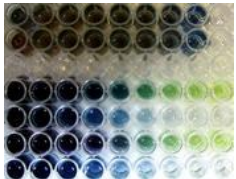


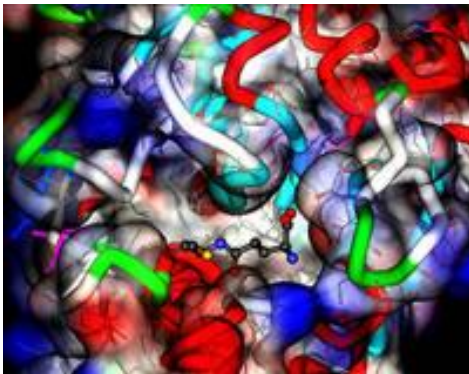
# 酵素分析方法 Enzyme Analysis (EA)

- 5 蛋白質定量 Protein Determination
- 6 酵素活性分析 Enzyme Activity Assay
- 7 電泳檢定法 Electrophoresis
- 8 蛋白質科技 Protein Technology

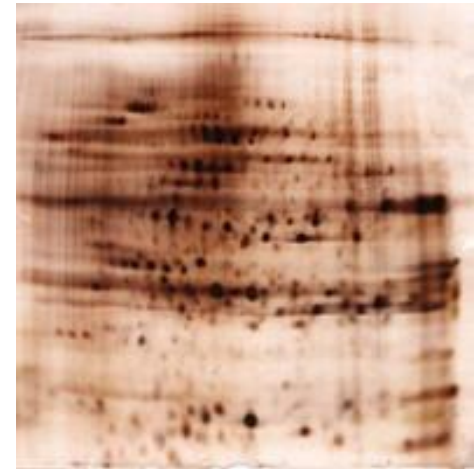
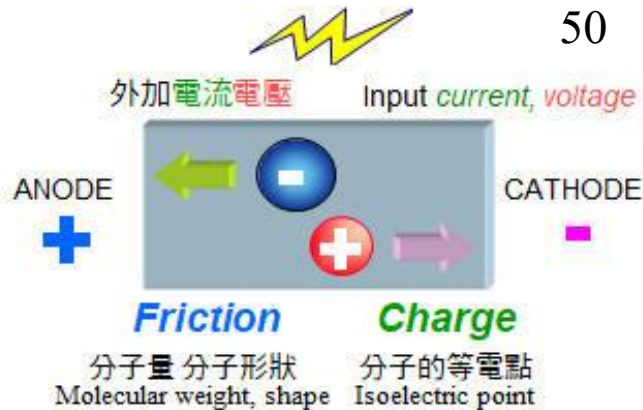
11



43



50



# 5 蛋白質定量 Protein determination methods

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## 5.1 Biuret method (雙縮脲反應)

