Systems Biology 551-1174-00L

Flux Balance Analysis

30 March, 2017

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Content:

- **Constraints and objectives** (US)
- Incorporating constraints and objectives (JS)
- FBA Applications (US)
  - FBA in Biotechnology (maximum theoretical yield)
  - Synthetic lethality - identification of non-obvious drug targets (eg cancer metabolism)

6. Flux Balance Analysis: Applications

Learning goals

- Explain how **constraints** and **objectives** are used to obtain answers from FBA models.
- Propose approaches to extend FBA to incorporate regulation, perturbations, and -omics data.
- Know examples of FBA applications: in biotechnology and cancer metabolism.

Exercise 7

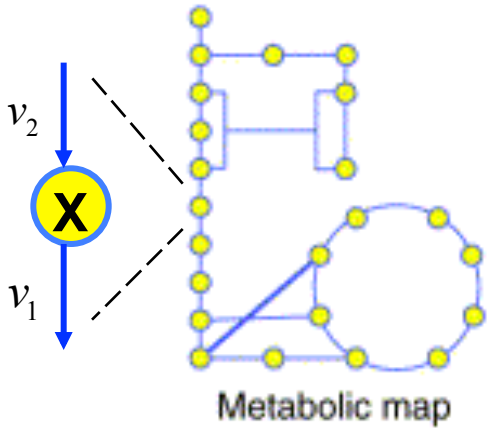
Use COBRA toolbox for genome-scale metabolic model of *E. coli* to

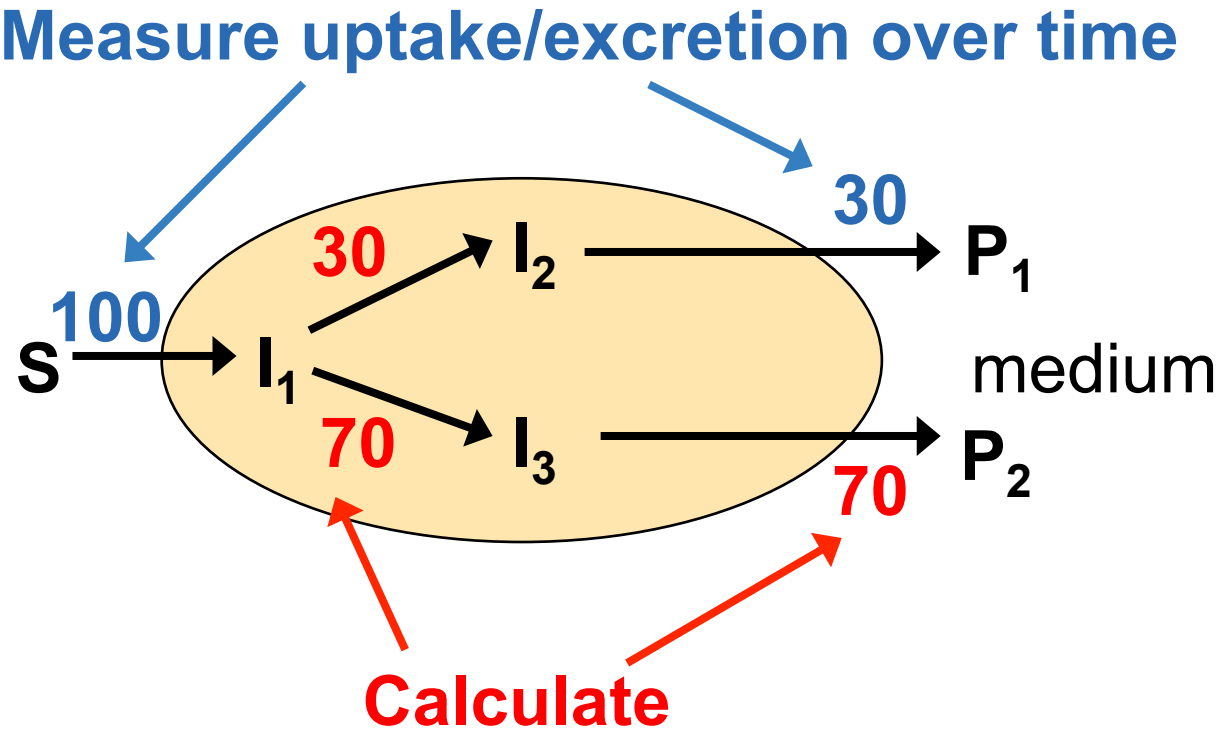
- **find optimal flux solution.**
- **identify essential reactions.**
- **identify epistatic interactions between selected genes and explain meaning of this interaction.**
- **interpret FBA model-based predictions.**

Reminder: we consider only steady state, and work with an underdetermined system of equations that has many possible solutions

