

Basics of metabolite profiling and metabolic flux analysis

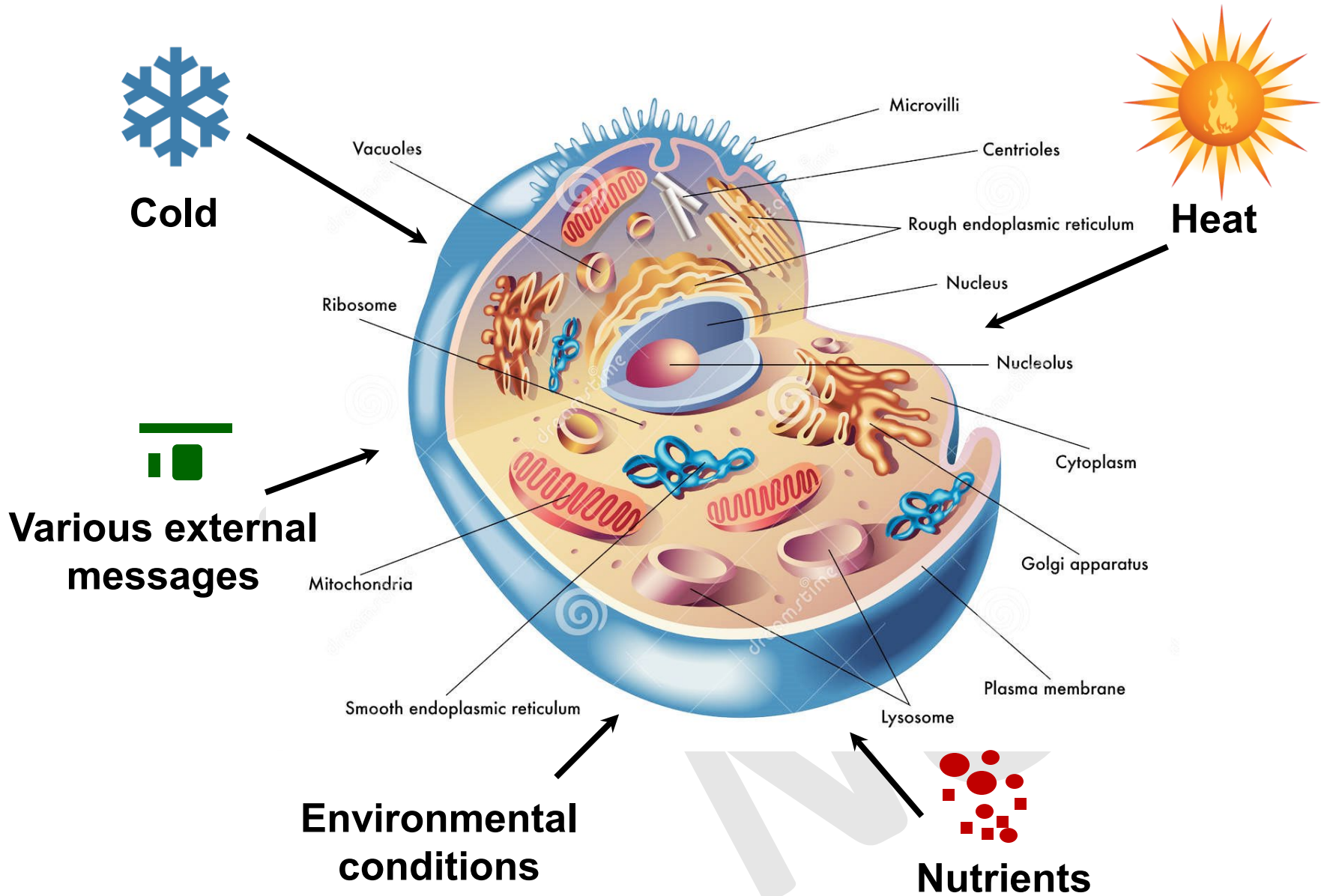
Issam Ben-Sahra

Membranes, Organelles & Metabolism

May 1st, 2020

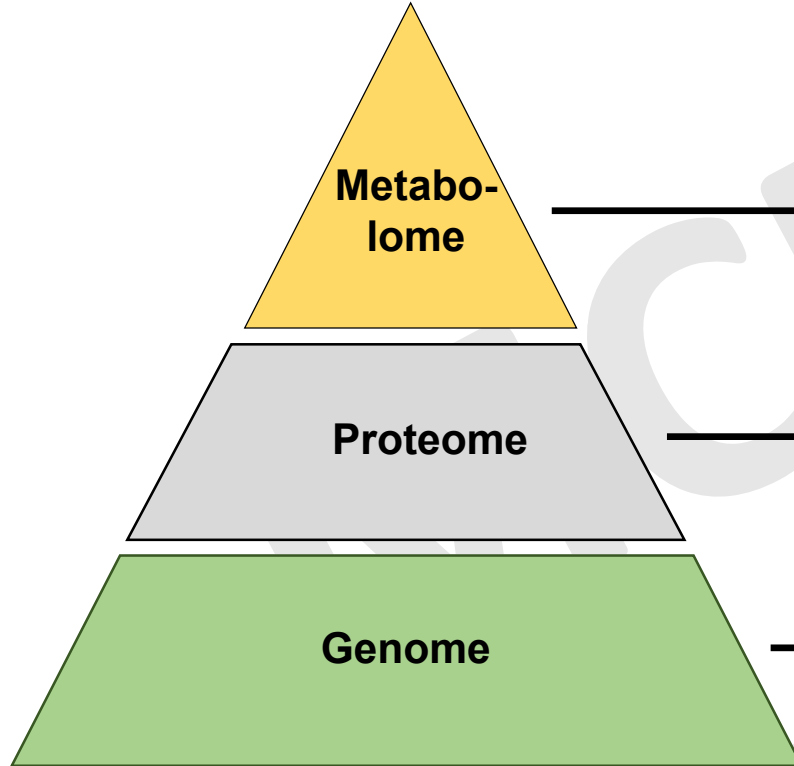
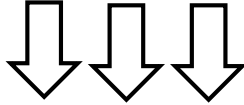
Contact: issam.ben-sahra@northwestern.edu Phone : 312-503-5318

Cells respond to environment

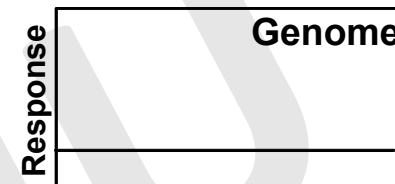
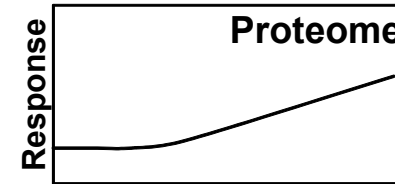
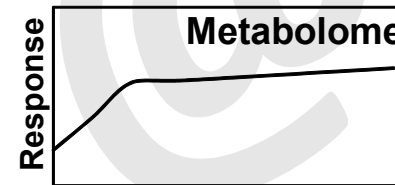


The Pyramid of Life

Signals (light, O₂, food, etc.)

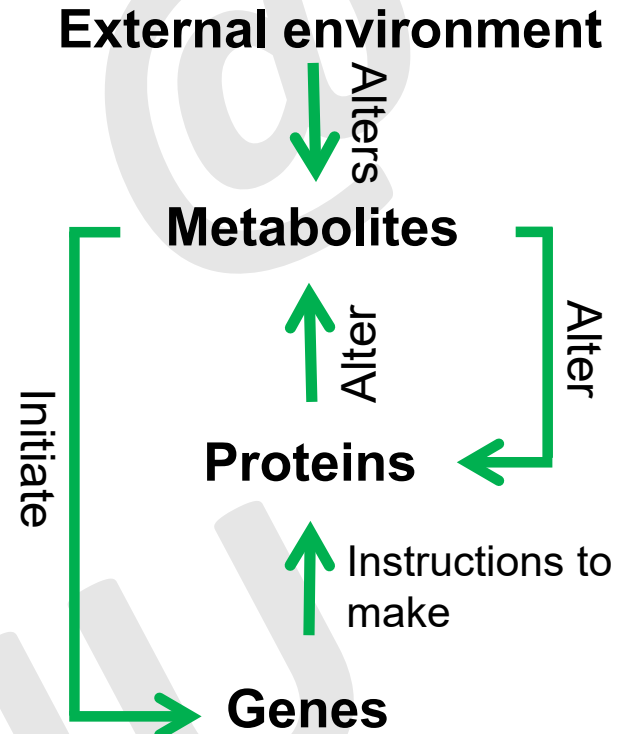
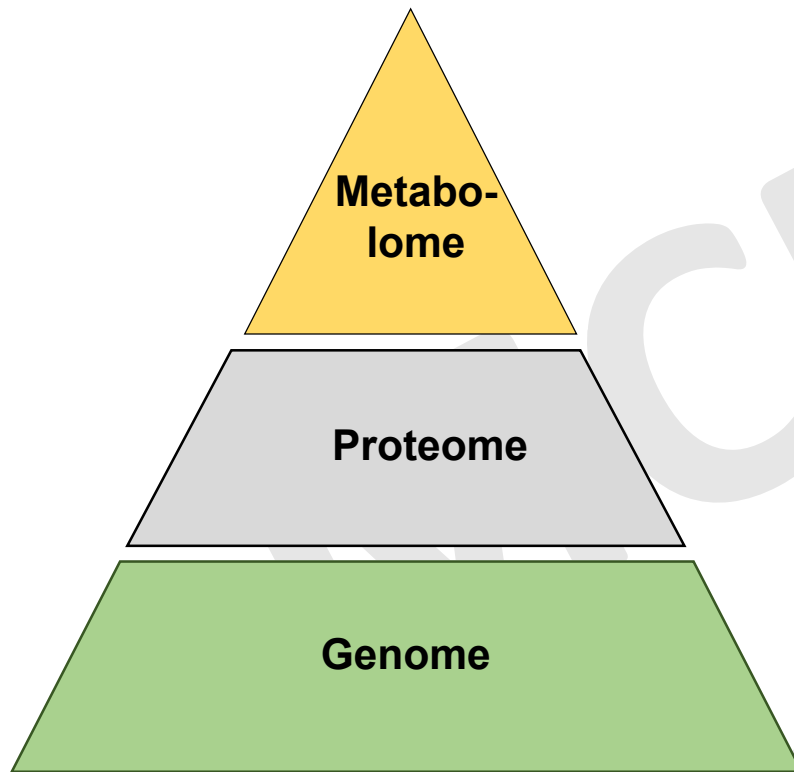


