PRACTICAL EXAM 4 - BIOCHEMISTRY
實作題 4 - 生物化學

Max. total points 100
滿分 100
Exam duration 90 minutes
操作時間 90 分鐘
20 questions
20 小題

INTRODUCTION
簡介

PURPOSE
目的

In this exam you will analyse enzyme kinetics with and without an inhibitor of the enzyme.
本測驗將分析在有無添加抑制劑時的酵素動力學。
The exam consists of two main parts, each of which contains three subparts.
本測驗將分成兩大部分，每個部分再分成三小部分。

Part 1 (57.5 points) 第一部分（57.5分）
1.1. Introduction to enzyme kinetics (theory) (0 point)
1.2. Enzyme kinetics experiment of an industrial α-galactosidase using a synthetic substrate analogue *p*NP-Gal (laboratory work) (Questions 1–2: 40 points)
1.3. Data analysis of enzyme kinetics of α-galactosidase (Questions 4–11: 17.5 points)
1.1. 簡介酵素動力學（理論）（0 分）
1.2. 利用合成受質模擬物 pNP-Gal 在實驗室來分析工業用的 α-半乳糖苷酶酵素動力學實驗，（問題 1-2；40 分）
1.3. 有關 α-半乳糖苷酶 酵素動力學數據分析（問題 4-11；17.5 分）

Part 2 (42.5 points) 第二部分（42.5分）
2.1. Introduction to enzyme inhibitors (theory) (Questions 11–13: 2 points)
2.2. Inhibition experiment of α-galactosidase (laboratory work) (Question 14: 27 points)
2.3. Data analysis of inhibition kinetics of α-galactosidase (Questions 15–20: 13.5 points)
2.1. 簡介酵素抑制劑（理論）（問題 11-13；2 分）
2.2. 有關 α-半乳糖苷酶 抑制實驗（實驗室階段）（問題 14；27 分）
2.3. 有關 α-半乳糖苷酶 抑制動力學數據的分析（問題 15-20；13.5 分）

Before you begin, we advise you to skim the entire exam to get an overview of the content. Since most points are earned on the lab part, **we recommend you to carry out parts 1.2 and 2.2, before starting with calculations and theoretical questions** (Parts 1.3, 2.1 and 2.3).
開始進行前，我們建議您將整個題目從頭至尾瀏覽一遍。由於實驗操作部分佔最大的得分，我們建議您先行操作 問題 1.2 與 2.2 後，再進行計算題與理論題部分。

MATERIALS & EQUIPMENT
材料與設備

First, verify that you have all items listed below in front of you. **Please raise your pink card immediately, if anything is missing – and no later than 15 minutes after the start of the exam.**
首先，確認在你面前所有的材料項目是否完備。如果有任何缺損，請舉手。實驗開始 15 分鐘後，將不予以補發。

A. 1 p200 pipette. Use pipette p200 for volume interval 20-200 µL, unless otherwise stated
一支 p200 微量吸管，範圍 20-200 µL
B. 1 p1000 pipette. Use pipette p1000 for volume interval 201-1000 µL, unless otherwise stated
一支 p1000 微量吸管，範圍 201-1000 µL
C. 96 tips for p200 in box. A pipette tip should be discarded after each pipetting, unless otherwise stated
一盒（96 支） p200 微量吸管用的吸管尖
D. 96 tips for p1000 in box. A pipette tip should be discarded after each pipetting, unless otherwise stated
一盒（96 支） p1000 微量吸管用的吸管尖
E. > 30 microcentrifuge tubes (1.5 ml)
多過 30 個 1.5 ml 的微量離心管
F. 1 rack for microcentrifuge tubes
一個 微量離心管試管架

G. 2 microtitre plates labelled with your country code + A or B
二個 微量滴定盤，上面有註記你的國家碼 +A 或 B
H. 1 microtitre plate template
一個 微量滴定盤位置表
I. 1 stopwatch
一個 計時器
J. 1 pencil
一支 鉛筆
K. 1 marker
一支 簽字筆
L. 1 calculator
一個 計算機
M. 1 ruler
一把 尺
N. Pink card for contact with exam personnel
粉紅色卡紙，與試務人員聯絡用
O. 9 ml 2 M (Molar=mole/Liter) Na_2CO_3 (**Stop**)
9 ml 2M Na~~2~~CO~~3~~ （停止液）
P. 6.5 ml 15 mM (milli-molar, milli=10^{-3}) *p*NP-Gal (**Substrate**)
6.5 ml 15mM pNP-Gal （受質）
Q. 15 ml Ultra pure water (**Water**)
15 ml 超純水（水）
R. 5 ml 1mM *p*NP (**Standard**)
5ml 1mM pNP （標準品）
S. 2 ml 0.024 mg/ml (**Enzyme**)
2 ml 0.024 mg/ml （酵素）
T. 5 ml 0.5 M (**Inhibitor**)
5 ml 0.5 M （抑制劑）
U. One touch pen for the tablet
一支 平板電腦觸控筆

1.1. INTRODUCTION TO ENZYME KINETICS
1.1. 簡介酵素動力學

α-Galactosidases catalyze the hydrolysis of terminal galactosyl residues in α-galactosides. Typically, the activity of these enzymes is assayed using the synthetic substrate analogue *para*-nitrophenyl-α-galactoside (*p*NP-Gal), which is hydrolyzed to galactose (Gal) and *para*-nitrophenyl (*p*NP) (Figure 1.1). *p*NP-Gal is colourless, while the *p*NP product is yellow and its concentration can be measured quantitatively by determining its absorbance A_{405} at 405 nm using a microtitre plate reader.
α-半乳糖苷酶 能催化水解 α-半乳糖苷 上終端的 半乳糖苷 殘基的反應。可以使用合成受質模擬物 para-nitrophenyl-α-galactoside (pNP-Gal) 分析這些酵

素的活性，酵素能將其分解成為 半乳糖 與 para-nitrophenyl (pNP)（圖 1.1)。pNP-Gal 是一無色物質，受分解後的 pNP 會呈現黃色，pNP 在 405 nm 具有一吸收峰。因此，能藉由 微量滴定盤分析儀讀取在 405 nm 下的吸收值，進行酵素定量的測試。