Infer a Non-Measurable Quantity

Flux analysis by flux balance analysis





Mass balance around metabolite *X*

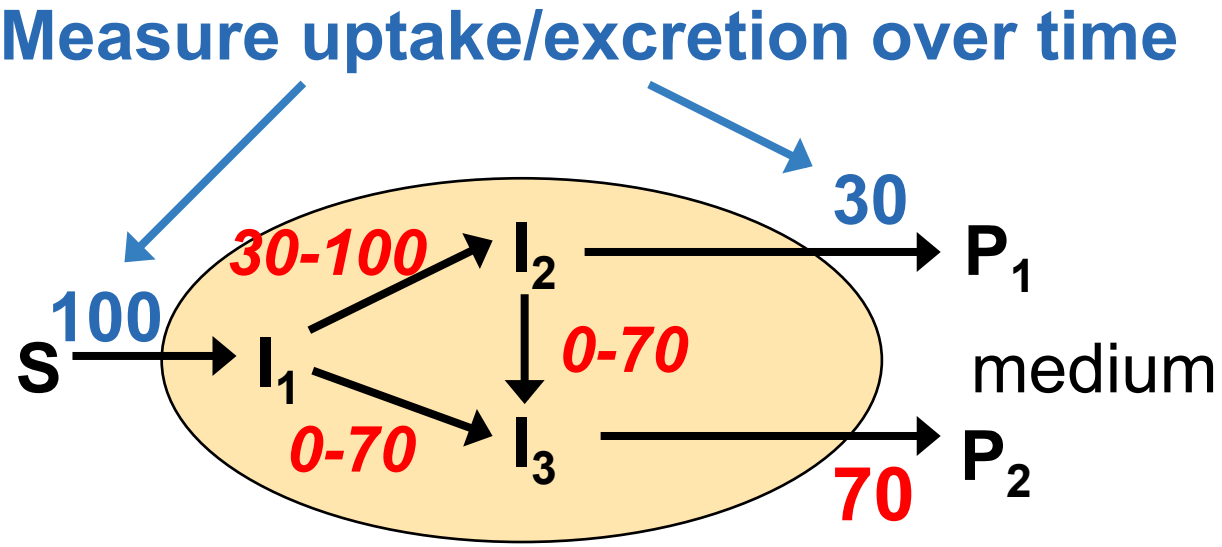
$$\frac{dX}{dt} = -1 \cdot v_1 + 1 \cdot v_2 = 0$$

↑

Steady state assumption

Infer a Non-Measurable Quantity

Flux analysis by flux balance analysis



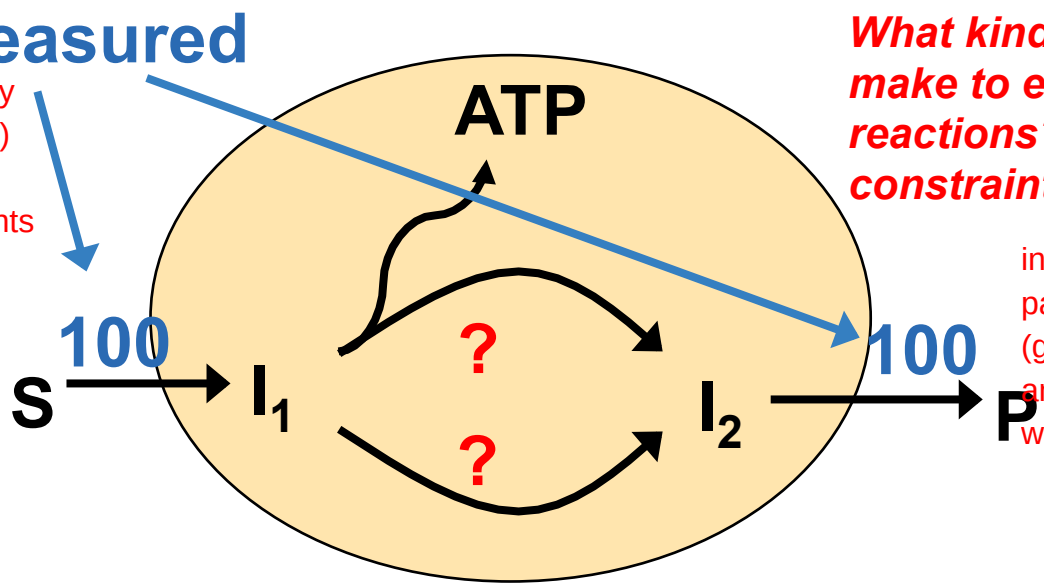
What could happen if not in steady state?

