Diffusional Contributions and Electrostatic Interactions during Ultrafiltration

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Diffusion Fundamentals IV

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Ultrafiltration

- Ultrafiltration is used extensively for protein concentration, buffer exchange, and desalting
  - Formulation of therapeutic proteins
  - Processing of food and beverage products
- Recent studies have demonstrated the potential of using ultrafiltration for protein separations based on different rates of protein transport across semi-permeable membranes

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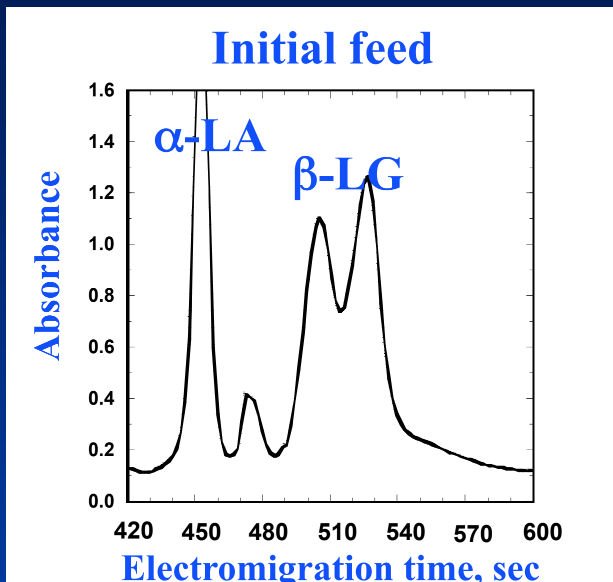
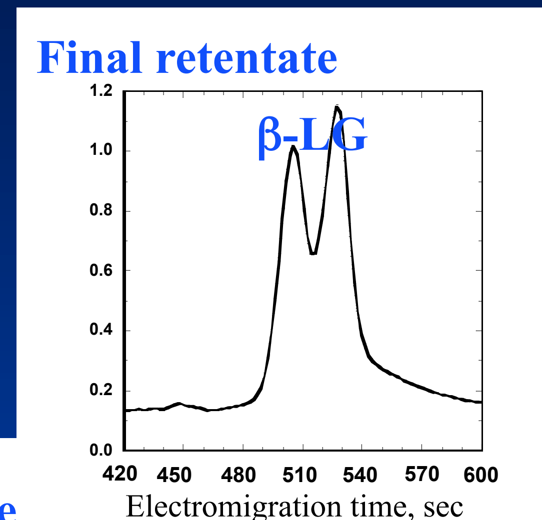
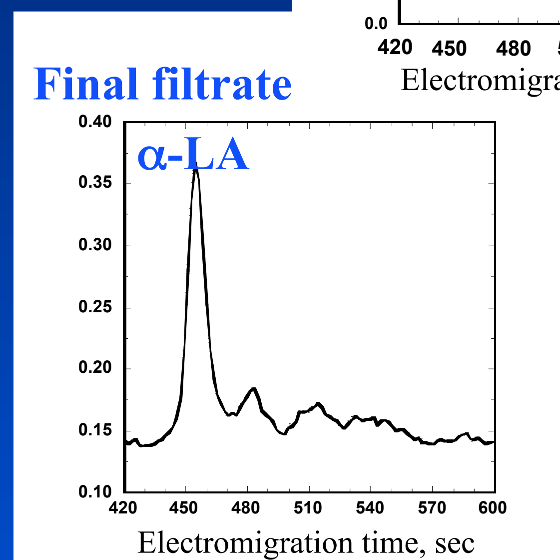
Whey Protein Purification



Ultrafiltration
at pH 5.5
50 mM phosphate

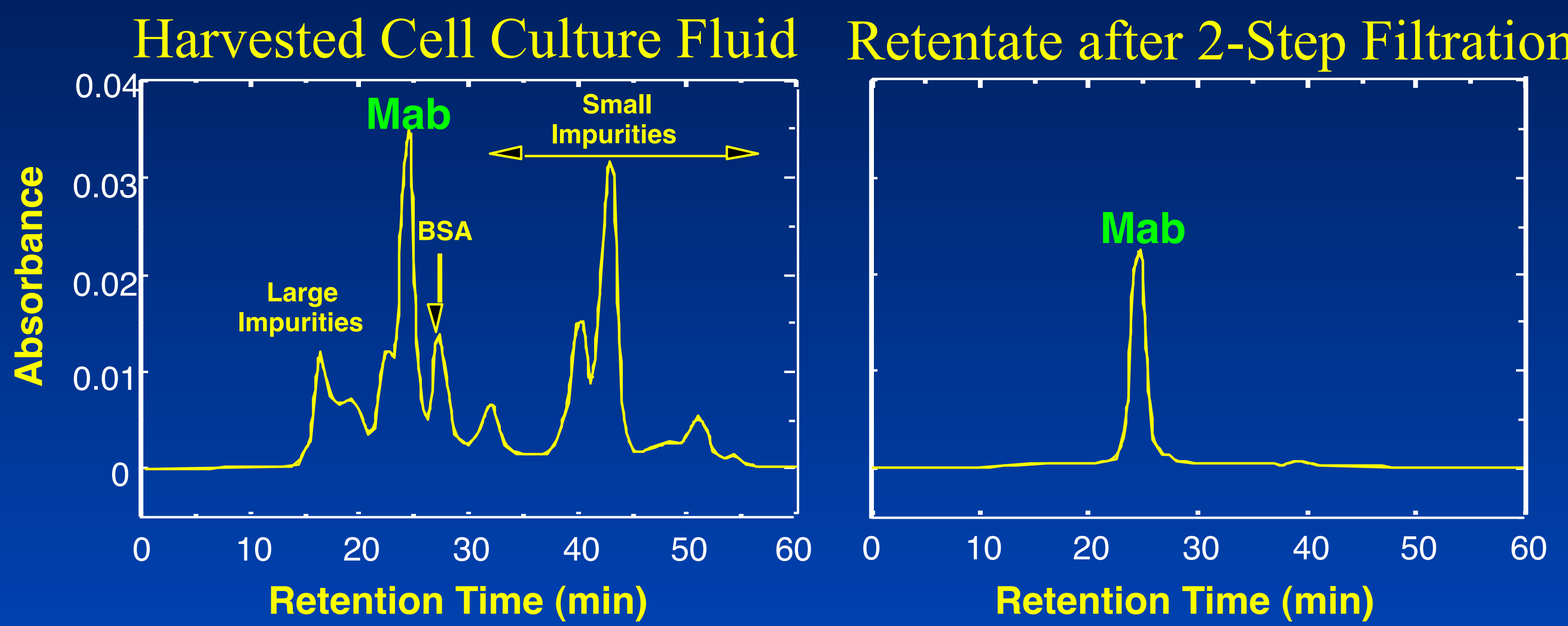




Protein	MW (kD)	pI
β-lactoglobulin	18	5.2
α-lactalbumin	14	4.6

Cheang and Zydney, J Membrane Sci, 231, 159 (2004)

Monoclonal Antibody Purification from Harvested Cell Culture Fluid



- **Purification with two membrane separations:**
  - Step 1: pH 7, 150 mM phosphate (Mab in permeate)
  - Step 2: pH 4, 10 mM acetate (Mab in retentate)

Membrane Transport

- Use of membranes for protein purification has generated renewed interest in understanding protein transport through semipermeable membranes
  - Diffusion / convection in highly constricted pores, including effects of pore geometry
  - Effects of electrostatic interactions (solution pH, ionic strength, membrane charge)

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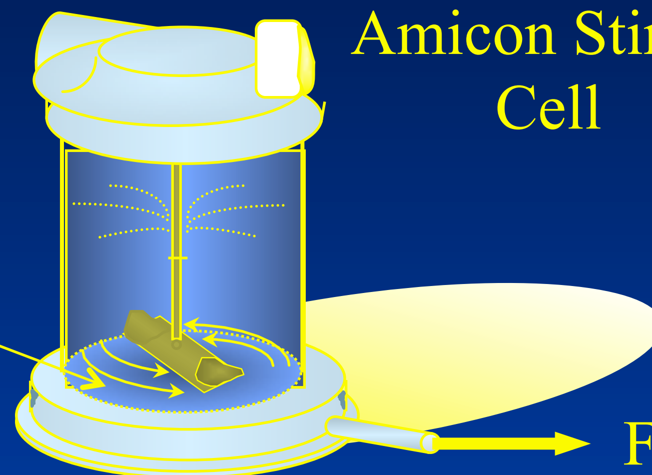
Stirred Cell Apparatus



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Filtrate Flux:

$$J_v = \frac{Q}{A}$$

Observed Sieving Coefficient:

$$S_o = \frac{C_{filtrate}}{C_{bulk}}$$

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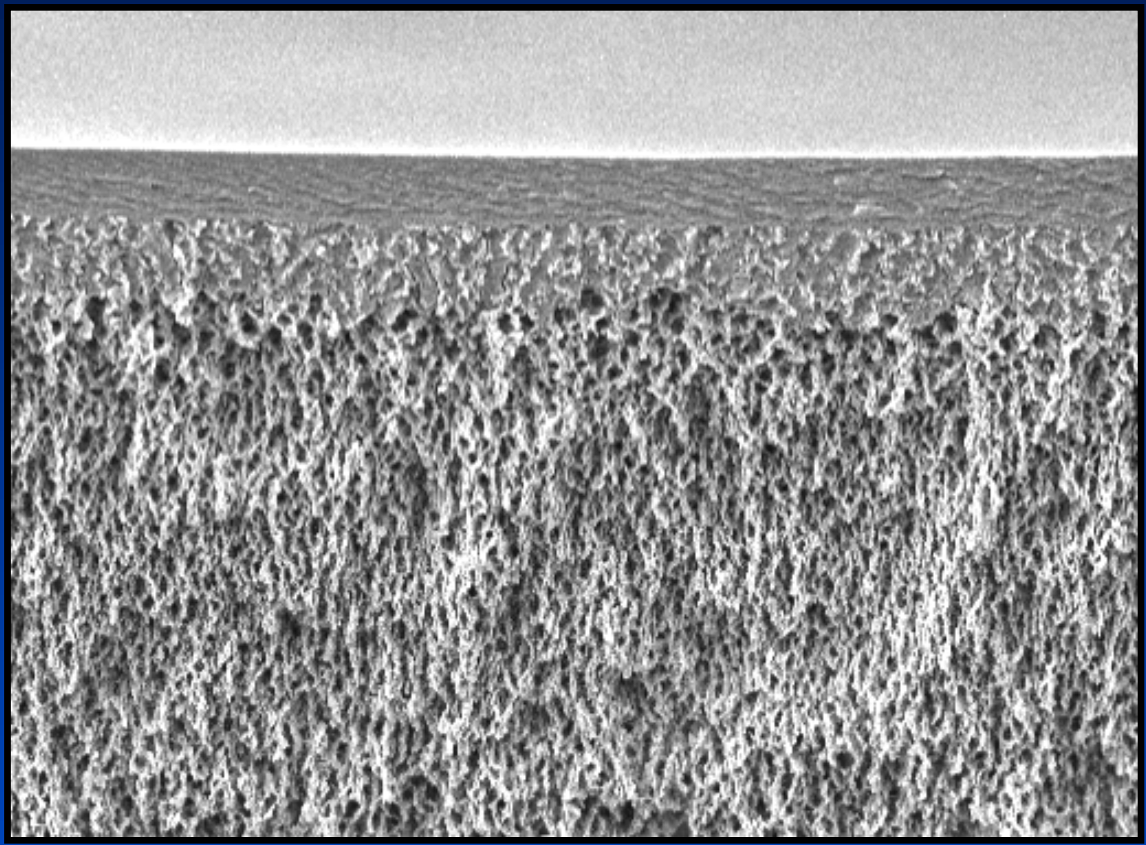
Ultracel Membrane



Skin

Support structure

- Composite regenerated cellulose membrane
- SEM cross-section provided by Millipore

