

The Nobel Prize in Physiology or Medicine 2006

Goes to



Professor Andrew Z. Fire, Ph.D.
Stanford University School of Medicine
Stanford, CA, USA



Professor Craig C. Mello, Ph.D.
University of Massachusetts Medical School
Worcester, MA, USA

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

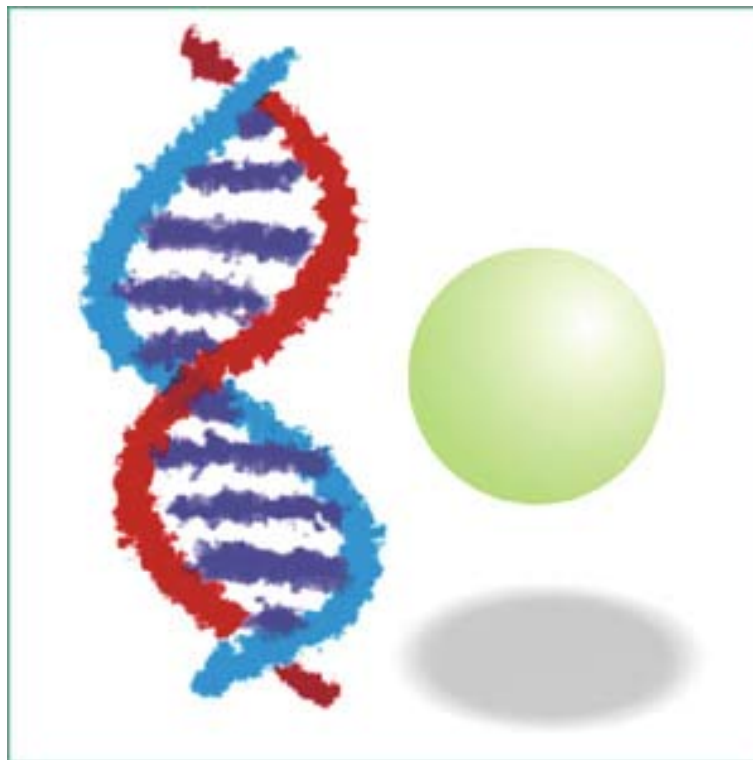
http://nobelprize.org/nobel_prizes/medicine/laureates/2006/

RNAi 動画: <http://www.nature.com/focus/rnai/animations/animation/animation.htm>



生化科技系
BST

BCX



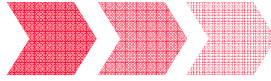
P1

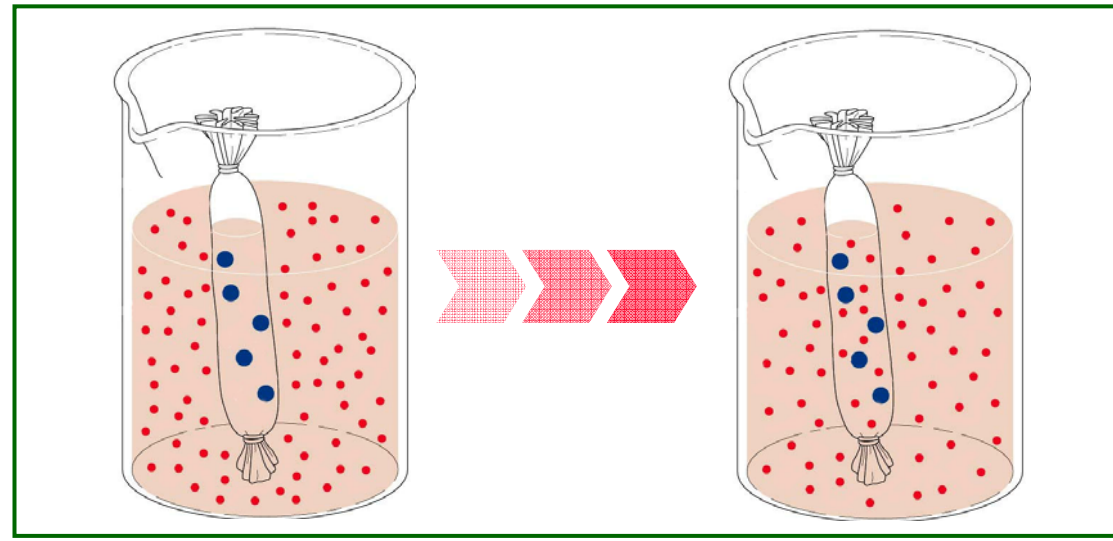
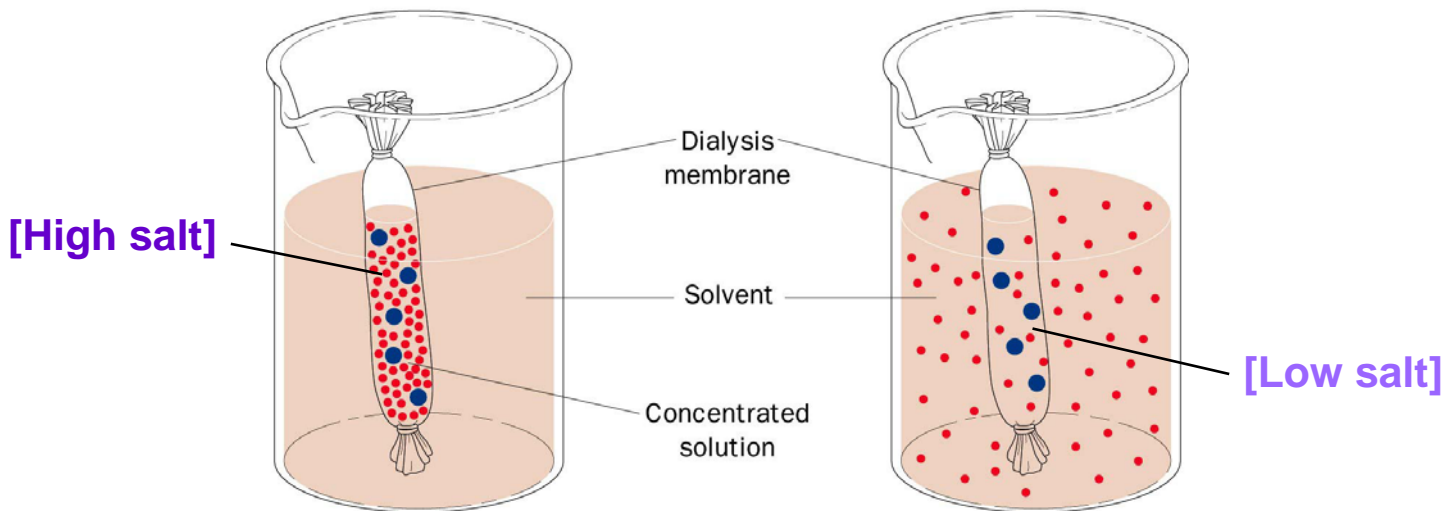
生物化學實驗

