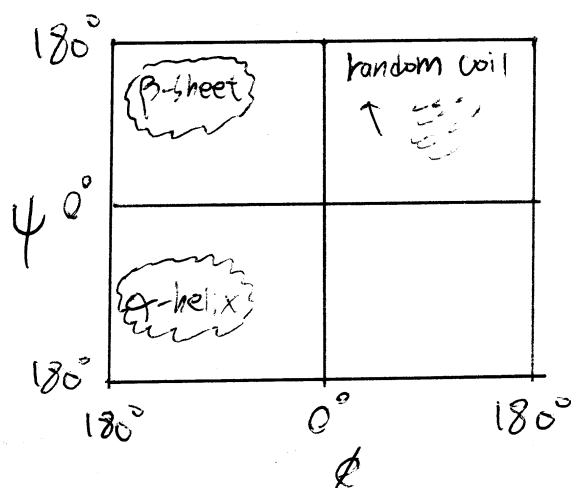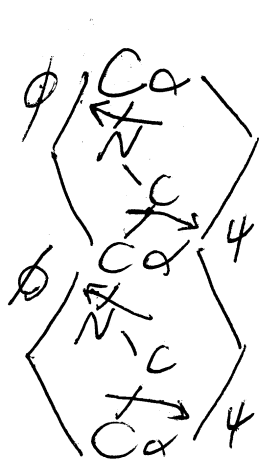1. Explain the following terminologies and describe their meaning in biochemistry (20%)

(a) Ramachandran plot (ϕ, ψ map)

Protein 中具有中和 ψ 的二面角, 作圖可得和 Protein 的二級結構

(b) Gel filtration column (可予略, 不會考)

(c) Henderson-Hasselbalk equation

$$pH = pka + \log\frac{[A^-]}{[HA]}$$

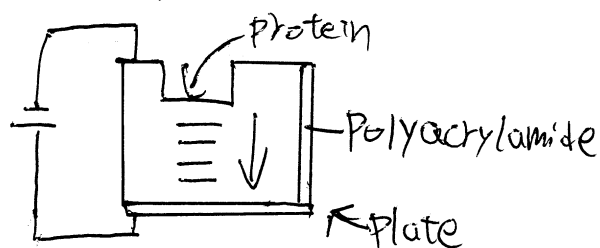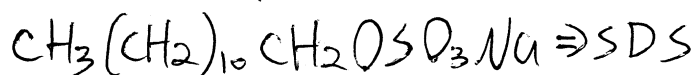⟨Explain⟩ For $HA + H_2O \rightleftharpoons H_3O^+ + A^-$

$$K_a = \frac{[H_3O^+][A^-]}{[HA]}$$

$$\Rightarrow \log K_a = \log[H_3O^+] + \log\frac{[A^-]}{[HA]} \quad , \quad By \begin{cases} \log K_a = -pka \\ \log[H_3O^+] = -pH \end{cases}$$
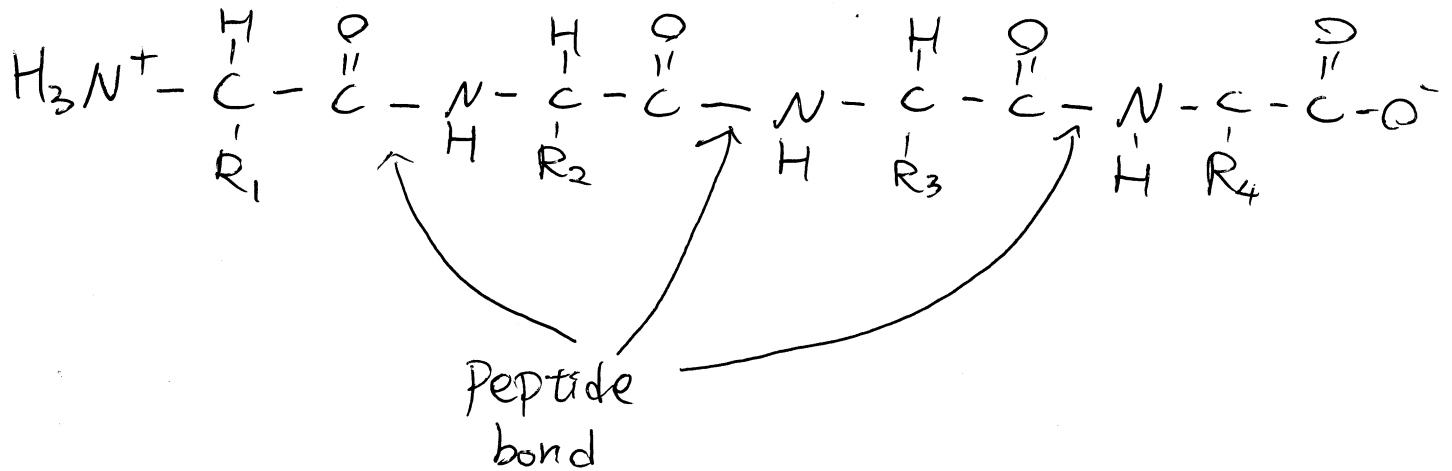
$$\Rightarrow -pka = -pH + \log\frac{[A^-]}{[HA]} \Rightarrow pH = pka + \log\frac{[A^-]}{[HA]}$$

