

# Integrated Electrochemical and Optical Methods for Studying Membrane Proteins

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## Introduction

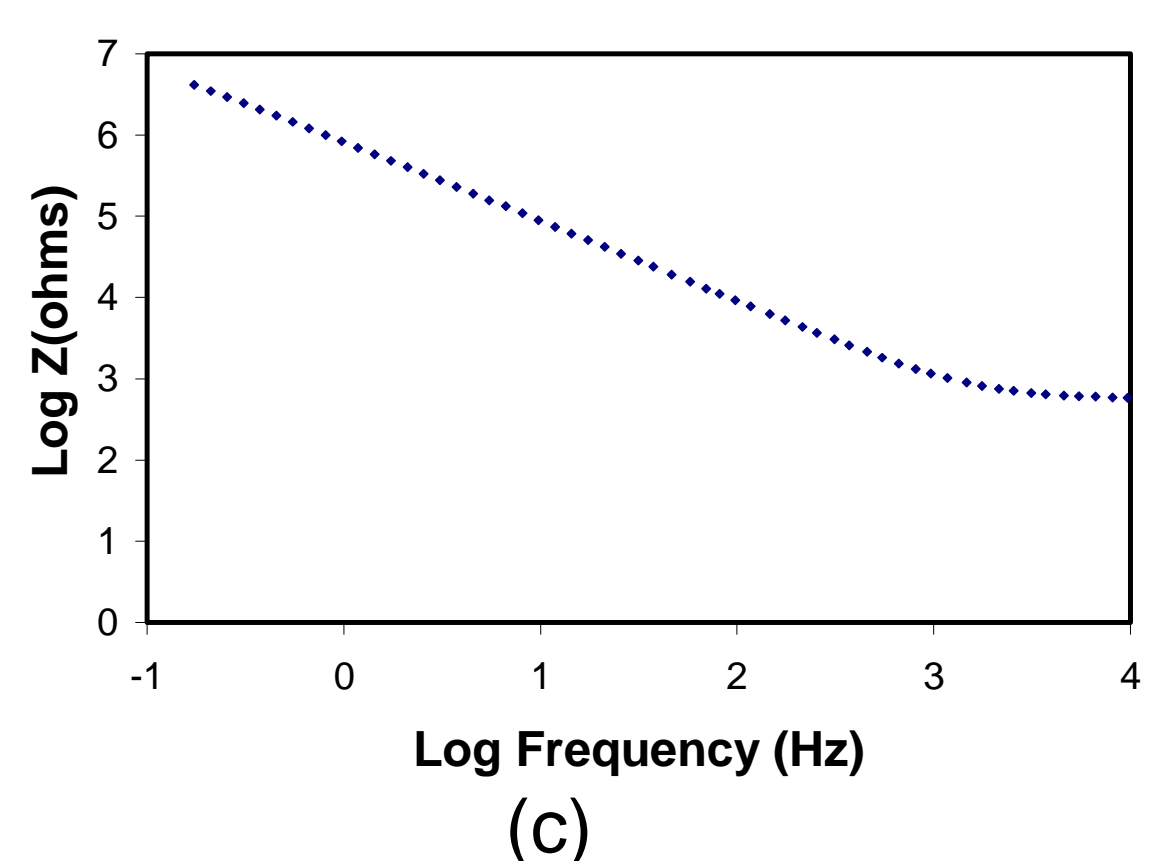
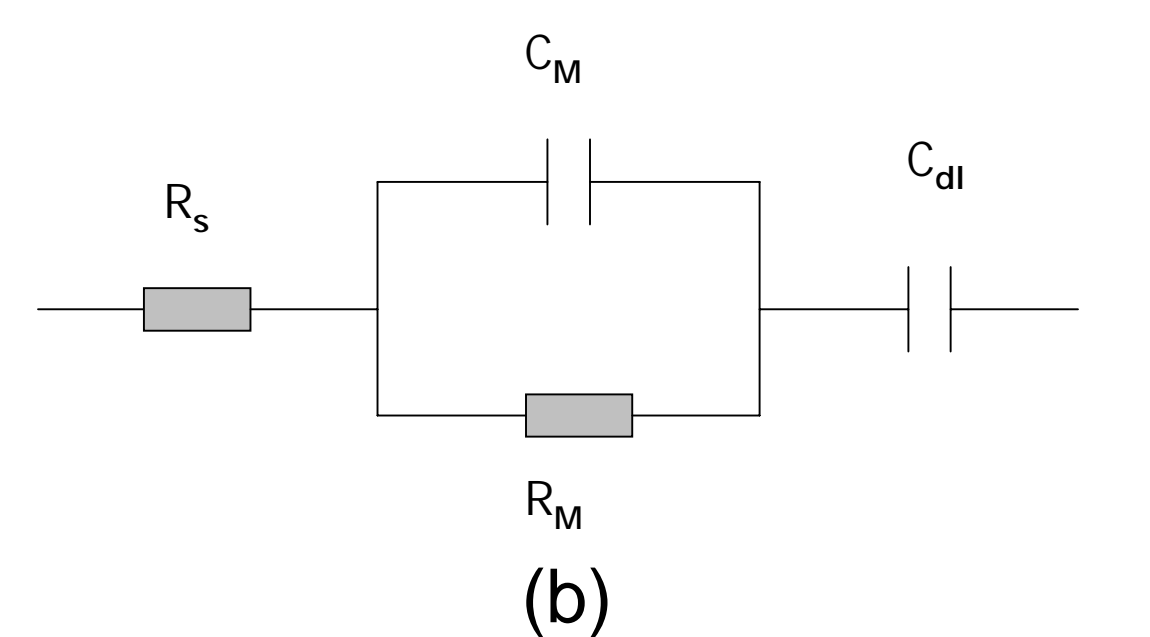
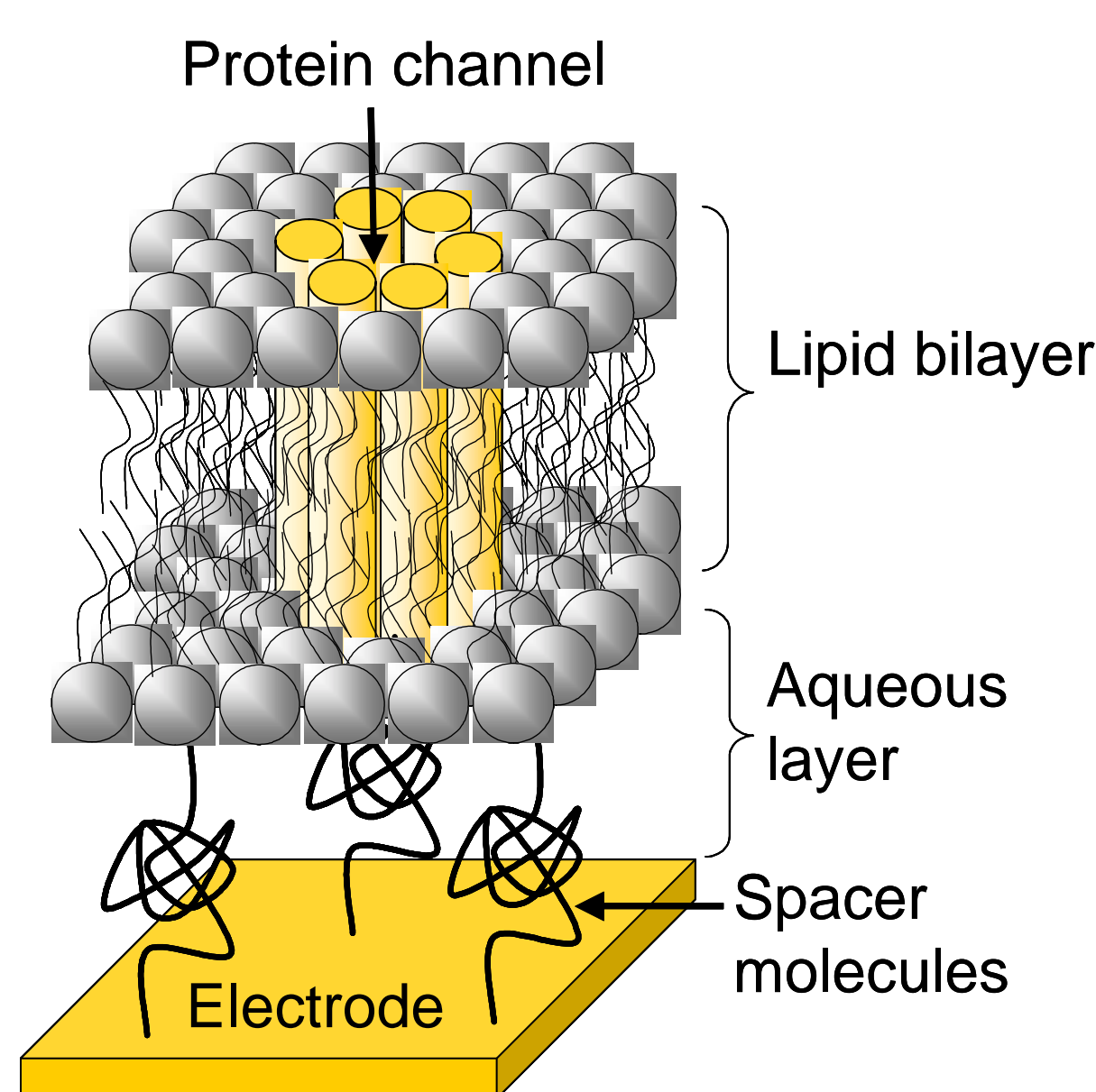
- Cell membranes use a bilayer lipid membrane (BLM) and membrane-bound protein(s) to efficiently perform a diverse array of vital molecular processes
- These processes can be mimicked in vitro by reconstituting membrane proteins into artificial BLM
- Recent progress in proteomics allows previously unknown membrane proteins having desired properties to be rapidly identified, cloned, mass produced and purified
- A Michigan Technology Tri-Corridor (MTTC) grant recently established a multi-disciplinary and multi-institutional center for excellence that broadens the scope of this expertise to include both the membrane proteins and the functional interfaces in which they are embedded
- The Center for Nanostructured Biomimetic Interfaces (CNBI) consists of 11 researchers from Michigan State University, the Michigan Molecular Institute and Neogen corporation

