7.013 Recitation 3 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lecture 4 (2/14):

Reaction kinetics: An endergonic reaction (one with a positive ΔG) cannot occur spontaneously, although it may be coupled to an exergonic reaction. In some cases exergonic reaction (one with a negative ΔG) can proceed spontaneously. The thermodynamics of the reaction are dictated by the difference in free energy between the substrate and the products. The kinetics of the reaction is determined by the transition stage and how much energy must be added to form the high energy intermediate. Enzymes lower the activation energy needed for a reaction to proceed, but do not change the free energy (ΔG) of either the reactants or the products. The following equation relates ΔG to enthalpy (ΔH, the available energy) and entropy (ΔS, unusable energy).

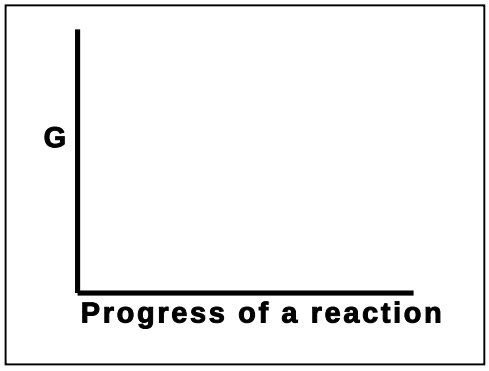
$$\Delta G = \Delta H - T\Delta S \text{ where T is the absolute temperature.}$$

Enzymes and energy: Enzymes are biological catalysts. Most biological catalysts are proteins, although RNA can also have enzymatic role (catalytically- active RNAs are known as ribozymes). Enzymes catalyze specific biological reactions and act by lowering the activation energy (E_{AC}) of the reaction that they catalyze. Each enzyme has a specific 3-D conformation and an active site(s) to which the substrate molecules can bind to form a transition state of enzyme-substrate complex (ES complex). The complex then gives rise to product (P) and the enzyme is released in its original form to catalyze the reaction once again.

Each enzyme is specific for a particular reaction. Enzyme function may be regulated by various factors. These factors may include prosthetic groups, metal ions/cofactors, coenzyme, substrate concentration, pH, temperature, inhibitors, competitive or noncompetitive inhibitors, allosteric modulators and the feedback inhibition by the end product of a biochemical reaction that involves multiple reaction steps.

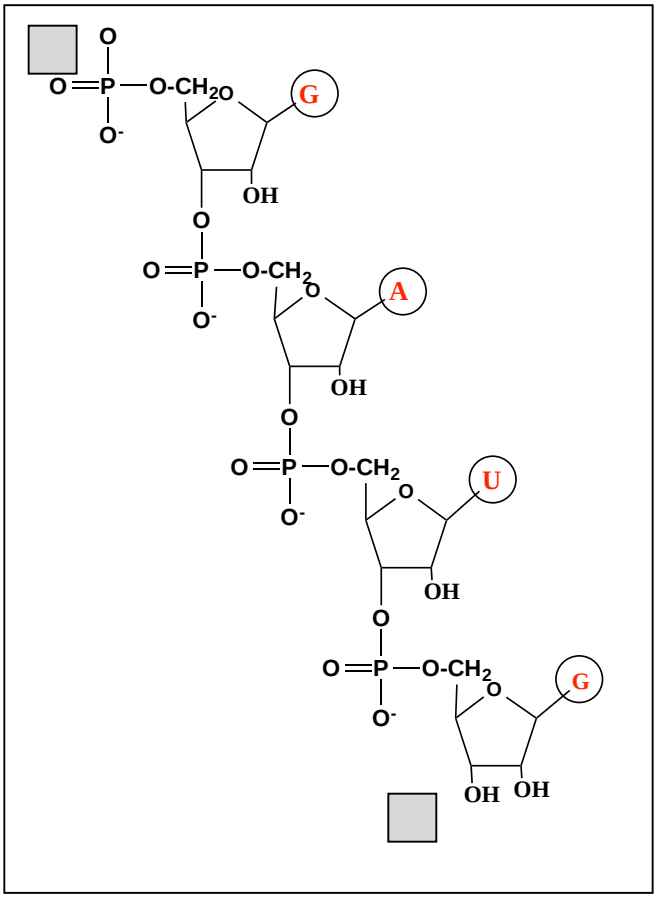
Questions

1. The formation of biological molecule usually requires energy (anabolic) with a $\Delta G>0$.



Draw the energy profile of an anabolic (energy requiring) reaction with a $\Delta G>0$. Label the ΔG, energy of activation (E_{AC}), reactants (R) and the products (P).

