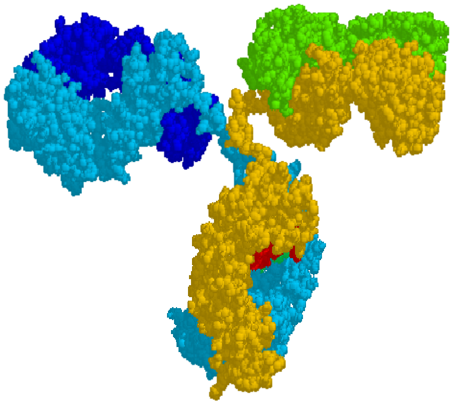
Development of biomimetic nanoporous membranes for the sensing and separation of proteins



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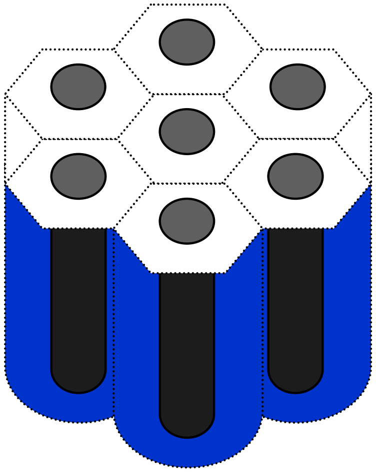
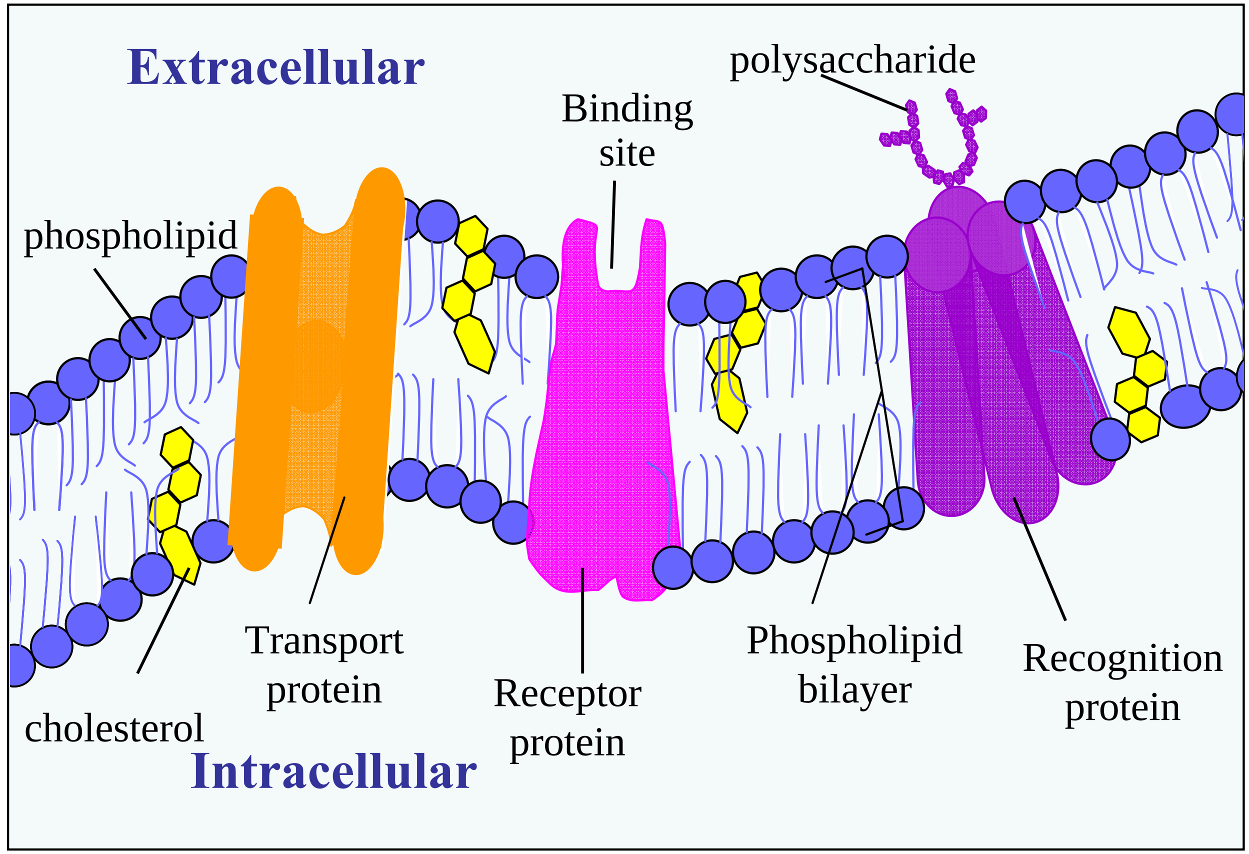


Outline

- Membranes in nature – Sensing and separation
- Biomimetic approach to sensing and separation
- Conclusion

Membranes in nature

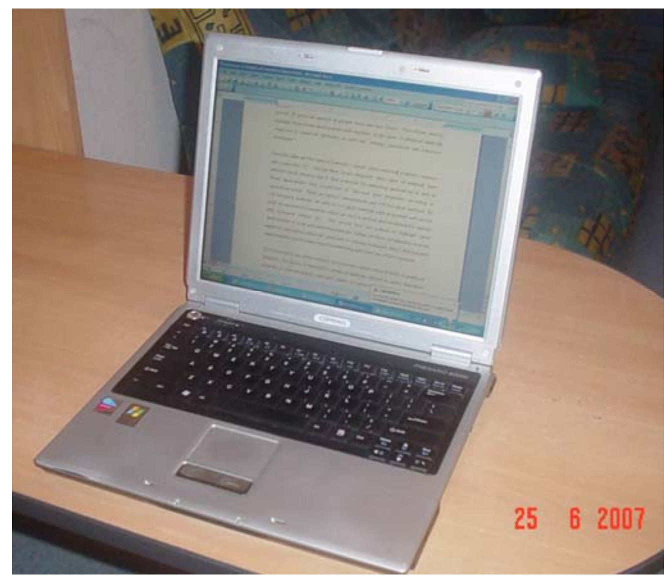
- Sensing
  - Recognition protein
  - Receptor protein
- Transport across membrane
  - Diffusion
  - Facilitated transport
  - Active transport
  - Osmosis



Materials-focused strategy

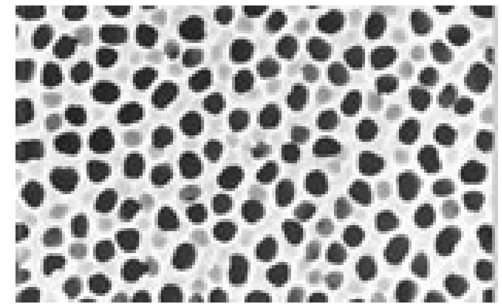
- **Classical Materials**
  - Plastics
  - Metals
  - Semiconductor
  - Ceramics
  - Composites

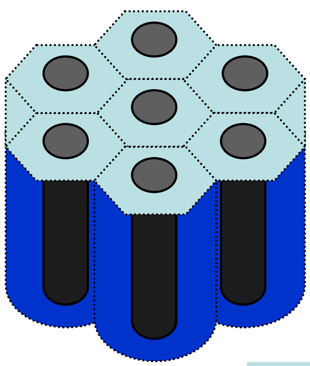
- **Advanced Materials**
  - Hybrids
  - Nanomaterials
  - Soft biomolecular materials



He L. and Toh C.S. *"Recent Advances in Analytical Chemistry – A Material Approach"*, Anal. Chim. Acta 556 1–15 (2006).

Materials-focused strategy



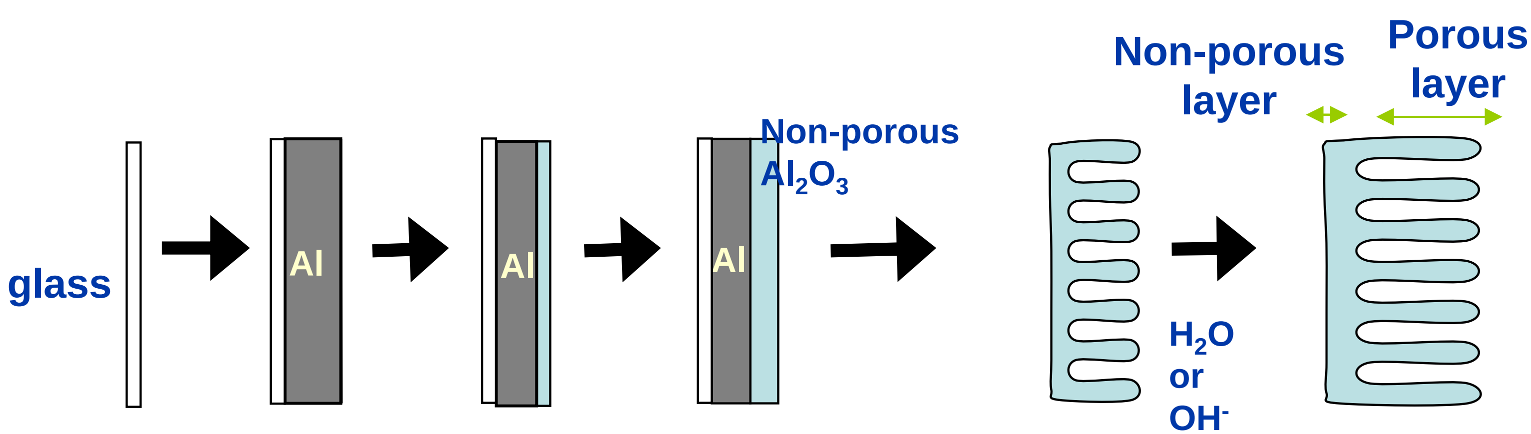


•Properties

- High mechanical strength
- High thermal and chemical stability
- Regular nano-sized porous structure (10 to 500 nm)
- Pore densities of about 10^{10} to 10^{11} pores per cm^2