Figure 1.1: *Schematic representation of the galactosidase activity assay: pNP is quantified using a microtitre plate reader (2) that measures the absorbance A at 405 nm. In order to measure enzymatic activity, a standard curve is used to convert absorbance to a product concentration. 1, microtitre plate (Material G).*
圖 *1.1*：*α*-半乳糖苷酶活性測試圖例。*pNP* 可以利用微量滴定盤分析儀 *(2)* 在 *405 nm* 波長下的吸收值進行定量分析。酵素活性測定可以藉由標準曲線的吸收值轉換成為濃度。實驗將在微量滴定盤 *(1)*（材料 *G*）上進行。

In Part 1, the dependency of the rate of hydrolysis on the substrate concentration will be investigated. In order to do so, a Michaelis-Menten plot (Figure 1.2), which describes this relationship is available to allow you to estimate the two important parameters V_{max} and K_m (see legend to Fig. 1.2).
在第一部分中，進行受質濃度與水解速率的研究。並用 Michaelis-Menten 曲線（圖 1.2）來描述兩個重要影響因子 *V*max 與 *K*m 的關係（見圖 1.2 說明）。

The initial reaction rate V_o can be determined from Δ[P]/Δt ,which is the change in product concentration ([P]) per time (Δt).
起始反應速率 V0 可以藉由 Δ[P]/Δt 來獲得。係指單位時間內 (Δt) 產物濃度的變化 ([P])。

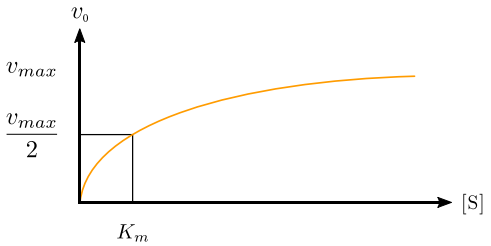

Figure 1.2: *Michaelis-Menten plot: Initial reaction rate V_o versus substrate concentration [S]. K_m is the substrate concentration at which the enzyme operates at half its maximum rate, V_{max}, which reflects the saturation of the enzyme active sites with substrate.*
圖 *1.2: Michaelis-Menten* 曲線，係參考最初反應速率 *V0* 與受質濃度 *[S]* 的圖形。*Km* 是最大酵素反應速率一半時的受質濃度，*Vmax* 則是反映了受質在飽和

狀態下，酵素的反應速率

Using another plot, the Lineweaver-Burk plot, the parameters V_{max} and K_m can be determined from the Y-axis and the X-axis intercepts, respectively (Figure 1.3). A Lineweaver-Burk plot is generated by plotting the inverse of the initial reaction rate ($1/V_o$) against the inverse of substrate concentration (1/[S]).
使用另一種 Lineweaver-Burk 曲線，可以從 X 與 Y 軸得截距算出 ~~Vmax~~ 與 ~~Km~~ 值（圖1.3）。 Lineweaver-Burk 曲線可以由 起始濃度 V0 與 受質濃度 [S] 的 倒數中（1/V0 與 1/[S]）繪得。

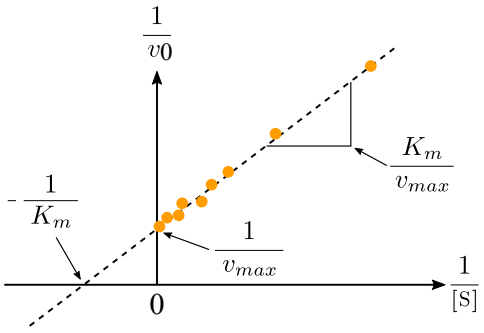

Figure 1.3: *Lineweaver-Burk plot: After determining V_o at different substrate concentrations, a Lineweaver-Burk plot is made. A straight line is fitted to the data to determine K_m from the inverse of the intercept of the line with the X-axis, and V_{max} from the inverse of the intercept of the line with the Y-axis. These intercepts are calculated from the equation of the line.*
圖 *1.3: Lineweaver-Burk* 曲線，可以藉由不同受質濃度下的 ***~~V0~~*** 來製作。***~~Km~~*** 值可以藉由 *X* 軸截距的倒數獲得，***~~Vmax~~*** 值可以藉由 *Y* 軸截距的倒數獲得。這些截距的數值可以經由線性的公式計算後得到。

1.2 ENZYME KINETICS EXPERIMENT OF AN INDUSTRIAL ALPHA-GALACTOSIDASE
1.2 工業用 A-半乳糖苷酶 的酵素動力學實驗

1.2.1 STANDARD CURVE
1.2.1 標準曲線

Begin by generating the standard curve that will be used to measure the product (*p*NP) concentration of the enzymatic reactions later on. To generate the standard curve, you will need to dilute the 1 mM pNP standard stock solution (**Standard**) in the stop reagent (**Stop**).
為了畫出標準曲線，稍後將進行酵素反應產物 pNP 的濃度測定。為了要得到標準曲線，利用（停止液）來稀釋 1mM pNP （標準品）。

Q. 1 Standard curve dilution scheme
標準曲線稀釋計畫

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The 1 mM pNP standard stock solution (Standard) will, when needed, be diluted in the stop reagent (Stop). Calculate the volumes of *p*NP and stop reagent needed to prepare the final standard concentrations in a total volume of 500 µl. Type your calculated values into the table below (Table 1.1).

問題：利用（停止液）來稀釋 **1mM pNP**（標準品），以得不同濃度的標準樣本。請計算標準樣本總體積為 **500 µl** 中 **pNP**（標準品）與（停止液）的體積，並將數據填入下表（表 **1.1**）

Tube label 編號	**St1**	**St2**	**St3**	**St4**	**St5**
[*p*NP] standard (mM) [pNP] 標準樣本濃度	0.2	0.4	0.6	0.8	1
Volume of standard stock solution (Standard) (µl) （標準品）體積 (µl)					
Volume of stop reagent (Stop) (µl) （停止液）體積 (µl)					

Preparation of the standard curve
Protocol
標準曲線製備步驟

a. With your marker, label five 1.5 ml microcentrifuge tubes according to the first row in Table 1.1: from St1 to St5.
取 5 支 1.5 ml 微量離心管，利用簽字筆參照表 1.1，分別標記上 St1 到 St5。

b. Transfer the different volumes of the 1 mM *p*NP standard solution (**Standard**) to the labelled 1.5 ml tubes according to your calculations in Table 1.1 (**use the same pipette tip**).
參照 表 1.1 的計算結果，分別將不同體積的 1 mM pNP（標準品）加到

