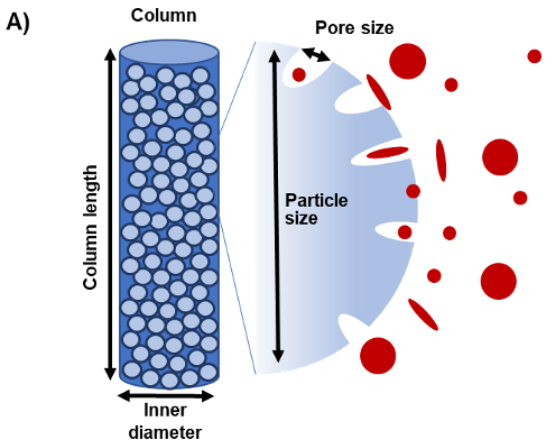
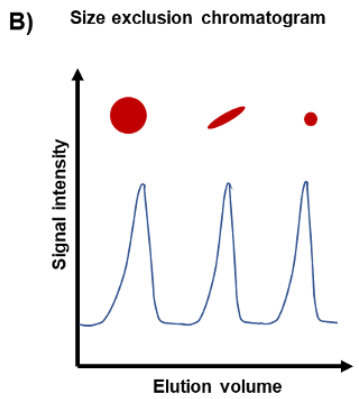
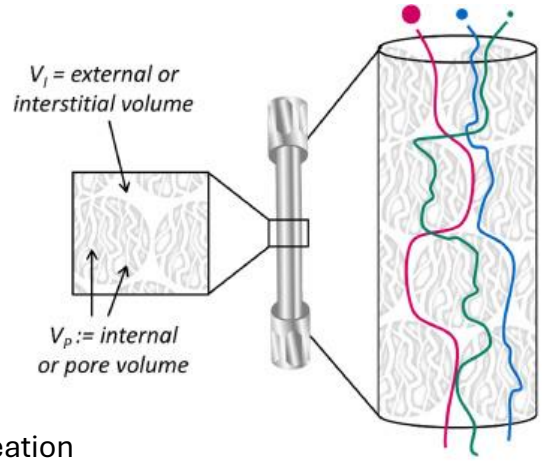
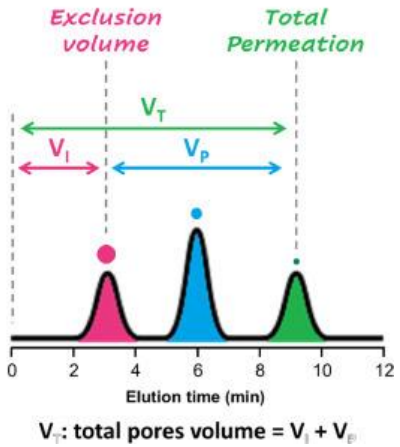
Size exclusion chromatography

