

# 酵素分析方法 Enzyme Analysis

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- 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

## 5 蛋白質定量 Protein determination methods

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**5.1 Biuret method** (雙縮脲反應)

**5.2 Lowry method**

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

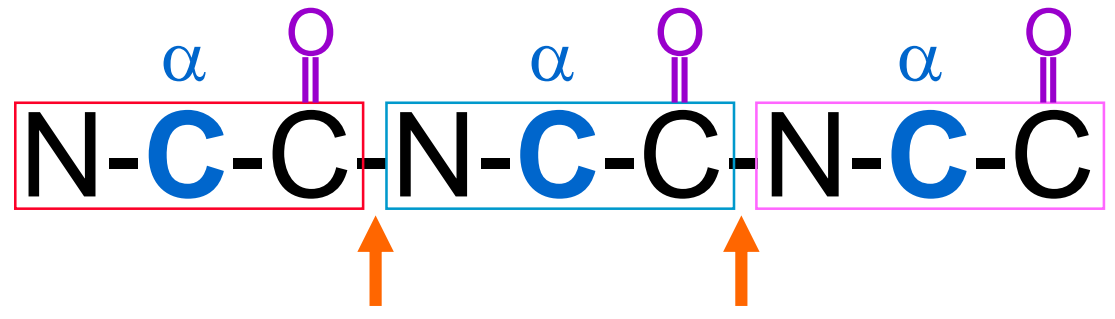
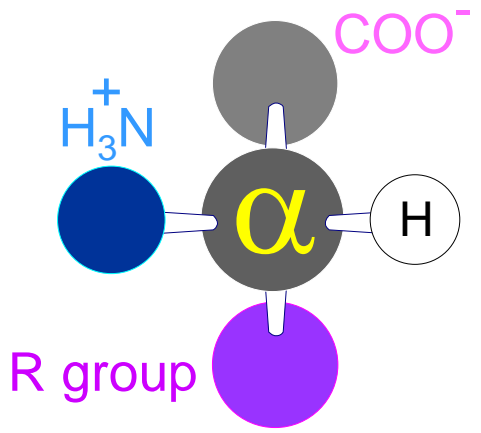
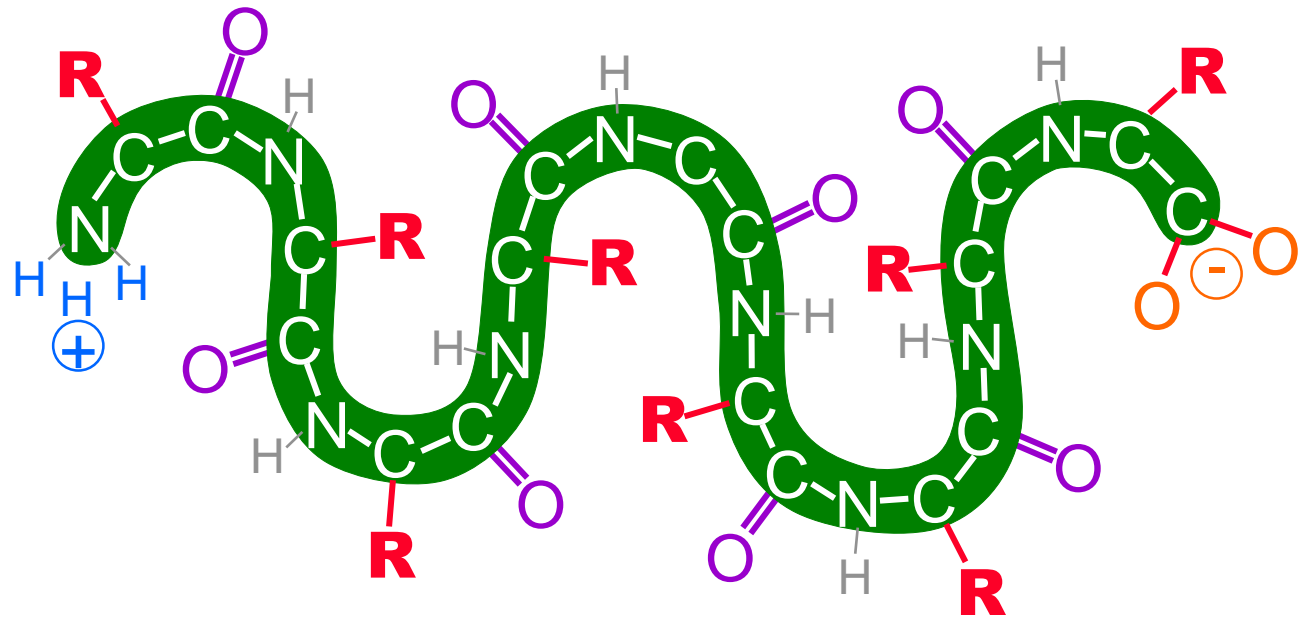
**5.4 Coomassie Blue (dye binding) method**