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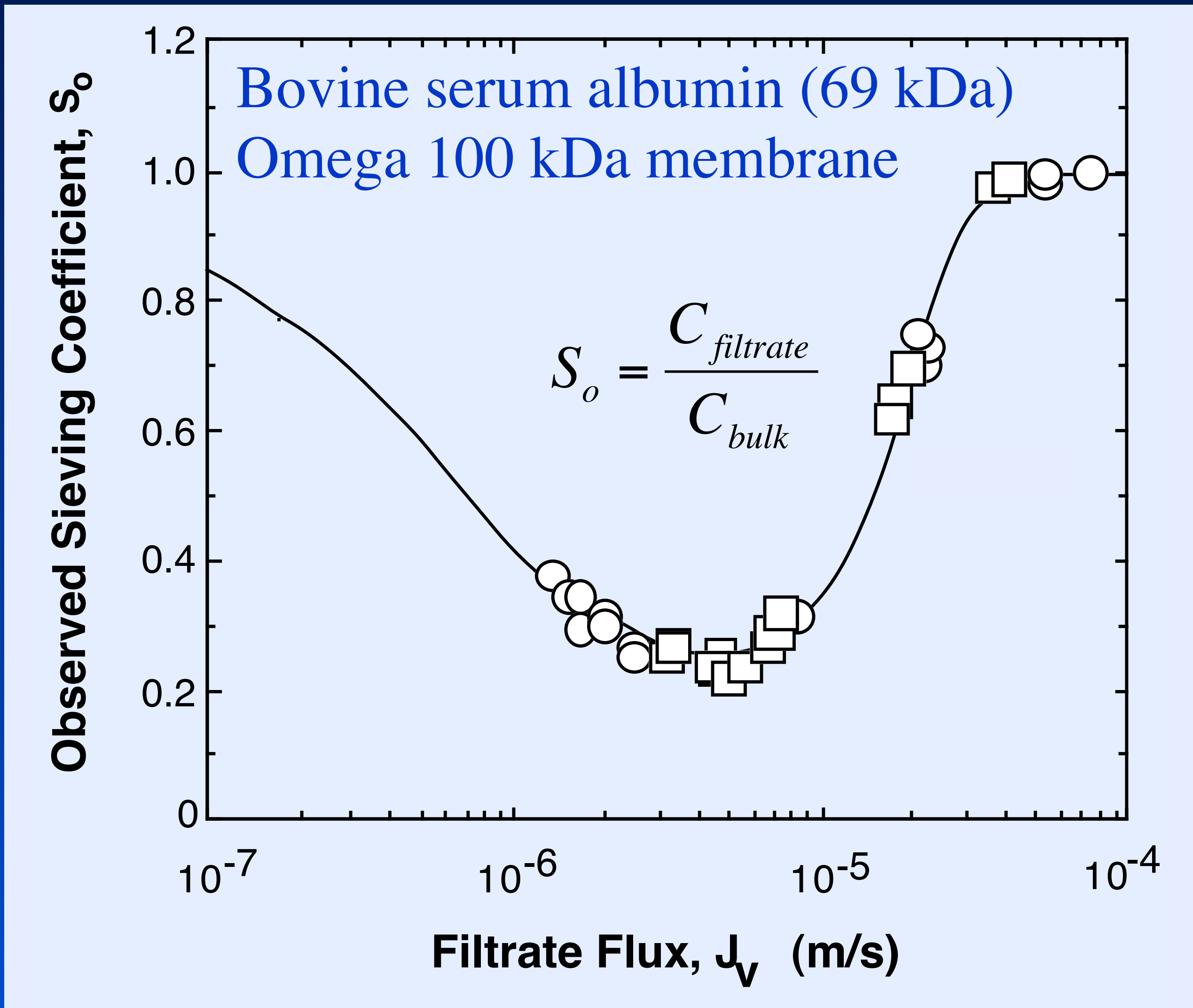
Sieving Coefficient vs Flux



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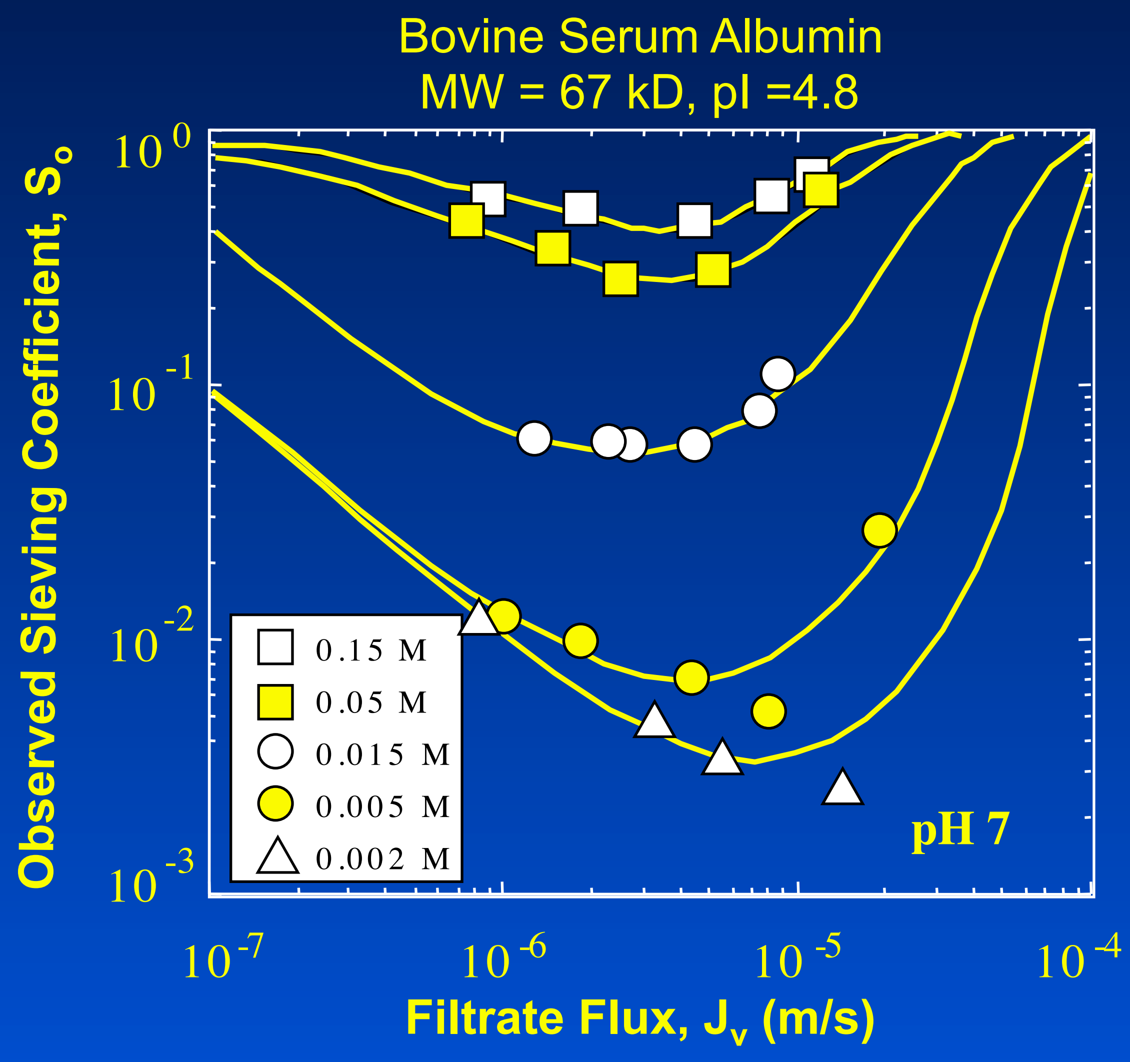
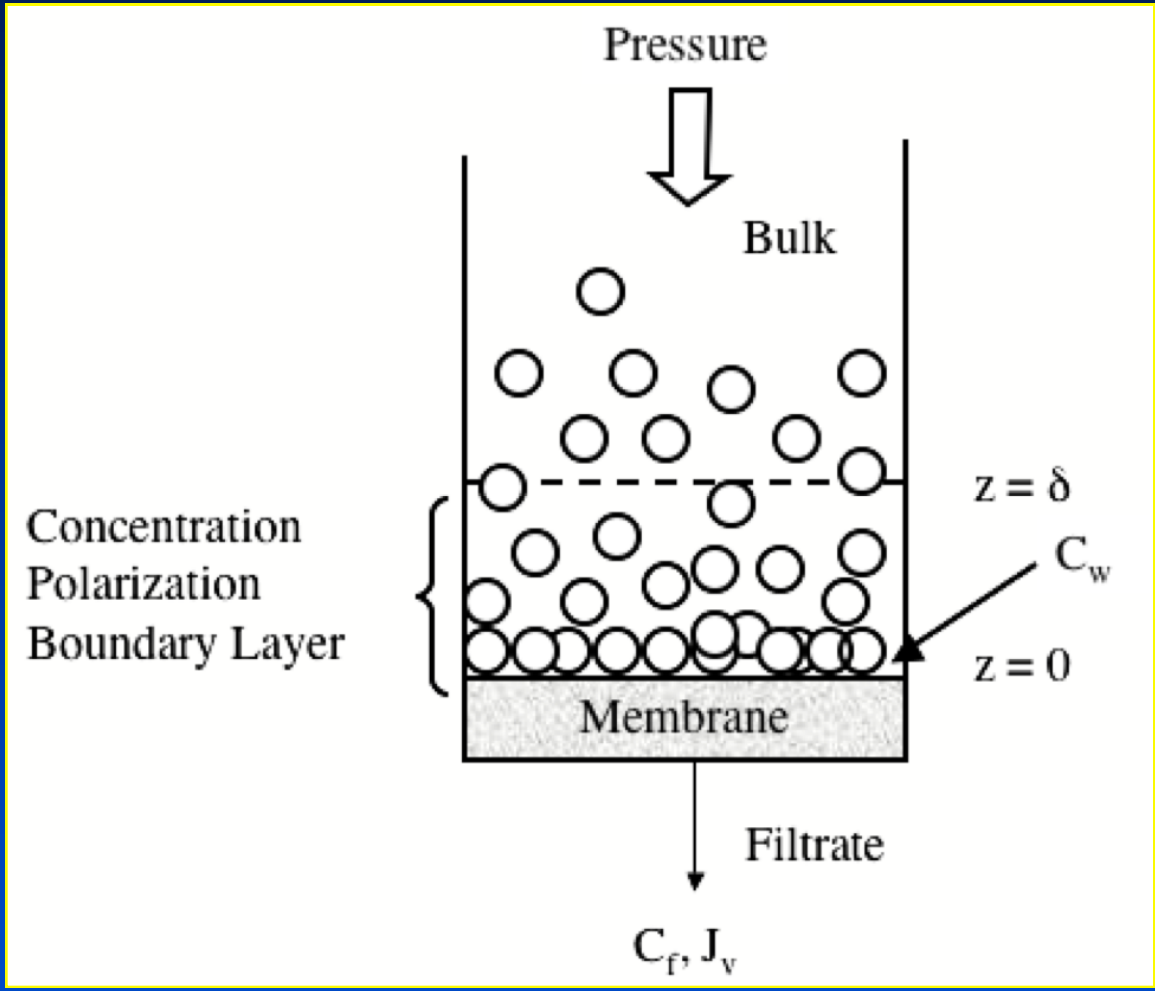
Effect of Ionic Strength



Physical Phenomena

- Protein sieving coefficient varies by over two orders of magnitude
  - Significant effects of both filtrate flux and solution ionic strength
- Protein sieving coefficient shows a distinct minimum with increasing filtrate flux, approaching a value of one at high flux
  - Concentration polarization (bulk transport)
  - Diffusion / convection through membrane

Concentration Polarization



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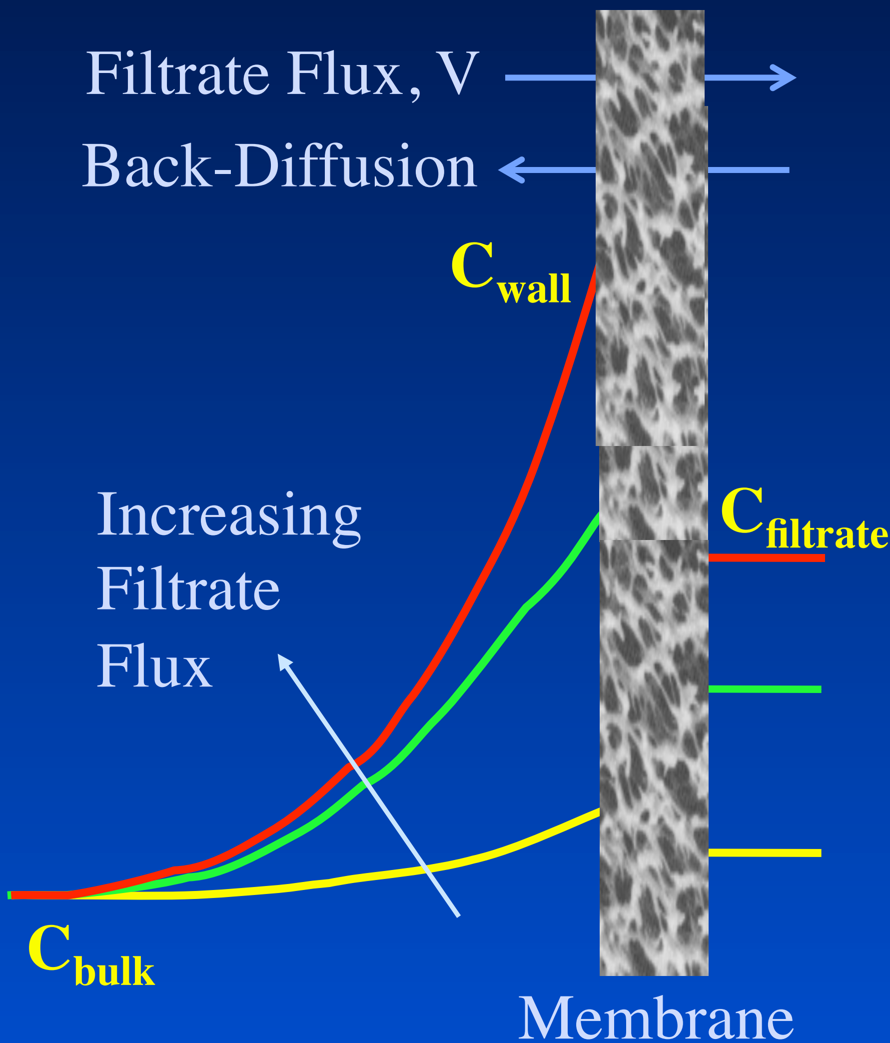
Concentration Polarization