上述的 1.5 ml 微量離心管（使用同一支吸管尖）中。

c. Transfer the different volumes of the stop solution (**Stop**) to the labelled tubes according to your calculations in Table 1.1. The standard solutions are mixed thoroughly by turning the microcentrifuge tubes upside-down 5 times.
參照 表 1.1 的計算結果，分別將不同體積的（停止液）加到上述的 1.5 ml 微量離心管中。請將這些標準液樣本的微量離心管蓋好，上下搖盪混合 5 次。

d. Transfer 100 µL ultra pure water (**Water**) into wells A1-A5 and B1-B5 of microtitre plate A (**use the same pipette tip**, see Fig. 1.4 and/or use the microtitre plate template to help you pipette in the correct wells).
分別取 100 µL 超純水（水）加到 微量滴定盤A 上 A1-B5 的位置（使用同一支吸管尖），參考 圖 1.4 或利用 微量滴定盤位置表 協助你添加到正確的位置。

e. Transfer 50 µL of each of the final *p*NP diluted standard solutions (Table 1.1) into the same microtitre plate wells**.** Each solution is pipetted in duplicates in two different wells (The subscripts I and II designate replicates of the same solution, Fig. 1.4).
參照 表 1.1 的計算結果，分別將 50 µL 不同濃度的 pNP 標準液樣本加到同一個微量滴定盤的孔洞中。（下標 I 與 II 係代表相同的溶液，但是進行重複實驗，見圖 1.4）

f. Add 100 µL stop reagent (**Stop**) using a p1000 pipette to each *p*NP standard, A1-A5 and B1-B5. Mix thoroughly by pipetting the mixture up and down two times.
利用 p1000 微量吸管吸取 100 µL（停止液）到 A1-A5 與 B1-B5 的每個 pNP 標準液樣本孔洞中。並利用上下吹吸溶液的方式進行混合，計兩次。

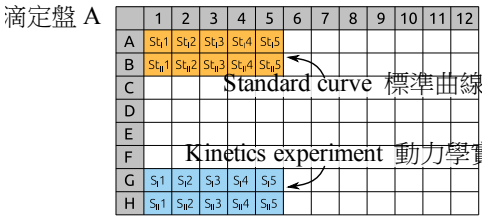

Figure 1.4: *Microtitre plate A: St, standards (see Table 1.1); S, reaction mixtures with different substrate concentrations (see Table 1.2 below).*
圖 *1.4*：微量滴定盤 *A*：*St*, 標準液樣本（表 *1.1*）；*S*, 不同受質濃度的反應混合物。（下表 *1.2*）

Proceed now with part 1.2.2, where you will set-up the enzymatic reaction mixtures to your microtiter plates.
進行 1.2.2 部分。至此，你已經完成酵素溶液混合物在微量滴定盤中。

Important note: the assistants will not accept any microtitre plates in the last

10 minutes of the exam. If you feel that you will not be able to complete part 1.2.2 in time, hand in your plate now by raising the pink card. You results will be shown in Question 2.
請注意，在考試結束前 10 分鐘將 不接受 任何的微滴定盤量量測。開始進行以下實驗，若你覺得無法及時完成實驗 1.2.2時，可舉起你的粉紅色卡片 ，並提交滴定盤。結果將會顯示在 問題 2 中。

1.2.2 ENZYME KINETICS EXPERIMENT
1.2.2 酵素動力學實驗

Protocol 步驟
Prepare the *p*NP-Gal substrate diluted solutions for the kinetics experiment.
製備 pNP-Gal 受質稀釋溶液，以利酵素動力學實驗進行

a. Label five 1.5 mL tubes with a marker with S1 through to S5 (Table 1.2).
取 5 支 1.5 ml 微量離心管，利用簽字筆參照表 1.2，分別標記上 S1 到 S5。

b. The 15 mM *p*NP-Gal substrate stock solution (**Substrate**) is diluted with ultra pure water (**Water**) in the labelled 1.5 ml tubes (see Table 1.2 below). The diluted solutions should be mixed thoroughly by turning the tubes upside-down 5 times.
參照下表 1.2 的說明，在 1.5 ml 微量離心管進行操作。將 15 mM pNP-Gal（受質）以超純水進行稀釋。製備好的受質稀釋溶液後，將微量離心管蓋好並上下搖盪混合 5 次。

Table 1.2: *Substrate dilution scheme for the kinetics assay.*
表 *1.2*：動力分析實驗所使用的受質溶液稀釋表。

Tube label 編號	**S1**	**S2**	**S3**	**S4**	**S5**
Volume (µl) of 15 mM *p*NP-Gal **(Substrate)** 15 mM pNP-Gal（受質）體積 (µl)	40	120	240	400	800
Volume (µl) of ultra pure water **(Water)** 超純水（水）體積 (µl)	960	880	760	600	200

c. Transfer 50 µL of each diluted substrate solution (Table 1.2) and 50 µL ultra pure water (**Water**) into microtitre plate A, wells G1-G5 and H1-H5. (see Figure 1.4 and/or the microtitre plate template).

參考表 1.2，分別取 50 µL 受質稀釋溶液 與 50 µL 超純水（水）加到 微量滴定盤A 上 G1-G5 與 H1-H5 的孔洞位置（參考 圖 1.4 或利用 微量滴定盤位置表 協助你添加到正確的位置）。

d. Set the timer at 5 minutes and start it immediately after you pipetted the enzyme solution to the first well to start the first enzymatic reaction (S_I1) as described below.
將計時器設定在 5 分鐘，當添加酵素溶液到第一個孔洞 (SI1) 時，便立即按下計時器。請參照以下說明進行。

e. Pipette 50 µL of the 0.024 mg/ml α-galactosidase enzyme (Enzyme) into wells G1-G5 and H1-H5 starting with S_I1 and $S_{II}1$, and continue in the same order and tempo throughout to $S_{II}5$ to start the enzymatic reactions in each well (hereafter referred to as the "enzymatic reaction mixture"). Ensure good mixing by quickly but gently pipetting 50 µl of the mixture up and down two times in each well.
分別吸取 50 µL 的 0.024 mg/ml α-半乳糖苷酶（酵素）到微量滴定盤 A 上 G1-G5 與 H1-H5 的孔洞位置，也就是 SI1 到 SII1，再以相似的次序與節奏，分別加到 SII5。至此開始酵素反應（以下稱為酵素反應溶液）。操作過中，必須確認混合是否均勻，可以吸取 50 µl 的混合液，利用上下吹吸溶液的方式進行混合，計兩次。

