PS#2: We will begin at 10 am with Problem 1 in BH212

Assignments
Problem 1- group 4
Problem 2- group 3
Problem 3- group 2
Problem 4- group 1

The beginning: Designate one person in each group to give a 5 min introduction: try to be succinct and remember to describe the biological significance of the question. You don’t need to describe the whole solution in these 5 min, but you can set the stage.

The discussion: Describe the solutions and the logic behind them.

The end: Designate one person in each group to give a short summary at the end of the problem discussion.
**Problem 1.** A new bacterium, *E. bioreg*, produces a key metabolite, called bioregia. A repressor, named Brr, controls the pathway producing bioregia. The operator sites were established by mutational analysis. The arrangement of regulatory sites is diagrammed below.

![Diagram of regulatory sites](image)

**Key:**
- **O1** = operator 1 (Brr binding site)
- **O2** = operator 2 (Brr binding site)
- Upward arrow: start site of transcription

Both operator sites are required for repression:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Arbitrary units of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1</td>
</tr>
<tr>
<td>brr-</td>
<td>100</td>
</tr>
<tr>
<td>O1-</td>
<td>100</td>
</tr>
<tr>
<td>O2-</td>
<td>100</td>
</tr>
<tr>
<td>O1-O2-</td>
<td>100</td>
</tr>
</tbody>
</table>

Propose several (2 or 3) hypotheses explaining why both operators are required. Describe experiments to test each hypothesis and indicate which experiments distinguish between the hypotheses.
Problem 2. *E. bioreg* produces another key metabolite, called hardwork. An activator, named Hda, controls the pathway producing hardwork; activation is observed when grit is present in the media. Grit is the ligand that binds to Hda, and is required for activation. The activator and its binding site were identified genetically. The arrangement of these regulatory sites is diagrammed below.

![Diagram of DNA regulatory sites](image)

Key:
Green: binding site of Hda
Upward arrow: start site of transcription.

The established genetic relationships are as follows:

<table>
<thead>
<tr>
<th>Strain</th>
<th>arbitrary units of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>10</td>
</tr>
<tr>
<td>WT + Grit</td>
<td>100</td>
</tr>
<tr>
<td>hda-</td>
<td>1</td>
</tr>
<tr>
<td>hda- + Grit</td>
<td>1</td>
</tr>
</tbody>
</table>

To determine whether binding of Hda is sufficient to activate transcription, you set up an *in vitro* transcription reaction. Hda is overexpressed in *E. coli* and then purified. The *in vitro* transcription reaction contains RNA polymerase, Hda, Grit, the double stranded DNA fragment encompassing the hardwork promoter and operator (shown below), and a standard transcription mix (salt, buffer, radioactively labeled ATP (ATP*), and the other 3 NTPs). RNA chains are precipitated with TCA and the radioactivity (cpm) determined. Although a template containing a control promoter transcribed well, Hda did not activate the hardwork promoter (results below). In the cell, the activated Hda promoter initiates as well as the control promoter.

<table>
<thead>
<tr>
<th>Template</th>
<th>radioactivity (cpm) in RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control promoter</td>
<td>10,000</td>
</tr>
<tr>
<td>Hardwork promoter</td>
<td>30</td>
</tr>
<tr>
<td>Hardwork promoter + Hda</td>
<td>30</td>
</tr>
<tr>
<td>Nla promoter + Hda + Grit</td>
<td>30</td>
</tr>
</tbody>
</table>

Propose 3 hypotheses for the inactivity of the *in vitro* system, and describe the experiments you would perform to test each hypothesis.
**Problem 3.** The abortive initiation assay, diagrammed to the right, is often used to characterize the parameters of promoters.

When you perform this assay, if RNAP and DNA are preincubated, and the NTPs are added 30 min later, the reaction is linear, and the steady state extrapolates to 0 at t=0. On the other hand, if RNAP, DNA and NTPs are added together, there is a lag to reach steady state. Both scenarios are diagrammed below.

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**Diagram:**

- **Initial binding:** R+P → RP<sub>c</sub>
- **Isomerization:** RP<sub>c</sub> → RP<sub>o</sub>
- **Abortive Initiation:** +2 NTPs

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**Questions:**

a. Why do you use only 2 NTPs in the abortive initiation assay?

b. Why do the cpm increase linearly for a long time?

c. What two factors account for the lag observed when NTPs are added together with RNAP and DNA?

d. What experiments would you do to determine the contribution of each factor to the lag?
Problem 4. *E. bioreg* also produces a 3rd important metabolite, stress, controlled by the Sta activator. Activation occurs in the presence of the trx ligand. The arrangement of regulatory sites is diagrammed below.

![Diagram of regulatory sites](image)

Key:
- Green: binding site of Sta
- Upward arrow: start site of transcription.

You have performed a careful *in vitro* analysis of the mechanism of activation, by determining the effect of the activator on $K_B$, the initial binding step, and $k_f$, the forward rate constant of the changes leading to open complex formation. You find that the activator exclusively affects $k_f$, without affecting $K_B$.

Propose several models for how an activator could work exclusively at $k_f$, and describe how you would test these models. Indicate whether the experiments proposed distinguish between the models.