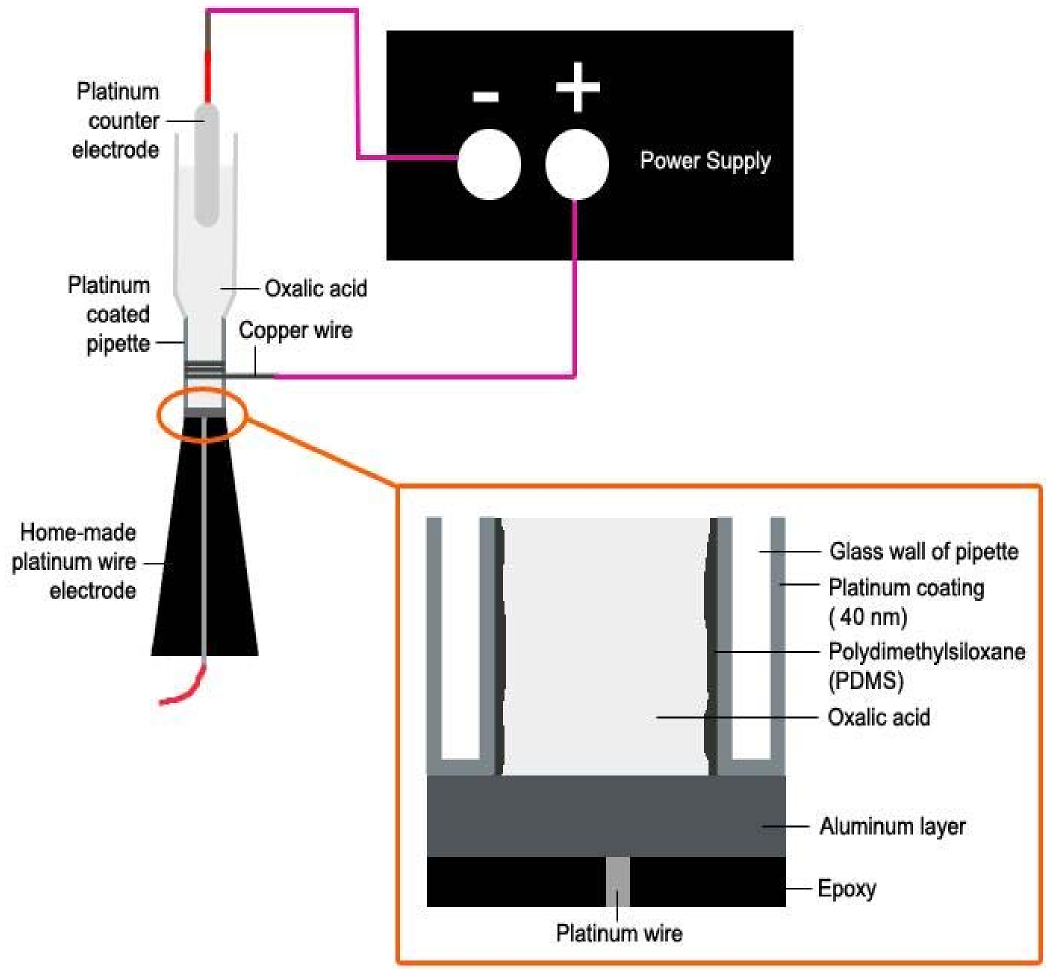
•Electrochemical Anodization

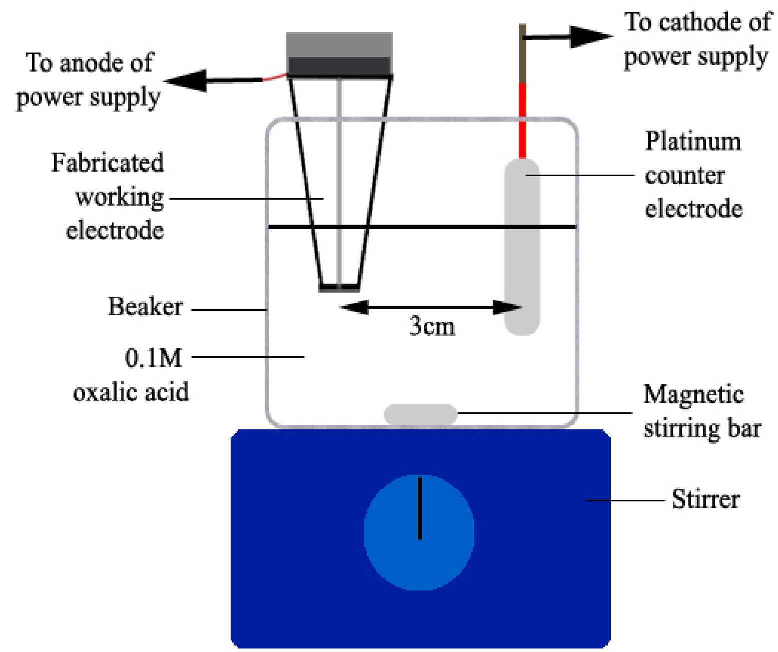
$$2Al + 3H_2O \rightarrow Al_2O_3 + 3H_2$$



Our biomimetic approach

- Fabrication of Electrode: Schematic & Approaches





Left: Schematic of fabrication of electrode using *surface contact pipette* anodization method

Top: Schematic of *conventional sub-surface* anodization method

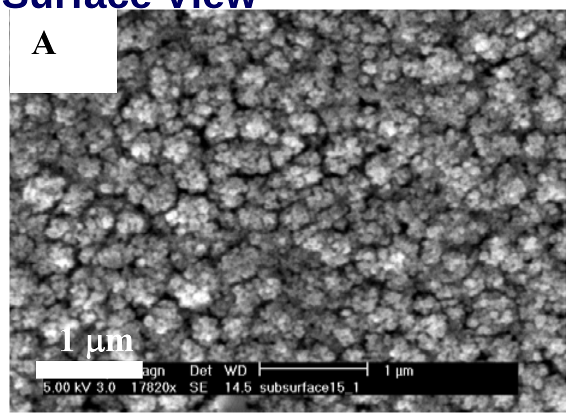
G.W. Koh, S. Agarwal, P.S. Cheow and C.S. Toh, *"Characterization of the barrier layer of nanoporous alumina films prepared using two different contact configurations",* Electrochimica Acta, 52(8), 2815-2821 (2007).

Our biomimetic approach

- **Alumina barrier layer**
  - Non-porous alumina layer between porous alumina and underlying conductive electrode
- **Removal of barrier layer**
  - Chemical etching method
  - Progressive step-down of the anodization voltage after formation of porous alumina layer *(Furneaux et. al. Nature 337,147, 1989)*
  - Re-anodization of alumina under constant current conditions *(Jagminas et. al. Appl. Surf. Sci. 405, 252,2006)*

