Problem 1

Name: _____

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 (≈ 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?

$L_c^{ds} = 48500\ \text{bp} \times 3.4\ \text{Å/bp} = 164900\ \text{Å}$

$= 16.5\ \mu\text{m}$

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?

$L_c^{ss} = 48500\ \text{nt} \times 0.5\ \text{nm/nt} = 24250\ \text{nm}$

$= 24.3\ \mu\text{m}$

This is longer than for dsDNA.
In the single strand the bases stack much less and therefore "reach further", i.e. the sugar-phosphate backbone is in a different conformation.

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

$\sqrt{\langle R_{ee}^2 \rangle} = \sqrt{N \cdot b^2}$ $\quad b$ = Kuhn length $\approx 100\,nm$ for dsDNA

$N = \frac{L_c^{ds}}{b} = \frac{16500\,nm}{100\,nm} = 165$ segments

$\sqrt{\langle R_{ee}^2 \rangle} = \sqrt{165 \cdot 100^2\,nm^2} = 1280\,nm \hat{=} 1{,}3\,\mu m$

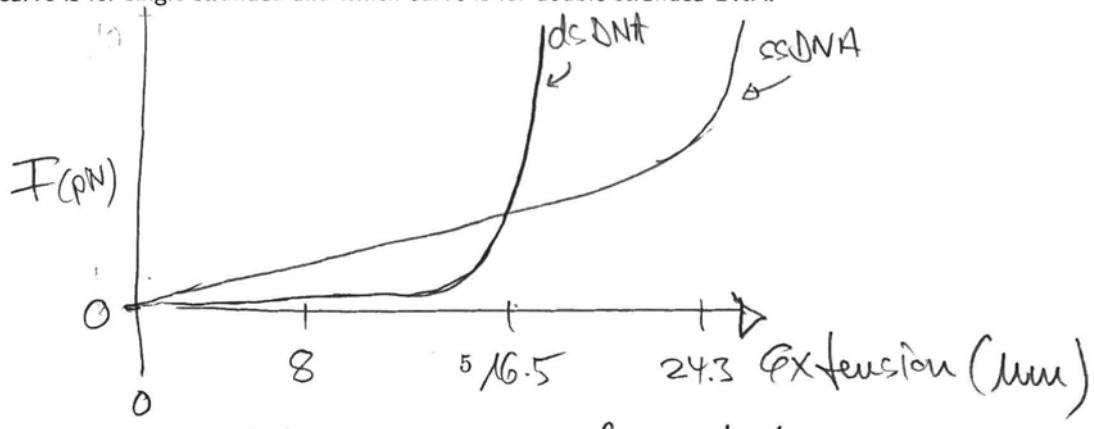
d) What is the average root-mean-squared end-to-end distance of single-stranded λ-DNA in free solution? You can assume that the segment or Kuhn length for single-stranded DNA is 1 nm.

$b = 1\,nm$ for ssDNA

$N = \frac{L_c^{ss}}{1\,nm} = \frac{24300\,nm}{1\,nm} = 24300$ segments

$\sqrt{\langle R_{ee}^2 \rangle} = \sqrt{24300\,(1\,nm)^2} = 156\,nm = 0.16\,\mu m$

