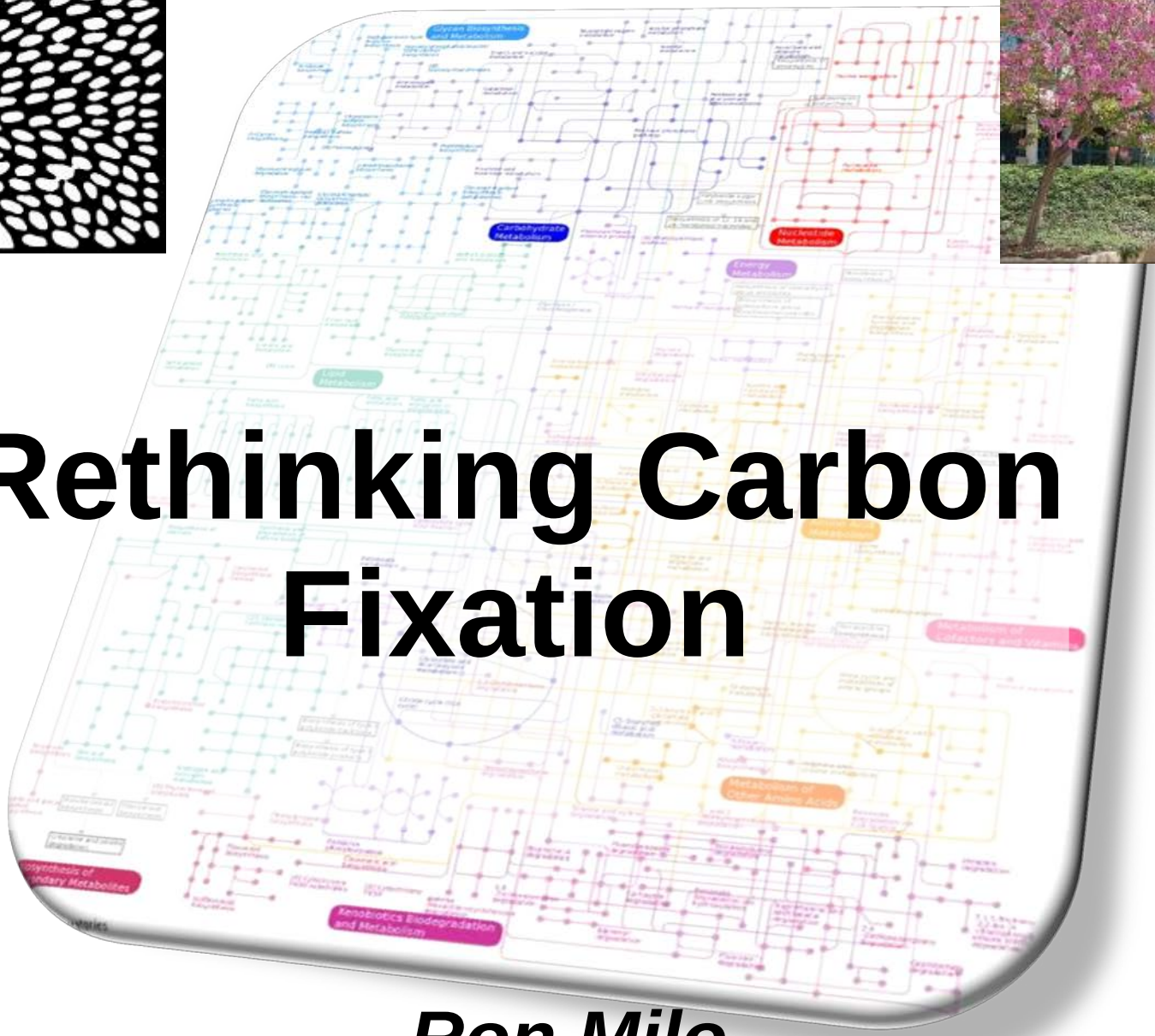


Rethinking Carbon Fixation

Ron Milo

Department of Plant Sciences

Weizmann Institute of Science


Elad
Noor
Arren
Bar-Even

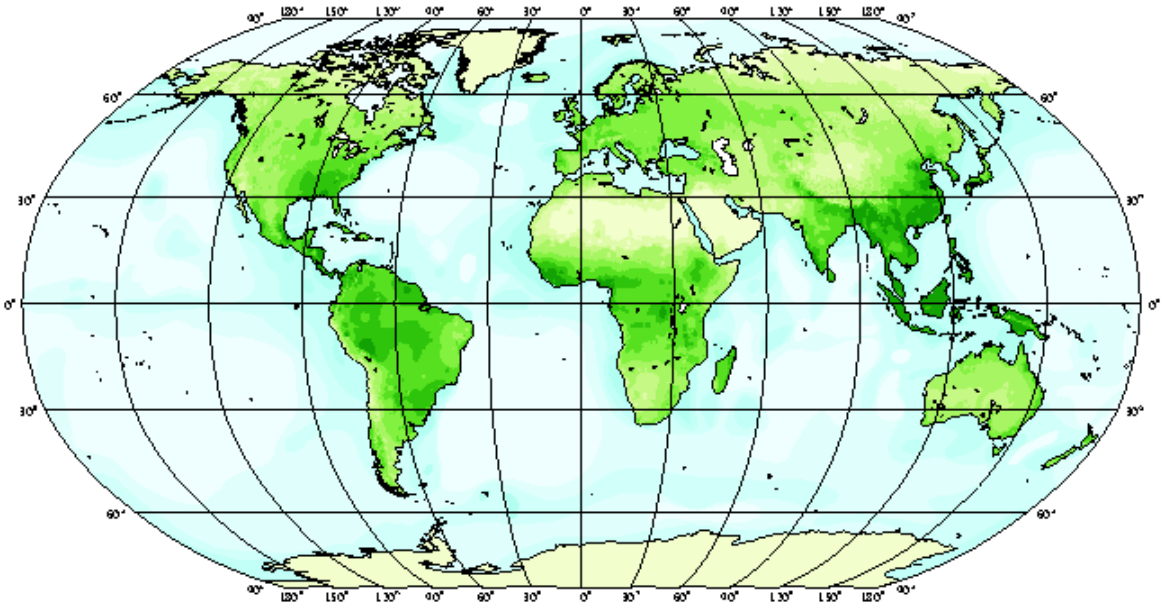
What limits maximal growth rates?

A major frontier for systems biology: Carbon Fixation

Requires majority of land and fresh water used by humanity

A molecular process that affects global climate

Major uncertainties about rates and limits



What governs the efficiency of photosynthesis and carbon fixation?

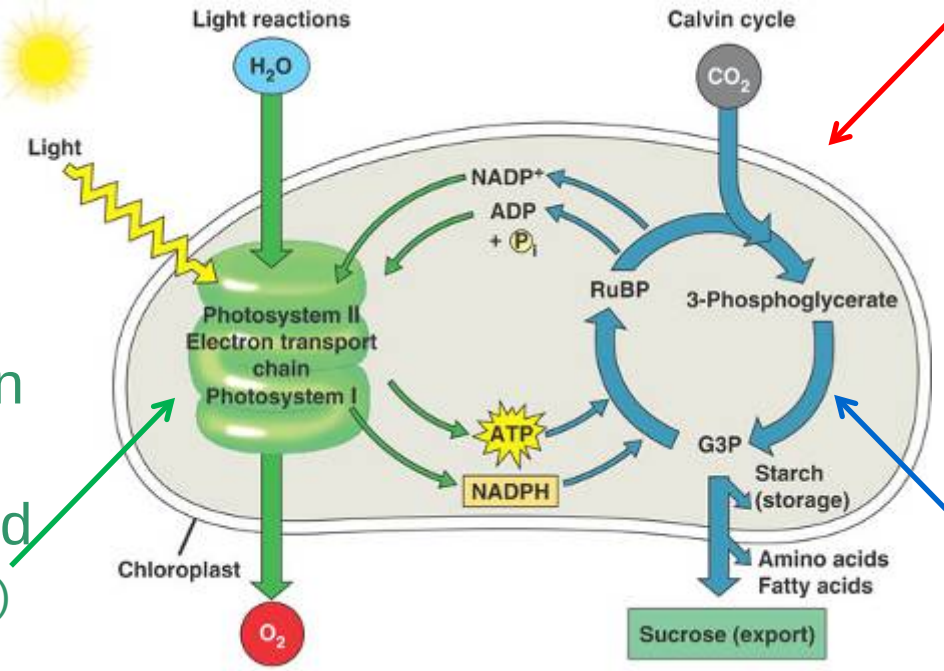
Constraints on metabolites concentrations, enzyme rates and pathways structure

(Noor et al, Mol. Cell 2010
Bar-Even et al, Biochemistry 2011
Bar-Even et al, PLOS CB 2011)

growth

Rubisco rate is at a limit

(Savir et al, PNAS 2010)



Design principles in photosynthesis: wavelengths utilized

(Milo, Photos. Res. 2009)

Synthetic carbon fixation pathways for higher productivity

(Bar-Even et al, PNAS 2010;
Bar-Even et al, JXB 2012)

The Calvin-Benson cycle drives carbon fixation

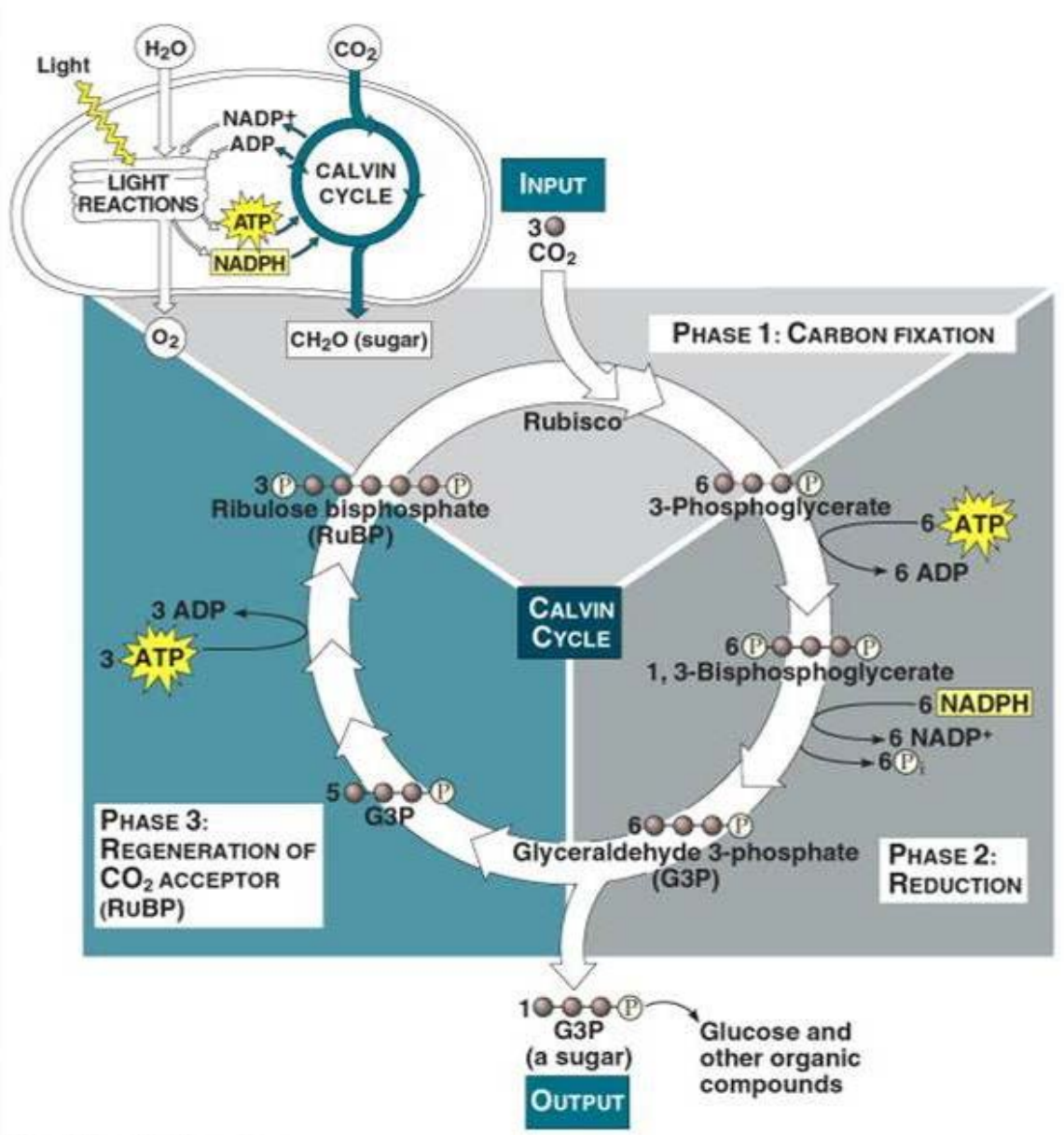
Photosynthesis fixates

carbon into organic compounds

Rubisco - key carboxylating

enzyme: capturing CO_2 and

producing 3-carbon sugars



Can we find novel ways to achieve carbon fixation?