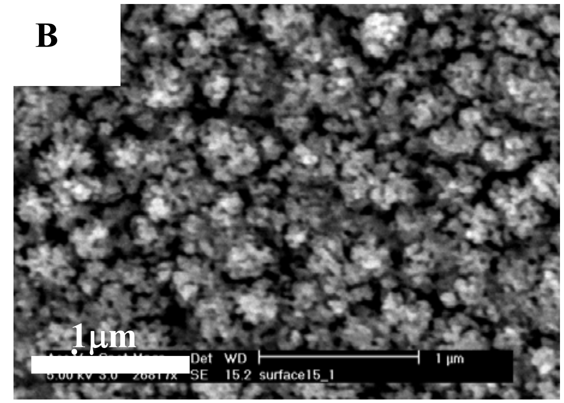
Conventional Sub-surface anodization

Surface View

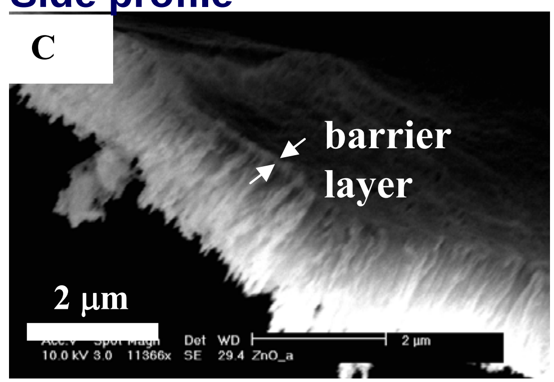


Pipette anodization

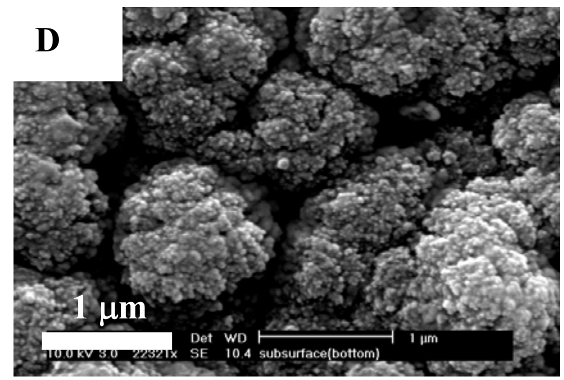
Surface View



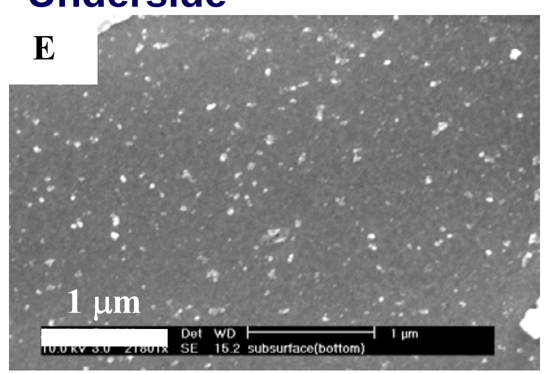
Side profile



Underside



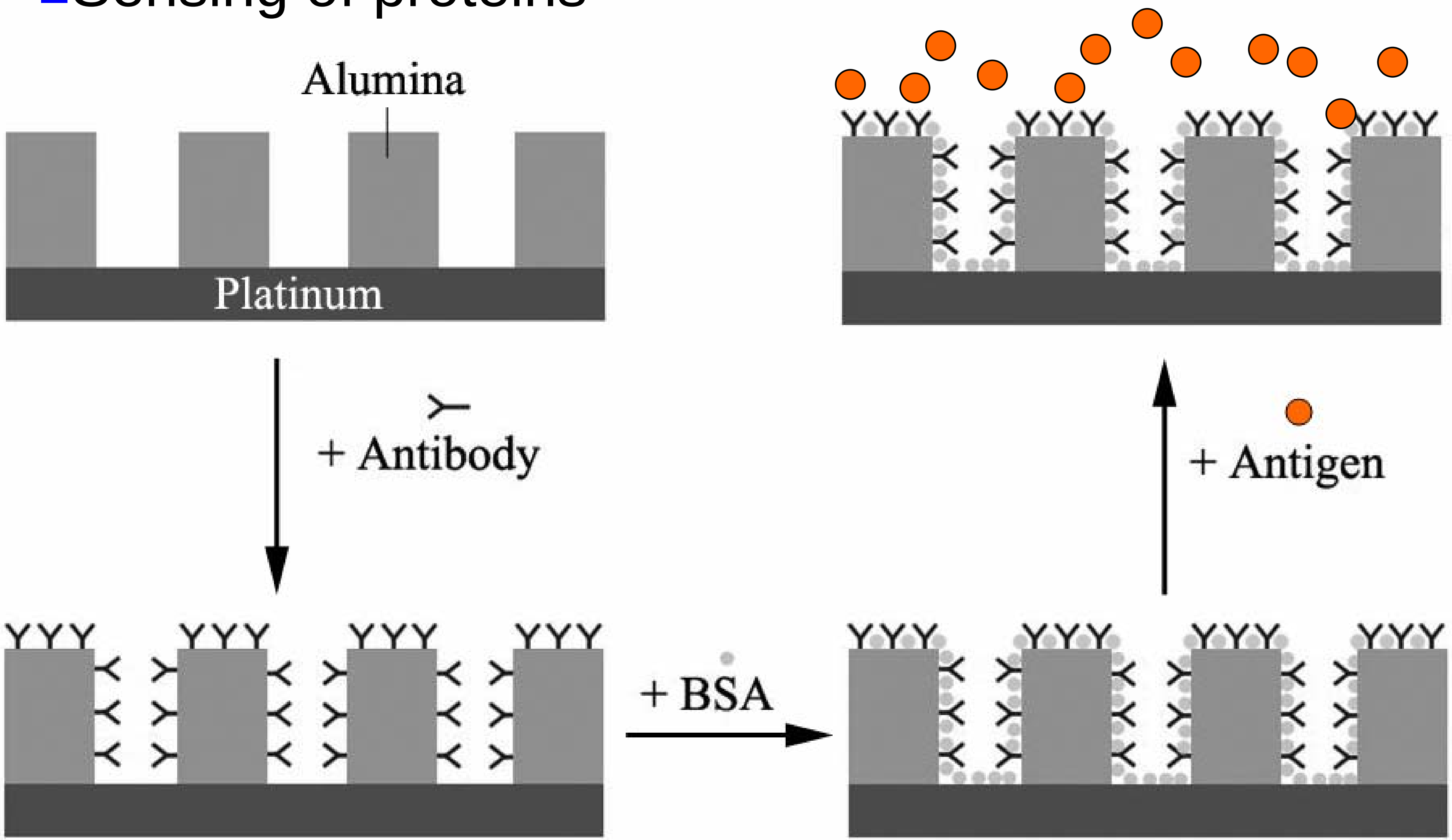
Underside



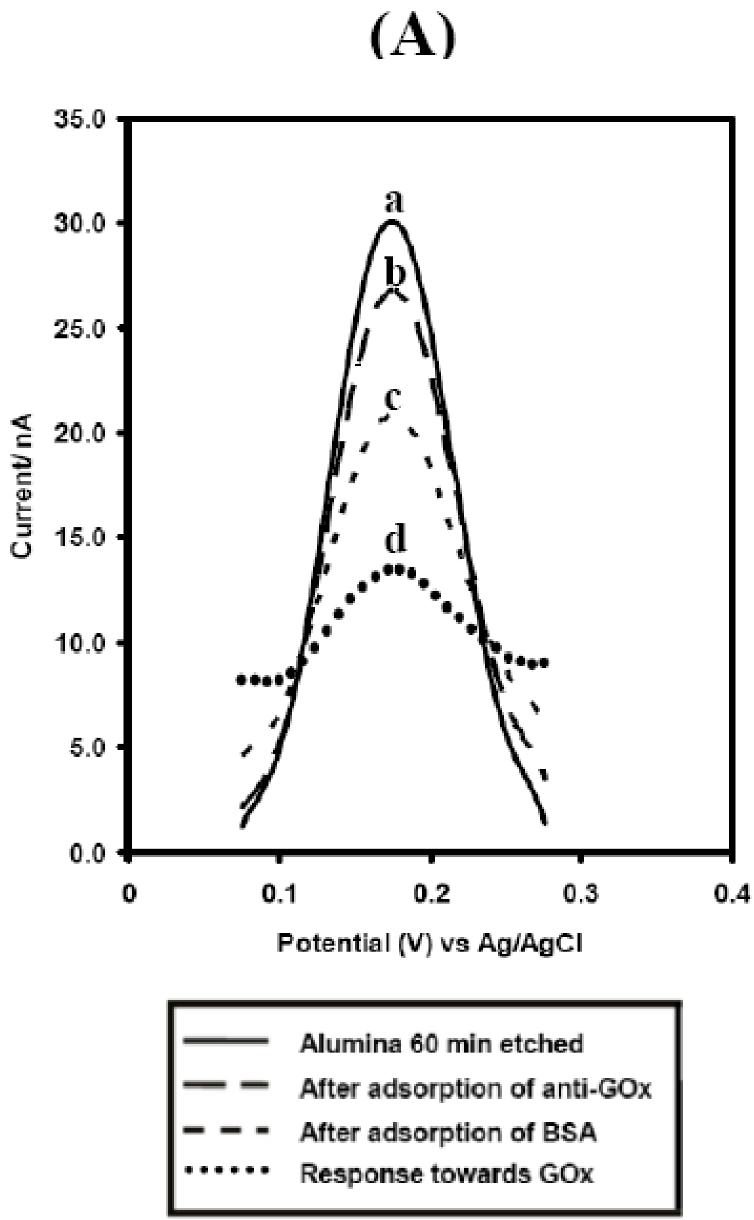
SEM images

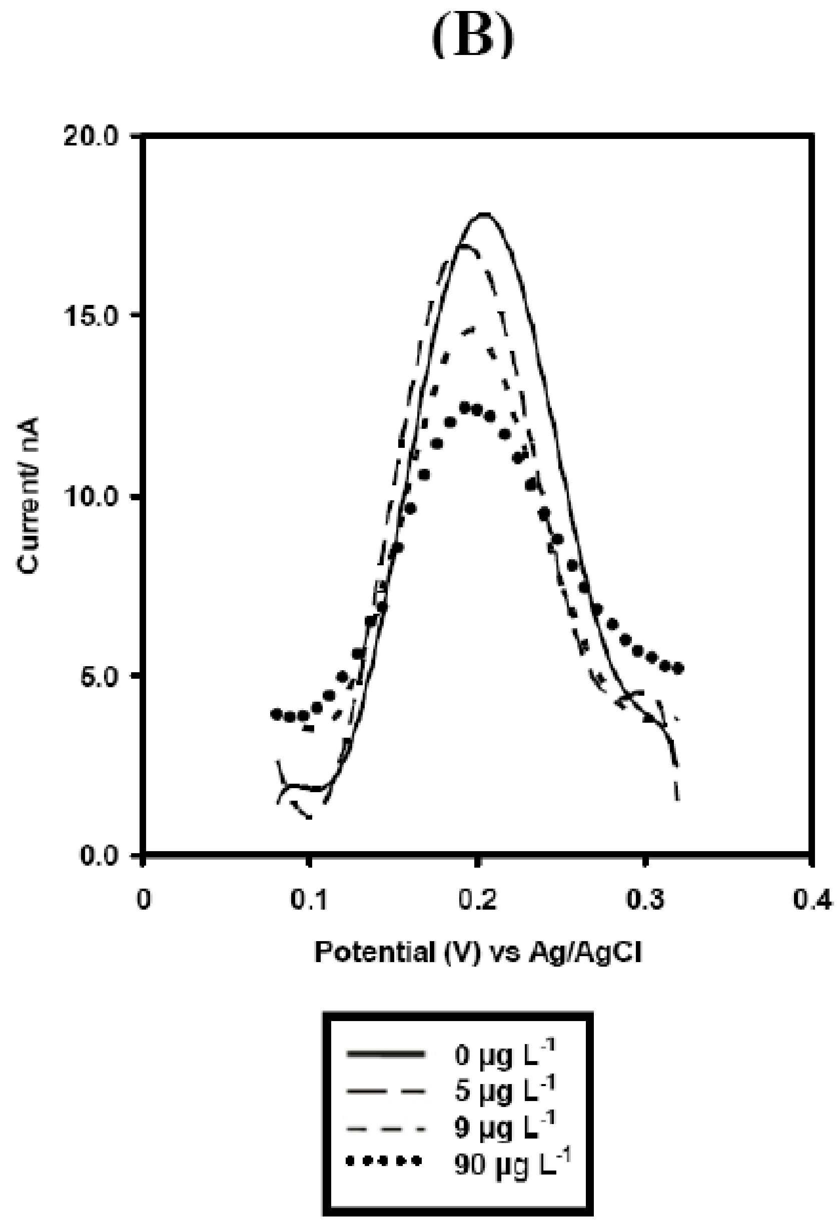
Our biomimetic approach

- Sensing of proteins



Our biomimetic approach





G.W. Koh, S. Agarwal, P.S. Cheow and C.S. Toh, *"Development of a membrane-based electrochemical immunosensor"*, Electrochimica Acta 53 (2), 803-810 (2007).

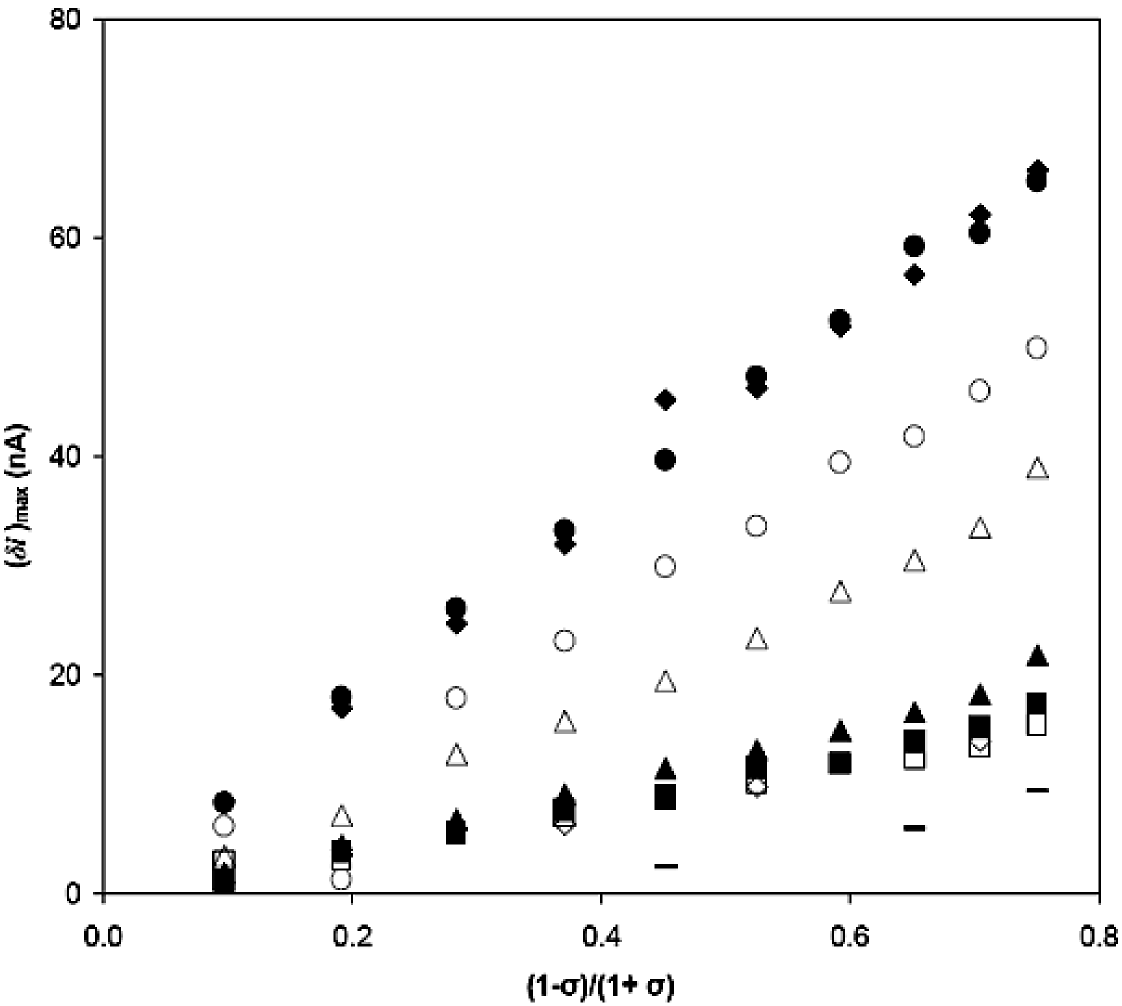
$$(\delta i)_{\max} = \frac{nFAD^{1/2}C_{\text{bulk}}}{\pi^{1/2}(\tau - \tau')^{1/2}}\left[\frac{(1-\sigma)}{(1+\sigma)}\right]$$