最古老的方法

## 5.2 Lowry method (JBC, 1951)

30 萬 citations

## 5.3 UV absorbance (206 nm, 280 nm)

最方便的方法

## 5.4 Coomassie Blue (Bradford) method

目前最常用的方法

## 5.5 Other methods (特殊基團、重金屬、**活性**)

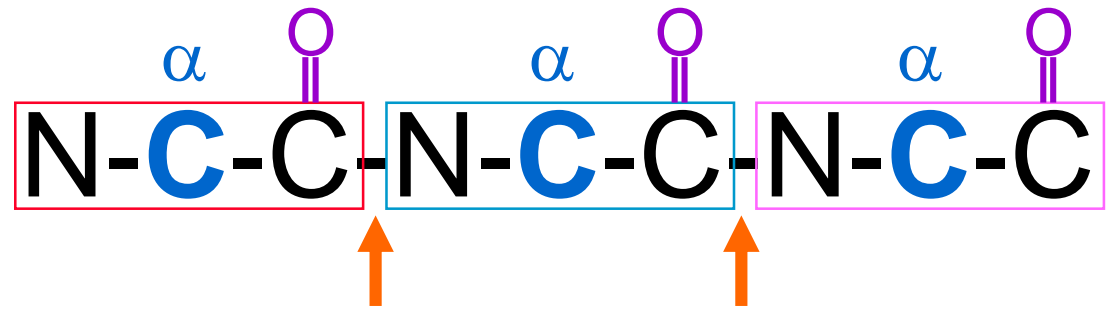
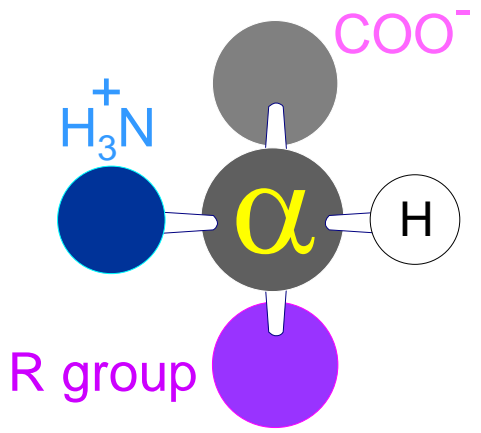
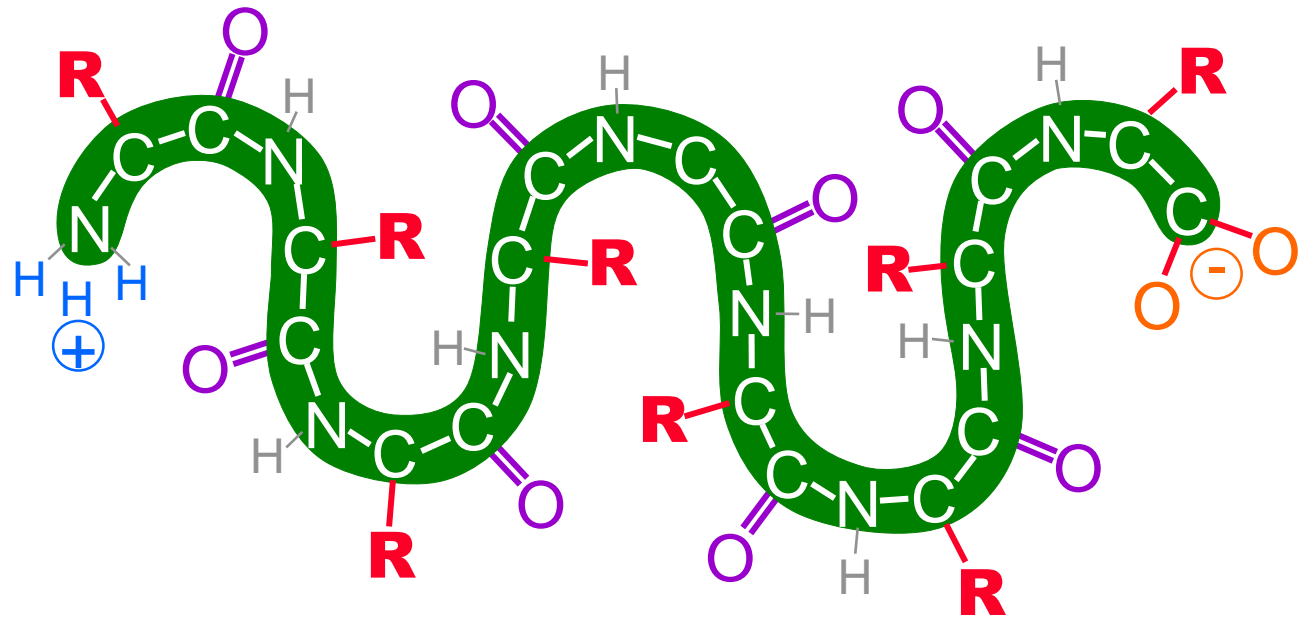
可遇不可求的方法

**所有定量法原理完全根據蛋白質的構造與性質**

# 蛋白質構造的骨架 Backbone of protein molecule

**Constant**

**Variable**

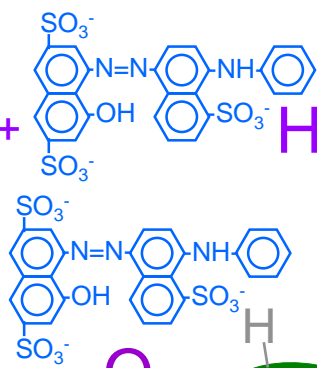


各種蛋白質定量法原理

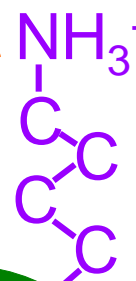
5 Specific Binding Group **Heme**



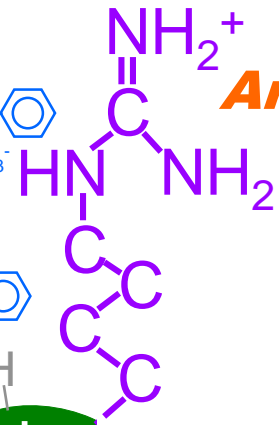
4 Coomassie Brilliant Blue G



Lys



Arg



3 UV Absorbance  
280 nm (aromatic)  
206 nm (carbonyl)

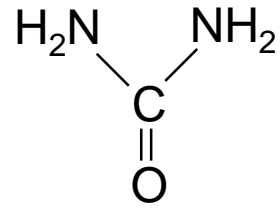
Phosphomolybdic-phosphotungstate

Biuret Methods (carbonyl)