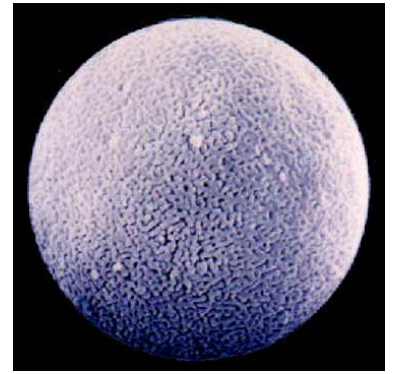
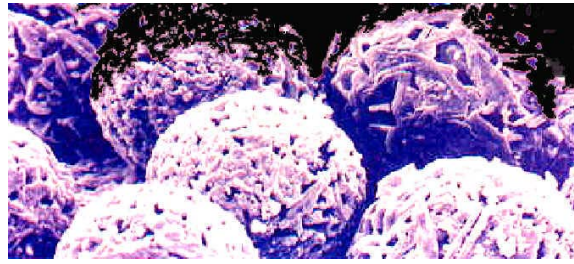
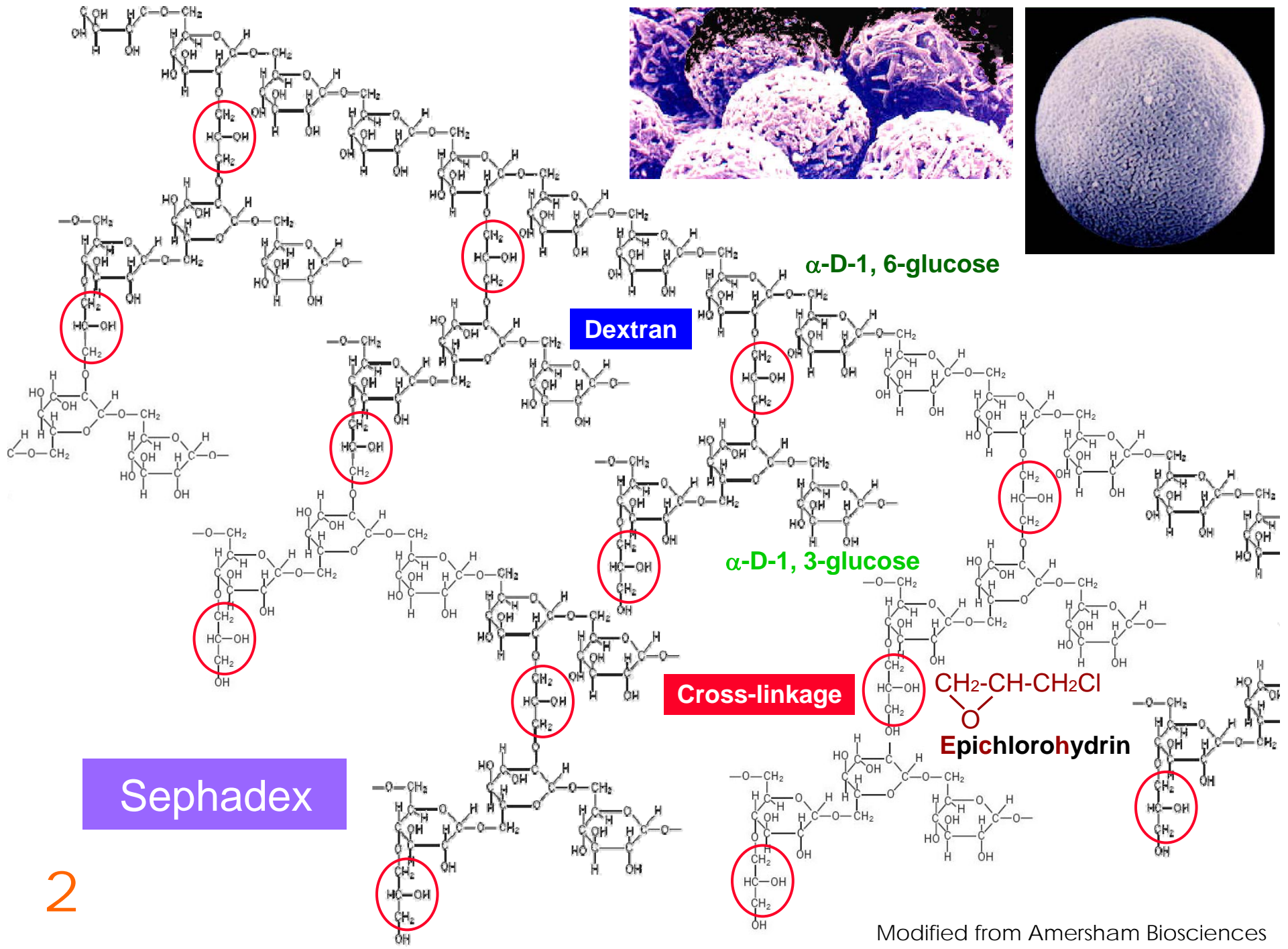
蛋白質定量