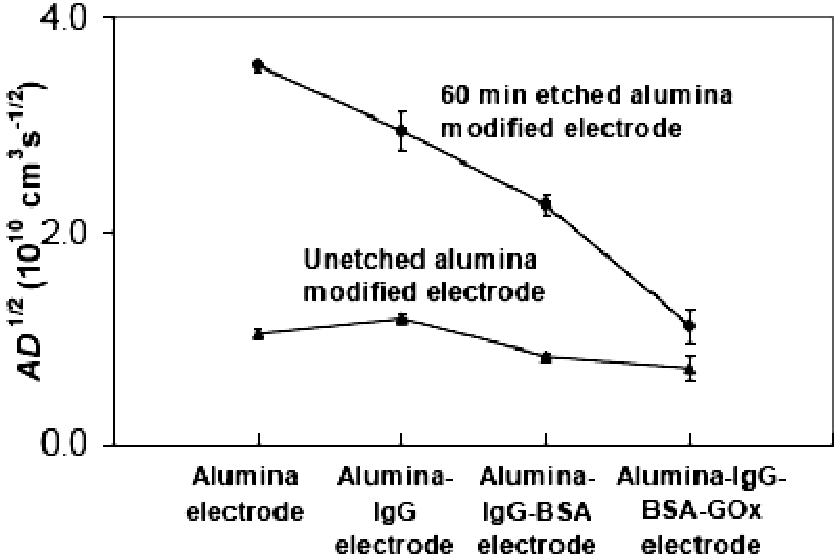
$(\delta i)_{\max}$ is the maximum peak height or DPV signal response

$(\tau - \tau')$= pulse duration

ΔE= pulse amplitude

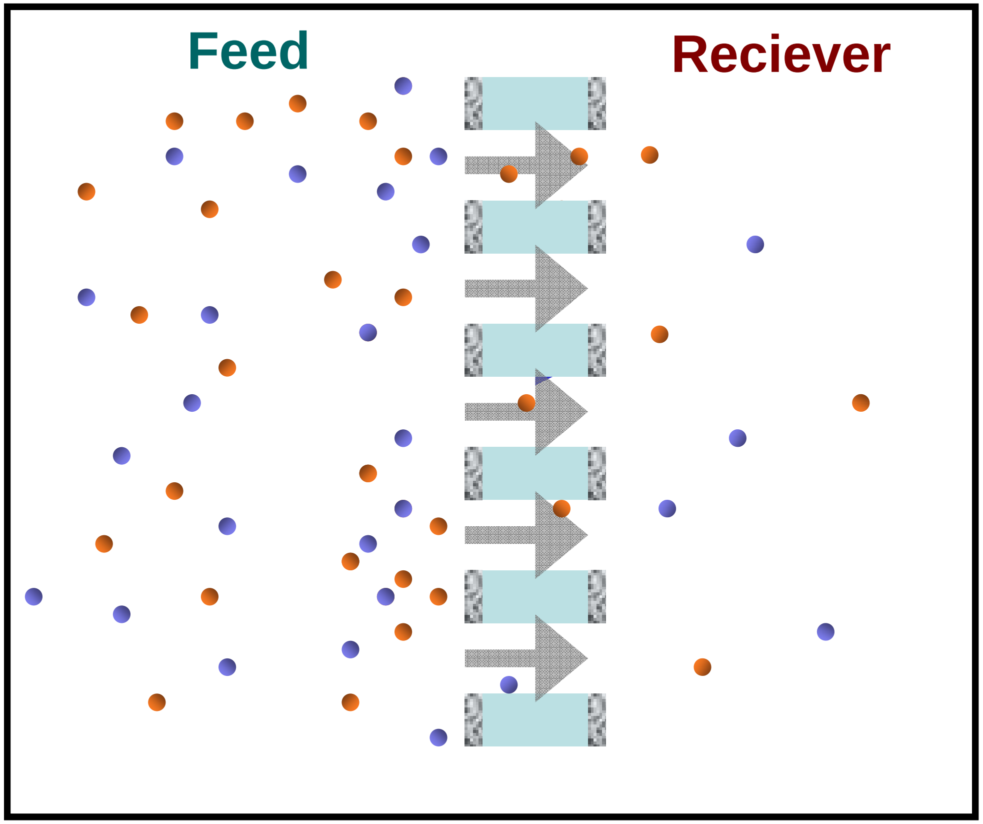
$\sigma = \exp(-nF\Delta E/2RT)$ for the oxidation process.