(d) SDS PAGE (2020 年考的時候送分)

$CH_3(CH_2)_{10}CH_2OSO_3Na \Rightarrow SDS$

為一種分離 protein 的方式。將 sample 注入凹槽中通以穩定的電流, 則 protein 會因分子量不同而有不同移動距離, M.W. 大在上方, M.W. 小在下方。

(e) peptide bond

$$H_3N^+ - \overset{\overset{H}{|}}{\underset{\underset{R_1}{|}}{C}} - \overset{\overset{O}{\|}}{C} - N - \overset{\overset{H}{|}}{\underset{\underset{R_2}{|}}{C}} - \overset{\overset{O}{\|}}{C} - N - \overset{\overset{H}{|}}{\underset{\underset{R_3}{|}}{C}} - \overset{\overset{O}{\|}}{C} - N - \overset{\overset{H}{|}}{\underset{\underset{R_4}{|}}{C}} - \overset{\overset{O}{\|}}{C} - O^-$$

Peptide bond

(f) Cooperativity of Hemoglobin

For $MbO_2 \rightleftharpoons Mb + O_2$ $\quad y = \dfrac{[MbO_2]}{[MbO_2] + [Mb]}$, $\quad k_d = \dfrac{[Mb][O_2]}{[MbO_2]}$

$\Rightarrow [MbO_2] = \dfrac{[Mb][O_2]}{k_d}$ $\Rightarrow y = \dfrac{([Mb][O_2]/k_d)}{([Mb][O_2]/k_d) + [Mb]}$

$$= \dfrac{[O_2]}{[O_2] + k_d}$$

For Hemoglobin: $Hb(O_2)_n \rightarrow Hb + nO_2$

$\Rightarrow y = \dfrac{P_{O_2}^n}{P_{O_2}^n + P_{50}^n}$ $\Rightarrow \dfrac{y}{1-y} = \dfrac{P_{O_2}^n}{P_{50}^n}$ $\Rightarrow \log\dfrac{y}{1-y} = n\log O_2 - n\log P_{50}$

n=2.8

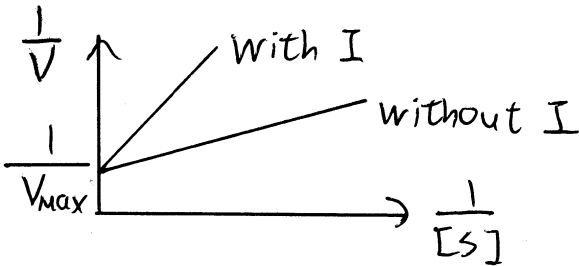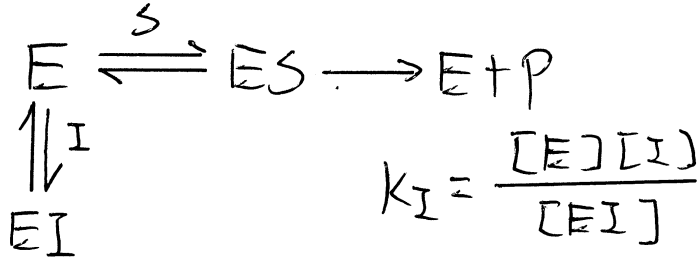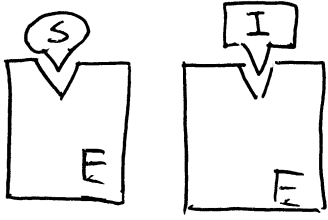For hemoglobin
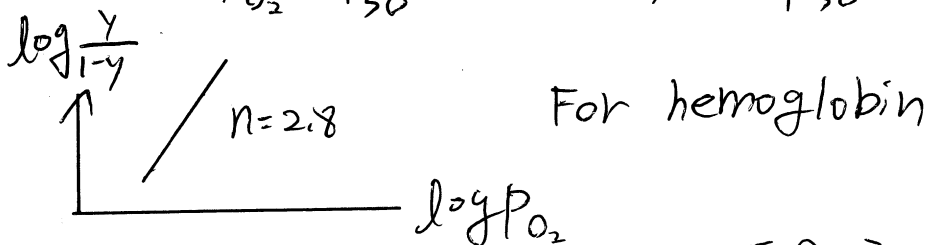
補 若是 Myoglobin , $y = \dfrac{[O_2]}{[O_2] + k_d} = \dfrac{P_{O_2}}{P_{O_2} + P_{50}}$