Lowry Methods

1

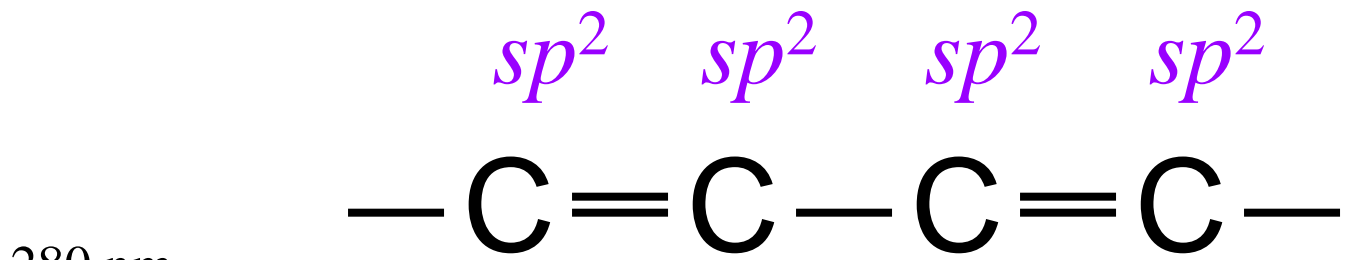
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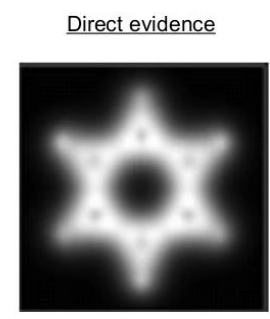
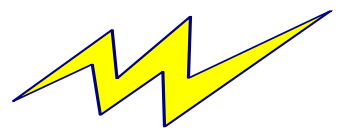
尿素 urea

圖 5.1

# ■ 共軛雙鍵 Conjugated double bonds

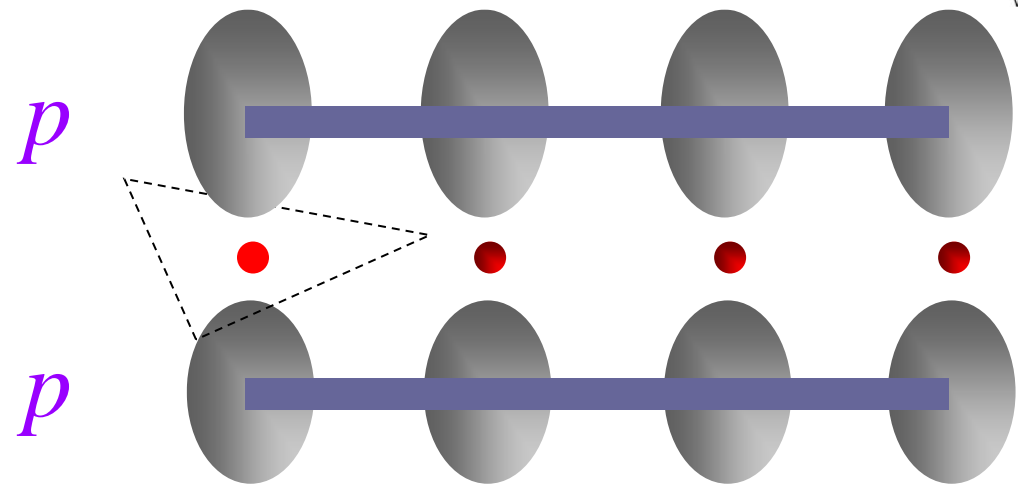


280 nm

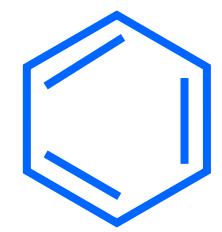


1981 an atomic surface probing technique was developed called Scanning Tunneling Microscopy (STM). The first published STM image showed benzene with an undistorted hexagonal shape.

