

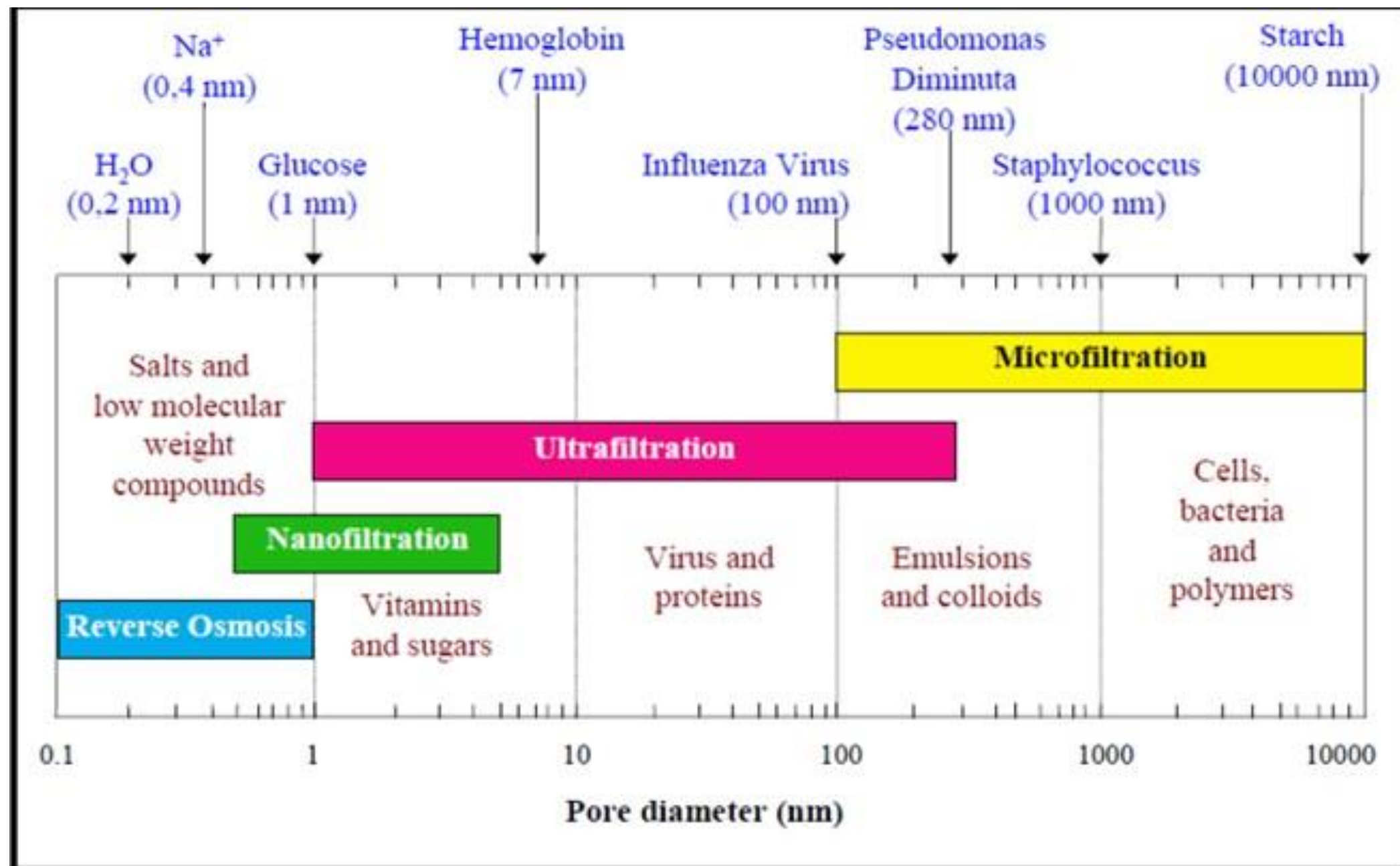
Preparation of Enzymes

FE 361 Enzymology

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Ultrafiltration

- Both dialysis and ultrafiltration are based on
 - Separation of molecules according to size
 - Employing a semi-permeable membrane with a defined range of a pore size