External stimuli

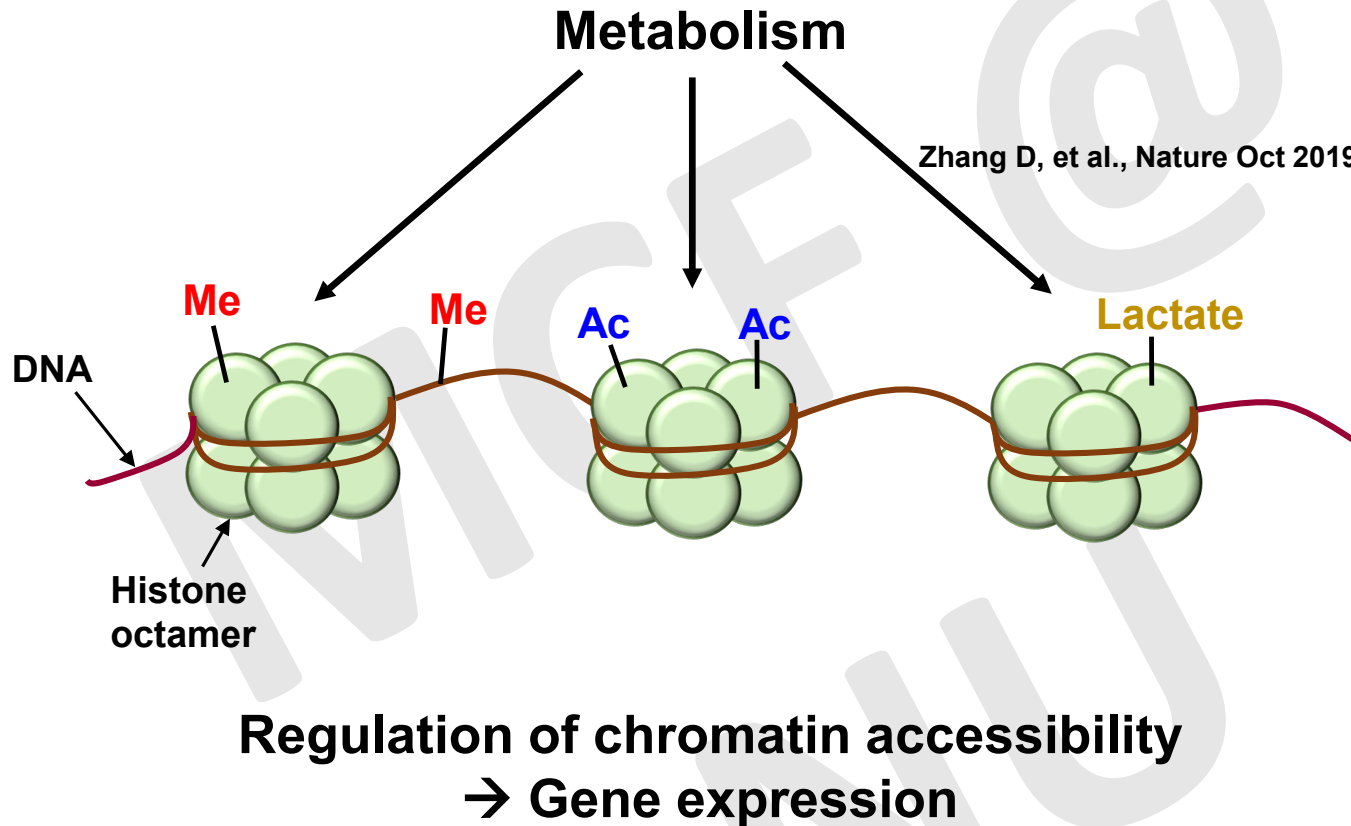


Time

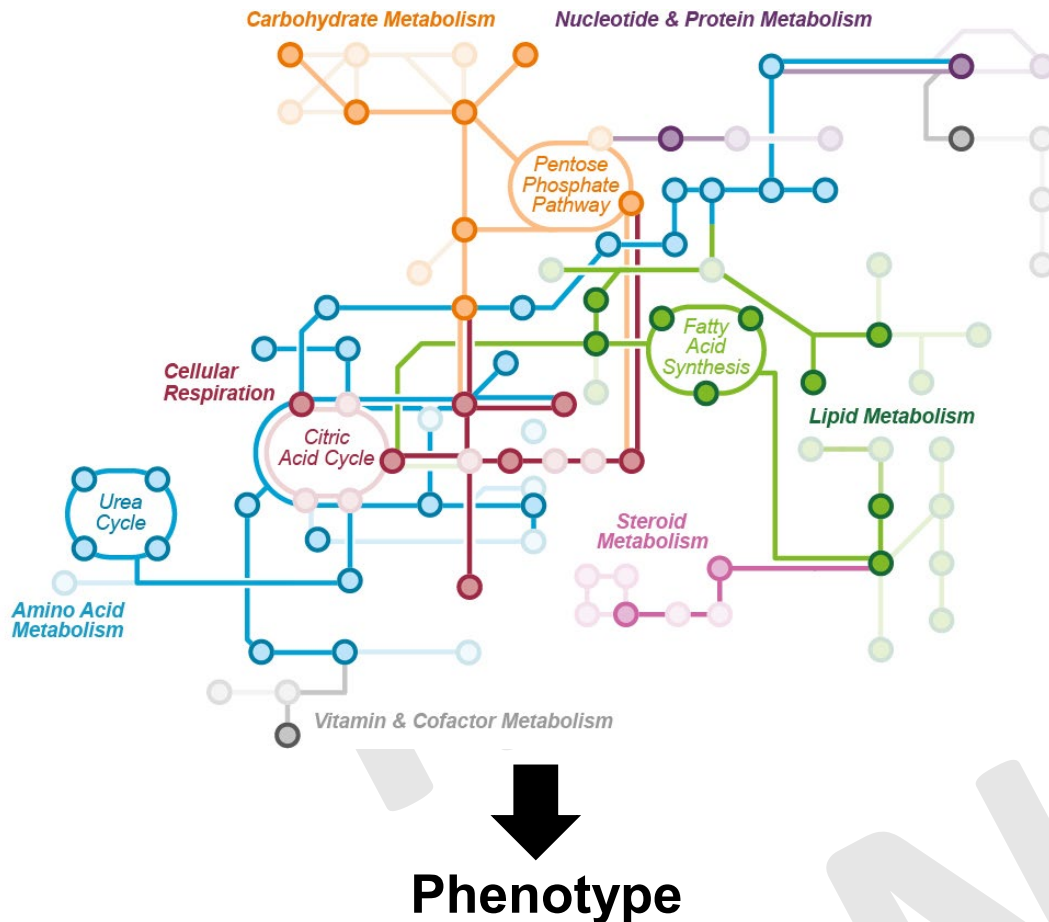
Metabolites are central in cell physiology