Principles; separate species such as proteins based on their hydrodynamic radius





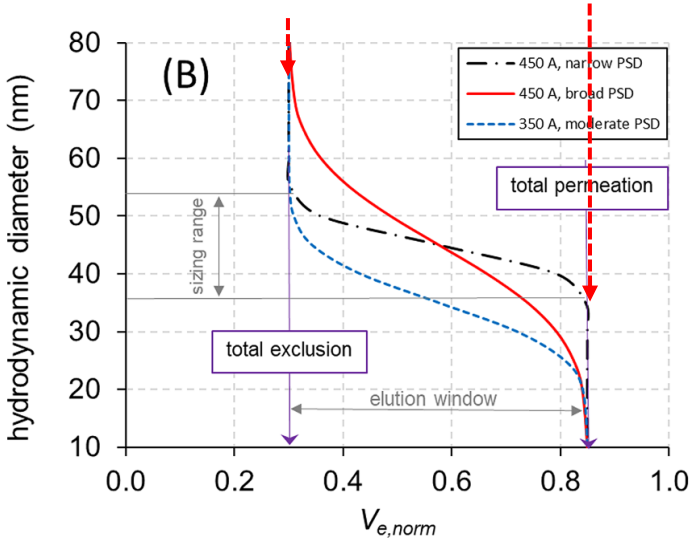




V_T: total pores volume = $V_I + V_P$

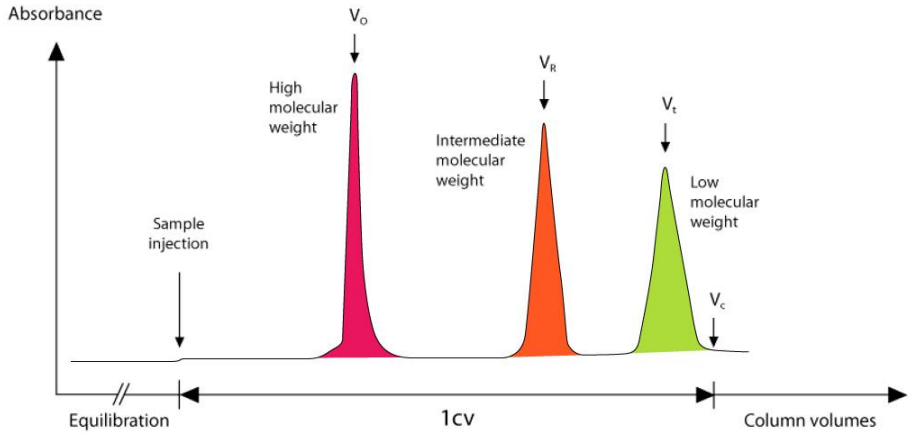
Exclusion volume
High molecular weight

Total permeation
Low molecular weight



CQ

Impurities
and identities

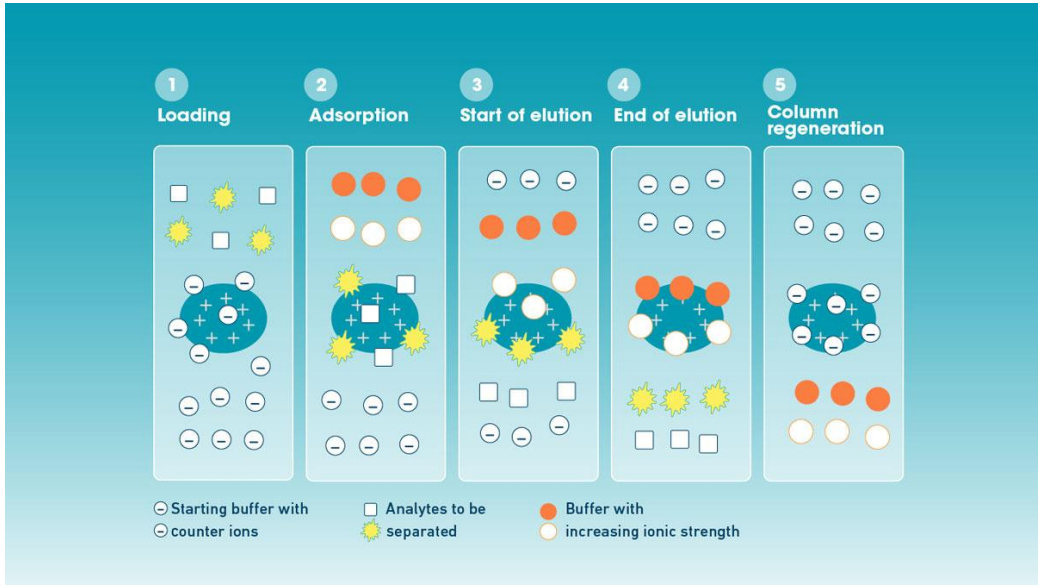


fractionation range : the elution window between the exclusion and permeation :

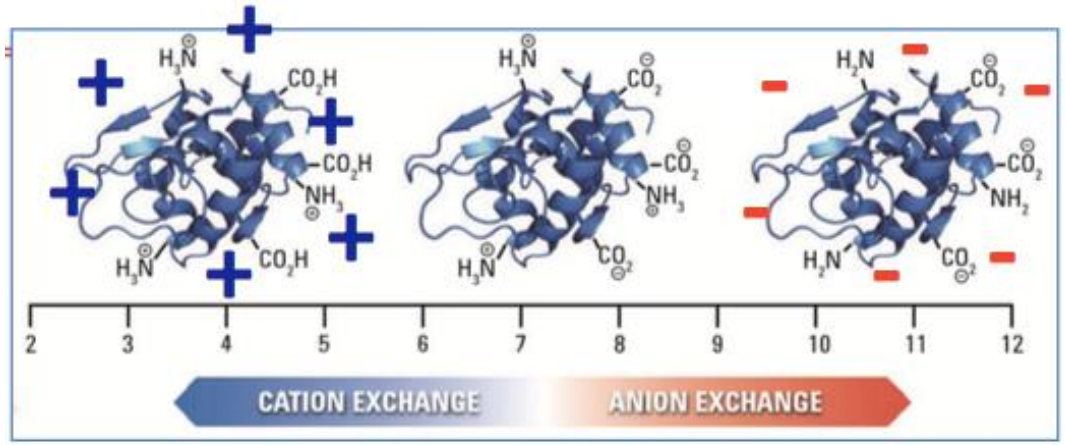
Ion exchange chromatography

Principles ; molecules are separated on the basis of their charge are eluted using a solution of varying ionic strength