■ 透析(dialysis) : desalting and exchange buffer

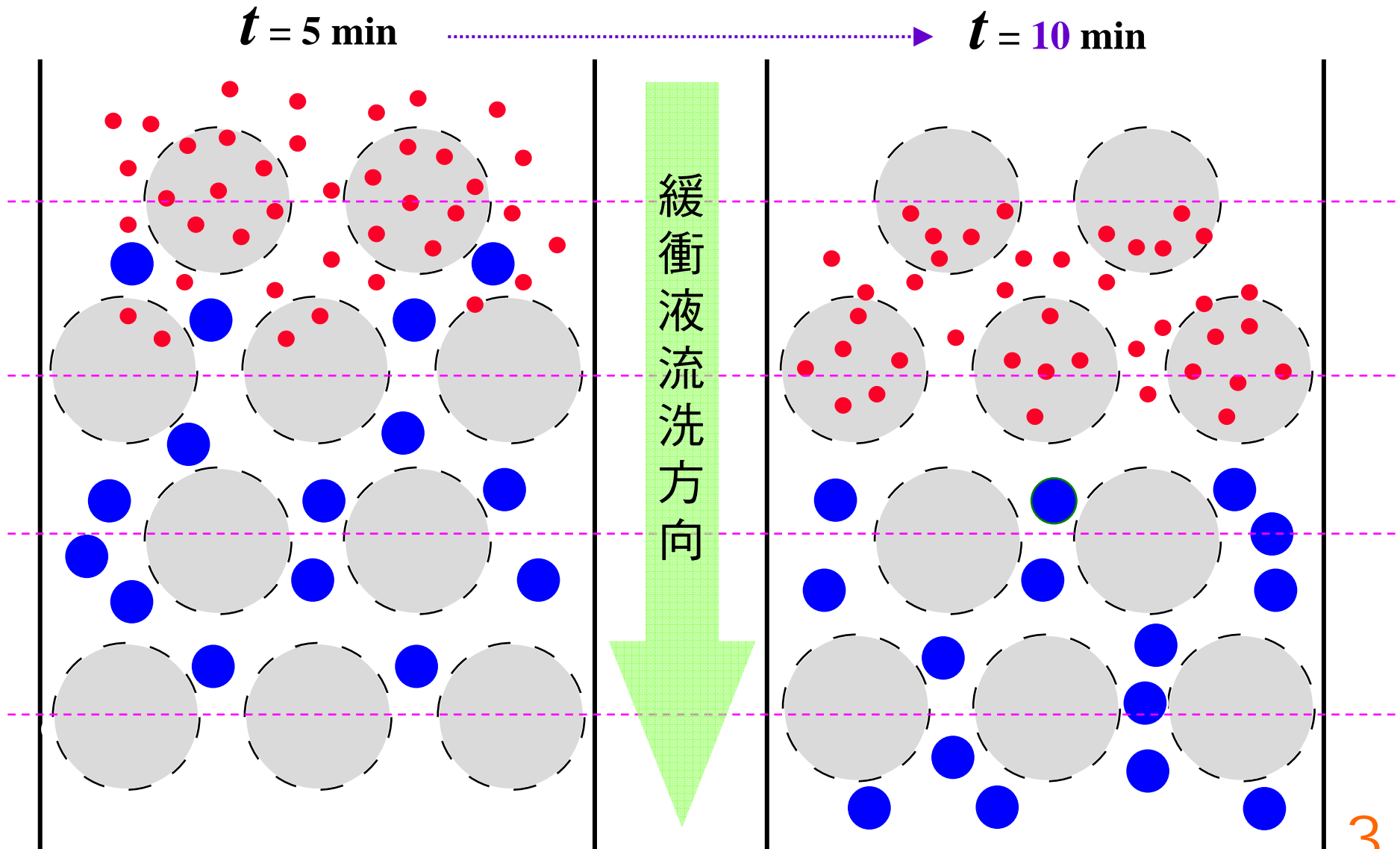


At start of dialysis  At equilibrium





膠體過濾法 : Separation, Desalting and Exchange buffer



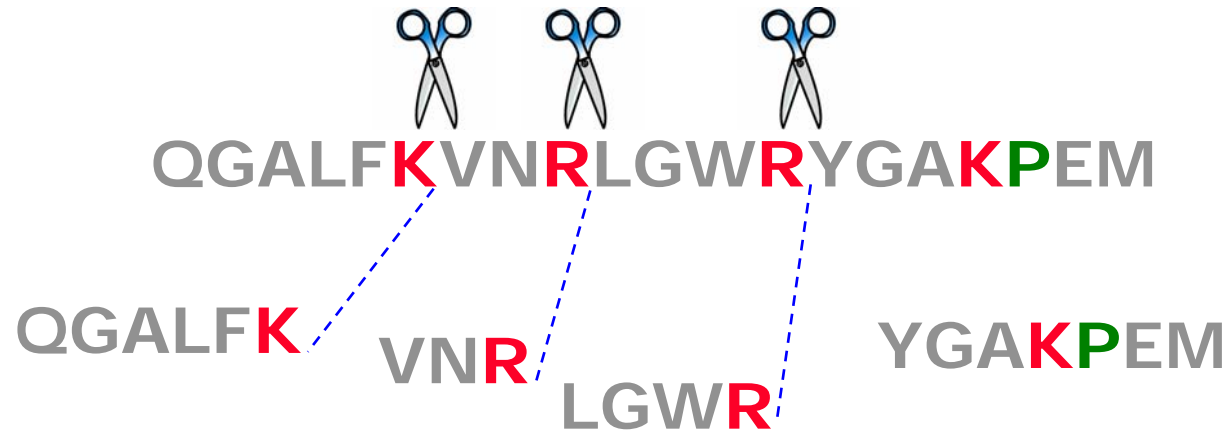
Trypsin (EC 3.4.21.4)

分子量：23.3 kD (porcine)

分類：Serine protease

作用方式：Endopeptidase

專一性：Cleaves proteins at the *carboxyl side* of **lysine** or **arginine**,
except when either is followed by **proline**



■ CHOM (chicken egg white ovomucoid)

含量：11% of all egg white proteins

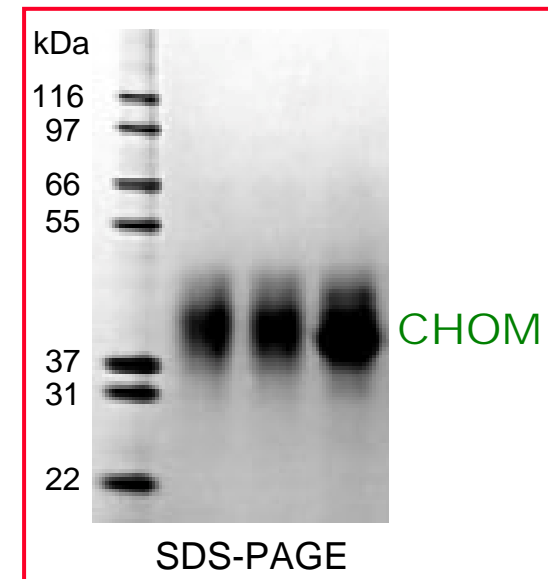
分子量：28 kDa, 186 a.a. (predicted MW: 20 kDa)