Metabolites can directly control gene expression

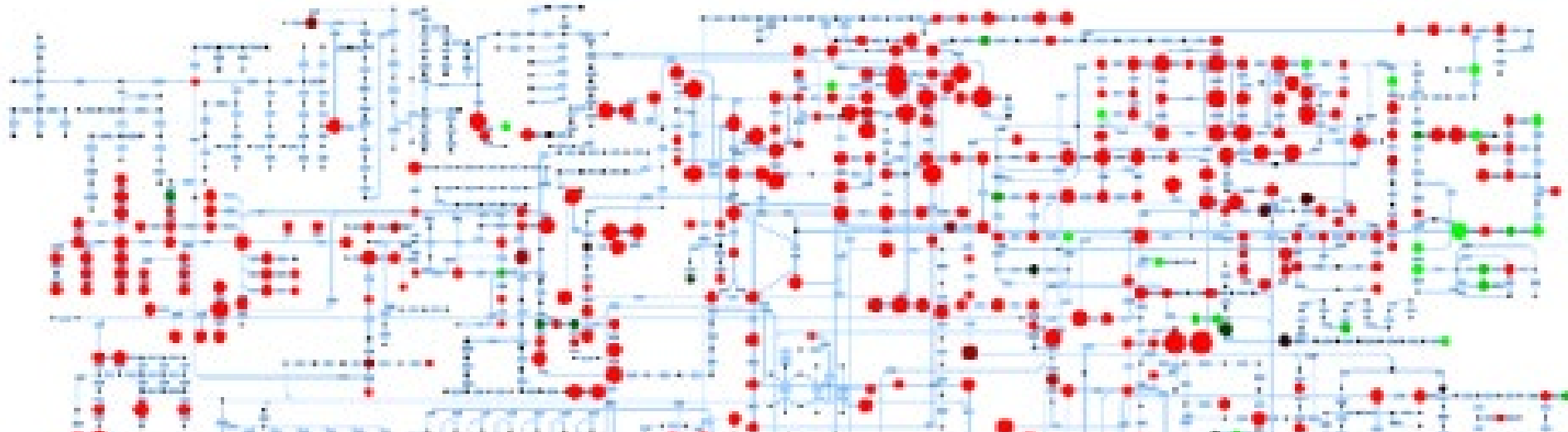


Metabolomics connects Proteome and Genome to Phenotype

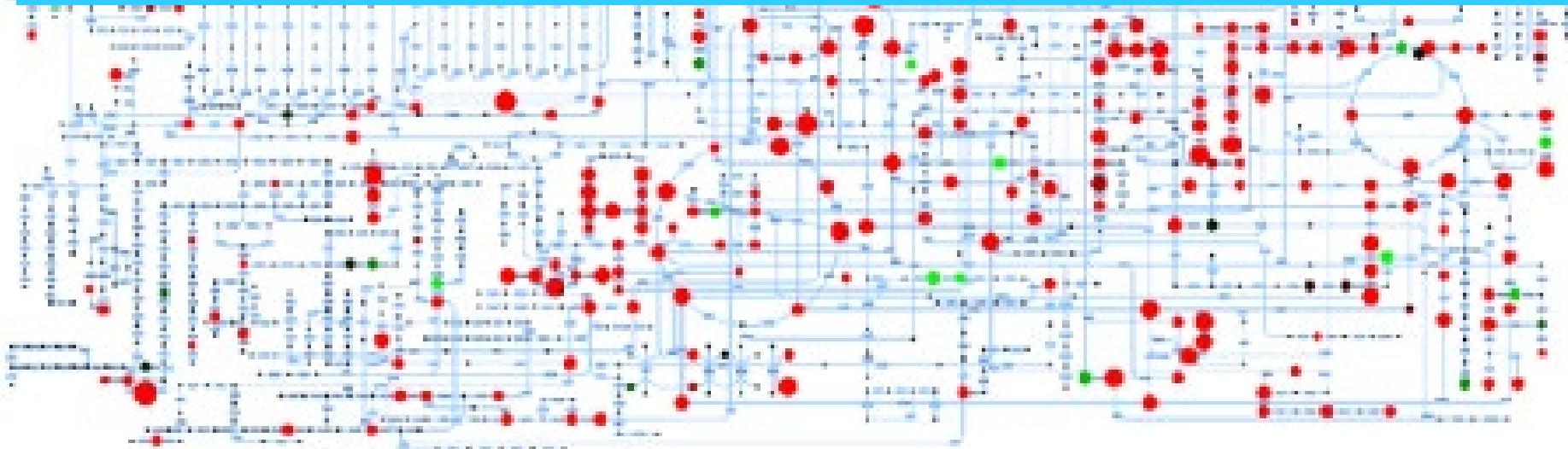


- **Metabolomics data provides insights into underlying biology**
- **Metabolomics data provides information behind the mechanisms by which genes function**
- **Multiple omics data pointing to the same biological pathways builds scientific hypotheses and bring us closer to translational science**

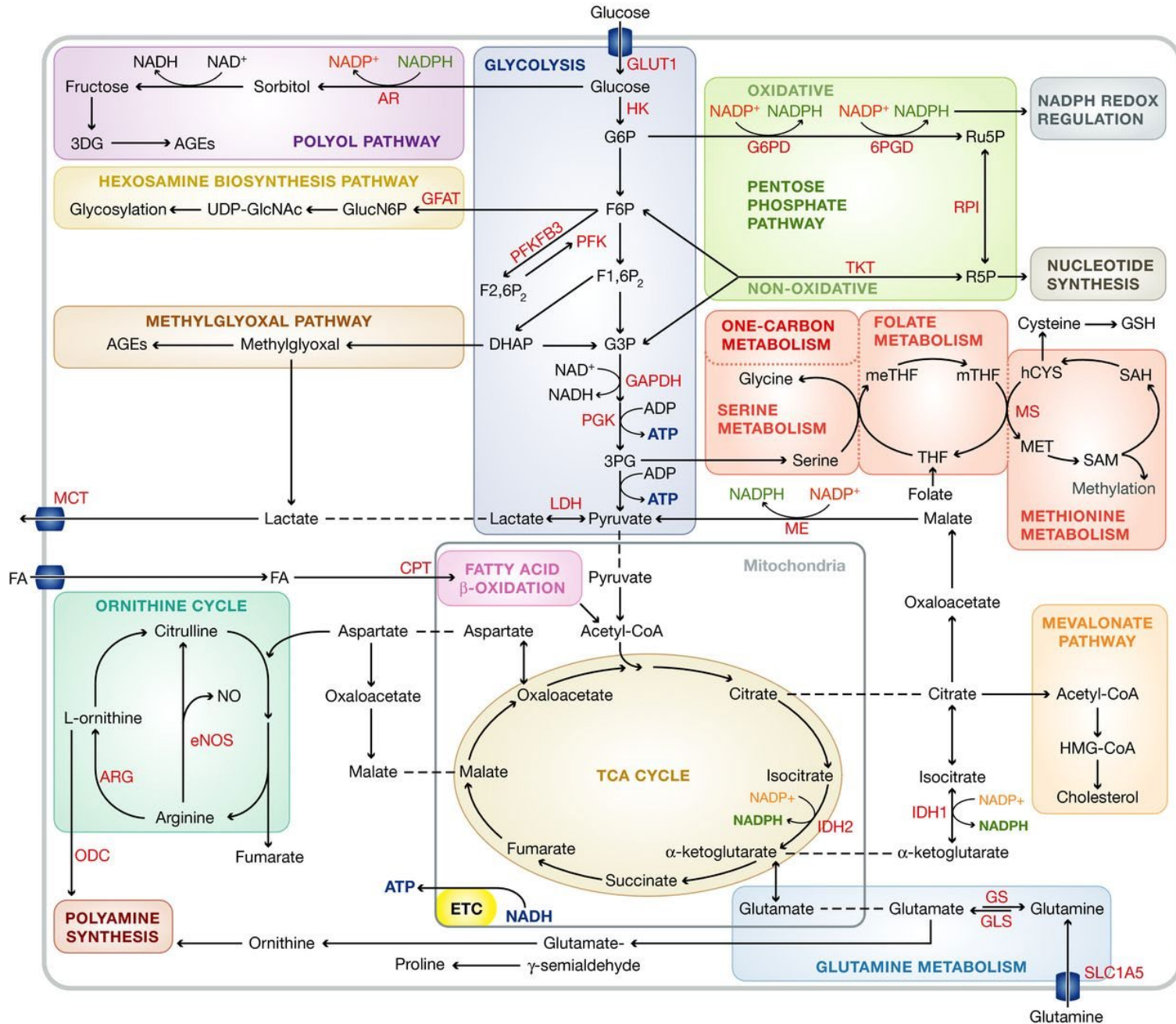
Steady-state Metabolomics



Quantitative Metabolomics: Measurement of metabolite levels at one instant t



Know your textbook



Metabolomics and Isotope Tracing

Cholsoon Jang,¹ Li Chen,¹ and Joshua D. Rabinowitz^{1,*}

¹Lewis Sigler Institute for Integrative Genomics and Department of Chemistry, Princeton University, Washington Rd, Princeton, NJ 08544, USA

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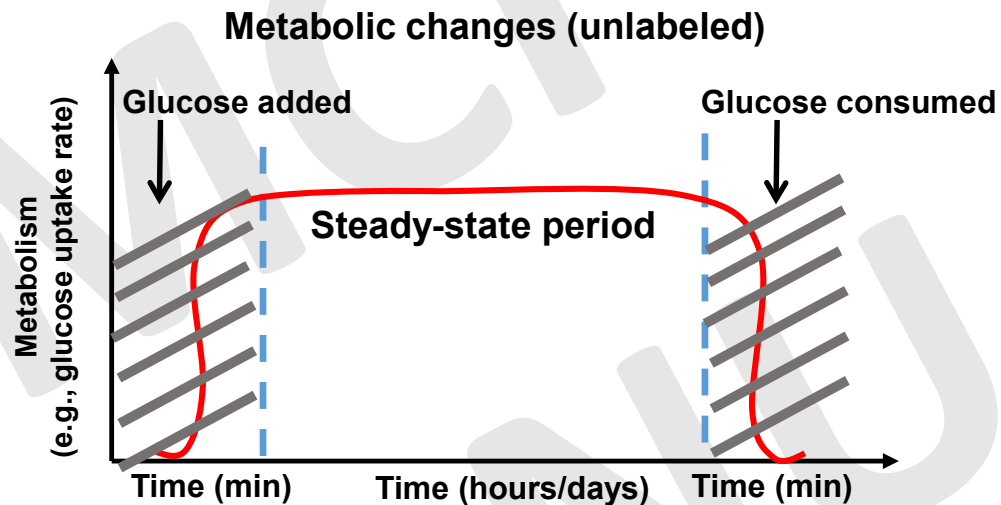
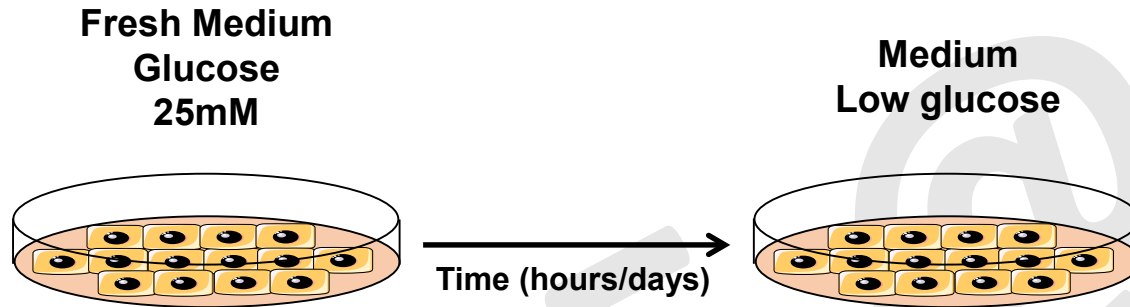
A roadmap for interpreting ¹³C metabolite labeling patterns from cells

Joerg M. Buescher¹, Maciek R. Antoniewicz^{2,*}, Laszlo G. Boros^{3,*}, Shawn C. Burgess^{4,*}, Henri Brunengraber^{5,*}, Clary B. Clish^{6,*}, Ralph J. DeBerardinis^{7,*}, Olivier Feron^{8,*}, Christian Frezza^{9,*}, Bart Ghesquiere^{1,*}, Eyal Gottlieb^{10,*}, Karsten Hiller^{11,*}, Russell G. Jones^{12,*}, Jurre J. Kamphorst^{13,*}, Richard G. Kibbey^{14,*}, Alec C. Kimmelman^{15,*}, Jason W. Locasale^{16,*}, Sophia Y. Lunt^{17,*}, Oliver D. K. Maddocks^{10,*}, Craig Malloy^{18,*}, Christian M. Metallo^{19,*}, Emmanuelle J. Meuillet^{20,*}, Joshua Munger^{21,*}, Katharina Nöh^{22,*}, Joshua D. Rabinowitz^{23,*}, Markus Ralser^{24,*}, Uwe Sauer^{25,*}, Gregory Stephanopoulos^{26,*}, Julie St-Pierre^{27,*}, Daniel A. Tennant^{28,*}, Christoph Wittmann^{29,*}, Matthew G. Vander Heiden^{30,*}, Alexei Vazquez^{10,*}, Karen Vousden^{10,*}, Jamey D. Young^{31,*}, Nicola Zamboni^{25,*}, and Sarah-Maria Fendt^{1,#}

