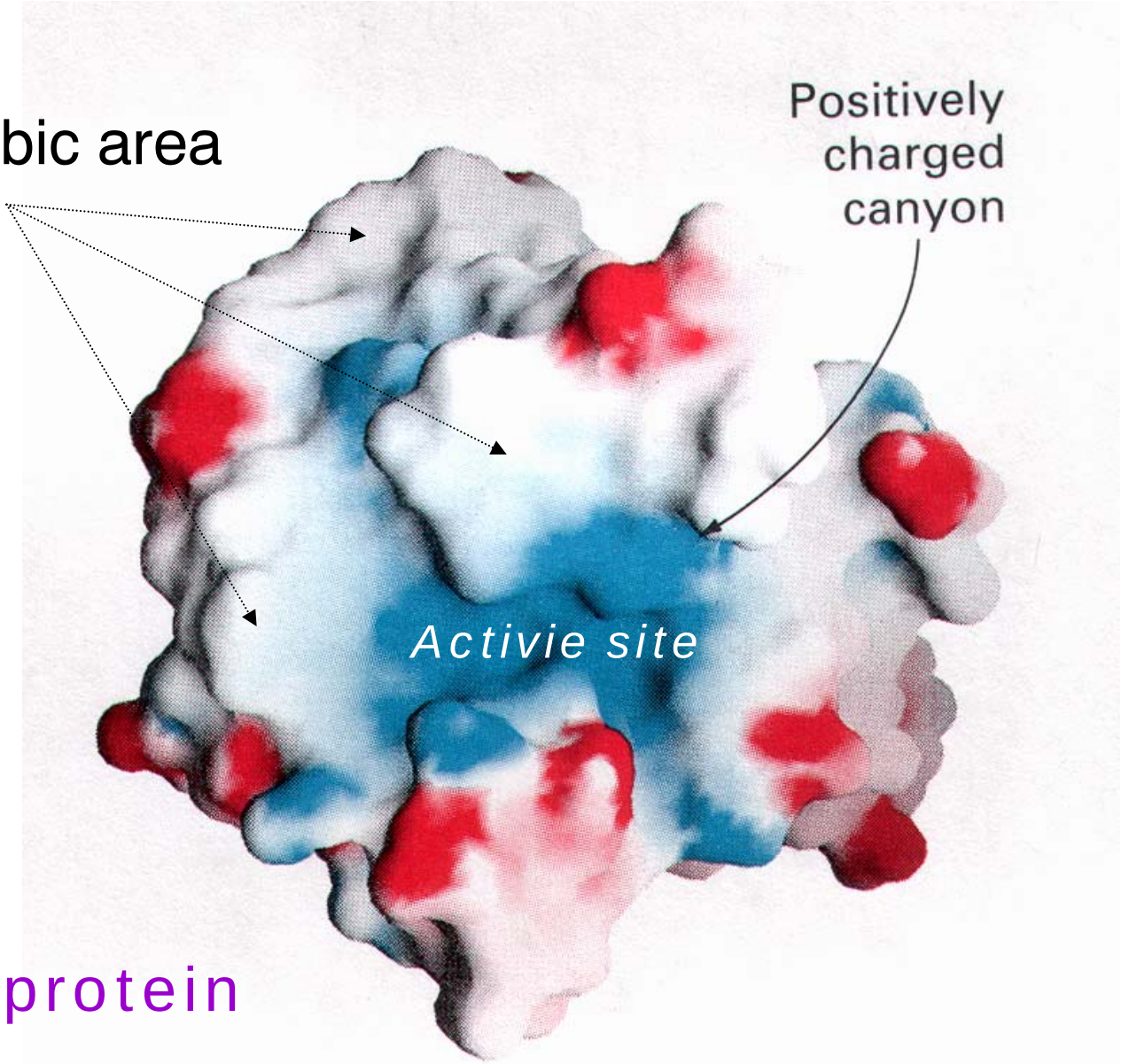
2 蛋白質抽取 Protein extraction

- 2.1 如何開始？ How to start?
  5W principles 基本原則
- 2.2 材料來源 Materials & sources
  材料取得與保存
- 2.3 均質及抽取 Homogenization & extraction
  確實做好第一步
- 2.4 鹽析及沉澱法 Salting-out & precipitation
  最經濟方便的方法