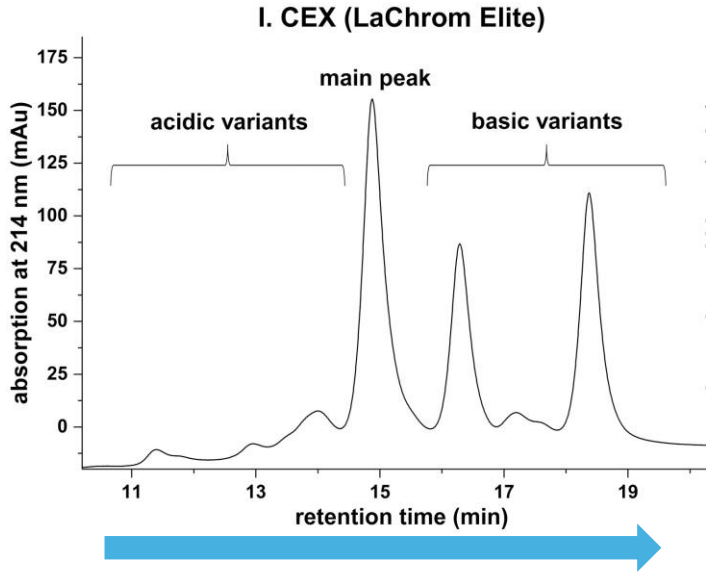
Principles



pI protein net charge



CEX AEX



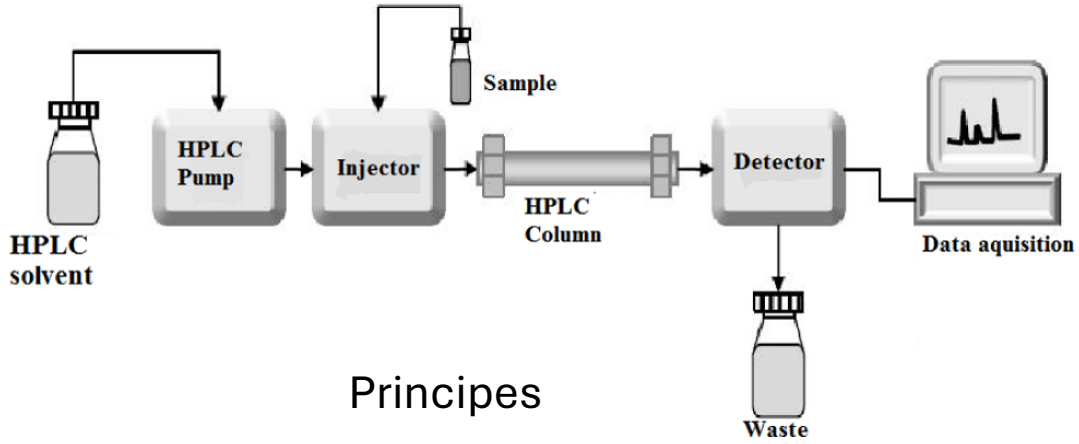
salt gradient

Reverse Phase Chromatography

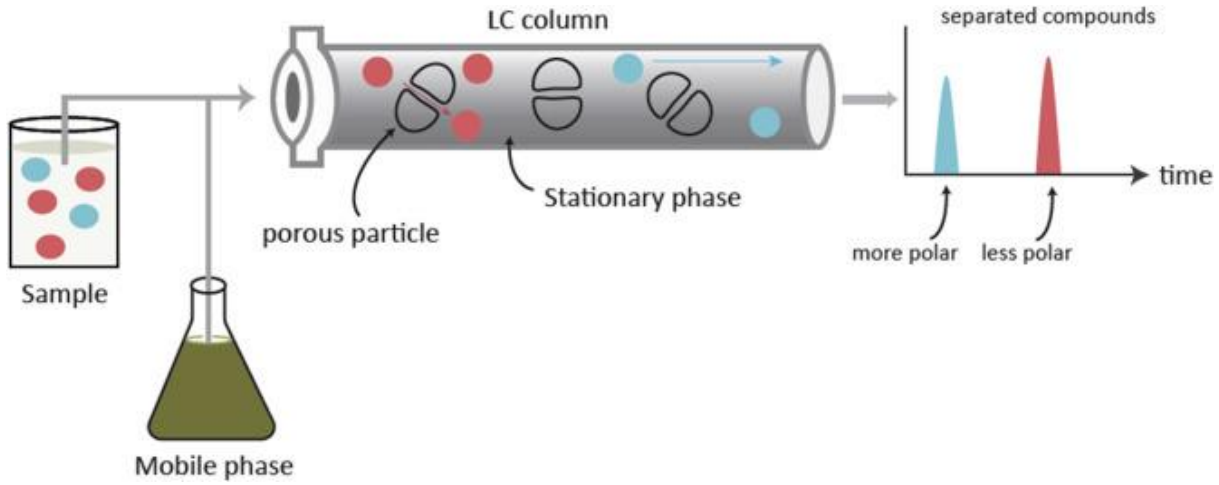
(RP-HPLC) involves the separation of molecules on the basis of hydrophobicity

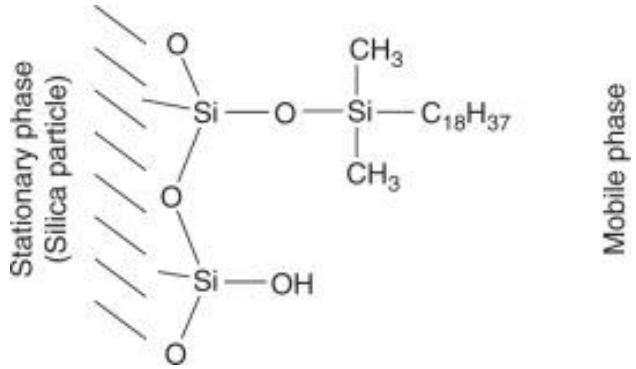
please also refer to the peptides mapping slides made by your classemate

