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

Chee-Seng Toh

Assistant Professor
Department of Chemistry
Faculty of Science
National University of Singapore
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

**Materials-focused strategy**

- 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**

$$2\text{Al} + 3\text{H}_2\text{O} \rightarrow \text{Al}_2\text{O}_3 + 3\text{H}_2$$
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

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 \( (Furneaux \text{ et. al. Nature 337, 147, 1989}) \)
  - Re-anodization of alumina under constant current conditions \( (Jagminas \text{ et. al.  Appl. Surf. Sci. 405, 252, 2006}) \)
Our biomimetic approach

- Sensing of proteins
Our biomimetic approach

(A)

(B)

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

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

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

\(\Delta E\) = pulse amplitude

\(\sigma = \exp\left(-\frac{nF\Delta E}{2RT}\right)\) for the oxidation process.
Biomimetic approach

Analytical Separation of proteins

Diffusion

Diffusion and migration
Biomimetic approach

Separation of proteins

Static system

Uncoated

Metal coated

Membrane Holder Contact

Platinum Layer

Alumina Membrane

Platinum

Permeate area

Feed Solution

Potentiostat

UV Detector
Biomimetic approach

At pH 7.0,

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

<table>
<thead>
<tr>
<th>Protein</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Serum Albumin (BSA)</td>
<td>4.9</td>
</tr>
<tr>
<td>Lysozyme (LYS)</td>
<td>11.0</td>
</tr>
<tr>
<td>Myoglobin (MYO)</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Change of protein concentrations in receiver solution over time

Applied potential = -1.5 V

(Receiver concentration is expressed as % of initial feed concentration)
Protein concentrations in receiver solution vs applied potential

Concentrations measured after 60 min
At pH 7.0, BSA is negatively charged.
Biomimetic approach

\[ J = -D \frac{\delta C}{\delta x} \]

Diffusion

0 V

Flux (\(\mu\)mol m\(^{-2}\) s\(^{-1}\))

- BSA (66 kDa)
- Lys (14.4 kDa)
- Myo (16.7 kDa)

Time (min)
Biomimetic approach

At favourable potentials

\[ J = -D \frac{\delta C}{\delta x} - \frac{zF}{RT} D C \frac{\delta \phi}{\delta x} \]  \quad (1)

Diffusion \hspace{1cm} Electrophoretic

\[ \varphi_x = \varphi_0 \exp(-\kappa x) \]

\[ \kappa = \sqrt{\frac{F^2 \sum c_i z_i^2}{\varepsilon_0 \varepsilon_r RT}} \]  \quad (2)

![Graph showing flux over time for different potentials](image)
Biomimetic approach

Electroosmotic movement

At unfavourable potentials

Surface charge ions

channel wall

Feed Receive

Feed Receive

Diffusion Migration Electro-osmosis
Biomimetic approach

Analytical Separation of proteins
Biomimetic approach

Separation of proteins

Flow system
Biomimetic approach

Applied potential of +2V

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.
Acknowledgements

- Guiwan Koh (graduate student)
- Shuchi Agarwal (former research assistant)
- Pui-Sze Cheow (graduate student)
- Eugene Ting (honours student)
- Mei-Qi Tan (honours student)
- Janice Ong (honours student)
- Thanh Binh Nguyen (graduate student)

- MOE (FRC) grant, the NUS Nanoscience and Nanotechnology Initiatives (NUSNNI), NUS scholarships for graduate students