Assumption: Steady state for intracellular metabolite concentrations

Underdetermined Systems have Multiple Solutions

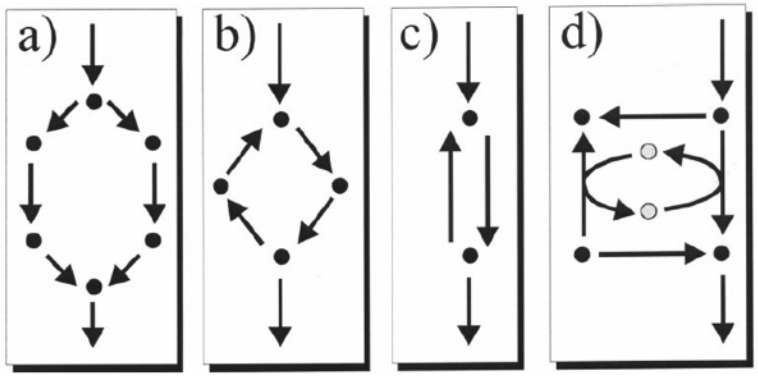
biologically, the reaction that produces ATP is commonly preferred (it's not a rule though)

Use assumptions and constraints to limit the possible solutions.



What kind of assumptions could we make to estimate flux through the 2 reactions? Think in terms of constraints and objectives!

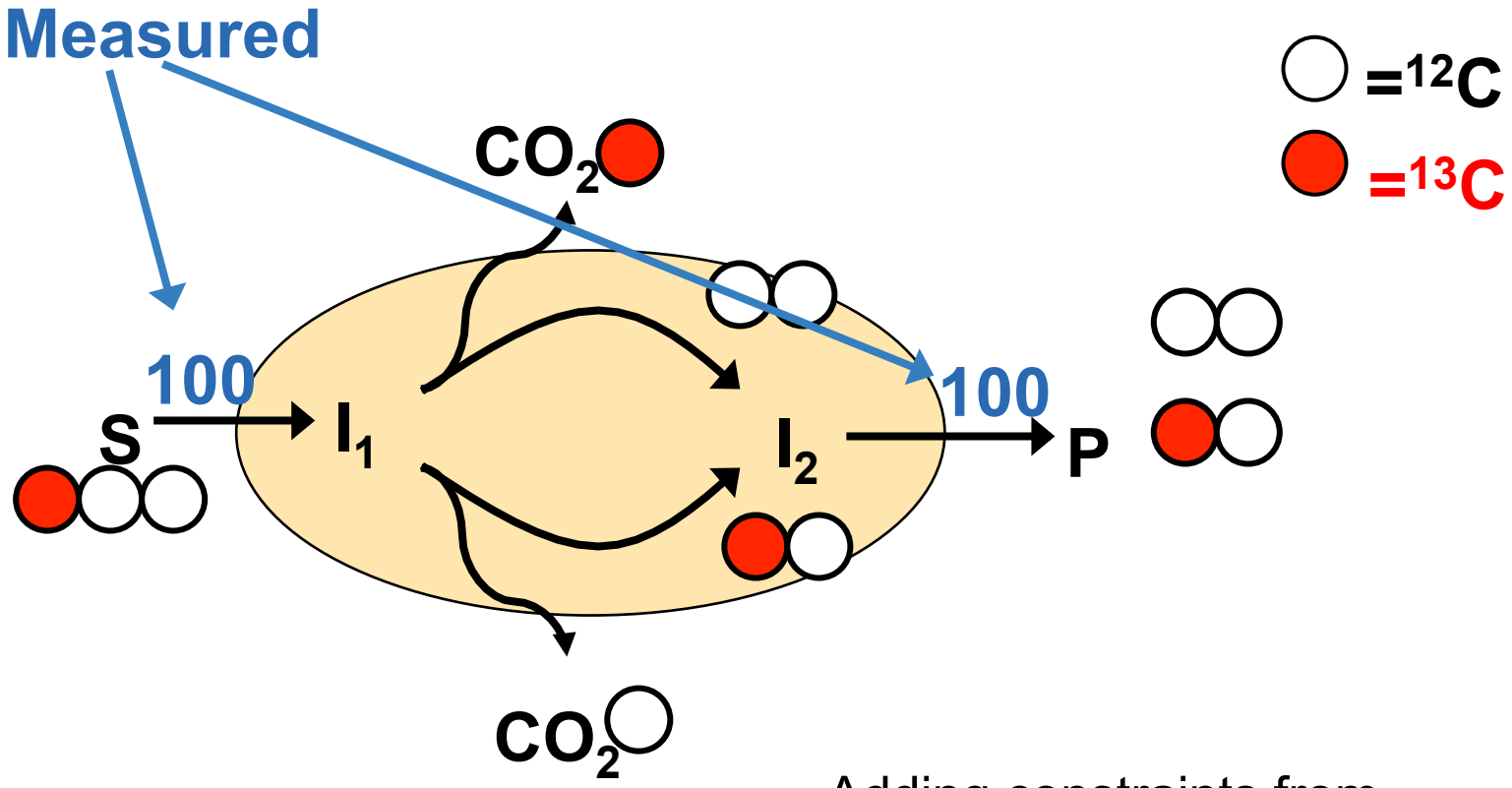
in this case, try genetic mutations so that the pathways are turned off one by one and observe P (genetic perturbations)
another way: use fluorescences on enzymes to see what kind of pathway is used to what degree