Constraints are different → productivity and rates might be higher (e.g. domestication)

Test our understanding of what limits Nature in evolving metabolic pathways

Finding synthetic alternatives to the Calvin-Benson Cycle

A

Nature uses six carbon fixation pathways

B

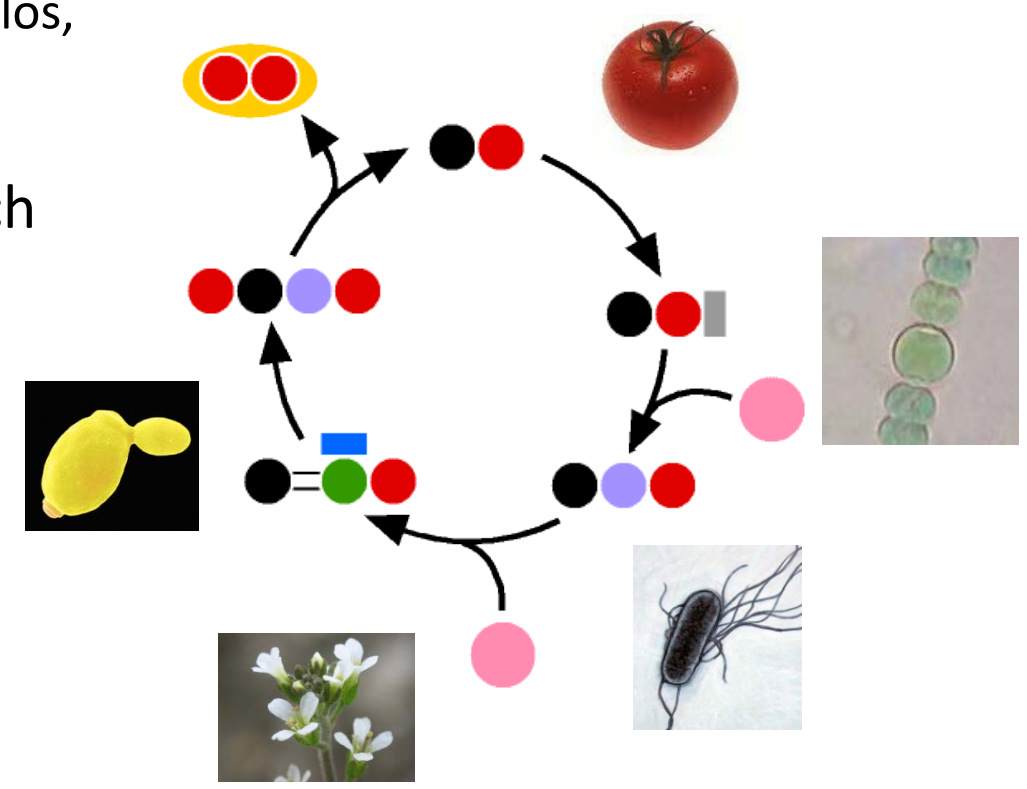
Synthetic carbon fixation pathways for increased productivity

C

KEGG database ~5000 naturally occuring enzymes

Synthetic biology & metabolic engineering
(following Kiesling, Stephanopoulos, Maranas, Hatzimanikatis,…)

A “mix and match” approach



How to compare the synthetic pathways?

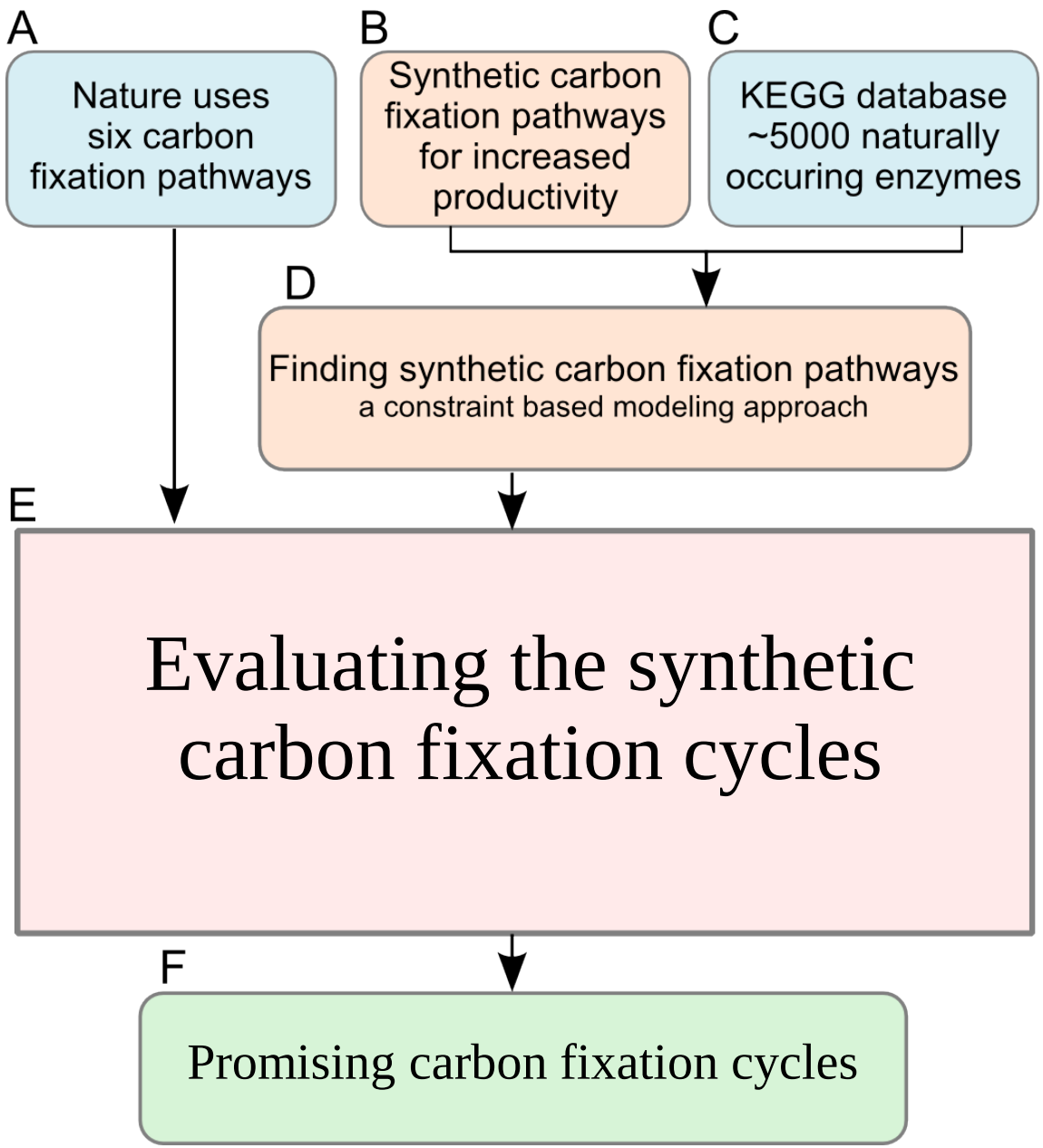
Exploration of $(\mathbf{5000})^{\mathbf{n}}$ possible networks

Novel visualization method of metabolic pathways

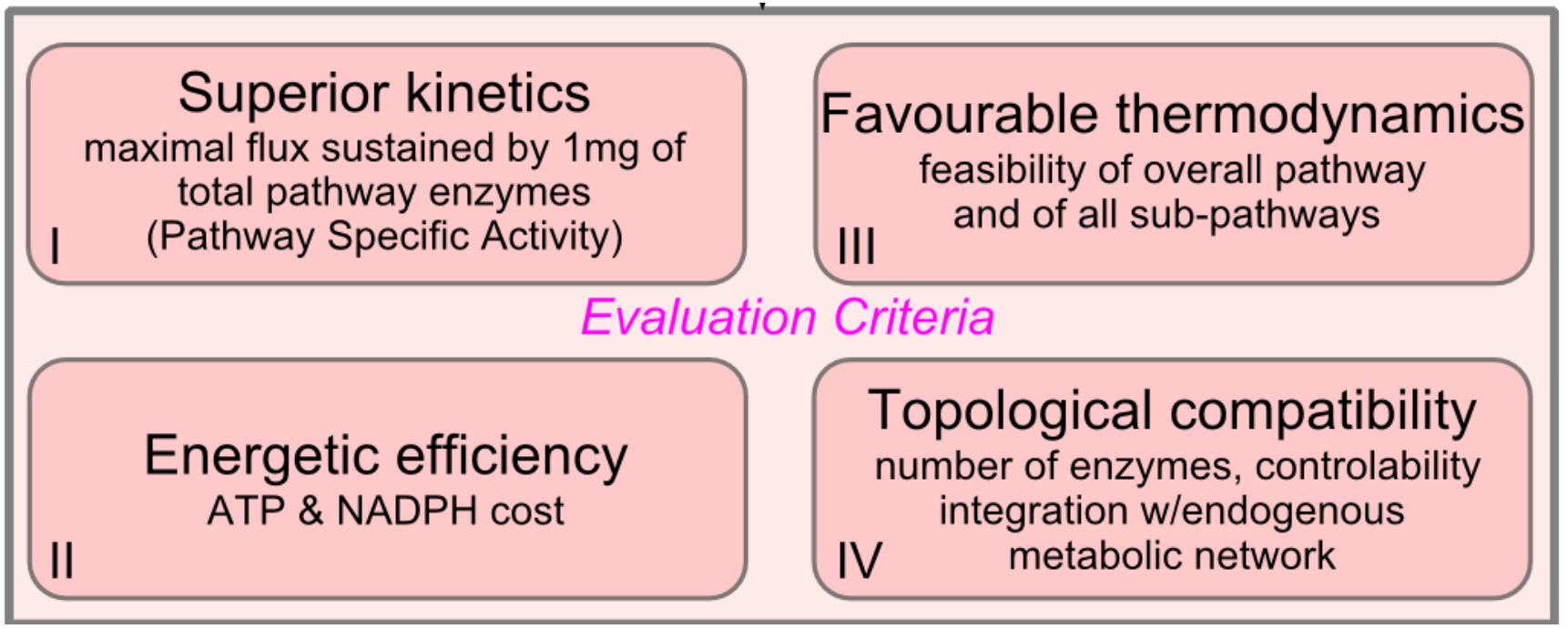
Manual curation
>1500 papers
>100 metabolic cycles

New methods to predict pathways rates

Integrated thermodynamic models

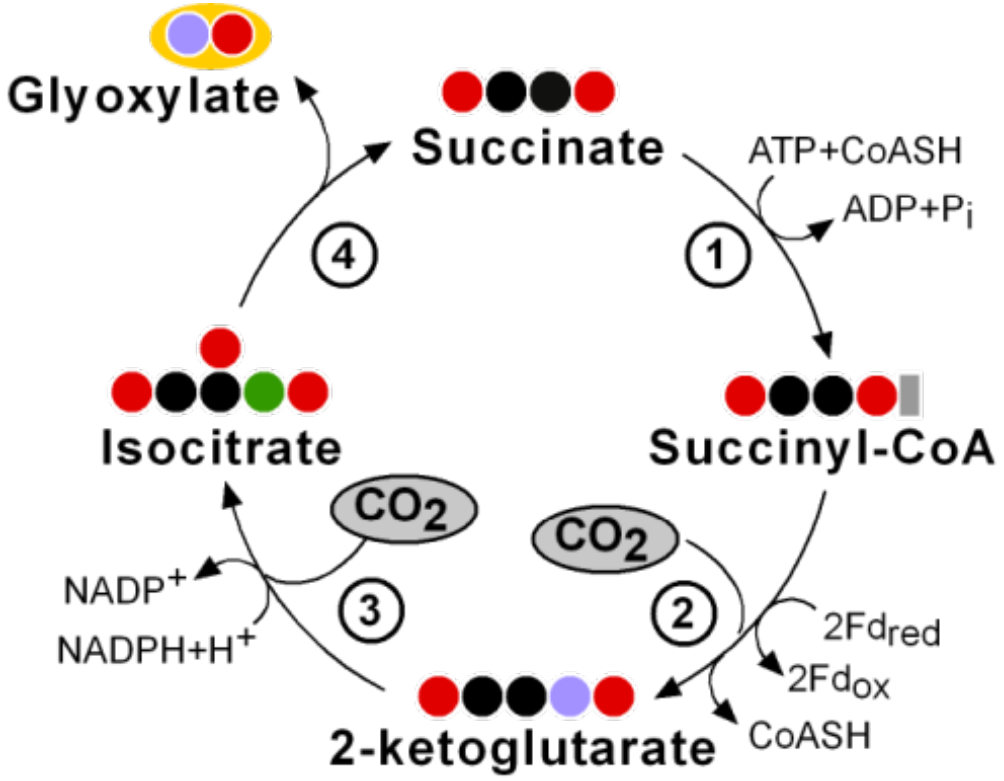


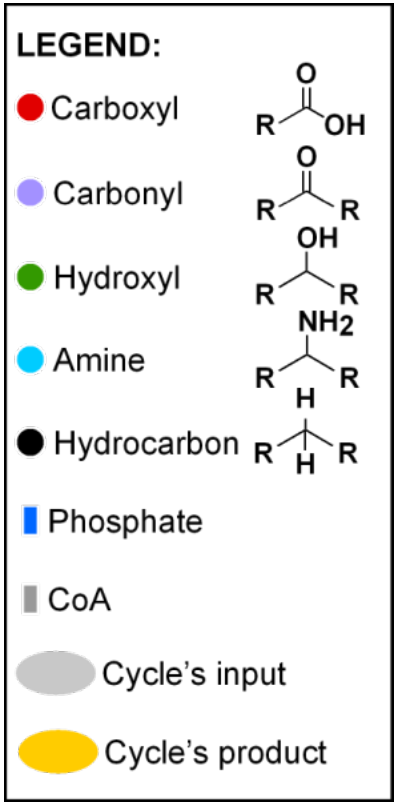
We systematically compare all possible synthetic carbon fixation pathways



