(1) Give brief definitions or unique descriptions of the following terms:

(a) exon
(b) K_M
(c) glycosidic bond
(d) Melting temperature
(e) nucleotide
(f) base tautomers
(g) poly A tail
(h) open complex

(2) Draw a stick diagram for each of the following:

(a) guanine
(b) cytidine
(c) deoxyadenosine in the syn conformation
(d) deoxythymidine-5-phosphate
(e) Watson-Crick G:C base pair (indicate H-bonds)

(3) What is catalytic perfection and how is measured?

(4) Draw an initial rates plot for an enzyme that catalyzes a first order reaction and has a V_{max} of 0.2 mM s^{-1}. Label all elements of the plot.

(5) How would you determine the K_M of an enzyme catalyzed reaction from a Lineweaver-Burk (double reciprocal) plot?

(6) Consider an enzyme with a Michealis-Menten mechanism, a K_M of 0.1 mM and a V_{max} of 0.01 mM s^{-1}. Answer the following:

(a) At what substrate concentration would the enzyme have an initial velocity of 1 μM s^{-1}?
(b) What fraction of all active sites are filled if the initial velocity is 8 μM s^{-1}?

(7) Give the proper name for the following nucleic acid fragments?

(a) pAp (b) d(TpC) (c) pppU (d) Gppp

(8) At what concentration will a Michaelis-Menten enzyme have an initial velocity of ¼ V_{max}?

(9) Answer the following question regarding a 1000 base pair (1 kb) dsDNA fragment:

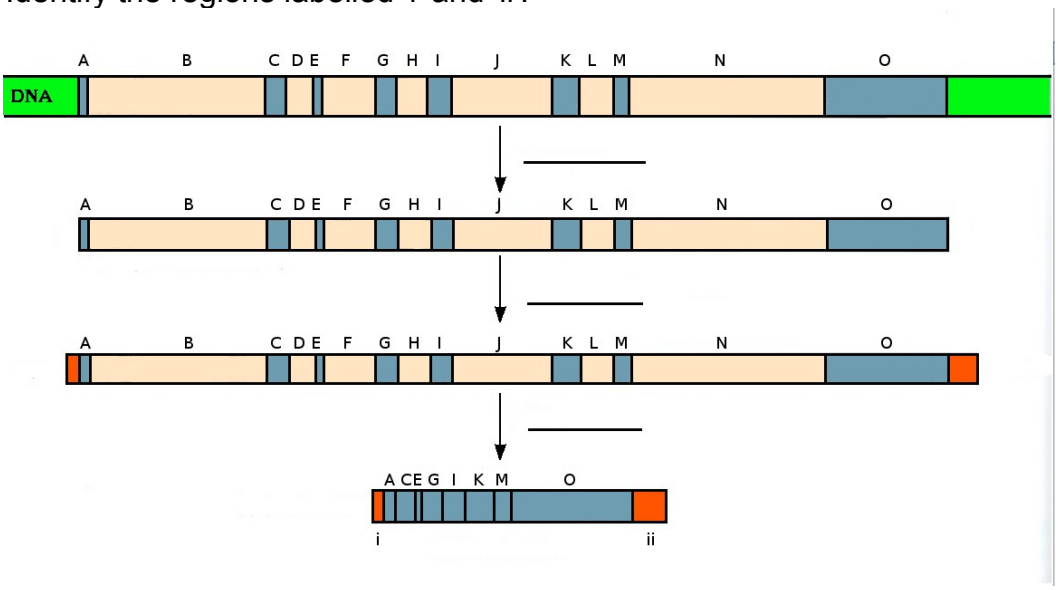
(a) If the fragment contains 22% G, what is the A content?
(b) If you slowly heat the dsDNA fragement while measuring the UV absorption, what will happen?

(10) Many DNA binding proteins have an approximate overall shape that resembles a 'U' or a horseshoe. The DNA binds inside the 'U' and makes extensive contacts with the protein. Answer the following questions:

(a) What is the most likely inside diameter of 'U' shaped DNA binding proteins?
(b) Would you expect 'U' shaped DNA binding proteins to bind to dsRNA with an equivalent sequence? Explain.

(11) The following figure indicates the typical steps associated with the production of eucaryotic mRNA.

(a) Fill in the blanks describing each of the steps in the production of mRNA.
(b) Identify each of the lettered regions of the figure.
(c) Identify the regions labelled 'i' and 'ii'.