Typical situations where stoichiometric flux analysis fails: (a) parallel pathways without any related flux measurement, (b) certain metabolic (futile) cycles, (c) bidirectional reactions, and (d) split pathways when cofactors (gray circles) are not balanced. To deal with such underdetermined systems, one can either:

- add constraints (how do we know that a reaction actually could take place?)
- make strong biological assumptions (e.g. on ATP production, cofactor balancing or evolutionary objectives), which severely limit the value of obtained results.

Generating Additional Experimental Information to Identify Real Flux Solution: **Principle of ^{13}C-based MFA**



Adding constraints from experimentally determined isotopic tracer pattern, using NMR or MS analysis

Flux Balance Analysis Deals with the Underdetermined Nature of the Model by:

- **adding principle-based constraints** (to reduce solution space)
  - reaction directionality (thermodynamics)
  - …….
- **adding data-based constraints**
  (difference between genome and what is expressed/active)
  - isotopic tracer on pathway usage
  - …..
  - …..

Which other data could one use to remove or constrain reactions ?

- consider only **optimal solutions** (ie using objective functions)
  - by using optimality for designing networks for biotechnology
  - assuming we know something about how evolution optimized metabolism

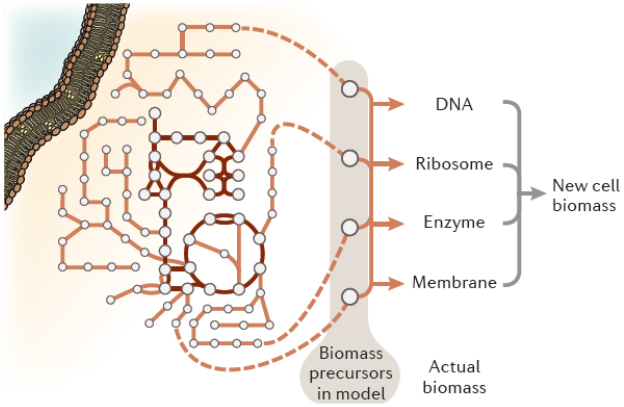
Possible Objective Functions

What could have been an evolutionary optimization of metabolism (in a stoichiometric sense)?

- Cells try to maximize ATP

Minimization of metabolic adjustment (MOMA)

- trying to reduce energy usage => minimization of overall fluxes => short pathways are mostly preferred over longer pathways when final energy/product is the same
- maximization of growth
- ((minimize toxic outputs such as ammoniak NH_3)

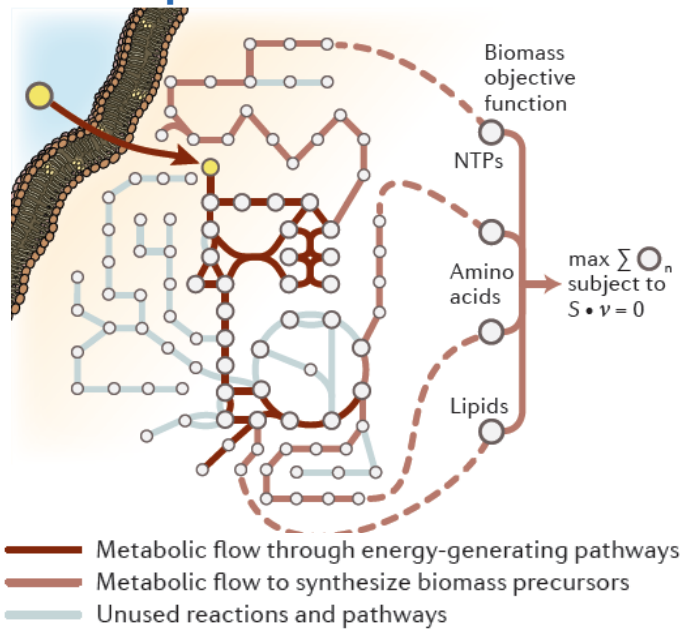


Biomass objective function

Maximize flux through the *pseudo* reaction to biomass !

Lewis et al 2012 Nat Rev Microbio

Optimal solution



Biomass vector

Describes the metabolic and energetic demands to make the basic building blocks for all cellular components.

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- **FBA Applications (US) 2 examples**
  - FBA in Biotechnology (maximum theoretical yield)
  - Synthetic lethality - identification of non-obvious drug targets (eg cancer metabolism)
  - .....