蛋白質表面的極性或非極性分布



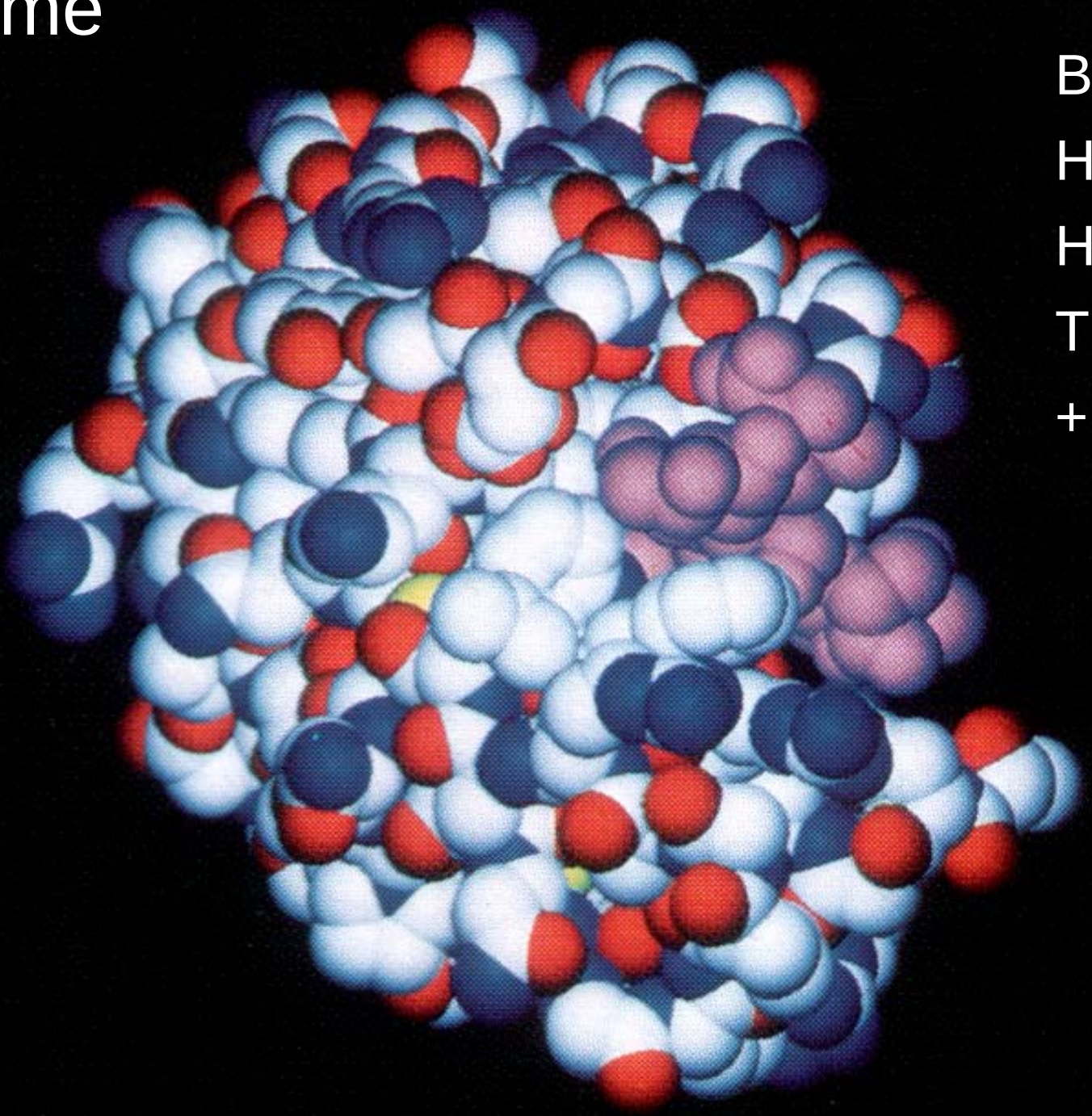
A typical protein

Superxoide dismutase (SOD)

Protein surface has both polar and non-polar patches.