The simplest carbon fixation cycles are not useful

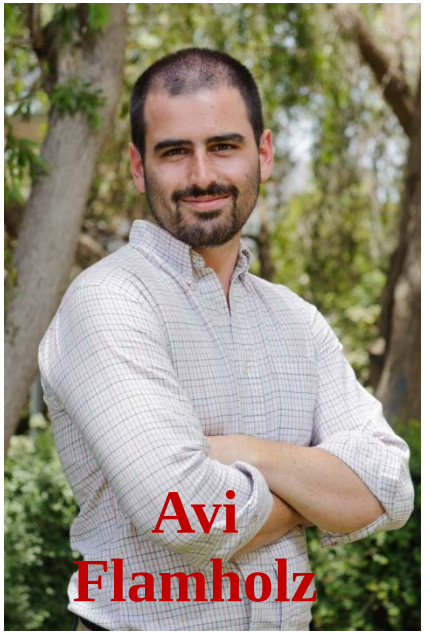
The simplest cycles are:
(1) Thermodynamically infeasible
(2) Kinetically slower
(3) Employing oxygen sensitive enzymes





Bar-Even et al., PNAS, 2010

Equilibrator - a web interface for thermodynamic analysis of biochemical systems



eQuilibrator

biochemical thermodynamics calculator

Search

Type a **compound name** or **reaction** or try an **example** below.

Examples

Reactions	Compounds	Enzymes
Glucose => 2 Ethanol + 2 co2	ATP	Rubisco
L-Malate + NAD+ => Oxaloacetate + NADH	Glucose	Aldolase
ATP + Water <=> ADP + Phosphate	Succinyl-CoA	Hexokinase

About eQuilibrator | Classic Reactions | Downloads | FAQ

Rational design converges with natural selection

Under constraint of using Rubisco as carboxylating enzyme:

-> Ideal solution is Calvin cycle

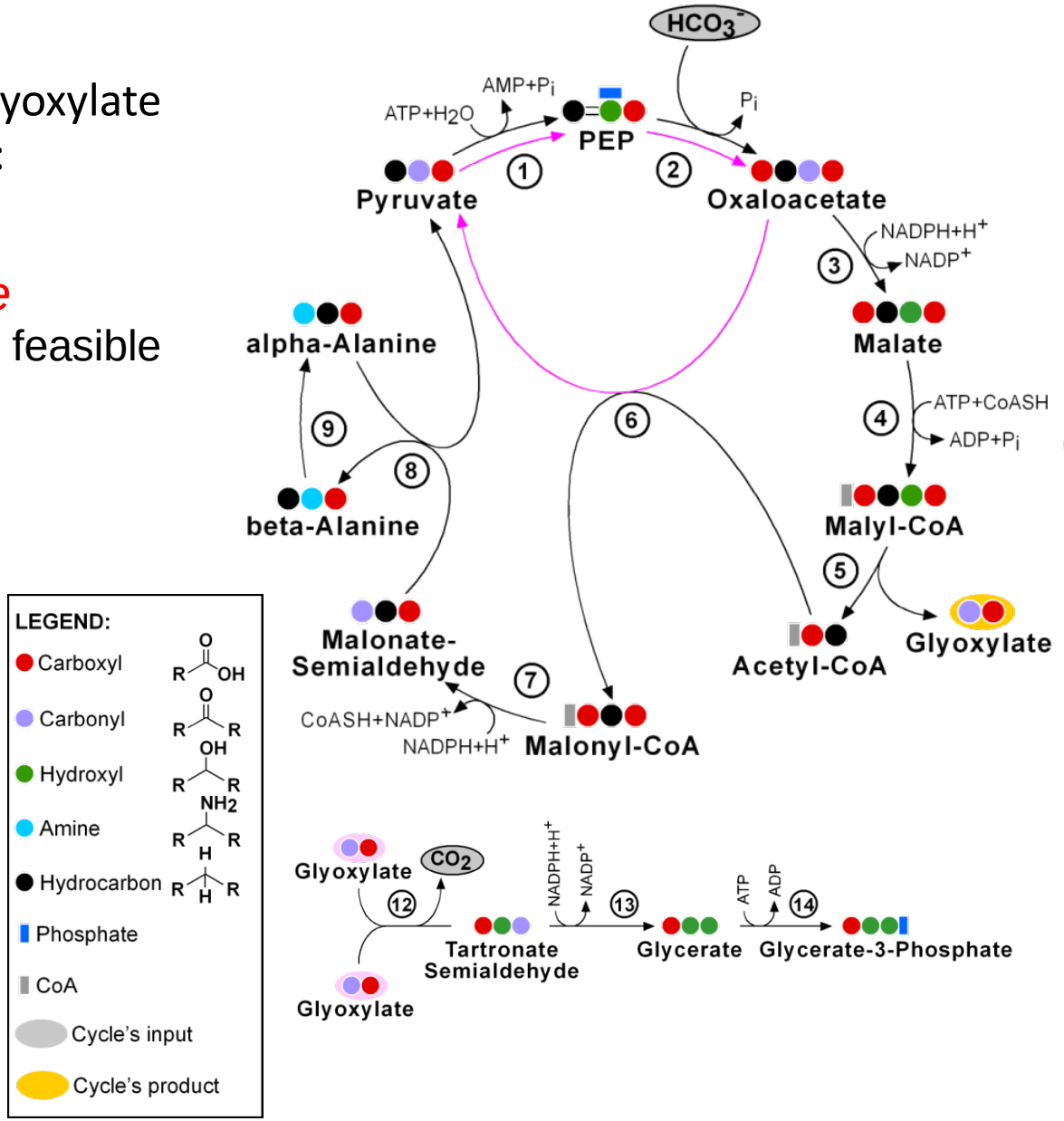
With no constraints other than using naturally occurring enzymes…

We find a family of promising novel pathways

The Malonyl-CoA – Oxaloacetate – Glyoxylate family of pathways is predicted to be:

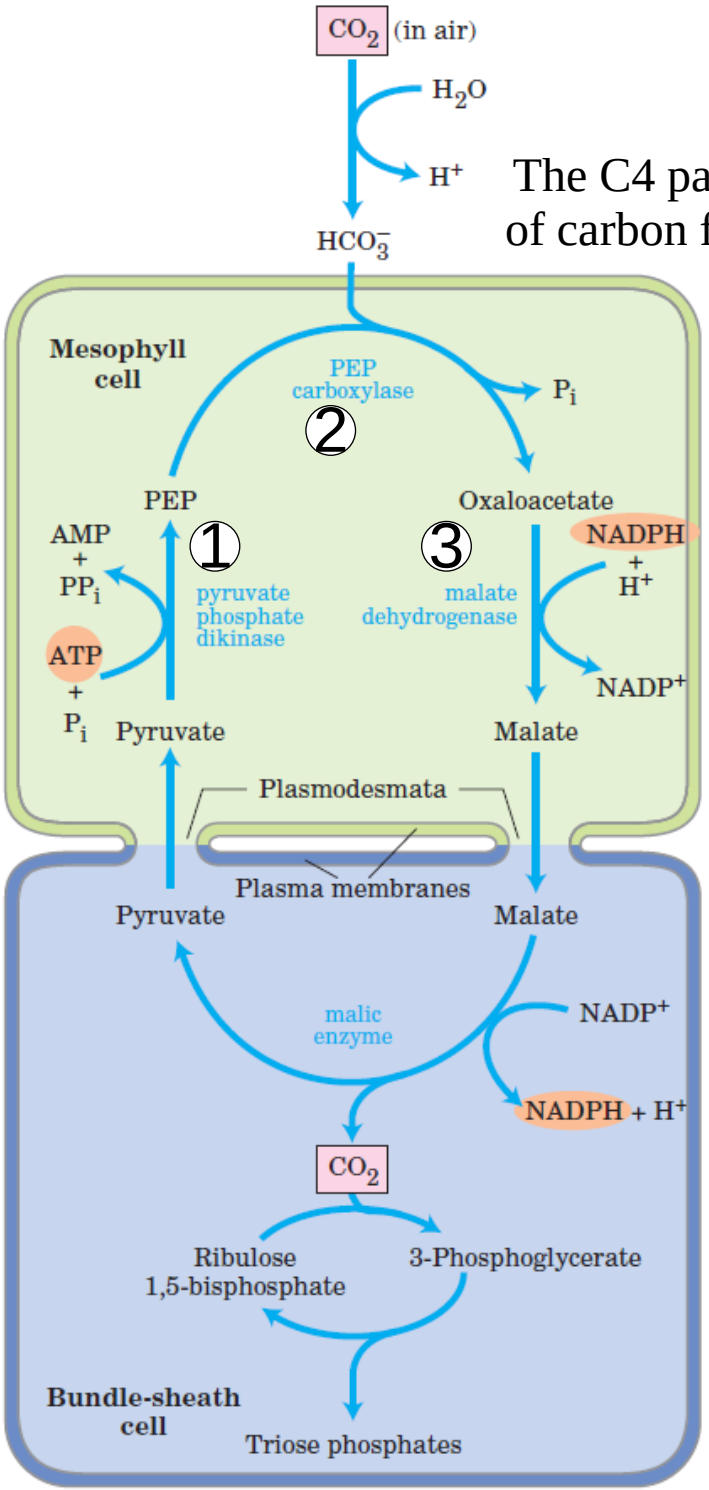
(1) Kinetically faster
2-3 fold faster than Calvin Cycle
(2) Thermodynamically & energetically feasible
(3) Impose least metabolic changes

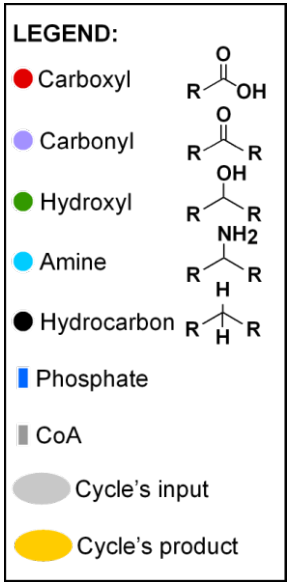
Can we achieve better results than natural pathway? Maybe if the constraints are different