$$\Rightarrow \dfrac{y}{1-y} = \log O_2 - \log P_{50}$$

n=1
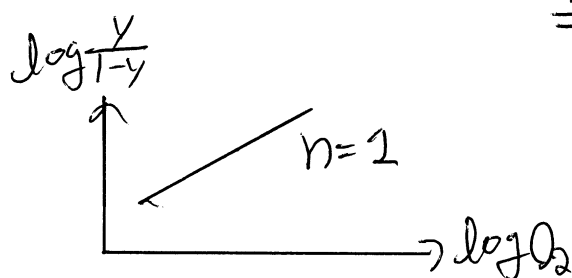
(g) Competitive inhibition

$$E \underset{S}{\rightleftharpoons} ES \longrightarrow E + P$$

$$K_I = \frac{[E][I]}{[EI]}$$

With I

without I

Without inhibitor:

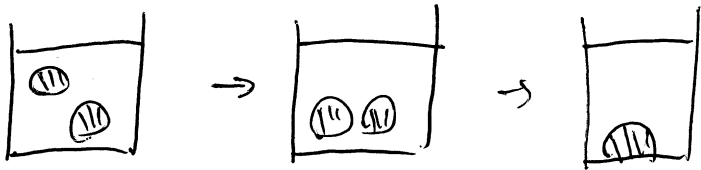$$\frac{1}{V} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

With inhibitor:

$$K_M^I \xrightarrow[a\ factor]{increase} \left(1 + \frac{[I]}{K_I}\right) K_M$$

$$\frac{1}{V} = \frac{\left(1 + \frac{[I]}{K_I}\right) K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$= \frac{K_M}{V_{max}} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

指 Inhibitor 會和 substrate 競爭結合位置，故 $K_M^I = \left(1 + \frac{[I]}{K_I}\right) K_M$

(h) Hydrophobic interaction
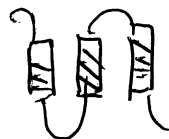

= Hexane as example

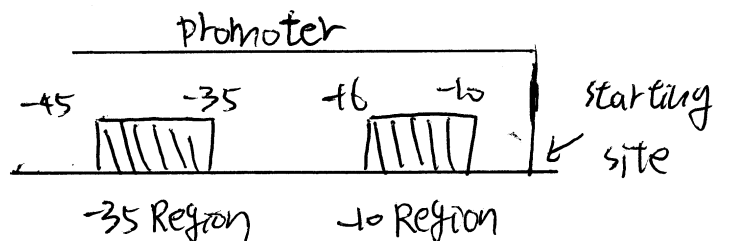疏水的分子在此效應下會受吸引而自我聚集。而在 protein 中，疏水性較高的胺基酸會彼此 interact，形成下一級的結構

Random Coil   Hydrophobic interaction

(i) DNA promoter contains three elements

Transcription starting site
-35 region
-10 region (Pribnow box)

Promoter

-45   -35   +6   -10   starting site

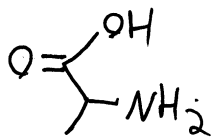-35 Region   -10 Region

(j) operon

　為具有相同 promotor 的一組基因，其受該 promotor 的
　調控。(a group of gene with the same promotor)

