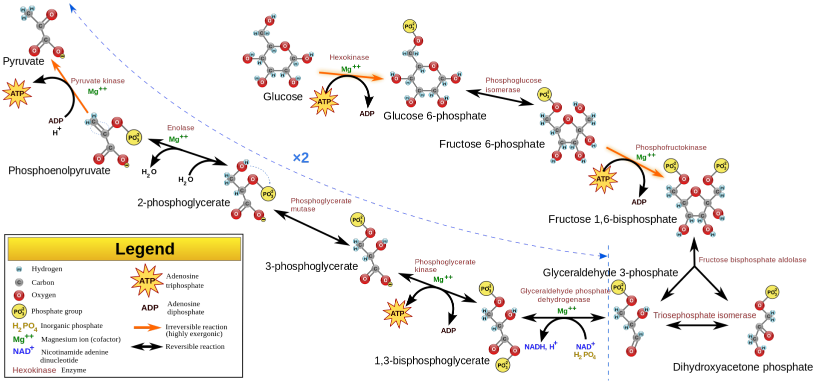
DM840 Algorithms in Cheminformatics: Multistep enzyme design with MØD

Daniel Merkle

October 30, 2019

Glycolysis


Pyruvate
Pyruvate kinase
Mg++
ATP
ADP
H+
Phosphoenolpyruvate
Enolase
Mg++
H2O
H2O
2-phosphoglycerate
×2
Phosphoglycerate mutase
3-phosphoglycerate
Glucose
Hexokinase
Mg++
ATP
ADP
Glucose 6-phosphate
Phosphoglucose isomerase
Fructose 6-phosphate
Phosphofructokinase
Mg++
ATP
ADP
Fructose 1,6-bisphosphate
Fructose bisphosphate aldolase
Glyceraldehyde 3-phosphate
Triosephosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde phosphate dehydrogenase
Mg++
NADH, H+
NAD+
H2 PO4
Phosphoglycerate kinase
Mg++
ATP
ADP
1,3-bisphosphoglycerate
Legend
Hydrogen
Carbon
Oxygen
Phosphate group
H2 PO4 Inorganic phosphate
Mg++ Magnesium ion (cofactor)
NAD+ Nicotinamide adenine dinucleotide
Hexokinase Enzyme
ATP Adenosine triphosphate
ADP Adenosine diphosphate
Irreversible reaction (highly exergonic)
Reversible reaction

Glycolysis

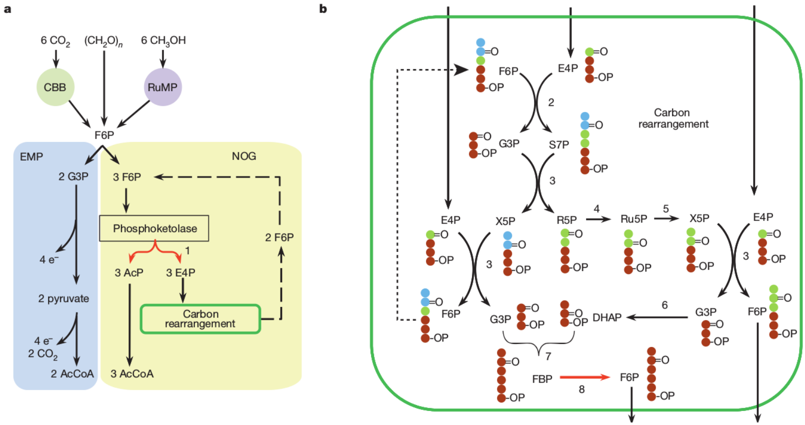
LETTER

doi:10.1038/nature12575

Synthetic non-oxidative glycolysis enables complete carbon conservation

Igor W. Bogorad[1,2], Tzu-Shyang Lin[1] & James C. Liao[1,3]

Glycolysis (classical and with 100% carbon yield)



Non-Oxidative Glycolysis (all 100% carbon yield)

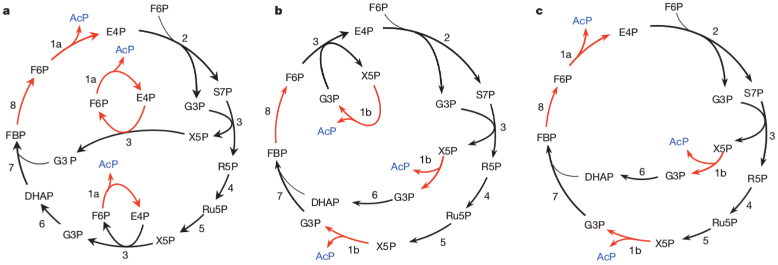
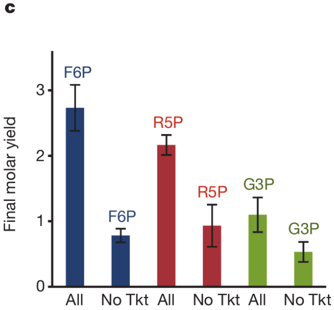


Figure 2 | Three FBP-dependent NOG networks. a–c, NOG using Fpk only (**a**), NOG using Xpk only (**b**) and NOG using F/Xpk (**c**). These configurations differ from those shown in Fig. 1 because the Xpk-linked Tkt has been integrated with carbon rearrangement. The red arrows in **a–c** indicate irreversible reactions that drive the cycle. Enzyme numbers are defined in Fig. 1 legend, except: 1a, Fpk; 1b, Xpk.