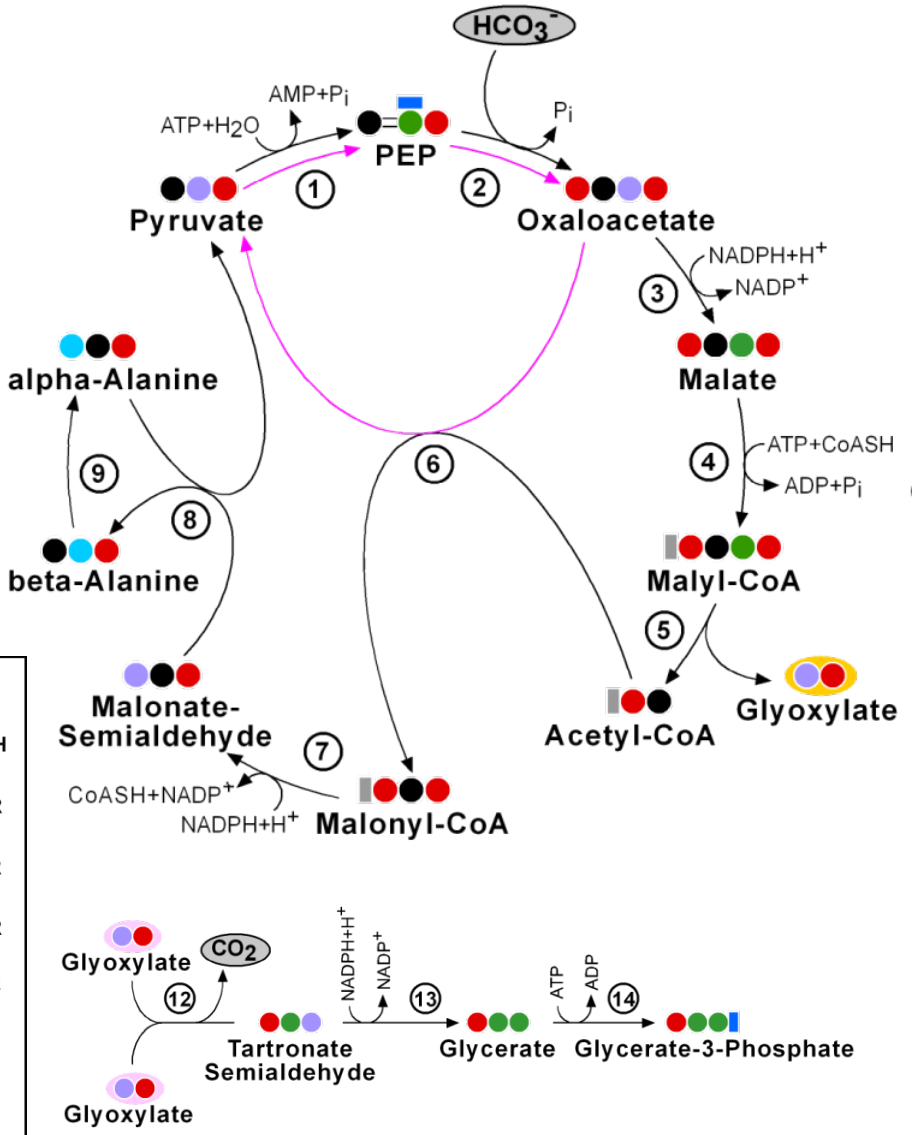


Bar-Even et al., PNAS, 2010

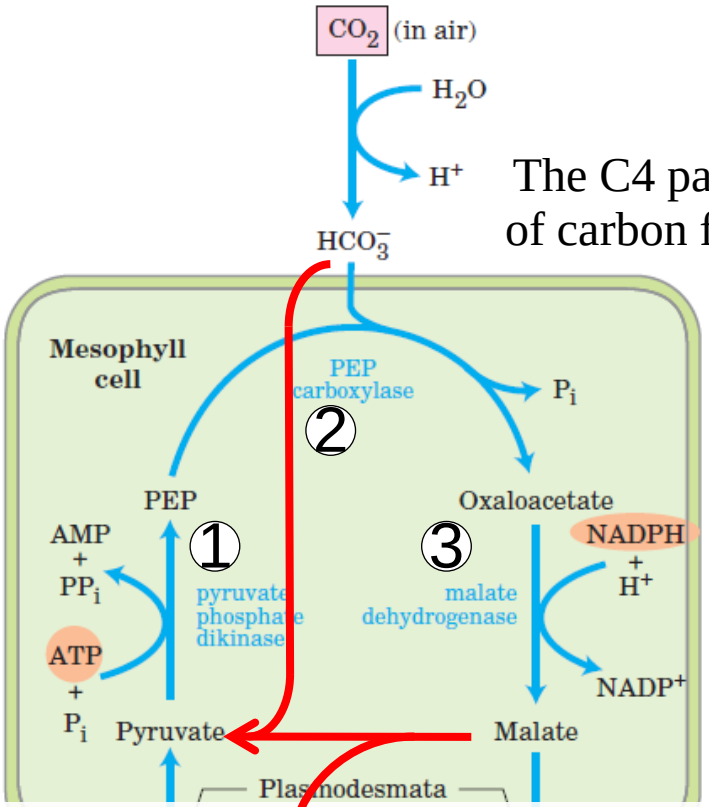
The C4 pathway of carbon fixation

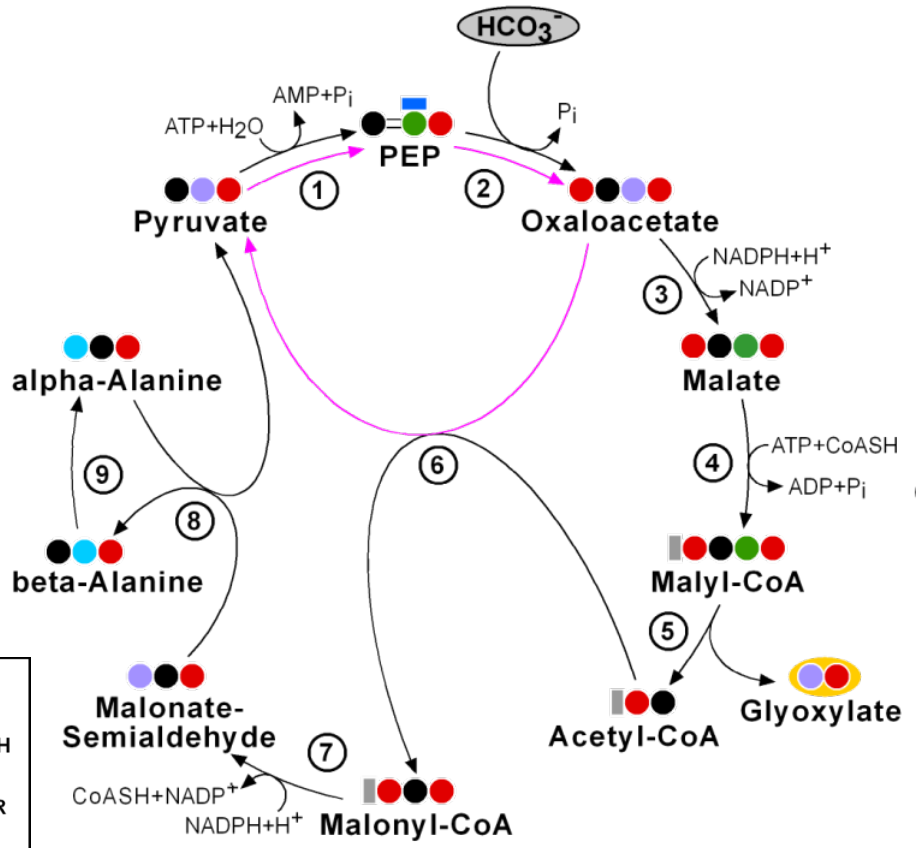


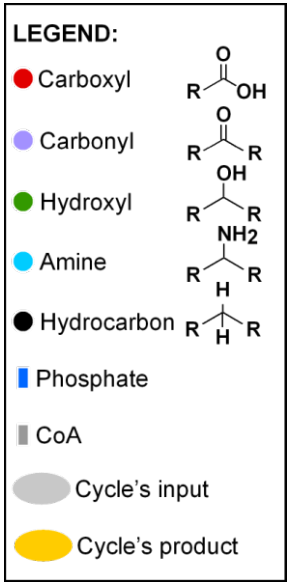


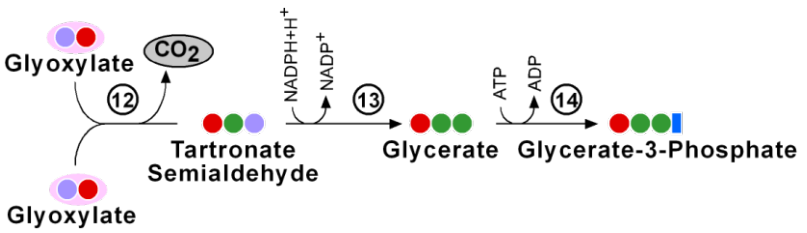


The C4 pathway of carbon fixation









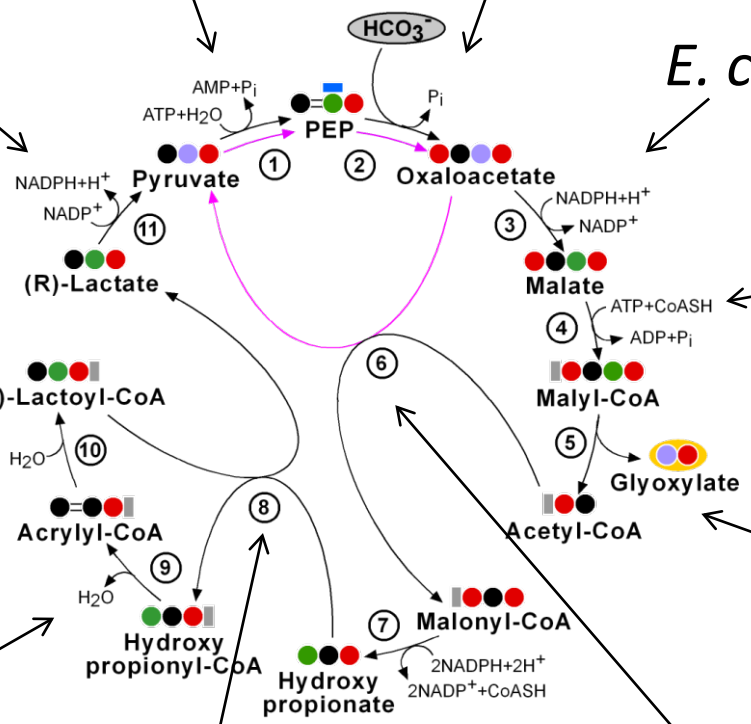
Testing the synthetic cycles by merging enzymes from several organisms

E. coli

E. coli

E. coli

E. coli



Methylobacterium
(sequence available)

Clostridium
(sequence not available)

Rhodobacter
(sequence available)

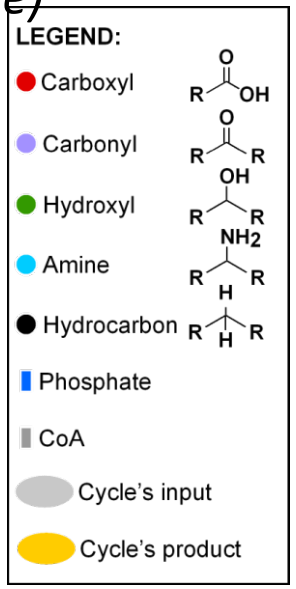
E. coli

Clostridium
(sequence available)

Chloroflexus
(sequence not available)

Propionibacterium
(sequence available)