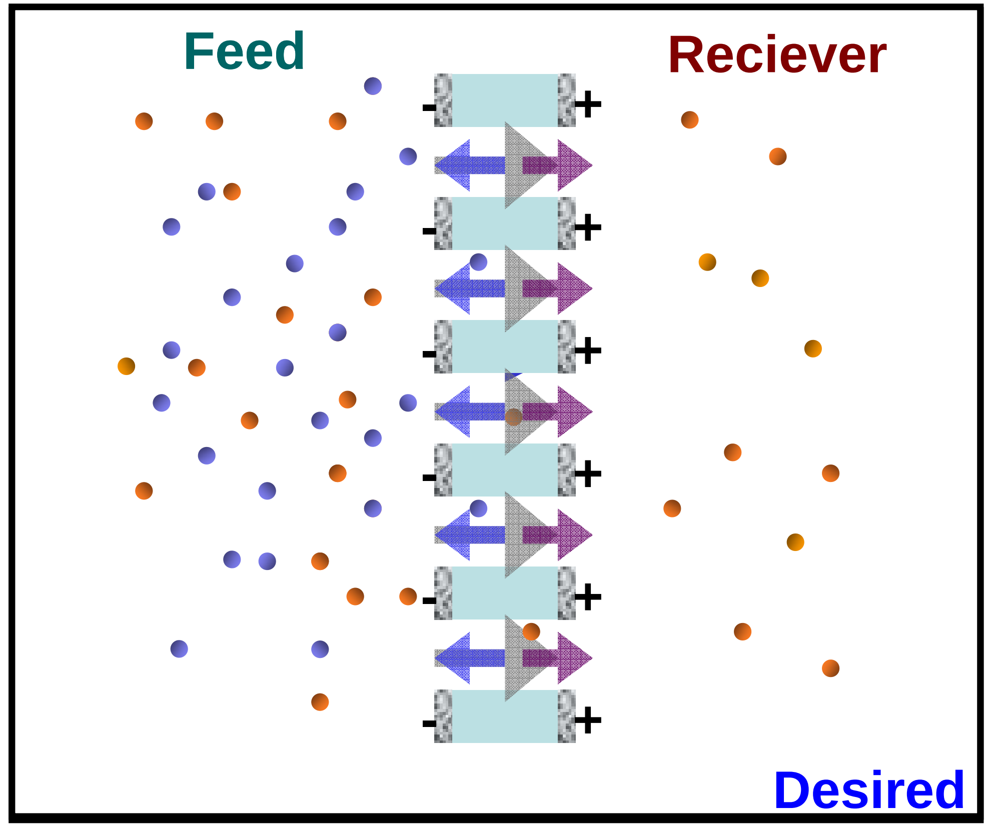




Biomimetic approach

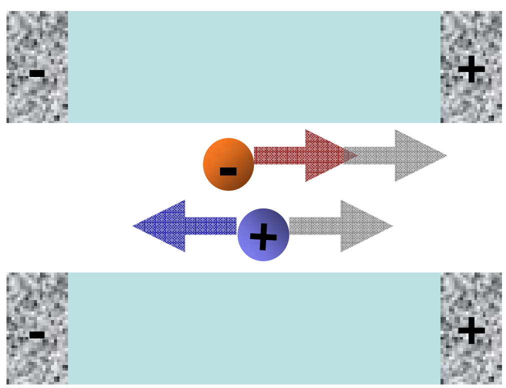
Analytical Separation of proteins

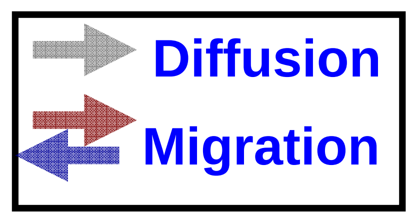




Diffusion

Diffusion and migration





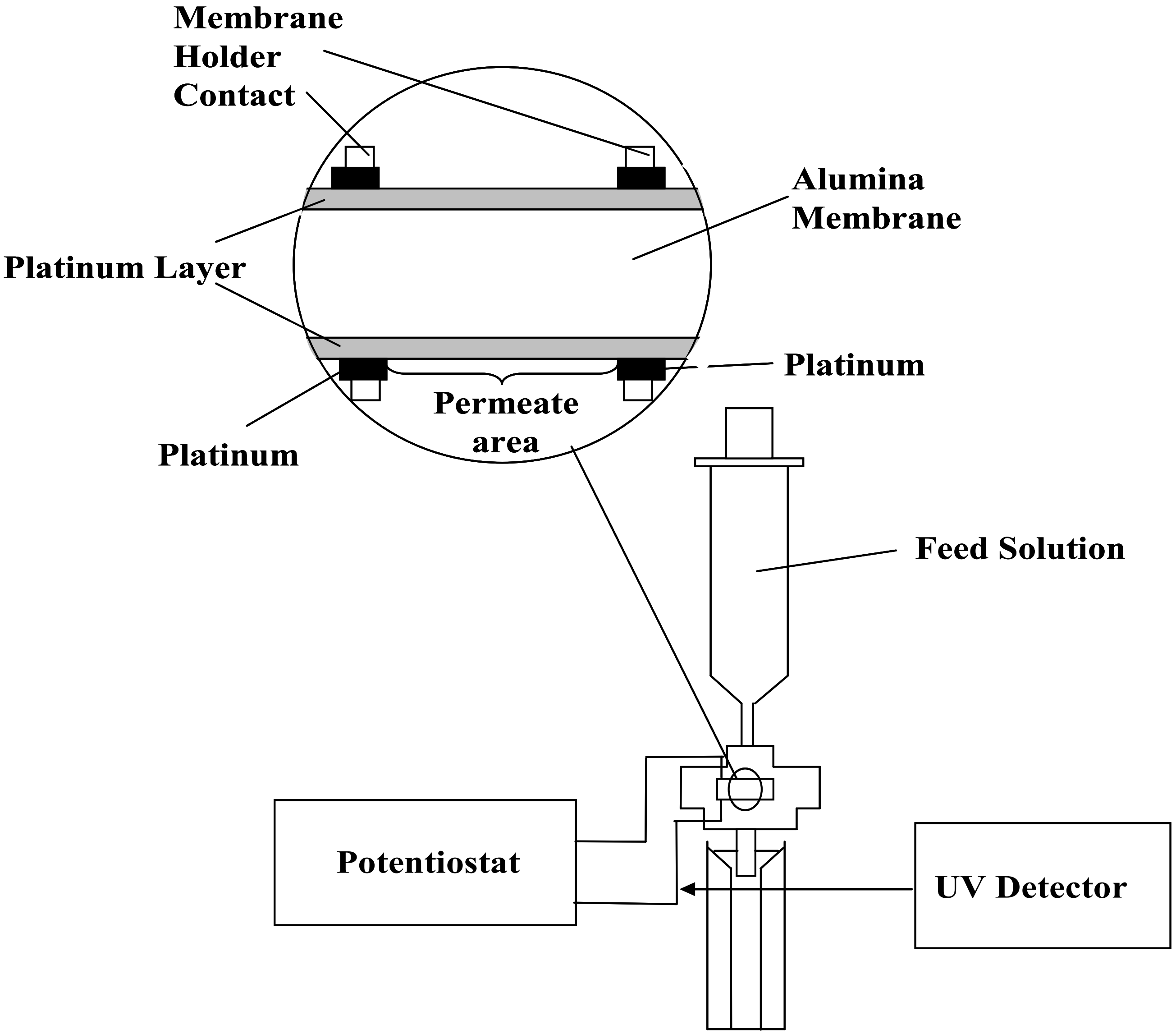
Biomimetic approach

- Separation of proteins

Static system

Uncoated

Metal coated



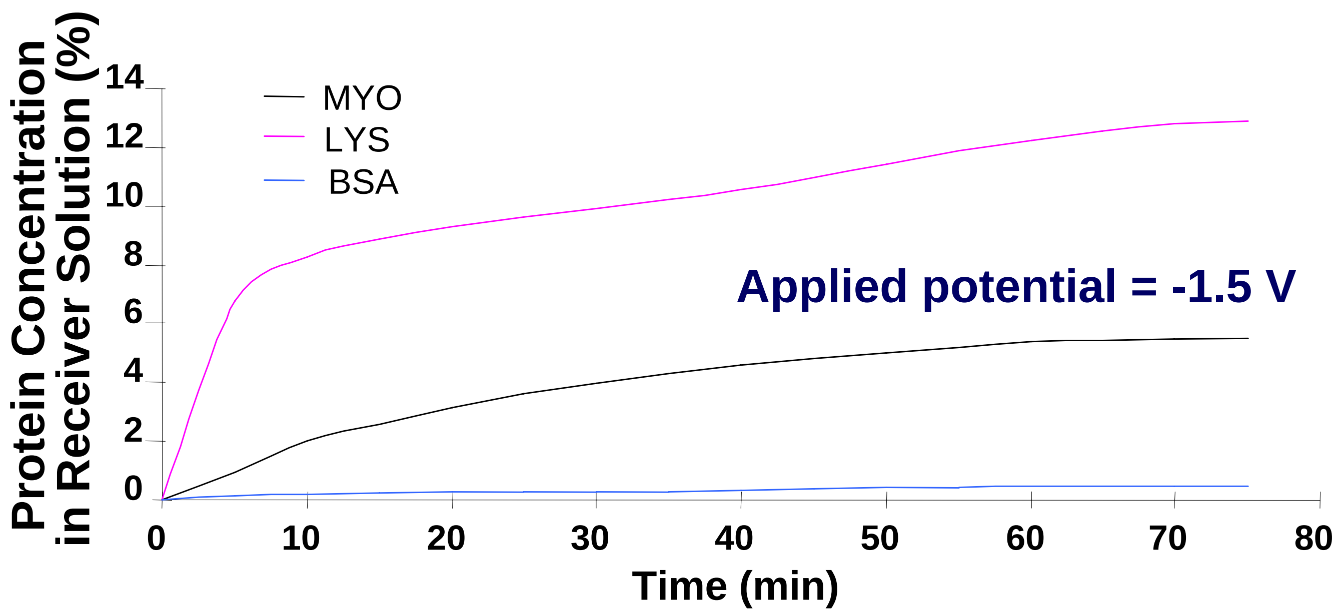
Biomimetic approach

Protein	pI
Bovine Serum Albumin (BSA)	4.9
Lysozyme (LYS)	11.0
Myoglobin (MYO)	6.9

At pH 7.0,

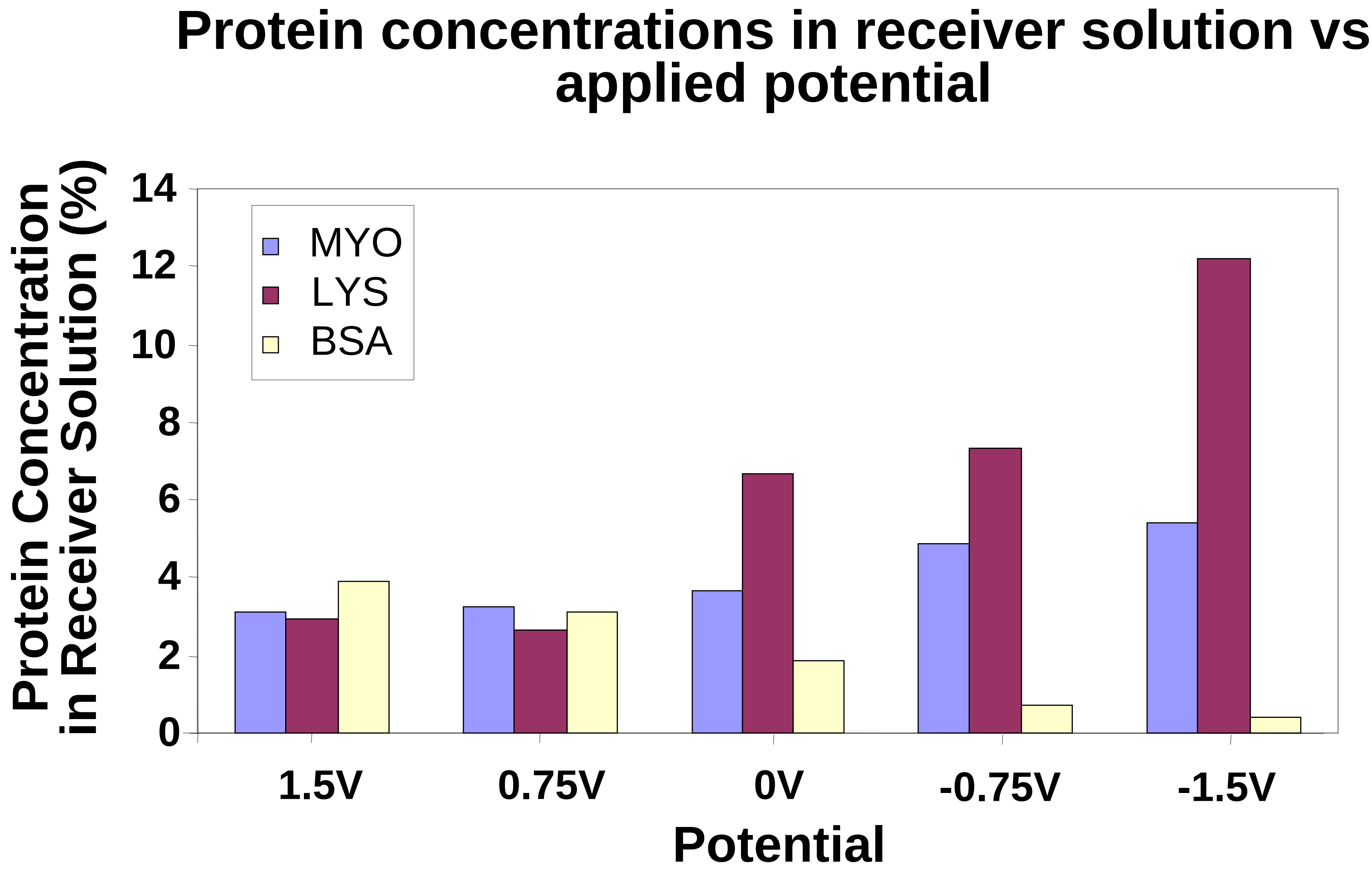
BSA: -ve charge
LYS: +ve charge
MYO: ~0 charge

Change of protein concentrations in receiver solution over time



(Receiver concentration is expressed as % of initial feed concentration)

