1. (30 Points) Definitions I. In the context of this course, define and distinguish between the following:

A. Interspecies Hydrogen Transfer vs. Hydrogen Cycling

In IHT, one species makes H₂ as a byproduct of their metabolism and requires a second species to consume it so that they may continue to live. In HC, H₂ is made in the cytoplasm and travels across the membrane uncharged, where it is oxidized during respirations, thereby producing ∆μ.

B. Sulfate assimilation vs. Sulfate dissimilation

In SA, sulfate is reduced to sulfide for purposes of incorporation into biomass. Since it must operate under very low sulfate concentrations, it uses a PAPS intermediate to increase the negative ∆G of the reaction. During SD, sulfate is used as an electron acceptor under high concentrations, so the PAPS intermediate is not utilized.

C. Cyclic Electron Transport vs. Linear Electron Transport

CET is used in photosynthetic bacteria; here, the same molecule (bacteriochlorophyll) acts as both the electron donor for respiration and as the terminal electron acceptor. IN LET, different molecules (like NADH and O₂) serve as electron donors and electron acceptors.

D. Chlorophyll a vs. Bacteriochlorophyll a

Bacteriochlorophyll a is used in photosystem I of GSB, PSB and cyanobacteria. Chlorophyll a is used in photosystem II of cyanobacteria to oxidize water.

E. RelA vs. SpoT

RelA senses uncharged tRNAs in the ribosome and create (p)ppGpp from GTP, initiating the stringent response. SpoT degrades (p)ppGpp to terminate the stringent response.

F. TCA Cycle vs. Reverse TCA Cycle

The TCA cycle is an aerobic cycle that completely oxidizes acetyl-CoA to CO₂ for the purpose of generating low-potential electrons for respiration. The reverse TCA cycle consumes energy (NADH and ATP) to incorporate CO₂ into a carbon skeleton for biosynthesis.
2. (5 points) **Methyltrophs.** Some methanogens grow by reducing CO₂ to form CH₄. Methyltrophs can grow by oxidizing CH₄ to form CO₂. When described this way, it appears that methyltrophs are growing by performing the exact opposite metabolism of the methanogens. Yet how can both processes have a negative ΔG? How can both organisms persist?

During methanogenesis, H₂ as the electron donor and CO₂ is the electron acceptor; this allows for a large negative ΔG. Methyltrophs share only one half reaction with methanogens, that is the CH₄/CO₂ redox couple. However, Methyltrophs use O₂ as an electron acceptor and CH₄ as the electron donor, which also provides a large negative ΔG. Therefore, the organisms are not really performing “opposite” metabolisms.

3. (15 Points) **Unfavorable Reactions.** The following processes involve transfer of electrons each with an ostensibly high positive ΔG₀. In specific physiological and/or thermodynamic terms, what allows each reaction to proceed favorably?

   - Electrons from NADH to H⁺ in butyrate fermenters
   - Electrons from S⁰ to NAD⁺ in *Thiobacillus ferrooxidans*
   - Electrons from H₂S to NAD⁺ in Green Sulfur Bacteria
   - Electrons from H₂O to NAD⁺ in cyanobacteria

   (a) The removal of the resulting H₂ gas by H₂-consumers (like methanogens) keeps the product concentration low, providing a net negative ΔG.
   (b) Reverse electron transport allows hydrogen ions to enter the cell; this release of energy allows NAD⁺ to be reduced.
   (c) Electrons from H₂S will reduce bacteriochlorophyll. The transfer of light energy via inductive resonance will alter the midpoint potential of the electrons so that they can be transferred to ferrodoxin and then to NAD⁺.
   (d) Electrons from H₂O reduce chlorophyll a, which is similarly activated by light to allow reduction of plastoquinones and then bacteriochlorophylla. Another round of light activation of bacteriochlorophyll a allows reduction of ferrodoxin and then NAD⁺.
4. (20 Points) PTS System. Consider cultures of *E. coli* strains growing in defined medium with either (a) lactose, (b) glucose, or (c) the combination of lactose and glucose. One strain is a “wild-type”, completely normal cell. Other strains contain lesions in particular genes that either (a) render the encoded protein completely nonfunctional (*null mutants*), or (b) create a *partially functional protein* that cannot perform some of its functions. Complete the table below and indicate the following:

(1) The amount of lactose in the cell. Acceptable answers include: “Low” and “High”

(2) The amount of expression seen for the *lacZYA* operon. Acceptable answers include “Low” and “High”