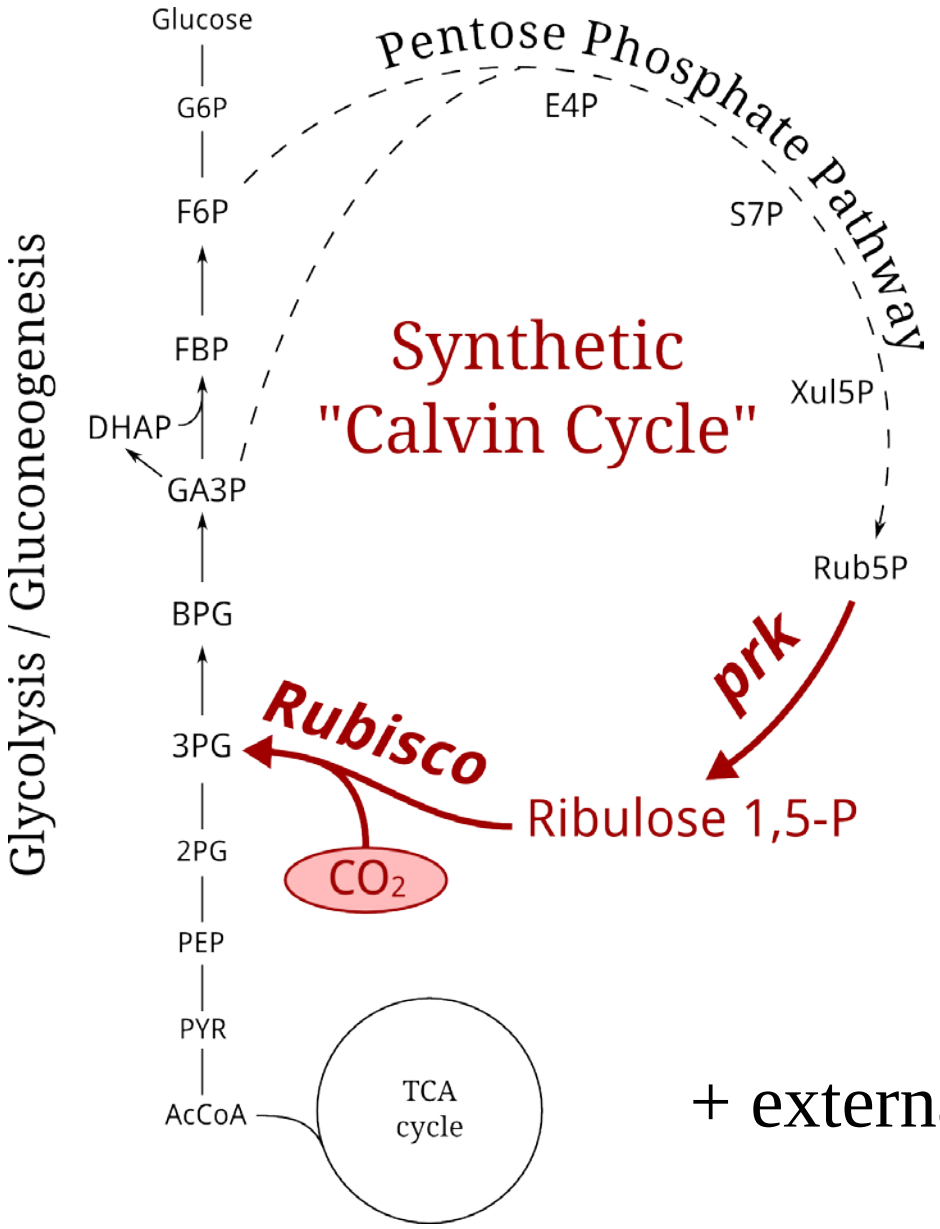
In Vitro



Testing computational predictions *in vivo*:

we develop a model system for comparing novel carbon fixation pathways

Only two genes are missing in *E. coli* to complete the Calvin-Benson cycle



See also:
Bonacci, …, Savage, PNAS 2012

+ external energy source

The real energy sources….

Toxicity hampers cloning efforts

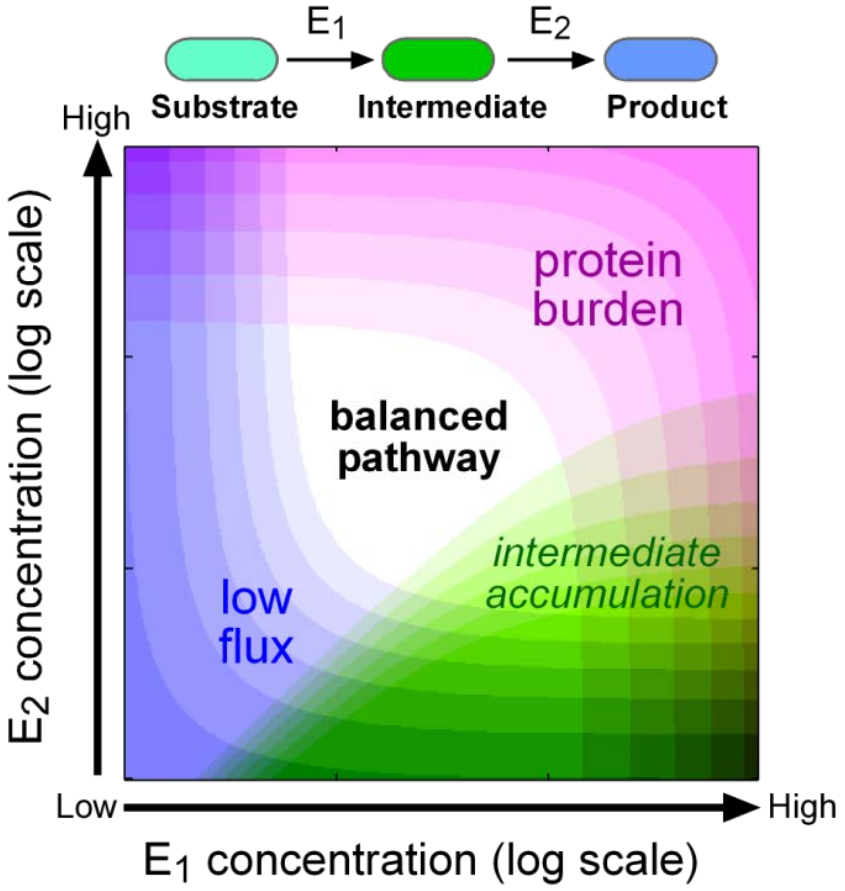
Enzyme levels are either too high or too low, or combination

Requires a novel method for exploring space of expression levels

Different expression levels in a synthetic metabolic pathway should be tested to find a balanced pathway

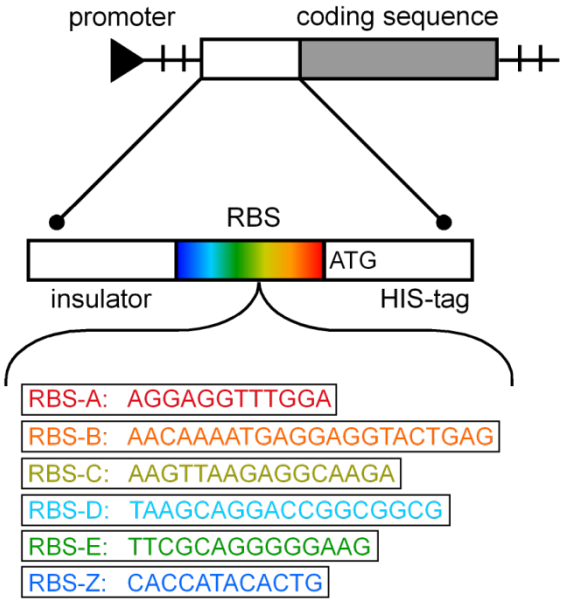
Location in expression space of the “ideal” balance is very difficult to predict

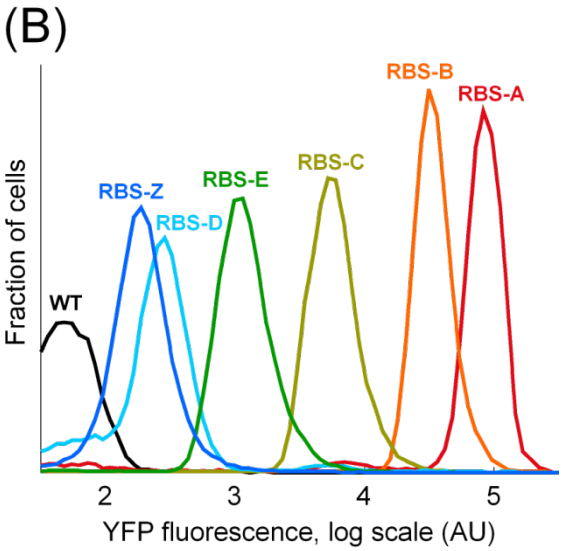
Requires experimental method to explore expression space efficiently



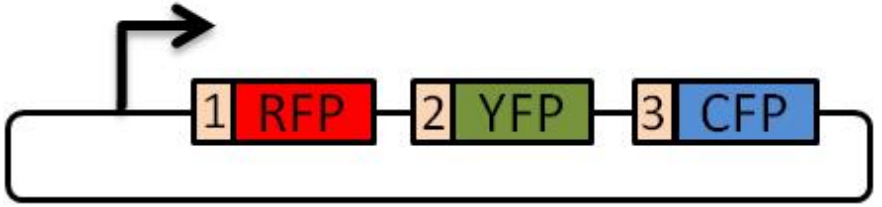
Ribosome binding site modulation explores expression space

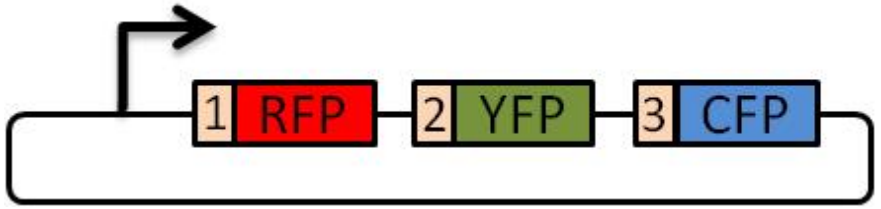
Following: *Salis, Mirsky & Voigt, NBT 2009*