Stryer L (1995) Biochemistry 4/e Fig. 21-36

Lysozyme

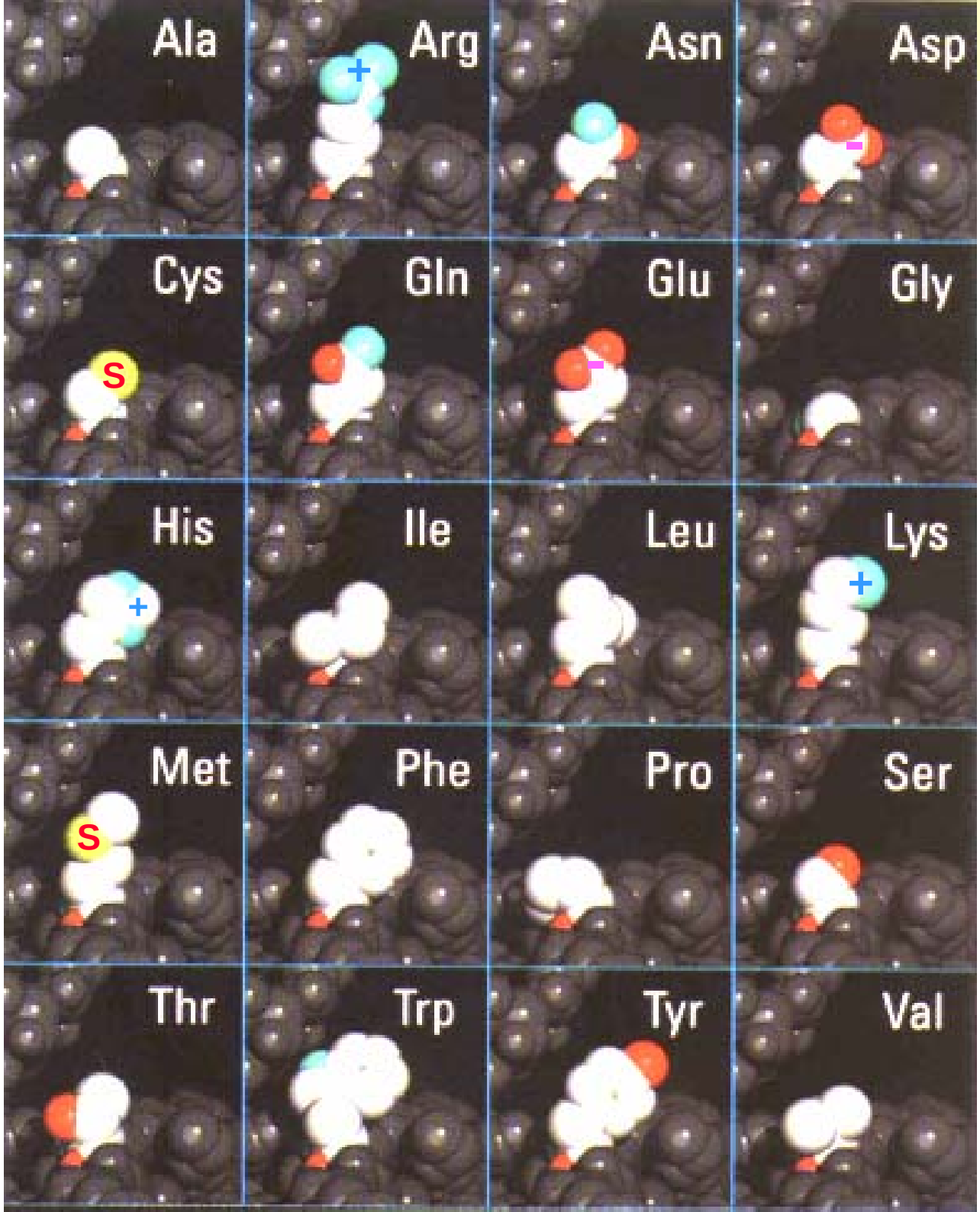


Backbone
Hydrophobic
Hydrophilic
Total
+ substrate

Watson et al. (1987) Mol Biol Gene, Plate 3