SDS-PAGE 之分子量：34~49 kDa

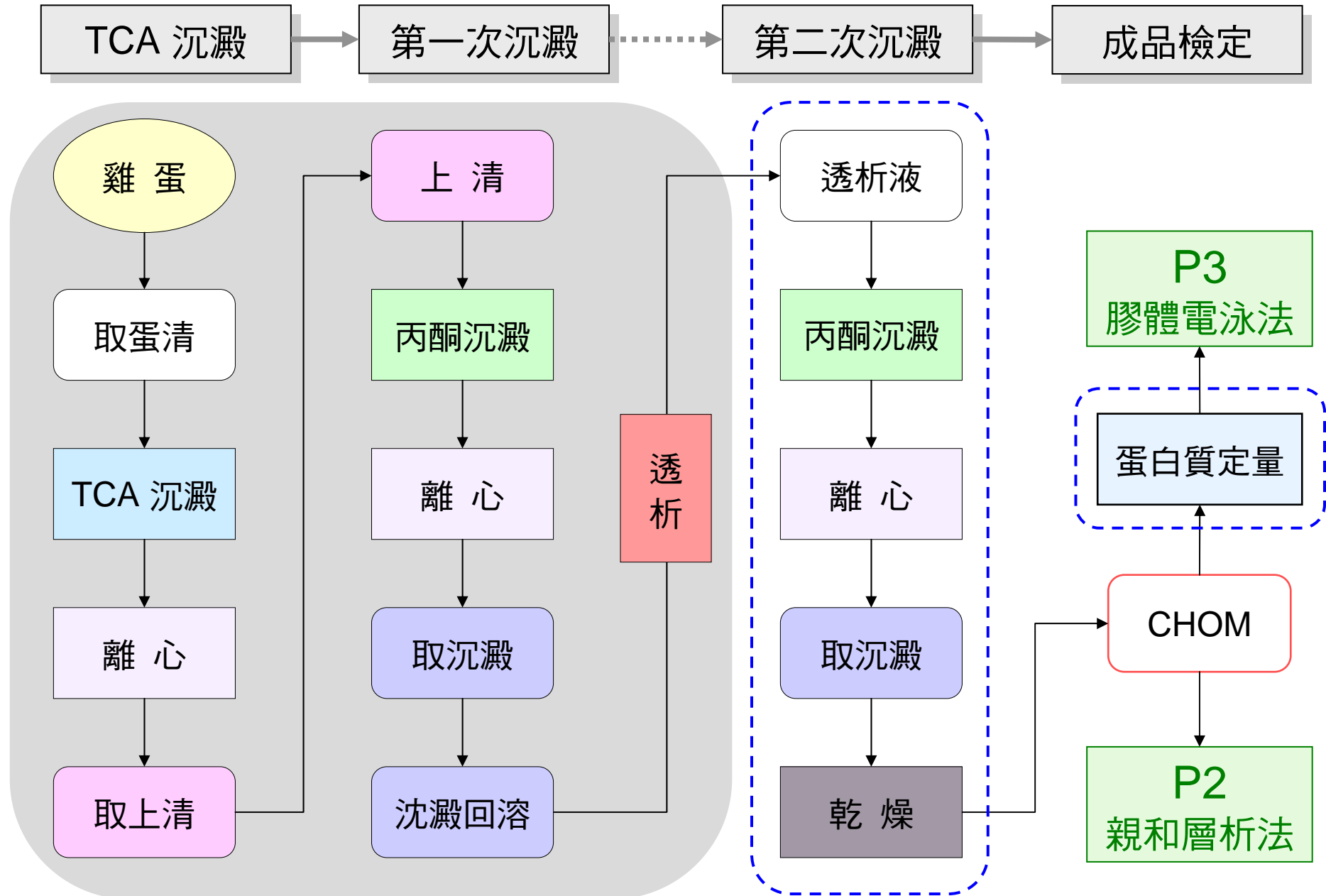
Carbohydrate content: 20~25%

糖基化：5 個 N-linked sugar chains

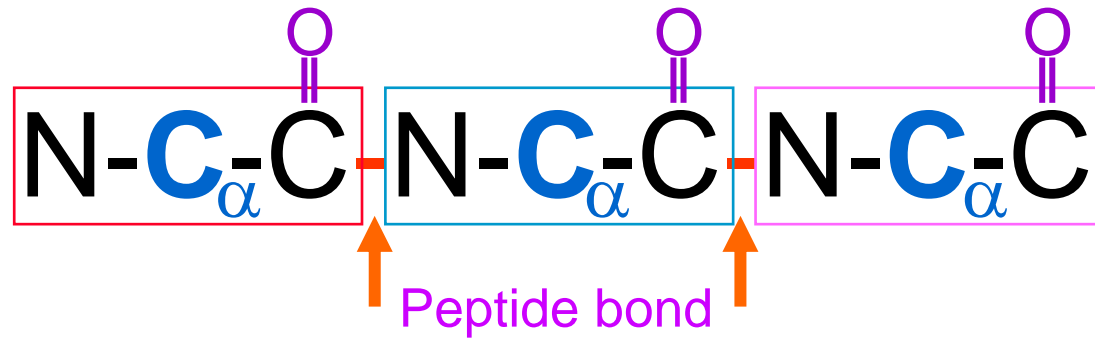
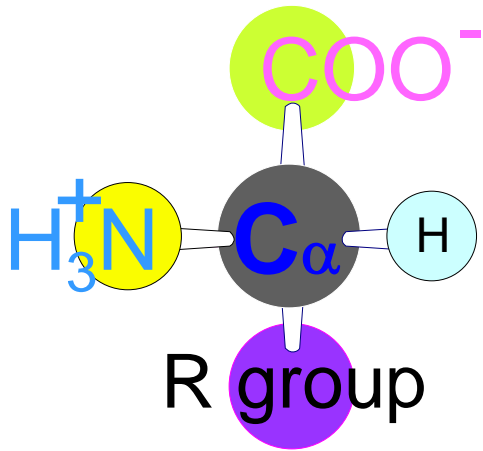
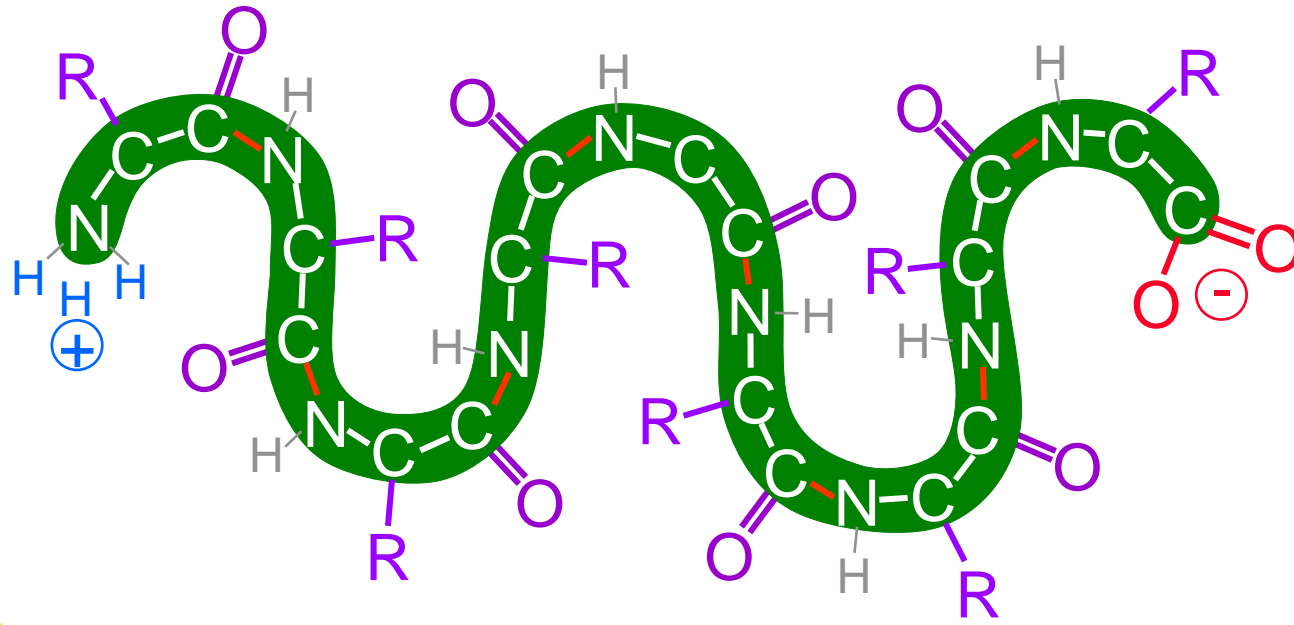
- Stable to digestion and heat
- Cooked eggs can cause allergic reactions in CHOM-specific allergic patients