These networks are **engineered**.

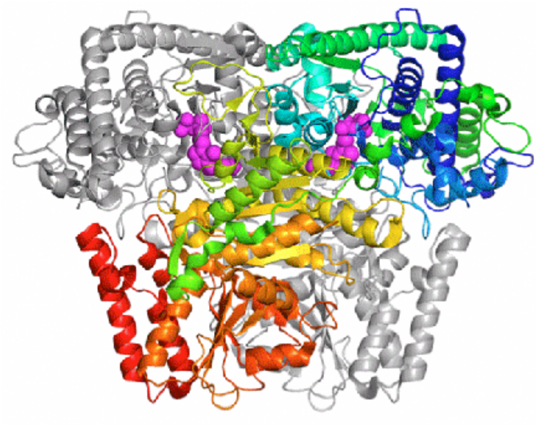
Non-Oxidative Glycolysis (*in vitro*, Results)



Depicted: (molar) yield of AcP (2 carbons).
Interpretation: all carbons of F6P are transformed into F6P (6 carbons).
in vivo (in bacteria): not perfect, but similar

In a nutshell: *It works*

Enzymes



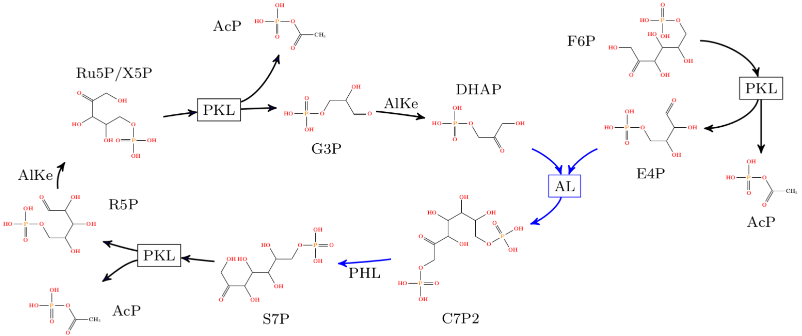
Different enzymes trigger/catalyze different reactions
(depicted: FPK, catalyzing F6P).

How a Computer Scientist does it

First, expand the network with Graph Grammars, then something like

```
def getBaseFlow(dg):
        flow = dgFlow(dg)
        for a in {Pi, water}:
                flow.addSource(a)
                flow.addSink(a)
        flow.addSource(F6P)
        flow.addConstraint(inFlow(F6P) == 1)
        flow.addSink(AcP)
        flow.transit.allowInOutReverse = False
        return flow
```

What you get (for example)



Now ... got in the lab, build it.

What you get (more examples)

Phosphoketolase Type	Only FBP					Other Bisphosphates									
	8 Unique Reactions					7 Unique Reactions					8 Unique Reactions				
	Reactions					Reactions					Reactions				
(XPK, FPK, SPK, OPK)	7	8	9	10	11	7	8	9	10	11	7	8	9	10	11
(0, 0, 0, 3)	–	–	–	–	–	–	–	–	–	–	–	–	–	4	16
(0, 0, 1, 2)	–	–	–	–	–	–	–	–	–	–	–	–	3	2	–
(0, 0, 2, 1)	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–
(0, 0, 3, 0)	–	–	–	1	2	–	–	1	2	–	–	–	–	9	20
(0, 1, 0, 2)	–	–	–	–	–	–	–	–	–	–	–	–	4	4	–
(0, 1, 1, 1)	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–
(0, 1, 2, 0)	–	–	1	–	–	–	1	–	–	–	–	–	8	2	–
(0, 2, 0, 1)	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–
(0, 2, 1, 0)	–	–	1	–	–	–	1	–	–	–	–	–	9	–	–
(0, 3, 0, 0)	–	–	–	2	4_a	–	–	2	4	–	–	–	–	14	24
(1, 0, 0, 2)	–	–	–	–	–	–	–	–	–	–	–	–	2	4	–
(1, 0, 1, 1)	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–
(1, 0, 2, 0)	–	–	1	–	–	–	1	–	–	–	–	–	6	2	–
(1, 1, 0, 1)	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–
(1, 1, 1, 0)	–	1	–	–	–	1	–	–	–	–	–	3	–	–	–
(1, 2, 0, 0)	–	–	2	–	–	–	2	–	–	–	–	–	10	–	–
(2, 0, 0, 1)	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–
(2, 0, 1, 0)	–	–	1	–	–	–	1	–	–	–	–	–	7	–	–
(2, 1, 0, 0)	–	–	2_c	–	–	–	2	–	–	–	–	–	10	–	–
(3, 0, 0, 0)	–	–	–	2_b	4	–	–	2	4	–	–	–	–	12	20