[www.newton.ex.ac.uk](http://www.newton.ex.ac.uk)



$p$  電子共振 resonance



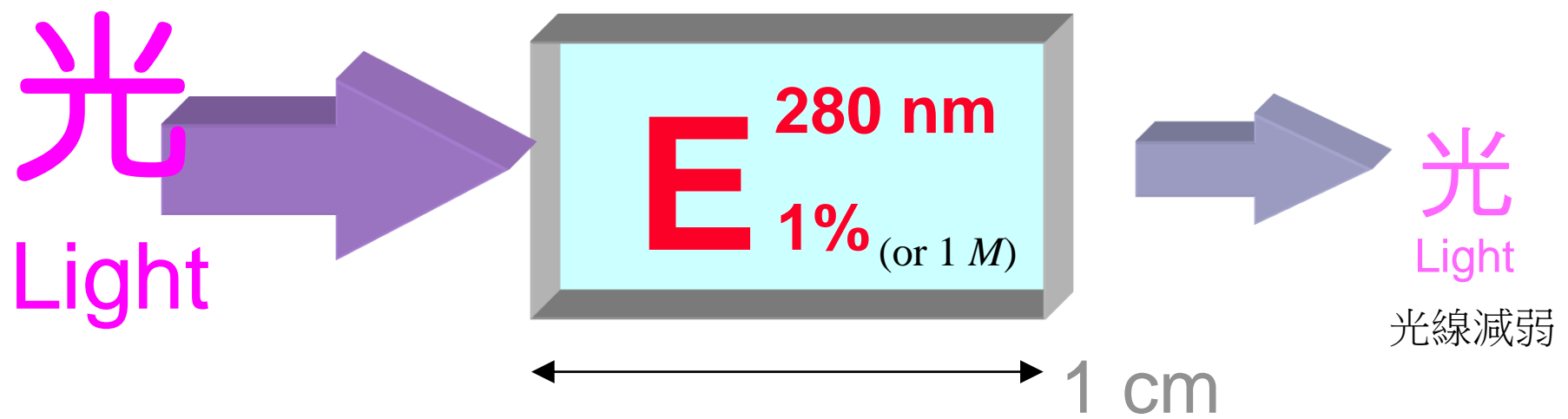
Benzene

# 分子消光係數 Molar extinction coefficient (E)

The constant indicating the capacity of light absorbance of a molecule

constant

蛋白質溶液



## 吸光值

光線減弱的程度

$$A = E \times b \times C$$

1

10

1

0.1%

↑ 實測

↑ 查表

↑ 推算

**Beer-Lambert Law**

## ■ 蛋白質消光係數 UV absorbance by proteins

● 280 nm - Aromatic Groups (Side chain)