**5.5 Other methods** (特殊基團、含重金屬)

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

**Constant**

**Variable**

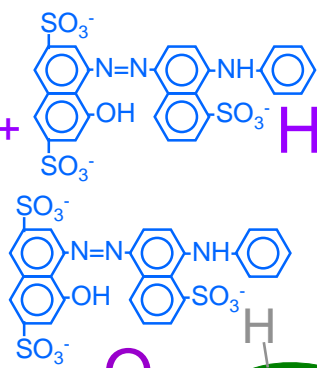


各種蛋白質定量法原理

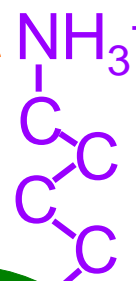
5 Specific Binding Group **Heme**



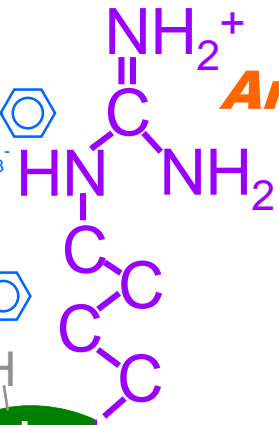
4 Coomassie Brilliant Blue G



Lys

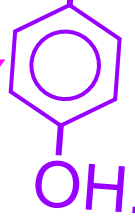


Arg



206 nm (carbonyl)

3 UV Absorbance 280 nm (aromatic)

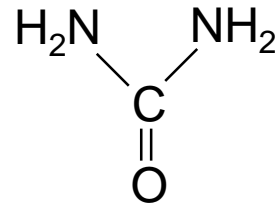


Phosphomolybdic-phosphotungstate

Biuret Methods (carbonyl)

