Problem set 7
(Discussion on July 1)

Problem 1

Single and double-stranded λ-phage DNA. The λ-phage (which is essentially a virus that preys on *E. coli*) has a 48.5 kbp double-stranded DNA genome. Purified λ-phage DNA ("λ-DNA") is frequently used in biophysical experiments. Let us consider some properties of both single- and double-stranded λ-DNA in aqueous solution at (roughly) physiological salt concentration ($\approx$ 150 mM monovalent salt) and pH. By single-stranded λ-DNA we mean that only one strand (of 48500 bases) is present. You can consider both single- and double-stranded λ-DNA to be well approximated by the FJC model in this problem.

a) What is the contour length of double-stranded λ-DNA?

b) What is the contour length of single-stranded λ-DNA? You can assume that the length per base for single-stranded DNA is 0.5 nm. Is the contour length for single-stranded λ-DNA shorter or longer than the length in a)? Why?

c) What is the average root-mean-squared end-to-end distance of double-stranded λ-DNA in free solution?

d) What is the average root-mean-squared end-to-end distance of single-stranded λ-DNA in free solution? You can assume that the segment or Kuhn length for single-stranded DNA is 1 nm.

e) Sketch the force-extension behaviour of both single- and double-stranded λ-DNA in a coordinate system with the force on the y-axis and extension on the x-axis. Clearly label which curve is for single-stranded and which curve is for double-stranded DNA.

Problem 2

Scanning Probe Techniques.

a) Briefly explain how an Atomic Force Microscope (AFM) works.

b) Let's assume the interaction potential between an AFM cantilever and the surface of a sample is $V(d) = 4\epsilon\left((\sigma/d)^{12} - (\sigma/d)^{6}\right)$ with d being the distance between the tip and the sample.

Name and sketch the potential, mark σ and ϵ and briefly describe the different regimes.

c) Sketch a force vs. distance curve obtained when performing a force spectroscopy experiment with a protein using an AFM and explain the different features observed.

Problem 3

Optimizing Michaelis-Menten. You are part of a team that is working on improving an industrial process for bioethanol production that used an enzyme E that is used in to break down its substrate (S) cellulose into the product (P) glucose. The overall reaction is well approximated by Michaelis-Mention kinetics and described by the following reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightleftharpoons} E + P \tag{1}$$

The process is currently run with a substrate concentration of 10 mM and an enzyme concentration of 1 μM. The standard enzyme currently used in the process has been characterized and the following parameters have been determined:

Parameter	Value
k_1	10^5 s^{-1} M^{-1}
k_{-1}	10^{-5} s^{-1}
k_2	10 s^{-1}

Your team members suggest several strategies to increase the rate of product formation in the reaction process. Which of these strategies would you pursue as promising candidates to significantly enhance the rate of product formation **and why**? With each change discussed below, we assume that the other parameters stay at their current values.

a) Make a mutation to the enzyme that increases k_1 10-fold.

b) Make a mutation to the enzyme that increases k_2 10-fold.

c) Increase the substrate concentration 10-fold.

d) Increase the enzyme concentration 10-fold.