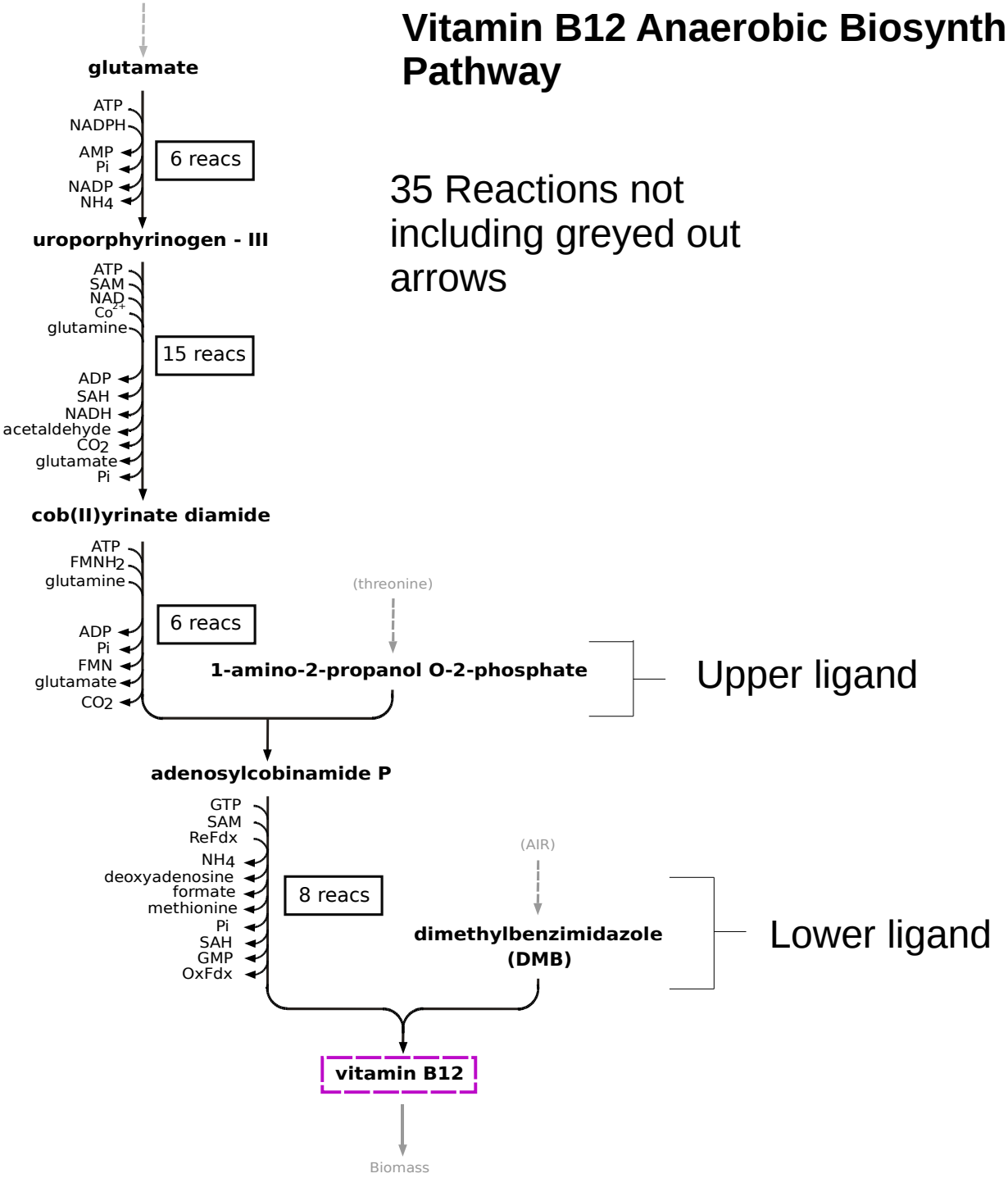
Why model Cobalamin Biosynthesis?

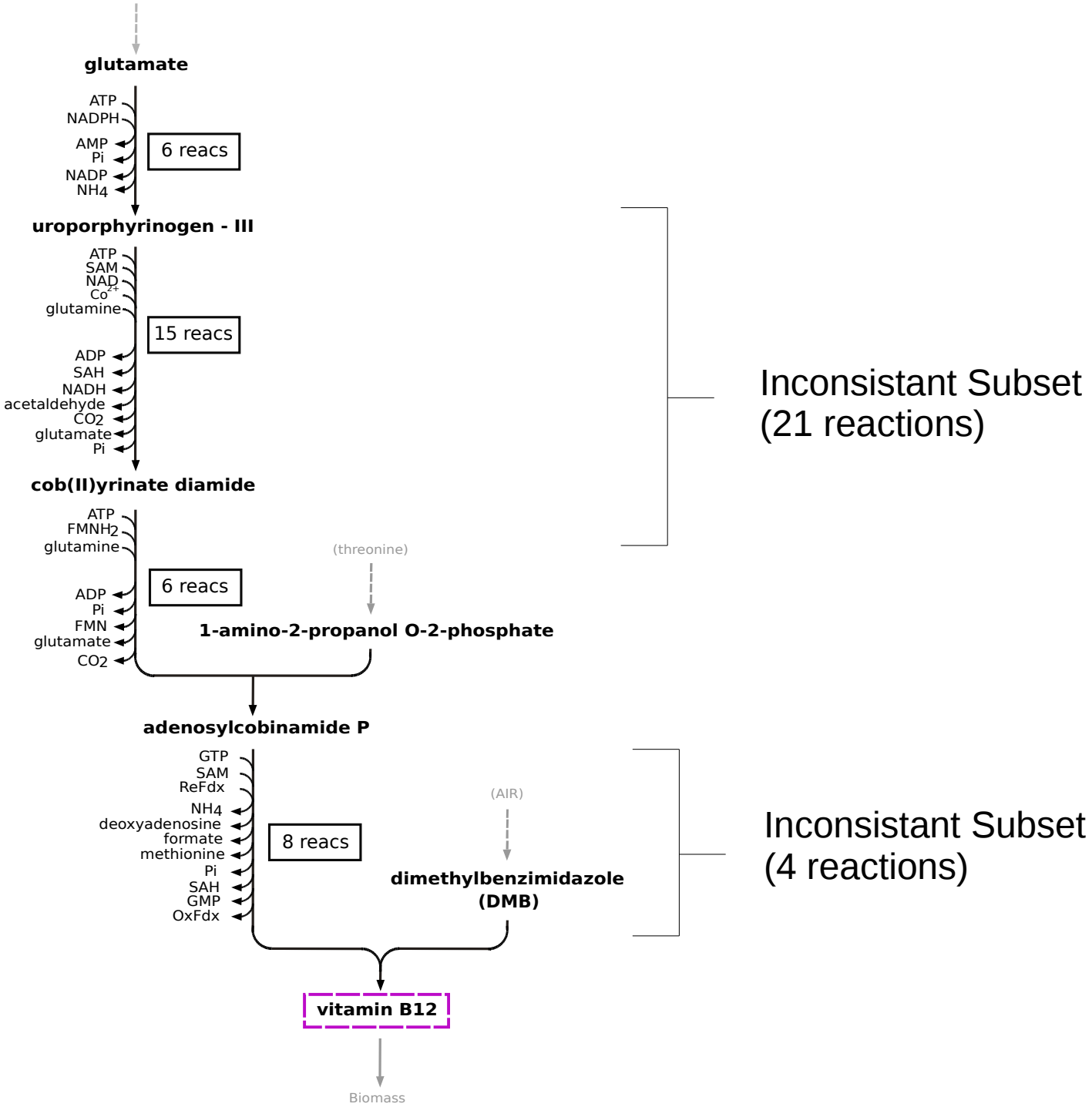
- Needed for the model biomass equation
- Cobalamin is a valuable commodity used in feed, food and pharmaceuticals.
- Recent reports complete long standing gaps in anaerobic cobalamin biosynthesis – DMB (lower ligand), precorrins.
- Cobalamin is the major fraction of corrinoids in *A.woodii.*
- Few (if any) *in silico* analysis of biosynthesis.