f. After 5 minutes incubation time add 100 µL 2 M Na_2CO_3 solution (**Stop**) using a p1000 pipette to stop each of the enzymatic reactions in wells G1-G5 and H1-H5 in the same order and tempo as you started them. Mix well by pipetting the mixture up and down two times.
經過 5 分鐘的作用時間後，利用 p1000 微量吸管 吸取 100 µL 2 M Na2CO3（停止液），分別以相似的次序與節奏加入 G1-G5 與 H1-H5 的孔洞中。利用上下吹吸溶液的方式進行混合，計兩次。

Q. 2 enzyme kinetics experiment
酵素動力學實驗

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Hand in your microtitre plate containing your samples from parts 1.2.1 and 1.2.2 by raising your pink card. After measurement, the obtained values will be display in the table below automatically.
問題：
當完成 **1.2.1** 與 **1.2.2** 的實驗後，請舉起你的粉紅色卡片 ，拿好你的微量滴定盤 請助教進行測量。結果將會自動顯示於平板的表中。

<u>Note:</u> no microtiter plates will be accepted in the last 10 minutes of the exam!
請注意：助教將不會在考試結束前 **10** 分鐘接受任何的微量滴定盤測量。

	1	2	3	4	5	6	7	8	9	10	11	12

A												
B												
C												
D												
E												
F												
G												
H												

1.3 DATA ANALYSIS OF ENZYME KINETICS
1.3 酵素動力學數據分析

Your task is now to determine the kinetics parameters of substrate hydrolysis by the *α*-galactosidase.
First the standard curve linear function for the product (*p*NP) should be determined using data from table 1.3
你的問題將由 α-半乳糖苷酶水解受質的動力學參數來決定。首先利用 表 1.3 進行產物 (pNP) 的標準曲線繪製。

Access to a standard curve enables you to calculate product concentrations in the reaction mixtures, which further allows the determination of the initial reaction rate (V_0) of the enzyme for each substrate concentration.
The standard data set resembling microtitre plate A and shown below (Table 1.3) should be used for the calculation. This will avoid error carry-over penalty from part 1.2. However, your own data will be measured and used in the evaluation of your exam.
利用標準曲線計算產物濃度，進一步將可以利用每個受質濃度獲得 起始反應速率 (V0)。有組類似的微量滴定盤 A 的標準數據在表 1.3，可以方便計算。這是用於避免在 1.2 部分發生錯誤使用。然而，你 親自 做出來的數據才會用於計算結果並評估你的考試成績。

Table 1.3: *Provided absorbance data for calculations (columns 1-5 in microtiter plate format).*
表 *1.3*：提供的吸光值數據（*1-5* 行的排列仿微量滴定盤）

	1	2	3	4	5
A	0.882	1.681	2.473	3.251	3.964
B	0.858	1.657	2.449	3.227	3.940

B	0.656	1.657	2.449	3.227	3.940
⋮					
G	0.304	0.728	1.049	1.272	1.512
H	0.307	0.716	1.009	1.234	1.466

Q. 3 Mean Absorbance of standards
標準溶液的平均吸光值

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Calculate the mean absorbance for each duplicate measurement for the standard curve given in Table 1.3. Enter all answers with three digits after the decimal point.
計算表**1.3** 的平均值，並用於標準曲線的繪製。將答案填入表中，到小數點後三位。

Tube label 編號	**St1**	**St2**	**St3**	**St4**	**St5**
[*p*NP] (mM)	0.2	0.4	0.6	0.8	1
Mean A_{405} nm of duplicates 重複吸光值的平均值					

Q. 4 standard curve linear function
標準曲線線性公式

In the figure below (Figure 1.5), the concentration of *p*NP (mM) is plotted against the absorbance (Mean A_{405} nm calculated in Question 3).
如下圖（圖 1.5），以 pNP (mM) 濃度 對 吸光值（A405 nm 平均值計算，如問題 4）

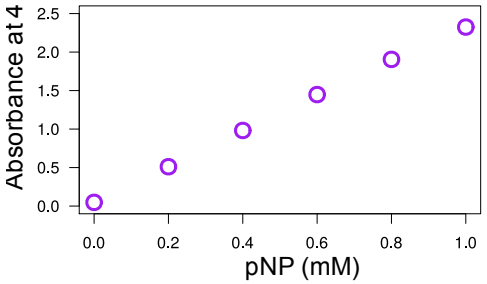


Figure 1.5: *Hypothetical pNP product standard curve. The purple circles represent means of measured absorbances and the black dashed line is a linear regression to them.*
圖 *1.5: pNP* 產物標準曲線。紫色圓圈代表平均吸光值，黑色虛線代表線性回歸結果。

Node Id: **1490bf7874a519200e9b4847**

Determine a and b of the standard curve linear function (see below) mathematically using only the mean absorbances of the two data points St1 and St5. Give a and b with three digits after the decimal point.:
問題：請利用兩組 **St1** 到 **St5** 的數值，利用數學方法計算標準吸光值線性公式中，**a** 與 **b** 的值。到小數點以下第三位。

A_{405} (absorbance units at 405 nm) = a·[*p*NP] (mM) + b, where a is the slope and b is the Y-axis intercept
A405（波長 **405 nm** 的吸光值單位） **= a [pNP] + b**，**a** 是斜率，**b** 是截距。

a (A_{405}/mM)	
b (A_{405})	

The volume of the enzymatic reaction mixture from the experiment in Part 1.2.2 is 150 µl.
酵素反應混合物體積在實驗 1.2.2 部分為 150 µl。

Q. 5 Reaction Time
反應時間

Node Id: **4a094717ade59a3249c9b494**

Convert the reaction time into seconds
問題：將反應時間單位轉換為秒.