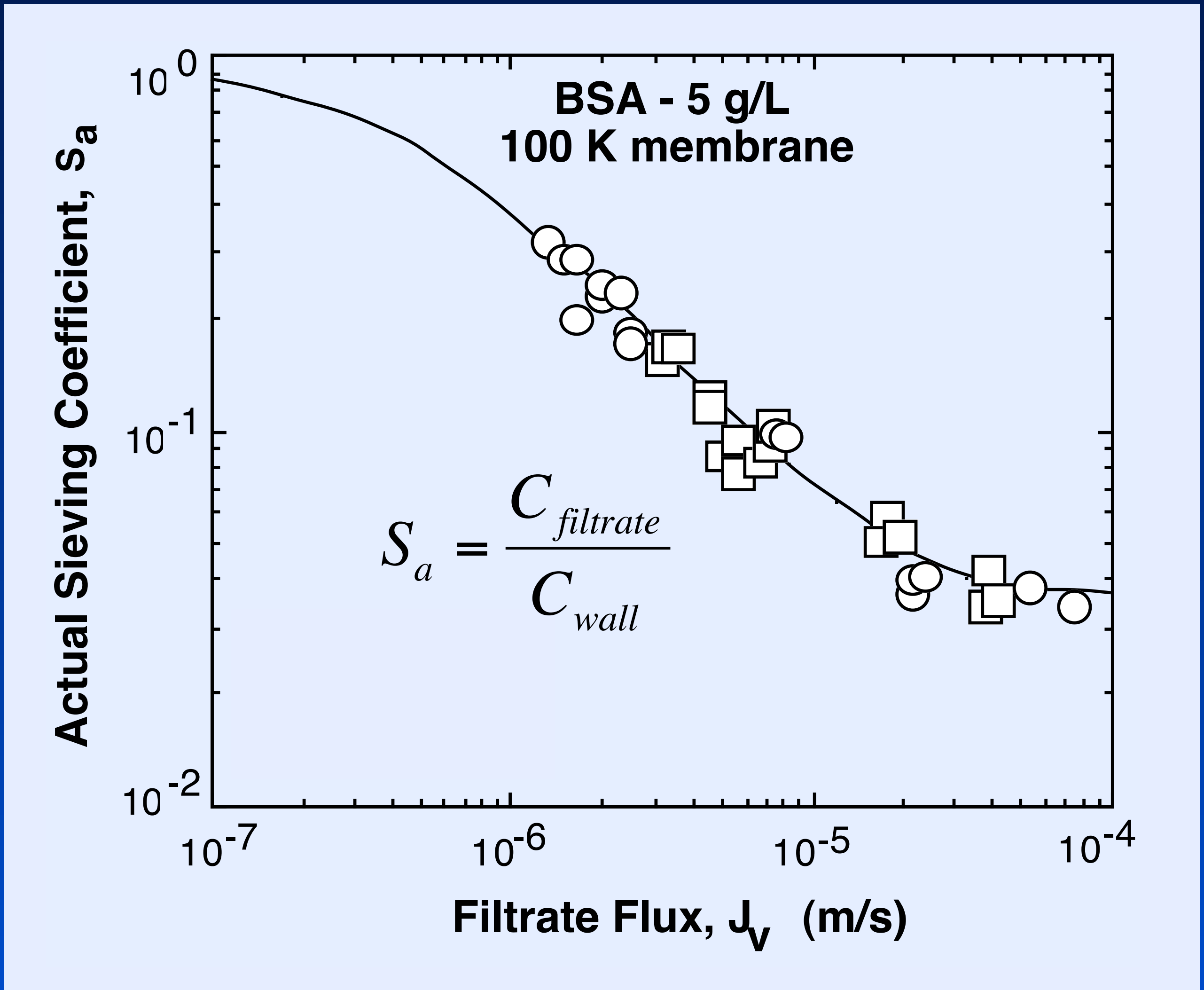


Accumulation of retained protein at upstream surface of membrane

– Extent of polarization determined by balance between convection towards membrane and diffusion back into bulk solution

$$VC - D_{\infty}\frac{\partial C}{\partial z} = VC_{filtrate}$$

Actual Sieving Coefficient



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Membrane Transport

Solute Flux:

$$N_s = K_c V C_{pore} - K_d D_\infty \frac{\partial C_{pore}}{\partial z}$$

K_c = hindrance factor for convection

K_d = hindrance factor for diffusion

Partition Coefficient:

$$\phi = \frac{C_{pore,z=0}}{C_{wall}} = \frac{C_{pore,z=\delta_m}}{C_{filtrate}}$$

Assumes equilibrium at pore entrance ($z = 0$) and pore exit ($z = \delta_m$)

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Actual Sieving Coefficient

$$S_a = \frac{C_{filtrate}}{C_{wall}} = \frac{S_\infty \exp\left(Pe_m\right)}{S_\infty + \exp\left(Pe_m\right) - 1}$$

Asymptotic Sieving Coefficient: $S_\infty = \phi \mathrm{K}_\mathrm{c}$

Membrane Peclet Number: $Pe_m = \frac{\mathrm{K}_\mathrm{c}}{\mathrm{K}_\mathrm{d}}\left(\frac{V\delta_m}{D_\infty}\right)$

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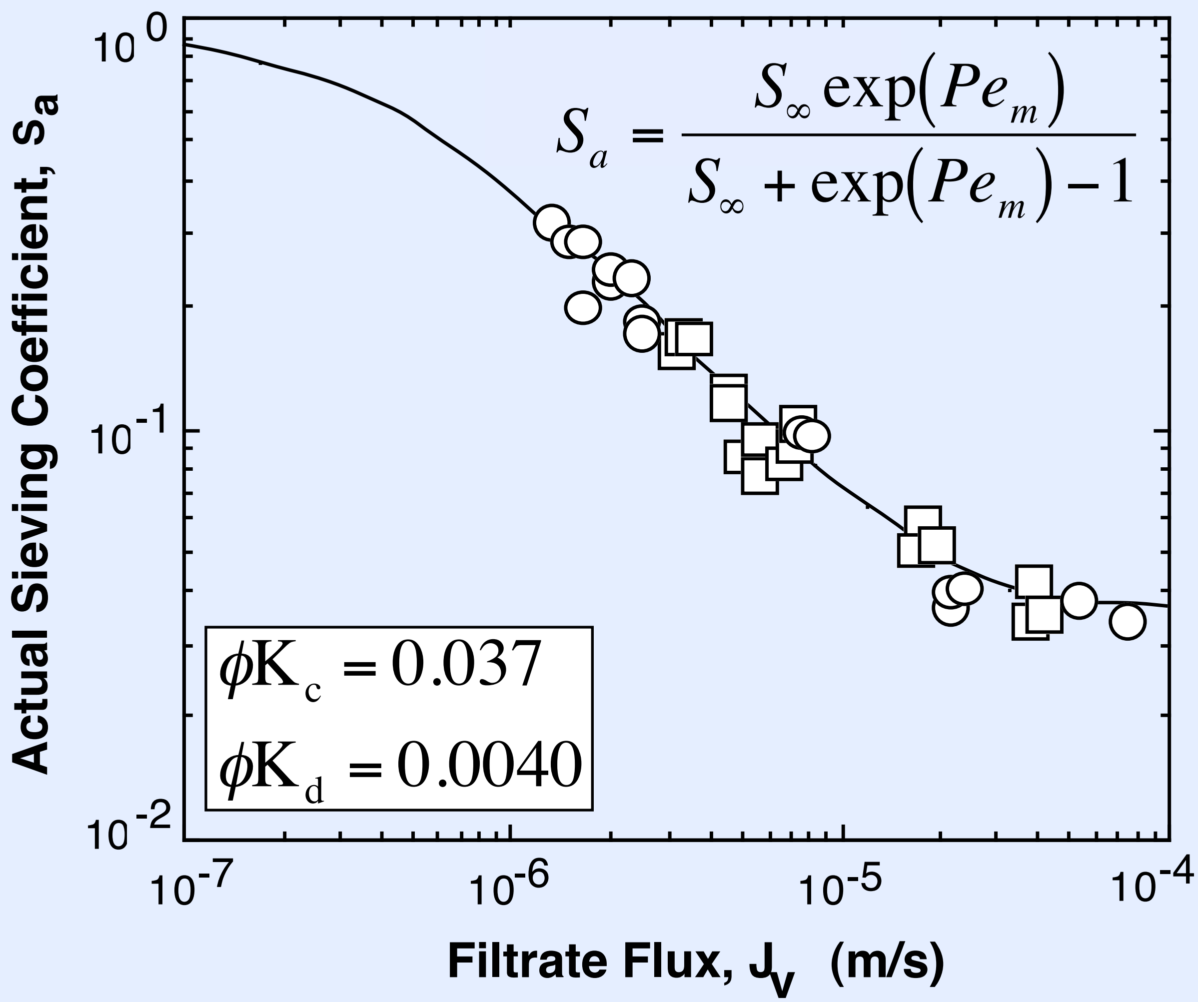
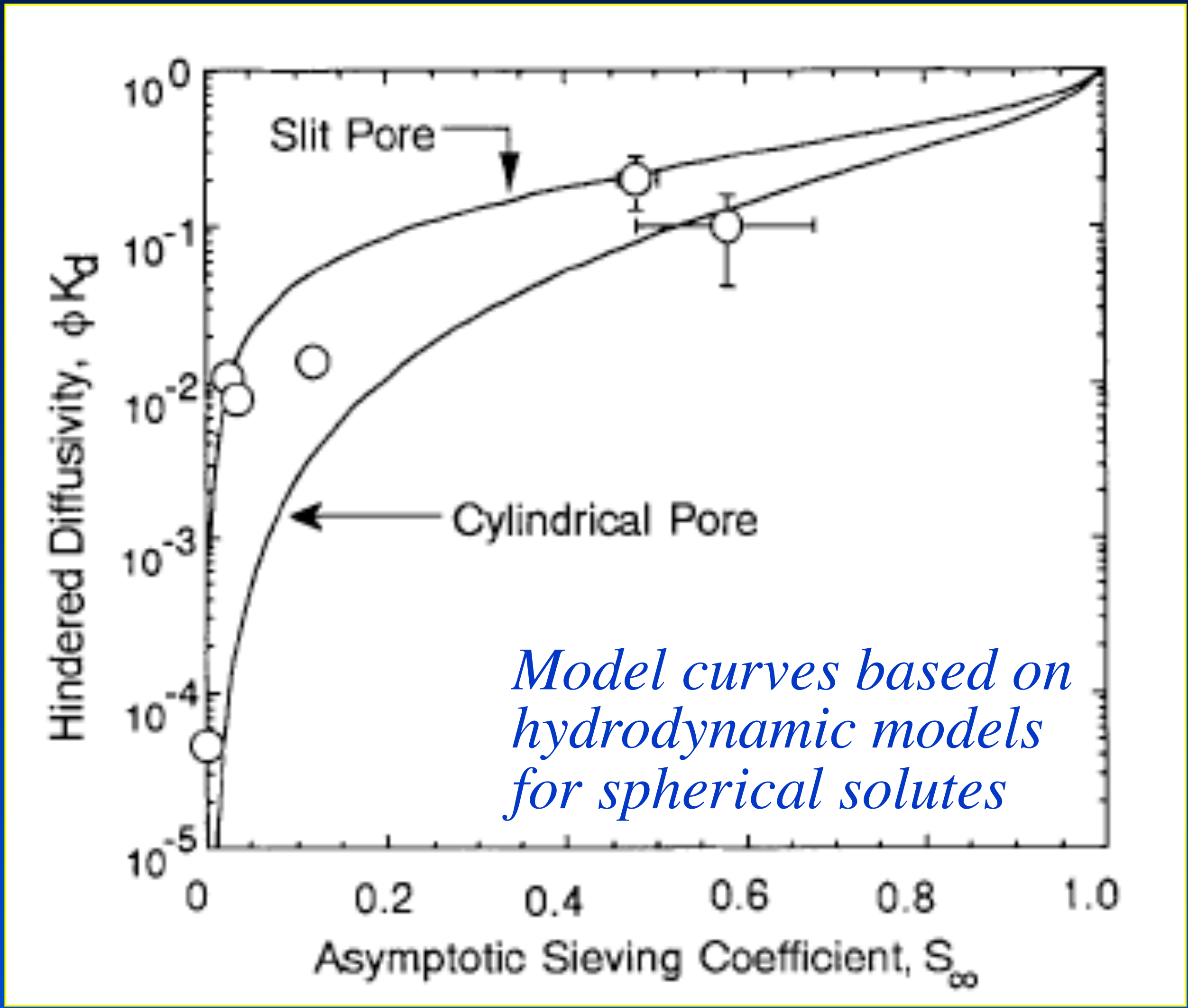
Actual Sieving Coefficient



