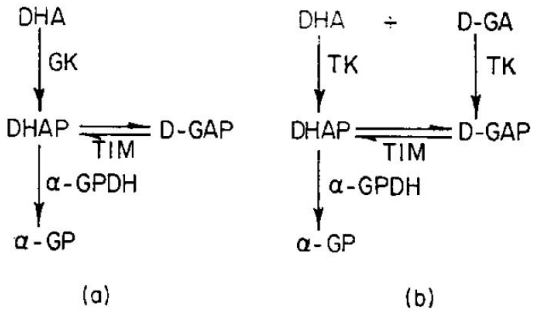
Seminar 4

Measuring metabolism

For mass spectrometry problems, you can look up exact masses of metabolites and their ions of metabolites in the METLIN database, metlin.scripps.edu; go to METLIN → Advanced to search by metabolite name.

1: The figure to the right shows a scheme for an enzymatic assay of the trioses dihydroxyacetone (DHA) and D-glyceraldehyde (D-GA) using the enzymes glycerol kinase (GK), triose kinase (TK), triose phosphate isomerase (TIM) and glyceraldehyde-phosphate dehydrogenase (a-GPDH). The readout is NADH produced by a-GPDH; GK is known to phosphorylate DHA only (panel a), while TK unspecifically phosphorylates both DHA and D-GA. How can the assay be used to quantify D-GA? What are the key assumptions?



2: Modern mass spectrometers can achieve very high mass accuracy. The error margin Δm is usually proportional to the mass/charge ratio m of the measured ion, $\Delta m = m / R$, where R is a constant called the mass resolution. If our instrument has R = 100,000, what is the error when measuring glutamate? Why is so high mass resolution useful?

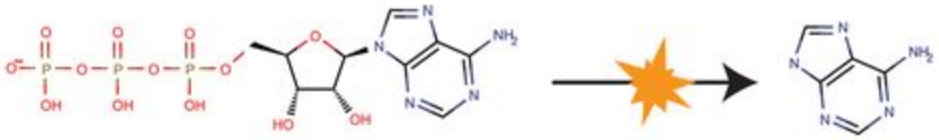
3: Mass spectrometry separates molecules by the mass/charge ratio (m/z) of the *ion* formed, which generally is somewhat different from the uncharged form of the metabolite M. For example, glucose ($C_6H_{12}O_6$) can form a $[M–H]^-$ ion $C_6H_{11}O_6^-$ by losing one proton during ionization, or a $[M–2H]^{2-}$ ion, $C_6H_{10}O_6^{2-}$ by losing two protons. What is the m/z for these two ions? Compare with the m/z of the $[M–H]^-$ ion of lactate. Note that "M" always means the uncharged form of the metabolite (protonated -COOH groups, etc).

4: When attempting to measure cysteine in a tissue extract, we fail to see any $[M–H]^-$ or ions (corresponding to the $-COO^-$ form of the carboxyl group) but we a observe a strong peak at the $[2M–3H]^-$ mass. Considering the redox chemistry of cysteine, what might this molecule be?

5: L-alanine and beta-alanine are related compounds, both of which are found in human metabolism. Look up their structure. Could these two species be distinguished using LC-MS? How / why?

6: D-serine is an unusual D-form amino acid present in the brain. Do you think this species could be identifiable / measurable using LC-MS (considering the presence of L-serine) ?

7: MS/MS is a technique where a metabolite can be fragmented by collision with an inert gas, and the m/z of the fragments generated are measured. An example fragmentation is shown below (McCloskey et al, Anal Chem 88:1362--1370). How can this technique help separate dGMP from AMP? (Look up their masses.)



Lipids

8: Look up the scheme for mitochondrial fatty acid beta-oxidation in humancyc (try identifier FAO-PWY). What classes of enzymes are involved? Which electron acceptors are used? Write down the net formula for one “round” of oxidation (one 2-carbon unit).

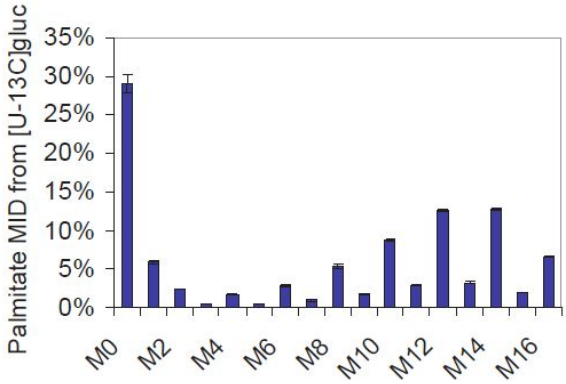
9: Compare fatty acid beta-oxidation with the fatty acid synthesis pathway (discussed in lecture 14). What similarities do you find?

10: Some fatty acids are oxidized in peroxisomes rather than mitochondria (see humancyc PWY66-391). What steps differ from the mitochondrial version? How are electrons handled in this case, without a respiratory chain nearby? What does this mean for energetics?

11: In general, fatty acids cannot be used for gluconeogenesis, because their oxidation generates only acetyl-CoA units, and there is no pathway that can generate pyruvate from acetyl-CoA. Why?
(Is it possible to produce oxaloacetate, the substrate for PEPCK, from acetyl-CoA?)

12: Is it possible to generate glucose from triacylglycerides?

13: The figure to the right shows the distribution of mass isotopomers in palmitate from cells grown on $^{13}C_6$-glucose. Can you explain the pattern? What might the pattern look like if no tracer is used? (Consider that ~1% of carbon i nature is ^{13}C). Data from Metallo et al, Nature 2011 (Figure S4).



14: Inhibitors of the HMG-CoA reductase (statins) are effective at blocking cell proliferation. Give two examples of metabolic products synthesized via this enzyme. Why might these products be important for proliferating cells?

15: Would the mass isotopomer distribution in cholesterol look similar to that of a fatty acid, given the same isotopomers of acetyl-Coa? Why / why not? (Hint: consider the fate of carbon in the mevalonate pathway.)

16: Cholesterol is rarely catabolized in nature. What is the fate of excess cholesterol in humans?

Nucleotides

17: What is the function of the highly phosphorylated sugar phosphoribosyl-diphosphate (PRPP) in nucleotide metabolism?

18: Considering the composition of biomass for an "average" cell, how much sugar is needed for nucleotide synthesis? For deoxynucleotide synthesis? Could you also estimate deoxynucleotide synthesis from the size of the genome?

19: What is the oxidation state of carbons uracil, the nucleobase first generated in pyrimidine synthesis? Is the the *de novo* synthesis of pyrimidines oxidative or reductive?

20: Look up the pathway of uracil degradation in humancyc. What is the energy yield of the pathway? What is the ultimate fate of the carbon? Why do you think this pathway has been retained in humans, while purine degradation has been lost?

21: Hypoxanthine-aminopterin-thymidine (HAT) medium is used in hybridoma cell culture to select for cells that have a functional thymidine kinase. Aminopterin (an "antifolate") blocks reactions that require folate-carried 1-carbon units, including those in *de novo* purine synthesis. What is the function of hypoxanthine and thymidine in HAT medium?

22: Consider a scenario where AMP is labeled +2 from a ^{13}C tracer. Can we conclude that there is *de novo* synthesis of AMP? What if we find a +7 mass isotopomer? Can MS/MS (see above) help demonstrate *de novo* synthesis of AMP in the case of a +2 mass isotopomer?