Equipement

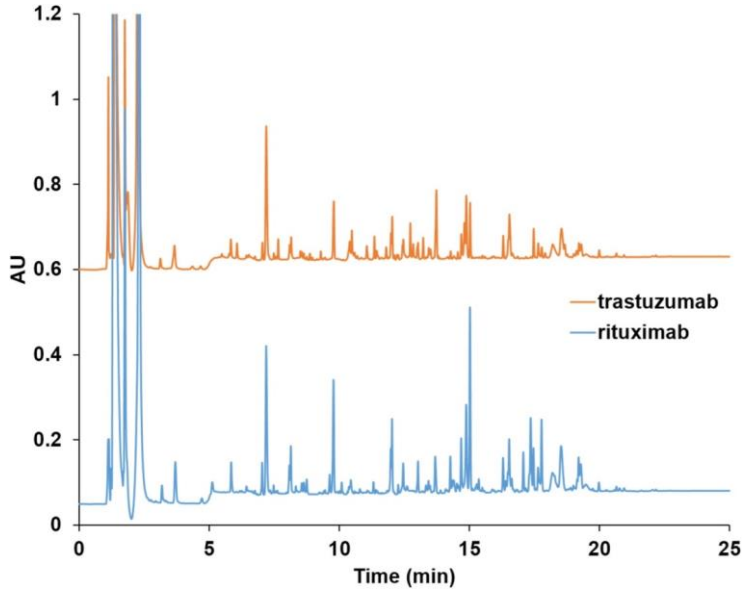


Principes



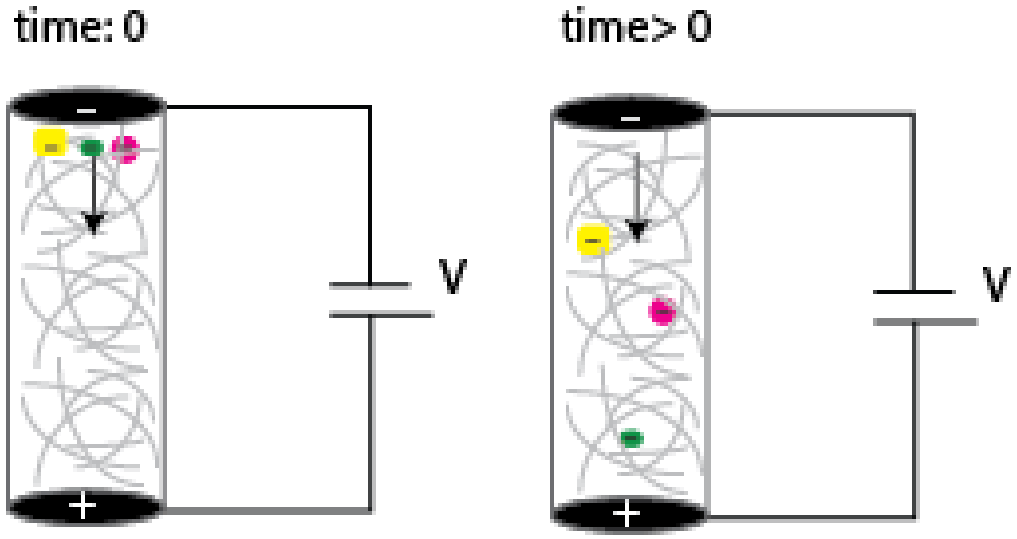


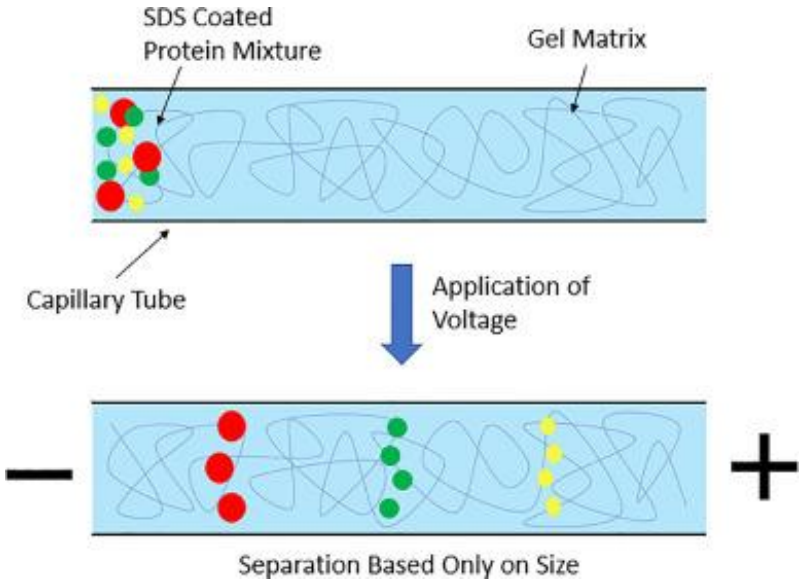
Example : peptide mapping of therapeutic monoclonal antibodies



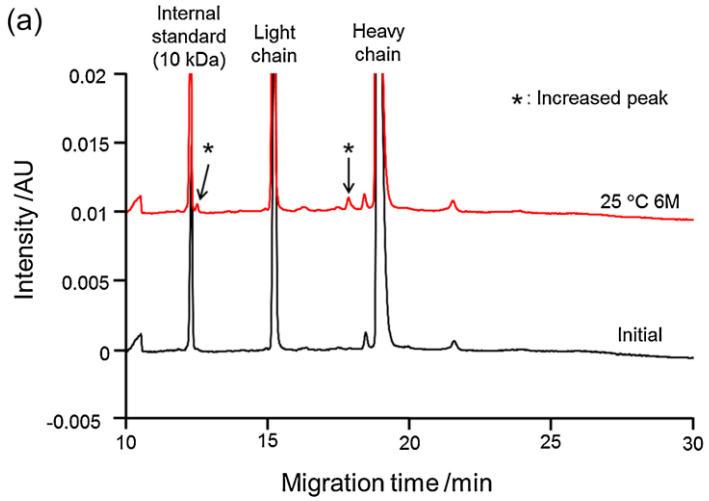
Capillary Gel Electrophoresis

1- Capillary conditioning
2- Filling with gel buffer
3-Injection of the analyzed samples (heating + SDS)
4-Electric field is applied
5-Similar electrophoretic migration
(similar charge to mass ratios)
electrophoretic mobility of the protein–SDS complex is proportional to the log (Mw)
6-Sieving through polymer networks
Proteins are separated in increasing size order
7- detection UV or fluorescence





Reduced monoclonal antibodies



Degradation sample

mAb initial sample