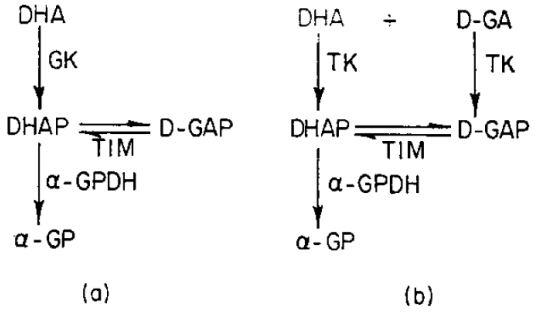
Seminar 4

Measuring metabolism

For mass spectrometry problems, you can look up exact masses of metabolites in the PubChem database, pubchem.ncbi.nlm.nih.gov

1: The figure to the right outlines an enzymatic assay of dihydroxyacetone (DHA) and D-glyceraldehyde (D-GA) using the enzymes glycerol kinase (GK), triose kinase (TK), triose phosphate isomerase (TIM) and glycerol-3-phosphate dehydrogenase (a-GPDH, 1.1.1.8). The readout is NADH produced by a-GPDH. GK can phosphorylate DHA, but not D-GA (panel a), while TK can phosphorylate both DHA and D-GA (panel b). 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 mass error 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 mass error when measuring glutamate? Why is so high mass resolution useful?

3: We want to investigate glutamine in an LC-MS data set. Wikipedia gives the mass of glutamine as 146.15 Da, so we look for the $[M+H]^+$ ion at 147.15, but find no signal at all. There should definitely be glutamine in the sample. What is wrong?

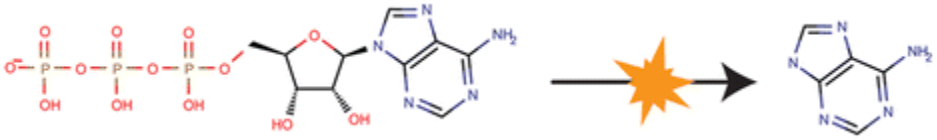
4: Mass spectrometry can only measure *ions* formed from metabolites. For example, glucose ($C_6H_{12}O_6$) can form a $[M–H]^–$ ion $C_6H_{11}O_6^–$ by losing one proton during ionization, or a $[M–2H]^{2–}$ ion, $C_6H_{10}O_6^{2–}$ by losing two protons. How does the m/z for these two ions compare with the m/z of the $[M–H]^–$ ions of lactate or glyceraldehyde? (Here "M" always means the uncharged metabolite: protonated -COOH groups, etc).

5: 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?

6: 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?

7: 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) ?

8: MS/MS is a technique where ions can be fragmented by collision with an inert gas, and the m/z of the fragments are then measured. An example fragmentation is shown below for ATP. (McCloskey et al, Anal Chem 88:1362--1370) Can this technique separate ATP from dGTP? (Look up their structures and masses.)



Lipids

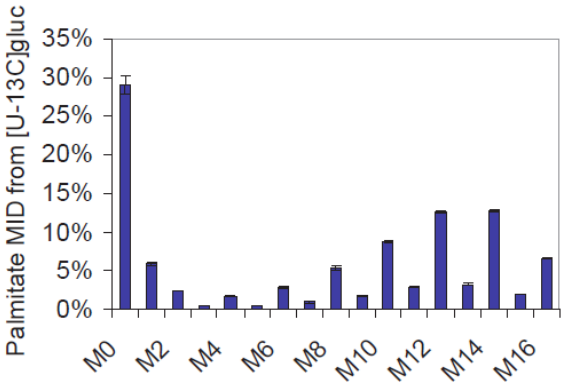
9: Look up the scheme for mitochondrial fatty acid beta-oxidation. What classes of enzymes are involved? Which electron acceptors are used? Write down the net formula for one “round” of oxidation (generating one acetyl-CoA). Why is it called *beta*-oxidation?

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

11: Some fatty acids are oxidized in peroxisomes rather than mitochondria. How does peroxisomal fatty oxidation differ from the mitochondrial version? How are electrons handled in this case, without a respiratory chain nearby? What does this mean for energetics? Why does the cell need two fatty acid oxidation systems?

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 would the distribution 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: Statins are inhibitors of HMG-CoA reductase, and are effective at blocking cell proliferation. Give two examples of metabolic products synthesized via this enzyme. Why do you think statins block cell proliferation?

15: Would the mass isotopomer distribution in cholesterol look similar to that of the fatty acid above, assuming the same isotopomers of acetyl-CoA? Why / why not?

16: Is cholesterol energy-rich? Can it be catabolized for energy? What is the fate of excess cholesterol in humans?

Nucleotides and one-carbon units

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

18: For a human cell, how much sugar is needed for ribose for DNA duplication? Is DNA synthesis a large biosynthetic cost for a cell?

19: 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? Compare uracil with a purine (like adenine). Why do you think pyrmidine degradation has been retained in humans, while purine degradation has been lost?

20: 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. What is the function of hypoxanthine and thymidine in HAT medium?