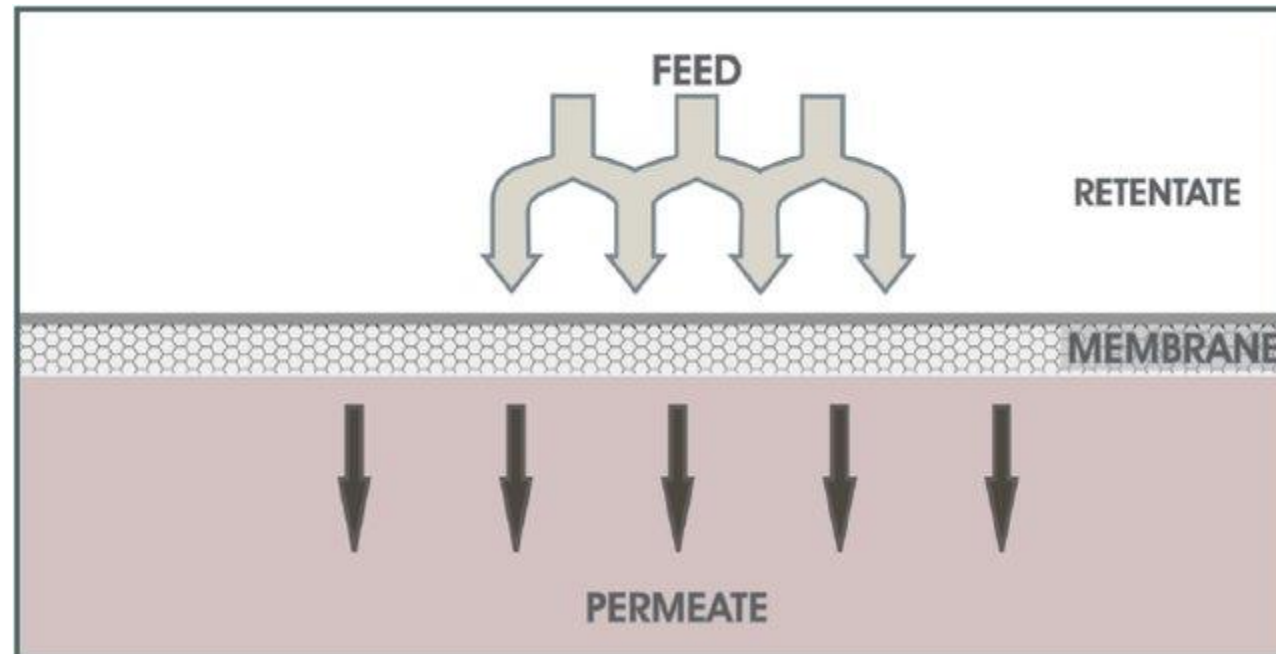


- Ultrafiltration will remove:
- High molecular weight substances
- Colloidal materials
- Organic polymeric molecules
- Inorganic polymeric molecules

- This separation process is used in industry and research for purifying and concentrating macromolecular ($10^3 - 10^6$ Da) solutions, especially protein solutions.
- Pore diameters in the 10-1000 Å range