P1 蛋白質抽取



■ 蛋白質構造的骨架：



■ 蛋白質定量法：

■ UV absorbance

■ Bradford dye-binding protein assay

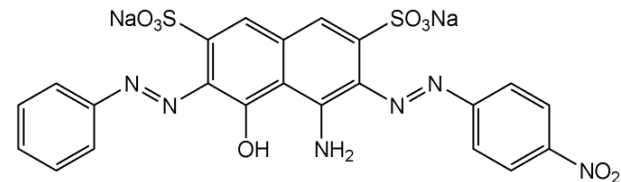
■ Biuret protein assay

■ Lowry protein assay

■ Bicinchoninic acid (BCA) protein assay

■ Amido black protein assay

used in criminal investigations to detect **blood** present with latent **fingerprints**

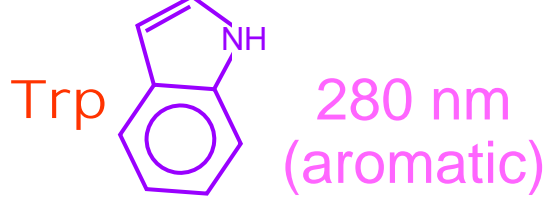
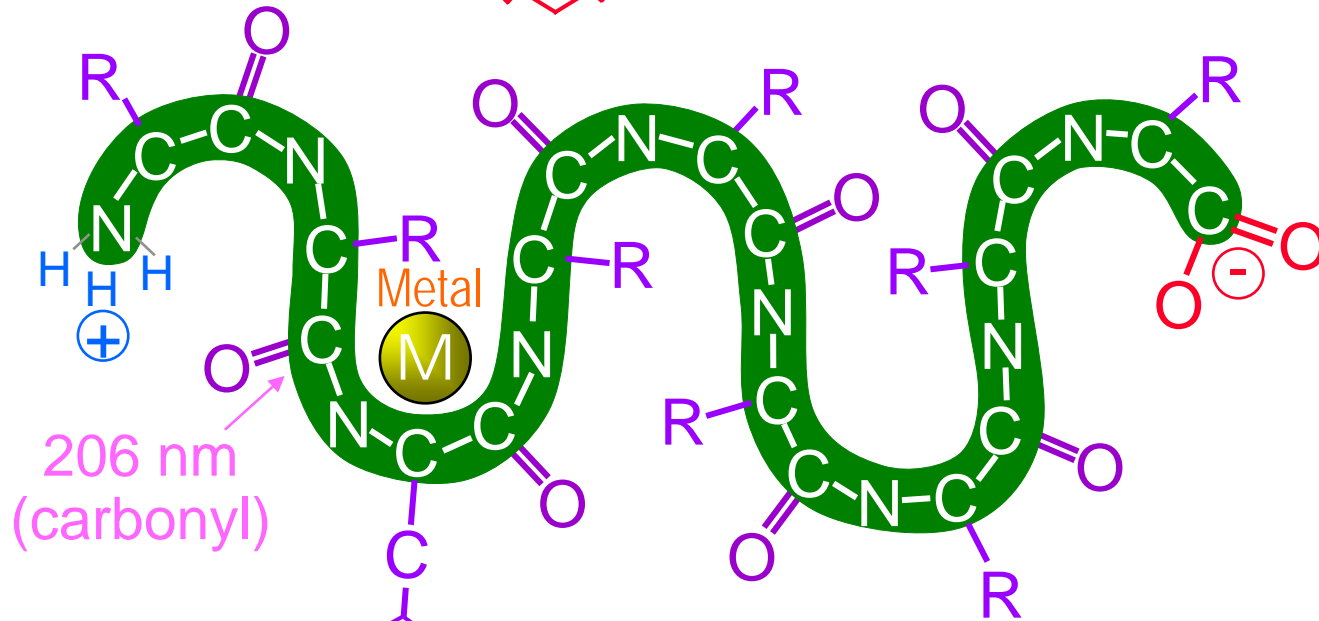
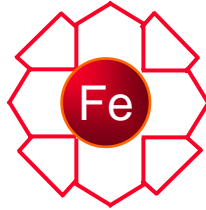


■ o-phthalaldehyde (OPA) protein assay

各種蛋白質定量法原理：

Specific Binding Group

Peroxidase
Heme



UV Absorbance

	Max. absorbance
Phe	260nm
Tyr	275nm
Trp	280nm

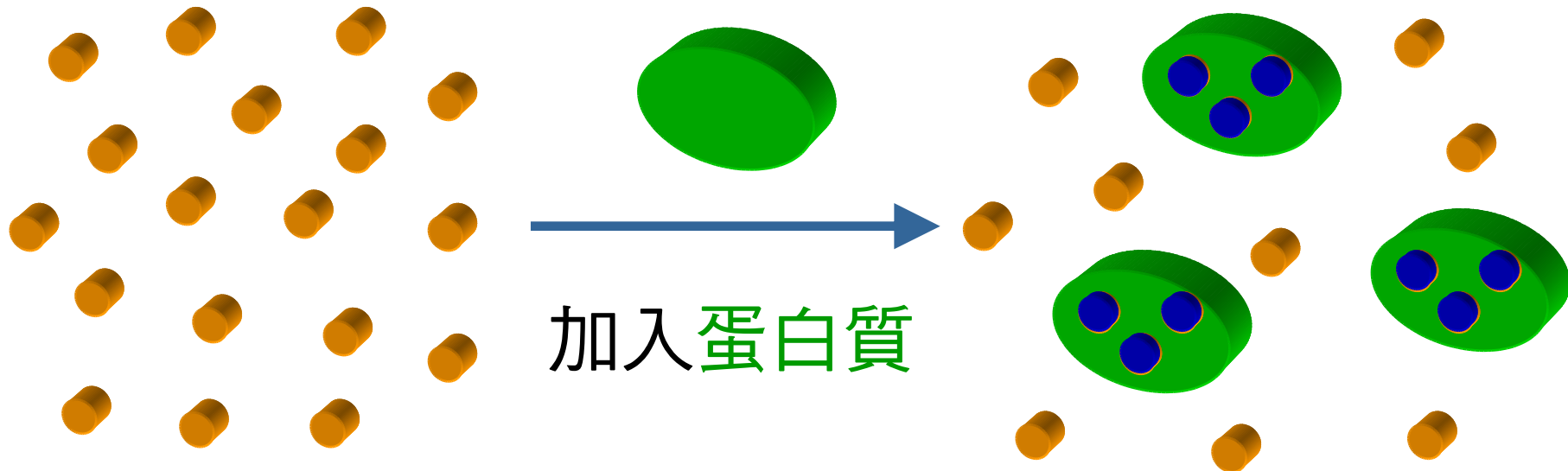
Bradford Method :