Reaction time (seconds) 反應時間（秒）	

Q. 6 Analysis of kinetics data (uninhibited enzyme)
分析動力學數據（未抑制酵素）

Node Id: **8af8b696e90b22d7e2054ab8**

Use the following standard curve equation to calculate the product concentration for each reaction mixture:
問題：利用下列標準曲線方程式分別計算每個反應混合物的產物濃度

A_{405} absorbance = 2.29*[*p*NP] (mM) + 0.058.

The initial reaction rate V_0 can be determined from Δ[Product]/Δtime, i.e. the change in product concentration per time. Give all numbers with three digits after the decimal point.
反應初始濃度 **V0** 可以被 **Δ[P]/Δt** 決定。即，單位時間內 **(Δt)** 所改變的產物濃度 **[P])**。

Tube label 編號	**S1**	**S2**	**S3**	**S4**	**S5**
Volume of Stock solution (Substrate) (µl) (from table 1.2) （受質）體積 (µl)（來自表 1.2）	40	120	240	400	80
Volume of ultra pure water (Water) (µl) (from table 1.2) 超純水（水）體積 (µl)（來自表 1.2）	960	880	760	600	20
Substrate concentration [S] prior to adding into the reaction mixture (mM) 製備前受質濃度 [S] (mM)					
Substrate concentration [S] in reaction mixture (mM) 製備後受質濃度 [S] (mM)					
Mean A_{405} absorbance, calculated from Table 1.3 來自表 1.3 的 405 nm					

平均吸光值					
[Product$_{mean}$] (mM) 產物平均濃度 (mM)					
V_0 (µM/second)					
1/[S] (1/mM)					
1/V_0 (second/µM)					

Q. 7 Michaelis-Menten PARAMETERS (graphical estimate)
Michaelis-Menten 參數（圖形估測）

Shown below (Figure 1.6) is a theoretical Michaelis Menten plot (V_0 versus [S]) resembling the reaction mixtures S1–S5 in Table 1.3.
下圖（圖 1.6）為 Michaelis Menten 的理論圖（V0 與 [S]）雷同於表 1.3 的 S1-S5。

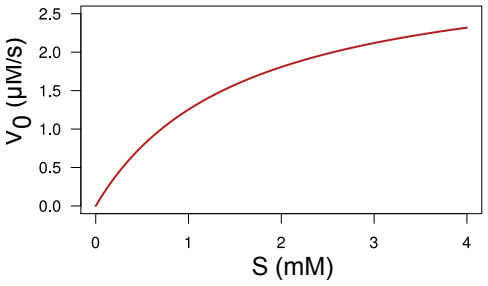


Figure 1.6: *Theoretical Michaelis-Menten plot for the kinetics experiment in the absence of inhibitor*
圖 *1.6 Michaelis Menten* 的理論圖。為缺乏抑制劑的動力學實驗。

Node Id: **cda3a6b0f57d7c91bc8e7f55**

Estimate V_{max} and K_m graphically from the Michaelis-Menten plot (Fig. 1.6). Give answers with one digit after the decimal point.
問題：利用 **Michaelis-Menten** 圖形（圖 **1.6**），推估 **Vmax** 與 **Km** 的值。請計算到小數點後一位。

V_{max} (µM/s)	
K_m (mM)	

Q. 8 Lineweaver-Burk linear function
Lineweaver-Burk 線性公式

Shown below (Figure 1.7) is the Lineweaver-Burk plot ($1/V_0$ versus 1/[S]) of the S1–S5 data point in Table 1.3.
下圖（圖 1.7）為利用表 1.3 S1-S5 所做出的 Lineweaver-Burk 圖形（1/V0 與 1/[S]）。

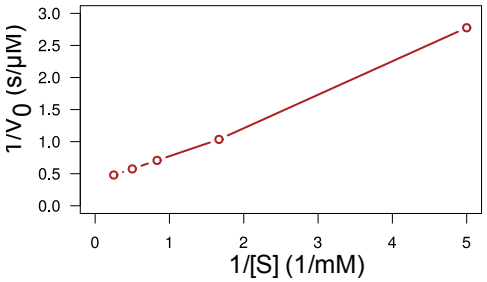


圖 *1.7*：無抑制型酵素數據的 *Lineweaver-Burk* 圖。

Node Id: **abe02ebea0fb571a968e4799**

Determine the linear function of in the Lineweaver-Burk plot (Figure 1.7) in the form shown below mathematically from the two data points for S1 and S5. Give a and b with three digit after the decimal point.
問題：請利用兩組 **S1** 到 **S5** 的數值，利用數學方法計算 **Lineweaver-Burk** 圖型的線性公式中，**a** 與 **b** 的值。到小數點以下第三位。

$1/[V_o] = a \cdot 1/[S] + b$,

a (mM·s/μM)	
b (s/μM)	

Q. 9 determination of Vmax and Km
Vmax 與 Km 值的計算

Node Id: **8ffaae0fa99b746314cfe790**

Using the linear function calculated above (Q. 8), determine K_m and V_{max} mathematically from the intercepts with the axes. Give numbers with three digits after the decimal point (no unit conversions should be done).
問題：以軸的截距計算上述（問題 **8**）的線性公式，並進行 **Km** 與 **Vmax** 的計算。到小數點以下第三位。（本題無單位）

V_{max}	
K_m	

Q. 10 Enzyme concentration in reaction mixture
反應混合物中的酵素濃度

Node Id: **02df7e4734e45ebc25743e41**

Calculate the enzyme concentration in the reaction mixture in µM from the enzyme stock concentration= 0.024 mg/ml and the enzyme's molar mass (75 000 gram/mole). Give the concentration with three digits after the decimal point.
計算酵素濃度（單位 **µM**），酵素濃度為 **0.024 mg/m**，酵素分子量為 **75 000 gram/mole**，到小數點以下第三位。

Enzyme stock (mg/ml)	0.024
[E] (µM) in reaction mixture (micro=10^{-6})	

Q. 11 Turnover rate constant
轉換率常數

The catalytic turnover rate constant k_{cat} (reaction rate of 1 enzyme molecule) has the unit 1/second and is calculated as follows:
請以以下公式計算催化轉換常數 kcal（一個酵素分子的反應速率），單位為單位／秒

$$k_{cat} = \frac{v_{max}}{[\mathrm{E}]}$$

Node Id: **e0caf4524c392ac095d6f406**

Determine k_{cat}. Give number with three digits after the decimal point.
計算 **kcat**，到小數點以下第三位。

k_{cat}(1/second)	