Hindrance Coefficients



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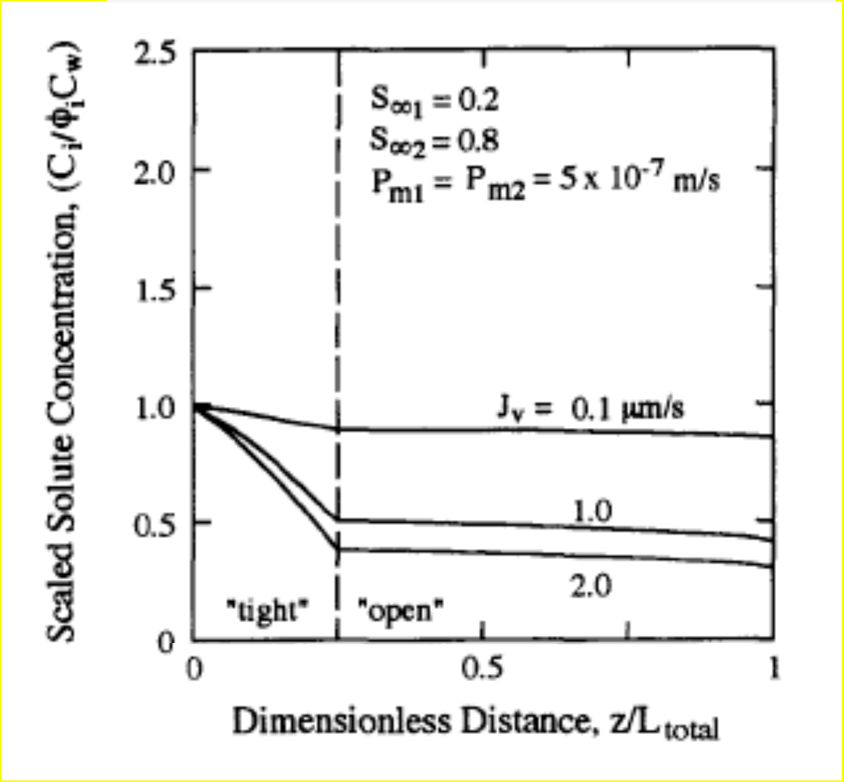
Multi-layer Membranes

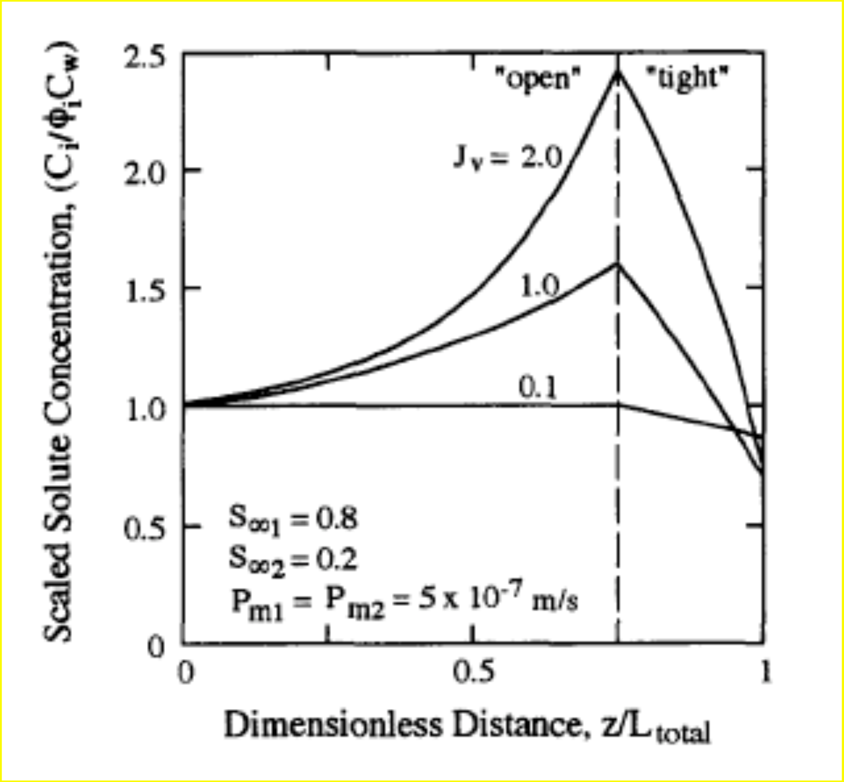
- Effects of diffusion / convection are significantly more complicated in multilayer and asymmetric membranes
  - Internal concentration polarization develops when flow is through the more open (larger pore size) region first
  - Net result is a directional dependence to solute transport / sieving coefficient

Directional Dependent Transport

Tight side up



Open side up



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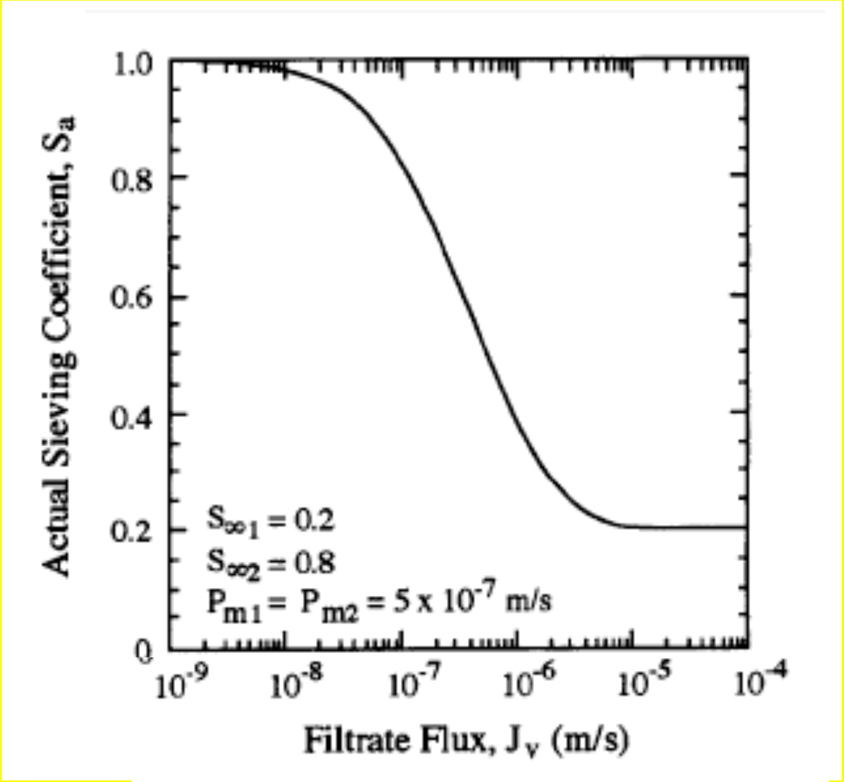
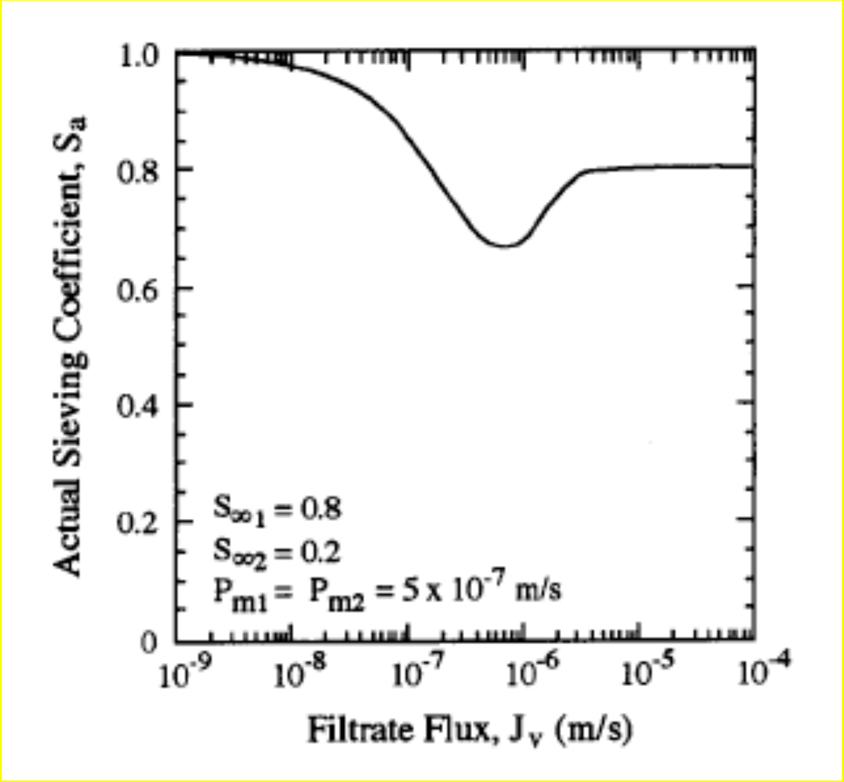
Actual Sieving Coefficient: Two-Layer Membrane