Coomassie Brilliant Blue G-250

470 nm

CBG 是一種指示劑

595 nm



酸性環境下呈茶色

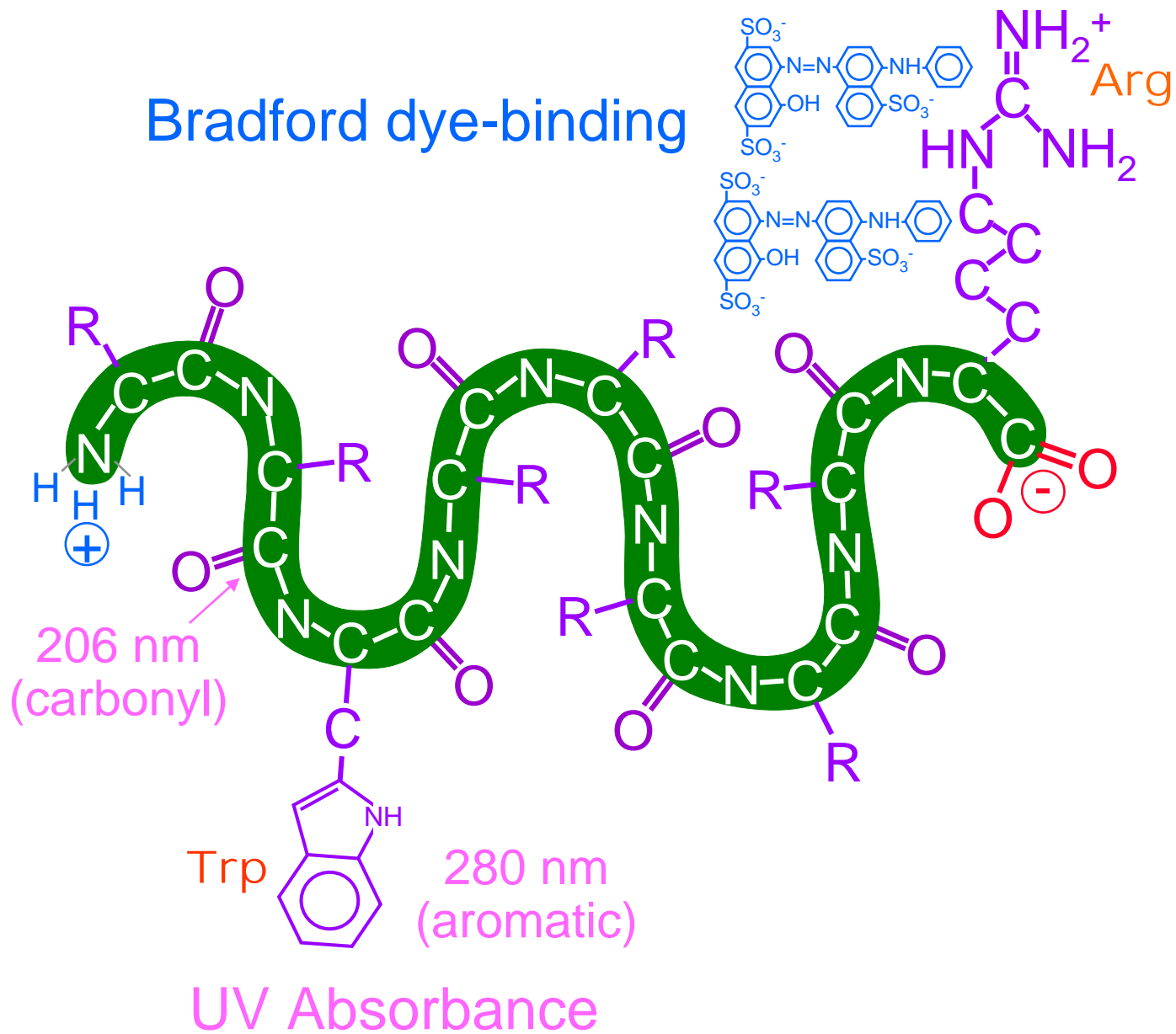
與蛋白質結合變藍色

9

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.

各種蛋白質定量法原理：

10



■ 蛋白質定量法：

■ Biuret method

Biuret 試劑乃鹼性硫酸銅溶液，銅離子在鹼性溶液中，會與蛋白質上的 peptide bonds 生成藍色或紫色的複合物，由於 biuret 是在鹼性環境中和硫酸銅發生呈色反應之最小單位，故稱此反應為 biuret 試驗。測定 550 nm 的吸光。

■ Lowry method (Folin-phenol 試劑法)

反應前半為 Biuret Method。二價銅離子在鹼性溶液中，會與蛋白質 peptide bonds 上的 nitrogen 結合，並還原為一價銅離子。一價銅離子與 Tyr、Trp 或 Cys 會使 Folin reagent 內的 phosphomolybdic-phosphotungstic acid (磷鉬酸與磷鎢酸) 還原為藍色，並在波長 660 nm 有很強的吸光。此法比紫外光吸收法靈敏 10-20 倍，較 Biuret method 靈敏 10-100 倍。