The Maximum Theoretical Yield Objective

To metabolically engineer a host strain, we need to know what the maximum theoretical yield for a product would be. For example, what would be the maximum theoretical yield of acetate from glucose?

Stoichiometric modeling allows to quantitatively consider different aspects for maximum yield:

-
-
-
-
- ……..

How would you describe the goal of FBA modeling in your own words here?

Flux Balance Analysis in Biotechnology

1. Design of a novel, synthetic pathway

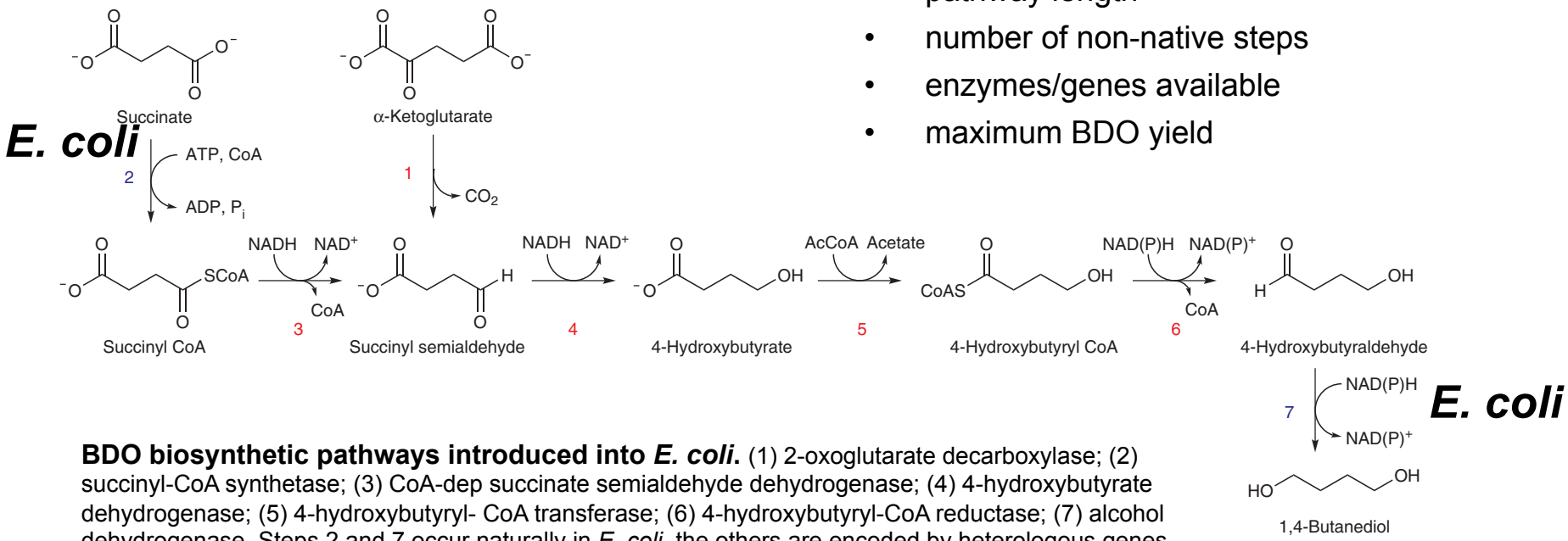
Metabolic engineering of *E. coli* for production of 1,4-butanediol (BDO)

Yim et al. 2011 Nature Chem Biol A non-natural product not synthesized in any know organism.

Search all enzymes from data base of all organisms (eg KEGG) or use chemical reaction rules. Yielded >10'000 pathways with 4-6 reactions to BDO. Then rank to select most promising –

Which principles could one use for this ranking?

- thermodynamic feasibility
- pathway length
- number of non-native steps
- enzymes/genes available
- maximum BDO yield



BDO biosynthetic pathways introduced into *E. coli*. (1) 2-oxoglutarate decarboxylase; (2) succinyl-CoA synthetase; (3) CoA-dep succinate semialdehyde dehydrogenase; (4) 4-hydroxybutyrate dehydrogenase; (5) 4-hydroxybutyryl- CoA transferase; (6) 4-hydroxybutyryl-CoA reductase; (7) alcohol dehydrogenase. Steps 2 and 7 occur naturally in *E. coli*, the others are encoded by heterologous genes.