二十種胺基酸的特徵

Structural features of 20 amino acids



Side chains

有大有小

Large or small

有正有負

Positively or negatively charged

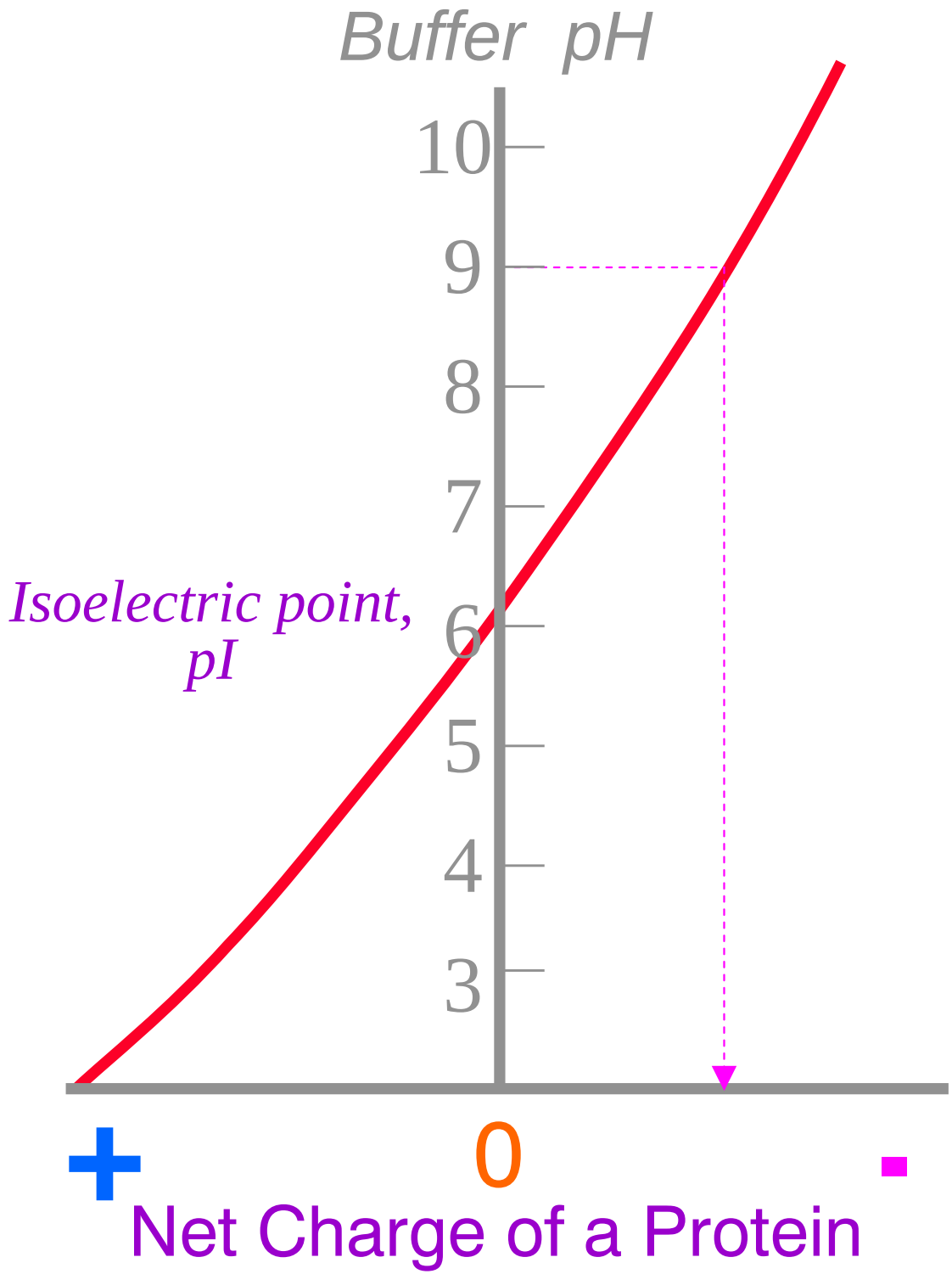
有極性

非極性

Polar or non-polar