**NOTE CAREFULLY:** Correct answers receive **POSITIVE** scoring; Incorrect answers receive **NEGATIVE** scoring. If you are merely guessing, it would be best to leave the square blank, which receives **ZERO** scoring.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Glucose Alone</th>
<th>Lactose Alone</th>
<th>Lactose + Glucose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>lacZYA</em> induction</td>
<td>Lactose in Cell</td>
<td><em>lacZYA</em> induction</td>
</tr>
<tr>
<td>Wild type</td>
<td>Low</td>
<td>High</td>
<td>High</td>
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<tr>
<td></td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td><em>lacI</em> null mutant</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td><em>ptsH</em> null mutant</td>
<td>LOW</td>
<td>LOW</td>
<td>LOW</td>
</tr>
<tr>
<td><em>ptsG</em> null mutant</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td><em>cya</em> null mutant</td>
<td>LOW</td>
<td>HIGH</td>
<td>LOW</td>
</tr>
<tr>
<td><em>crr</em> null mutant</td>
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<td>HIGH</td>
<td>LOW</td>
</tr>
<tr>
<td><em>crp</em> mutation, where CRP* acts as if cAMP is always bound</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td>Crr protein that cannot be phosphorylated</td>
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<td>LOW</td>
</tr>
<tr>
<td>Crr protein that cannot be dephosphorylated</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
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</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Encoded Enzyme</th>
<th>Gene</th>
<th>Encoded Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>lacI</em></td>
<td>LacI repressor</td>
<td><em>ptsG</em></td>
<td>PTS Enzyme II-BC for glucose</td>
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<tr>
<td><em>lacZ</em></td>
<td>β-Galactosidase</td>
<td><em>crr</em></td>
<td>PTS Enzyme II-A for glucose</td>
</tr>
<tr>
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<td>Lactose permease</td>
<td><em>crp</em></td>
<td>Crp, the catabolite repression protein</td>
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<tr>
<td><em>ptsI</em></td>
<td>PTS Enzyme I</td>
<td><em>cya</em></td>
<td>Adenylate cyclase</td>
</tr>
<tr>
<td><em>ptsH</em></td>
<td>PTS Hpr “histidine protein”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5. (10 Points). Sensing. For each protein listed below, describe, in the context of this course, (a) under what circumstances does the protein become active, describing pertinent signals where appropriate and (b) what is the action of the active protein.

A. CheY-P

CheY-P is activated by CheA-P when repellants increase or attractants decrease in concentration. CheY-P binds to the flagellar switch to induce tumbling.

B. CheA-P

CheA-P is activated when the helices of the MCPs rotate in response to increasing repellant or decreasing attractant. It kinases CheY and CheB, which adds methyl-groups to the MCPs, decreasing their sensitivity.

C. Fnr

Fnr is activated when O₂ concentration becomes very low, essentially anaerobic; the oxidation state of bound iron allows for the conformational change. Active Fnr acts as both a transcriptional activator or genes involved in anaerobic fermentation and respirations and as a transcription repressor of genes involved in aerobic respiration and the TCA cycle.

D. LexA

LexA becomes active when it binds to RecA which is itself bound to ssDNA (indicating DNA damage). Once active, LexA cleaves itself, relieving repression of genes involved in DNA damage repair.

E. ArcA-P

ArcA becomes active when it is phosphorylated by ArcB-P, which detects excess reduced ubiquinol. ArcA-P represses genes involved in aerobic respiration and activates genes involved in fermentation or low-oxygen respiration.

A. Connecting each step logically to the preceding step, explain the presence of excess methionine leads to lower rates of sulfate reduction and cysteine synthesis in *Escherichia coli*.

- Excess methionine leads to increased pools of S-Adenosyl-Methionine (SAM), which
- Is bound to the MetJ repressor, which now is able to
- Repress the transcription of methionine biosynthetic genes, leading to
- Less formation of cystathione from cysteine, which
- Leads to an increase in the cysteine pool which, whereby
- Excess cysteine feedback inhibits the CysE protein,
- Leading to the accumulation of less o-acetyl-serine,
- Which leads to lower pools of N-acetyl-serine,
- Which is the ligand of the CysB protein, which, without its ligand
- Fails to activate transcription of the genes involved in sulfate reduction,
- Leads to lower rates of sulfate reduction.

B. Connecting each step logically to the preceding step, explain the presence of excess histidine leads to lower rates of histidine synthesis in *Escherichia coli*.

- Excess histidine leads to high levels of charged tRNA^{His},
- Which allows the ribosome to easily translate the HisL leader peptide,
- Which prevents the his mRNA from folding into an a particular secondary structure,
- Which thereby allows the mRNA to fold into an alternative secondary structure
- Which interferes with RNA polymerase, causing transcription termination,
- Which prevents the production of more enzyme for histidine biosynthesis.
• (10 Points) Methanogenesis.

A. Consider methanogenesis from methanol. What are the identities and amounts of carbon-containing waste products that are produced from the consumption of 1 mole of methanol.

\[ \frac{3}{4} \text{ mol CO}_2 \text{ and } \frac{1}{4} \text{ mol CH}_4. \]

B. Why do some organisms couple electron transfer during methanogenesis to the extrusion of sodium ions, and not to the extrusion of hydrogen ions?

If the \( \Delta G \) of methanogenesis is insufficient to allow for proton extrusion, but can allow for sodium ion extrusion, sodium ions will be pumped out and multiple sodium ions may be allowed to re-enter the cell, whereby their combined energy release may allow for simultaneous pumping hydrogen ions via a Na/H antiport; the H\(^+\) may re-enter the cell via the F\(_0\)F\(_1\)ATPase to allow for ATP biosynthesis.