Bi1X 2013
Single Molecule Digest Homework

Sample preparation

1) Read the Materials and Methods section in the attached paper by Han *et al.*, 2009. Explain what reactions were involved when you tethered the DNA and beads to the surface.

Biology of restriction enzymes

2) Most restriction enzymes have binding sites that are about 6 bp long. How many times would a particular 6-bp sequence appear in a 100 kbp of random DNA? (A rough estimation is enough. Recall the calculation you did in assignment 1.)

3) What organisms make restriction enzymes? How does that relate to the function of restriction enzymes? Note that (double-stranded DNA) bacteriophages have genome size of around 100 kbp.

Comparing bulk and single-molecule rates

4) We will compare the digestion rate of the single molecule experiment you did in class with the standard bulk reaction condition.

(a.) For an enzymatic reaction like the following:

$$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{\text{cat}}} E + P$$

(E: Enzyme, S: Substrate, P: Product.)

The Michaelis-Menten equation describes the reaction rate (change of reactant or product concentration per unit of time) as a function of substrate concentration, enzyme concentration, and two constants, k_{cat} and K_m.

$$v = \frac{d[P]}{dt} = V_{\max}\frac{[S]}{K_m + [S]} = k_{\text{cat}}[E]_0\frac{[S]}{K_m + [S]}$$

($[E]_0$ stands for total amount of enzyme.)

Write down the reaction rate associated with the DNA restriction digest experiment.

(b.) The NEB website gives the following definition of enzyme activity:
"*One unit (of enzyme) is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.*"
Figure out the bulk digestion rate, substrate concentration (convert to nM) and enzyme concentration (in "unit"per micro-liter) from this description. Assume the K_m is 5 nM (Typical value for restriction enzymes is 1-10 nM). Calculate the value of k_{cat}.

(c.) What are the substrate concentration (convert to nM) and enzyme concentration (in “unit”per micro-liter) in the single molecule digestion experiment you did? (Hint: There is only one layer of single DNA molecules on the surface. Think about how many beads are there in the field of view, the area, height and volume of the flow chamber.) What would the digestion rate be based on these concentrations, and the k_2 and K_M given in part (b.) ?

(d.) Do a similar image analysis as what you learned in the Matlab session. Plot the number of beads in the field of view in your movie as a function of time, for both the control movie and the digestion movie. What is the digestion rate based on your plot? How does that compare to the theoretical value in (c.)? (Remember to convert to the same units as in (c.) in order to have a fair comparison.)

(e.) List some reasons why the bulk and single molecule rates might differ. Think about reaction conditions as well as the geometry of the two settings.