2.1 INTRODUCTION TO INHIBITORS
2.1 抑制劑的介紹

Inhibitors are compounds that can specifically bind to enzymes, thereby reducing their activity and resulting in apparent changes in either K_m, V_{max} or both. Change in apparent kinetic parameters can be determined from the Lineweaver–Burk plot of an enzymatic reaction performed in the presence of an inhibitor. Reversible inhibitors can be competitive, non-competitive, or uncompetitive, depending on the mode of binding to their enzyme targets.
抑制劑是能專一性結合酵素因而降低其活性的化合物，會改變反應之 Km、Vmax 或二者。這些表觀動力參數的改變，可藉由酵素反應在抑制劑存在下的 Lineweaver-Burk 作圖決定。可逆性的抑制劑可能是競爭性、非競爭性、或無競爭性，決定於它與目標酵素結合的方式。

The inhibition of enzyme activity and apparent change in kinetic parameters can also be visualised in Michaelis-Menten and Lineweaver-Burk plots (Fig. 2.1).
酵素活性的抑制及動力參數的改變可由 Michaelis-Menten 及 Lineweaver-Burk 作圖看出（圖2.1）

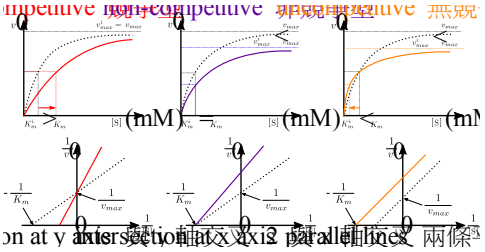


Figure 2.1: *Inhibition of enzyme activity in Michaelis-Menten and Lineweaver-Burk plots. Dashed black curves are without an inhibitor and solid curves in the presence of inhibitor. v_0 is initial reaction rate.*
圖*2.1 Michaelis-Menten* 及 *Lineweaver-Burk* 作圖與酵素活性的抑制。黑色的虛線為無抑制劑，實線為有抑制劑。*V0* 為起始反應速率。

Inhibitors are characterized by their inhibition equilibrium constant K_i, defined as
抑制劑的特徵可以由各自的抑制平衡常數 Ki 來決定，其定義如下：

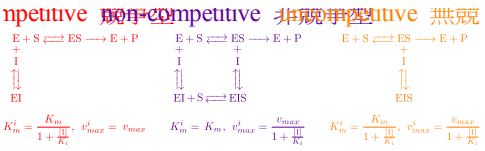
$$K_i = \frac{[\mathrm{I}]\,[\mathrm{E}]}{[\mathrm{EI}]}$$

Where [I], [E] and [EI] are the concentrations of the free inhibitor, free enzyme and enzyme-inhibitor complex, respectively.
此處 [I]、[E] 及 [EI] 分別代表游離抑制劑、游離酵素及酵素－抑制劑複體的濃度。

For **competitive inhibition**, the apparent K_m in the presence of inhibitor is designated as K_m^i. The chemical equilibrium for substrate (S) and inhibitor (I) binding to the enzyme (E) is shown below. K_m and K_m^i are related according to the equation below:
對競爭性抑制而言，抑制劑存在時的表觀 Km 被定為 Kmi。受質 (S) 和抑制劑 (I) 結合到酵素 (E) 的化學平衡式如下所示。Km 與 Kmi 的相關性根據下面的公式：

For **non-competitive inhibition**, the apparent V_{max} in the presence of inhibitor is designated as V_{max}^i. V_{max}^i and V_{max} are related according to the equation below:
對非競爭性抑制而言，抑制劑存在時的表觀Vmax被定義為與Vmaxi。Vmaxi與Vmax的相關性根據下面的公式：

For **uncompetitive inhibition**, the apparent K_m and V_{max} in the presence of inhibitor are designated as K_m^i and V_{max}^i, respectively. K_m and V_{max} are related to K_m^i and V_{max}^i according to the equations below:
對無競爭性抑制而言，抑制劑存在時的表觀K_m及V_{max}被定義為與$K_m{}^i$與及$V_{max}{}^i$的相關性根據下面的公式：



Equation 2.1: *The chemical equilibria for substrate (S) and inhibitor (I) binding to the enzyme (E) is shown in the top part of the figure for different inhibition types. The lower part shows the equations relating the change in apparent kinetic parameters to the inhibitor concentration and to the inhibition equilibrium constant.*
公式*2.1*：受質 *(S)* 和抑制劑 *(I)* 結合到到酵素 *(E)* 的化學平衡式，圖形上半部顯示在不同類型抑制圖。下半部分則顯示表觀動力參數變化與抑制劑濃度和抑制常數平衡方程式的關係。

Q. 12

factors affecting inhibition
影響抑制的因素

Node Id: **e73dd9526a3687dcfece83b2**

For all inhibition types, the degree of inhibition, *i.e.* reduction in enzymatic reaction rate, is dependent on: (choose the best of the answers below).
問題：對於所有的抑制類型，抑制的程度(即酵素反應速度的降低)是依賴於下列何者：(選擇下面最好的答案)。

1. INHIBITOR CONCENTRATION [I]
抑制劑的濃度 [I]

2. SUBSTRATE CONCENTRATION [S]
受質濃度 [S]

3. K_I OF THE INHIBITOR
抑制劑的KI

4. CONCENTRATION OF [ES]
[ES] 的濃度

5. STATEMENTS 1, 2 AND 3
敘述1, 2 及 3

6. STATEMENTS 1 AND 3
敘述1 及 3

Q. 13 Competitive Inhibition signature
競爭性抑制特徵

Node Id: **895c70dd92f56ff77a0f683c**

Indicate if the following statement is true or false
問題：指出下列的敘述正確或錯誤

TRUE 正確 FALSE 錯誤

In competitive inhibition, the increase in substrate

concentration [S] reduces or overcomes inhibition.
在競爭性抑制作用，受質濃度 [S] 增加時會減少或克服抑制效果