Flux Balance Analysis in Biotechnology

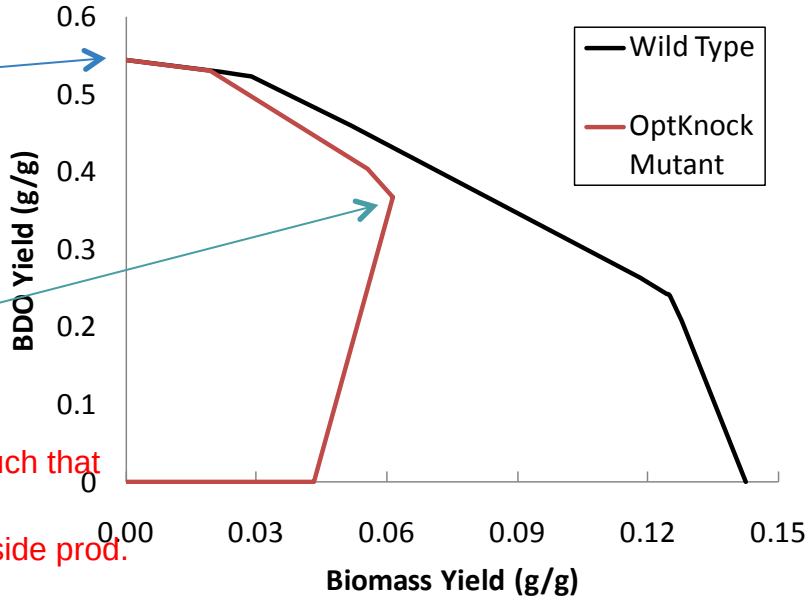
2. Metabolic Engineering of Host Strain

Problem: channel maximum carbon/energy to product but still allow cell growth! Tradeoff between biomass and product yield, hence many optimal combinations exist! **But how to practically restrict cell growth to force product formation????**

The OptKnock search algorithm identifies gene deletions that couple BDO production to cell growth, but maintain high theoretical BDO yield. ***What is basis for a point in below chart?***

Maximum theoretical yield 0.54 g BDO per g glucose – no growth!

Realistic maximum theoretical BDO yield with growing cells.



OptKnock mutant has deletions in *adhE, ldhA, pflB*, and *mdh*. It must produce some BDO to grow! *In this case, under anaerobic conditions formation of the natural fermentation products ethanol, formate, lactate and succinate is blocked.*

looking for optimal combinations of deletions such that product is optimized and cell still grows.
therefore, try to KO all pathways for anaerobe side prod.
so that BDO is the only anaerobe side prod.

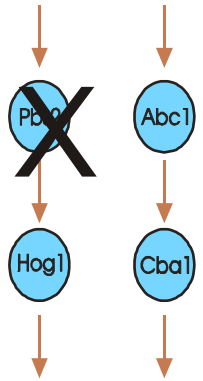
Tradeoff between maximum theoretical BDO and biomass yield in OptKnock strain designs on glucose.
Both strains have BDO pathway reactions shown on previous slide. Plot shows maximum attainable BDO yield as a function of the maximum growth yield.

Cancer Metabolism

- Cancer cells have a very distinct metabolism
  - Warburg effect (ie aerobic lactate fermentation)
  - rapid growth
  - glutamine addiction, etc
- Cancer is not a defined state, but a continuous process of accumulating genetic defects
- Cancer cells often evade drug treatment through evolutionary adaptation
- *How can they adapt?*

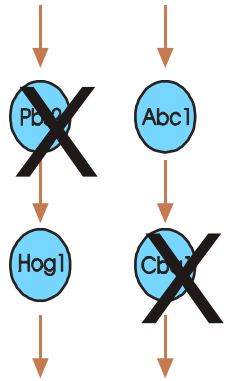
The Concept of Synthetic Lethality in Evolving Drug Resistance

How could it help a cancer cell to evade drug treatment after adaptation ?
How could this knowledge help developing therapy ?

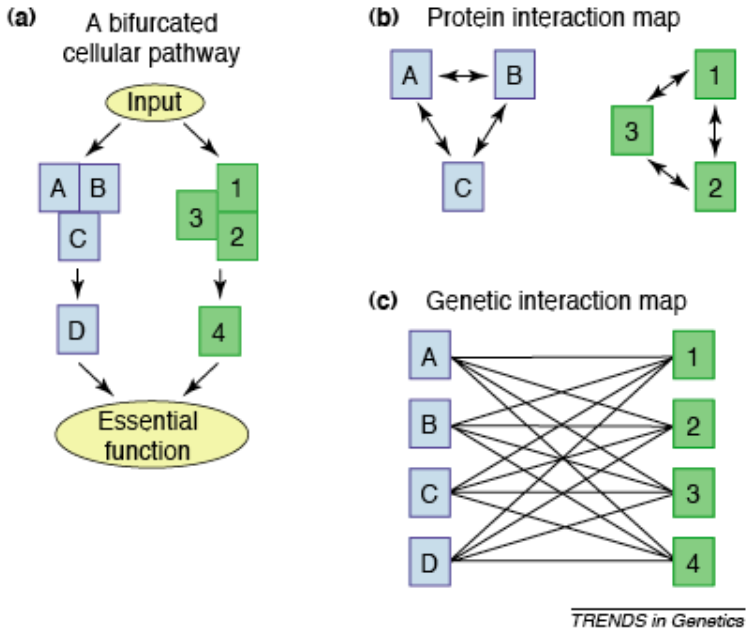


common target

The two pathways control some common targets; mutation of *PBS2* alone causes only a moderate phenotype. The second mutation in the parallel pathway leads to lethality



