

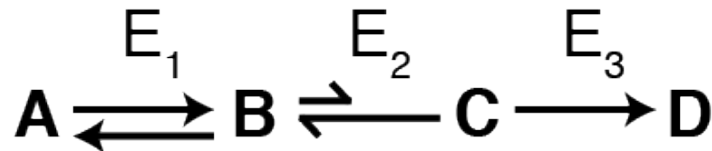
1. The Stanley Miller experiment looked at applying a spark to a container filled with inorganic compounds to mimic what was thought to be the environment on primordial earth. What types of molecules were determined to be formed? Hint: Think of what the goal of this experiment was. (4 pts).

Amino acids or organic molecules

2. RNA is rapidly hydrolyzed in alkaline solution to yield a mixture of nucleotides whose phosphate groups are bonded to either the 2' or 3' positions of the ribose residues. DNA is resistant to alkaline degradation. Explain with an arrow-pushing mechanism using the structure of any RNA polymer (i.e., can be composed of any appropriate base of your choosing). (5 pts).

arrow-pushing mechanism

3. You add A, E₁, E₂, and E₃ to a test tube and incubate at the optimal temperature for E₁, E₂, and E₃ (37°C) for 24 hours. E₁, E₂, and E₃ are all "good enzymes" (i.e., good catalysts and stable for >24 hours). Would you expect there to be more A or D after this 24 hour period? Why? (5 pts).

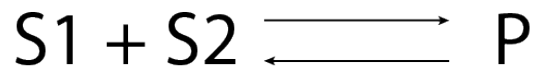


- A) More A
- B) More D

E₃ pulls C towards D such that more C is produced as E₂ attempts to bring this reaction to equilibrium. Ultimately, A will be consumed as D accumulates.

+4pts correct answer B)
+1pts correct explanation

4. Explain how an enzyme affects the equilibrium constant (K_{eq}), describing K_{eq} in terms of concentrations of each metabolite, of the following reaction (6 pts):



It does not affect the K_{eq}.

5. What are the 3 properties an enzyme must have to catalyze chemical reactions. (6 pts).

1. Enzyme binding the substrate, 2. Binding and stabilizing the transition state, and 3. Dissociating from the product.

+2pts Enzyme binding the substrate
+2pts Binding and stabilizing the transition state

+2pts Dissociating from the product

6. Enzymes are able to catalyze reactions with considerably higher catalytic efficiencies than typical chemical catalysts. Name the four mechanisms enzymes use to achieve these catalytic efficiencies. (8 pts).

1. Covalent catalysis, 2. Metal ion catalysis, 3. Electrostatic catalysis, 4. Proximity and orientation effects.

+1pts Covalent catalysis

+1pts Metal ion catalysis

+1pts Electrostatic catalysis

+1pts Proximity and orientation effects

7. Redox balance is extremely important for achieving high yields, titers, and production rates. Achieving redox balance is a challenge when a fermentation is grown anaerobically. Yeast is a beloved strain for its ability to produce ethanol at unusually high yield when grown anaerobically. Explain chemically why yeast produces ethanol when under anaerobic conditions (but not under aerobic conditions unless fed very high concentrations of glucose) despite ethanol being toxic to the yeast cell at high concentrations. Use specific chemicals in your explanation. (6 pts).

Under anaerobic conditions, yeast cannot use oxygen as an electron sink as it accumulates the reduced NADH. It cannot maintain glycolytic flux without having NAD⁺ co-substrate. It needs to oxidize NADH somehow and thus it converts pyruvate to ethanol as a means of oxidizing NADH to NAD⁺. Thus, production of toxic ethanol is better for the cell than the inability to maintain glycolysis flux.

+2pts Oxidize NADH to NAD⁺

+4pts Maintain glycolysis flux

8. A graduate student, Tammy, wants to build an *E. coli* strain capable of biosynthesizing the pigment indigo as a sustainable alternative to the conventional, dirty chemical synthesis. She targets mutagenesis of the limiting enzyme by conducting error-prone PCR. Tammy transforms her diverse library into *E. coli*, plates onto agar plates and picks the colonies with the most blue color. Is this isolation of the most blue colonies an example of a (4 pts.):

A. Selection

or

B. Screen?

Screen

9. The chemical strategy of glycolysis includes the following (circle all that apply) (6 pts.):
- A. Adding phosphoryl groups
 - B. Reducing glucose ultimately to pyruvate
 - C. Transfer phosphate groups to create higher energy bonds

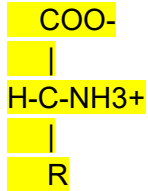
D. Couple hydrolysis of reactive intermediates with ATP synthesis

A, C, and D

10. One of the chief outcomes of glycolysis is to form a net of 2 molecules of ATP for each molecule of glucose consumed. What is the utility of ATP to the cell? Be precise in your answer. (6 pts.)

ATP contains the high energy bonds in the triphosphate. Energy released upon hydrolysis can be coupled to energetically unfavorable reactions to drive them forward.

11. Draw structure of tyrosine as it would exist at pH=7. (6 pts.)



12. Compartmentalization of individual library members is essential for sequencing the “hits” of the screen or selection. Plasmid transformation is a method resulting in the compartmentalization of a single library member in the cell. How does a plasmid transformation accomplish only having a single plasmid in a cell? (8 pts.)

Transformation is very inefficient/low probability event. Thus, the odds of successfully transforming more than one plasmid is extremely low.

13. What type of interaction stabilizes an A) alpha helix? B) beta sheets? C) What type of interaction is most important for stabilizing a protein fold? (7 pts.)

A) And B) both H-bonding (2pts each)

C) hydrophobic interactions (3pts)

14. Pete Schultz theorized that antibodies could be raised to act as an enzyme. He termed these “catalytic antibodies.” Explain why Prof. Schultz theorized antibodies could catalyze a reaction. (6 pts.)

Antibodies are molecules that can tightly bind molecules of diverse structures. Prof. Schultz hypothesized that antibodies could be raised to tightly bind a transition state analog of a reaction desired to be catalyzed and thereby stabilize this transition state.

15. Explain how the negative charge of the triphosphate does not electrostatically block nucleophiles from attacking the phosphate electrophile in the cell? (4 pts.)

Shielded by metal ions (specifically Mg⁺²).

16. When conducting error-prone PCR, engineers typically target 1-5 mutations per gene. Provide the explanation why this range has empirically been shown to be ideal. Why is not more than 5 mutations? (8 pts.)

As you increase the number of mutations, you increase the probability of picking up a deleterious mutation. Greater than 5 increases your odds of obfuscating a beneficial mutation with a deleterious mutation more than the benefits of increased diversity.

17. RNA polymerase is able to very rapidly catalyze messenger RNA formation largely because this reaction is highly exergonic. Explain why this reaction is highly exergonic. (5 pts.)

Hydrolysis of high energy triphosphate bond

Name: _____ Major: _____ S18BioE11 Biochem Midterm March 22,
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ATP