Bio-Lab 廣告 Cell 83 (4), 1995

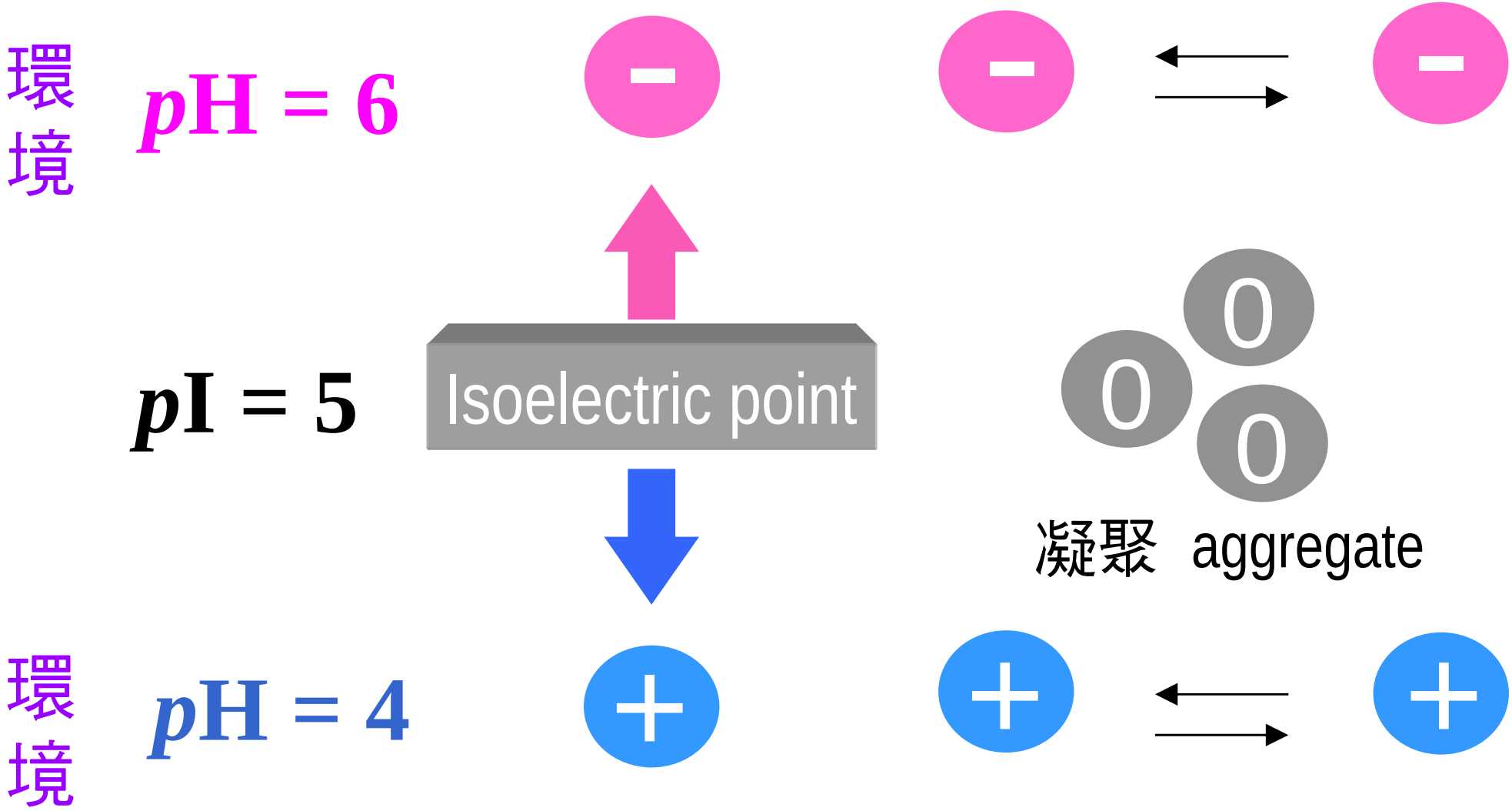
環境影響分子的帶電性質



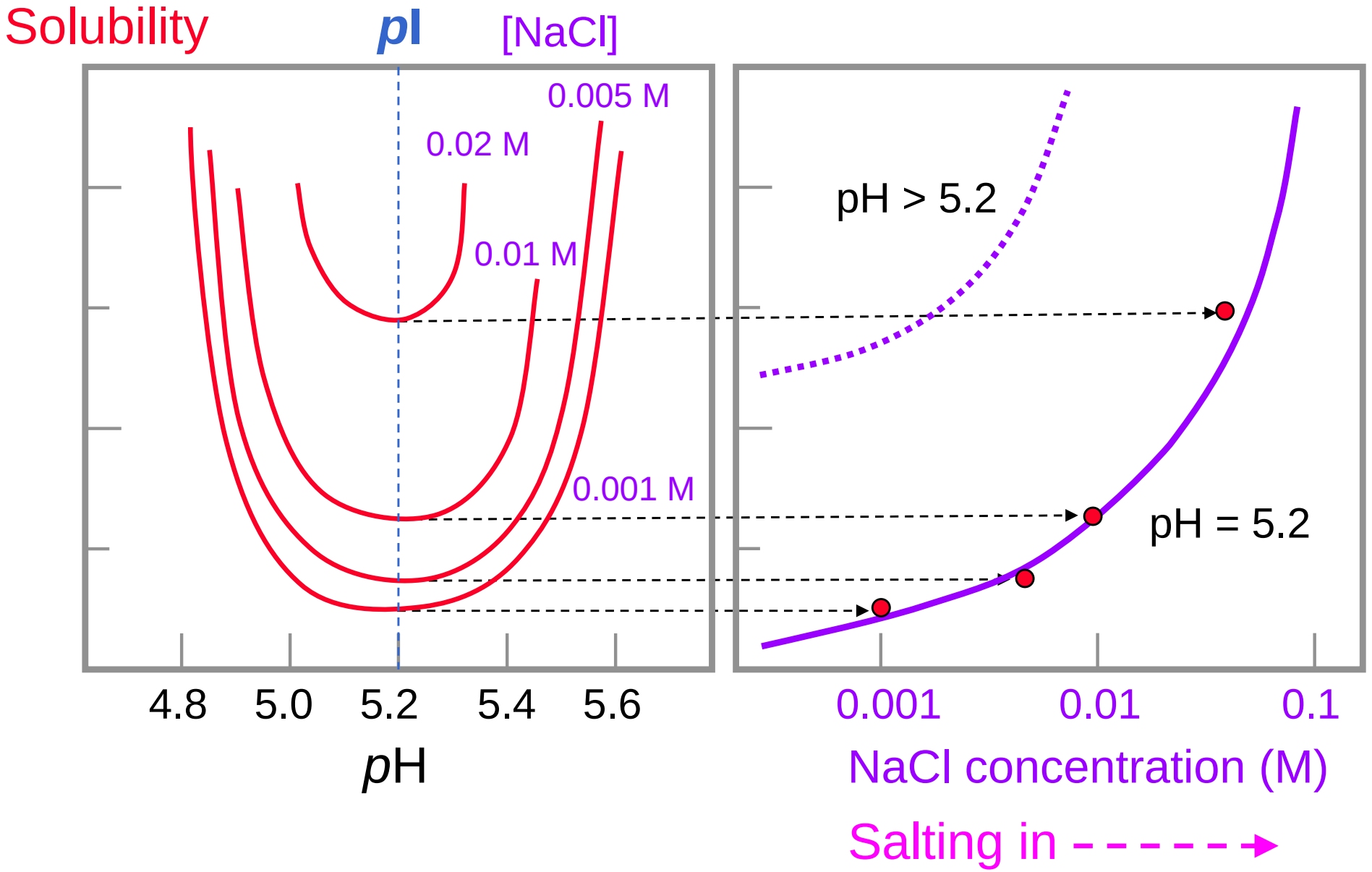
Environmental pH effects the charge properties of a protein