common target

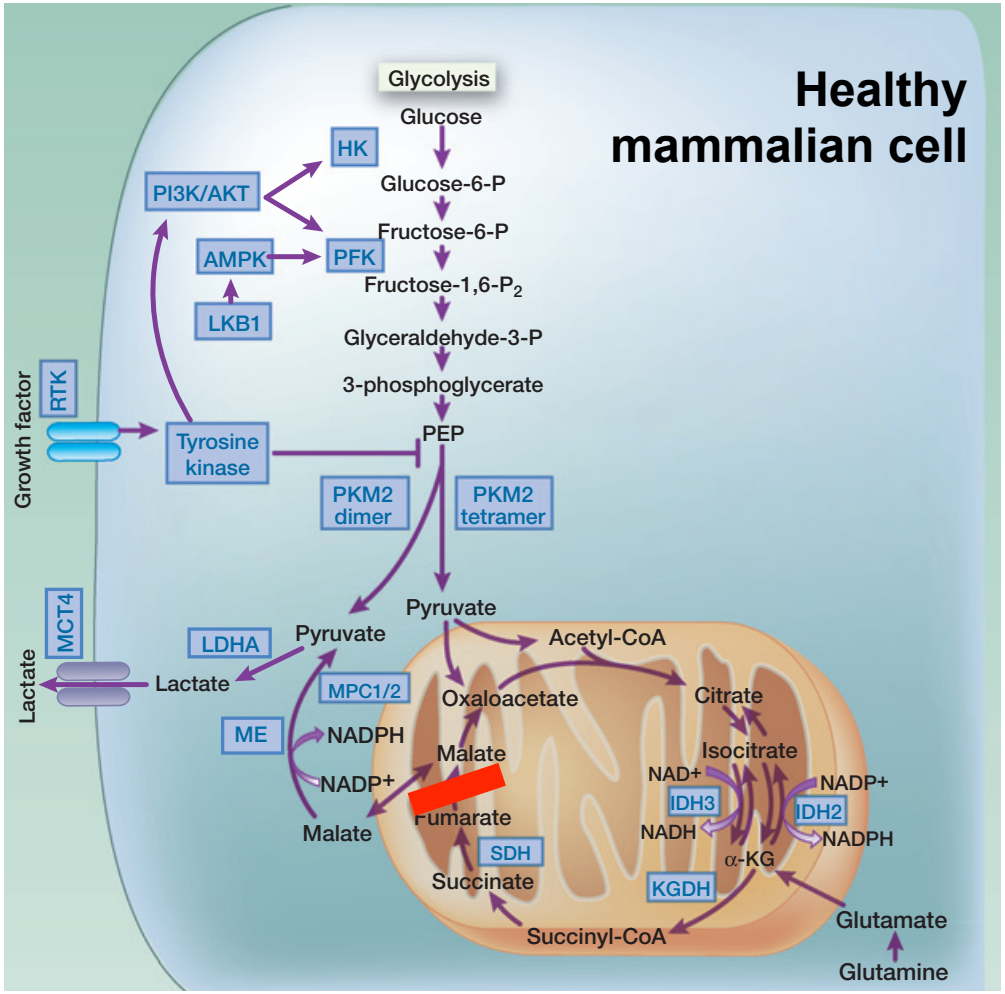


Genetic versus physical interaction maps. (a) A bifurcated cellular pathway will have distinct networks on genetic- and physical interaction maps. Proteins A, B, C and D (blue) and proteins 1, 2, 3, and 4 (green) are members of two functionally redundant pathways required to perform an essential function. Proteins A, B and C interact with each other physically, so do proteins 1, 2 and 3. (b) A protein interaction map, or physical interaction map, identifies interactors based on protein–protein interactions, whereas genetic interaction map (c) identifies 'interactors' based on functions without the requirement that the proteins must interact. The combination of these two complementary approaches can be used to deduce a cellular pathway and, in principle, enable the construction of a 'wiring diagram' of the yeast cell.