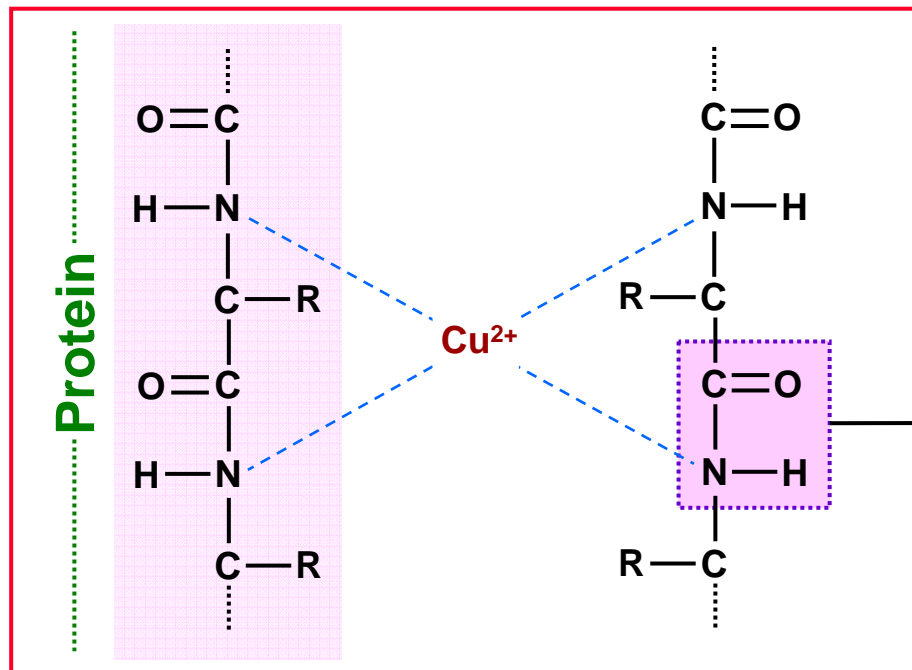
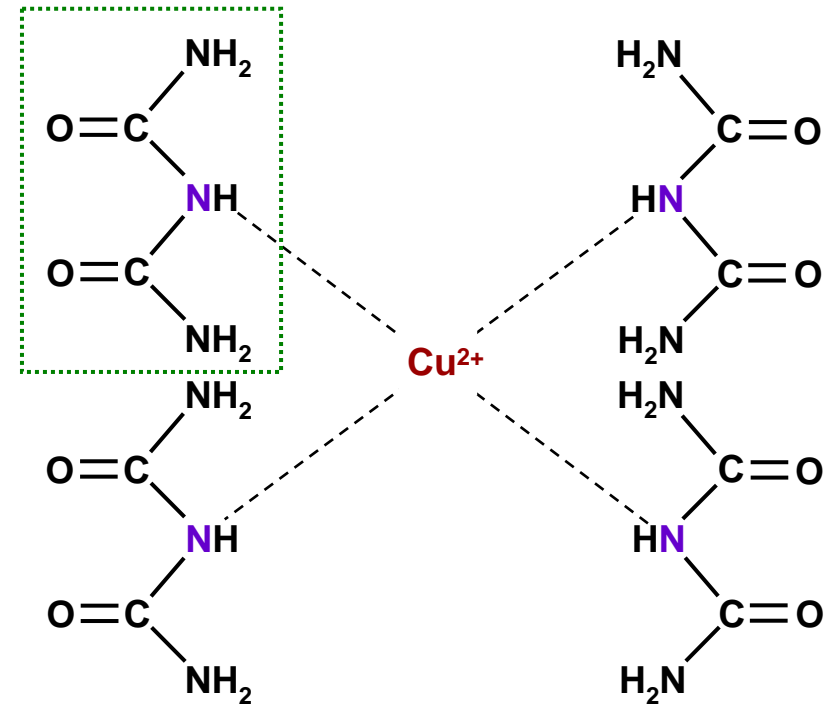
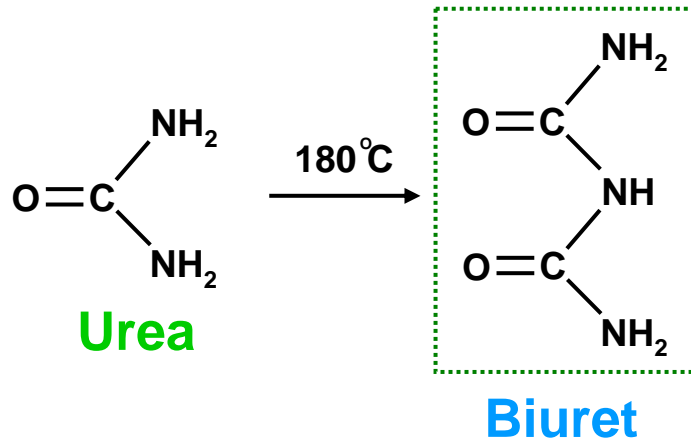
Lowry et al. (1951). Protein measurement with the Folin-Phenol reagents. J. Biol. Chem. 193: 265-275.

■ Bicinchoninic acid (BCA) protein assay

亦是 Biuret 法的延伸。在鹼性溶液中，蛋白質使銅離子由二價還原成一價後，進一步的以 BCA 與一價銅離子結合產生很深的紫色，在波長 562 nm 有很強的吸光。本法的優點在於鹼性溶液中 BCA 比 Folin reagent 穩定，並且只需一個操作步驟。

Smith et al. (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76-85

■ 蛋白質定量法：Biuret method



Peptide bond

■ 蛋白質定量法：

Biuret method 加強版

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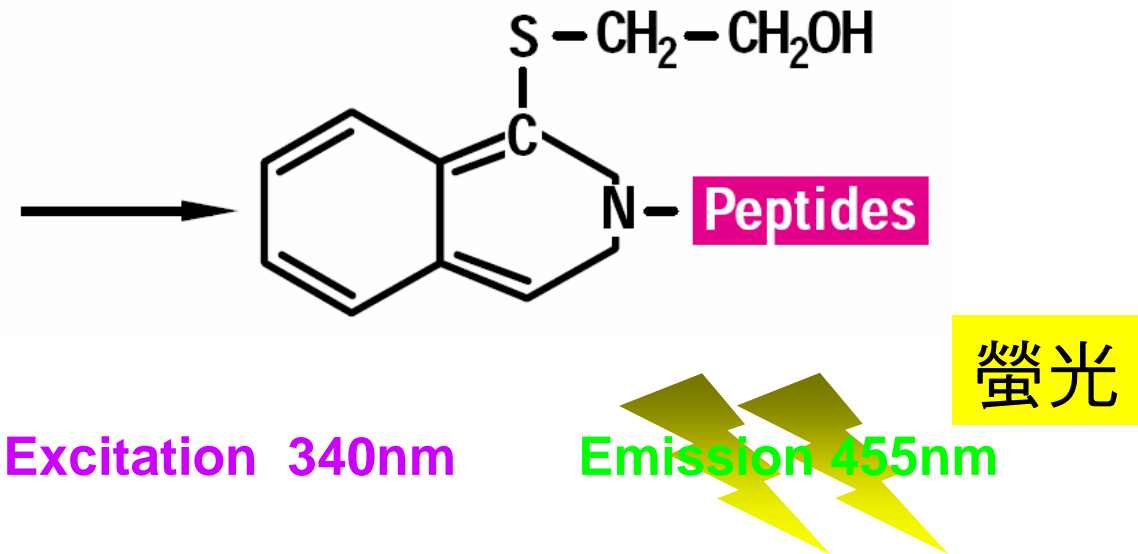
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蛋白質定量法：o-phthalaldehyde (OPA) protein assay