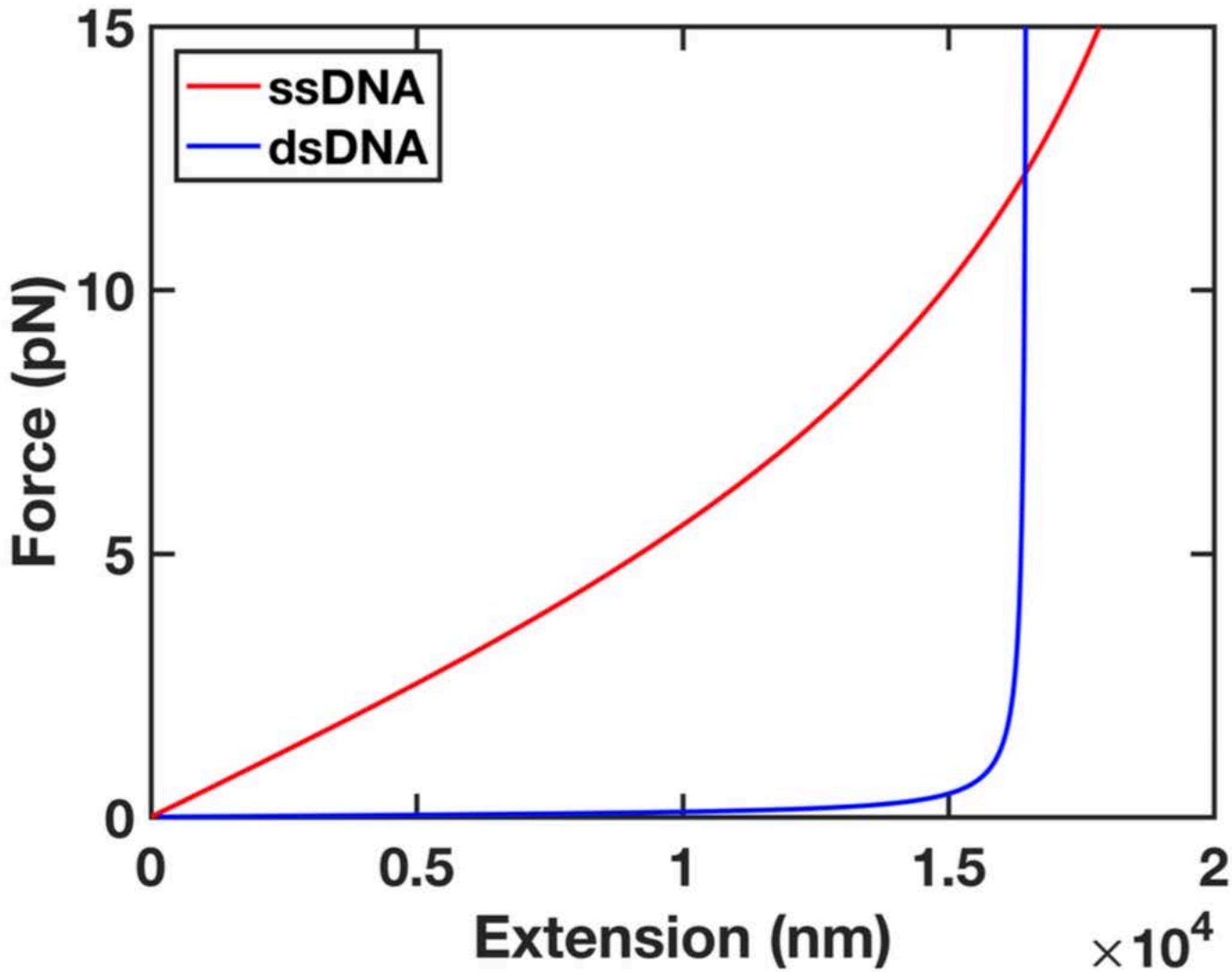
e) Sketch the force-extension behaviour of both single- and double-stranded λ-DNA in a coordinate system with the force on the y-axis and extension on the x-axis. Clearly label which curve is for single-stranded and which curve is for double-stranded DNA.



See "Additional plots" for a better representation.

FJC for ss and dsDNA

The plot shows the FJC model for ss and dsDNA with the parameters given in the problem.



Problem 3

Name: _____

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.

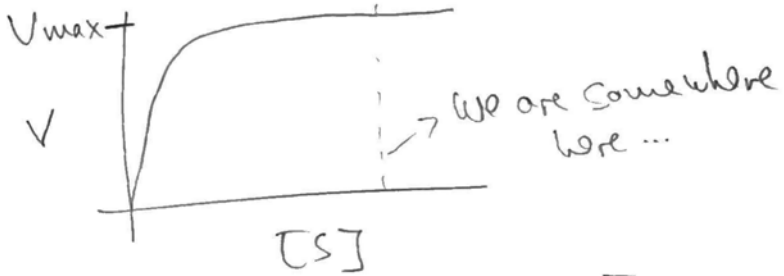
With the parameters given: $K_M = \frac{k_{-1} + k_2}{k_1} = \frac{10^{-5}\frac{1}{s} + 10\frac{1}{s}}{10^5 \frac{1}{M \cdot s}}$

$V = k_2[E]\frac{[S]}{[S]+K_M} = V_{max}\frac{[S]}{[S]+K_M}$

$= 9.9 \cdot 10^{-6}$ M/s

$= 10^{-4}$ M $= 0.1$ mM

Given that $[S] = 10$ mM, the reaction is in the saturation regime:



See "Additional plots" for a better representation

Increasing k_1 10-fold will decrease K_M to 10^{-5} M; but since we are already at saturation, this will increase V only to $9.99 \cdot 10^{-6}$ M/s

$\Rightarrow$ Not a promising strategy.

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

Increasing k_2 10-fold increases $V_{max} = k_2[E]$ 10-fold and will increase the reaction rate (almost) 10-fold, since we are at saturation.

$\Rightarrow$ with the mutation $V = 9 \cdot 10^{-5}$ M/s

$\Rightarrow$ This is a promising strategy!

c) Increase the substrate concentration 10-fold.

Increasing [S] 10-fold has a similar affect as the mutation in a). We go even further into the saturation regime, and $V = 9.99 \cdot 10^{-6}$ M/s is only slightly increased.

$\Rightarrow$ Not a promising strategy!

d) Increase the enzyme concentration 10-fold.

Increasing [E] 10-fold increases $V_{max} = k_2[E]$ 10-fold and since we are at saturation increases the overall rate (almost) 10-fold to $V = 9.9 \cdot 10^{-5}$ M/s

$\Rightarrow$ This is a promising strategy.