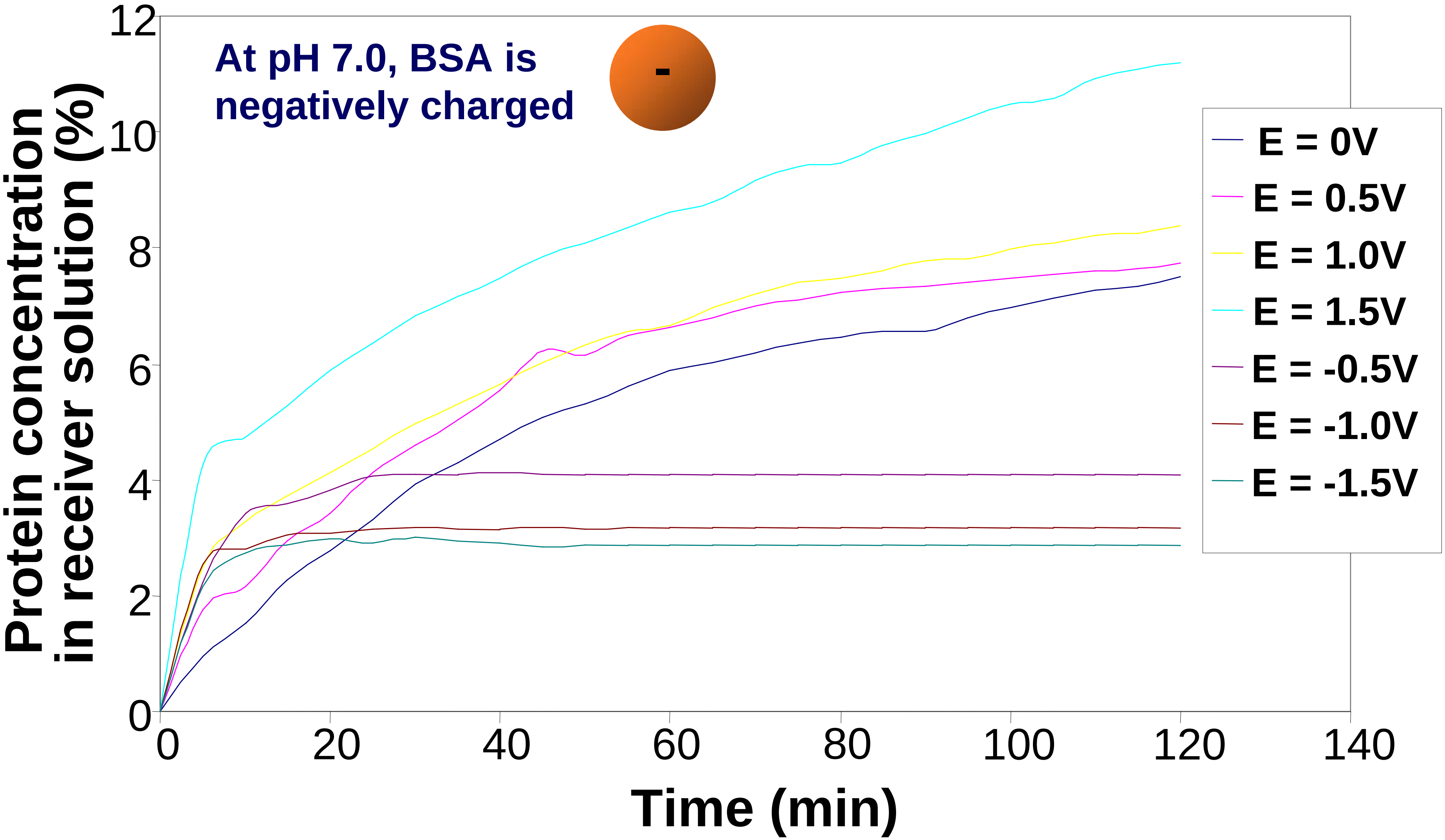
Biomimetic approach



Concentrations measured after 60 min

Biomimetic approach

Single Protein – Bovine Serum Albumin (BSA)

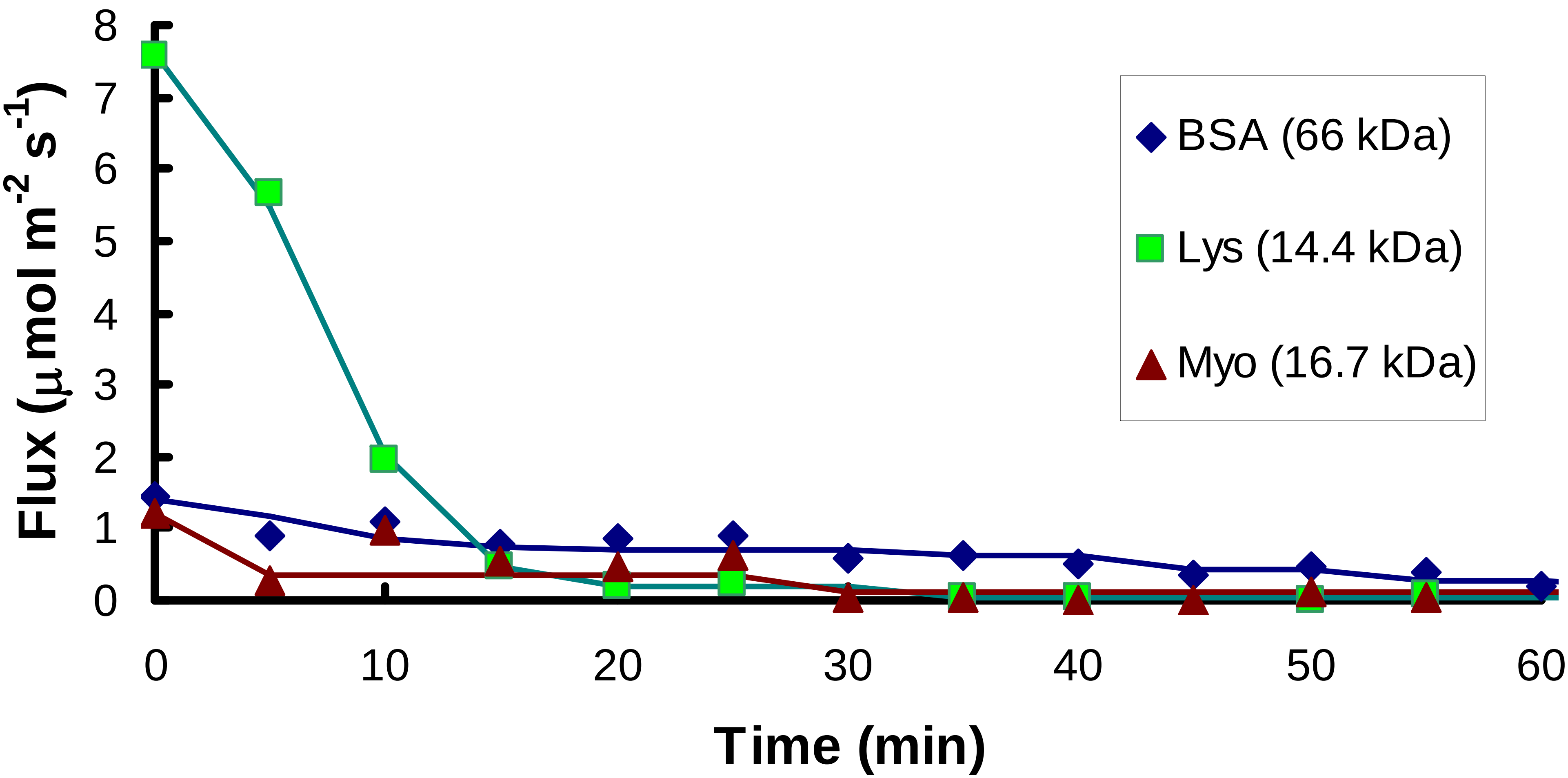


Biomimetic approach

0 V

$$J = -D\frac{\delta C}{\delta x}$$

Diffusion



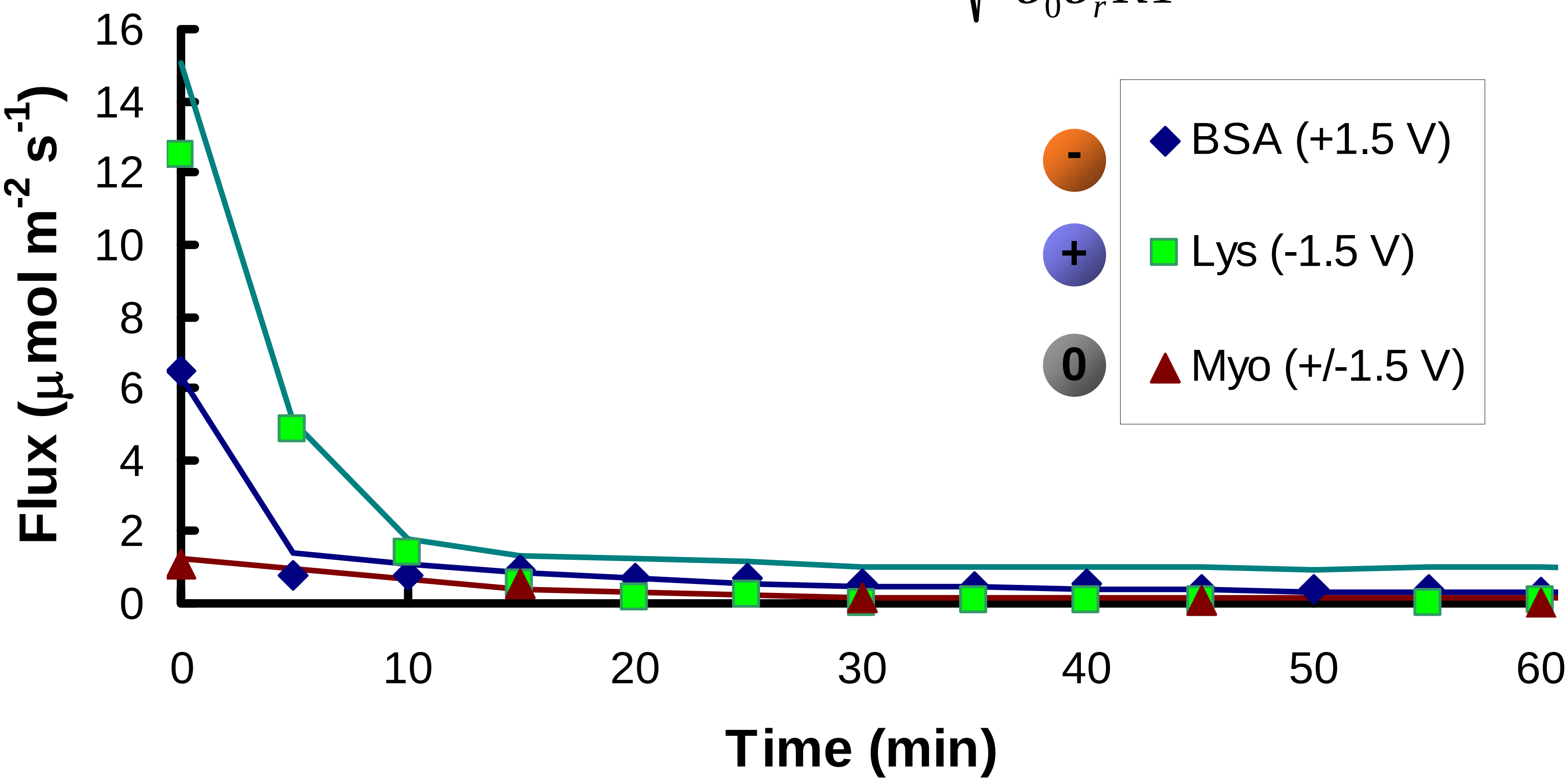
Biomimetic approach

At favourable potentials

$$J = -D\frac{\delta C}{\delta x} - \frac{zF}{RT}DC\frac{\delta \phi}{\delta x} \qquad \textbf{(1)}$$