1 mg/mL 溶液 → 吸光度 (280 nm) = 1 約值

● 192 nm - Carbonyl Groups (Backbone) 專一

1 mg/mL 溶液 → 吸光度 (192 nm) = 60 靈

理想中的夢幻定量法！但是... (206 nm) = 29 敏

200 nm UV light is interfered heavily by O<sub>2</sub>

**比色法 Spectrophotometry**

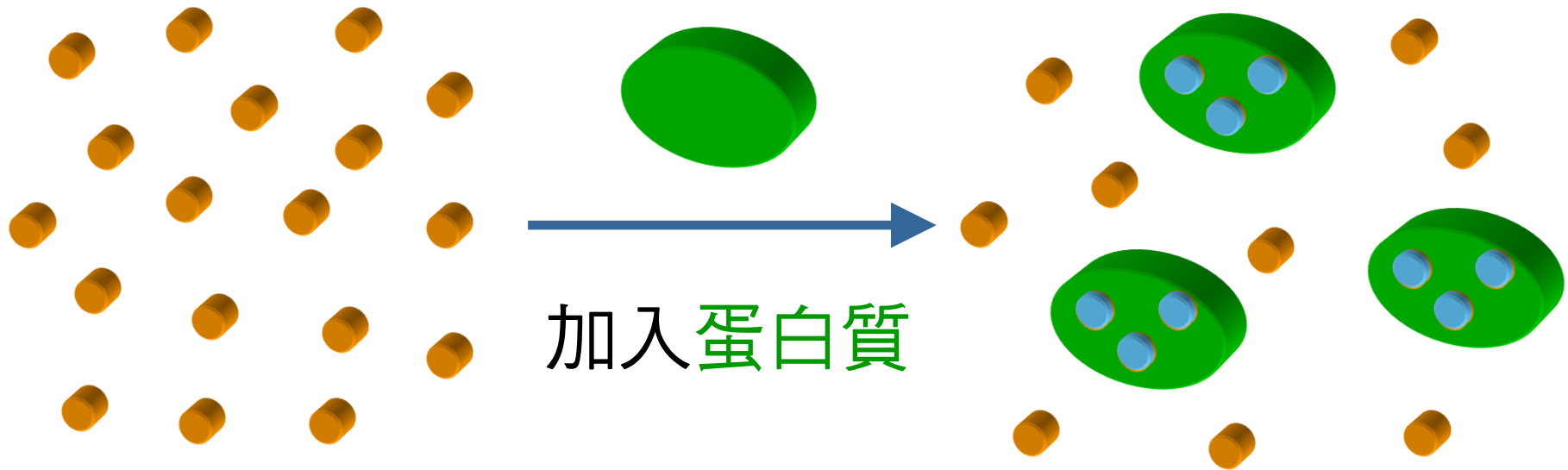
# Bradford Dye-binding Method

## Coomassie Brilliant Blue G-250

470 nm

CBG is an *indicator*

595 nm



酸性環境下呈茶色

Brown (acidic)

加入蛋白質

呈色與蛋白質量成正比

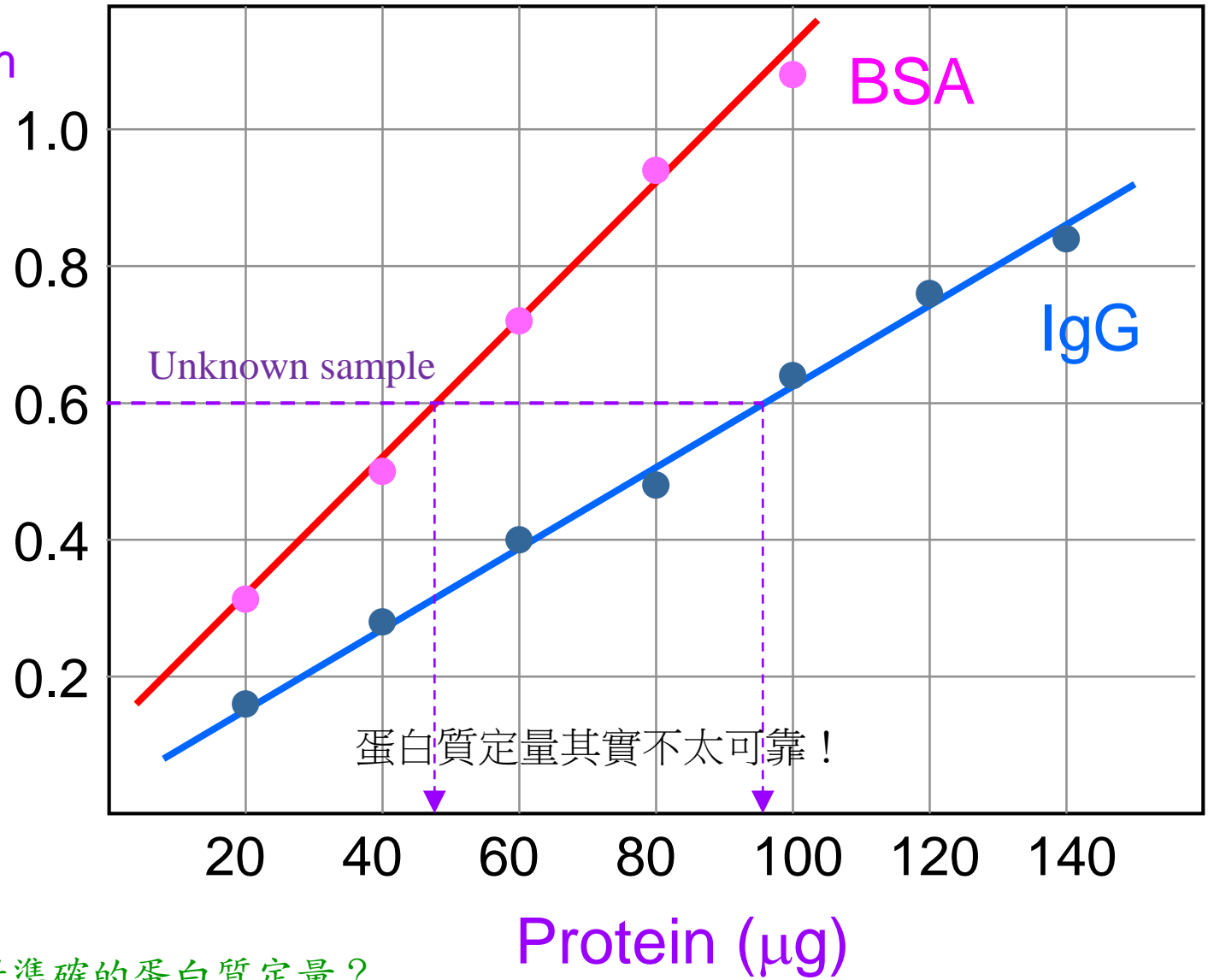
與蛋白質結合變藍色

Blue (pH↑)