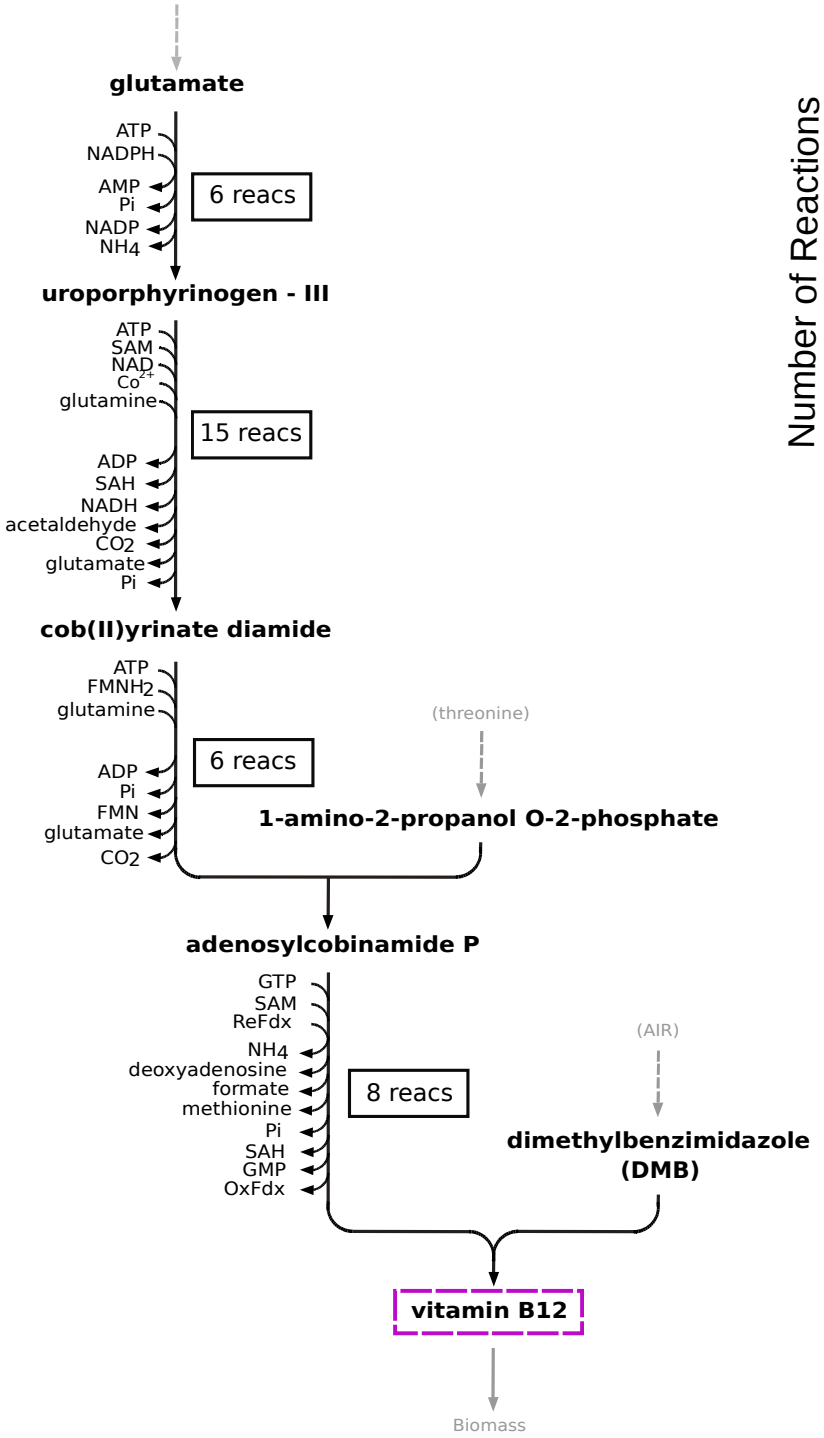
Can cobalamin be produced as part of biomass in *A.woodii* GSM?

Vitamin B12 Anaerobic Biosynthesis Pathway

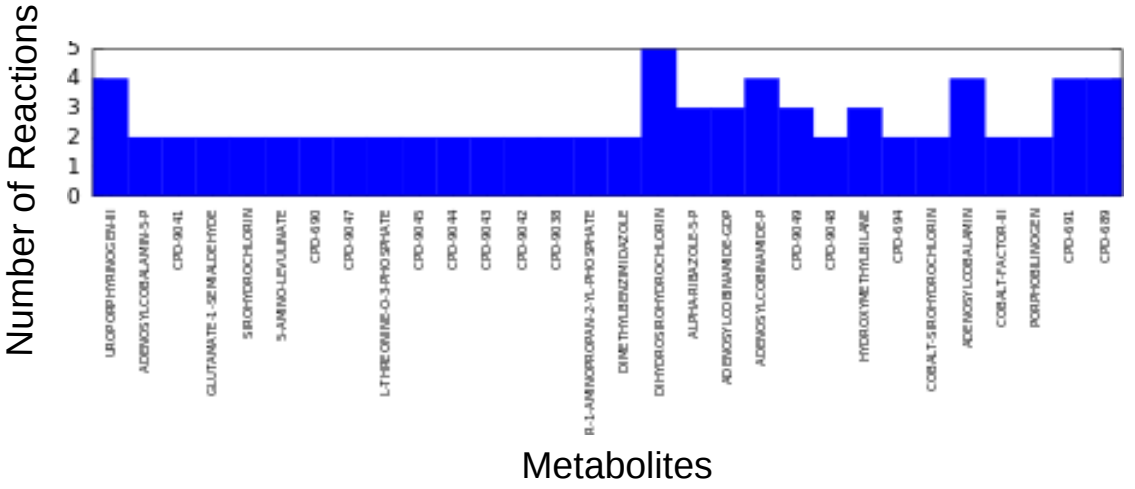
35 Reactions not including greyed out arrows



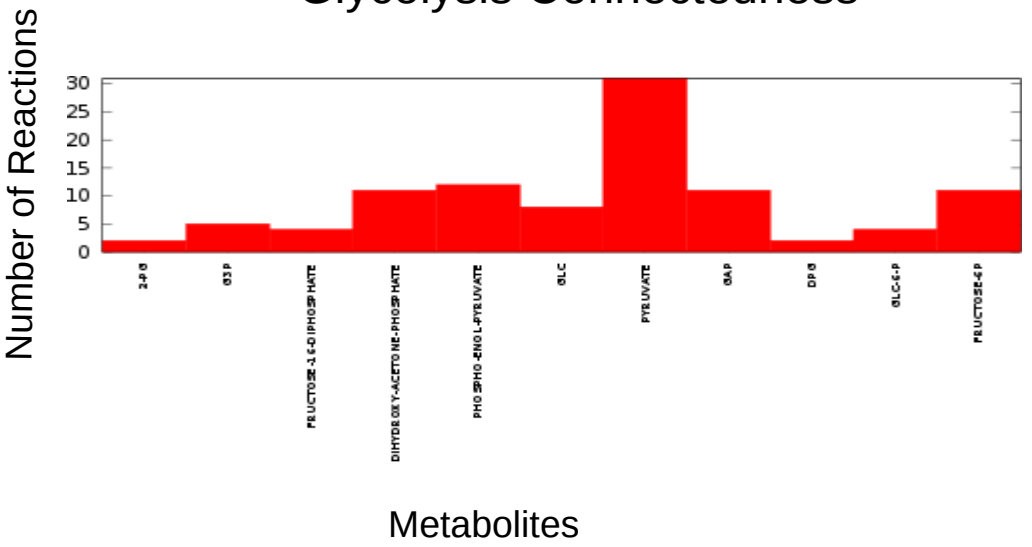

glutamate
ATP
NADPH
AMP
Pi
NADP
NH4
6 reacs
uroporphyrinogen - III
ATP
SAM
NAD
Co2+
glutamine
15 reacs
ADP
SAH
NADH
acetaldehyde
CO2
glutamate
Pi
cob(II)yrinate diamide
ATP
FMNH2
glutamine
6 reacs
ADP
Pi
FMN
glutamate
CO2
(threonine)
1-amino-2-propanol O-2-phosphate
adenosylcobinamide P
GTP
SAM
ReFdx
NH4
deoxyadenosine
formate
methionine
Pi
SAH
GMP
OxFdx
8 reacs
(AIR)
dimethylbenzimidazole
(DMB)
vitamin B12
Biomass
Inconsistant Subset
(21 reactions)
Inconsistant Subset
(4 reactions)



