Section A:

1D	**5D**
2A	**6D**
3C	**7C**
4B	**8C (1 pt given for A)**

B1.

i) Since [S] << K_M for both cases, we can use the approximation (3 pts)

$$V = E_t(k_{cat}/K_M)[S],$$

therefore the product formation is proportional to (k_{cat}/K_M) NOT k_{cat}. Therefore, the Tyr-Ala substrate would produce more product (3 pts)

ii) The Arginine-Ala substrate binds with the lowest affinity since it has the higher K_M. (+2). Chymotrypsin has a non-polar binding pocket in its active site, hence it prefers non-polar amino acids and will bind charged ones with lower affinity (+3).

B2. (5 pts) What is the *general* mechanism for the acceleration of a chemical reaction by an enzyme? You may use a specific reaction mechanism in your discussion if desired.

The key word here was 'general', i.e. applicable to all enzymes. All enzyme lower the energy of the transition state (+4.5 pts) by forming bonds with the transition state (+1/2) or by bringing catalytic groups together in close proximity to the substrate (+1/2).

Partial credit was given for the equation [E]+[S] --> [ES] --> EP, as this is a general description of the mechanism, but it does not allude to why the rate increases.

B3.

i) This is a competitive inhibitor, it binds in the active site and is similar in structure to the substrate (+4). It is lacking a peptide bond and therefore cannot be cleaved (+4).

ii) V_{MAX} is given by 1/y-intercept = 1/0.01 = 100 umole/sec (1/2 for units)

iii) α= 1 + (10 nM/10 nM) =2. Since the ratio of the slopes is equal to α, the slope will be twice as steep. Since it is a competitive inhibitor, V_{MAX}, and therefore the Y-intercept does not change.

iv)

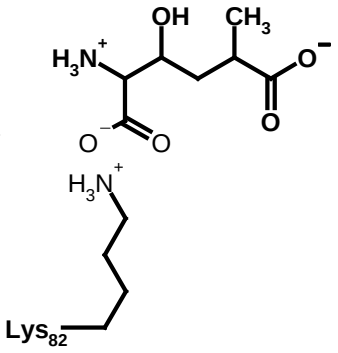
Choice A:

Part a. Since the slope of a lnK versus 1/T plot gives you the enthalpy, the binding enthalpies must be the same.

Part b: Since the K_D of the Val-wildtype interaction is lower, it binds tighter, ΔG^o for binding is larger. Since the enthalpies (ΔH^o) are the same, there must be a more favourable entropic term for the wild-type. This is due to the non-polar interaction between the Val on the enzyme and the Phe on the substrate. This generates a hydrophobic interaction with a large change in ΔS^o due to the release of ordered water.

Choice B:

Lysine has a positively charged amine group on the end (1 pt), therefore to get the drug to bind with high affinity you would include a negative charge on the drug (3 pts). This would generate an electrostatic interaction (1 pt) with a favourable entropy of binding.



B4.

i)

n_h = slope at log(Y/(1-Y)) = 0. $\Delta Y/\Delta X = (5-0)/(-5-(-6)) = 5$

K_D is always the [L] that gives Y=0.5, or log(Y/(1-Y)) =0. In this case:

$$\log K_D = -6$$

$$10^{\log K_D} = 10^{-6}$$

$$K_D = 10^{-6} = 1\mu M$$

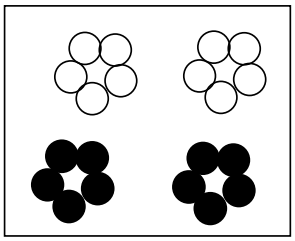
ii)

Since [L]=K_D, Y=0.5. Therefore [ML]/([M]+[ML])=0.5, or [M]=[ML]=2μM. (1 pt)

Since $[L]_{TOT}$ = [L]+[ML]

$[L]_{TOT}$ = 1μM + 2 μM = 3 μM.

iii) Since the Hill coefficient equals the number of binding sites (n_h = 5, 1 pt) , the system is infinitely cooperative (1 pt), thus only fully liganded or completely empty pentamers will be found. (1 pt). 1 pt for a correct diagram.



iv)

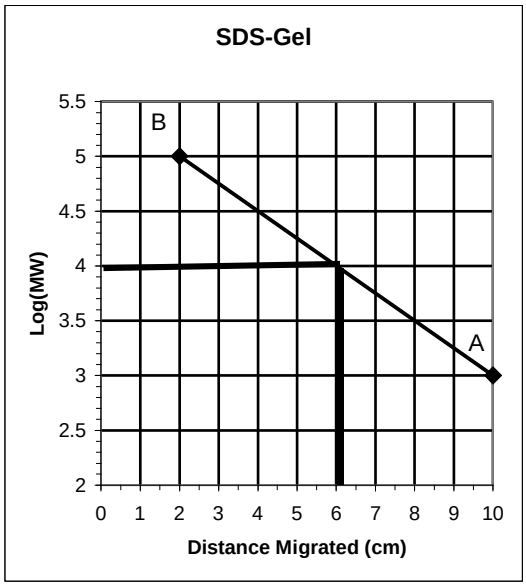
Choice A: Since the binding becomes non-cooperative, a straight line with a Hill coefficient or slope =1 will be observed. Since the affinity increased, the K_D will drop to 0.1μM. Log($1x10^{-7}$) = -7, so the line will cross the x-axis at -7.

Choice B: Non-cooperative binding gives *linear* Scatchard plots. The slope of the plot is $-1/K_D$, or $-K_A$. In this case, the slope would be -10^7 M^{-1}.

v)

Part a: A distance migrated of 6 cm corresponds to a log MW of 4, or a molecular weight of 10,000.

Part b: Since SDS denatures proteins (+2) the molecular weight is of the subunit (2 pts).



B5.

A. The binding curve is still S-shaped, but shifted to the right. Therefore the oxygen binding is less at any given O_2 concentration. At high altitudes hemoglobin+ BPG binds less oxygen in the lungs, but because of the shape of the binding curve, it releases more oxygen in the tissue than hemoglobin with low BPG levels, thus the oxygen delivery is about the same.

B. The binding curve is hyperbolic and shifted far to the left. Indicative of non-cooperative binding and a low K_D. This allows myoglobin to bind and store the oxygen that is released by hemoglobin at low oxygen pressures.

C. The shape of the binding curve is S-shaped, but shifted to the left, almost as far as the myoglobin curve (the K_D values are similar). This allows the fetal blood to bind the oxygen that is released by the material blood at the placenta.

D. Both cleave a peptide bond, both use water as a nucleophile, both use a base to activate the nucleophil. The differences are that Serine proteases have an acyl-intermediate, use serine as the first nucleophile and use His instead of Asp to activate the nucleophil. HIV proteases use Asp to activate the nucleophile and do not form an acyl intermediate.

E

Gel filtration	size	Just wash the column
Cation Exchange	Proteins have a positive charge	Change in pH to change the charge on the protein. Change in salt concentration to interfere with electrostatic interaction.
Anion exchange	Proteins with a negative charge.	
Affinity Chromatography	Specific protein-ligand interaction	Excess free ligand, or a change in pH or salt concentration.