Steady-state Metabolomics



Nutrient and the response of metabolism



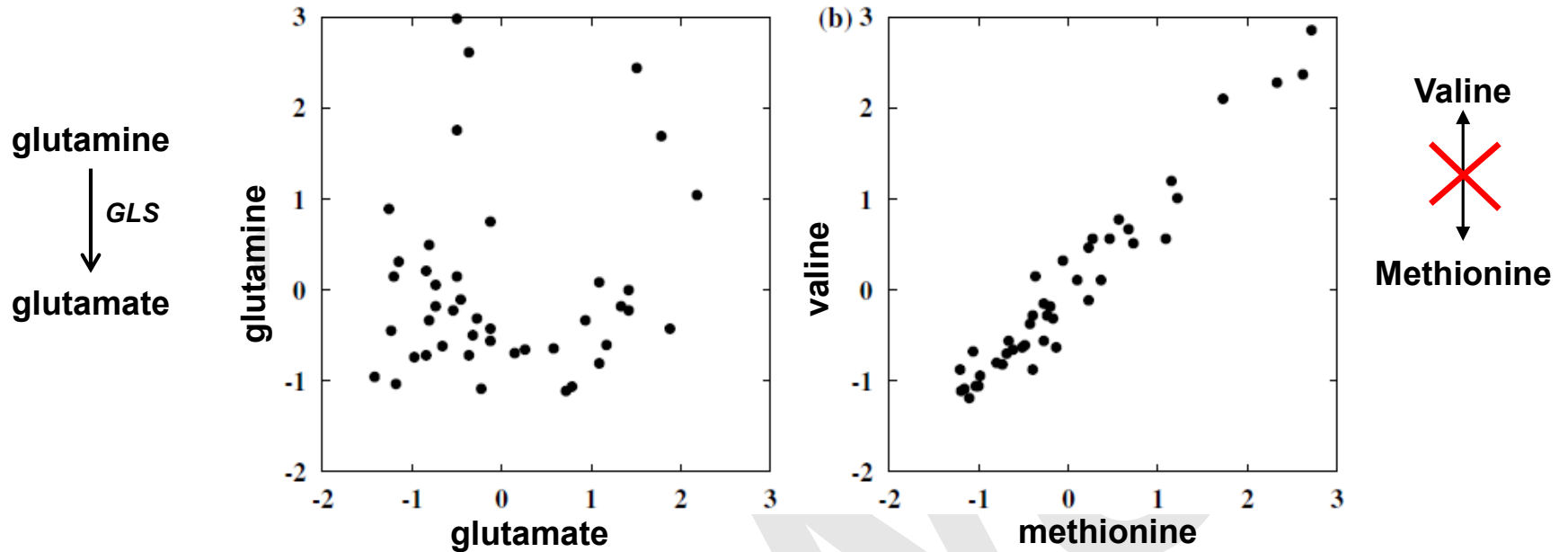
The quantitative inflows and effluxes from each metabolite must be balanced

The origin of correlations in metabolomics data

Diogo Camacho, Alberto de la Fuente, and Pedro Mendes*

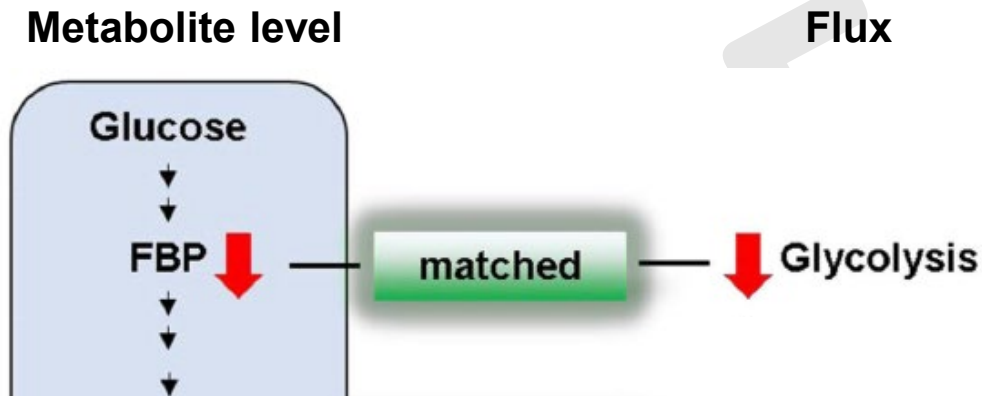
Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, MC 0477, Washington St., Blacksburg, VA, 24061, USA

Received 19 August 2004; accepted 15 September 2004



Metabolite levels Vs Metabolic flux

Glucose removal decreases flux though glycolysis but some glycolytic intermediates increase (e.g., PEP)