2. There are four families of amino acid. Write down the
20 different structures of amino acid according to their
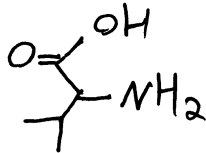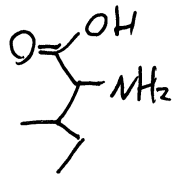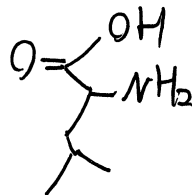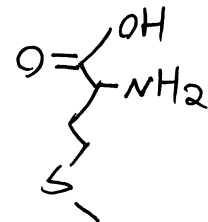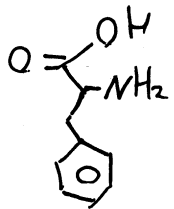families. Label them with one-letter code. (10%)
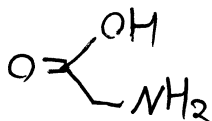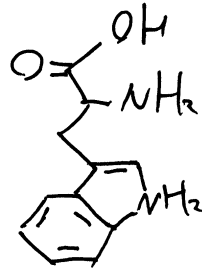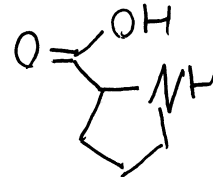〔不要懷疑，背下來〕
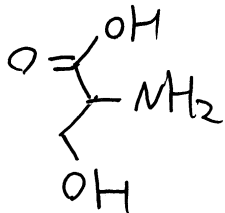
A. Non - polor

Ala (A)　　　Val (V)　　　ile (I)　　　Leu (L)　　　Met (M)

Phe (F)　　　Gly (G)　　　Trp (W)　　　Pro (P)
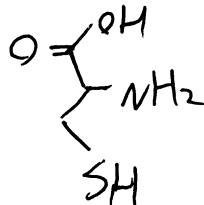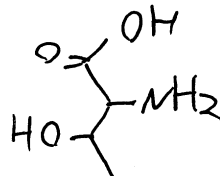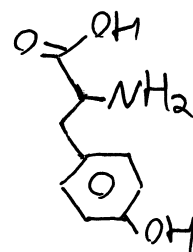

B. Polar

Ser (S)　　　Cys (C)　　　Thr (T)　　　Tyr (Y)　　　Asn (N)
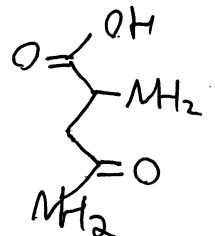
Gln (Q)
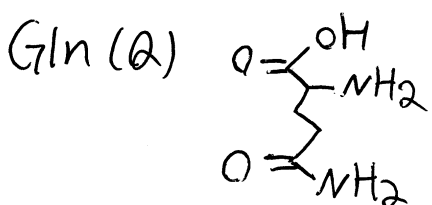
C. Acidic:

ASP (D)
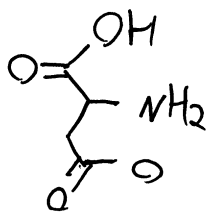
Glu (E)
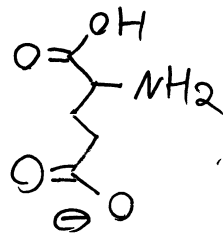
D. Basic

His (H)
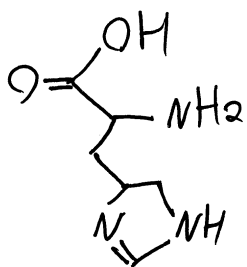
Lys (K)
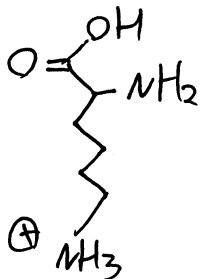
Arg (R)
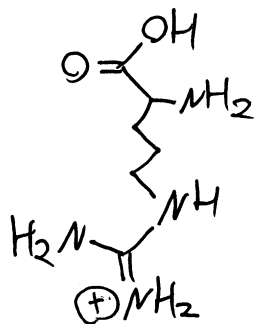
3. A sample of a peptide of unknown sequence was treated with trypsin; another sample of the same peptide was treated with chymotrypsin. The sequences (N-terminal to C-terminal) of the smaller peptides produced by trypsin digestion were

Met - Val - Ser - Thr - Lys
Val - Ile - Trp - Thr - Leu - Met - Ile
Leu - Phe - Asn - Glu - Ser - Arg

the sequence of the smaller peptides produced by chymotrypsin digestion were

Asn - Glu - Ser - Arg - Val - Ile - Trp

Thr - Leu - Met - Ile
Met - Val - Ser - Thr - Lys - Leu - Phe

(2020 有出)