Tight side up



Open side up



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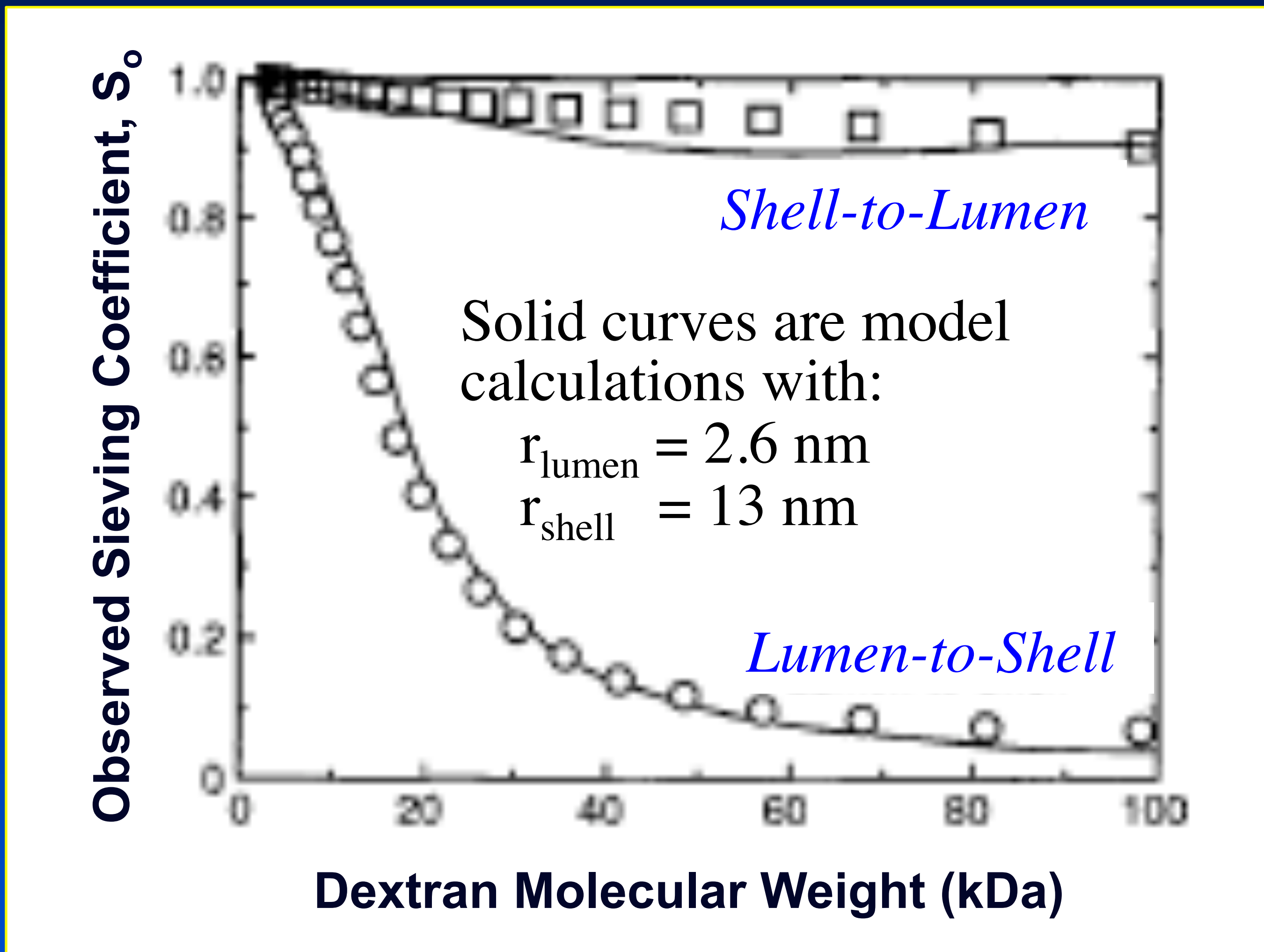
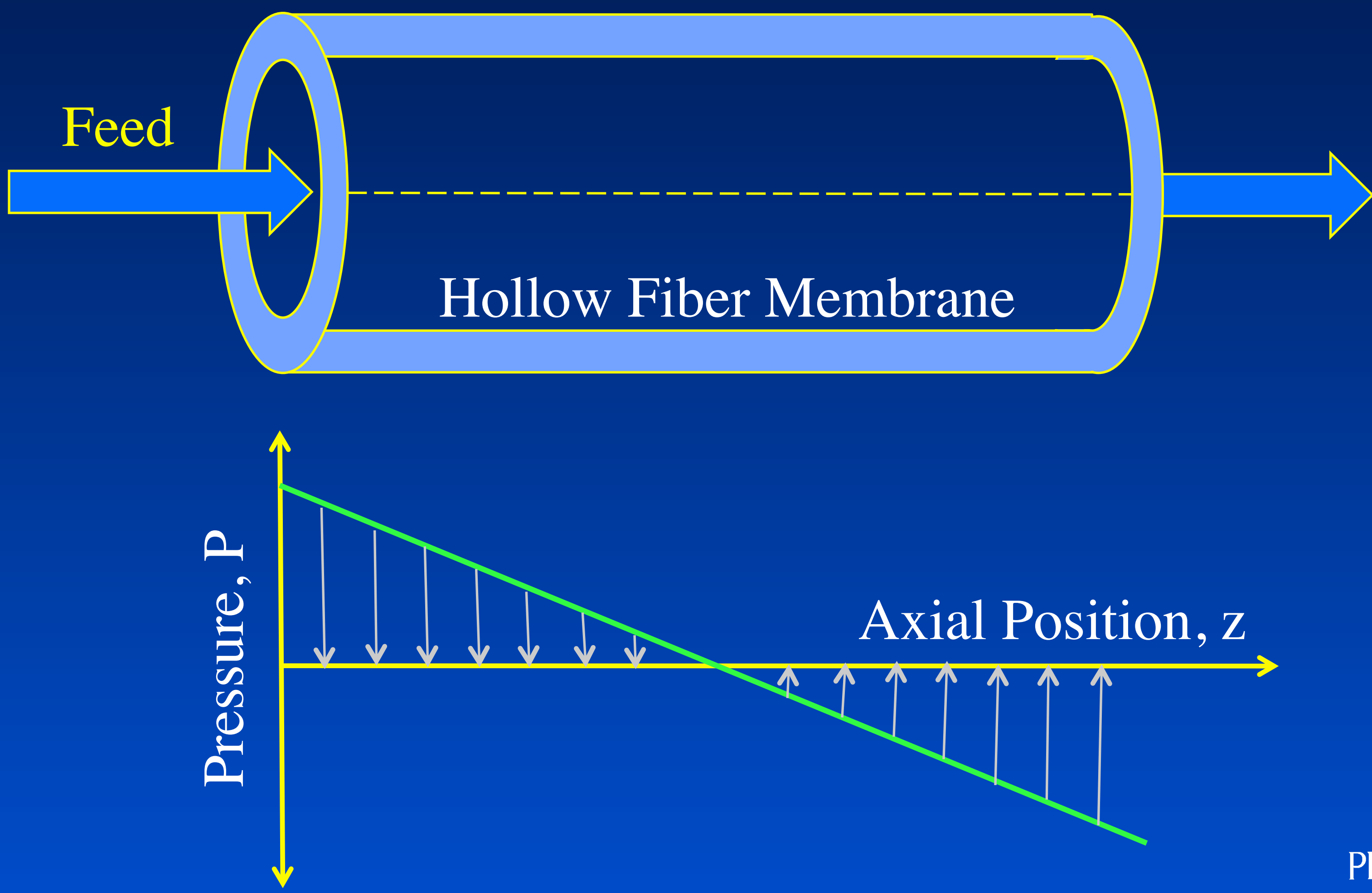
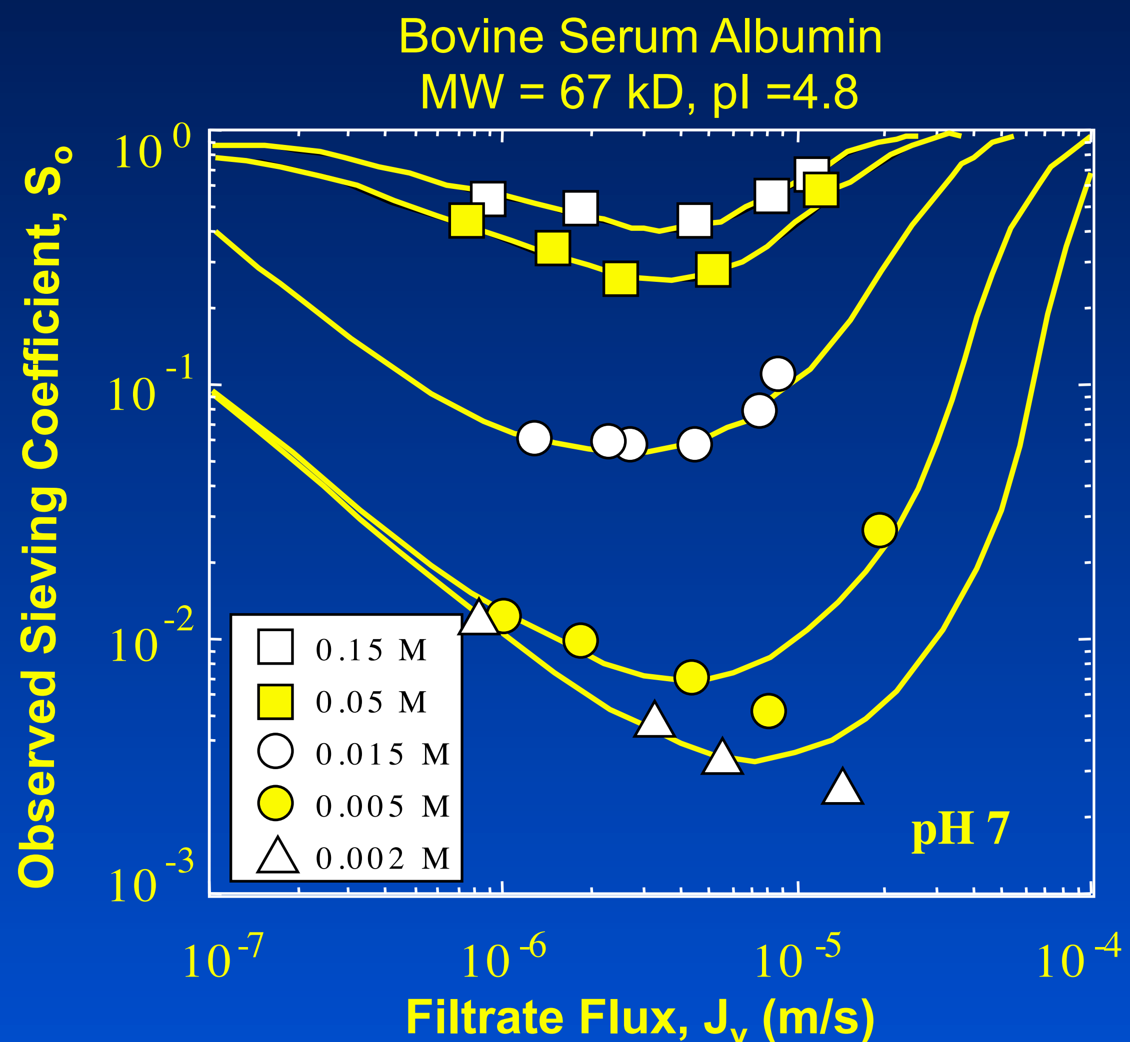
Dextran Sieving – Dual Skinned Hollow Fiber Membrane



Starling Flow - Backfiltration



Electrostatic Interactions

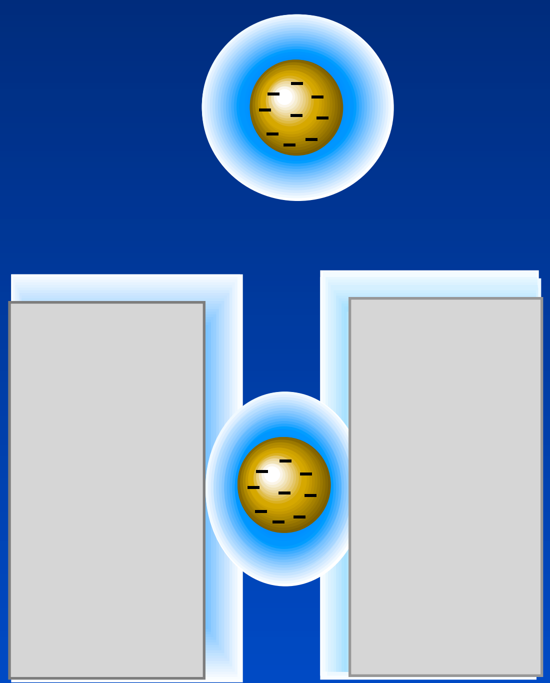


Electrostatic Interactions

$$\phi = \frac{C_{pore}}{C_{solution}} = (1-\lambda)^2 \exp\left(-\frac{E}{kT}\right)$$

steric interactions

electrostatic interactions



$$\lambda = \frac{r_{solute}}{r_{pore}} = \text{solute to pore size ratio}$$

$$\frac{E}{kT} = \text{dimensionless energy of interaction}$$

Energy of Interaction

$$\frac{E}{kT} = A_1\sigma^2_{protein} + A_2\sigma^2_{pore} + A_3\sigma_{protein}\sigma_{pore}$$

σ_{protein} = surface charge density of protein

σ_{pore} = surface charge density of pore wall

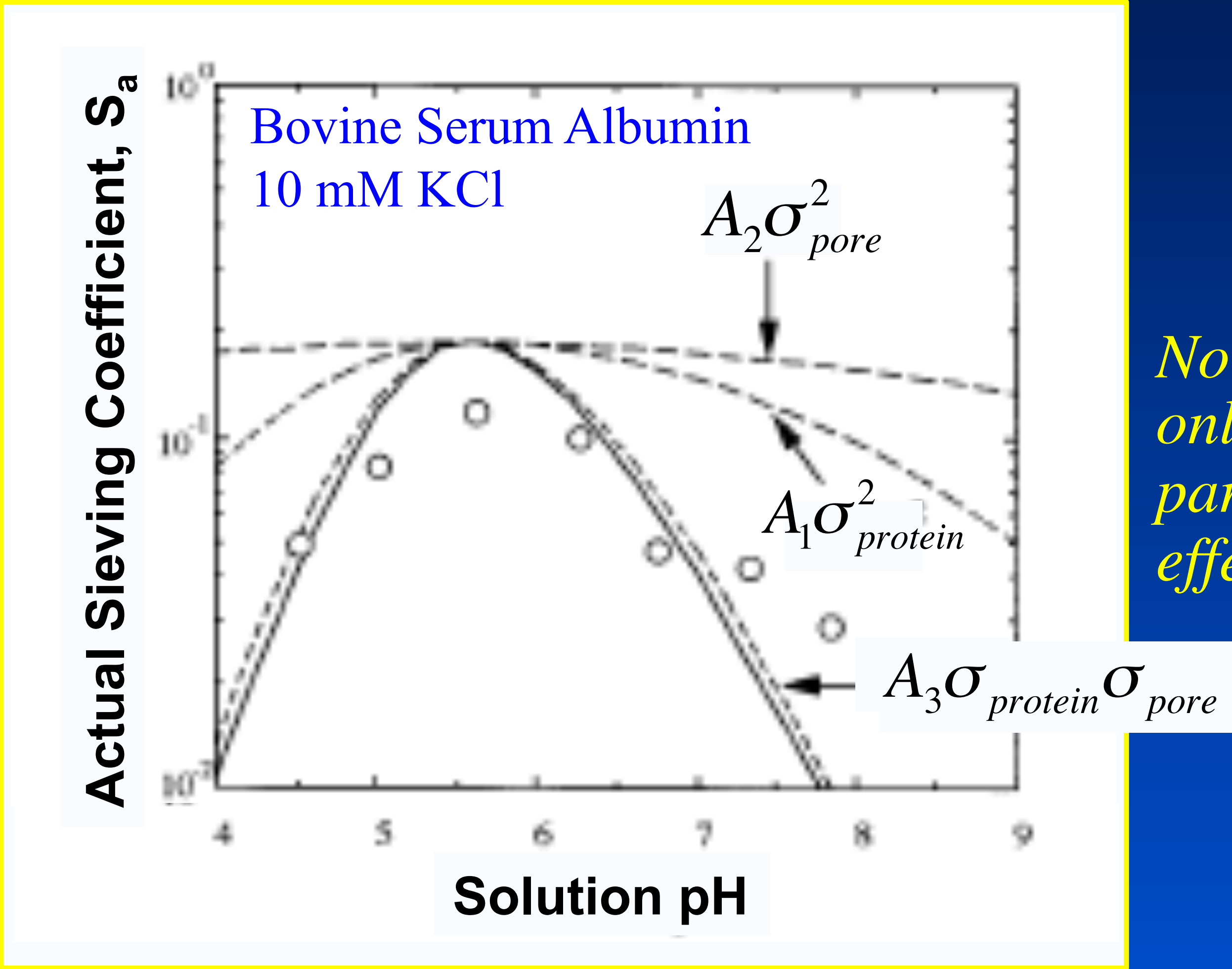
Three terms associated with:

(1) distortion of protein double layer

(2) distortion of double layer in pore

(3) direct charge-charge interactions

Protein Sieving -- pH Effects



Note: Model only includes partitioning effects

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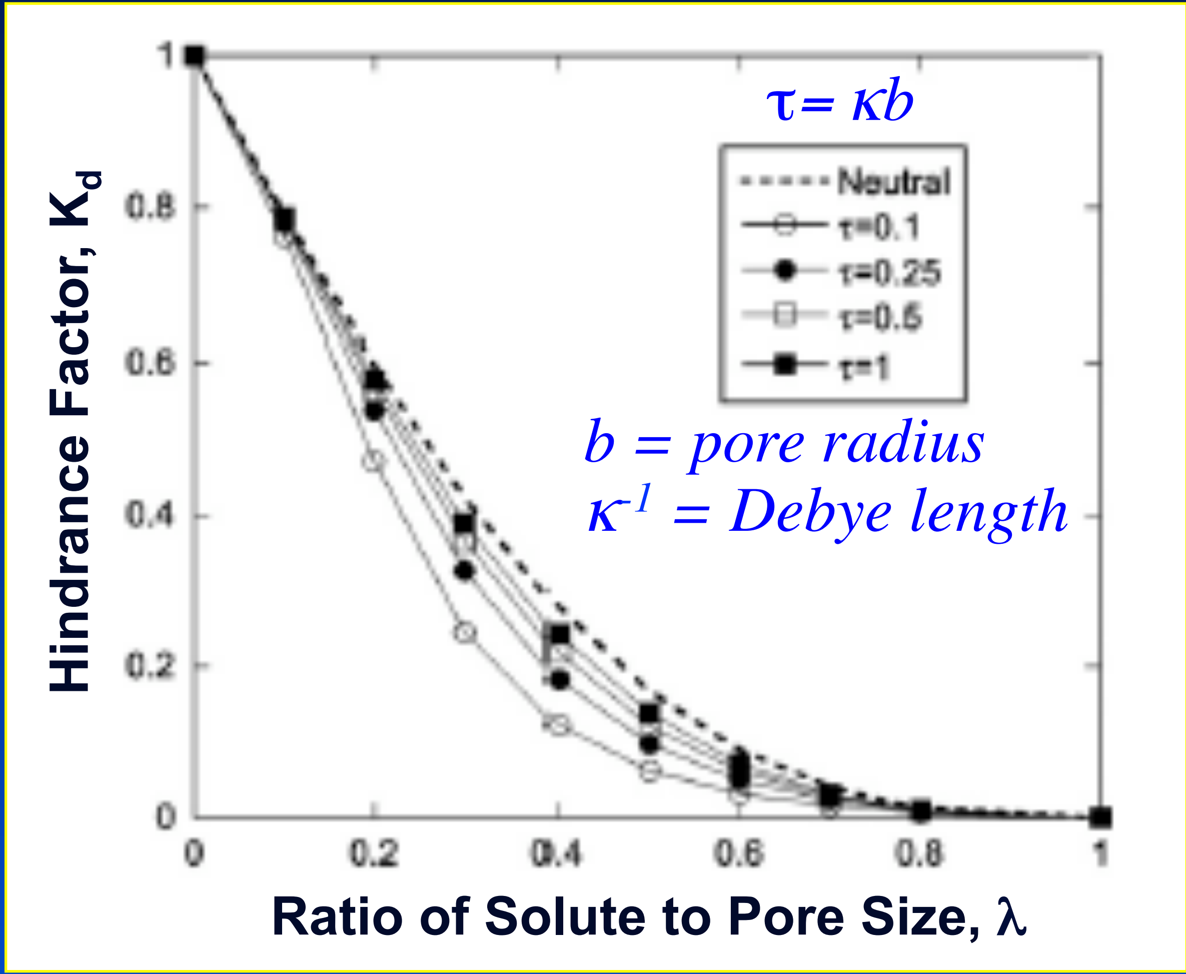
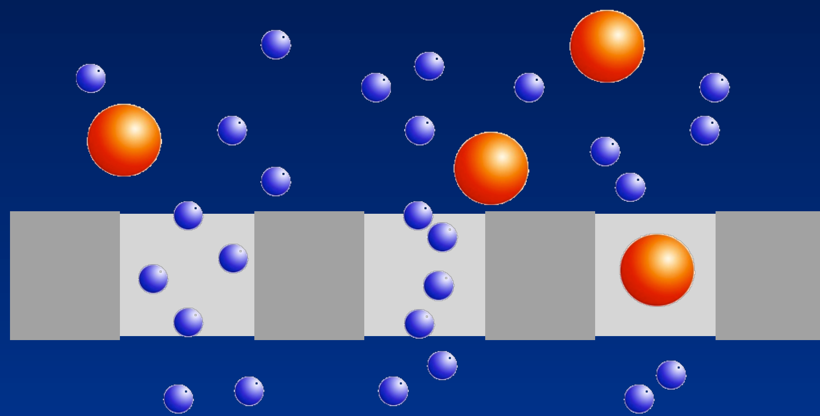
Hindrance Factor - Diffusion



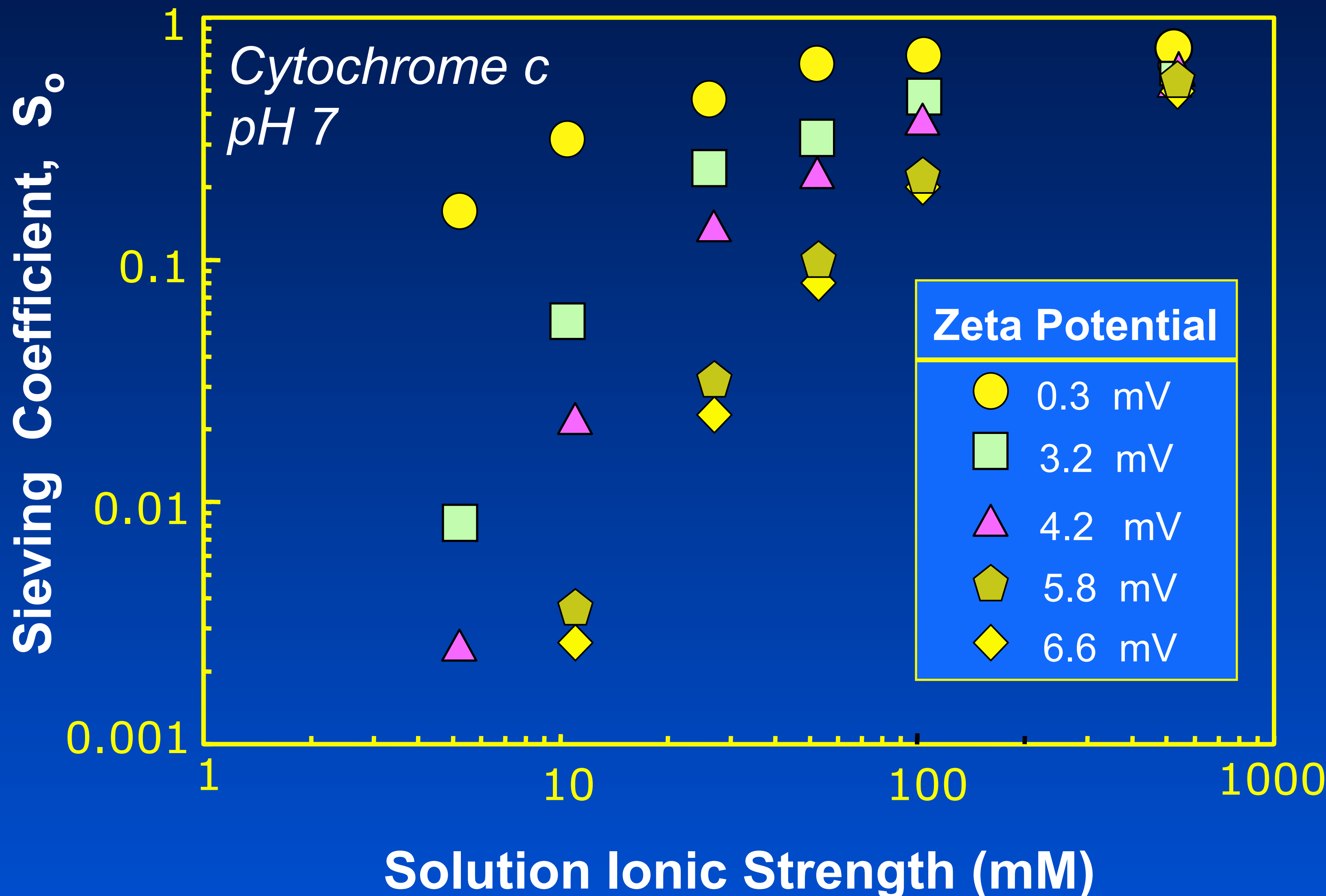
from Dechadilok and Deen, J Membrane Sci, 336, 7 (2009)

Charged Membranes

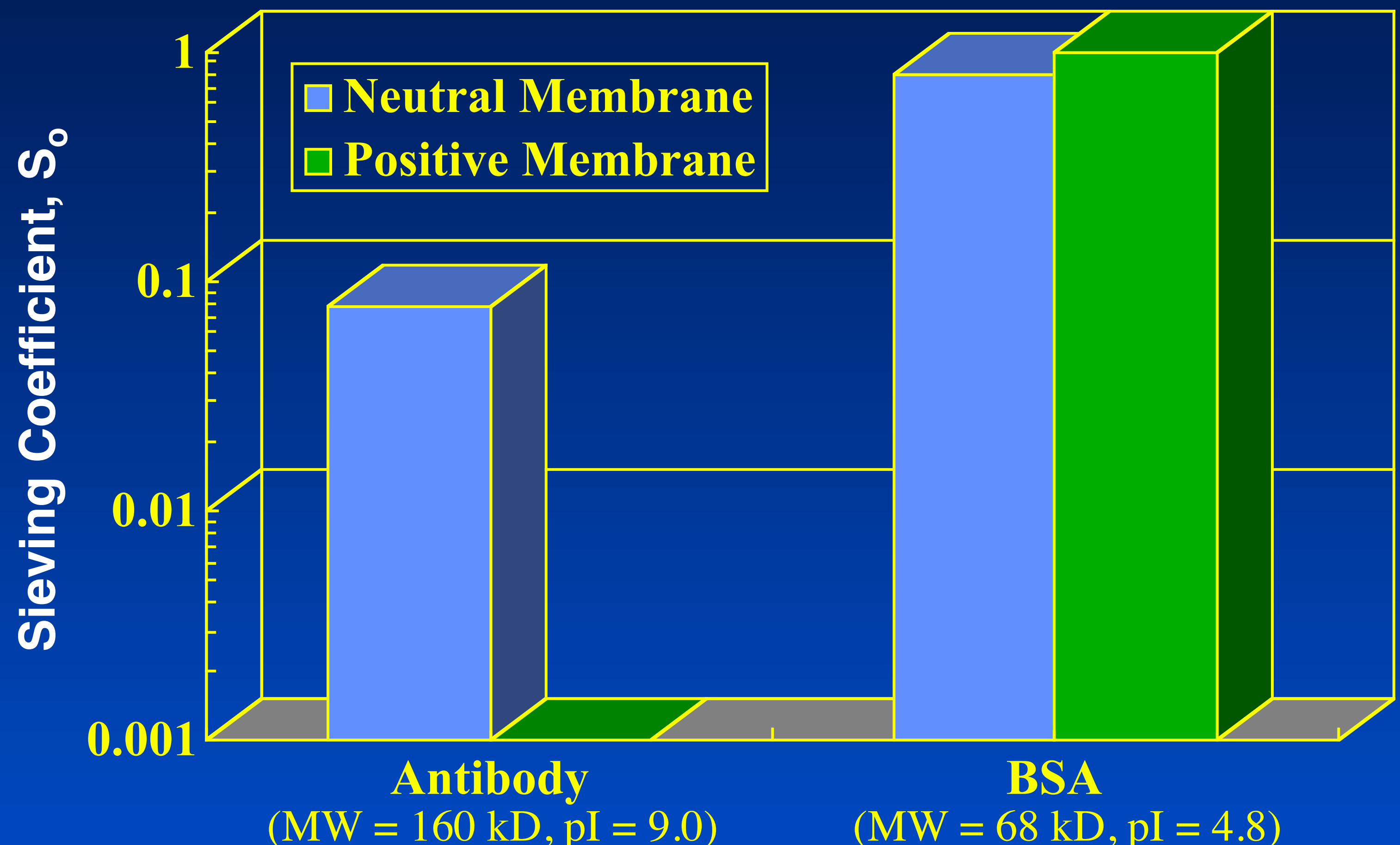


- Opportunity to improve performance of traditional ultrafiltration processes by increasing protein retention
- Opportunities for highly-selective protein separations