Lowry Methods

1



尿素 urea

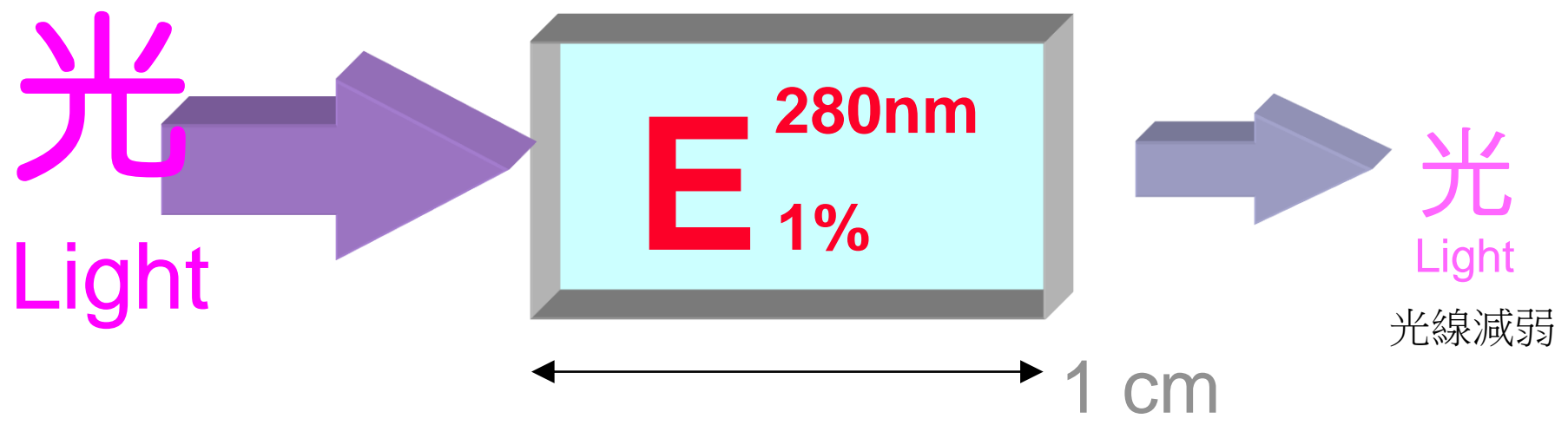
2

圖 5.1

# ■ 分子消光係數 Molar extinction coefficient

● The constant indicating the capacity of light absorbance for a molecule

constant 蛋白質溶液



吸光值

$$A = E \times b \times c$$

1 = 10 × 1 × 0.1%

# ■ 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

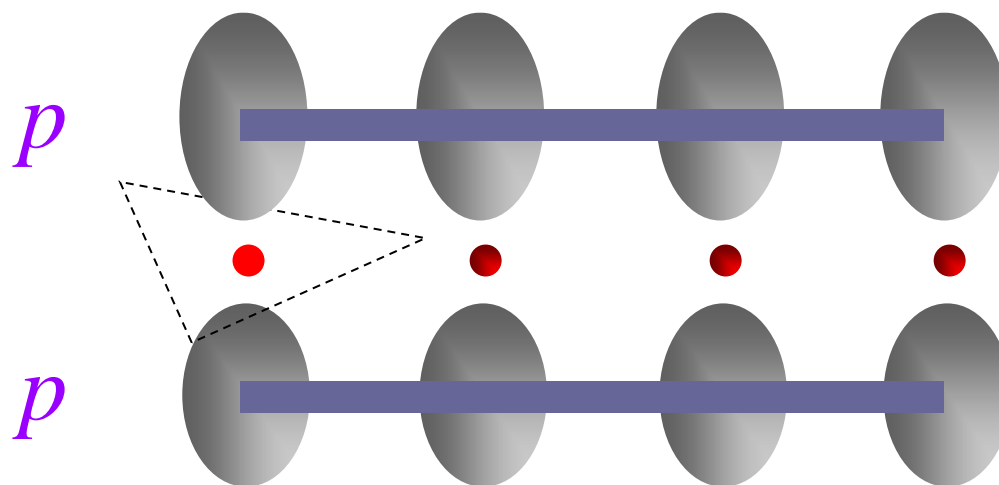
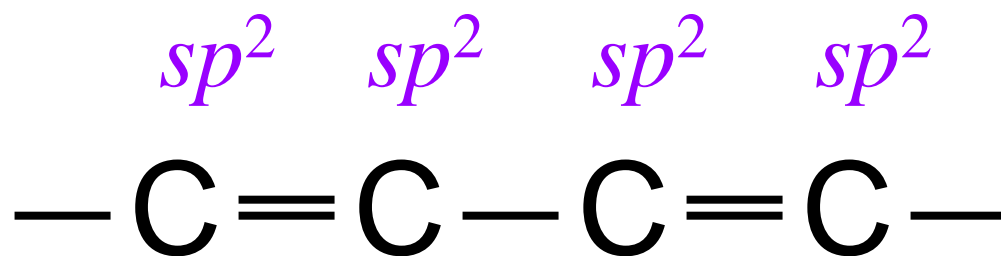
● **192 nm** - Carbonyl Groups (Backbone)