Find the amino acid sequence of unknown peptide (100%)

Ans: Trpsin 會切 Lys、Arg 的 C 端，所以有可能是：

Met ... Lys ⎯ Leu ... Arg ⎯ Val ... Ile ... ①
        ↑斷   ↑斷

or Leu ... Arg ⎯ Met ... Lys ⎯ Val ... Ile ... ②
          ↑斷   ↑斷
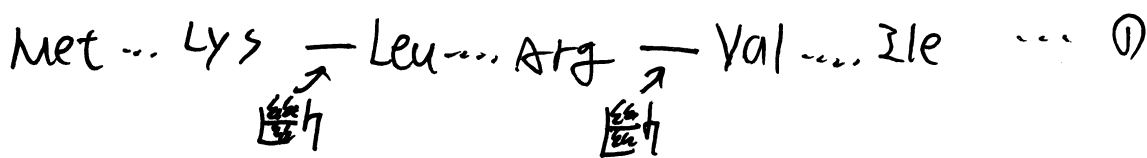
而 chymotrypsin 會切 Tyr、Trp、Phe 的 C 端，所以可能是

Asn ... Trp ⎯ Met ... Phe ⎯ Thr ... Ile ... ③
        ↑斷   ↑斷

or Met ... Phe ⎯ Asn ... Trp ⎯ Thr ... Ile ... ④
         ↑斷   ↑斷
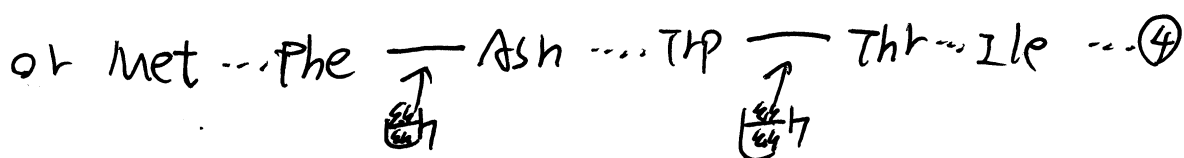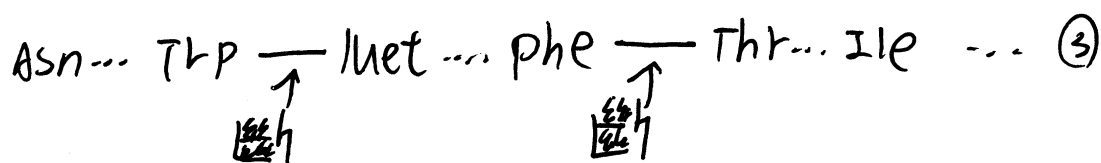
綜合比較 ①、④ 相同 ⇒ 得解

Met - Val - Ser - Thr - Lys - Leu - Phe - Asn - Glu - Ser - Arg - Val - Ile
- Trp - Thr - Leu - Met - Ile

4. Anfisen use Ribonuclease to do the folding-unfolding experiments, and he concluded that "3D structure of protein is encoded in its amino acid sequence". Describe Anfinsen's experiment on Ribonuclease.

Native ribonuclease

denatured (reduction) → reconnect →

if random, $9 \times 5 \times 3 \times 1 = 105$ possible structure ⇒ (X)