2. The schematic below shows a growing RNA polymer. ***Note:*** *The nucleotide bases are shown in red.*



i. Label the 5' and 3' ends in the shaded boxes.

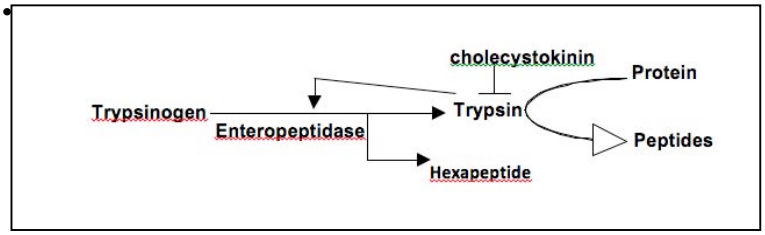
ii. Show the direction of polymerization of RNA by drawing an arrow and give the sequence that is complementary to the RNA sequence that is provided.

5'_____**3'**

iii. **Box** the nucleotide base that is uniquely a part of RNA and not DNA.

3. Trypsin is a protease. This enzyme breaks a protein into peptides by hydrolyzing the peptide bonds that have amino acids lysine and arginine at the carboxyl (-COOH) side of the peptide bond. The steps involved in the production and regulation of trypsin are outlined and shown in the schematic below. *Please note that a "T" represents inhibition and an "->" represents activation.*

- **Reaction 1:** Trypsin is produced as inactive trypsinogen.
- **Reaction 2:** Trypsinogen is cleaved to active trypsin and a hexapeptide by enteropeptidase enzyme.
- **Reaction 3:** Trypsin hydrolyzes the peptide bonds that have lysine and arginine at the carboxyl (-COOH) side of the cleaved peptide bond.
- **Reaction 4:** Trypsin then undergoes feedback inhibition by cholecystokinin.



You mimic Reaction #3 in five separate test tubes (1-5) as described below. You allow the reaction to proceed for 30 minutes in each tube and measure the amount of protein hydrolyzed.

- **Tube #1 (Control):** You perform the reaction at **37^{O}C** and **pH 7.4 in the presence of Ca^{2+} ions** and measure 100% hydrolysis of the protein into peptides.

- **Tube #2:** You perform the reaction at 5**0^{O}C** and **pH 7.4** and observe 0% hydrolysis of the protein substrate. However, if the temperature is brought to **37^{O}C,** you observe 100% hydrolysis of the protein as seen in tube #1.

- **Tube #3:** You perform the reaction at **37^{O}C** and **pH 7.4** for and in the presence of EGTA, a Ca^{2+} ion chelator (absorbs Ca^{2+} ions) and observe 0% hydrolysis of the protein substrate. You add excess of Ca^{2+} ions to the tube and observe 100% hydrolysis of the protein.

- **Tube #4:** You perform the reaction at **37^{O}C** and a **pH of 7.4** in the **presence of soybean trypsin inhibitor (SBI)** and do not detect any measurable hydrolysis of the protein. You increase the substrate concentration by 4 fold and observe 100% hydrolysis of the protein.

- **Tube #5:** You perform the reaction at **37^{O}C** and a **pH of 7.4** in the **presence of di- isopropyl fluorophosphate (DFP),** which binds covalently to trypsin. You observe 0% hydrolysis of the protein. You increase the substrate concentration by 4 fold but do not observe a measurable hydrolysis of protein substrate.

a) Explain the effect of the changed reaction parameters in the following test tubes on **structure** and **function** of trypsin and its protein substrate.

Reaction parameters	Affects **Trypsin (***Yes/No***)? Explain.**	Affects **Trypsin substrate** (*Yes/ No*)? **Explain.**
50^{O}C in tube #2		
EGTA in tube #3		

b) Based on the information provided, would you characterize…

i. SBI as a *competitive / allosteric / reversible/ irreversible inhibitor*? **Explain** why you selected this option.

ii. DFP as a *competitive/ non- competitive / allosteric/ reversible/ irreversible inhibitor?* Choose **all possible options** and give an explanation for the option(s) that you selected.

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