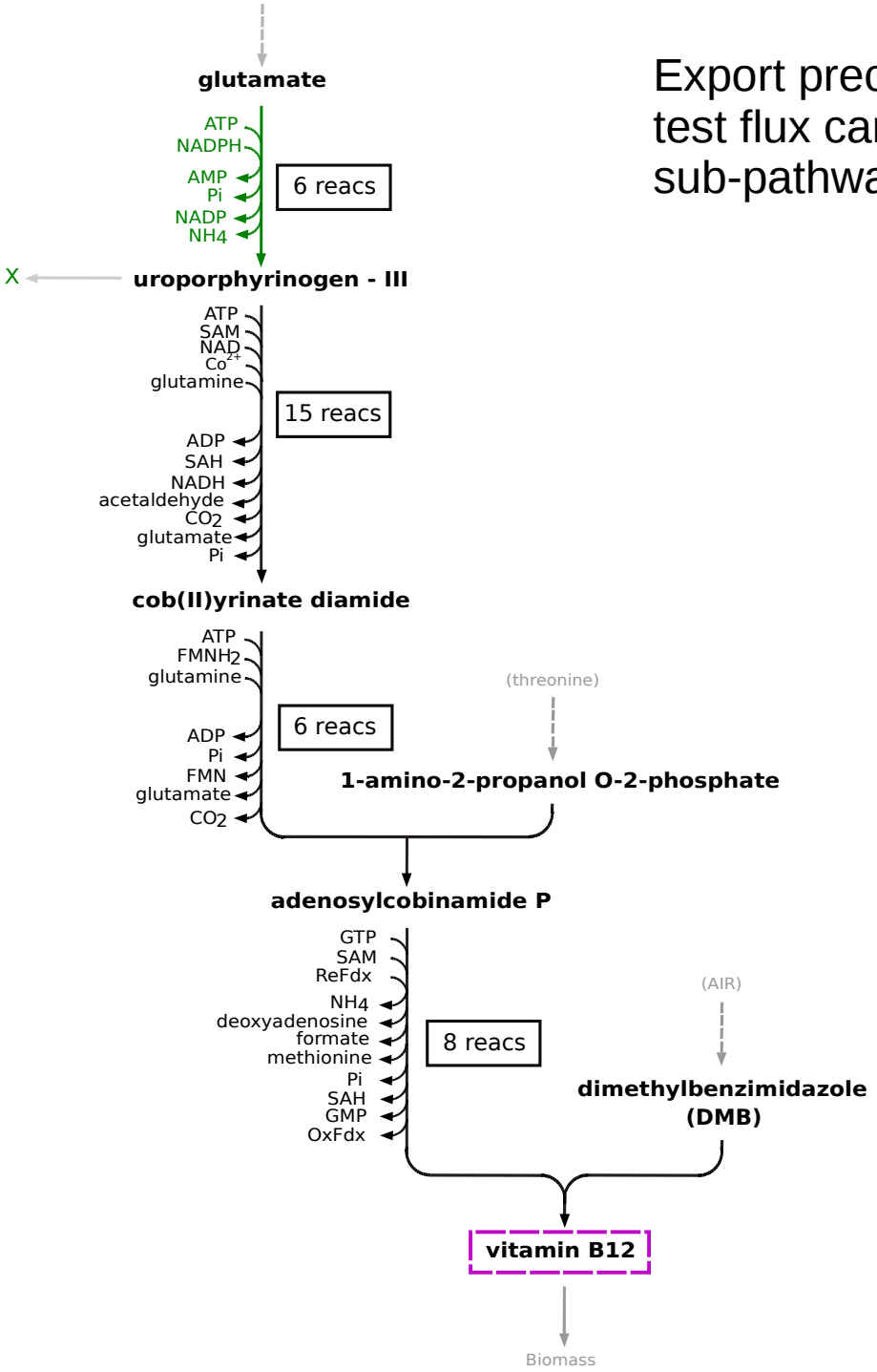
B12 Biosynthesis Connectedness



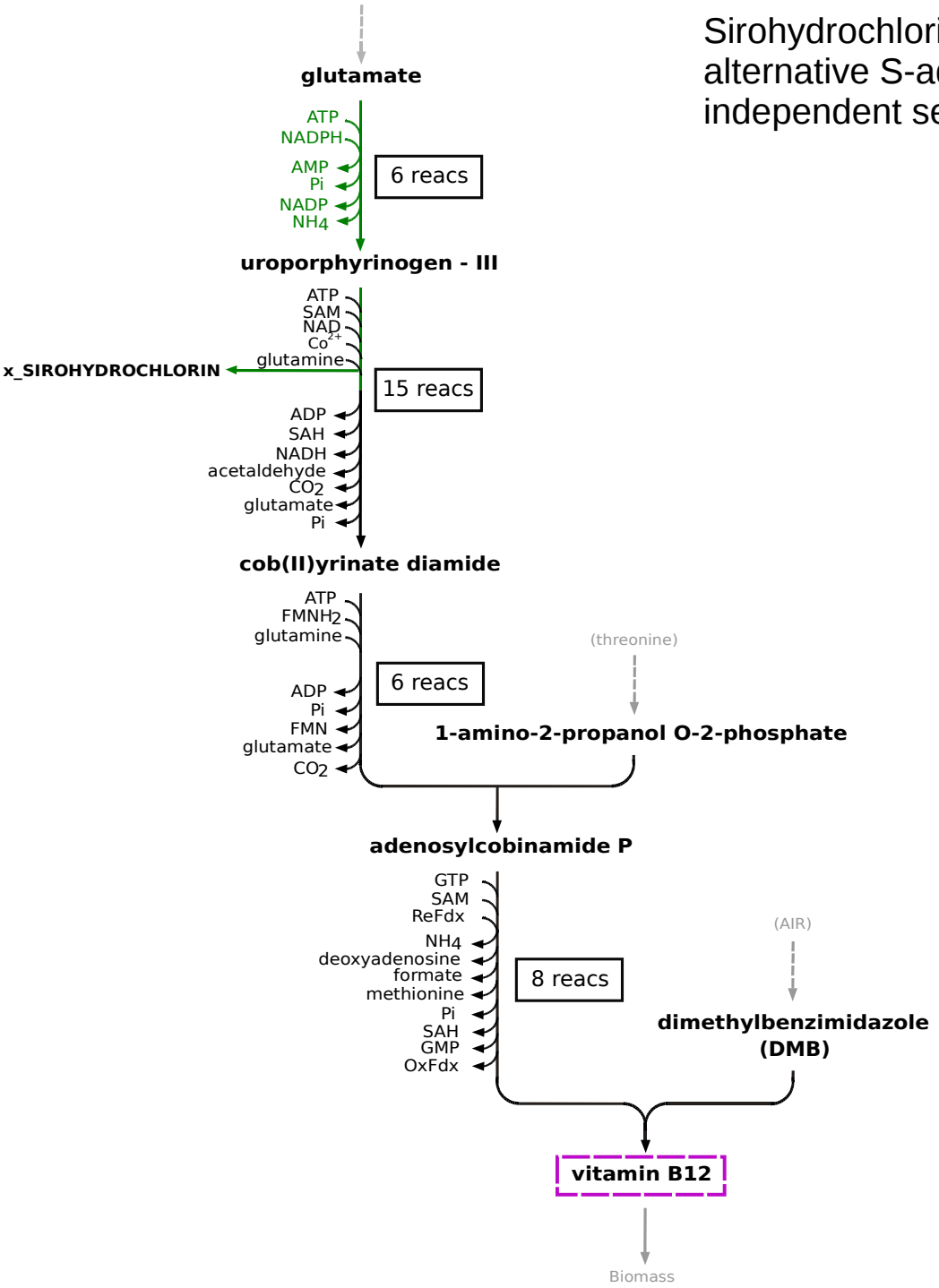
Glycolysis Connectedness

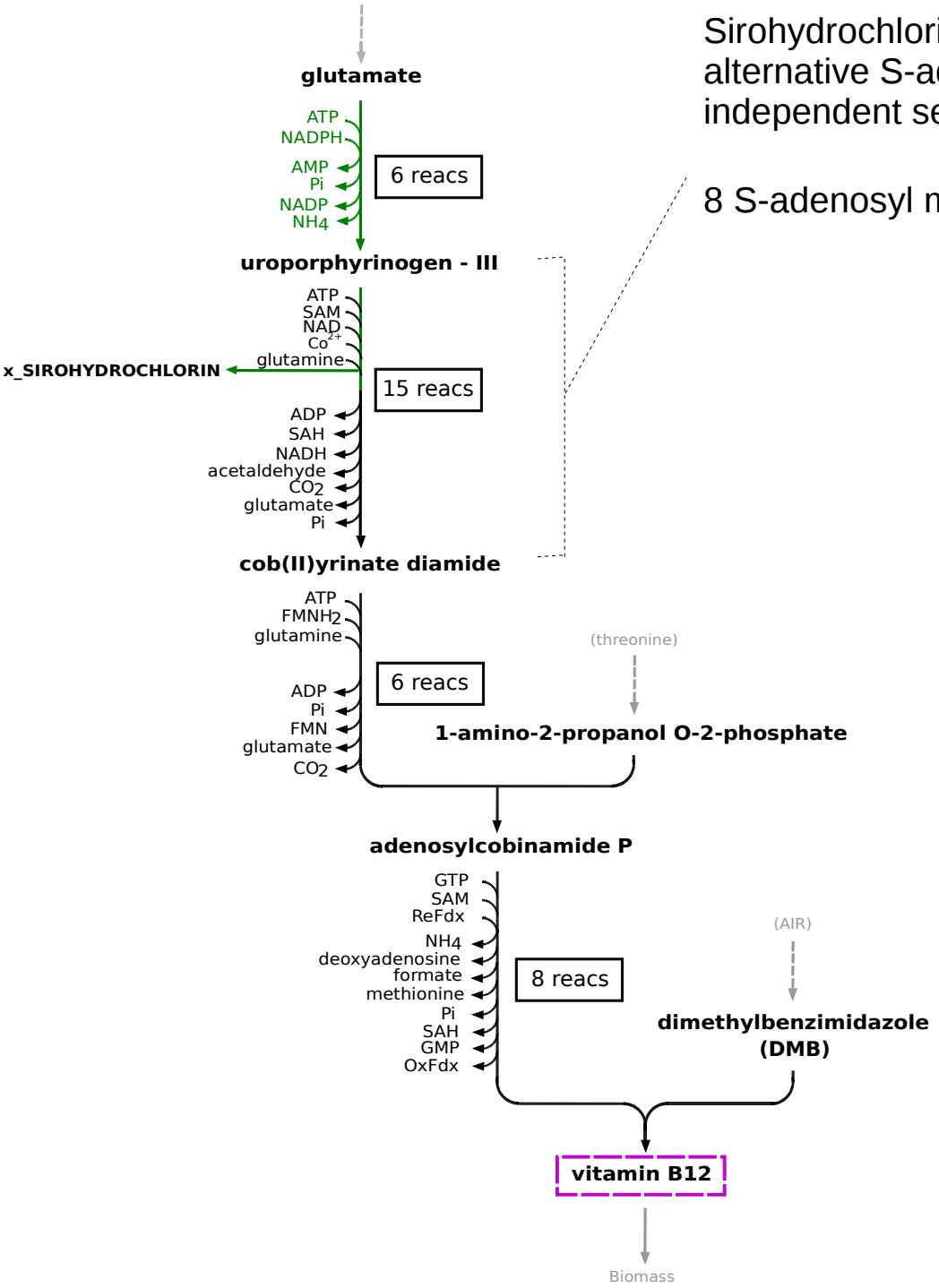


Export precursor intermediates to test flux carrying capability of sub-pathways