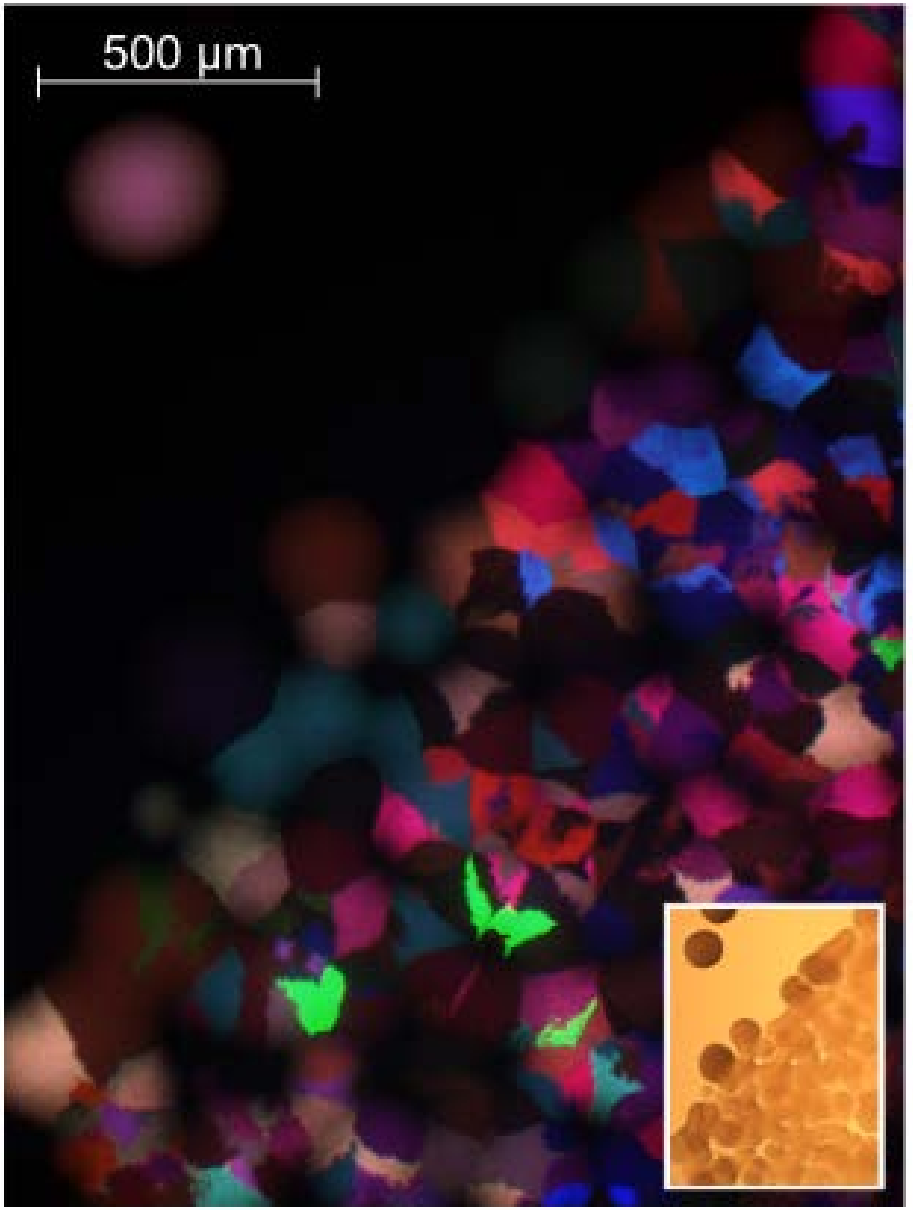


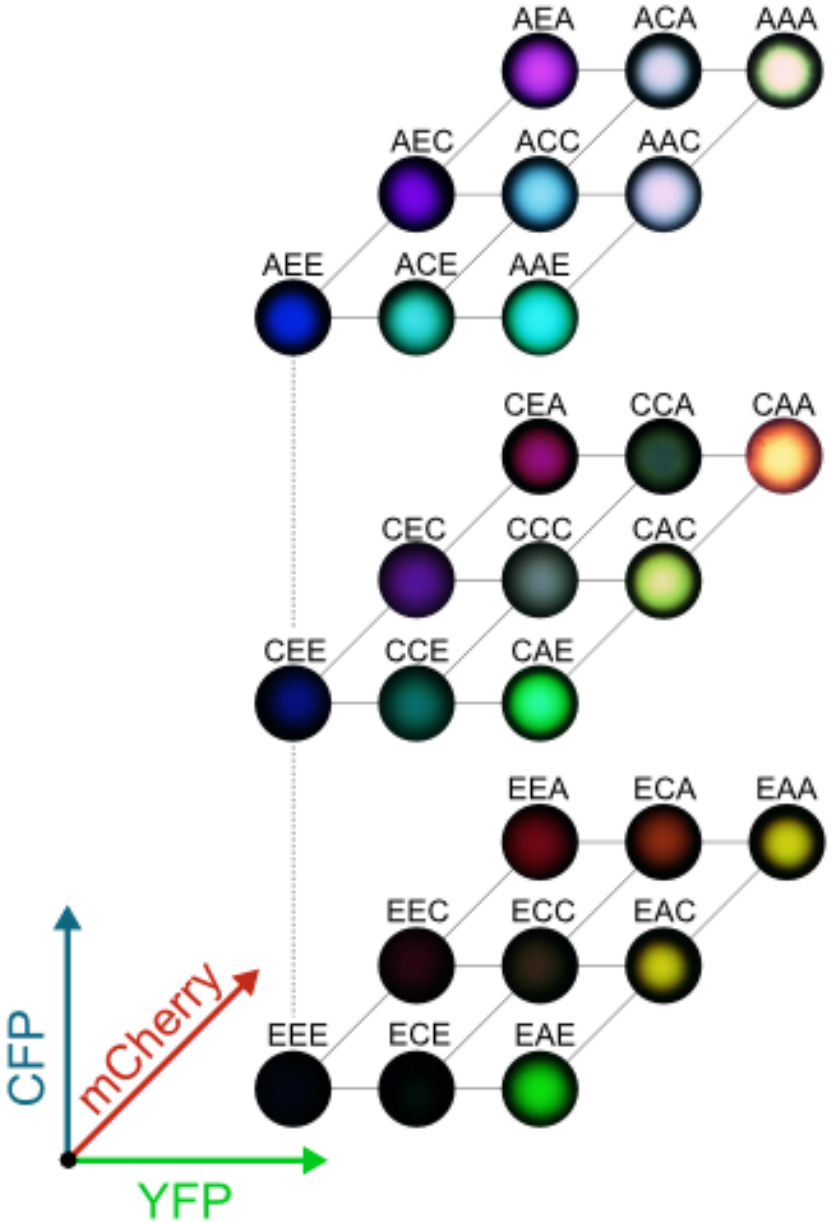


Proof of concept – using ribosome binding sites to span fluorophores expression space

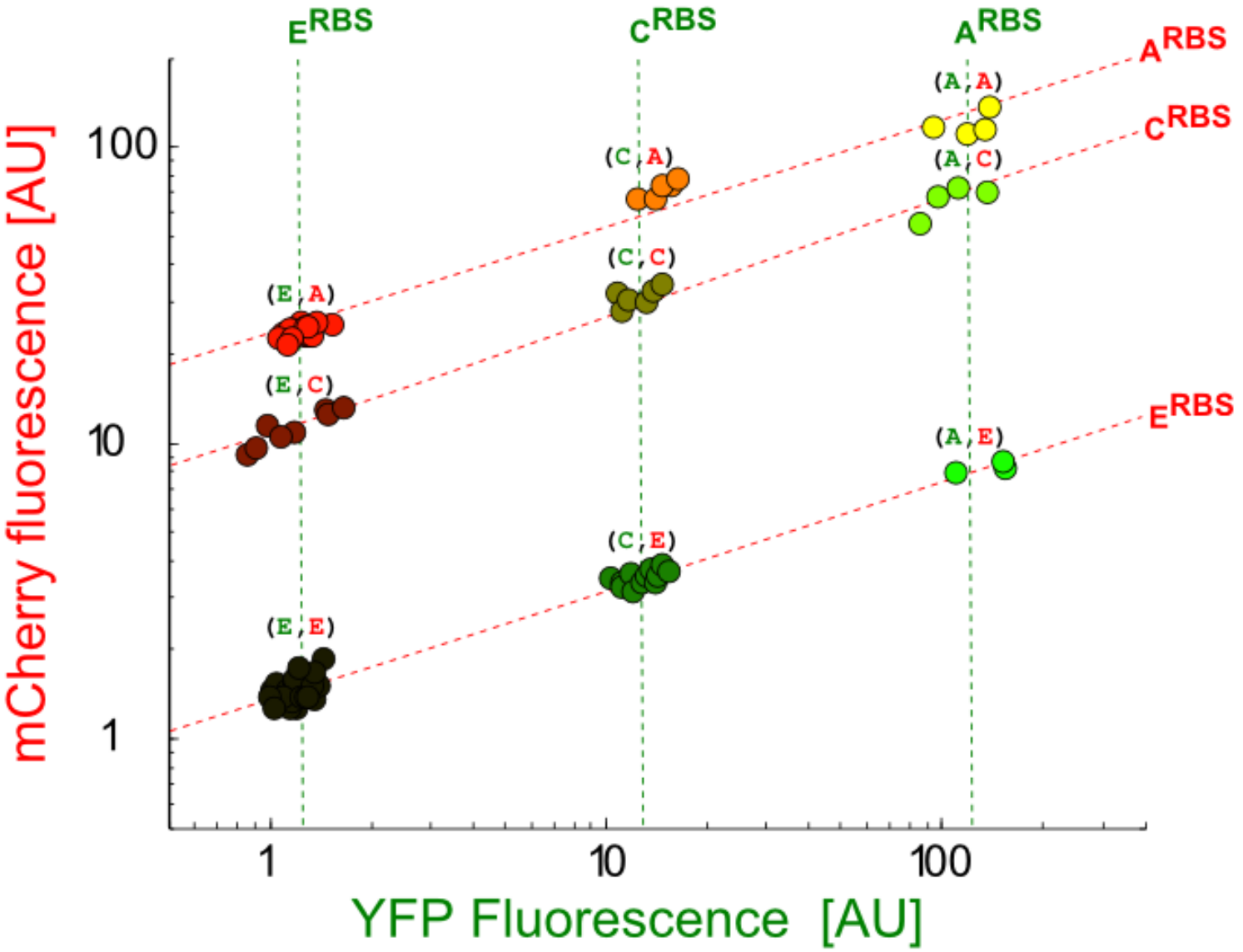



1 RFP
2 YFP
3 CFP


500 µm


AEA
ACA
AAA
AEC
ACC
AAC
AEE
ACE
AAE
CEA
CCA
CAA
CEC
CCC
CAC
CEE
CCE
CAE
EEA
ECA
EAA
EEC
ECC
EAC
EEE
ECE
EAE
CFP
mCherry
YFP

Expression space is spanned in a combinatorial manner over ~2 orders of magnitude




• Enabled us to solve the toxicity problem in bacteria
• Useful for optimizing pathways

Expressing & testing key steps of carboxylation

Ribose-5-P

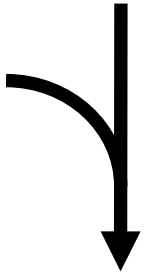
(Ribose-5-phosphate isomerase, *E. coli*)

Ribulose-5-P

PRK (Phosphoribulokinase, *S. elongatus)*

Ribulose-1,5-BP

CO_2



Rubisco (R. rubrum)

Glycerate-3P (x2)

in-vitro carbon fixation assay using *E.coli* extracts

Ribose-5-P

(Ribose-5-phosphate isomerase, *E. coli*)

Ribulose-5-P

ATP

PRK (Phosphoribulokinase, *S. elongatus)*

Ribulose-1,5-BP

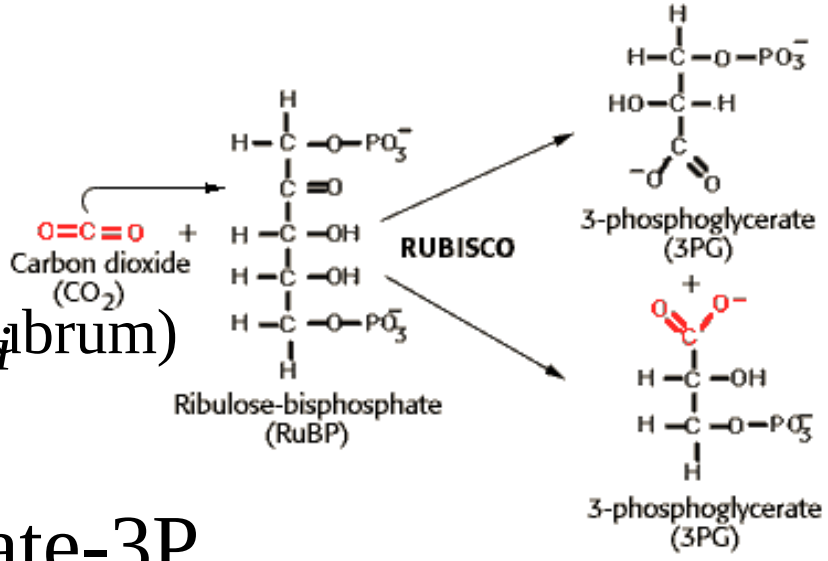
$^{13}CO_2$

Rubisco (*R. rubrum*)

"Carbon fixating" *E. coli* cell extract

^{13}Glycerate-3P + Glycerate-3P

?



Carbon fixation assay using LC-MS

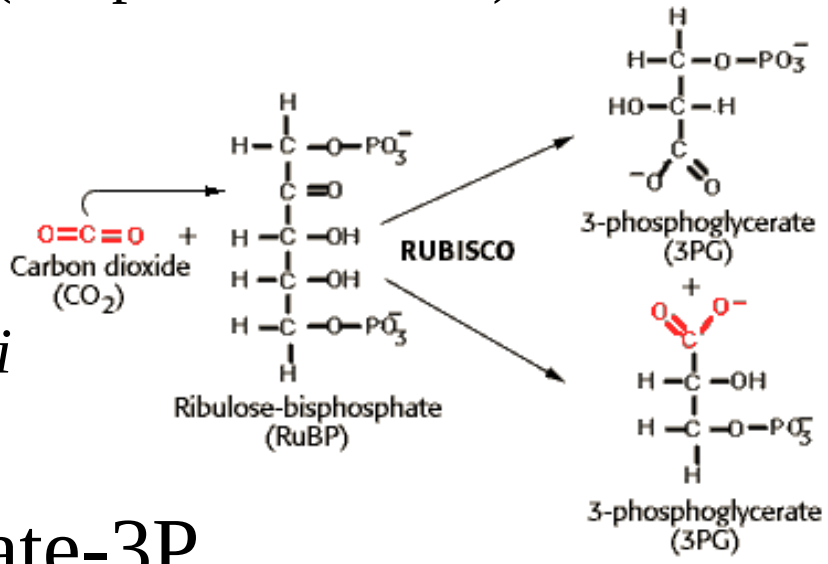
Ilana Rogachev and Sergey Malitsky (Asaph Aharoni lab)