2.2. INHIBITION OF ALPHA-GALACTOSIDASE (27 POINTS)
2.2. ALPHA-半乳糖苷酶的抑制 (27 分)

This part is experimentally similar to Part 1b. An inhibition kinetics experiment of α-galactosidase will be conducted in the presence of 50 µL inhibitor, which has a concentration of 0.5 M (mole/Liter).
這部分實驗與Part 1b相似。在50 µL抑制劑存在下進行α-半乳糖苷酶抑制動力學實驗，其濃度為 0.5 M (mole/Liter)。

Protocol 步驟
Substrate preparation for inhibition kinetics experiment
抑制動力學實驗受質的製備

a. Prepare the substrate solutions according to Table 2.1, similarly to what you have done in Part 1.2.2. Remember to mix the solutions by turning the tubes upside down 5 times.
根據表 2.1 製備受質溶液，與你做的 1.2.2 部分相似。記住要左右顛倒轉動試管 5 次混合溶液。

Table 2.1: *Substrate dilution scheme for kinetic assay.*
表 *2.1*：動力學分析的受質稀釋計畫

Tube label 編號	**IS1**	**IS2**	**IS3**	**IS4**	**IS5**
Volume (µl) of substrate stock solution (Substrate) （受質）體積 **(µl)**	80	160	320	600	840
Volume (µl) of ultra pure water (Water) 超純水（水）體積 **(µl)**	920	840	680	400	160

b. Transfer 50 µL inhibitor (**Inhibitor**) into the microtitre plate B wells A1-A5 and B1-B5 using the same pipette tip. (See Figure 2.2 and/or the microtitre plate template).

使用相同的吸管尖，在微量滴定盤 B 的A1到A5和B1到B5各孔洞中加入 50 µL 的（抑制劑）。（參考 圖 2.2 或利用 微量滴定盤位置表 協助添加到正確的位置）。

c. Transfer 50 µL of each final substrate solution (Table 2.1) to the same well positions (A1-A5 and B1-B5).
將每個最後受質溶液 50 µL 加入到相同孔洞位置（A1-A5 和 B1-B5）中（表 2.1）。

滴定盤 B

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS_I1	IS_I2	IS_I3	IS_I4	IS_I5							
B	$IS_{II}1$	$IS_{II}2$	$IS_{II}3$	$IS_{II}4$	$IS_{II}5$							
C												
D												
E												
F												
G												
H												

Figure 2.2: *Microtitre plate B: IS, samples are reaction mixtures in the presence of inhibitor at different substrate concentration (see Table 2.1 above).*
圖*2.2*：微量滴定盤*B*：*IS*為樣本為在不同受質濃度的抑制劑存在下的反應混合物（見上述的表 *2.1*）。

d. Set the timer at 5 minutes and start it immediately after you start the first enzymatic reaction by adding the enzyme solution to the first well (IS_I1) as described below.
將計時器設定在 5 分鐘，當添加酵素溶液到第一個孔洞 (ISI1) 時，便立即按下計時器。請參照以下說明進行。

e. Pipette 50 µL of the α-galactosidase (**Enzyme**) into the wells A1-A5 and B1-B5, starting with IS_I1 and $IS_{II}1$, and continue in the same order and tempo throughout to $IS_{II}5$ to start the enzymatic reaction in each well.
分別吸取 50 µL 的 α-半乳糖苷酶（酵素）到微量滴定盤 A 上 A1 到 A5 和 B1 到 B5 的孔洞位置，也就是 ISI1 到 ISII1，再以相似的次序與節奏，分別加到 ISII5。至此開始酵素反應。

Ensure good mixing by quickly but gently pipetting 50 µl of the mixture up and down two times in each well immediately after you pippette the enzyme.
加入酵素後，立即將 50 µl 的混合物以快速但溫柔的上下吹吸兩次，以確保均勻的混合。

f. After 5 minutes incubation, add 100 µL stop reagent (**Stop**), using a p1000 pipette, to stop each of the enzymatic reactions in the wells A1-A5 and B1-B5 in the same order and tempo as you started them.
經過 5 分鐘的作用時間後，利用 p1000 微量吸管 吸取 100 µL（停止液）停止各酵素反應，分別以相似的次序與節奏加入A1 到 A5 和 B1到 B5 的孔洞中。利用上下吹吸溶液的方式進行混合，計兩次。

Mix thoroughly by pipetting the mixture up and down two times immediately after you pipette the stop solution.

在你加入停止試劑後，立即上下吹吸溶液兩次以將其混合均勻。

Q. 14 ENZYME inhibition KINETICS EXPERIMENT
酶抑制動力學實驗

Node Id: **426d12c4f9e170efdd86247a**

Hand in your microtitre plate containing your samples from part 2.2 by raising your pink card. After measurement, the obtained values will be displayed in the table below automatically. Please use the standard data given below (Table 2.2) for your calculations
問題：舉起你的粉紅色卡片，繳交含有你 **2.2** 部分樣品的微量滴定盤。測量過後，所得的數值將自動顯示在平板的表中。請使用下表所給的標準資料（表 **2.2**）計算。

Note: no microtitre plates will be accepted in the last 10 minutes of the exam!
注意：考試的最後**10** 分鐘內將不再接受微量滴定盤！

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

2.3. DATA ANALYSIS OF INHIBITION KINETICS OF ALPHA-GALACTOSIDASE
2.3 有關 A-半乳糖苷酶 的抑制動力學數據分析

In this section you will utilize the theory of Part 2.1 and the supplied inhibition data from Part 2.2 (see Table 2.2 below) to calculate enzyme kinetic parameters in the presence of inhibitor.
本小節將利用理論部分 2.1 與來自實驗部分 2.2 的抑制數據（見下表 2.2）計算在添加抑制劑下酵素動力學的參數。

The Lineweaver-Burk equation for the inhibited data will be compared to the supplied hypothetical Lineweaver-Burk equation for the uninhibited reaction to deduce the type of inhibiton.
Lineweaver-Burk 公式的抑制數據將與題目所提供的假設無抑制反應下演繹之抑制類型的理論 Lineweaver-Burk 公式進行比較。

When you identify the type of inhibition, you will use these two Lineweaver-Burk equations (supplied hypothetical uninhibited and in the presence of inhibitor) to determine the change in the relevant kinetic parameters and to use the relevant equation to determine the inhibition equilibrium constant (K_i).
當要確定抑制類型時，你將會使用兩個 Lineweaver-Burk 公式（假設的環境為無抑制並添加抑制劑）進行動力參數以及抑制平衡參數 (Ki) 的測定。