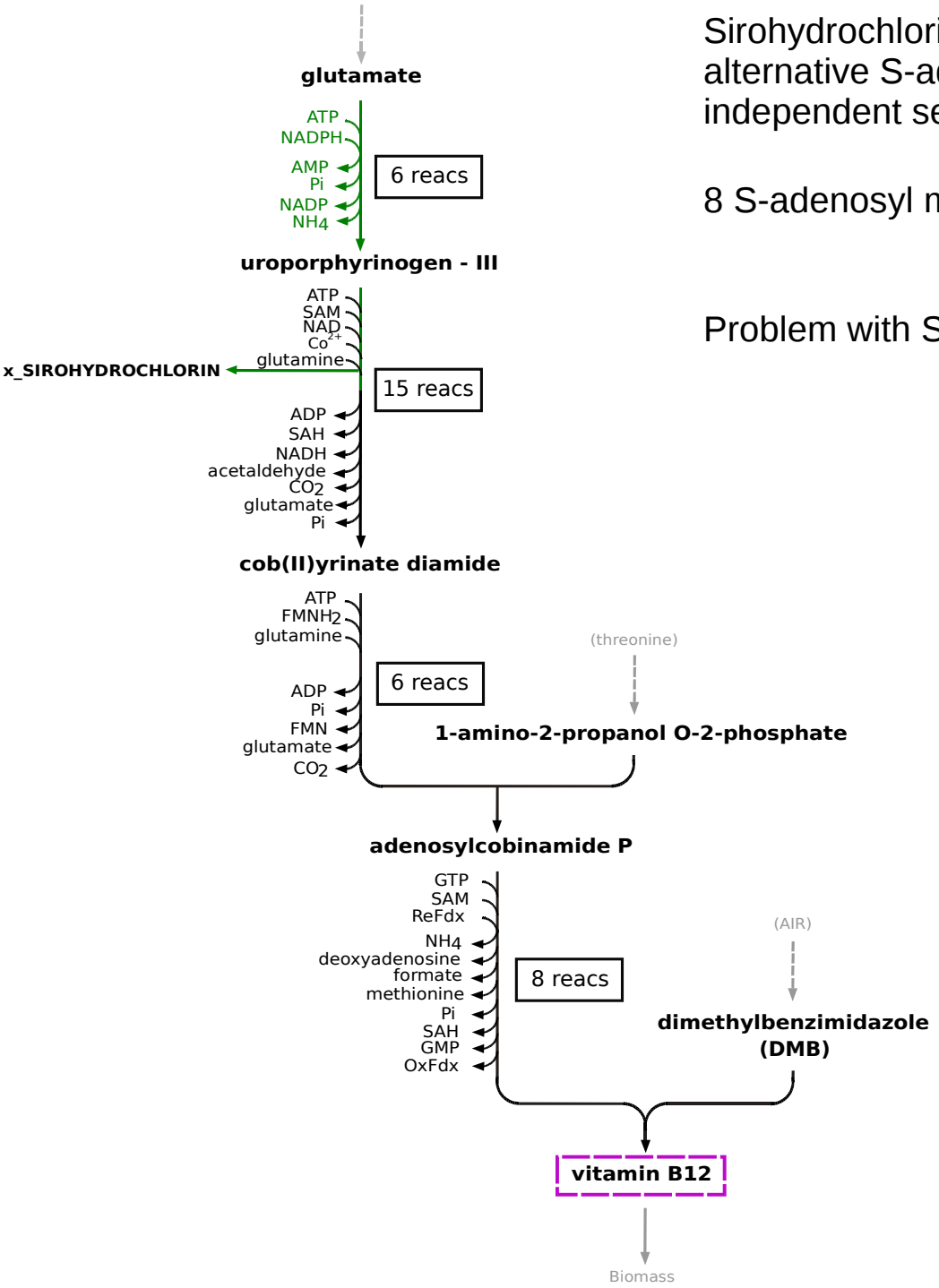
Sirohydrochlorin can be exported with an alternative S-adenosyl methionine (SAM) independent set of reactions.





Sirohydrochlorin can be exported with an alternative S-adenosyl methionine (SAM) independent set of reactions.