僅有原本的 native structure (26-84, 40-95, 58-110 65-72)
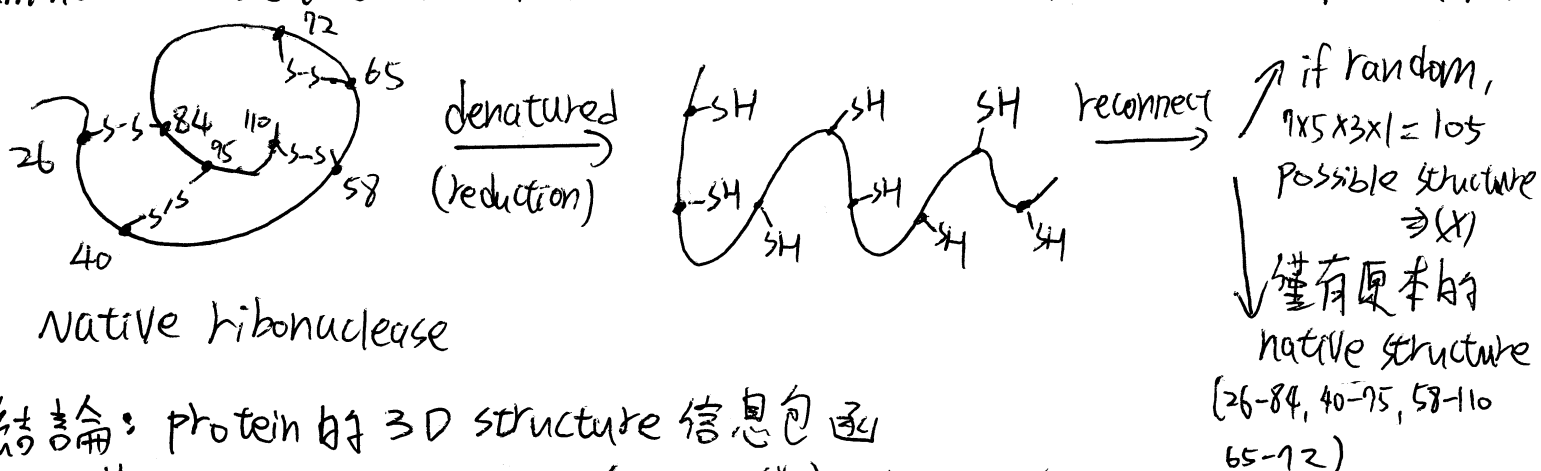
結論：protein 的 3D structure 信息包函 在其 amino acid sequence (一級結構) 之內

(2020 有出)

5. Describe the Edam degradation. (5%)

将蛋白質和 phenylisothiocyanate 結合, 得到 PTH-R₁, PTH-R₂
..., 可知胺基酸排列的順序
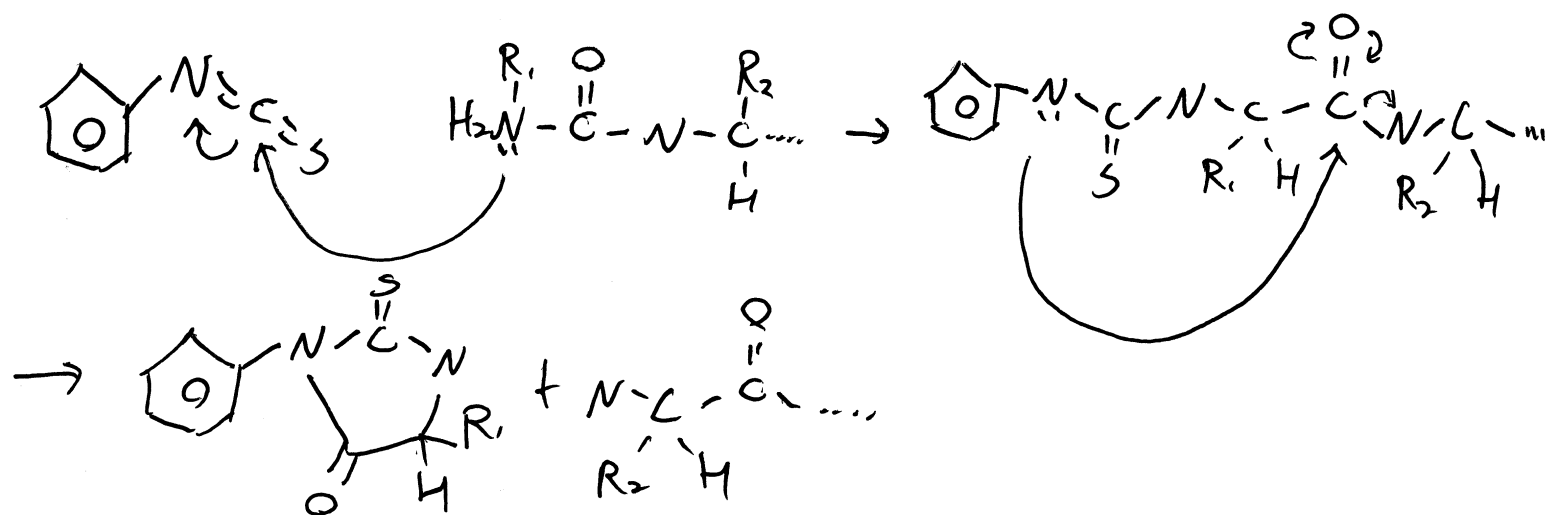
6. write down the structure of the following RNA (100%)
pACGU

幹, 到底三小破課

2020有出

7. write down the hydrogen bond between base pair AT, and between G C ?

