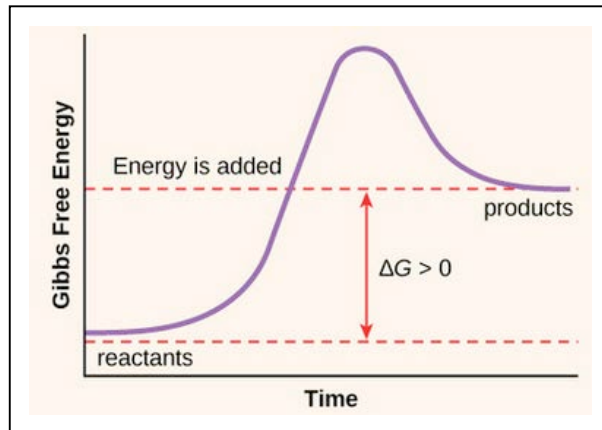


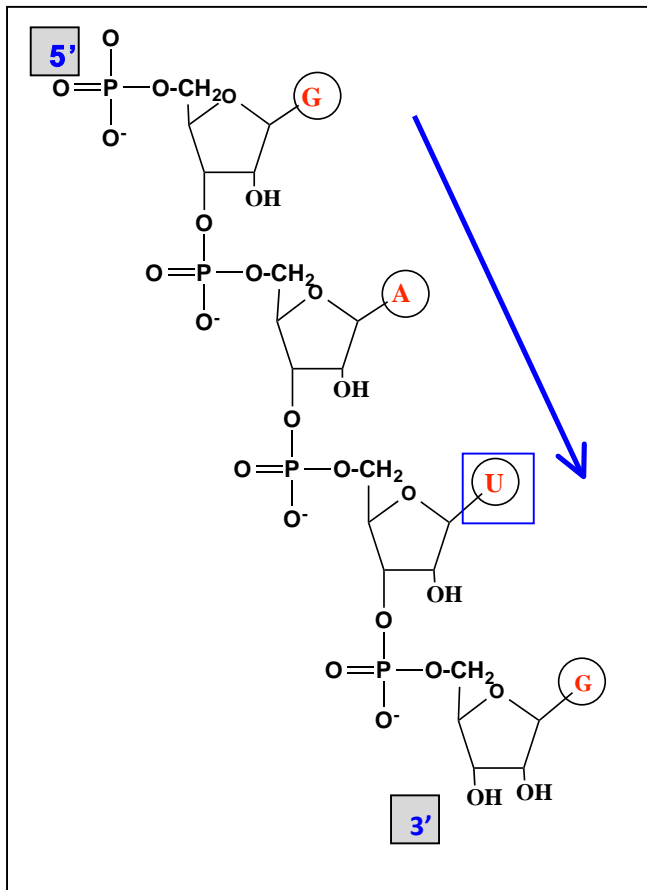
## The key: 7.013 Recitation 3 – Spring 2017

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  $\Delta G$ , 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.

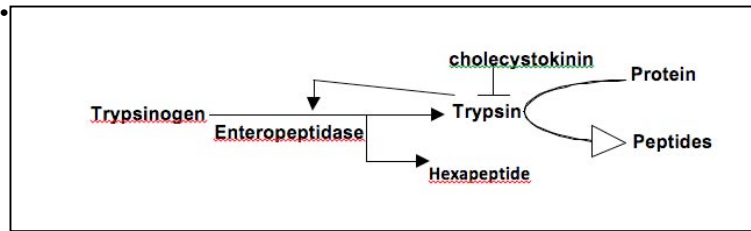
ii. Show the direction of polymerization of RNA by drawing an arrow and give the sequence that is complementary to the RNA sequence that is provided.

5'CATC3'

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°C** and **pH 7.4** in the **presence of Ca<sup>2+</sup> ions** and measure 100% hydrolysis of the protein into peptides.
- **Tube #2:** You perform the reaction at **50°C** and **pH 7.4** and observe 0% hydrolysis of the protein substrate. However, if the temperature is brought to **37°C**, you observe 100% hydrolysis of the protein as seen in tube #1.
- **Tube #3:** You perform the reaction at **37°C** and **pH 7.4** for and in the presence of EGTA, a Ca<sup>2+</sup> ion chelator (absorbs Ca<sup>2+</sup> ions) and observe 0% hydrolysis of the protein substrate. You add excess of Ca<sup>2+</sup> ions to the tube and observe 100% hydrolysis of the protein.
- **Tube #4:** You perform the reaction at **37°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°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 <b>Trypsin</b> (Yes/No)? <b>Explain.</b>	Affects <b>Trypsin substrate</b> (Yes/ No)? <b>Explain.</b>
50°C in tube #2	<i>Yes, it may denature the enzyme i.e. disrupt its active 3D- dimensional conformation.</i>	<i>Yes, since the substrate is a protein itself, an alteration in temperature may disrupt its 3D folding.</i>
EGTA in tube #3	<i>Ca<sup>2+</sup> ions may act as a prosthetic group for trypsin. Their chelation by EGTA may prevent trypsin from catalyzing this reaction.</i>	<i>No. However, if you make the assumption that the Ca<sup>2+</sup> ions are needed for the proper folding of protein substrate then your answer is "yes".</i>

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

i. SBI as a competitive / allosteric / reversible/ irreversible inhibitor? **Explain** why you selected this option. *The effect of the inhibitor may be reversed by increasing the substrate concentration i.e. it competes with the substrate to bind to the active site of the enzyme. So SBI is a competitive, reversible inhibitor.*

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. *The effect of DFP cannot be reversed by increasing the substrate concentration, suggesting that it binds to a site on an enzyme other than the substrate-binding site. This makes DFP either a noncompetitive inhibitor (which is usually a pharmaceutically made chemical) or an allosteric inhibitor (which is usually produced within the cell). Alternatively, based on the information you can also argue that this is competitive reversible inhibitor. You need more data to make a definite conclusion.*

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