8 S-adenosyl methionine → 8 S-adenosyl homocysteine



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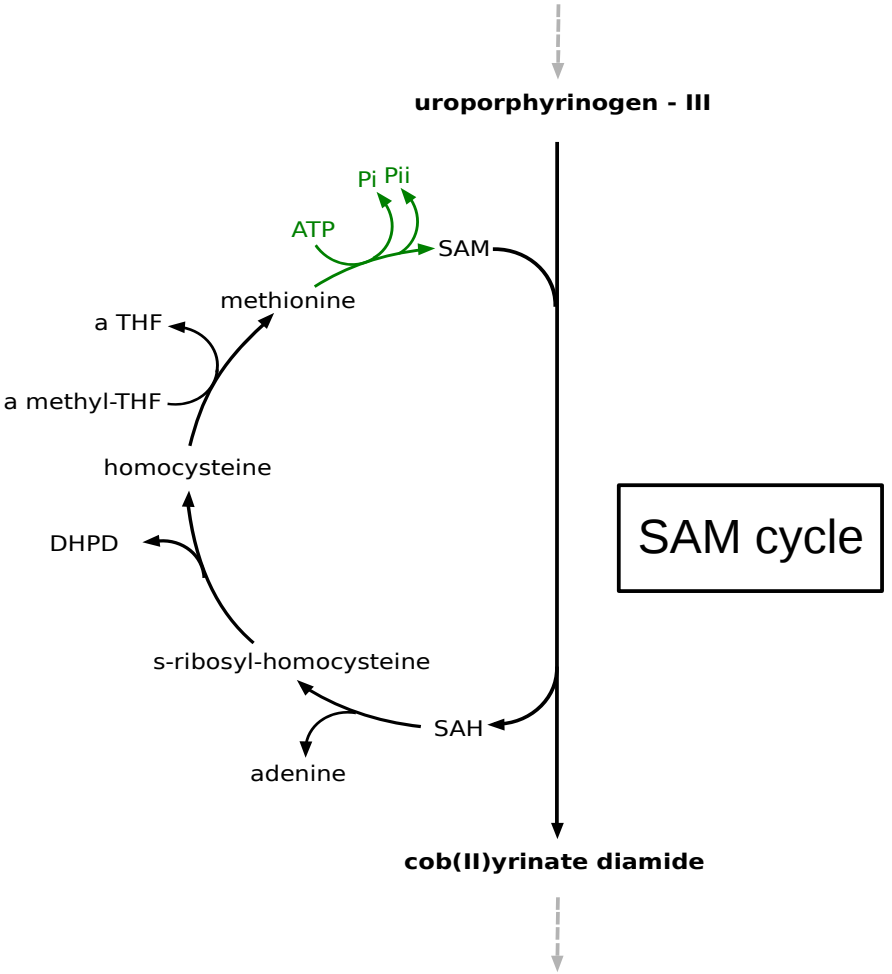
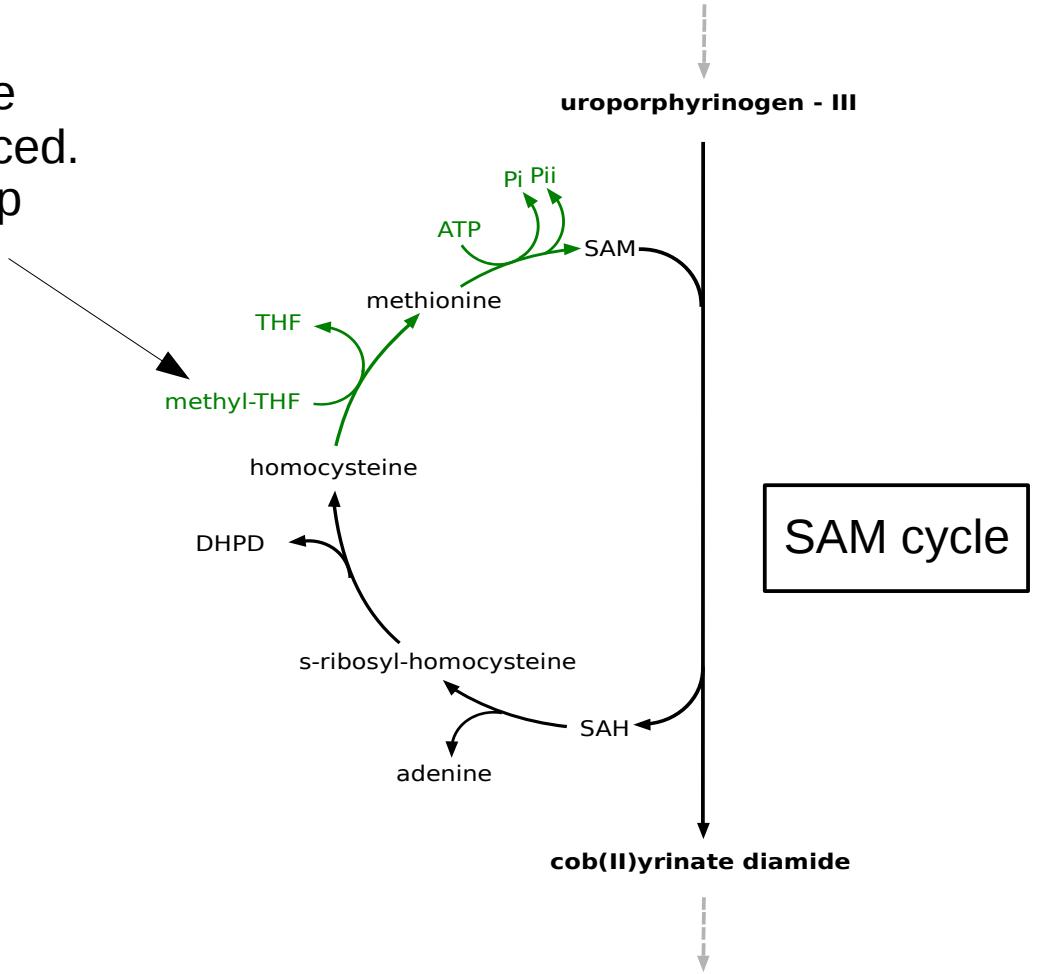
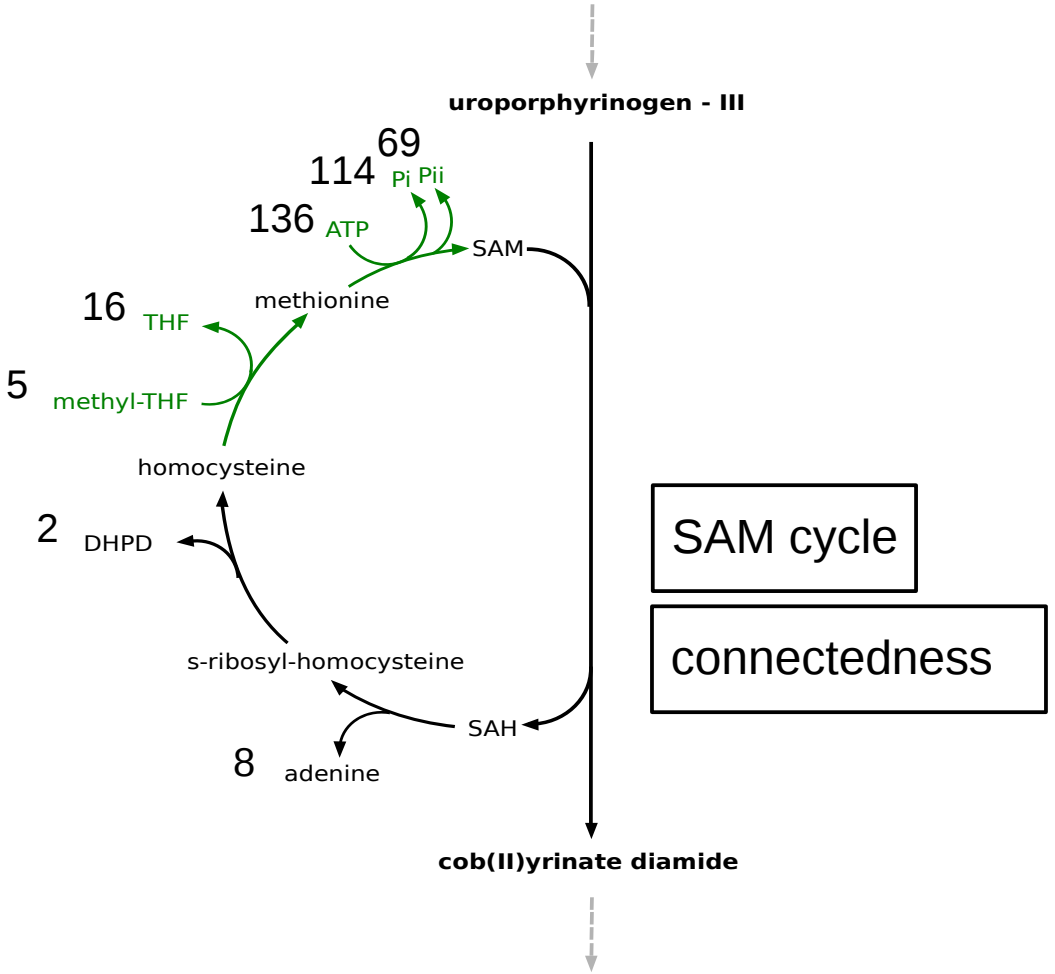
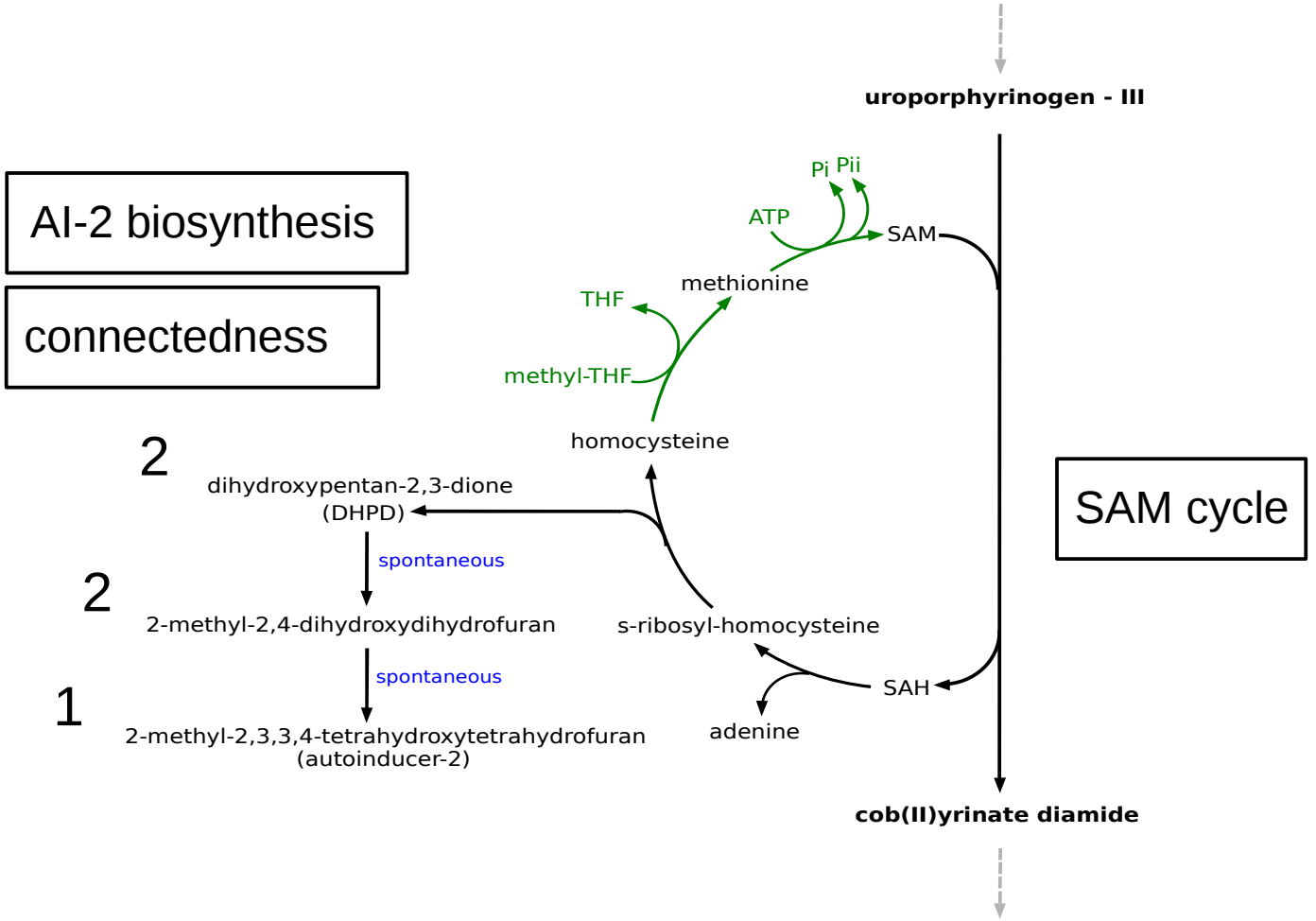
Problem with SAM regeneration?