Diffusion   Electrophoretic

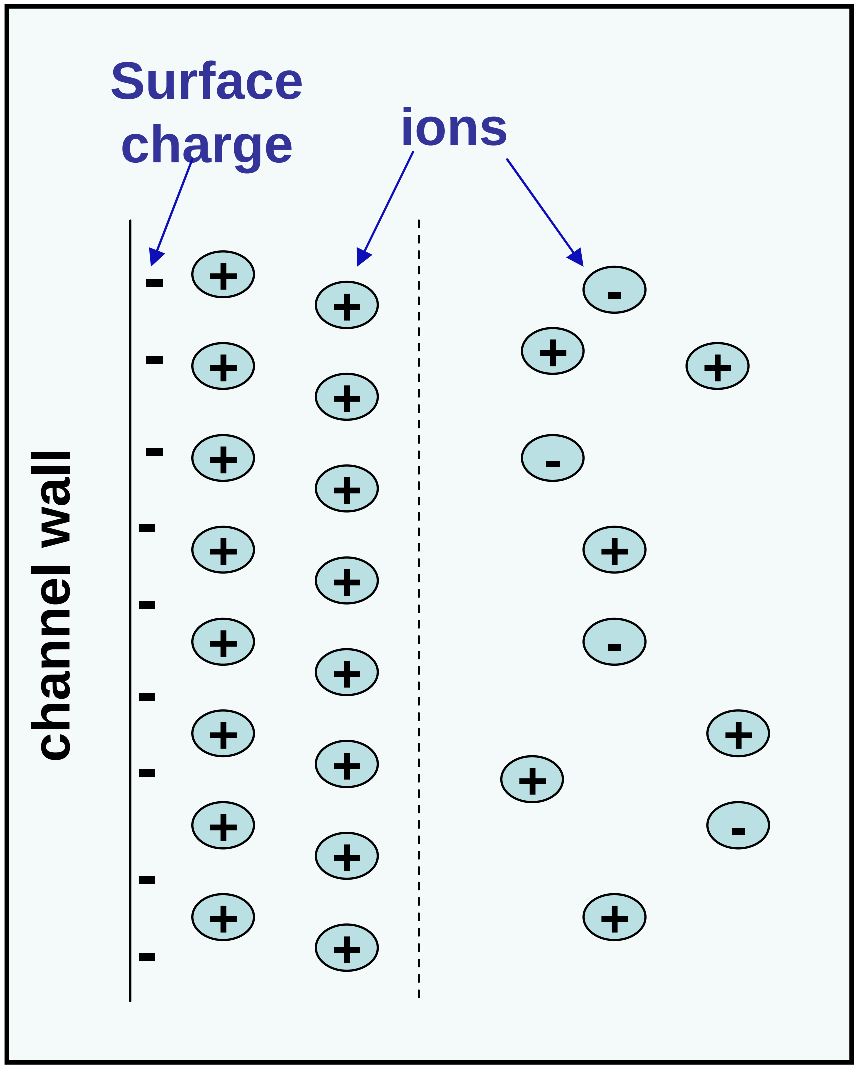
$$\varphi_x = \varphi_0 \exp(-\kappa x) \qquad \kappa = \sqrt{\frac{F^2 \Sigma c_i z_i^2}{\varepsilon_0 \varepsilon_r RT}} \qquad \textbf{(2)}$$

