Seminar 2

Isotope tracing

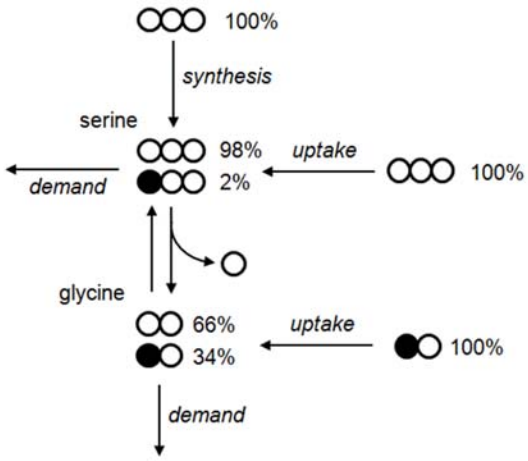
1: How many possible ^{13}C isotopomers are there for glucose? How many mass isotopomers? How many isotopomers correspond to the +5 mass($^{13}C_5$) isotopomer?

2: Pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) both feed pyruvate carbon into the TCA cycle, but PDH cleaves off one carbon atom, while PC adds one atom. Which carbon is lost in the PDH reaction? If we can measure mass isotopomers of citrate, what pyruvate tracer(s) would be suitable to analyse PDH versus PC activity?

3: In a study using a $^{13}C_5$-glutamine tracer (that is, all 5 carbon atoms are ^{13}C), 70% of the intracellular glutamine pool is +5 (mass isotopomer $^{13}C_5$), while 30% of the glutamate pool is +5. What fraction of the glutamate pool is derived from glutamine in this case?

4: Fructose biphosphate aldolase cleaves fructose-1,6-diphosphate (fdp) into two 3-carbon sugars, dihydroxyacetone phosphate and glyceraldehyde phosphate. If the isolated enzyme (in vitro) is given a mix of 50% +6 and 50% unlabeled (+0), what isotopomers would you expect after the reaction has reached steady state? Would you expect the same results in cells?

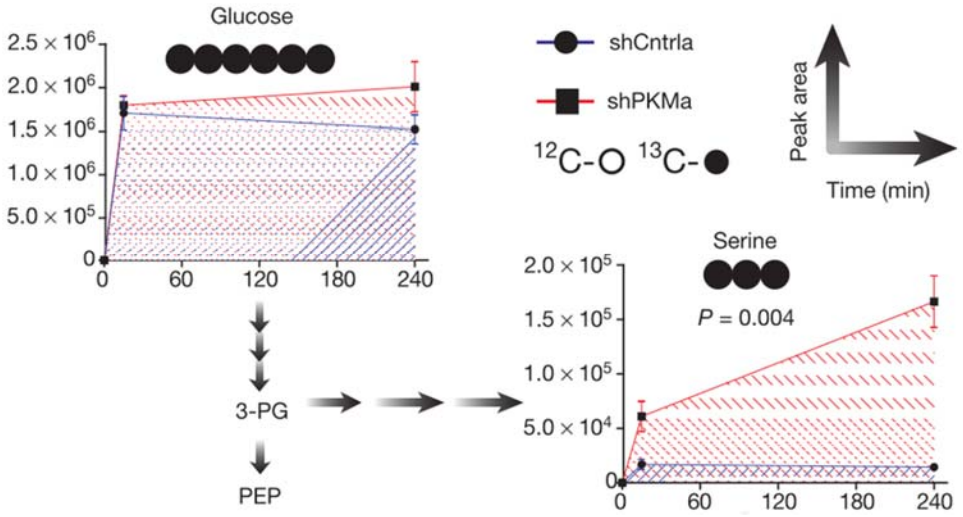
5: The figure on the right describes measured isotopomers of serine and glycine in melanoma cells (Jain & Nilsson et al, Science 2012). What fraction of glycine is derived from serine in this case? If the glycine uptake flux is 4 fmol / cell / h, what is the flux of glycine synthesis from serine? If you also know the serine uptake rate, is it possible to determine the absolute rate of serine synthesis? Why / why not?



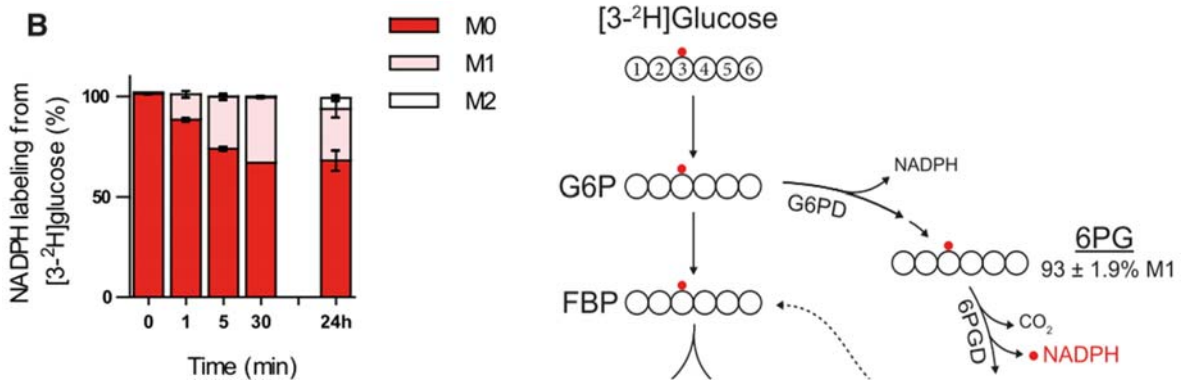
6a: In a time-series experiment, an intracellular metabolite X is initially unlabeled at time 0. In cell type A, metabolite X reaches 50% labeling after 2 hours of incubation with a 100% labeled tracer. In cell type B, 50% labeling is reached after 4 hours. What can we say about the rate of synthesis of X from the tracer in A and B?