1 mg/mL 溶液 → 吸光度 (192 nm) = **60**

(206 nm) = **29**

200 nm UV light is interfered heavily by  $O_2$

# ■ 共軛雙鍵 Conjugated double bonds



$p$  電子共振 resonance



Benzene

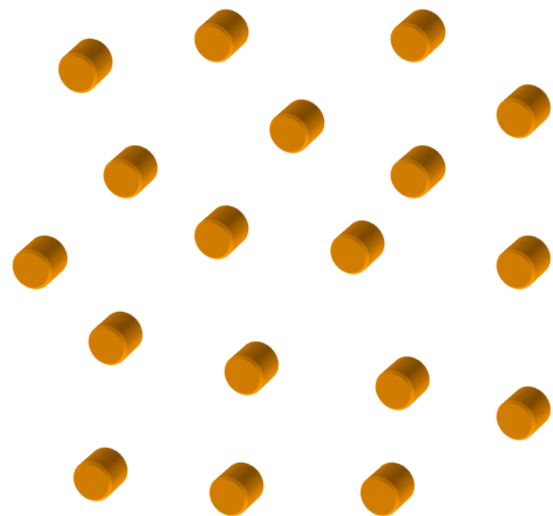
# Bradford Method

## Coomassie Brilliant Blue G-250

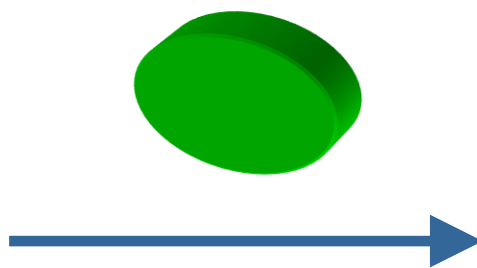
470 nm

CBG is an *indicator*

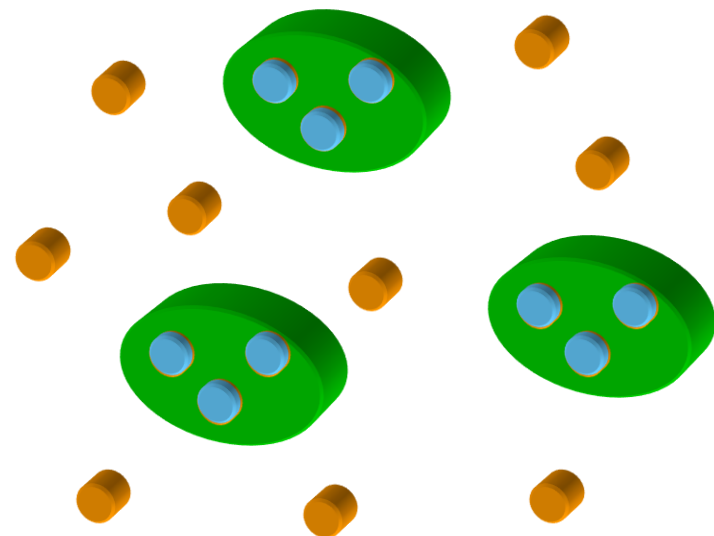
595 nm



酸性環境下呈茶色  
Brown (acidic)



