

Course 2851 Principles of Metabolism  
Metabolism and endocrinology programme, Karolinska Institutet

Lecture 6  
Isotope tracing

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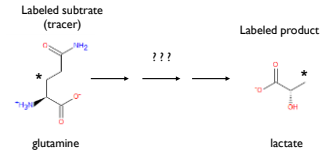
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Karolinska Institutet



Basic principle of isotope tracing

- Detecting isotopes in metabolic products from known tracers reveals metabolic pathways

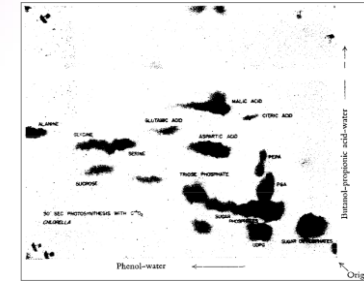
Example: glutaminolysis



- Requires a recognizable "label" (isotope) that does not itself interfere with metabolism



Use of isotope tracing to discover metabolic pathways



Calvin, 1961 nobel lecture  
[www.nobelprize.org](http://www.nobelprize.org)

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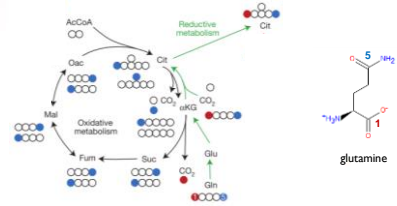
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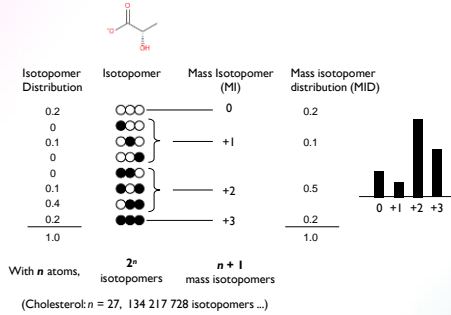
### Measuring pathway activity using stable isotope (<sup>13</sup>C) tracing

Example: reductive carboxylation in the TCA cycle

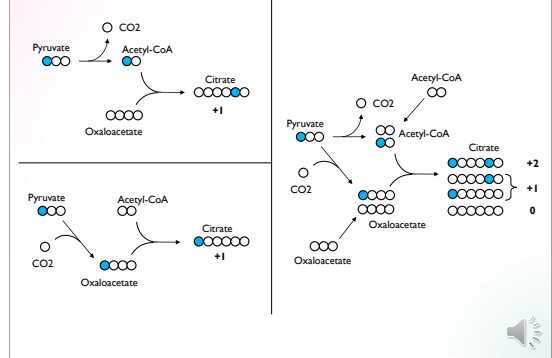


Metallo et al. Nature 2011 (modified)

### Isotomers, mass isotomers, distributions



### Example: citrate synthesis in the TCA cycle




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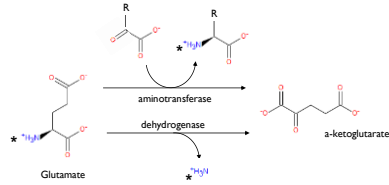
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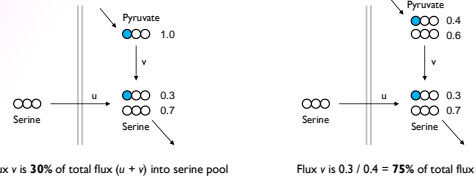
Not all reactions & enzymes are distinguishable



Depends on (1) choice of tracer and (2) measurable metabolites

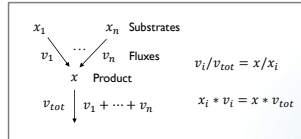
Quantitative flux analysis with stable isotopes

Determining relative fluxes from MIDs

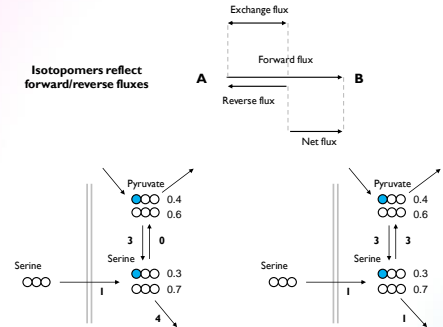


General case

For every (mass) isotopomer, at steady-state



Caveat: isotopomers may not yield net fluxes




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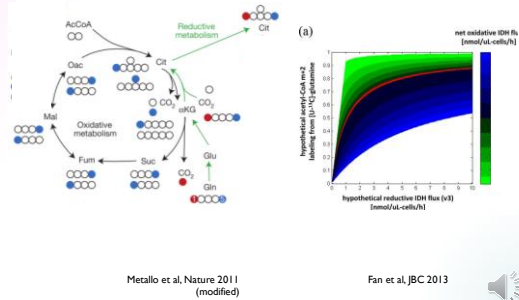
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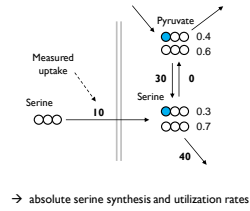
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### Reductive carboxylation, revisited



### Combining isotopomer and uptake / release data

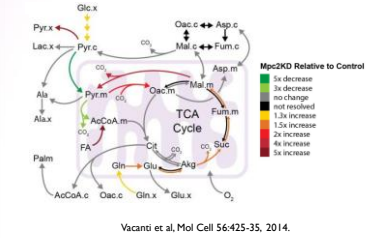
Allows determining absolute fluxes



### Systematic flux analysis methods (steady state)

- Metabolic network model + atom mappings
- Collect flux ratio equations for all measured isotopomers
- Combine with uptake / release data if available
- Solve equation system (fit data to model)

Wiechert et al. Metab Eng 3:265–283, 2001.  
Antoniewicz et al. Metab Eng 9:68–86, 2007.




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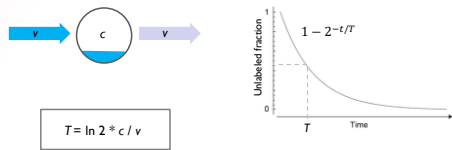
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### Flux analysis via pool turnover rates

- Flux "through" a metabolite pool (mol / s) relative to pool size (mol)
- The turnover rate can be described by half-time  $T$

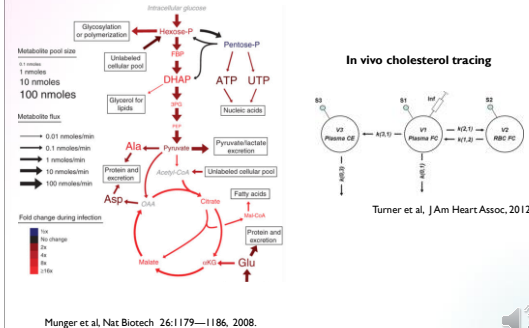


$$T = \ln 2 \cdot c / v$$

- Isotopomer dynamics can be measured with pulse label / time series
- If pool size (concentration)  $c$  is known, flux  $v$  can be determined



### Flux analysis via pool turnover rates



Munger et al. Nat Biotech 26:1179-1186, 2008.




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