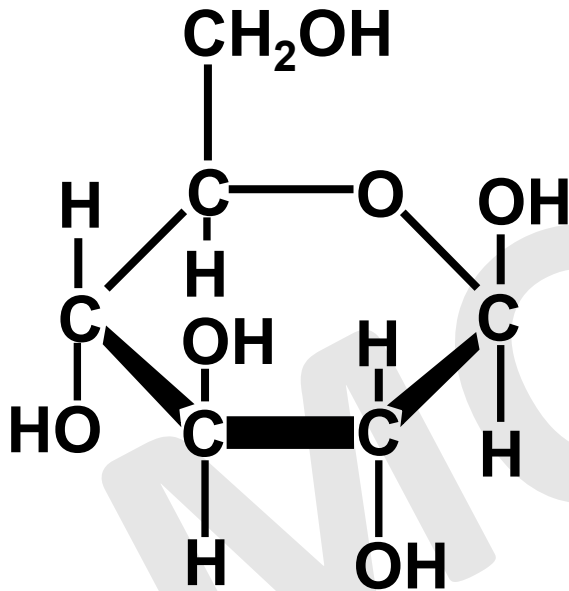


Adapted from Jang C, et al., *Cell* 2018

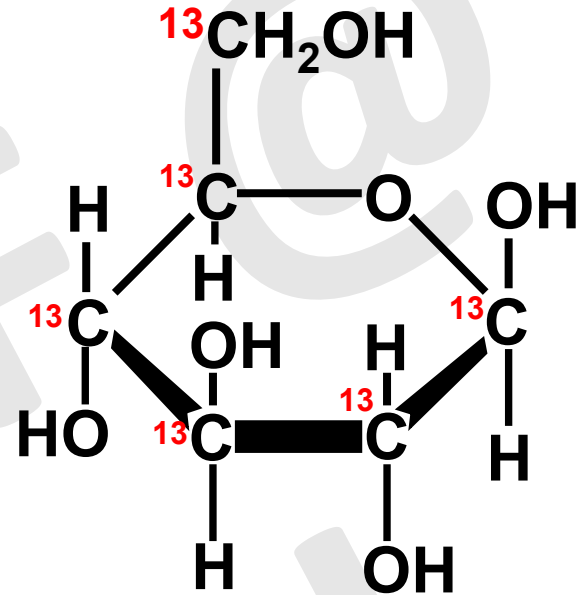
Metabolic flux



Isotopic tracer



Glucose



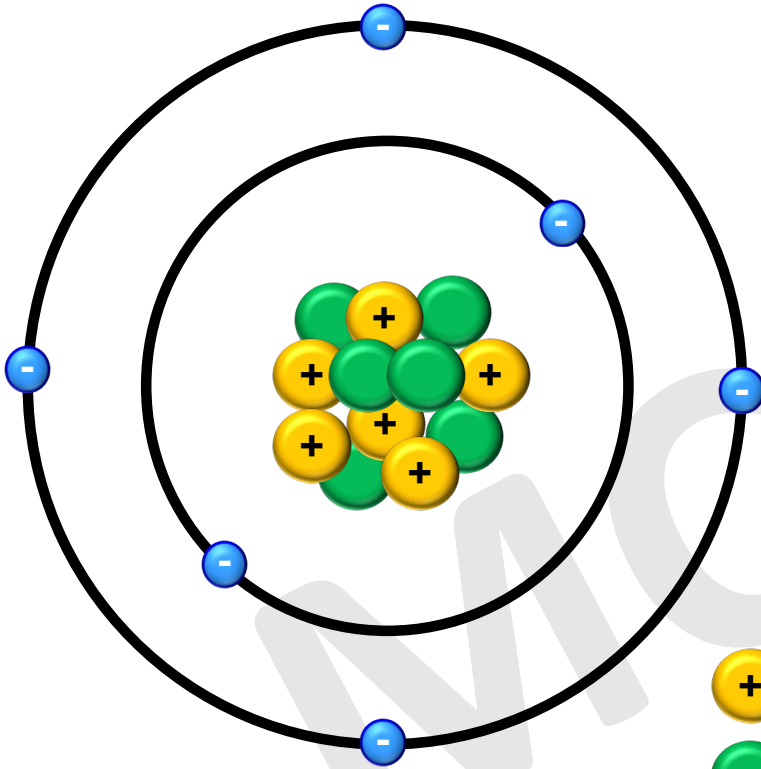
¹³C₆-Glucose

U-¹³C-Glucose

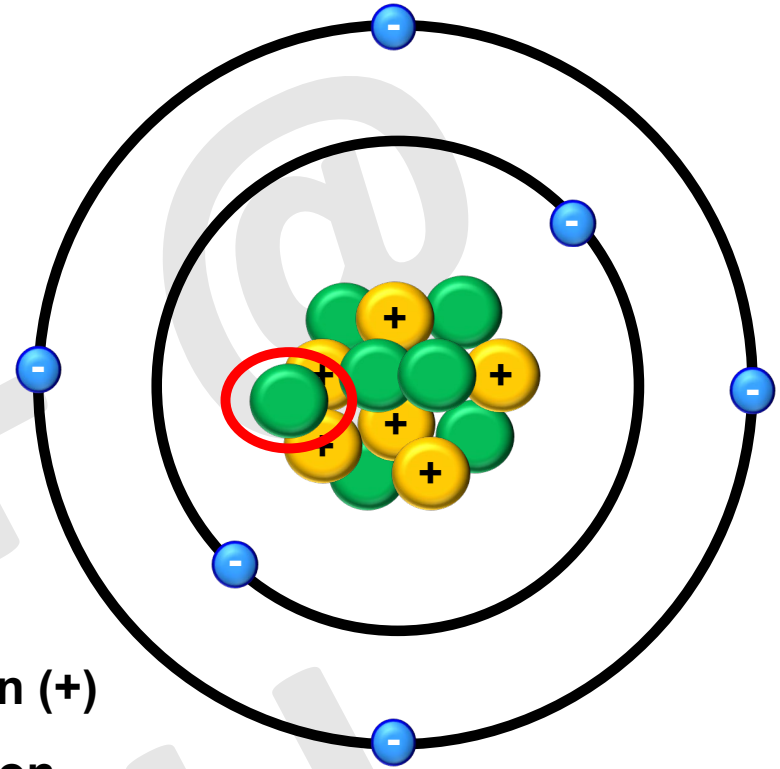
- Heavy isotope
- Non radioactive
- Similar physical properties

Isotopic tracer

Carbon 12



Carbon 13



 Proton (+)

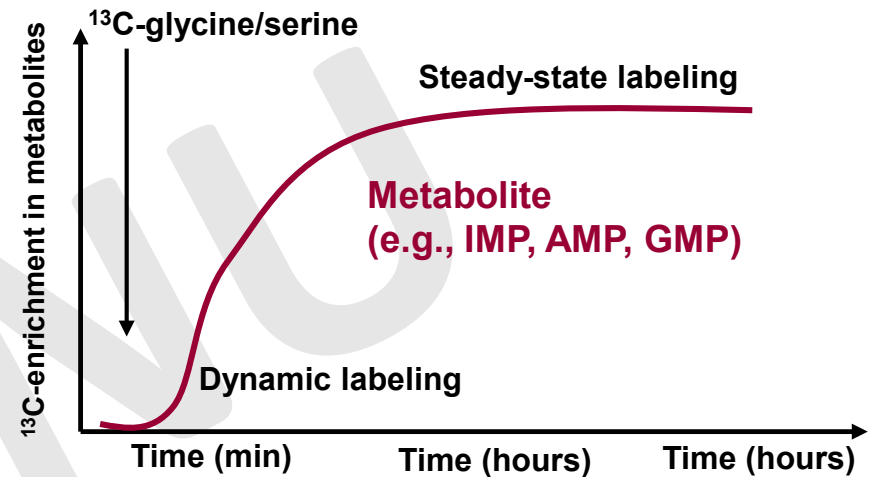
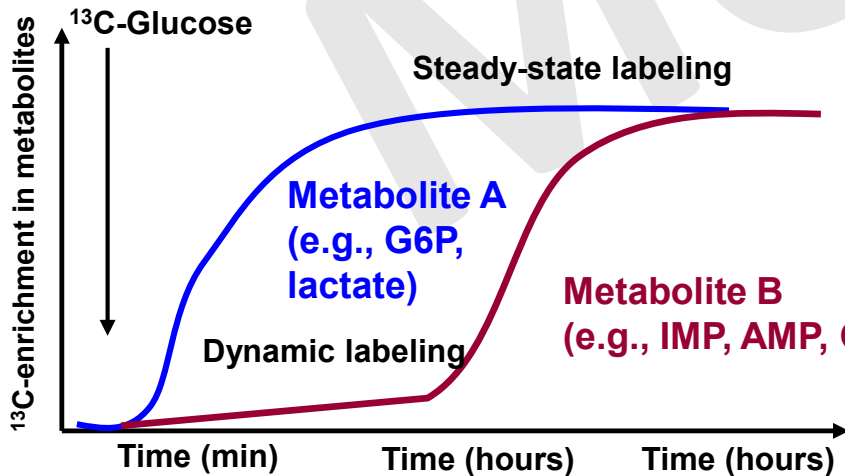
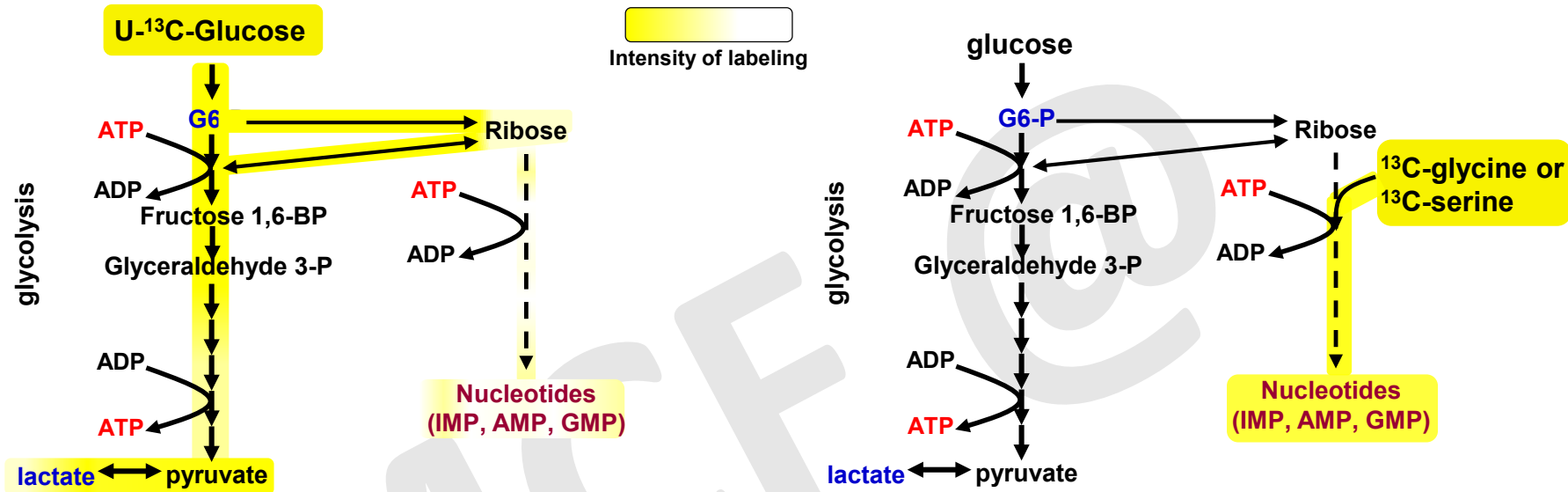
 Neutron

 Electron (-)

Carbon 13 has an extra neutron

Because the labeled atom has the same number of protons, it will behave in almost exactly the same way as its unlabeled counterpart and, will not interfere with the reaction under investigation.

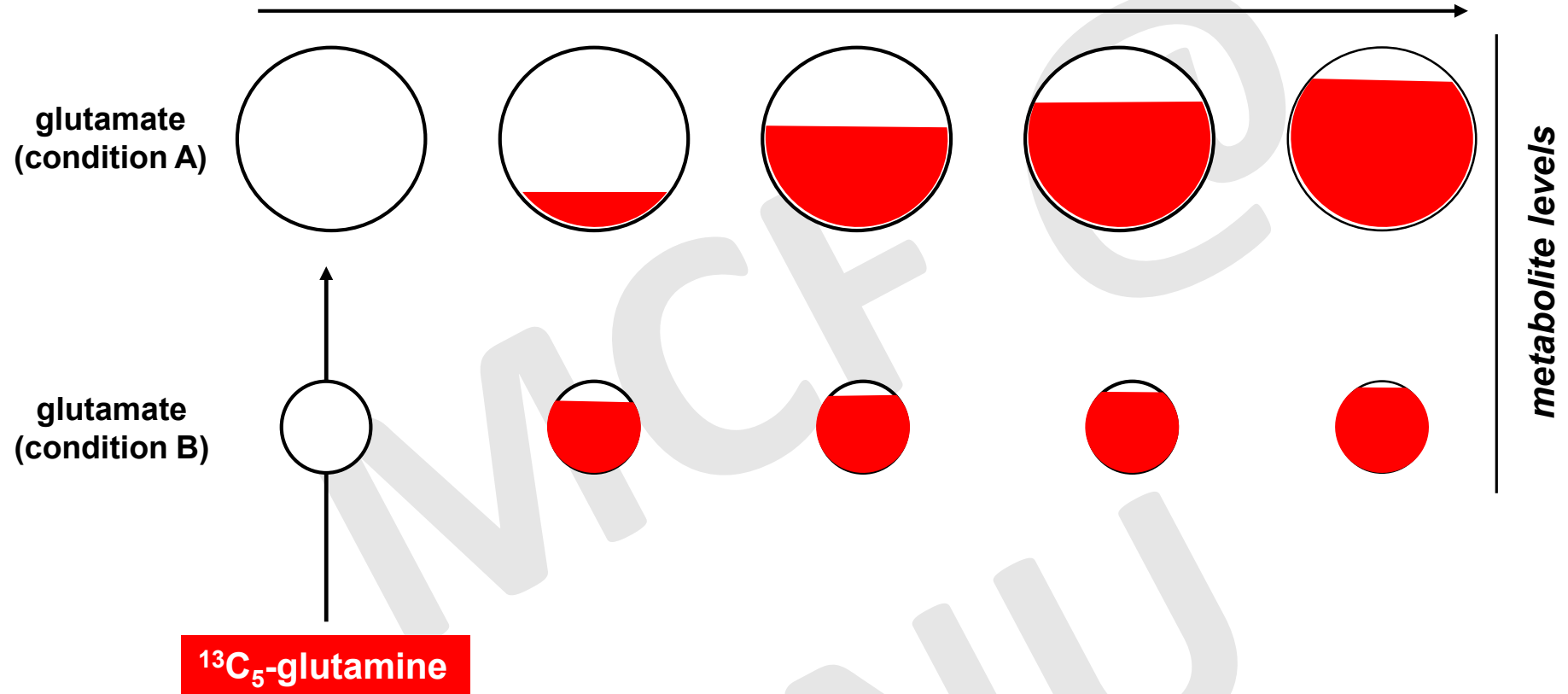
Isotopic tracing to study the activity of metabolic pathways in cells