Answers

(1)

a – The coding sequence that is retained in mature mRNA and directs eucaryotic protein synthesis.

b – The concentration of substrate at which the enzyme has ½ its maximal initial velocity.

c – The covalent bond between the C1' of D-ribose and the N1(or N9) atom of the nucleic acid base.

d – The temperature at which 50% of dsDNA or dsRNA has separated into single strands (denatured).

e – A nucleoside phosphate or the repeating unit of a nucleic acid polymers.

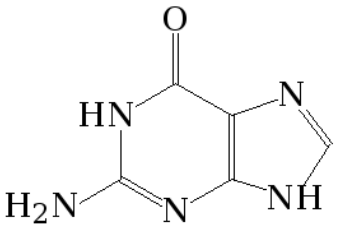
f – Nucleic acid base structures that differ in the location of single hydrogen atom. These structures have altered covalent and hydrogen bonding patterns.

g – A stretch of 10s to 100s of adenylic acid nucleotide added to the 3' terminii of eucaryotic mRNA.

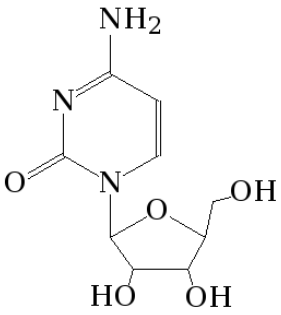
h – The transcription initiation complex in which the DNA strands of the bacterial promoter (-10 - +4) are single stranded.

(2)

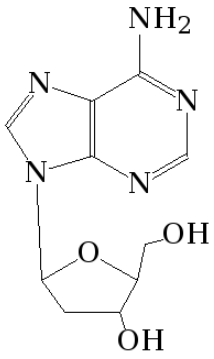
a –



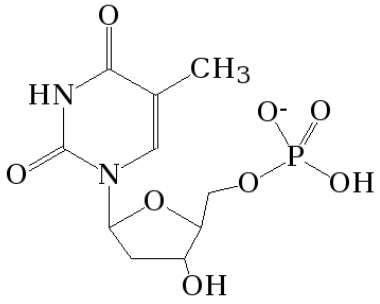
b –



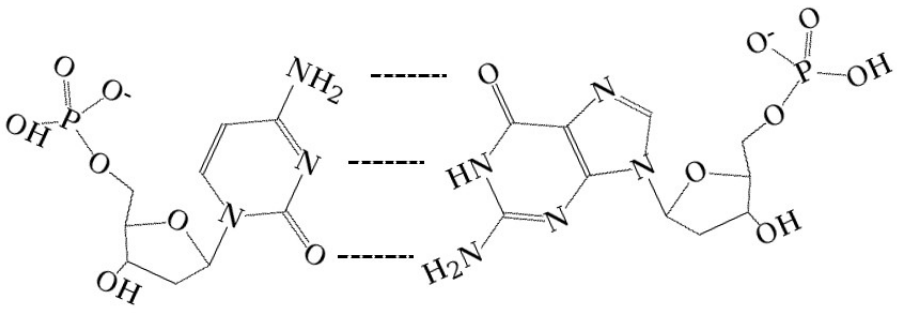
c –



d –



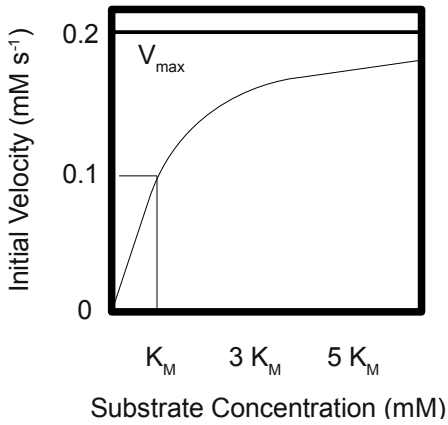
e –



(3) The theoretical maximum efficiency of any catalyst occurs when the catalytic rate is limited by diffusion of the substrate and enzyme. In this case, $k_2 >>> k_{-1}$ and every "collision" between substrate and catalyst results in the formation of product.

Catalytic perfection is measured using k_{cat}/K_M, where k_{cat} represents the "turnover number" or the rate constant for product formation (typically equivalent to k_2 for Michaelis-Menten enzymes) and K_M is the Michaelis constant indicating the concentration of substrate at which the initial velocity is ½ maximal. This value is ~$10^8 – 10^9$ M^{-1} s^{-1} for catalytically perfect enzymes.
Note: Recall that enzyme efficiency depends upon both its maximal rate AND the concentration of substrate.

(4)



This question does not indicate magnitude of K_M so we cannot numerically quantify the substrate concentrations along the x-axis.