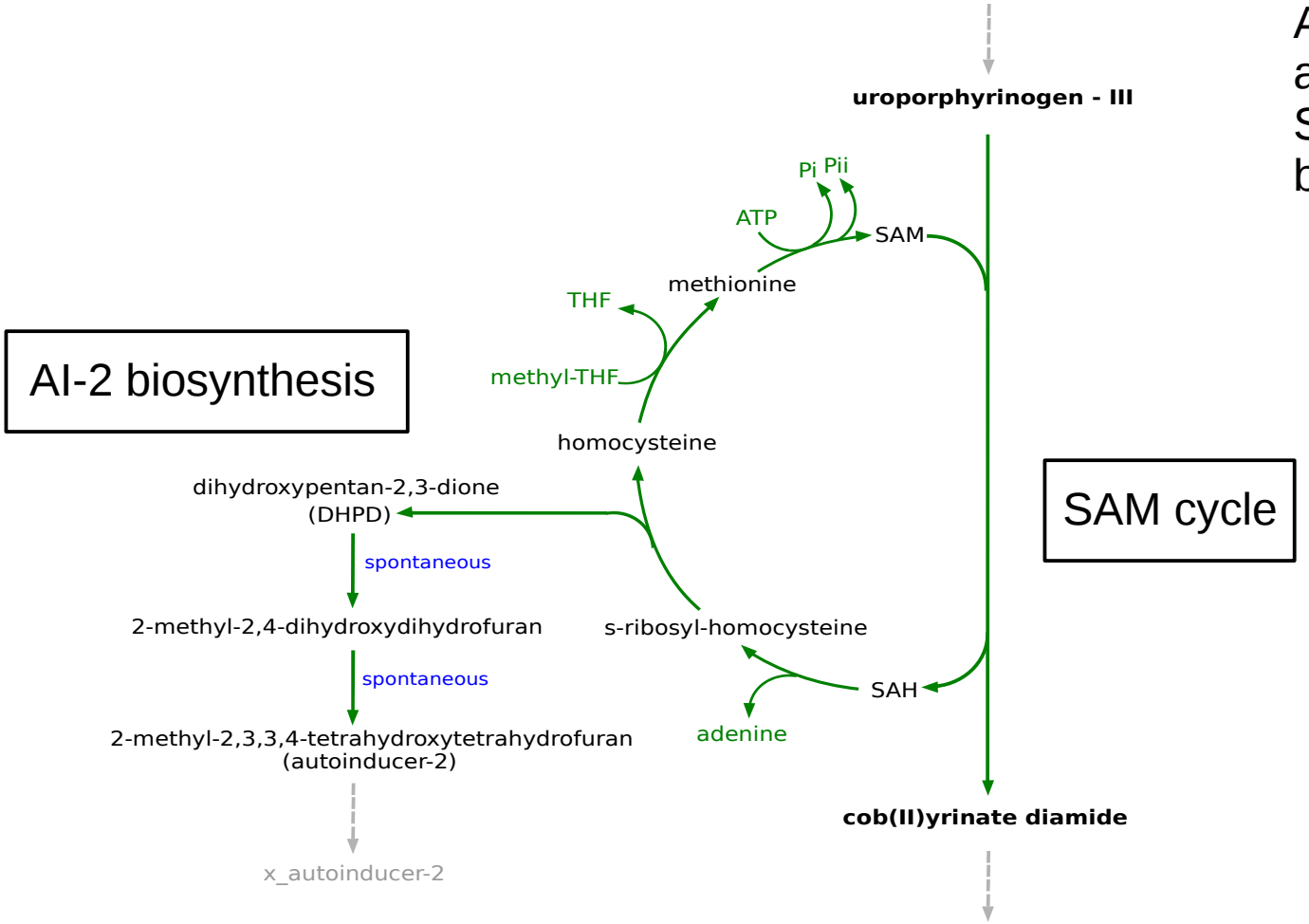

uroporphyrinogen - III
Pi Pii
ATP
SAM
methionine
a THF
a methyl-THF
homocysteine
DHPD
s-ribosyl-homocysteine
SAH
adenine
cob(II)yrinate diamide
SAM cycle

Generic metabolite names. Not balanced. Corrected with help from PRIAM

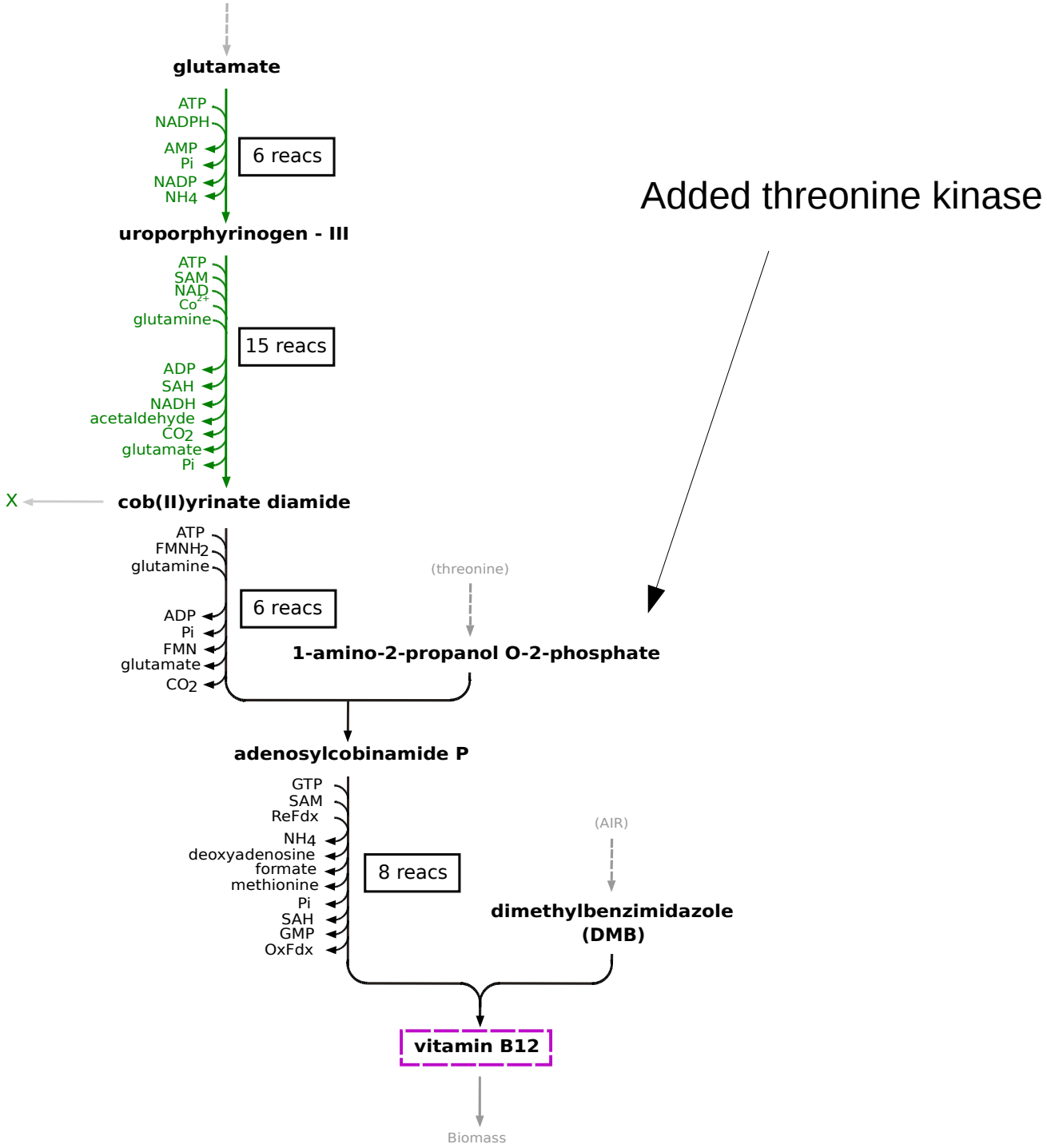



uroporphyrinogen - III
69
114
Pi Pii
136
ATP
SAM
methionine
16
THF
5
methyl-THF
homocysteine
2
DHPD
s-ribosyl-homocysteine
SAH
8
adenine
cob(II)yrinate diamide
SAM cycle
connectedness