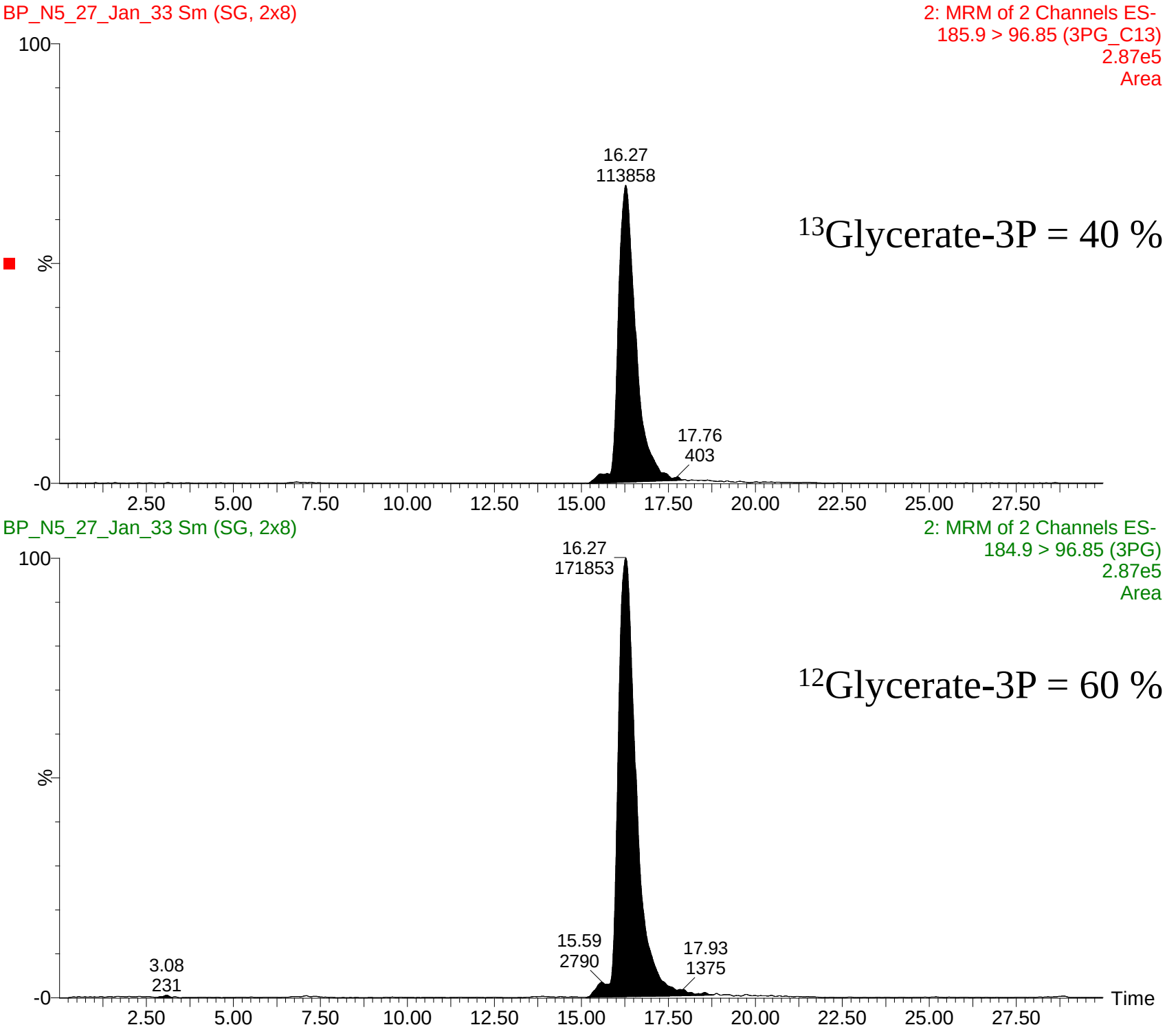
Ribulose-1,5-BP

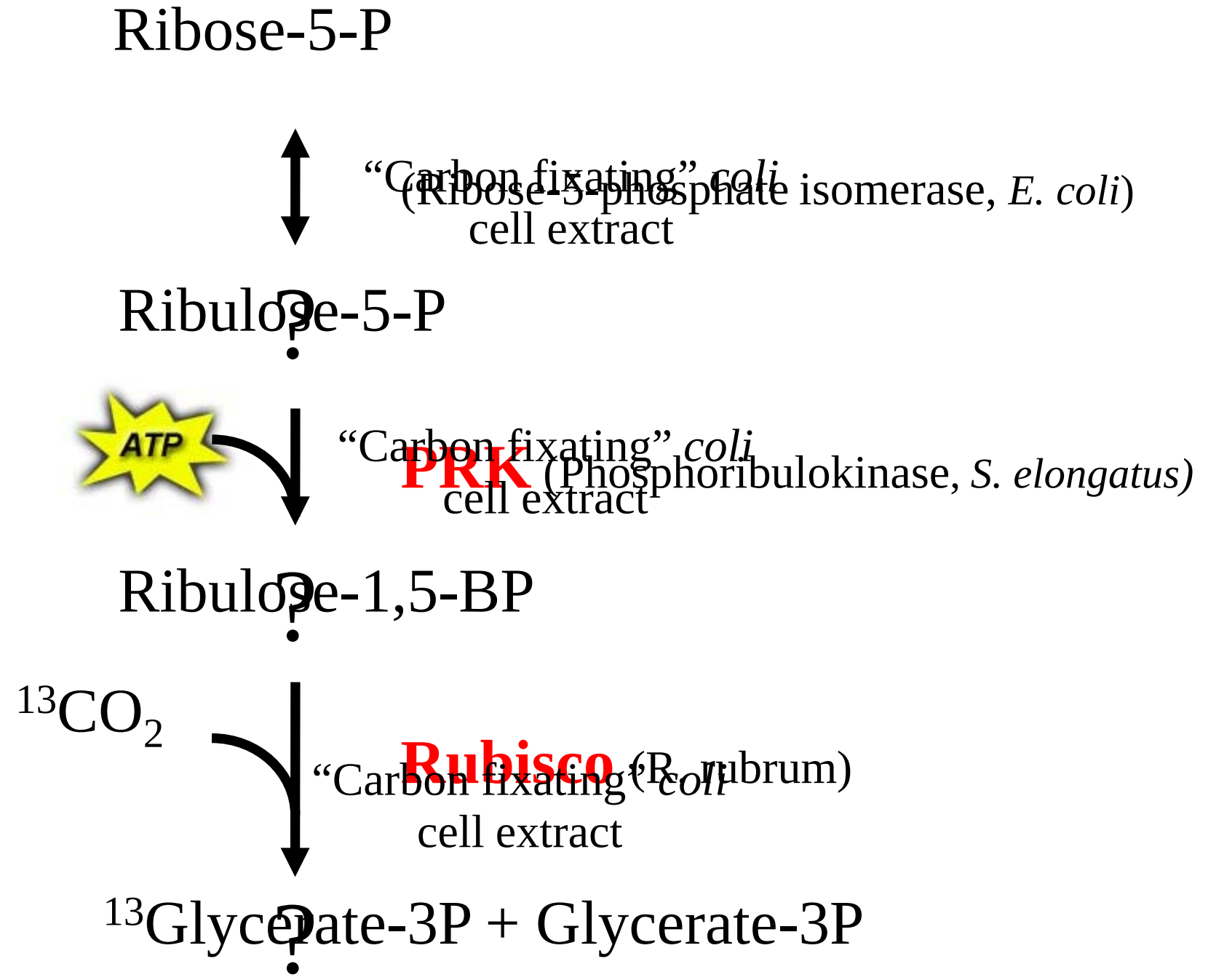
$^{13}CO_2$

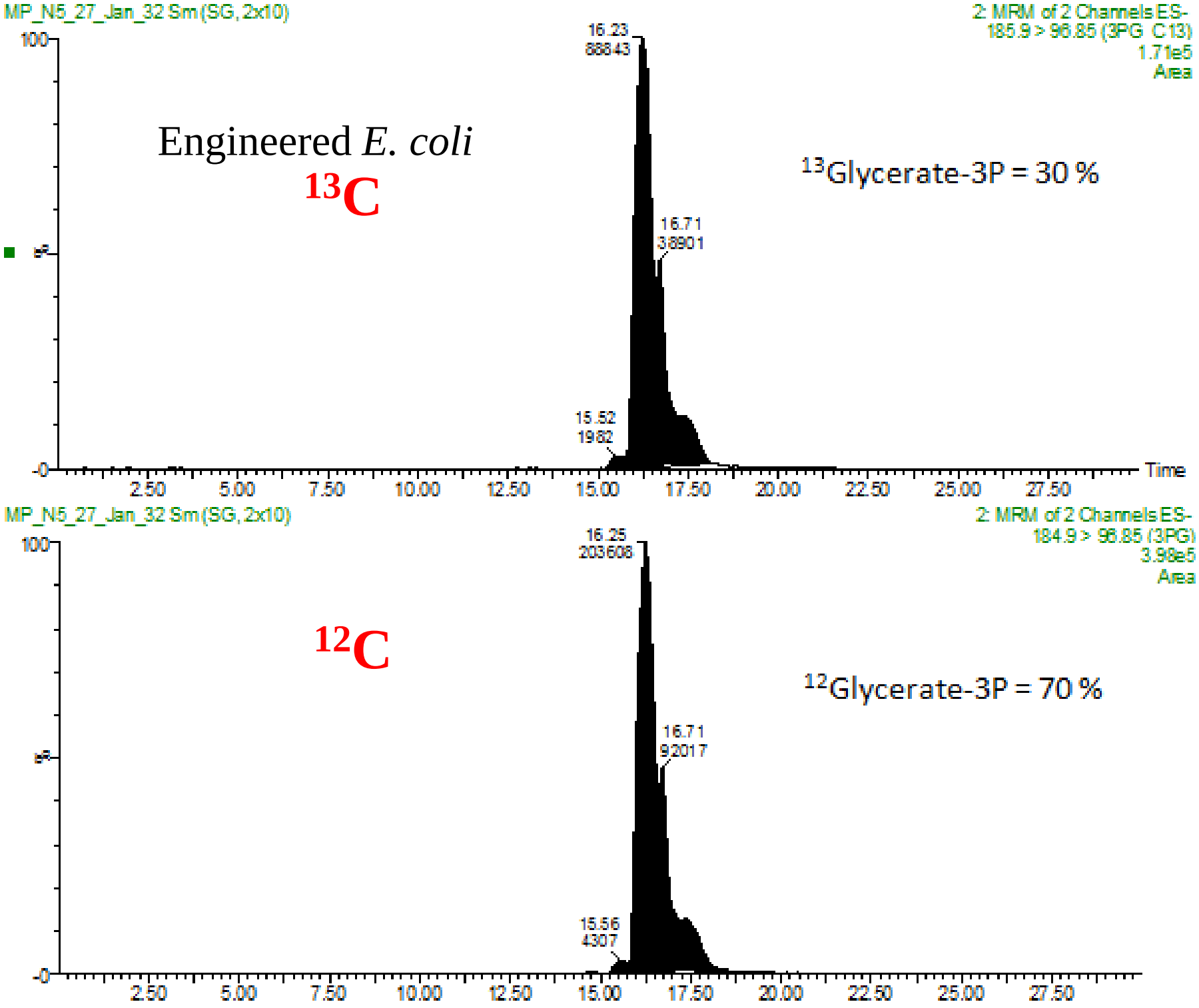
"Carbon fixating" *coli* cell extract

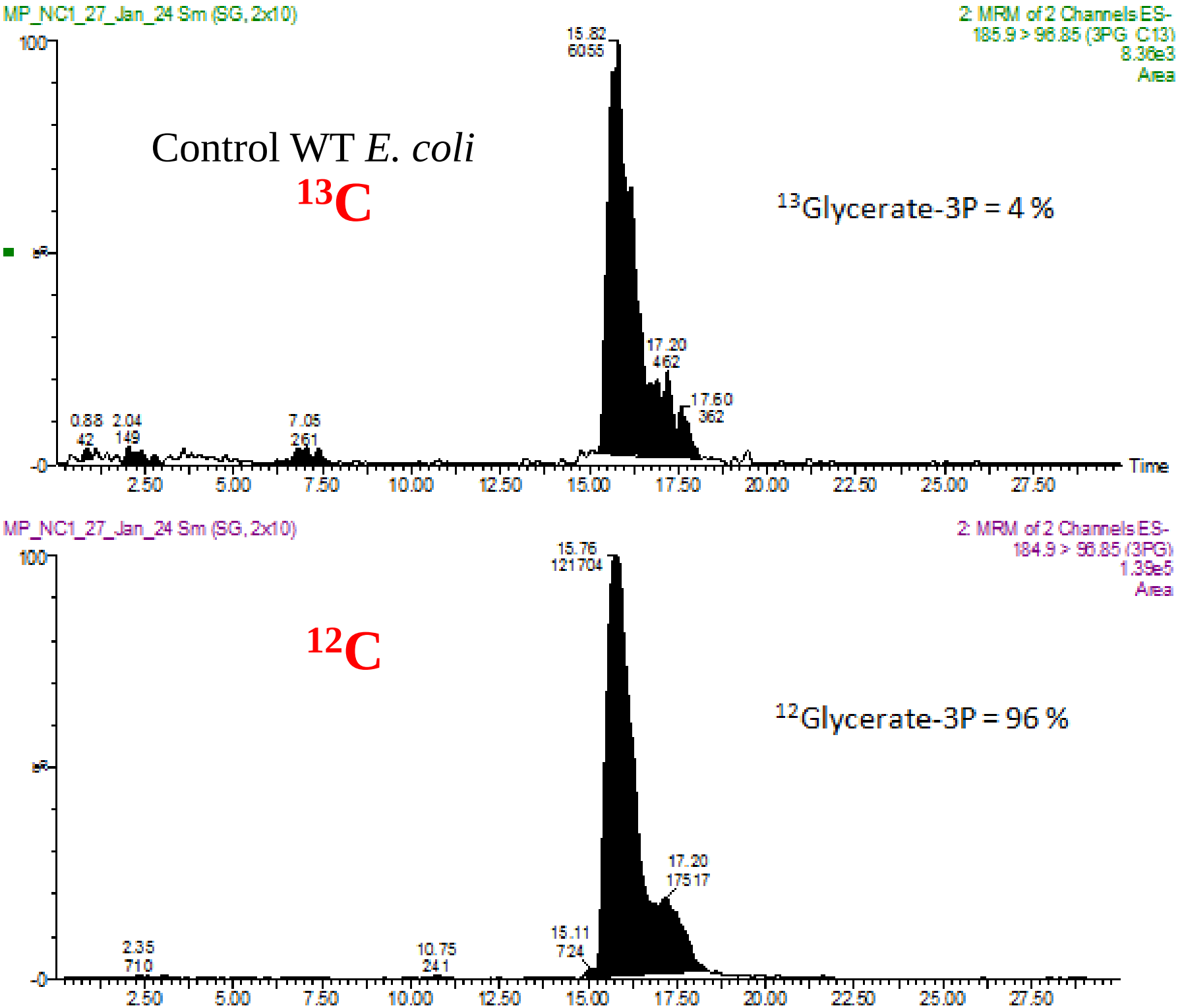
^{13}Glycerate-3P + Glycerate-3P



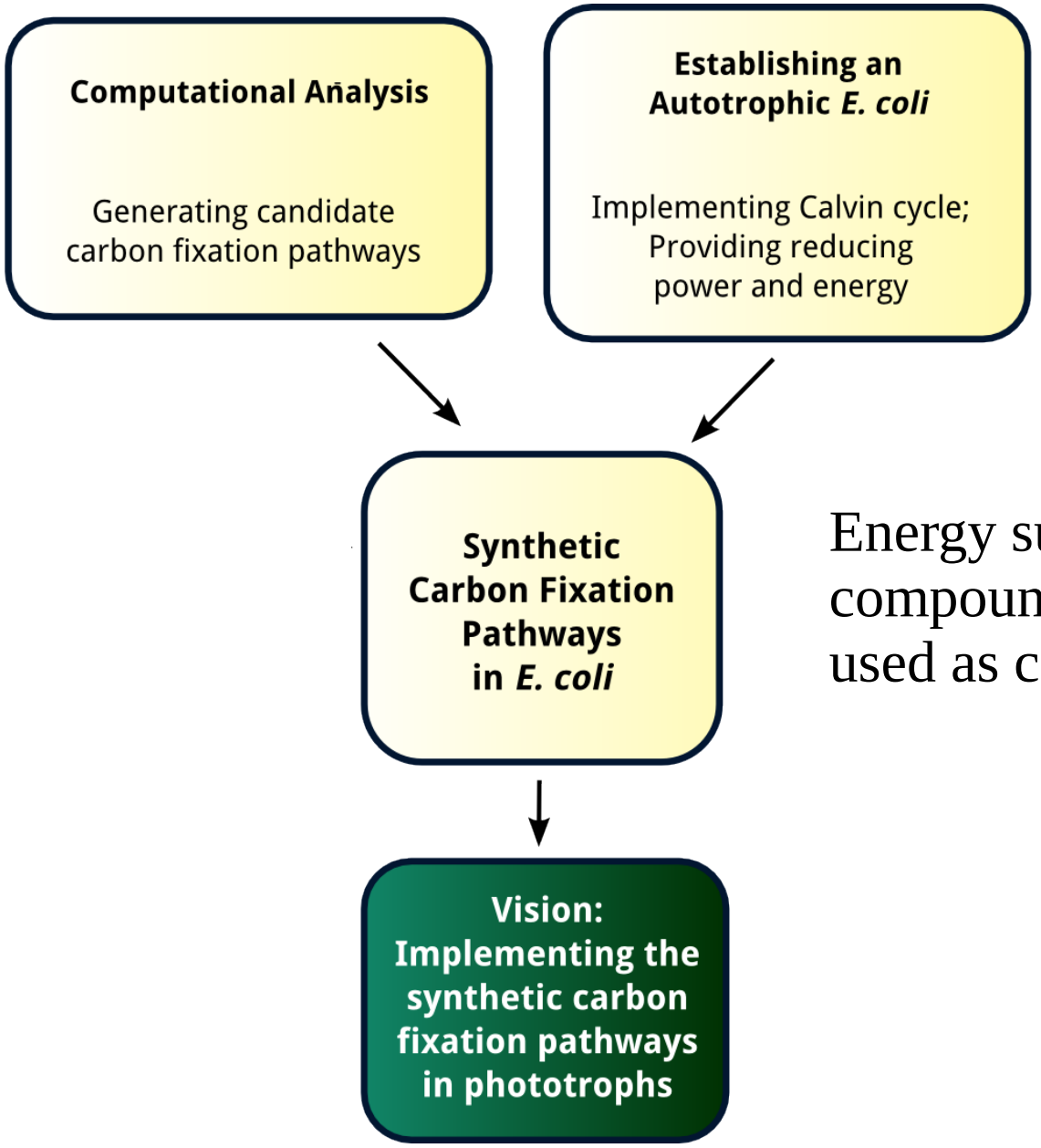

BP_N5_27_Jan_33 Sm (SG, 2x8)
2: MRM of 2 Channels ES-
185.9 > 96.85 (3PG_C13)
2.87e5
Area
16.27
113858
17.76
403
13Glycerate-3P = 40 %
BP_N5_27_Jan_33 Sm (SG, 2x8)
2: MRM of 2 Channels ES-
184.9 > 96.85 (3PG)
2.87e5
Area
16.27
171853
15.59
2790
17.93
1375
3.08
231
12Glycerate-3P = 60 %
100
%
-0
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
Time


Ribose-5-P
"Carbon fixating" coli cell extract
(Ribose-5-phosphate isomerase, E. coli)
Ribulose-5-P
ATP
"Carbon fixating" coli cell extract
PRK (Phosphoribulokinase, S. elongatus)
Ribulose-1,5-BP
13CO2
"Carbon fixating" coli cell extract
Rubisco (R. rubrum)
13Glycerate-3P + Glycerate-3P


MP_N5_27_Jan_32 Sm (SG, 2x10)
2: MRM of 2 Channels ES-
185.9 > 96.85 (3PG C13)
1.71e5
Area
16.23
88843
16.71
38901
15.52
1982
Engineered E. coli
13C
13Glycerate-3P = 30 %
Time
MP_N5_27_Jan_32 Sm (SG, 2x10)
2: MRM of 2 Channels ES-
184.9 > 96.85 (3PG)
3.98e5
Area
16.25
203608
16.71
92017
15.56
4307
12C
12Glycerate-3P = 70 %
100
%
0
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50


MP_NC1_27_Jan_24 Sm (SG, 2x10)
2: MRM of 2 Channels ES-
185.9 > 96.85 (3PG C13)
8.36e3
Area
Control WT E. coli
13C
13Glycerate-3P = 4 %
15.82
6055
17.20
462
17.60
362
0.88
42
2.04
149
7.05
261
Time
MP_NC1_27_Jan_24 Sm (SG, 2x10)
2: MRM of 2 Channels ES-
184.9 > 96.85 (3PG)
1.39e5
Area
12C
12Glycerate-3P = 96 %
15.76
121704
17.20
17517
15.11
724
2.35
710
10.75
241

Experimentally engineer carbon fixating *E. coli* as part of grand plan



Energy supplied by reduced compound that cannot be used as carbon source

We are aware that

“evolution is smarter than you are”

(Orgel’s law)

We expect to learn about horizontal gene transfer, constraints on metabolic networks and limits to productivity

B10NUMB3R5

THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

The number you need, with reference in just a minute

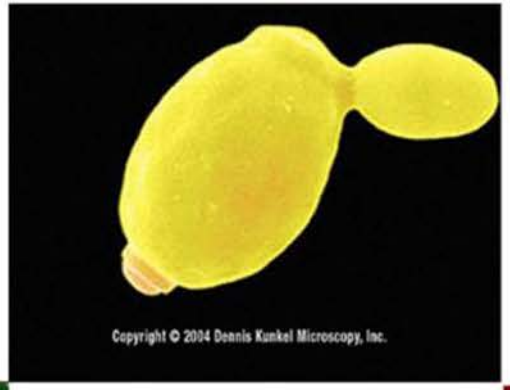
BioNumbers – Useful biological numbers database

Wiki-like, users edit and comment

Over 3500 properties & 5000 users/month

www.BioNumbers.org

ATP to make one cell: ~55 billion
Volume occupied by RNA: 6%
Number of tRNA/cell: ~200,000
Speed: 50 μm/sec
Ribosomes: 6,800 - 72,000
Proteins: ~3.6×10^6
Translation rate: 12 - 21 aa/sec
Volume occupied by water: 70%



Generation time: 4 days
Cells in an adult male: 1031
Number of genes: 20,621
Eggs laid during lifetime: 300
Size of Genome: 100Mbp
Life span: 2-3 weeks
Run speed at 20°C: 0.13mm/sec
Cells in hatched larvae: 556

Median haploid volume: 42 μm^3