Juang RH (2005) EPA

等電點與環境 *p*H 的關係 pI versus pH



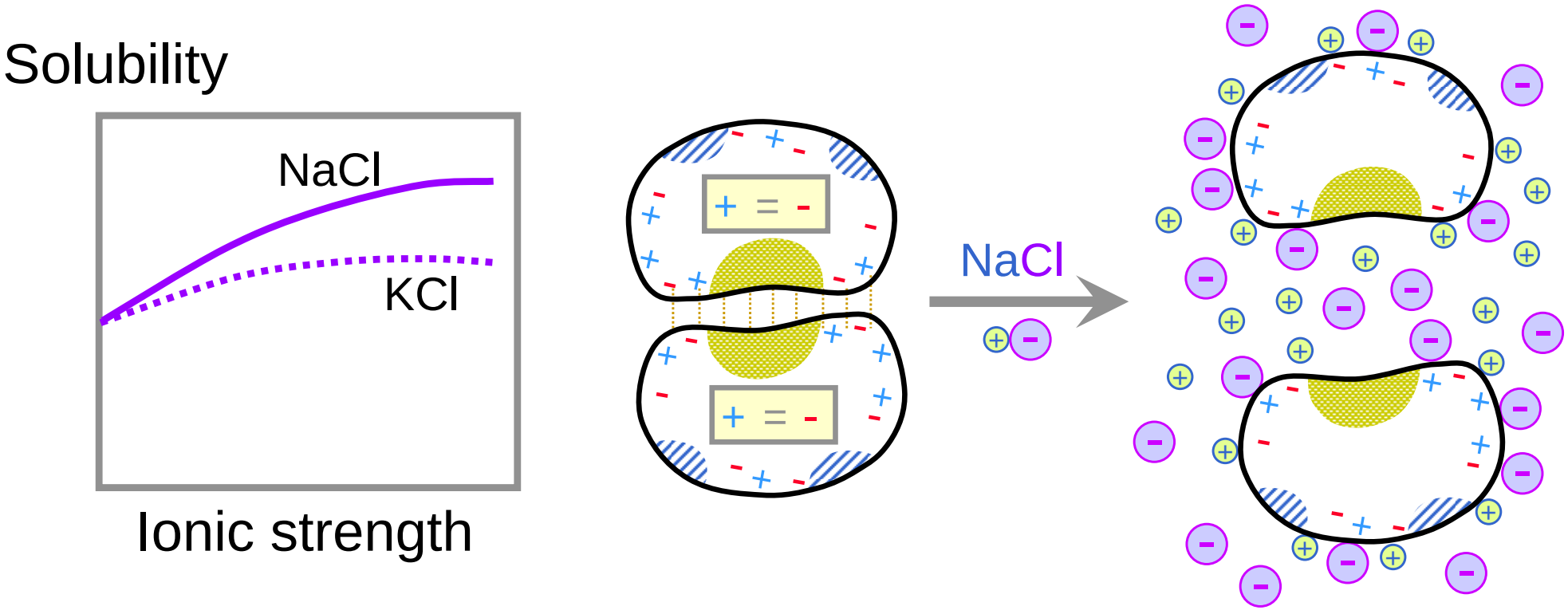
提高鹽濃度增加蛋白質溶解度



Higher salt concentration increases the solubility of a protein

Juang RH (2005) EPA

鹽溶 Salting-in effect

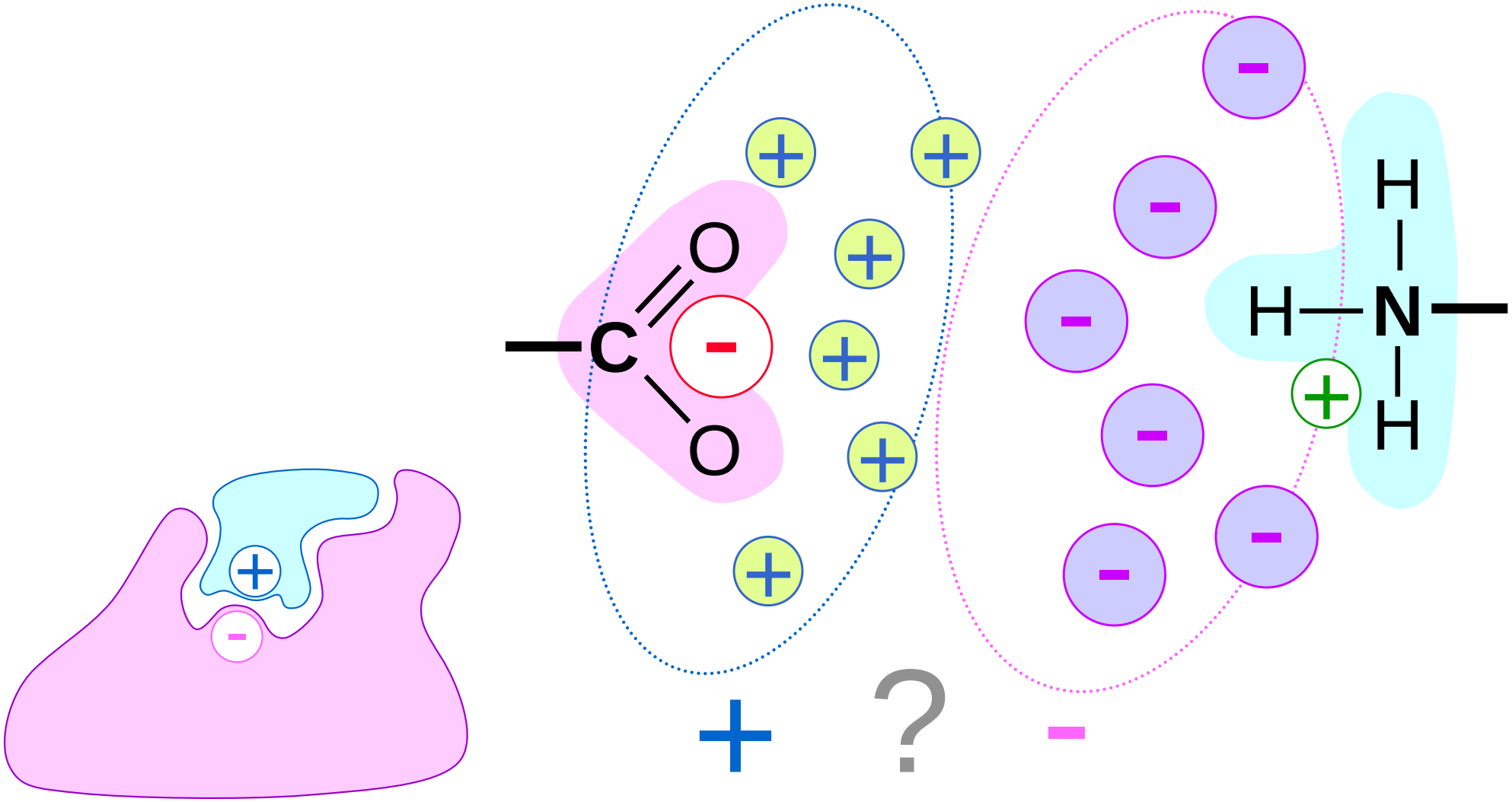


分子在其等電點時，容易互相吸引，聚合成沈澱；加入鹽離子會破壞這些吸引力，使分子散開，溶入水中。

Juang RH (2005) EPA

離子鍵在鹽溶液中不易形成

Adapted from Alberts et al (2002) Molecular Biology of the Cell (4e) p.115



- 水中的酵素與基質還是可以生成離子鍵

But the enzyme-substrate binding can still depend on ionic interactions (why?)