8. Describe how to use NMR to determine the 3D structure of a protein ?

將 protein 配製成 solution, 相鄰的 H 小於 5Å 時會有 NDE 效果, 可解出 3D 結構。

9. For the reaction:

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Use steady-state approximation (for ES) and materials balence, derive Michaealis-menten equation: (10%)

a. Steady-state approximation of [ES]:

$$V_{form} = V_{destruct} \Rightarrow K_1 [E][S] = K_{-1}[ES] + k_2[ES]$$
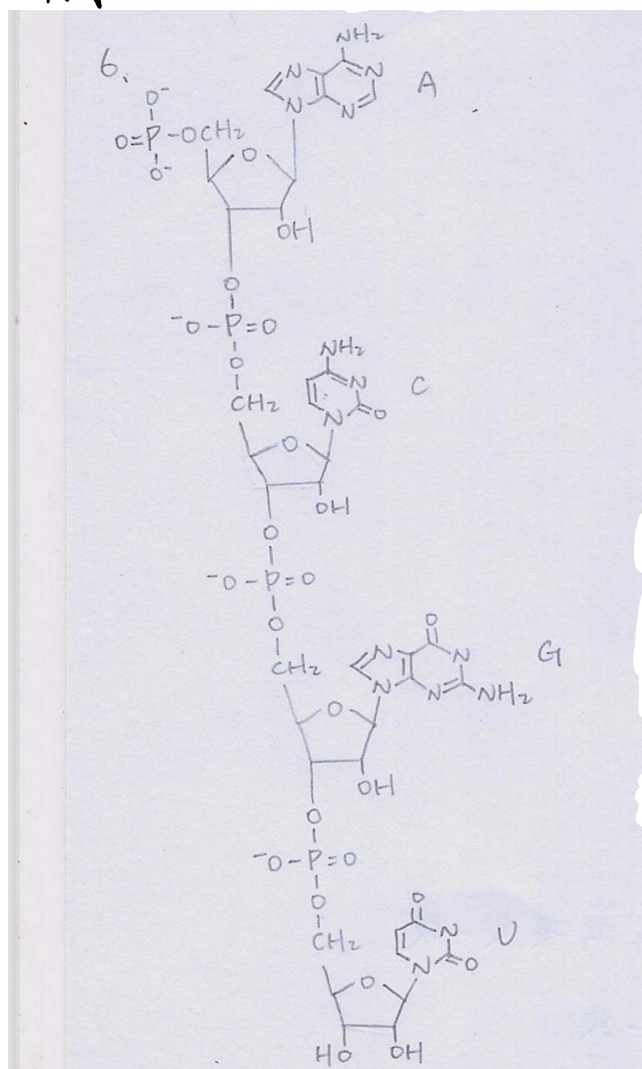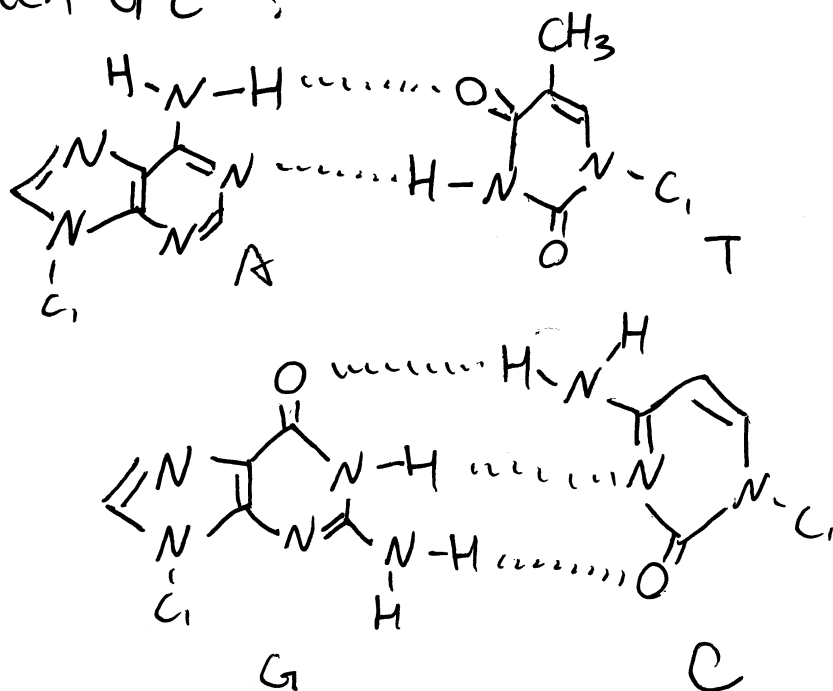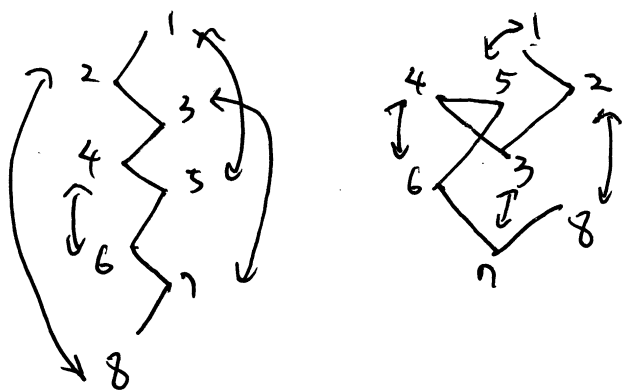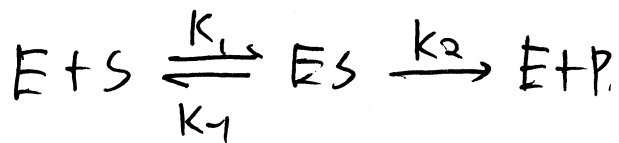
b. material balence: $[E]_T = [ES] + [E]_{free} = [ES] + [E]$