Number of ribosomes: ~200,000
Nucleus volume: 7% of cell
mRNA out of total RNA: 5%
mRNA in cell: 15,000
Kcat of Pyruvate kinase: 71,400/min
Cell diameter: ~5μm
RNA to DNA ratio: 50

Total number of taste buds: 10,000
Cell divisions in a life-time: 10^{17}
Abundance of p53 per cell: ~160,000
Average brain weight: ~1350g
Hairs on the head: 90,000-150,00
Diameter of erythrocytes: 7.5μm
Weight of skin: 4.1 Kg
Average time between blinks: 2.8 Sec

B10NUMB3R5
THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

SnapShot: Key Numbers in Biology

Cell

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Cell size

Bacteria *(E. coli):* ≈0.7-1.4 μm diameter, ≈2-4 μm length, ≈0.5-5 μm^3 in volume; 10^8-10^9 cell/ml for culture with OD_{600}≈1

Yeast *(S. cerevisiae):* ≈3-6 μm diameter ≈20-160 μm^3 in volume

Mammalian cell volume: 100-10,000 μm^3; HeLa cell: 500-5000 μm^3 (adhering to slide ≈15-30 μm diameter)

Length scales inside cells

Nucleus volume: ≈10% of cell volume

Cell membrane thickness: ≈4-10 nm

"Average" protein diameter: ≈3-6 nm

Base pair: 2 nm (D) x 0.34 nm (H)

Water molecule diameter: ≈0.3 nm

Energetics

Membrane potential ≈70-200 mV → 2-6 k_BT per electron ($k_BT \equiv$ thermal energy)

Concentration

Concentration of 1 nM:
in *E. coli* ≈1 molecule/cell;
in HeLa cells ≈1000 molecules/cell

Characteristic concentration for a signaling protein: ≈10 nM-1 μM

Water content: ≈70% by mass; general elemental composition (dry weight) of *E. coli:* ≈$C_4H_7O_2N_1$; Yeast: ≈$C_6H_{10}O_3N_1$

Composition of *E. coli* (dry weight):
≈55% protein, 20% RNA, 10% lipid, 15% other

Protein concentration: ≈100 mg/ml = 3 mM. 10^6-10^7 per *E. coli* (depending on growth rate); Total metabolites (MW < 1 kDa) ≈300 mM

Division, replication, transcription, translation, and degradation rates

at 37°C with a temperature dependence (Q10) of ≈2-3

Cell cycle time (exponential growth in rich media): *E. coli* ≈20-40 min; budding yeast 70-140 min; HeLa human cell line: 15-30 hr

Diffusion and catalysis rate

Diffusion coefficient for an "average" protein: in cytoplasm D≈5-15 $\mu m^2/s$ → ≈10 ms to traverse an *E. coli* → ≈10 s to traverse a mammalian HeLa cell; small metabolite in water D≈500 $\mu m^2/s$

Diffusion-limited on-rate for a protein: ≈10^8-10^9 $s^{-1}M^{-1}$ → for a protein substrate of concentration ≈1 μM the diffusion-limited on-rate is ≈100-1000 s^{-1} thus limiting the catalytic rate k_{cat}

Genome sizes and error rates

Genome size:
E. coli (enterobacteria) ≈5 Mbp
S. cerevisiae (budding yeast) ≈12 Mbp
C. elegans (nematode) ≈100 Mbp
D. melanogaster (fruit fly) ≈120 Mbp
A. thaliana (plant) ≈120 Mbp
M. musculus (mouse) ≈2.5 Gbp
H. sapiens (human) ≈2.9 Gbp
T. aestivum (wheat) ≈16 Gbp


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