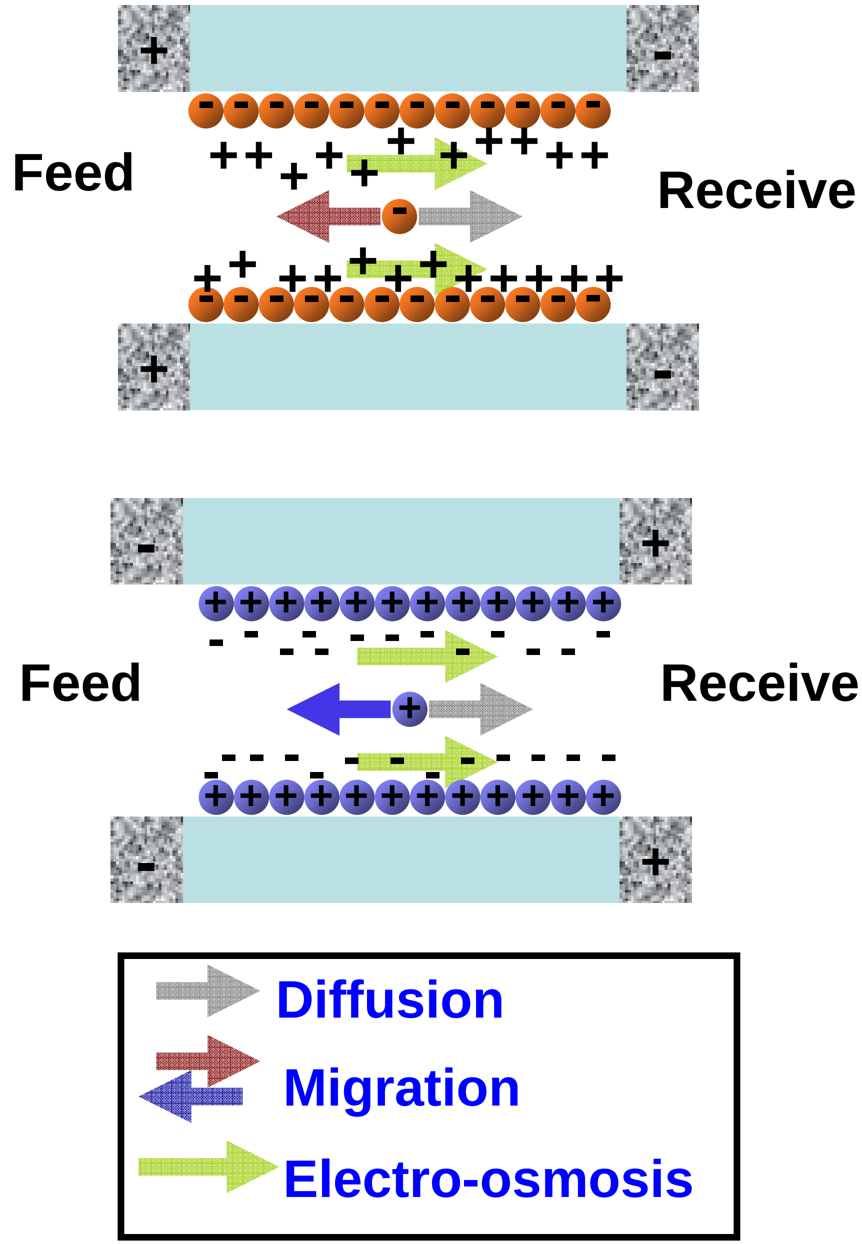


Biomimetic approach

Electroosmotic movement

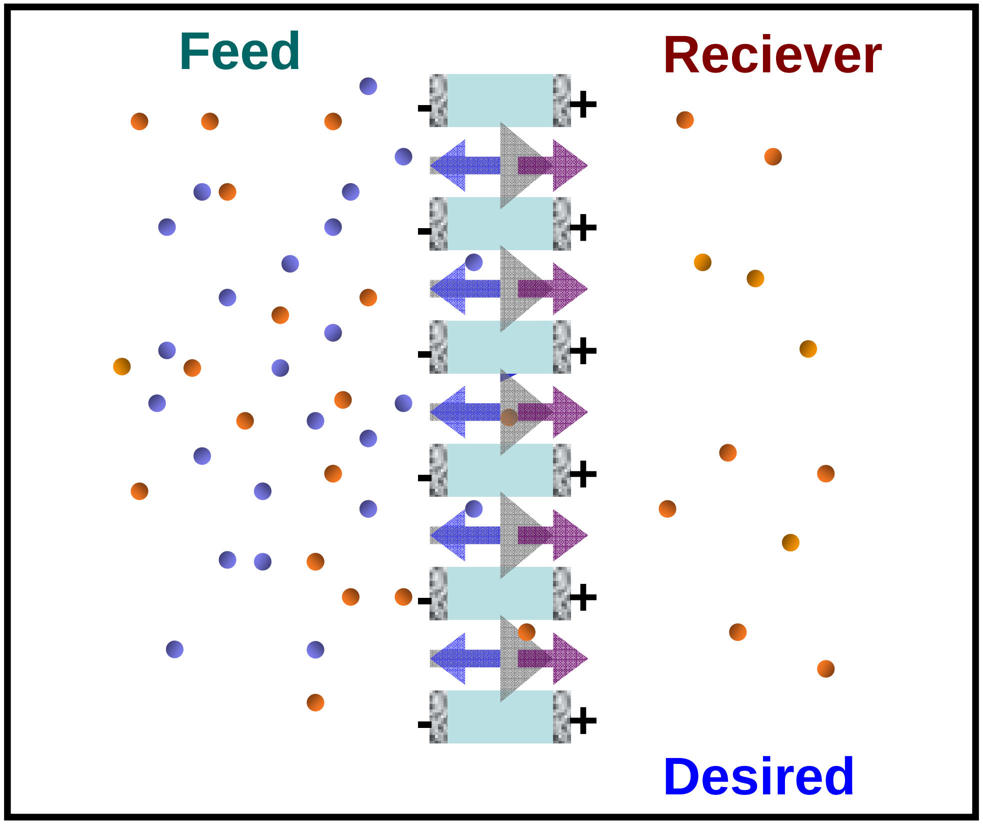
At unfavourable potentials

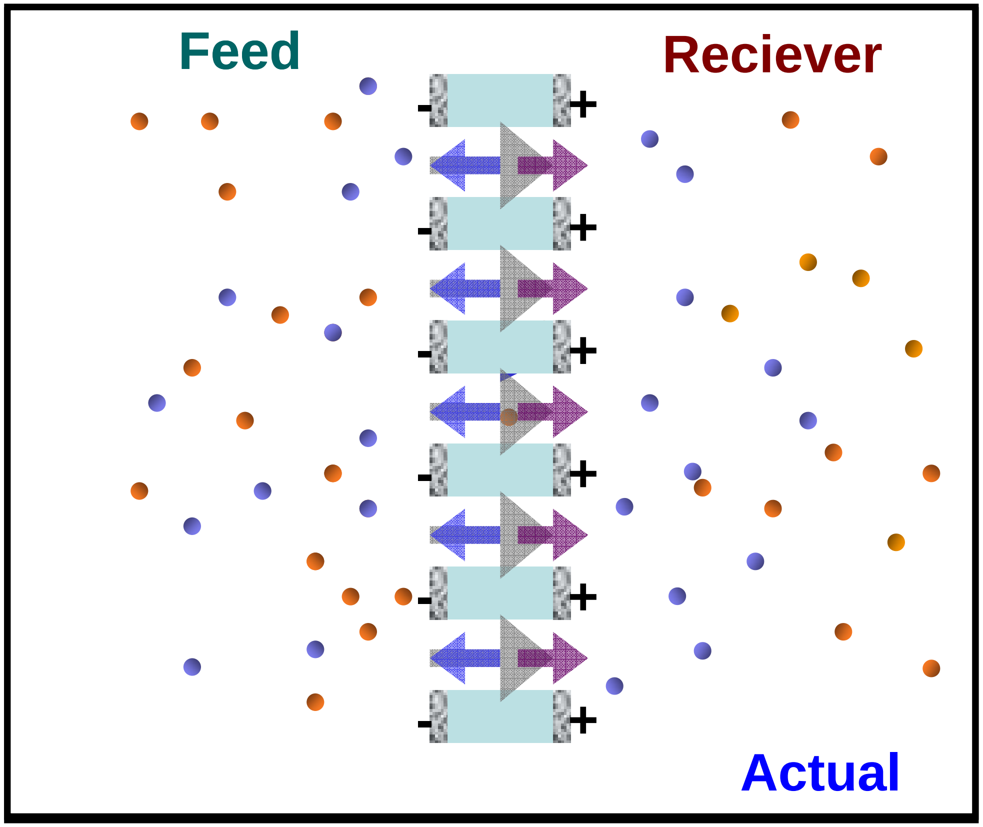




Biomimetic approach

Analytical Separation of proteins

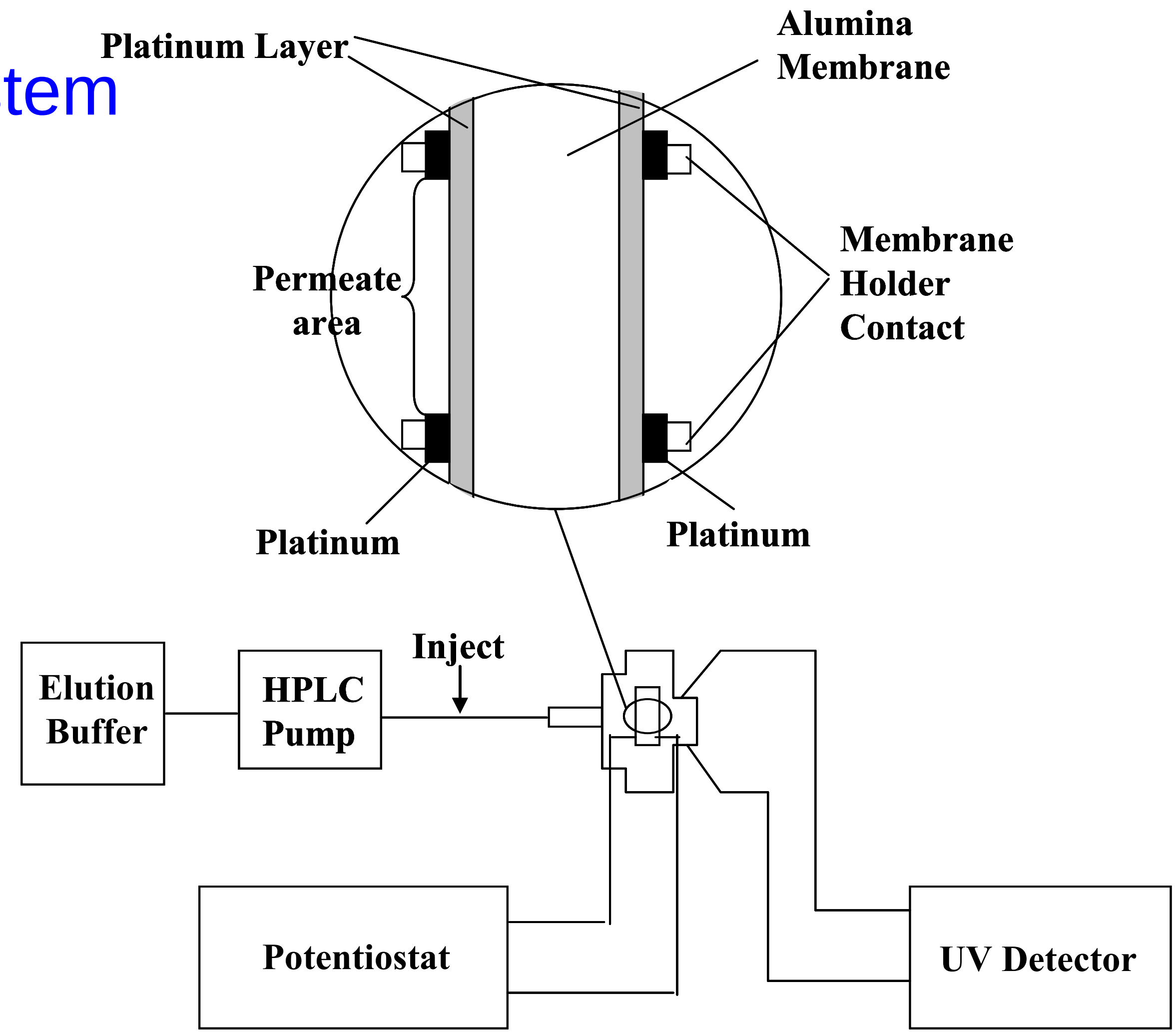




Biomimetic approach

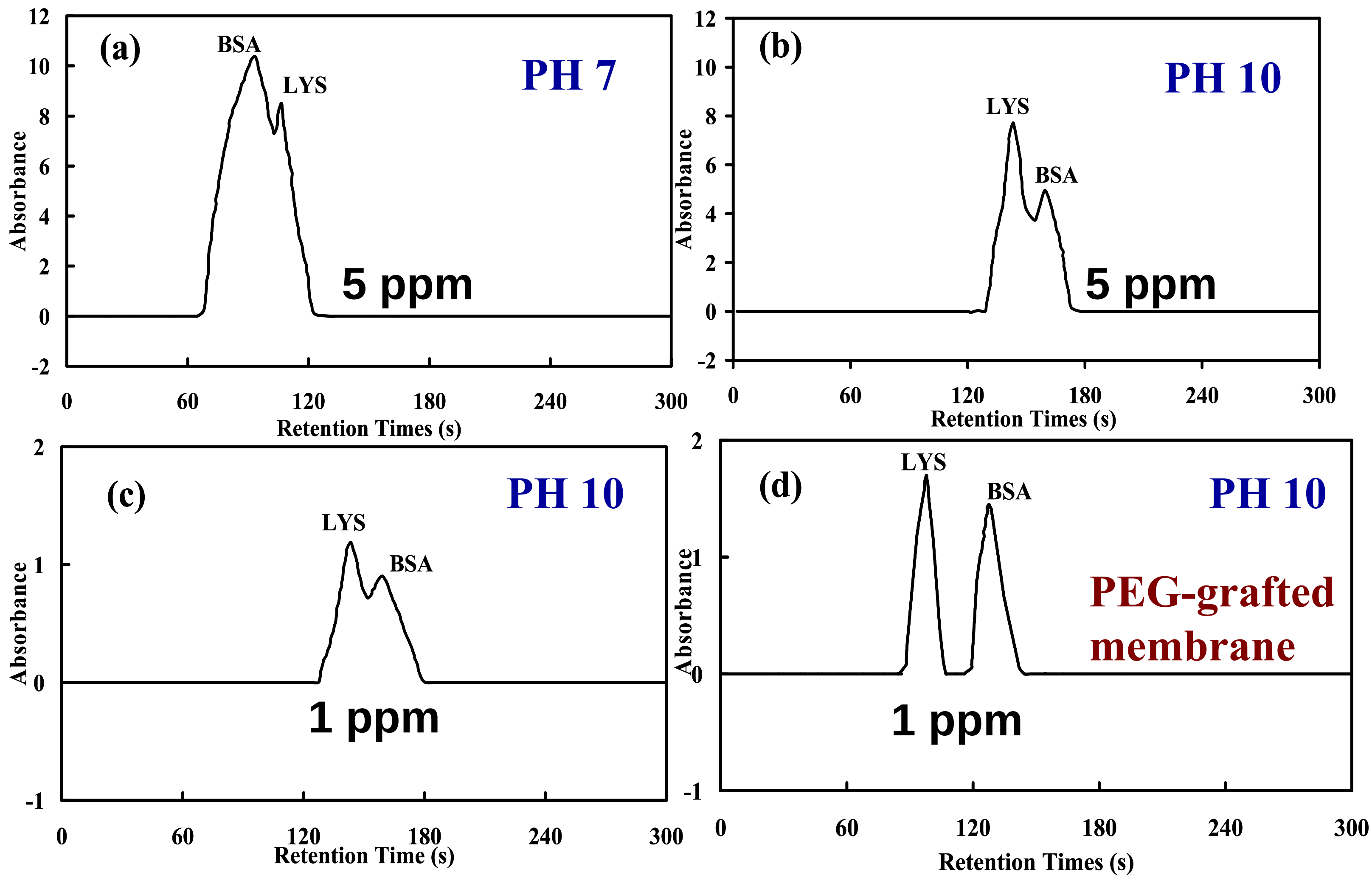
- Separation of proteins

Flow system



Biomimetic approach

Applied potential of +2V



P.S. Cheow, E. Ting, M.Q. Tan and C.S. Toh, *"Separation of proteins using nanoporous alumina membrane"*, submitted.
P.S. Cheow and C.S. Toh, *"Electromembrane for analytical separation of proteins"*, invention disclosure.

Conclusion

- Development of a protein sensor based on selective binding of protein to ‘receptor’ lined along walls of nanochannels. Signaling is induced based on extent of blockage of channels.
- Protein separation using facilitated transport strategy. Proteins are selected based on charge-to-size ratio.
- On-going work to reduce size of channels so to ‘fit’ the size of smaller molecules.

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