$$\Rightarrow [E] = [E]_T - [ES] \text{ st } \boxed{E}$$

$$K_1 ([E]_T - [ES])[S] = k_2[ES] + k_1[ES] = (k_2 + k_{-1})[ES]$$

$$\Rightarrow \frac{([E]_T - [ES])[S]}{[ES]} \simeq \frac{k_2 + k_{-1}}{k_1} = K_M$$

$$K_M[ES] = ([E]_T - [ES])[S] \Rightarrow [ES] = \frac{[E]_T[S]}{K_M + [S]}$$

$$rate = V = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}, \text{ when } [S] \to \infty$$

$$[E]_T = [ES], \text{ rate has it's maximum}$$

$$\therefore V = \frac{k_2[ES][S]}{K_M + [S]} = \frac{V_M[S]}{K_M + [S]}$$

10. The hydrolysis of a phenylalanine - containing peptide is catalyzed by α-chymotrypsin with the following results. Calculate K_M and V_{max} for the reaction. (10%)

peptide concentration (M)	velocity (M/min)
2.5×10^{-4}	2.2×10^{-6}
5×10^{-4}	3.8×10^{-6}
10.0×10^{-4}	5.9×10^{-6}
15×10^{-4}	7.1×10^{-6}

By $\quad \dfrac{V_{MAX} [S]}{K_M + [S]} = V \Rightarrow \dfrac{1}{V} = \dfrac{K_M + [S]}{V_M [S]} = \dfrac{K_M}{V_M} \dfrac{1}{[S]} + \dfrac{1}{V_M}$

以 $\dfrac{1}{[S]}$ 當 x 軸, $\dfrac{1}{V}$ 當 Y 軸

$\dfrac{1}{[S]}$ (M)	$\dfrac{1}{V}$
4000	454545.45
2000	263157.89
1000	169491.53
666.67	140845.07

$\dfrac{K_M}{V_M} = \dfrac{\dfrac{1}{3.8 \times 10^{-6}} - \dfrac{1}{2.2 \times 10^{-6}}}{2000 - 4000}$

$= 95.69$

$\Rightarrow \dfrac{1}{V} = 95.69 \times \dfrac{1}{[S]} + \dfrac{1}{V_M}$

代入 $\left(\dfrac{1}{[S]}, \dfrac{1}{V}\right) = \left(\dfrac{1}{2.5 \times 10^{-4}}, \dfrac{1}{2.2 \times 10^{-6}}\right)$

得 $V_M = 1.39 \times 10^{-5}$ M/min

$\Rightarrow K_M = 1.33 \times 10^{-3}$

$\dfrac{1}{V}$ ↑ slope = $\dfrac{K_M}{V_M}$, → $\dfrac{1}{[S]}$