Membrane Charge Effects



Membrane Charge Effects



pH 5.0, 10 mM acetate buffer

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Charged UF Membranes

- **Number of Charge Groups**
  - Surface density of ligands
  - Ligands with multiple charge groups
- **Location of Charge Groups**
  - Effect of spacer arm length / branching
- **Nature of Charge Groups**
  - Weak versus strong acid / base
  - Detailed ligand and linkage chemistry

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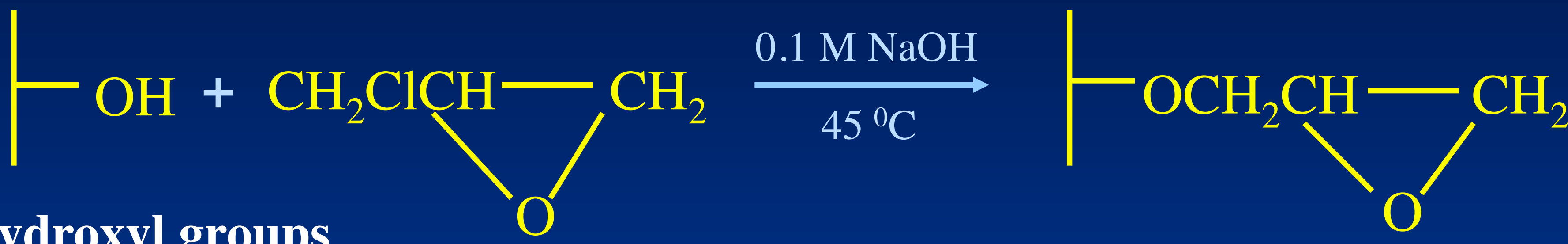
Epichlorohydrin Activation



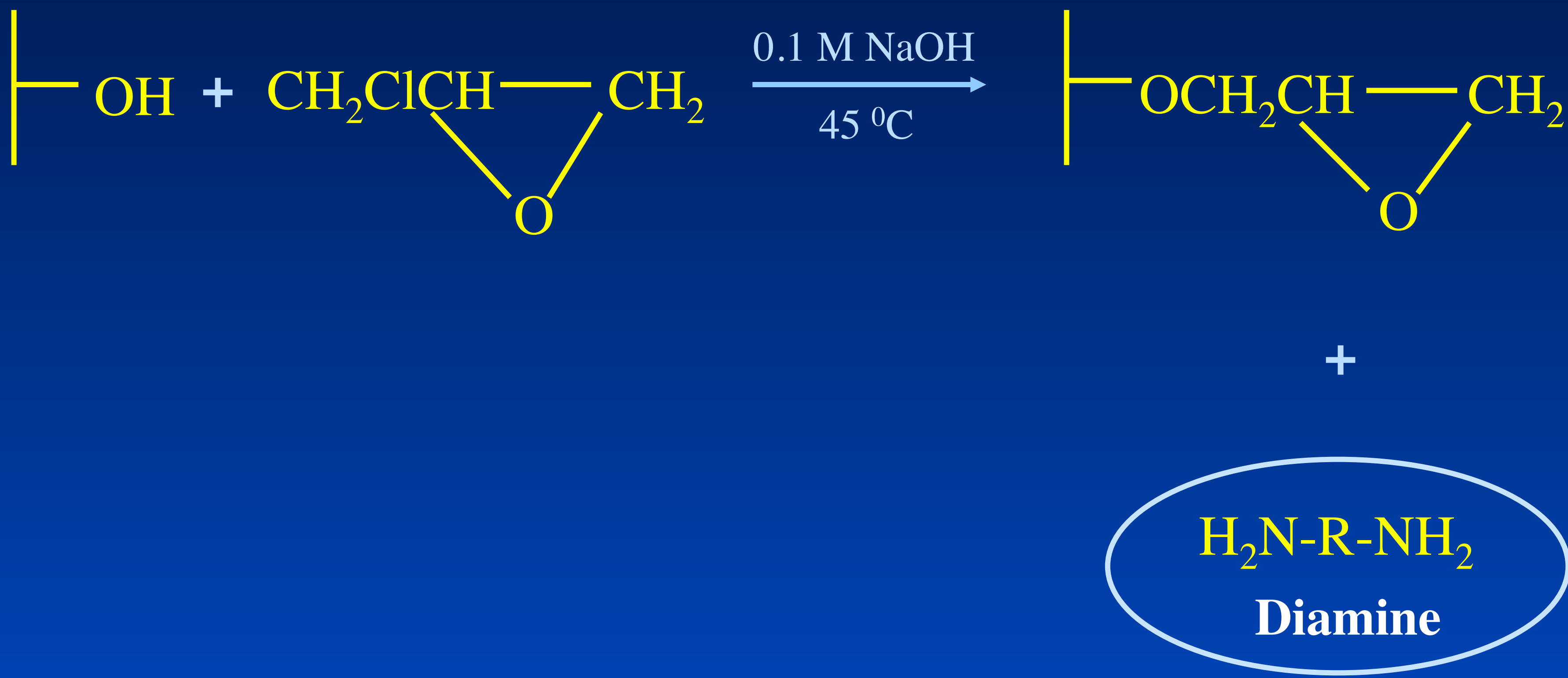
Hydroxyl groups on cellulose

Epichlorohydrin

Activated Membrane

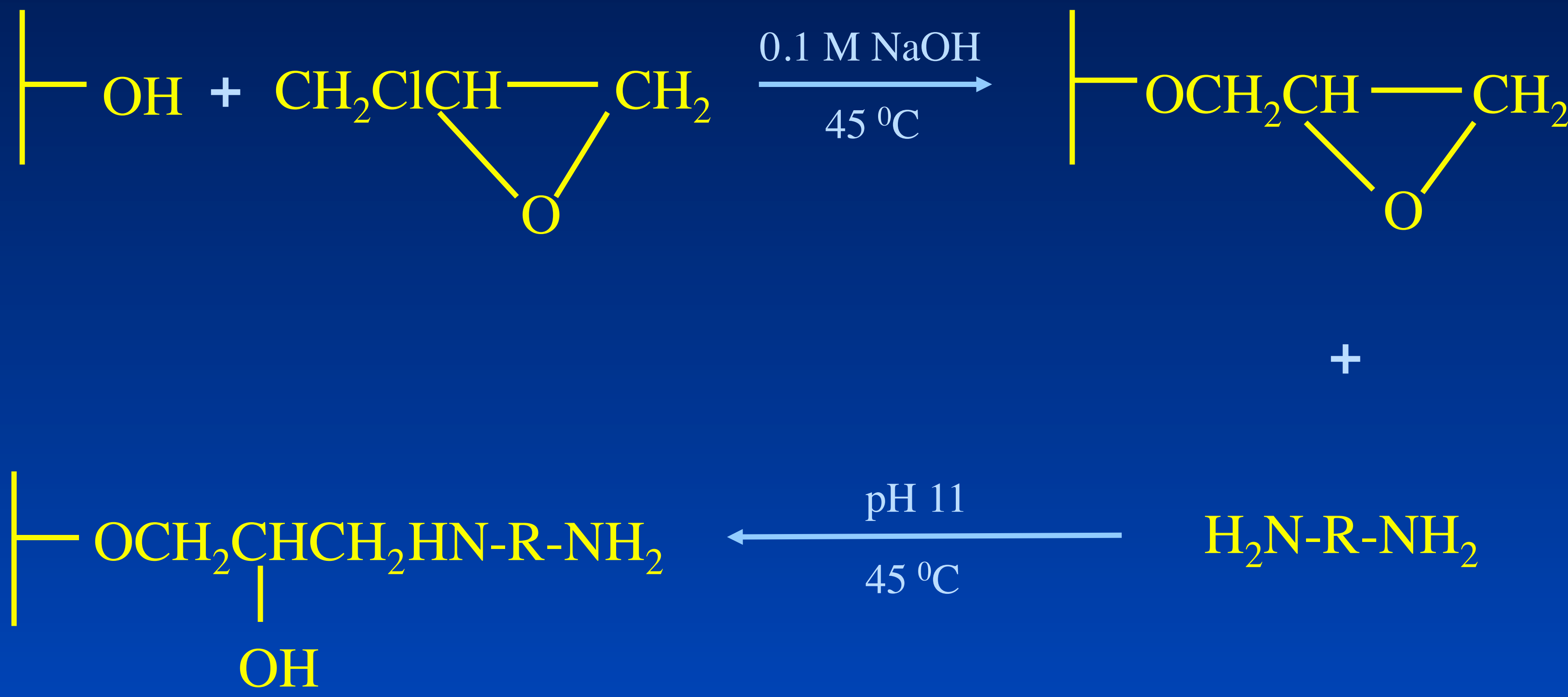
Degree of modification controlled by reaction time

Diamine Addition



Multiple chemistries and ligand lengths

Charge - Modified Membrane



Charge-Modified Membrane

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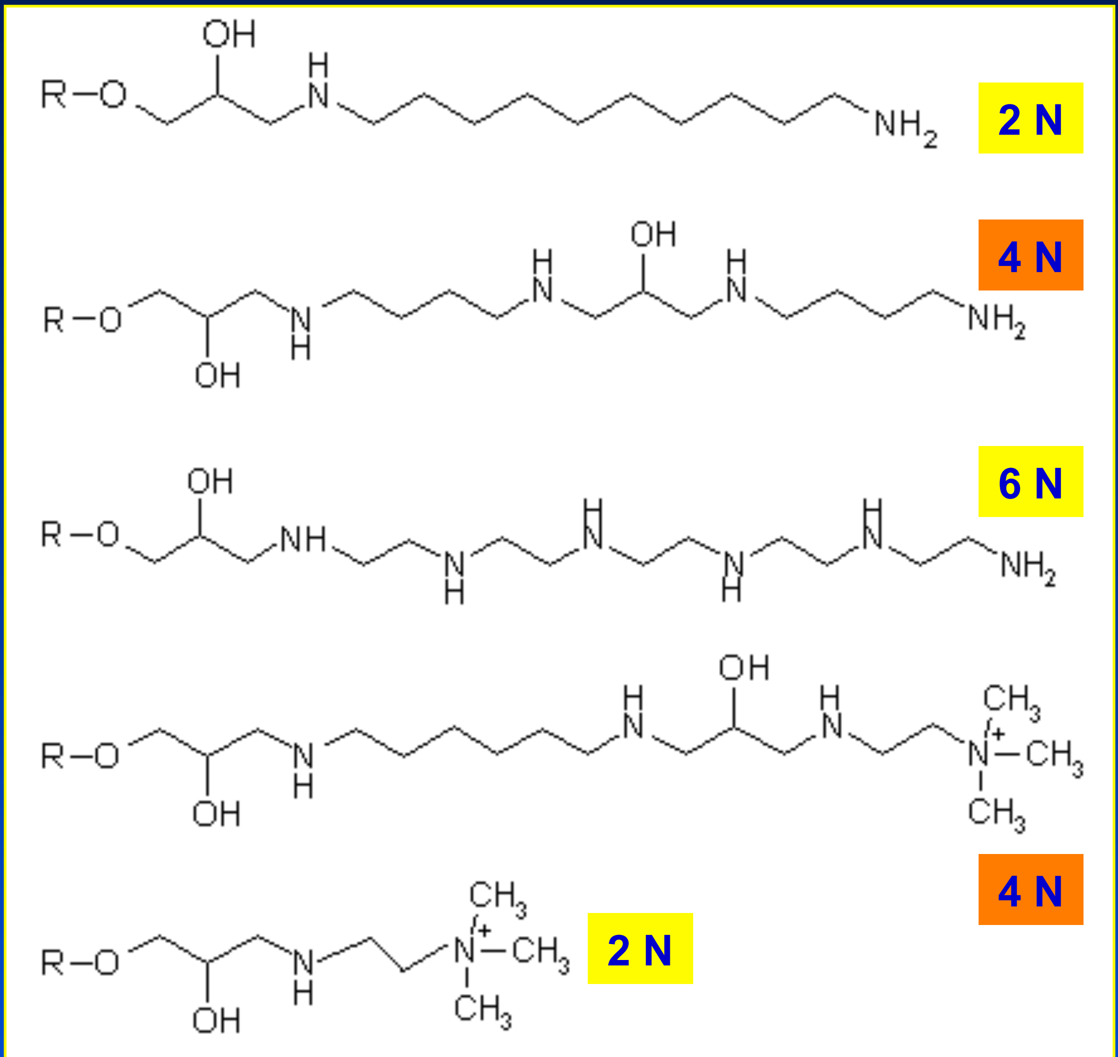
Ligand Chemistry