# 不同蛋白質的定量差異 Deviation of standards

$A_{600\text{ nm}}$



蛋白質定量其實不太可靠！

設計一個絕對準確的蛋白質定量？

# ■ 各種蛋白質定量法的比較 Comparison

Methods	Precision	Accuracy	Remarks
Biuret	0.05 - 5 mg	High	Rapid, Corrosive, Interference
Lowry	0.05 - 0.5 mg	Medium	Slow, Interference
Absorbance 280 nm	0.05 - 2 mg	Low	Sample recoverable, Interference
Absorbance 205 nm	0.01 - 0.05 mg	High	Sample recoverable, O <sub>2</sub> interference
Bradford Dye-binding	0.01 - 0.05 mg	Medium - High	Rapid, Interference, Color staining

# Precise + Accurate

Bradford Method

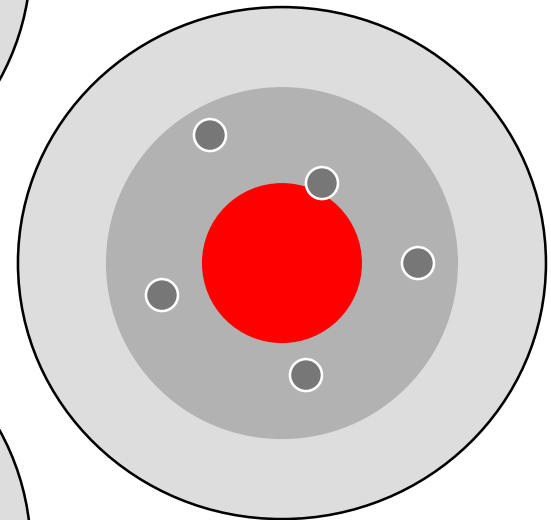
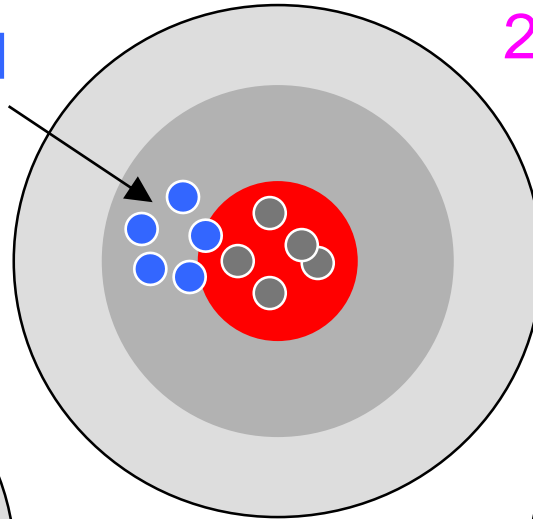
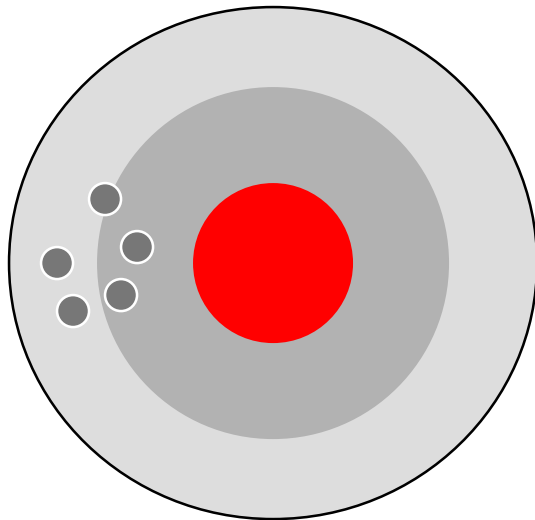
206 nm absorbance

Precise

Accurate

精  
確

準  
確



Lowry Method

Biuret Method

280 nm absorbance

# Imprecise + Inaccurate