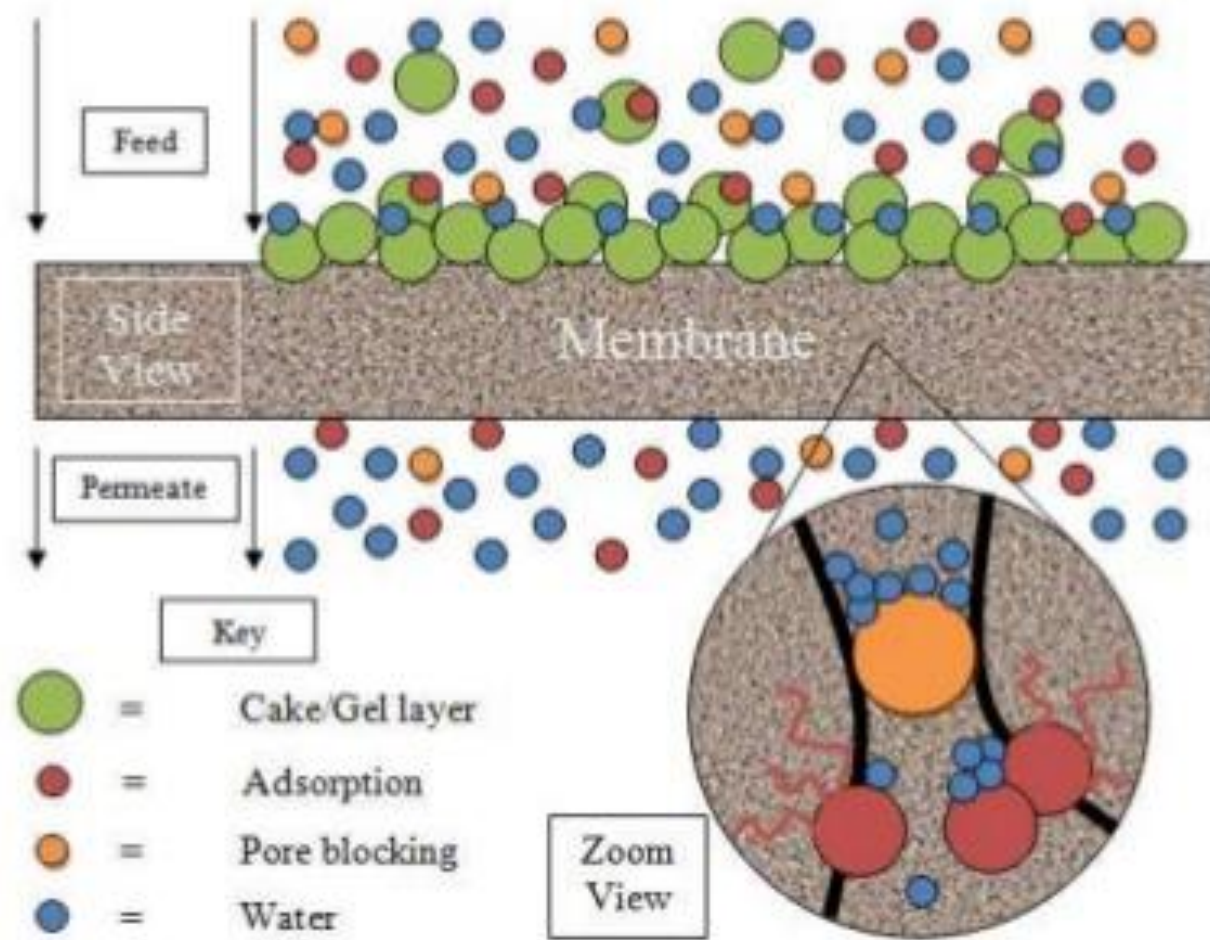
Principle of Ultrafiltration

- Ultrafiltration concentrates a protein solution using a selective permeable membranes. This membrane allows the solvent and small molecules mass through while retaining the protein.
- Ultrafiltration requires a force to drive solutes through the membrane. The solution is forced against the membrane by pump, gas pressure or centrifugation.



- The ability of an ultrafiltration membrane to retain macromolecules is traditionally specified in terms of its molecular cut-off (MWCO).
- A MWCO value of 10 kDa means that the membrane can retain from a feed solution 90% of the molecules having molecular weight of 10 kDa.
- Ultrafiltration separates solutes in the molecular weight range of 5 kDa to 500 kDa. UF membranes have pores ranging from 1 to 20 nm in diameter.

- The main problem in ultrafiltration of proteins in the vicinity of the membrane. This problem may cause reduction in flow rates. This effect is called as 'concentration polarization' and this problem occurs generally if the protein solution become more concentrated.
- The easiest way to reduce this effect is mixing or stirring of the protein solution by a magnetic stirring device in close proximity to the membrane.
- After concentration of sample once, it is advisable to repeat ultrafiltration by adding fresh solvent.



Differences between dialysis and ultrafiltration

- Similarities:
 - Separate small molecules
 - Concentrate enzyme/protein
 - Membrane separation
- Difference is the driving force