(a) **Diaminodecane**
(1.4 nm), 13 C

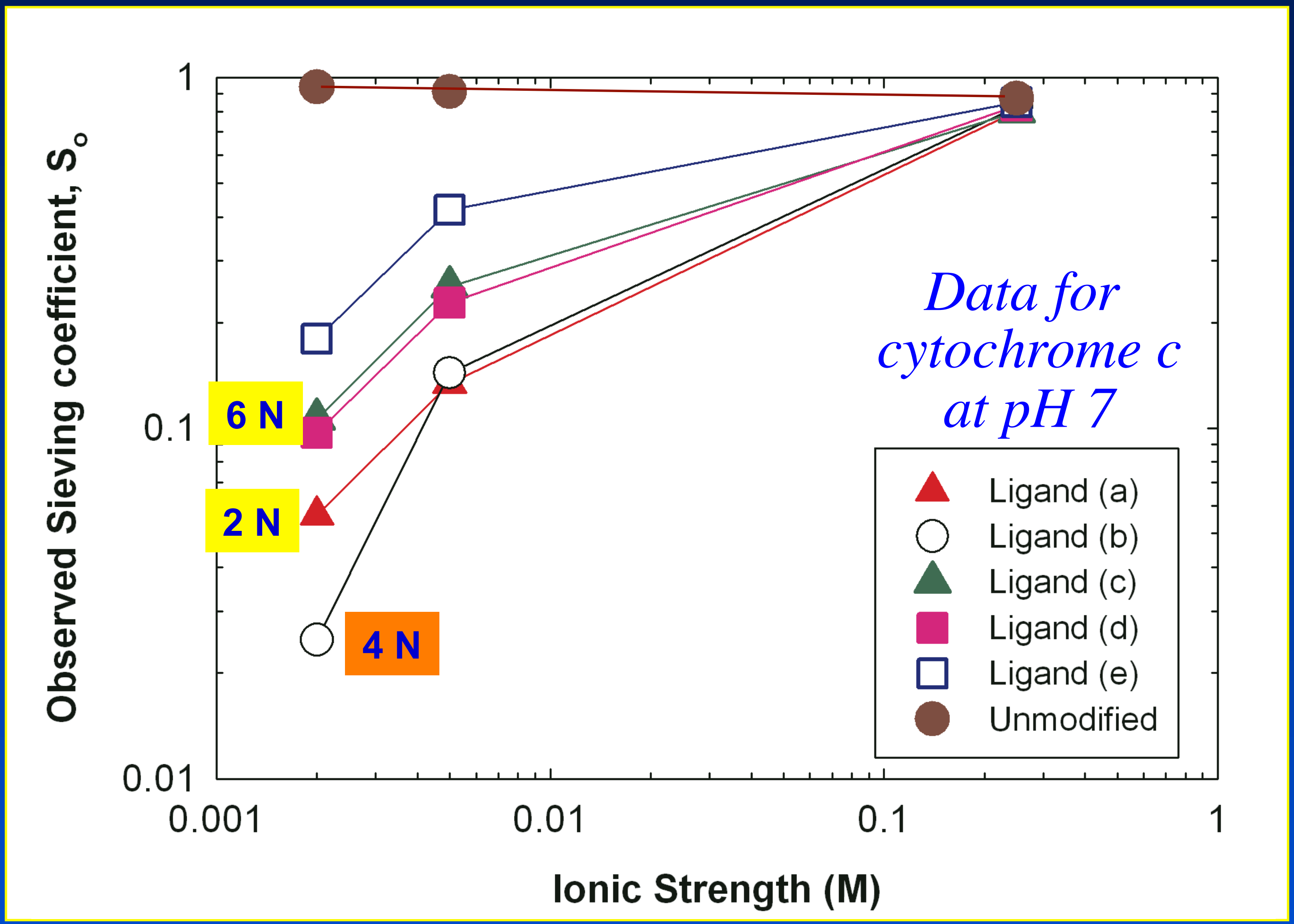
(b) **Diaminobutane**
(1.8 nm), 14 C
Sequential reaction

(c) **Pentaethylenehexamine**
(1.8 nm), 13 C

(d) **Quaternary amine**
(1.7 nm), 14 C
Sequential reaction

(e) **Short quaternary amine**
(0.4 nm), 5 C

Effects of Ligand Chemistry



Separation of Protein Variants

- Protein variants differ at only a single amino acid residue (e.g., deamidation, oxidation of methionine, carbamylation, etc)
- Resulting variants can have different activity and immunogenicity --> very challenging separation
- **Example:** myoglobin variants produced by chemical modification of a single lysine (conversion of amine to carboxylic acid)

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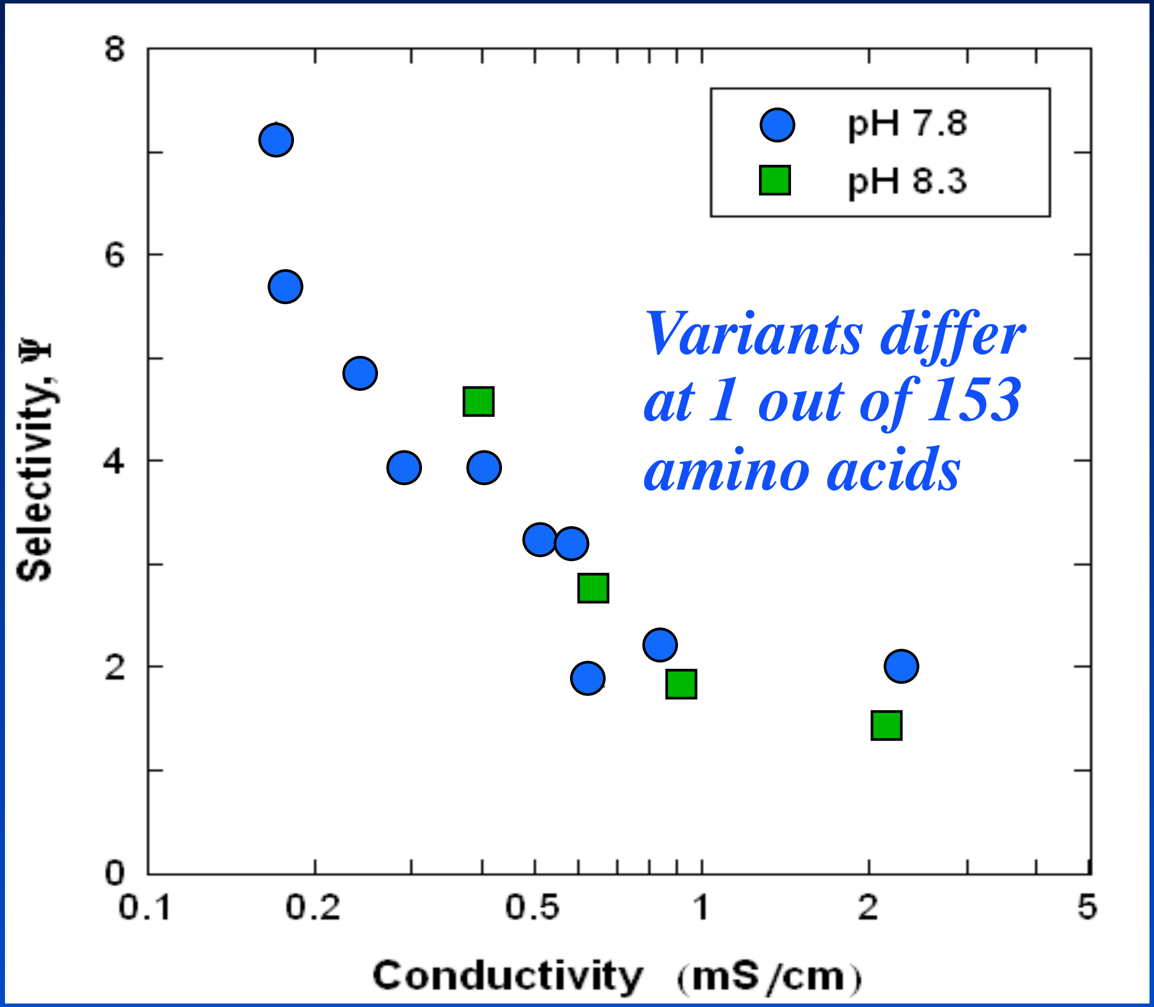
Myoglobin Sequence

GLSDGEWQQV
IAGHGQEVLI
EKFDKFKHLK
LKKHGTVVLT
HHEAELKPLA
IKYLEFISDA
GDFGADAQGA
DIAAKYKELG

LNVWGKVEAD
RLFTGHPETL
TEAEMKASED
ALGGILKKKG
QSHATKHKIP
IIHVLHSKHP
MTKALELFRN
FQG

Chemical modification of a single lysine amino acid out of 153 residues

Selectivity for Myoglobin Variants



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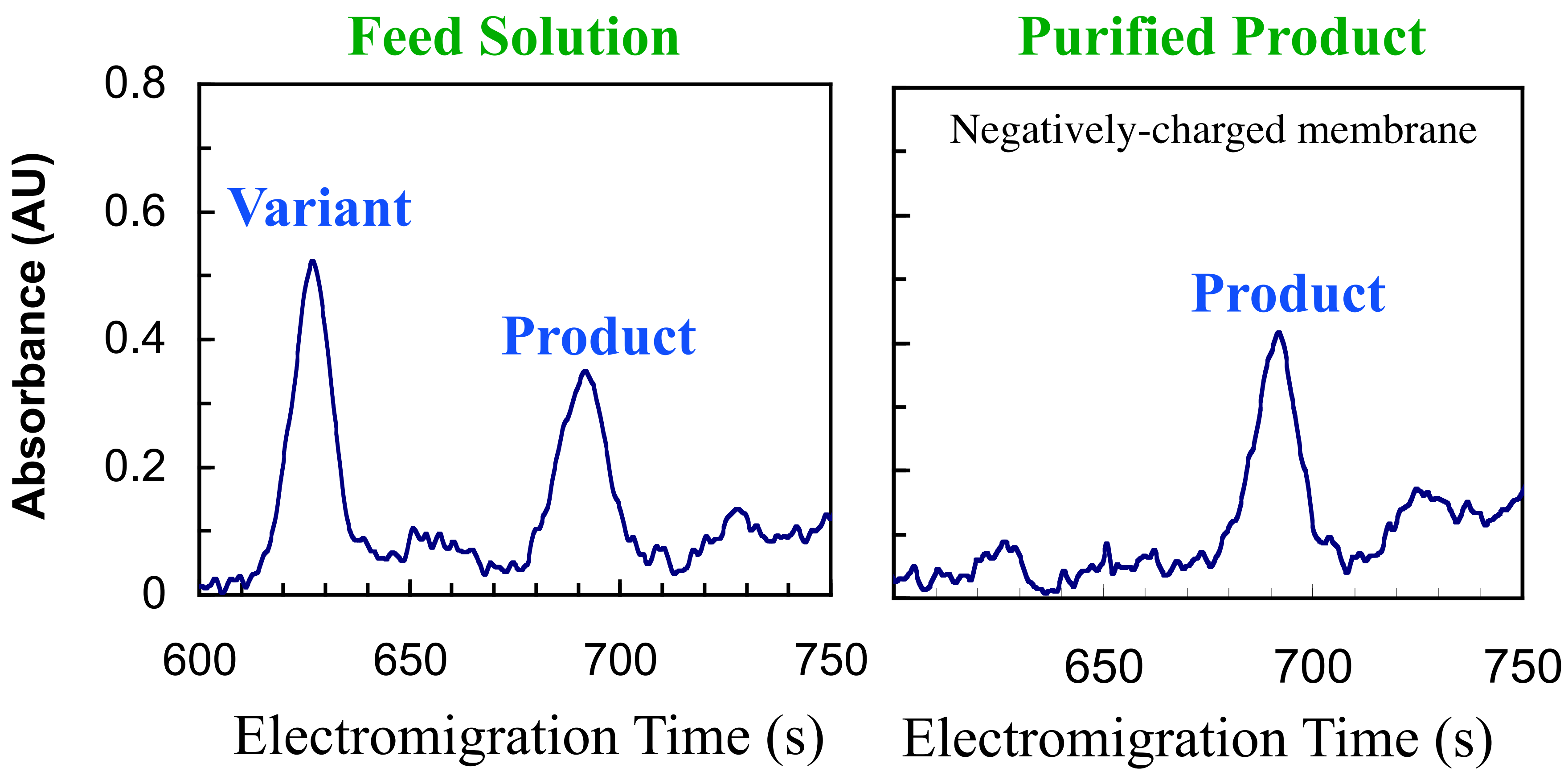
Separation of Myoglobin Variants



Summary

- Diffusive transport can significantly alter protein transmission during ultrafiltration
  - Bulk mass transport (concentration polarization)
  - Enhanced transmission at low filtrate flux
  - Directional dependence for multilayer membranes
- Electrostatic interactions provide unique opportunities for enhanced performance
  - Repulsive interactions cause strong rejection
  - Role of ligand structure still being explored

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