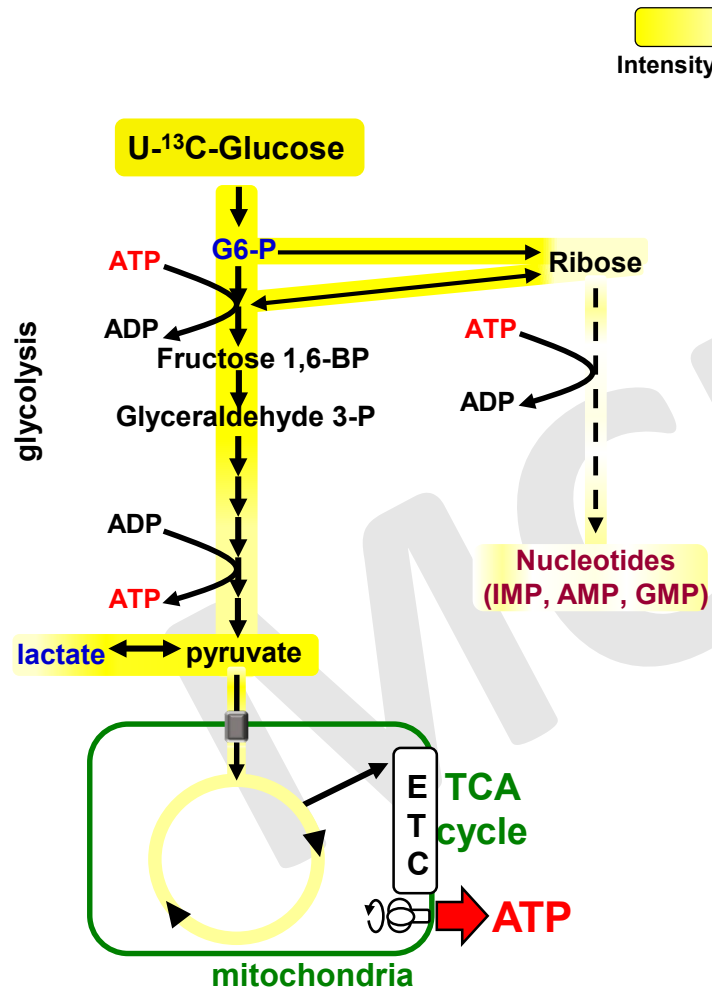
Isotopic tracing: Consider the size of the unlabeled pool

Dynamic labeling data

¹³C enrichment over time



Isotopic tracing to study the activity of metabolic pathways



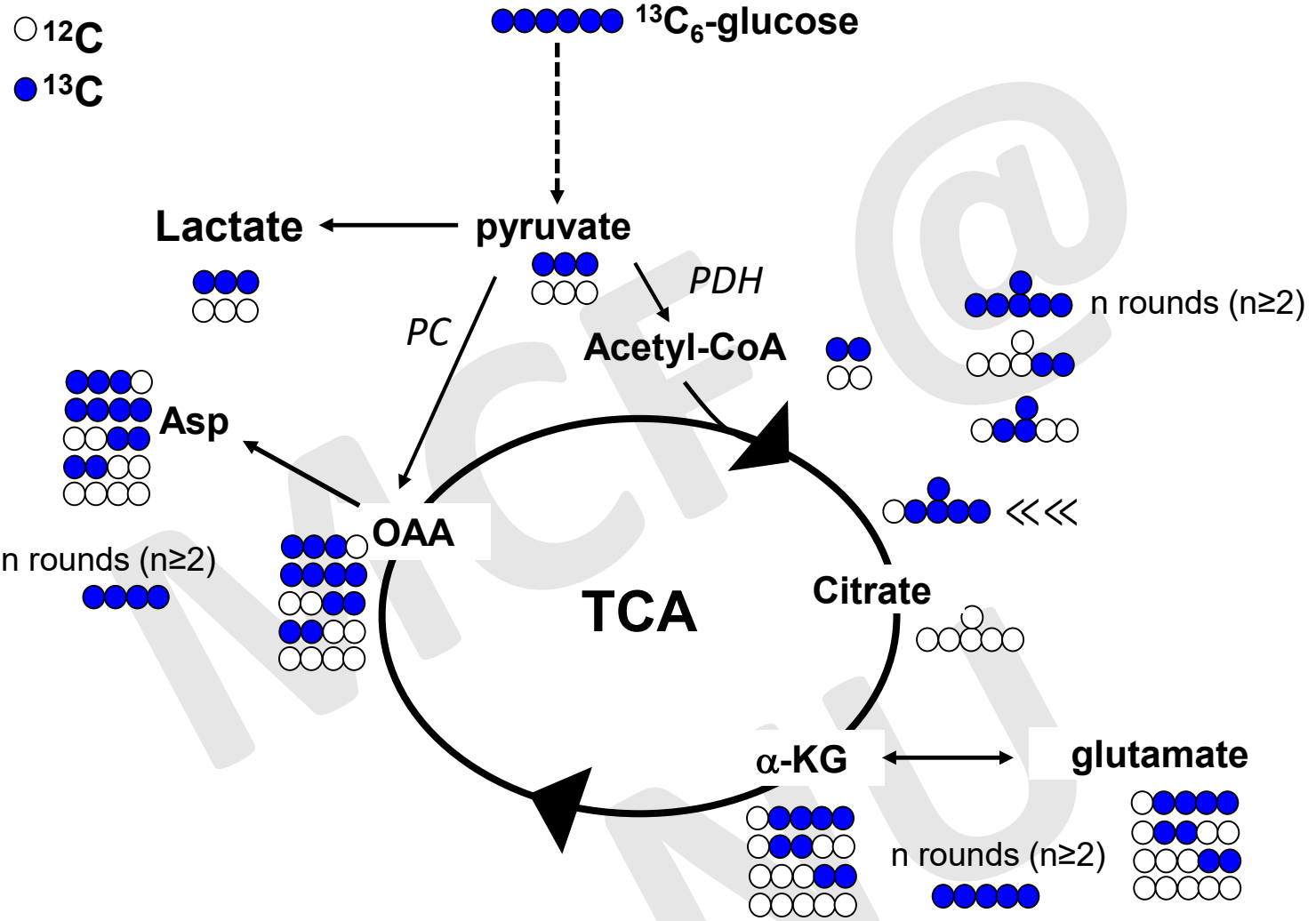
Recommendation for tracing experiments:

- Consider tracer uptake (e.g., glucose uptake) before performing the tracing
- Perform the flux in tracer-free medium (e.g., glucose-free medium)
- Timing of labeling matters:

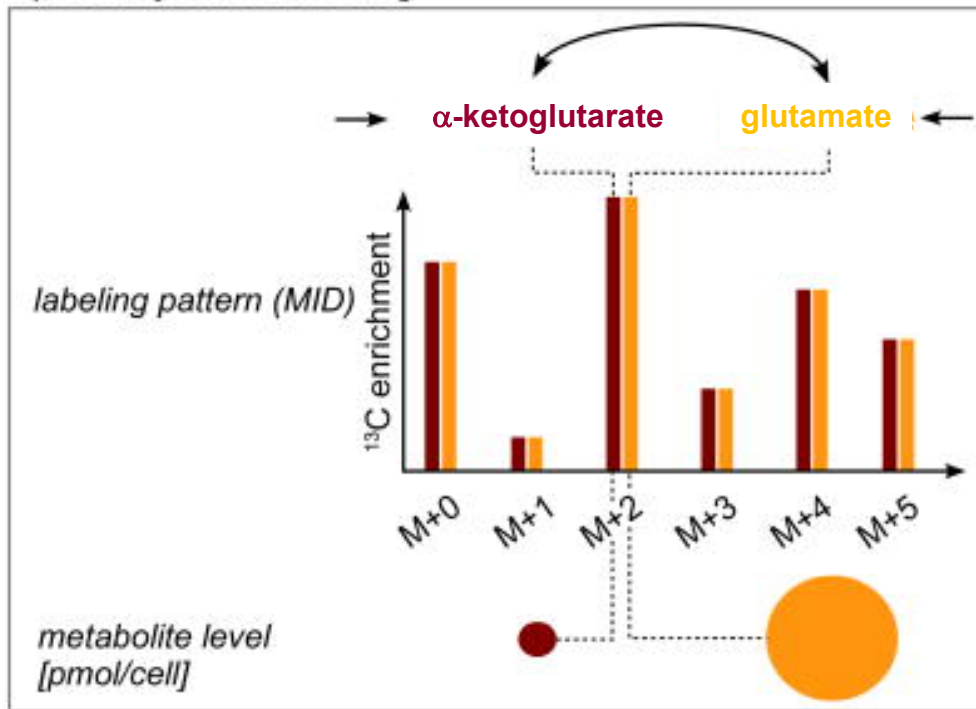
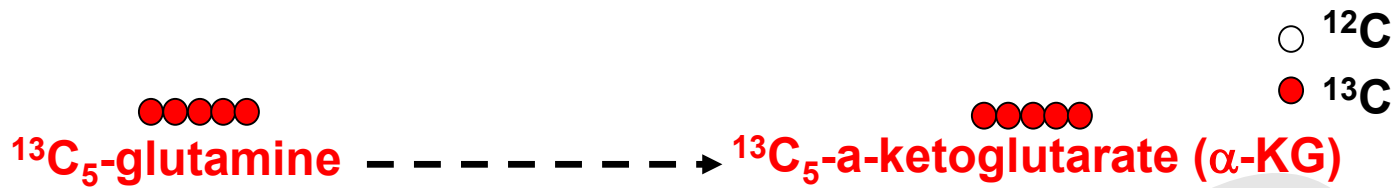
For ¹³C-glucose:

- ~15-30 min of labeling enables to label glycolytic intermediates at the steady-state level
- 2-4h of labeling is required to label the TCA cycle
- 6-15h of labeling is required to label nucleotides

Representation of the metabolic tracing diagram



Isotopic tracing: Impact on scientific research



α -KG



M+0



M+1



M+2



M+3



M+4

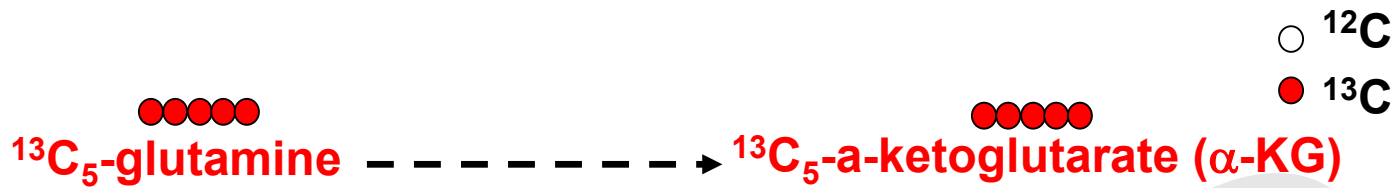


M+5

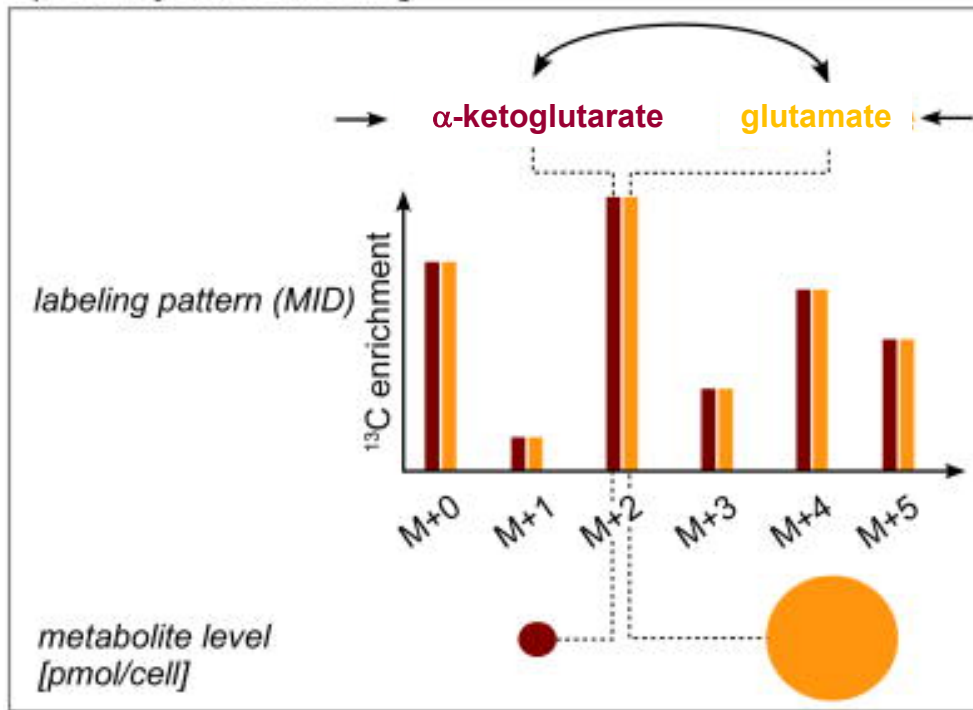
Isotopologues

Adapted from Buescher JM et al., *Curr Opin Biotechnol* 2015

Isotopic tracing: Impact on scientific research



○ ^{12}C
 ● ^{13}C



α -KG Ion counts

○○○○○ M+0 815000

○○○○● M+1 215000

○○○○●● M+2 35000

○○●●●● M+3 25000

○●●●●● M+4 95000

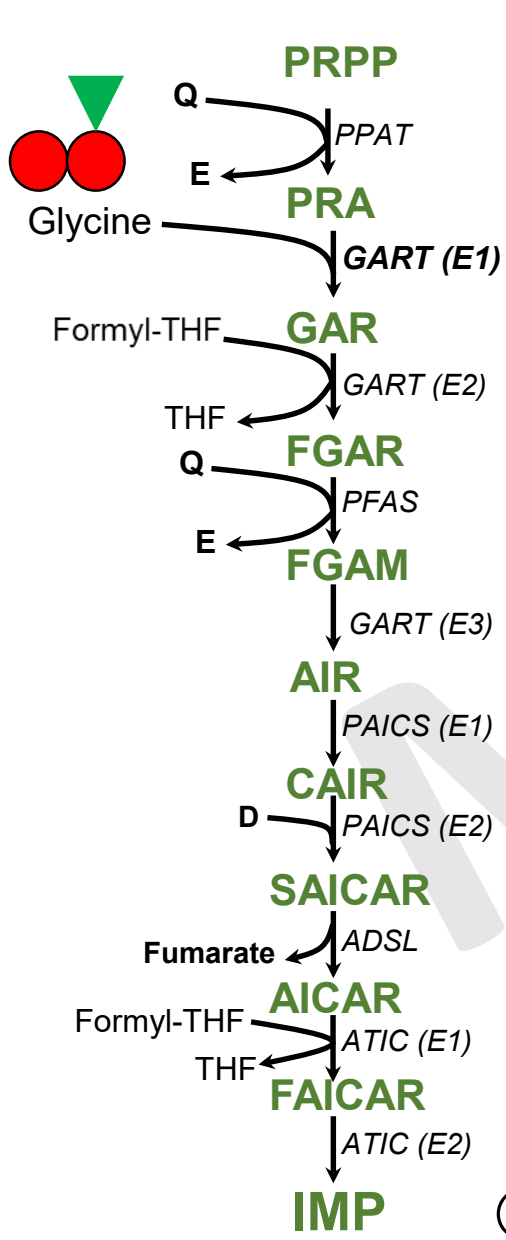
●●●●●● M+5 5615000

= 6800500

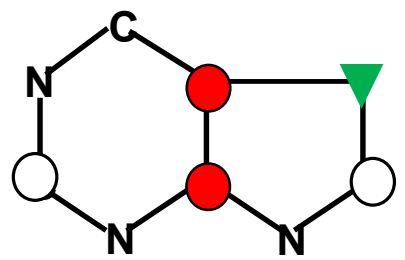
Adapted from Buescher JM et al., *Curr Opin Biotechnol* 2015

$$\text{Fractional abundance } \alpha\text{-KG (M+5)} = \frac{5615000}{6800500} \sim 0.82$$

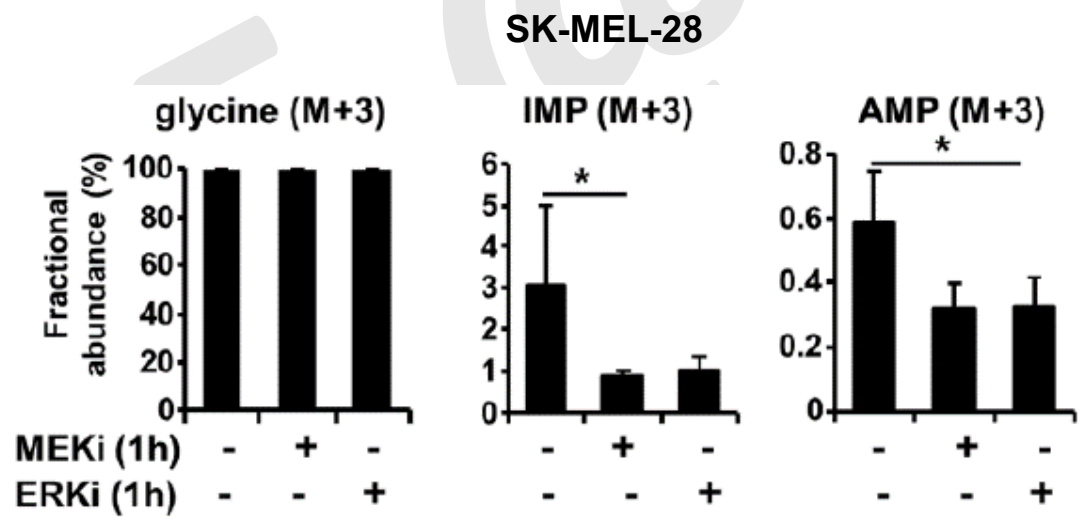
Isotopic tracing: Ben-Sahra lab



De novo purine synthesis

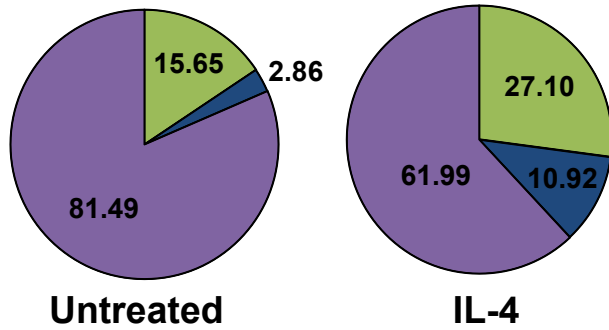


Tracer: $^{13}\text{C}_2$ - ^{15}N -glycine



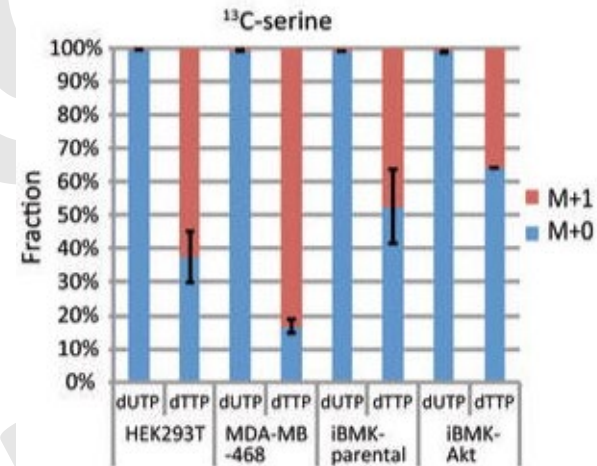
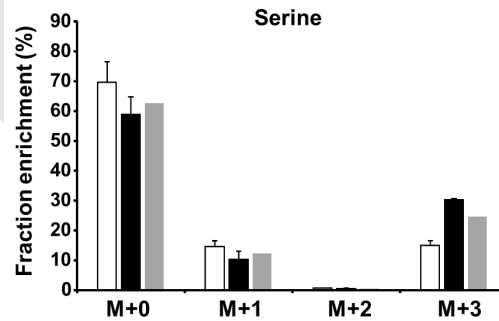
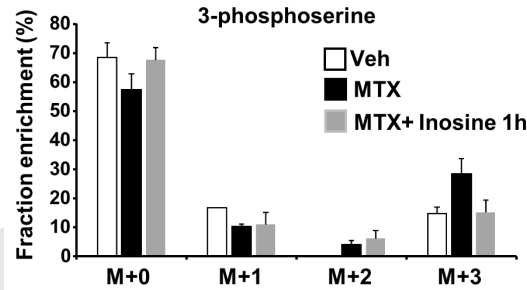
Presentation of the tracing data