Table 2.2: *Provided absorbance data for inhibition experiment (the first two rows and columns 1-5 in microtiter plate format).*
表 *2.2*：抑制實驗的吸光值數據（前兩行與列雷同於微量滴定盤上的數據）

	1	2	3	4	5
A	0.251	0.375	0.507	0.596	0.634
B	0.252	0.380	0.501	0.598	0.635

Q. 15 Analysis of Inhibition kinetics Data
抑制動力學數據分析

Node Id: **fc546fd4d7128cbd27680699**

Calculate and fill in the table below. In order to calculate product concentrations in mM, use the standard equation given in Q6:
問題：計算並填入下表。為了計算產物濃度 **(mM)**，請使用問題 **6** 的標準公式。

Absorbance A_{405} = 2.29 * [*p*NP] (mM) + 0.058

Tube label 編號	IS1	IS2	IS3	IS4	IS5
Volume (µl) of Stock solution (Subtrate) (from table 2.1) （受質）體積 (µl)（表 2.1）	80	160	320	600	840
Volume of (µl)					

ultra pure water (Water) (from Table 2.1) 超純水（水）體積 (µl)（表 2.1）	920	840	680	400	160
Substrate concentration [S] prior to adding into the reaction mixture (mM) 調製前受質濃度 [S](mM)					
Substrate concentration [S] in reaction mixture (mM) 調製後受質濃度 [S](mM)					
Mean A_{405} absorbance from table 2.2 A405 平均吸光值（表 2.2）					
[$Product_{mean}$] (mM) 產物平均濃度 (mM)					
V_0 (µM/second)					
1/[S] (1/mM)					
1/V_0 (second/µM)					

A Lineweaver-Burk plot is produced based on the inhibition kinetics data IS1-IS5 in table 2.2. The hypothetical Lineweaver-Burk equation of the uninhibited reaction is: **1/[*Vo*] = 0.363·1/[S] + 0.908**, and this line is plotted in Fig. 2.3. This supplied equation should be used for the calculations below, and **NOT the equation you have determined in part 1.3**

(Fig. 1.7).
利用 表 2.2 中 IS1-IS5 的數據進行 Lineweaver-Burk 圖形的繪製。未抑制反應下的假設 Lineweaver-Burk 公式為 1/[Vo] = 0.363·1/[S] + 0.908，圖形請見 圖 2.3。所提供的公式僅供以下計算使用，並 不能 作為 題目部分 1.3 的測定（圖 1.7）。

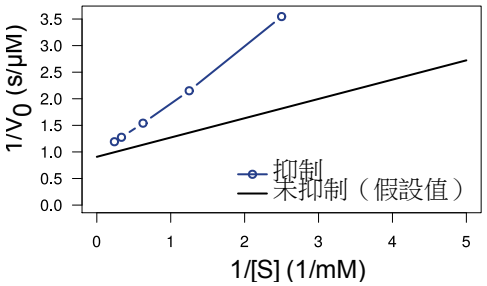


圖 *2.3 Lineweaver-Burk* 圖形，抑制數據與假設抑制數據。

Q. 16 Lineweaver-Burk linear function (inhibited reaction)
Lineweaver-Burk 線性公式（抑制反應）

Node Id: **9cdfa5cfc4afcaa4b2b2537b**

Determine the linear function of the Lineweaver-Burk plot (Fig. 2.3) in the presence of inhibitor in the form shown below mathematically using only the data from IS1 and IS5. Give a and b with three digits after the decimal point.
問題：利用 **IS1** 到 **IS5** 的數據，有抑制劑存在的條件下，以數學方式計算出 **Lineweaver-Burk** 圖形的線性方程式。求出 **a** 與 **b** 的值，到小數點後第三位。

1/[*Vo*] = a·1/[S] + b,

a (mM·s/μM)	
b (s/μM)	

Q. 17 apparent Kinetic Parameters with inhibitor
添加抑制劑的表觀動力學參數

Node Id: **3b5a63b8ad2e148c37a696e5**

Determine the apparent kinetic parameters in the presence of inhibitor from the Lineweaver-Burk plot of the inhibited reaction. Give the parameters with three digits after the decimal point (no unit conversions should be done in this calculation).
問題：自 **Lineweaver-Burk** 圖形中測量添加抑制劑的表觀動力學參數。請

到小數點後三位。（本題無單位）

V_{max}^{i}		
K_m^{i}		

Q. 18 Type of Inhibition
抑制的類型

Node Id: **965ea5209993e7cab506a43a**

What type of inhibition does the inhibitor exert on the α-galactosidase? Choose the most likely type of inhibition based on the size of changes in kinetic parameters in the presence of the inhibitor as compared to the hypothetical data for the uninhibited enzyme

問題：有關 α-半乳糖苷酶 抑制劑屬於何種類型？基於有抑制劑參與時動力參數的改變，並與未被抑制酵素的理論值進行比較，選出最類似的抑制類別。

1. COMPETITIVE 競爭型
2. NON-COMPETITIVE 非競爭型
3. UNCOMPETITIVE 無競爭型

Q. 19 Effect of Substrate concentration
受質濃度的影響

Node Id: **cdfb4878a0cb0206b185bf18**

Based on the type of inhibition you have chosen above, how would an increase in substrate concentration influence inhibition? Choose one of the statements below.

問題：基於上述所選擇的抑制類型，當受質濃度增加時，對於抑制效果的影響為何？請自以下敘述中選出正確答案。

1. LESS INHIBITION 輕微抑制
2. NO CHANGE 沒有改變

3.	MORE INHIBITION 嚴重抑制

Q. 20 Inhibition Constant
抑制常數

Node Id: **d9e44291cbc8ad927f078e05**

Determine the inhibition constant (K_i) if the concentration of the 50 µL inhibitor added to the reaction mixture is 0.5 M. Give numbers with three digits after the decimal point (no unit conversions should be done in this calculation).

問題：測定抑制常數 **(Ki)**，假設抑制劑體積為 **50 µL** 並加入反應混合物為 **0.5M**。請到小數點後三位。（本題無單位）

Inhibitor concentration in the reaction mixture (mM) 反應混合物的起始濃度 (mM)	
K_i (mM)	

END
結束