# Seminar 3

## Energetics

**1:** Draw the Δ*G* diagrams (substrate, energy barrier, product) for (1) an irreversible reaction (2) a spontaneous reaction. What are the important feature of the energy diagram in each case?

**2:** What is the effect of enzyme catalysis on forward / reverse / net / exchange fluxes?

**3:** Find the Δ*G* for oxidation of glucose to lactate (glycolysis). Is the reaction favorable? How many ATP could you theoretically synthesize with this amount of energy? Compare with the actual ATP yield of glycolysis.

**4:** What is the Δ*G* for complete oxidation of glucose to CO2 (balance the reaction with O2 and H2O) ? How does it compare to the glucose to lactate conversion? Compare this Δ*G* with the number of ATP typically obtained from complete oxidation of glucose; what fraction of the energy in glucose is captured as ATP?

**5:** Pyruvate kinase is one of the few reactions that is capable of "driving" ATP synthesis. Does this mean that the Gibb's energy of phosphoenolpyruvate is higher than that of ATP?

**6:** Would you expect the Δ*G* of deamination reactions to be positive or negative, considering the oxidation numbers? For example, check the oxidation numbers in the reaction

$$\text{Glutamine} + H_2O \rightarrow \text{Glutamate} + NH_3$$

Compare your results with the Δ*G* of the reaction.

**7:** Transamination can be viewed as the coupling of an amination and deamination reaction. For example, a transaminase might couple

$$\text{Alanine} + H_2O \rightarrow \text{Pyruvate} + NH_3$$

$$\text{2-oxoglutarate} + NH_3 \rightarrow \text{Glutamate} + H_2O$$

What is the resulting reaction and its Δ*G* ?

**8:** Are "high-energy" metabolites like ATP typically unstable in water? Why / why not?

**9:** If all cells run the metabolism towards lower G, how are energy-rich molecules synthesized?

# Respiration

**10:** How many $O_2$ are required to oxidize one molecule of pyruvate? How many ATP are obtained? What factors are involved in determining these values? Are they exact?

**11:** For any substrate, the respiratory quotient R is defined as the molar ratio (CO2 produced) / (O2 consumed) for complete oxidation of the substrate. What chemical property does this ratio reflect? What values of R would you expect for glucose oxidation? Why is the R value useful?

**12:** Can you calculate the respiratory quotient for palmitate (C16 fatty acid, sum formula $C_{16}H_{32}O_2$) without knowing the details of palmitate oxidation? Can you tell how many NADH (or equivalent carriers) will be used? Can you use this value to estimate the ATP production from palmitate?

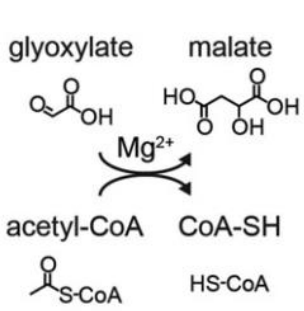
**13:** Are there other functions of the respiratory chain besides ATP synthesis?

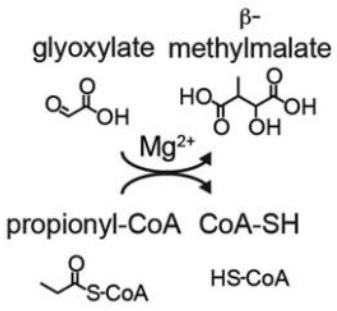
# Enzyme classes

**14:** Which EC classes are used in the TCA cycle? Which ones are used in the non-oxidative arm of the pentose phosphate pathway (transaldolase, transketolase, etc) ? What are the major differences?

**15:** Look up the EC classification of ATP-citrate lyase (2.3.3.8) at enzyme.expasy.org. This particular enzyme is a bit complicated. What EC classes are involved?

**16:** Knowing EC classification can be helpful for discovering the function of previously uncharacterized enzymes. For example, Strittmater et al (Hum Mol Genet 23:2313-2323, 2014) studied the function of the *CLYBL* gene product, which is sequence-related to ATP-citrate lyase (ALCY), and experimentally determined that it catalyzes the below reactions. Could you have predicted this from the EC classification of ATP-citrate lyase (*ACLY*) ?



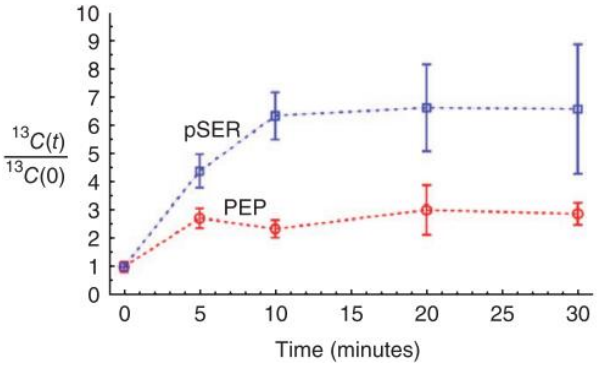



# Amino acid metabolism & isotope tracing

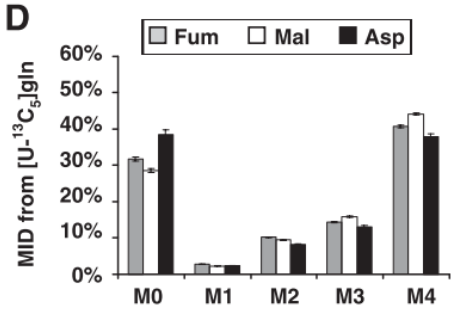
**17:** All amino acids except leucine and lysine can be used as substrates for gluconeogenesis in some way. Why are leucine and lysine exceptions? Can they still give useful products in case of glucose starvation?

**18.** During starvation, catabolism of muscle protein does not produce equal amounts of all protein-coding amino acids, but mainly glutamine and alanine. Why do you think these particular amino acids are the major products?

**19:** The figure to the right is from Locosale et al. Nat Genet 43:868-874, 2011. The authors state that "13C incorporation into pSER (13C-pSER) reaches steady state at a time scale comparable to the time for phosphoenolpyruvate (PEP) to reach steady state, suggesting that the relative fluxes [of serine and pep synthesis] are comparable". Do you agree with this conclusion?




**20:** The figure shows labeling of fumarate, malate, and aspartate from U-$^{13}C_5$-glutamine in cells cultured in medium containing unlabeled aspartate (Gaglio et al, Mol Sys Bio 7:523, 2011). How is aspartate synthesized in these cells, and can you explain the mass isotopomers observed? Can you think of a reason why aspartate is being synthesized even though aspartate is available in the medium?



**21:** Some cells can engulf and digest protein from their surroundings to obtain amino acids. The figures are from Commisso et al, Nature 497:633-637, 2013, showing uptake of fluorescent labeled albumin (left), and the MID of alanine from $^{13}C$-labeled protein (right). Can we tell what fraction of alanine is obtained from protein consumption?