Ionic bonding is not stable in water solution

鹽影響蛋白質溶解度 Salt effects protein solubility

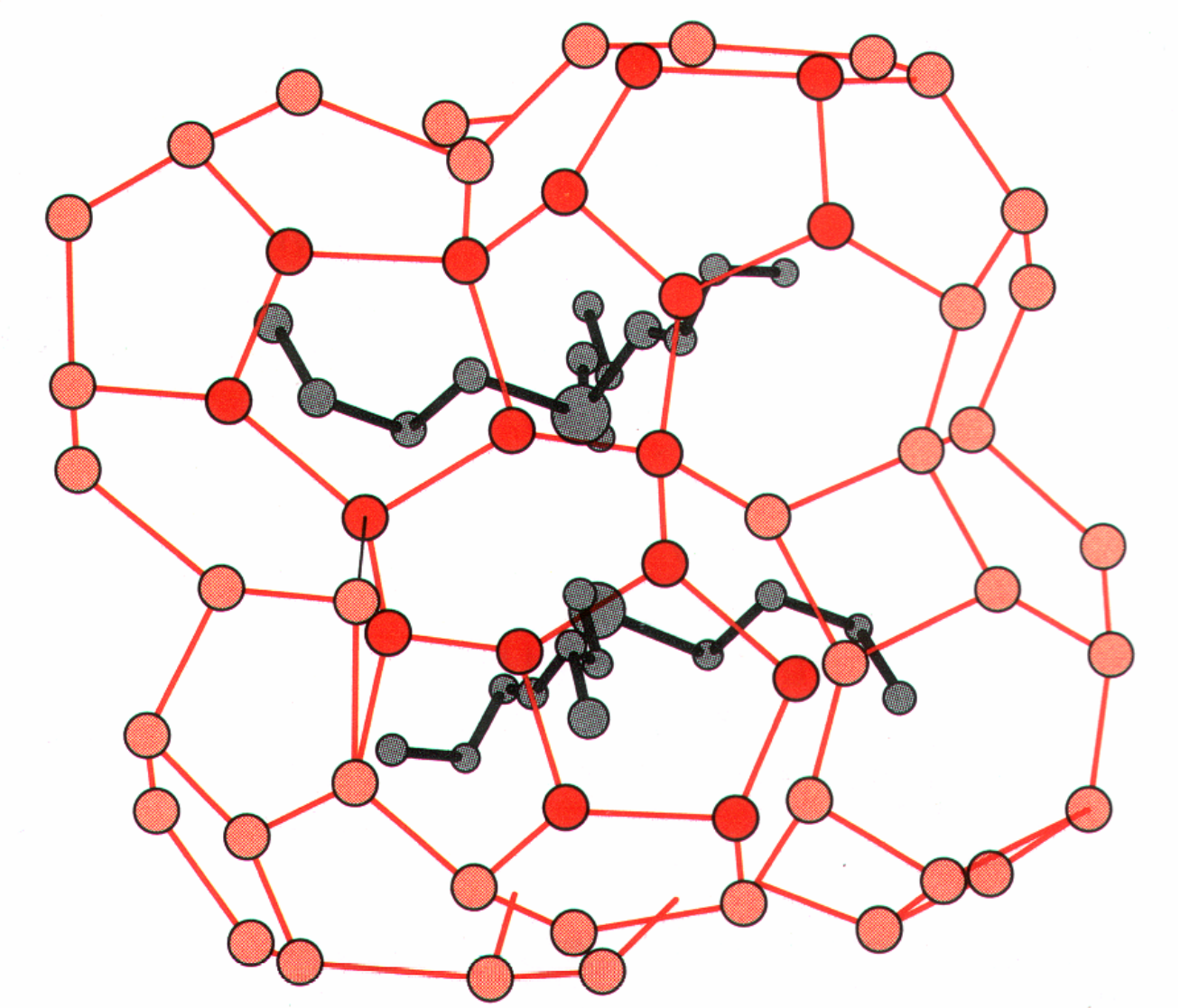
- **鹽溶 Salting-in:**

  加鹽使蛋白質溶入水溶液中

- **鹽析 Salting-out:**

  加鹽使蛋白質由水溶液中沉澱出來

疏水性物質間的親和力 水籠 Clathrate

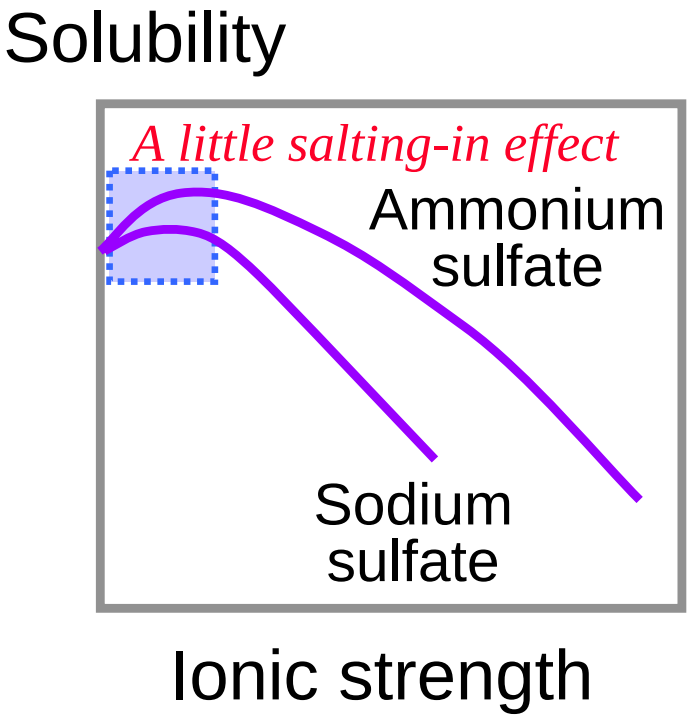


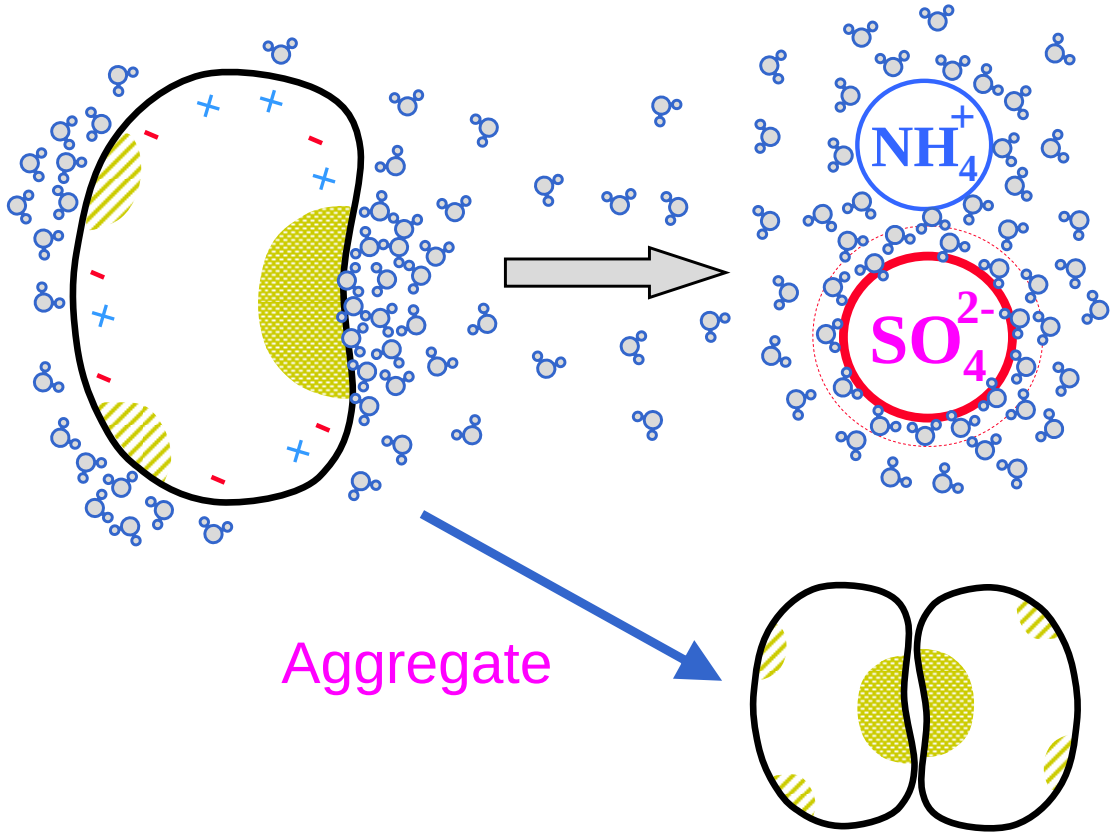
- 水分子會包圍在非極性分子四周，形成類似竹籠的構造，隔離非極性分子，水分子本身的流動性因此而降低。

Water molecules surrounding the outer surface of non-polar molecules are “immobilized” and form a “cage of water” to isolate these non-polar interface