(5) – There are two solutions.
a – Measure the x-intercept which is $(-1/K_M)$
b – Determine V_{max} from the y-intercept and then determine K_M from the slope (K_M/V_{max})

(6)

a – Starting with the Michaelis-Menten equation, $v_o = (V_{max}[S]) / (K_M + [S])$
We substitute the given values for v_o, V_{max} and K_M, which yields:
$1\ \mu M\ s^{-1} = (0.01\ mM\ s\text{-}1\ [S]) / (0.1\ mM + [S])$
Converting μM to mM and solving for [S] produces:
$0.001\ mM\ s^{-1}\ (0.1\ mM + [S]) = (0.01\ mM\ s^{-1}\ [S])$
$0.0001\ mM^2\ s^{-1} + 0.001\ mM\ s^{-1}\ [S] = 0.01\ mM\ s^{-1}\ [S]$
$0.0001\ mM^2\ s^{-1} = 0.009\ mM\ s^{-1}\ [S]$
[S] = 0.011 mM

b – V_{max} is 0.01 mM s^{-1} or 10 μM s^{-1}. At V_{max}, E_T = ES (all enzyme has substrate bound). If the initial velocity of the enzyme is 8 μM s^{-1}, the fraction of sites filled is simply 8 μM s^{-1}/10 μM s^{-1} or 0.80 (80%).

Note: Part b only holds for enzymes with a Michaelis-Menten mechanism.

(7)

a – adenosine – 3', 5' – diphosphate
b – deoxythymidyl – 3',5' – deoxycytidine
c – uridine – 5' – triphosphate
d – guanosine – 3' – triphosphate

(8) Starting with the Michaelis-Menten equation, $v_o = (V_{max} [S]) / (K_M + [S])$.
Substitute $v_o = ¼ V_{max}$, and solve for [S]
$¼ V_{max} = (V_{max} [S]) / (K_M + [S])$
$K_M + [S] = (V_{max} [S]) / ¼ V_{max}$
$K_M + [S] = 4 [S]$
$[S] = K_M / 3$

(9)

a – Using Chargaff's rule, G = C and G + C = 44%
A + T = 100% - 44% = 56% and A = 28%

b – The UV absorption will remain constant and relatively low until the melting temperature is reached. The UV absorption will rapidly rise over a narrow temperature range as the dsDNA cooperatively denatures and will then remain constant and relatively high.
Note: Denature and Unwind are <u>not</u> equivalent or interchangable.

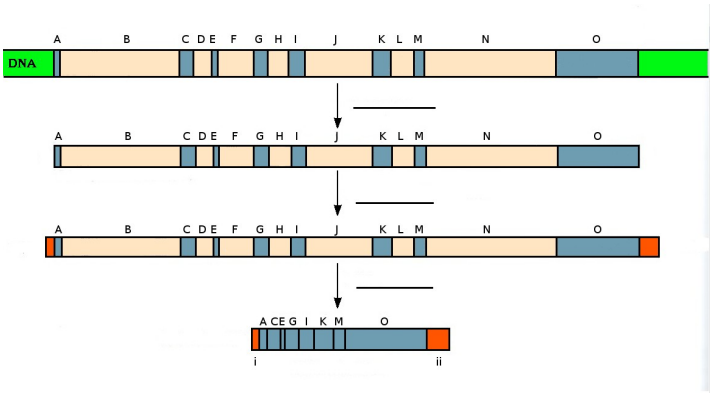
(10)

a – Virtual all dsDNA in cells is B-DNA and has a diameter of 2.0 nm. The 'U' shape would require a closely similar diameter to maintain geometric complementarity.

b – The dsRNA is unlikely to bind as it adopts an A-DNA like conformation. The overall size and shape of dsRNA is significantly different from B-DNA and generally disrupts the geometric complementarity required for protein-ligand interactions. In particular, the double helix is wider (2.6 vs 2.0 nm) and there are significant difference in the size of the major and minor grooves. The chemical differences (2'-OH and U) between RNA and DNA may also adversely affect specific binding interactions.

Note: The difference in helix diameter and groove size are intimately related to the differences in other structural parameters (*eg.* helix pitch, rise, base pair twist, ribose conformation, *etc.*).

(11)



a –
Transcription
Processing or Capping
Splicing

b –
A, C, E, G, I , K, M, O are exons
B, D, F, H, J, L, N are introns

c –
'i' is the 5' cap (7-methyl guanosine)
'ii' is the 3' polyA tail