Chart



- citrate_13C1
- citrate_13C2
- citrate_13C3
- citrate_13C4
- citrate_13C5
- citrate_13C6
- Unlabeled citrate

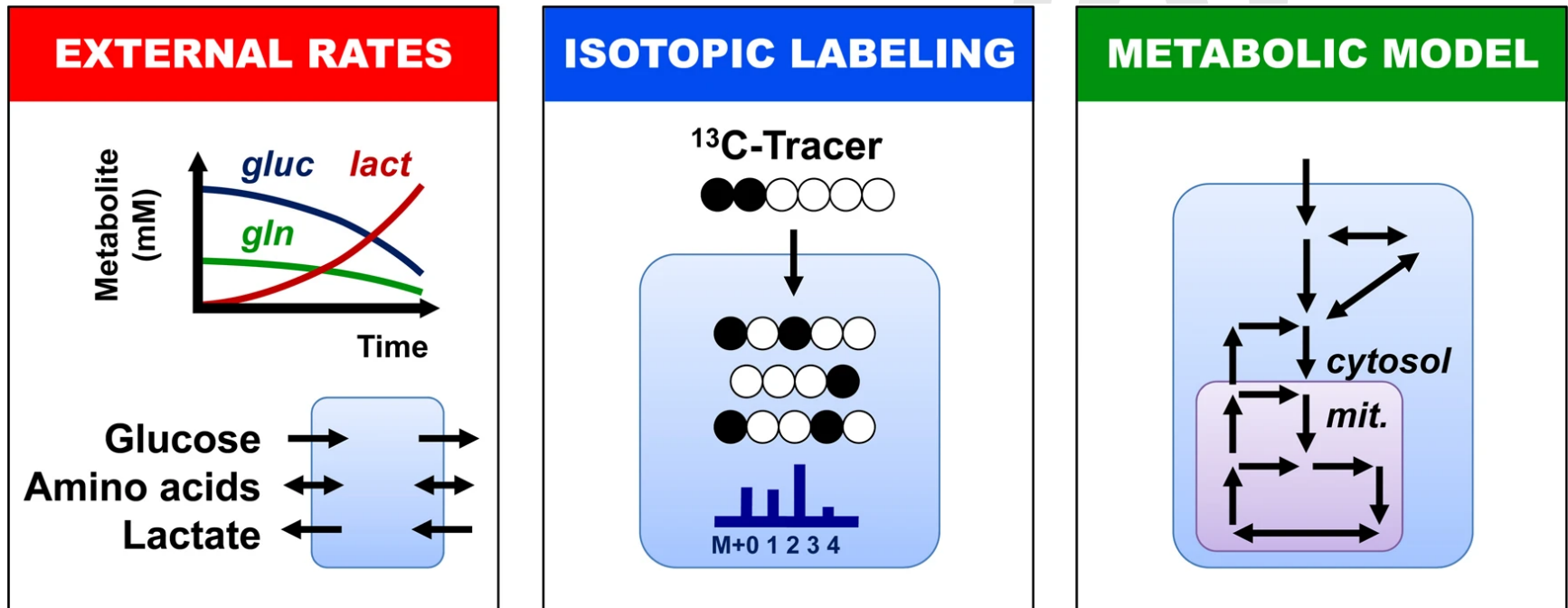
Histogram



Fan J et al, Nature 2014

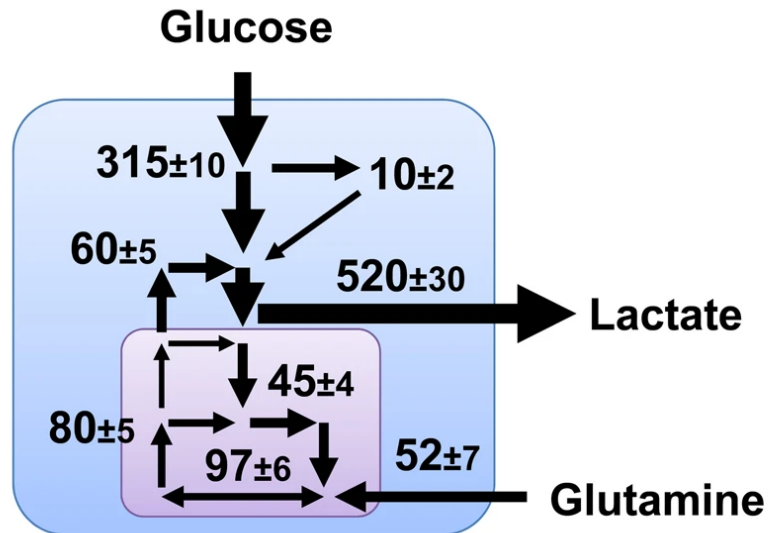
Metabolic Flux Analysis (MFA)

^{13}C metabolic flux analysis (MFA) is a mathematical approach for quantifying intracellular metabolic fluxes in cancer cells.

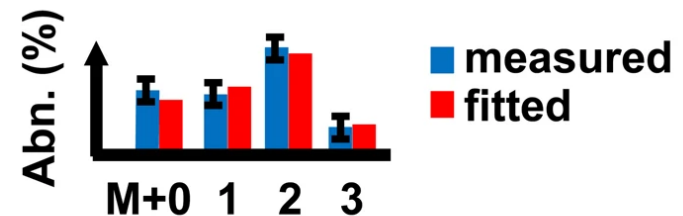


Metabolic Flux Analysis (MFA)

¹³C METABOLIC FLUX ANALYSIS (software tools e.g. Metran, INCA)



Statistical Analysis of Goodness-of-Fit



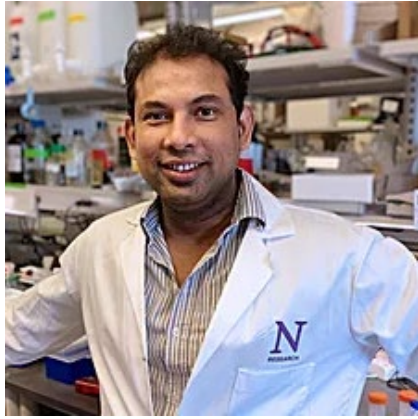
$$SSR = \sum \frac{(\text{measured} - \text{fitted})^2}{(\text{stdev})^2} < \chi^2$$

Antoniewicz MR et al., Exp & Mol Med 2018

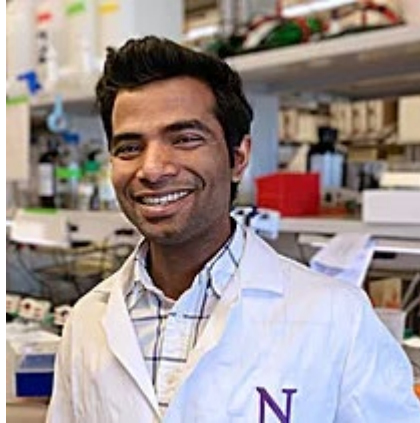
The software INCA can be used to perform MFA calculations.

Acknowledgements

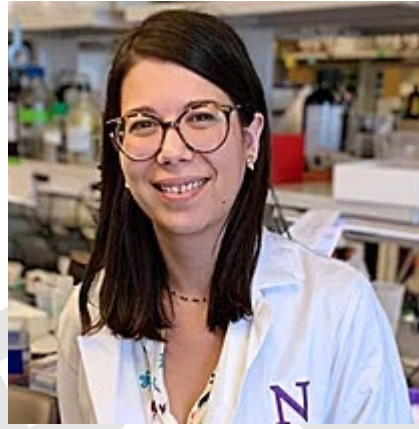
Lab members:



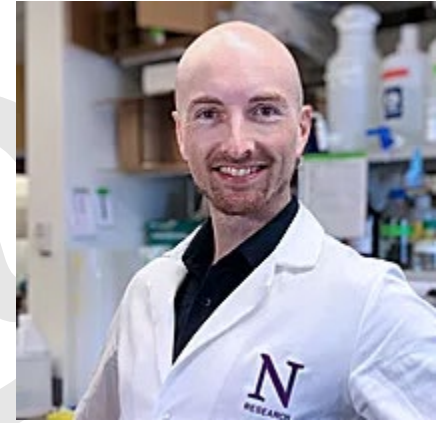
Eunus Ali



Umakant Sahu



Elodie Villa

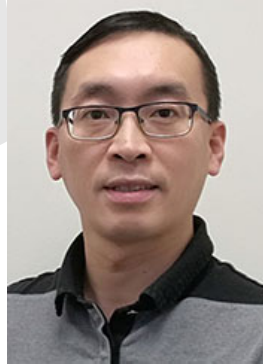


Brendan O'Hara

Metabolomics Developing Core Facility



Nav Chandel



Peng Gao



The LAM Foundation
A BREATH OF HOPE



LYNN SAGE
CANCER RESEARCH
FOUNDATION



**National Institutes
of Health**

“How to Conduct in vivo Metabolomics”