Alberts et al (1994) Molecular Biology of the Cell (3e) p.49

鹽析 Salting-out





蛋白質分子表面的疏水性區域，都聚集許多水分子，當鹽類加入時，這些水分子被抽出，以便與鹽離子進行水合，暴露出來的疏水性區域互相結合，形成沈澱。

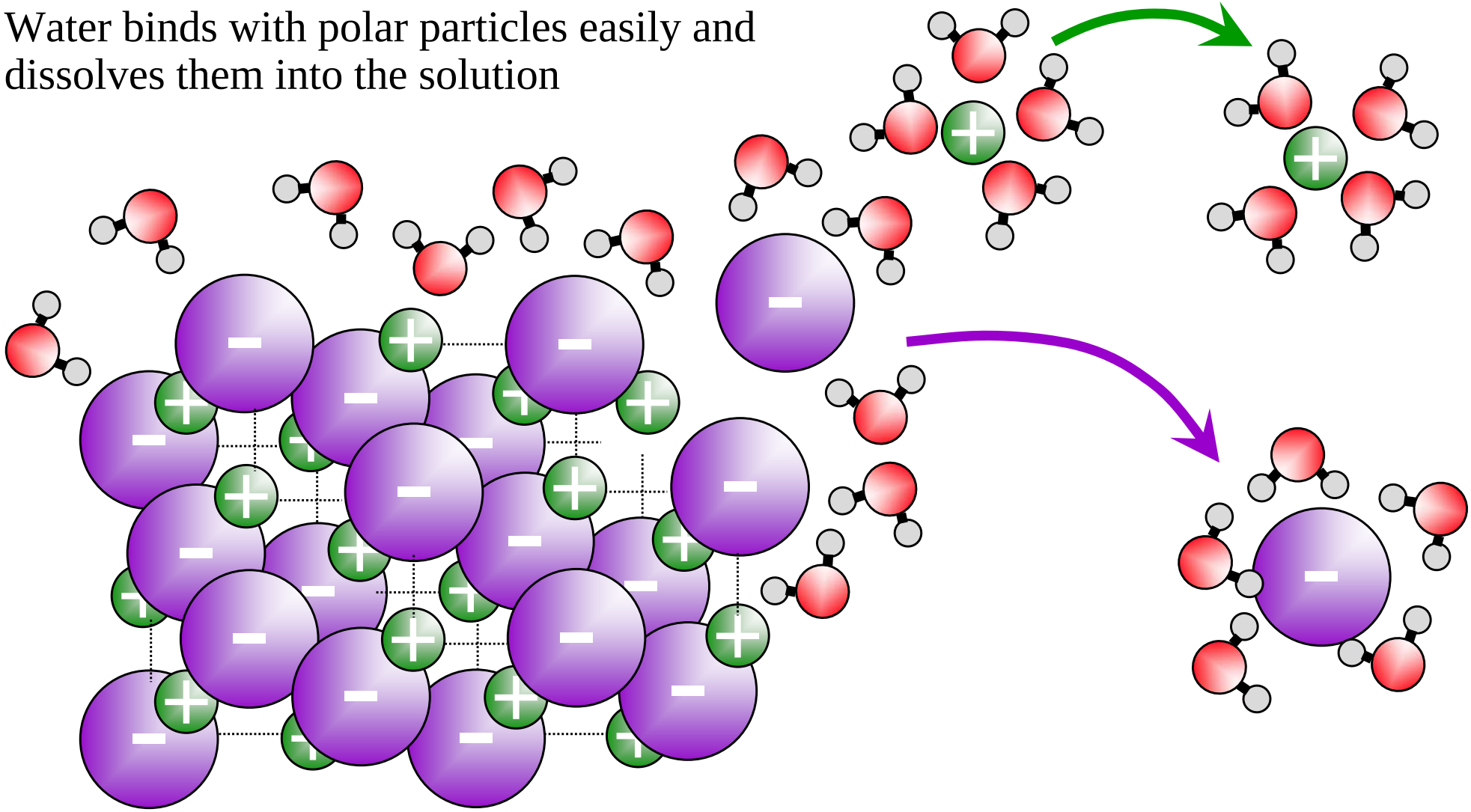
= hydrophobic

Juang RH (2005) EPA

無所不在的水合作用 Hydration is everywhere

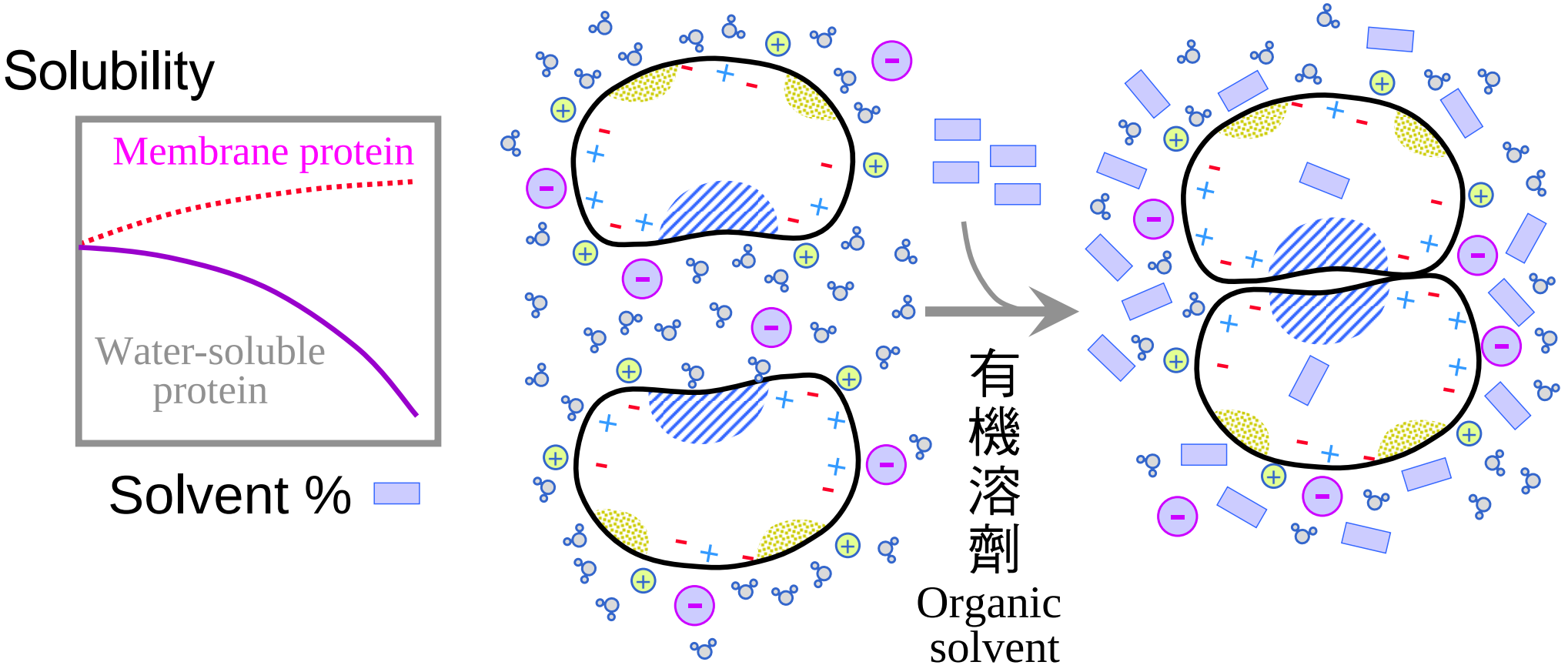
- 水分子易與極性粒子發生水合

Water binds with polar particles easily and dissolves them into the solution



Adapted from Nelson & Cox (2000) Lehninger Principles of Biochemistry (3e) p.87

有機溶劑沈澱法 Precipitation by organic solvent



降低水活性，使溶液的介電常數下降，增加蛋白質溶質分子之間的作用力，因而聚集在一起。

= hydrophilic

Juang RH (2005) EPA

各種鹽析沉澱法比較 Comparison of methods

	Salting-in 鹽溶	Salting-out 鹽析	Organic solvent
Factors	Ionic interactions on protein surface	Non-polar area of protein surface	All interaction forces on protein surface except hydrophobic
Reagents	NaCl (monovalent)	$(NH_4)_2SO_4$ (divalent)	Methanol, acetone
Mechanism	Protein has no net charge at its pI, that leads to the binding between proteins via ionic interactions, and precipitation. Salt can interfere these ionic interactions and separate bound protein molecules.	Big divalent ions attract water molecules immobilized on the protein surface, expose the non-polar surface, which then interacts with other proteins to form precipitate.	Organic solvent decreases the water activity and the dielectric constant of the solution, which then decreases the solubility of the protein and precipitates it.
Fig	Fig 2.3	Fig 2.4	Fig 2.5
Remarks	The reverse process of salting-in is not salting-out, it is the dialysis process against a dilute solution.	1) Non-polar proteins will be precipitated earlier. 2) Protein is very stable in ammonium sulfate.	1) Some proteins might be denatured by heat produced. 2) Factors facilitate precipitation: larger protein, pH close to protein pI. 3) Lipophilic protein might be dissolved more readily.