AI-2 biosynthesis
connectedness
uroporphyrinogen - III
Pi Pii
ATP
SAM
methionine
THF
methyl-THF
homocysteine
2
dihydroxypentan-2,3-dione
(DHPD)
spontaneous
2
2-methyl-2,4-dihydroxydihydrofuran
s-ribosyl-homocysteine
spontaneous
1
2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran
(autoinducer-2)
SAH
adenine
SAM cycle
cob(II)yrinate diamide



Adding transporter for autoinducer-2 allows both SAM cycle and autoinducer-2 biosynthesis to carry flux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formate
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GMP
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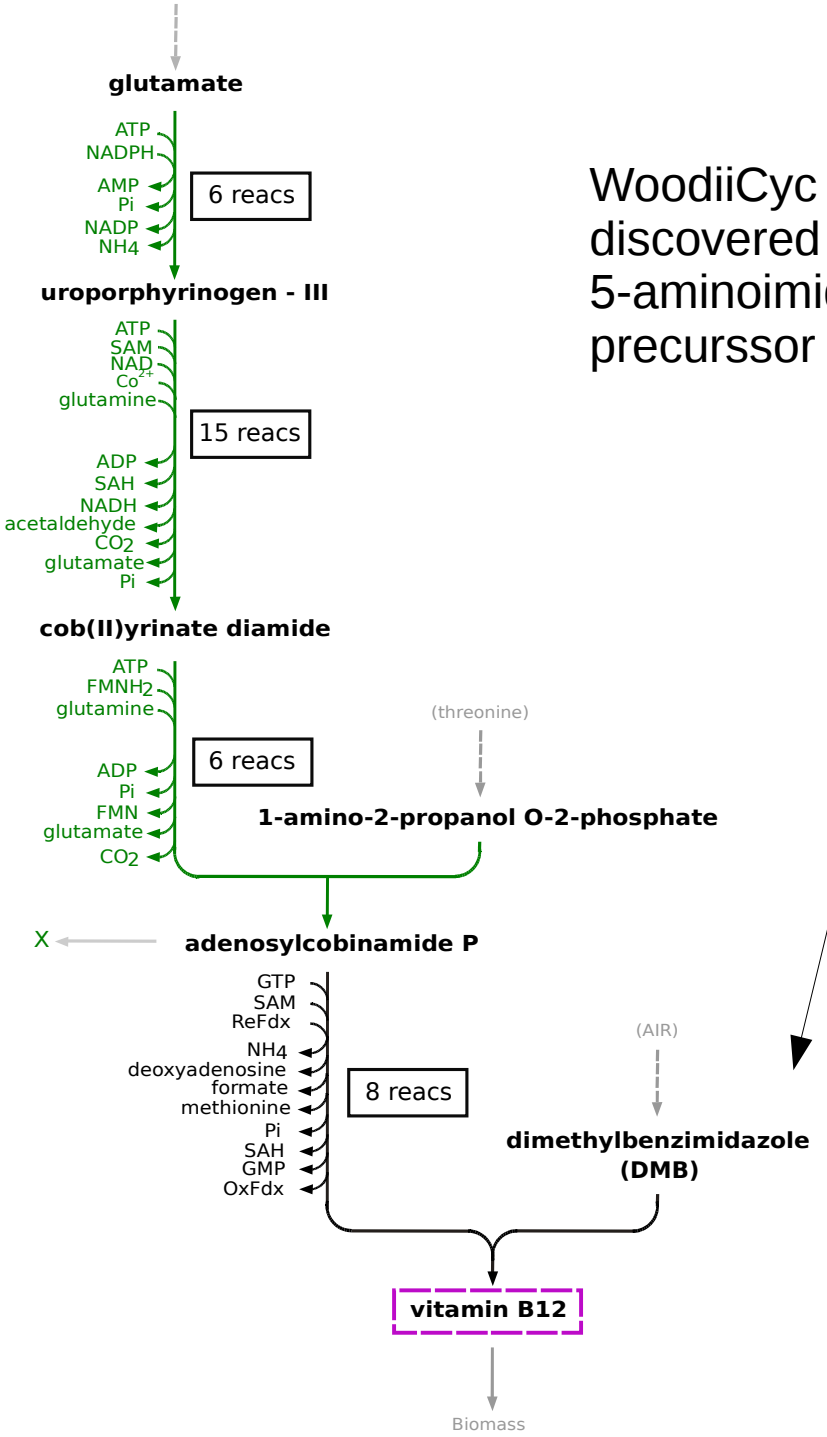
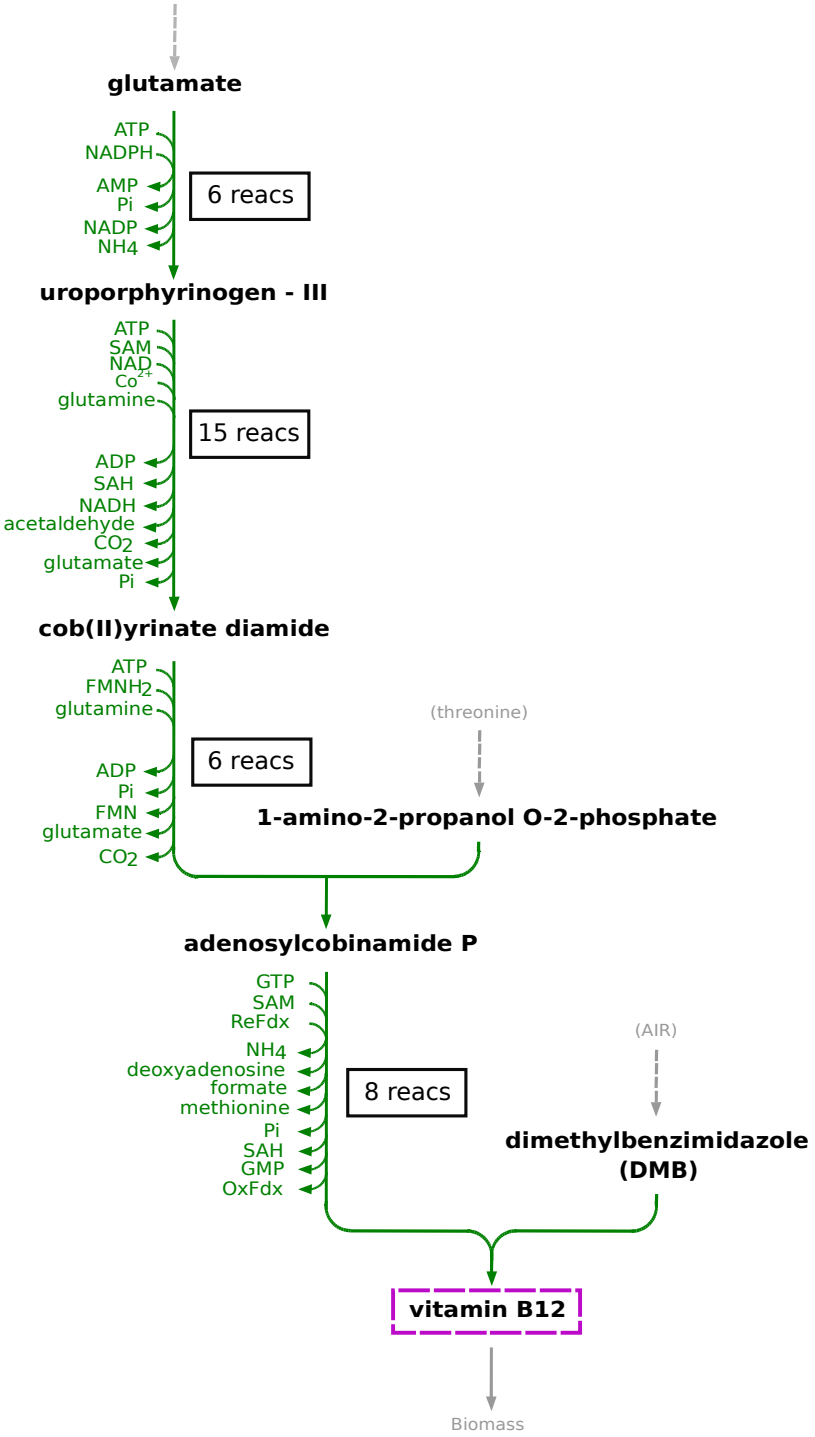
8 reacs
(AIR)
dimethylbenzimidazole
(DMB)
vitamin B12
Biomass
Added threonine kinase