6b: For the experiment in 6a, say we also know that the pool size of X is twice as large in cell type A than in cell type B (relative quantification). Does this help determine the flux?

7: The figure below (from Chaneton et al, Nature 491:458-462, 2012) shows relative abundances (in arbitrary units) of the indicated mass isotopomers of glucose and serine in control cells (shCntrla, blue) and in pyruvate kinase knockdown cells (shPKMa, red). From these data, the authors conclude that there is “an increased metabolic flux into the serine and glycine biosynthetic pathway” in the shPKMa cells. Is this a valid interpretation? Why/why not?



8: The below figure shows ^{2}H labeling in NADPH from 3-^{2}H-glucose over time, reflecting labeling from the pentose phosphate pathway (from Lewis et al, Mol Cell 55:263-263, 2014). What is the half-time of the NADPH pool (roughly) ? Can the 24h time point be considered to be steady state? Why is there an M2 ($^{2}H_2$) mass isotopomer in NADPH? What fraction of cellular NADPH is obtained from the pentose phosphate pathway according to these data?



TCA cycle

1: Write down the complete reaction formula for pyruvate oxidation in the TCA cycle. How many NADH, QH2, ATP are generated from one molecule pyruvate?

Solution: pyr + 2 H20 + 2 NAD + Q + ADP → 3 CO2 + 3 NADH + QH2 + ATP.

2: Why do you think so many steps are needed to oxidize two carbons (from acetyl-CoA) to CO_2?

Solution: There is a lot of energy to extract from one pyruvate molecule; the carbons change several oxidation numbers (to end up as +4). Therefore several enzymes are needed to capture the energy in several reduced carriers (and one ATP).

3: Are there any steps in the TCA cycle that could (theoretically) run without a functioning respiratory chain (when electron carriers cannot be reoxidized) ? Could ATP be generated without respiration?

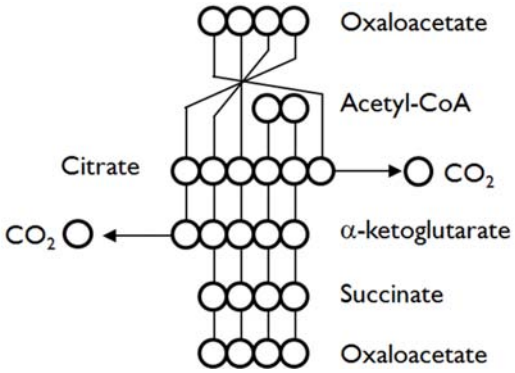
Solution: a-ketoglutarate could be oxidized to succinate, since this generates ATP, not NADH. (Succinate does in fact accumulate in hypoxic conditions.)

4: Consider a situation where 10 fmol / cell / h of acetyl-CoA enters the citrate synthase reaction, and 5 fmol / cell / h of citrate is drained from the cycle for fatty acid synthesis. Show how to balance the fluxes in the cycle with an anaplerosis reaction.
See: Owen et al, JBC 277:30409-30412, 2002.

Solution: 10 fmol / h acetyl-CoA requires 10 fmol / h oxaloacetate for the citrate synthesis reaction to run. To compensate, 5 fmol / h oxaloacetate could generated by pyruvate carboxylase; alternatively, a-ketoglutarate could be replenished with 5 fmol / h.

5: The figure to the right shows one "turn" of the TCA cycle at the atom level. What happens to carbon 3 of a-ketoglutarate? Are we missing something?

Solution: it appears that carbon 3 always stays in the cycle. However, succinate (and fumarate) are rotationally symmetric metabolites, so this carbon actually has a 50% chance of exiting the cycle in each turn.



6: Consider the TCA cycle oxidizing only glutamate as a substrate (entering as alpha-ketoglutarate). Show how to balance fluxes with a cataplerosis reaction. What is the net yield of NADH, QH2, ATP from 1 molecule alpha-ketoglutarate in this case?

Solution: in this case half of the oxaloacetate must be removed and used to regenerate pyruvate (and hence acetyl-CoA). The simplest solution is using PEPCK and pyruvate kinase. Then, half of the alpha-ketoglurate in the cycle derives from citrate, and half from glutamate, so the reactions from alpha-ketoglurate to succinate runs at twice the rate of the others. The net yields becomes 1 akg → 4 NADH + 2 QH2 + 5 CO2 (ATP cancels out).