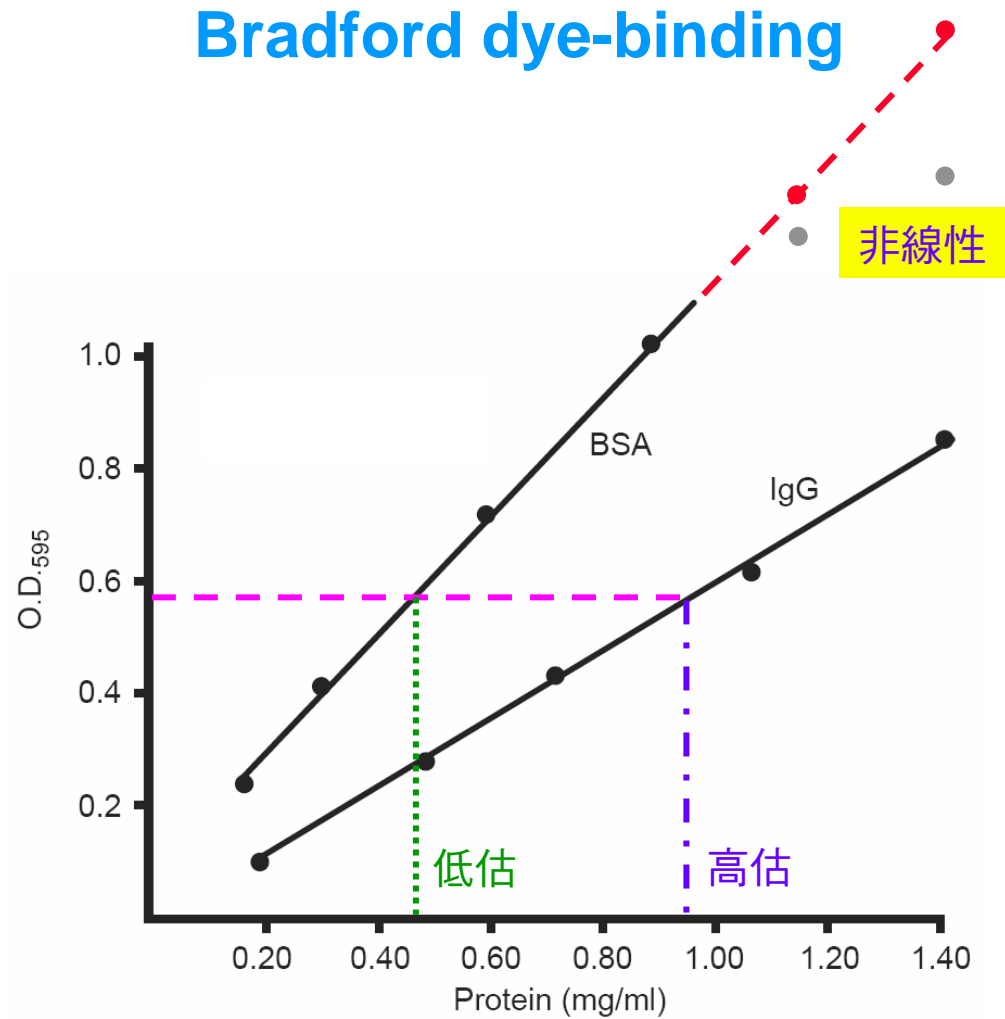


不同蛋白質定量法的測定值差異：

Assay Results (protein mg/mL)

	Biuret	Lowry	Bradford
Alcohol dehydrogenase	5.8	5.0	7.8
α -amylase	6.8	6.0	8.3
Bovine serum albumin	9.7	8.4	21.1 高估
Carbonic anhydrase	8.8	8.9	13.0
Catalase	7.6	6.3	9.7
α -Chymotrypsin	9.4	11.6	7.8
Cytochrome C	25.7	11.3 低估	25.3
β -Galactosidase	9.5	9.9	7.9
Hemoglobin (bovine)	16.2	8.3 低估	19.9
Histones	9.7	9.2	15.8 高估
Lysozyme	10.4	12.6	9.9
Myoglobin	13.7	7.9	20.7
Ovalbumin	10.2	10.1	9.4
Ovomucoid	7.8	8.3	19.9 高估
Pepsin	9.8	12.4	4.1 低估
Ribonuclease	11.8	15.9	5.3 低估
Trypsin inhibitor (soy)	9.1	10.3	6.1
Transferrin	8.5	9.0	12.6
Trypsin	11.4	15.5	4.9 低估
Average	10.2	9.8	12.1

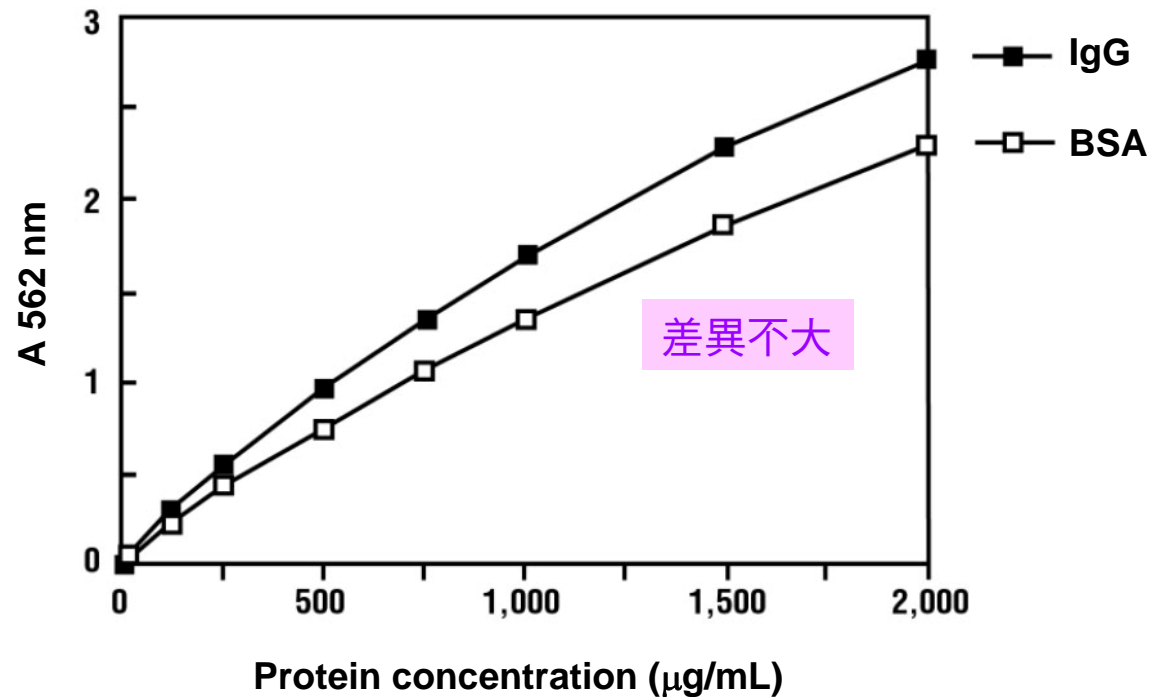
蛋白質定量法：不同標準品的標準曲線不同



■ 蛋白質定量法：不同標準品的標準曲線不同



BCA Protein Assay



■ 蛋白質定量法：溶液的適用性

Reagents Compatible With *Bio-Rad* Bradford Dye-Binding Protein Assay

Acetate, 0.6 M

Acetone

Ammonium sulfate, 1 M

Boric acid

ATP, 1 mM

DTT, 1 M

Eagle's MEM

EDTA, 0.1 M

EGTA, 0.05 M

Ethanol

Formic acid, 1 M

Fructose

Glucose

Glutathione

Glycerol, 99%

Glycine, 0.1 M

HEPES, 0.1 M

KCl, 1 M

Mercaptoethanol, 1 M

MgCl₂, 1 M

NaCl, 5 M

Phenol, 5%

Phosphate, 1 M

SDS, 0.1%

Sodium phosphate

Tris, 2 M

Triton X-100, 0.1%

Urea, 6 M

■ 各種蛋白質定量法的比較：

定量方法	靈敏度	準確度	其它說明
A 280nm	0.05-2.0 mg	低 高	操作簡便 樣本可回收 其它物質干擾大 樣品最好成分單一
A 205nm	0.01-0.05 mg	低 高	操作簡便 樣本可回收 許多物質皆具有carbonyl group
Biuret	0.05-5.0 mg	高	呈色快速 有腐蝕性 銨離子干擾大(如Tris、硫酸銨)
Lowry	0.05-0.5 mg	中	操作步驟多 呈色形成較慢 多種離子有干擾
BCA	0.02-0.5 mg	中 高	呈色較快 生成物穩定
Bradford	0.01-0.05 mg	中 高	立即呈色 易受多種物質干擾 短時間內可重複操作

P1 蛋白質抽取