WoodiiCyc was missing the recently discovered reactions linking 5-aminoimidazole ribotide (AIR) precurssor for DMB synthesis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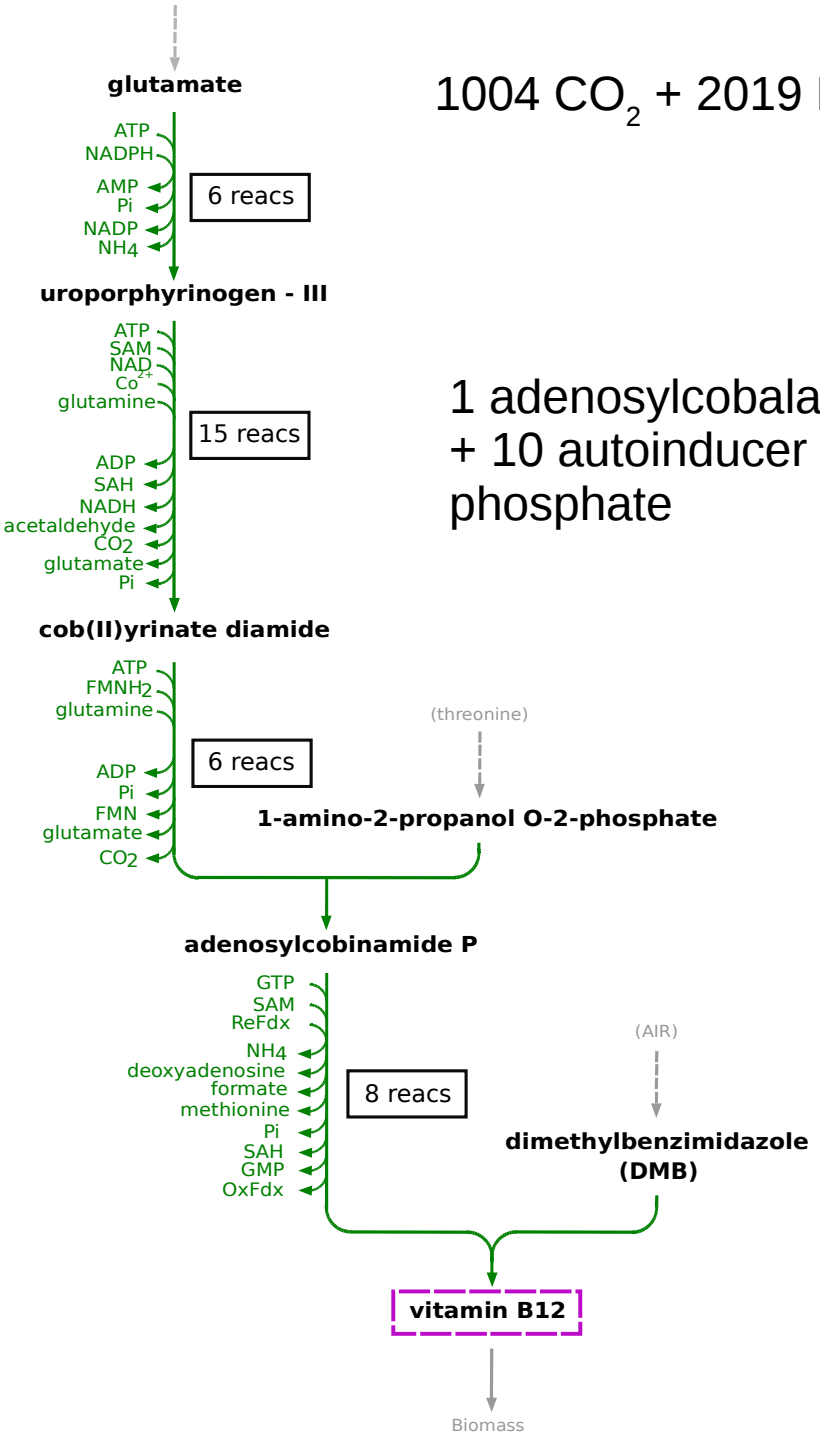

$1004\ CO_2 + 2019\ H_2 + Co^{2+} + Pi + 18\ NH_3$

1 adenosylcobalamin (B12) + 439 acetate + 10 autoinducer + deoxyribose-5-phosphate

Remaining Questions

Why does the yield of cobalamin per gDW cell range 5.5 fold when growing on different substrates?

$CO_2 + H_2$	→ 650 nMol/gDW cell
Fructose + N_2	→ 1210 nMol/gDW cell
Methanol + CO_2	→ 2672 nMol/gDW cell
Methanol + Formate	→ 3564 nMol/gDW cell

B vitamins in *A.woodii* GSM

B Vitamins	Cofactor	Producible
Ribof avin (B2)	FMN, FAD	yes
Pantothenate (B5)	CoA	no
Pyridoxin (B6)	PLP	yes
Niacin (B3)	NAD(P), NAD(P)H	yes
Cobalamin (B12)	MeCbl, AdoCbl	yes
Biotin (B7)	CoR	–
Thiamine (B1)	ThDP	–

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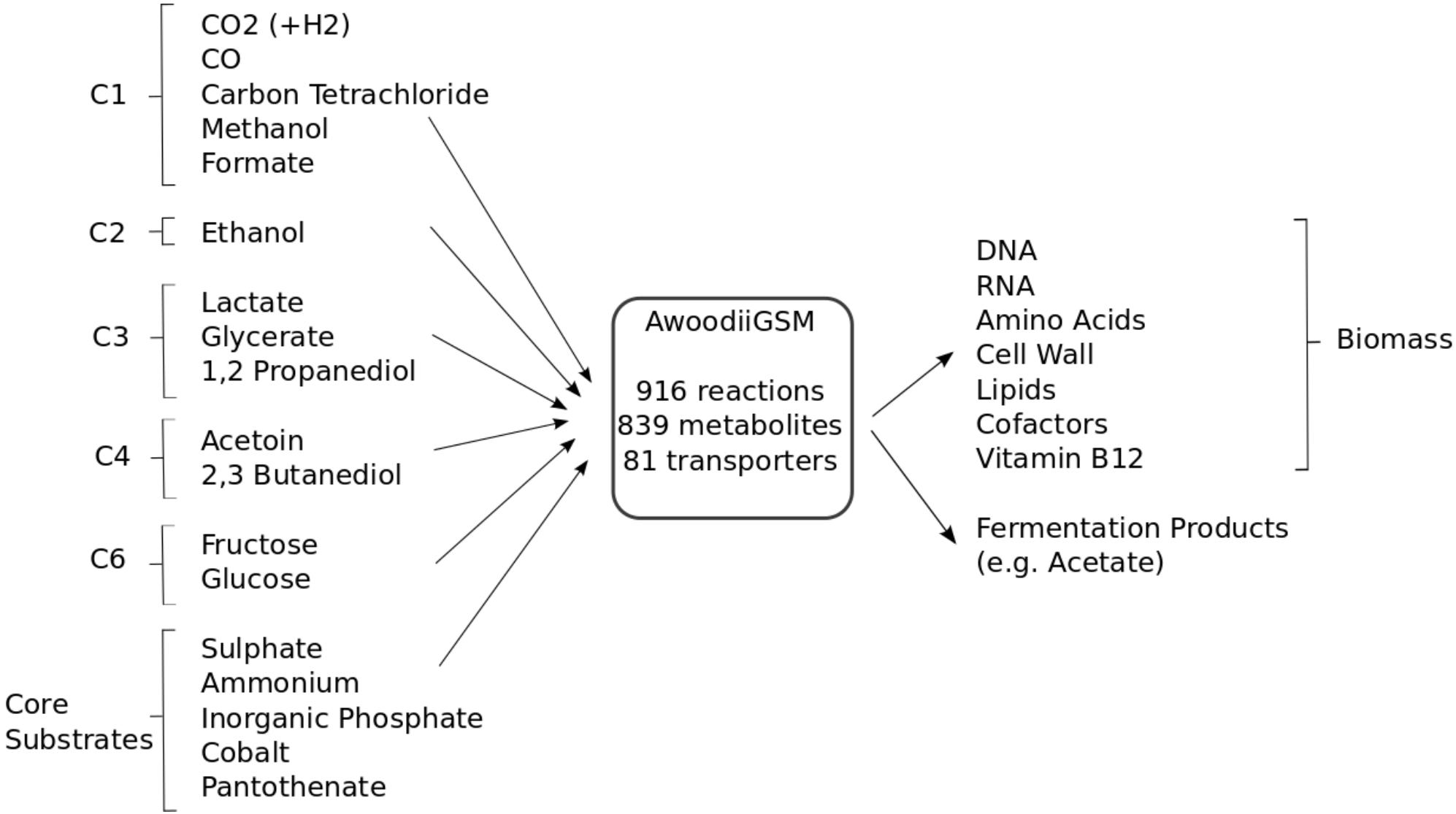
Cobalamin (B12)	MeCbl, AdoCbl	yes
Biotin (B7)	CoR	–
Thiamine (B1)	ThDP	–

Balch, W. E.; Schoberth, S.; Tanner, R. S.; Wolfe, R. S. (1977). "Acetobacterium, a New Genus of Hydrogen-Oxidizing, Carbon Dioxide-Reducing, Anaerobic Bacteria". International Journal of Systematic Bacteriology. 27 (4): 355.

Model Overview



Conclusions

- Cobalamin biosynthesis can be incorporated into *A.woodii* GSM biomass equation
- Biosynthesis of quorum sensing compounds is coupled to growth via cobalamin biosynthesis

Thanks for listening