Ooi et al 2006, Trend Genetics 22: 56

Finding Drugs to Intervene with Cancer Metabolism

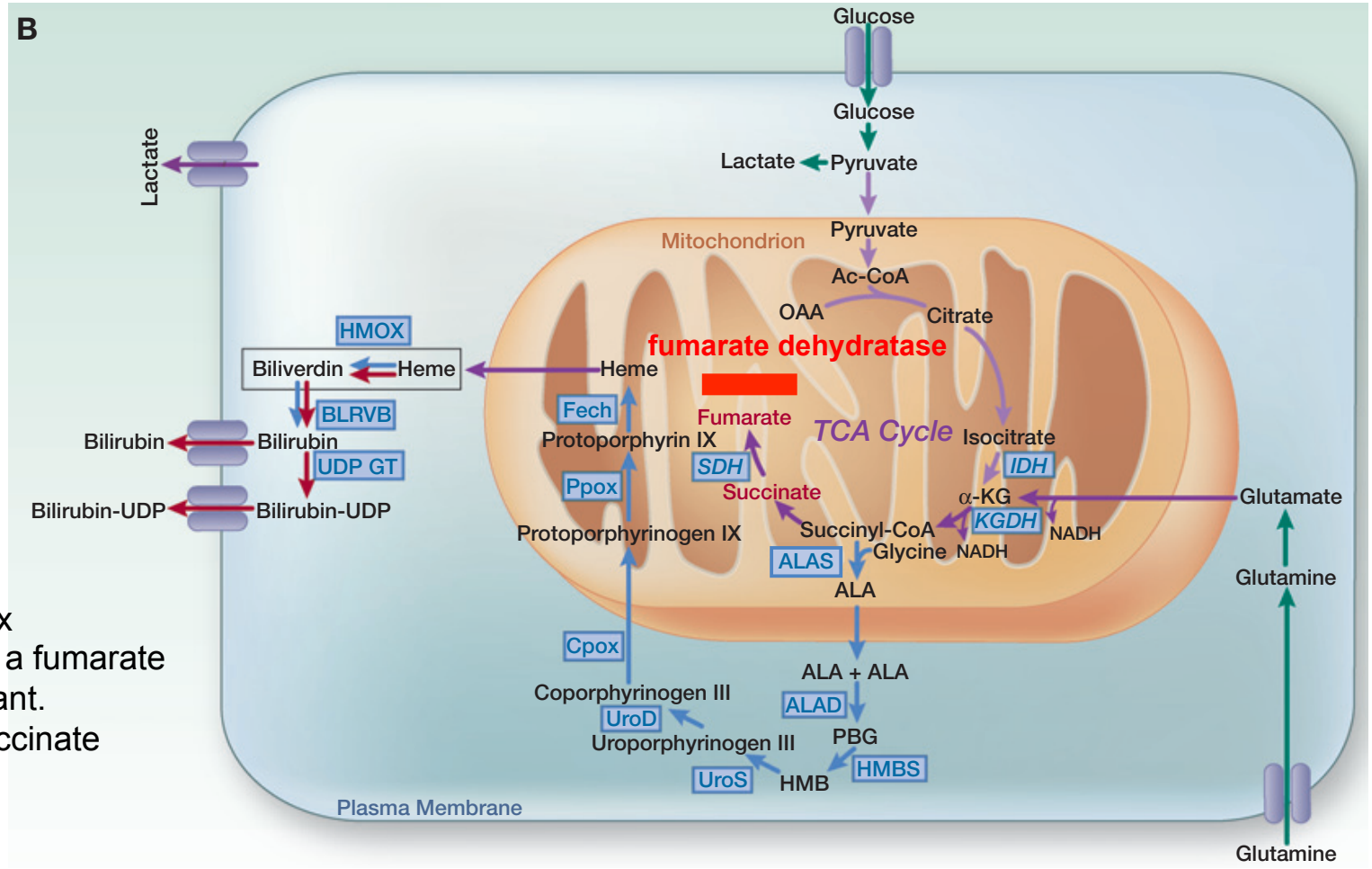
- Germline mutations in fumarate hydratase are responsible for renal-cell cancer
- How can cancer cells without a TCA cycle survive
- Are there any non-obvious drug targets for such cancer cells?
- **FBA with genome-scale human renal cancer model**
- **Which *in silico* deletion are synthetic lethal in a fumarate hydratase mutant?**



Frezza et al 2011 Nature; Jerby & Ruppin 2012 Clin Cancer Res

An Unexpected Metabolic Bypass

FBA predicted partial mitochondrial NADH production through a linear pathway from glutamine to bilirubin (by combining heme biosynthesis and degradation)



FBA predicted flux rearrangement in a fumarate dehydratase mutant. Fumarate and succinate accumulate.

Non-Obvious Drug Targets

- *How could one use FBA modeling in finding potential new drug targets in cancer metabolism ?*
- Improve models by using tissue (or patient) specific cell line data, for example transcriptomics, proteomics etc
  to do what with the FBA model ?

Exercise 7: *E. coli* Central Metabolism

Goal

- Use FBA to simulate a more realistic model of central carbon metabolism in *E. coli*
- Predict synthetic lethality
  When a combination of mutations in two or more genes leads to cell death, whereas a mutation in only one of these genes does not.