加入蛋白質

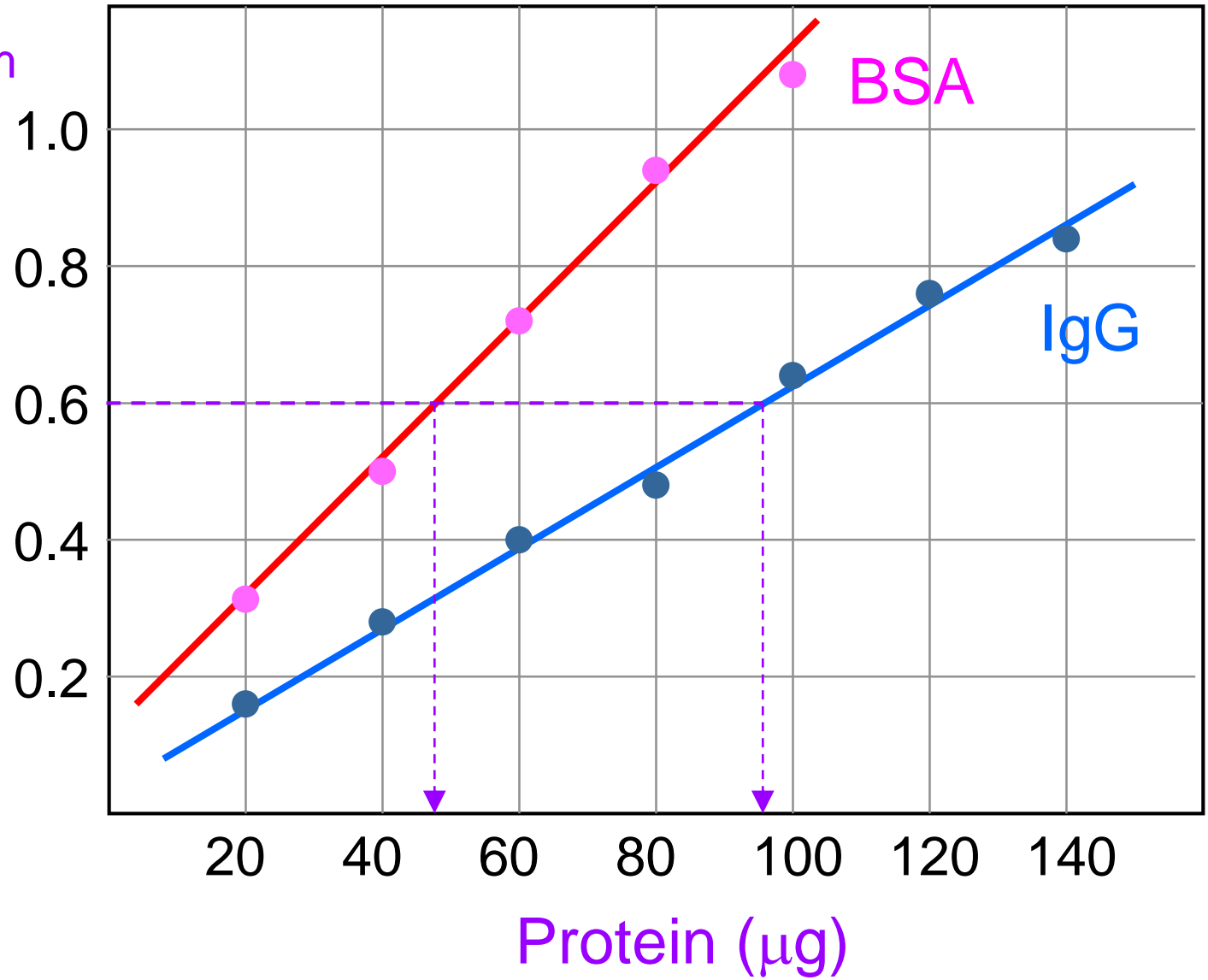


與蛋白質結合變藍色  
Blue (pH↑)



# 不同蛋白質的定量差異 Deviation of standards

$A_{600\text{ nm}}$



# ■ 各種蛋白質定量法的比較

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

# Precise + Accurate

Bradford Method

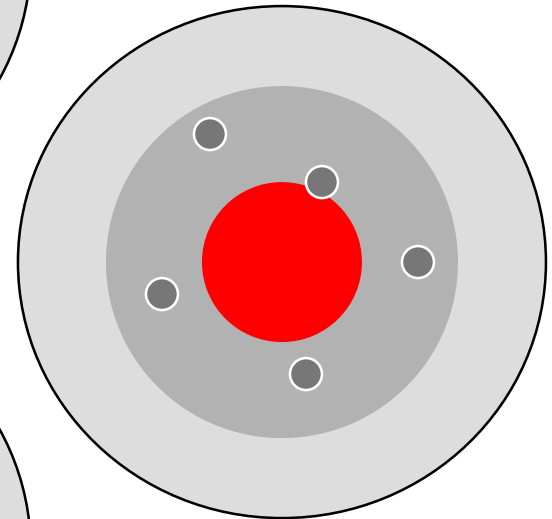
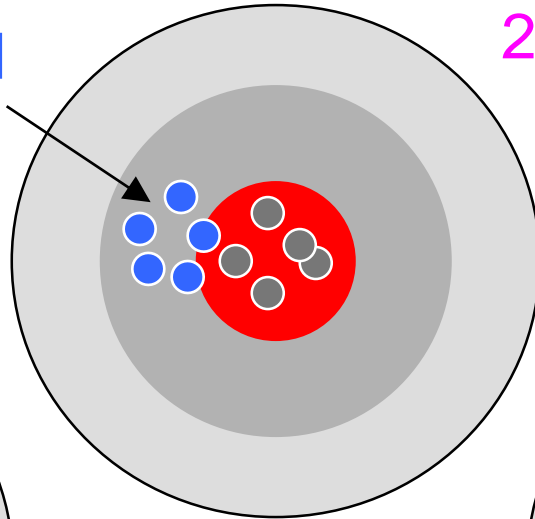
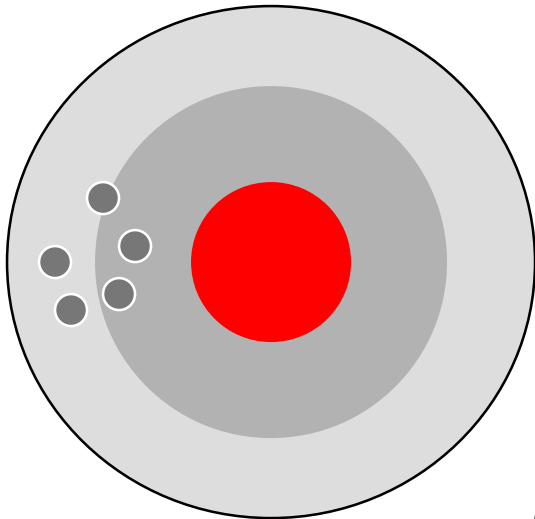
206 nm absorbance

Precise

Accurate

精  
確

準  
確



Lowry Method

Biuret Method

280 nm absorbance

# Not precise + Inaccurate