## Application to Pharmaceutical Research

- Produce membrane proteins having medical relevance
  - Protein Expression Laboratory
  - REF Center: Structural Biology of Membrane Proteins
- Measure protein activity in lipid bilayer
  - Electrochemical methods
  - Optical methods
- Membrane proteins under investigation:
  - Transient response potential vanilloid (TRPV) family
  - Neuropathy target esterase (NTE)
  - Cytochrome C oxidase
  - Cytochrome P450s
- Silicon-based, high-density biosensor arrays
- Silicon-based, high-throughput drug screening systems

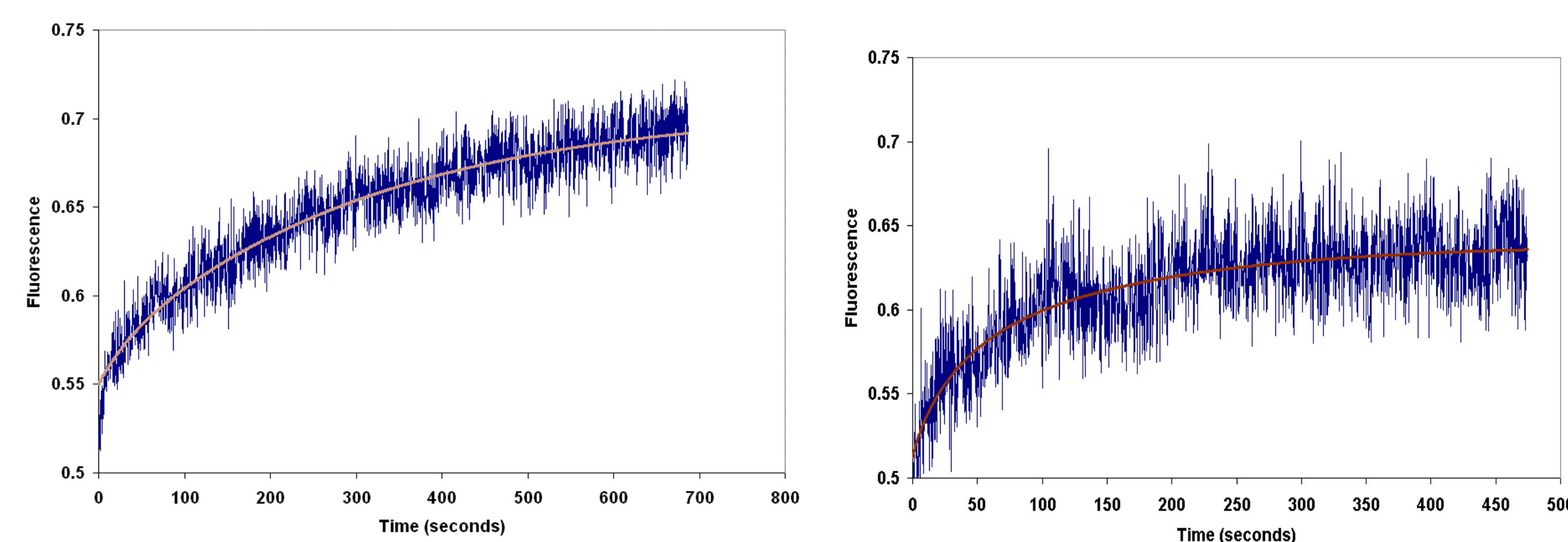
## Electrochemical Techniques

- AC Impedance spectroscopy
  - Tethered lipid bilayer is assembled on the gold electrode
  - Ion channel protein is embedded inside the lipid bilayer (see fig. a)
  - Impedance is measured as a function of frequency (see fig. b)
  - Data are fitted to an equivalent circuit (see fig. c)
  - Approach allows high throughput screening of compounds that interact with membrane proteins
- Cyclic Voltammetry
  - Membrane protein is embedded inside the tethered lipid bilayer
  - Voltage is cycled, and resulting current gives useful information about protein function
  - Approach is useful for biosensors



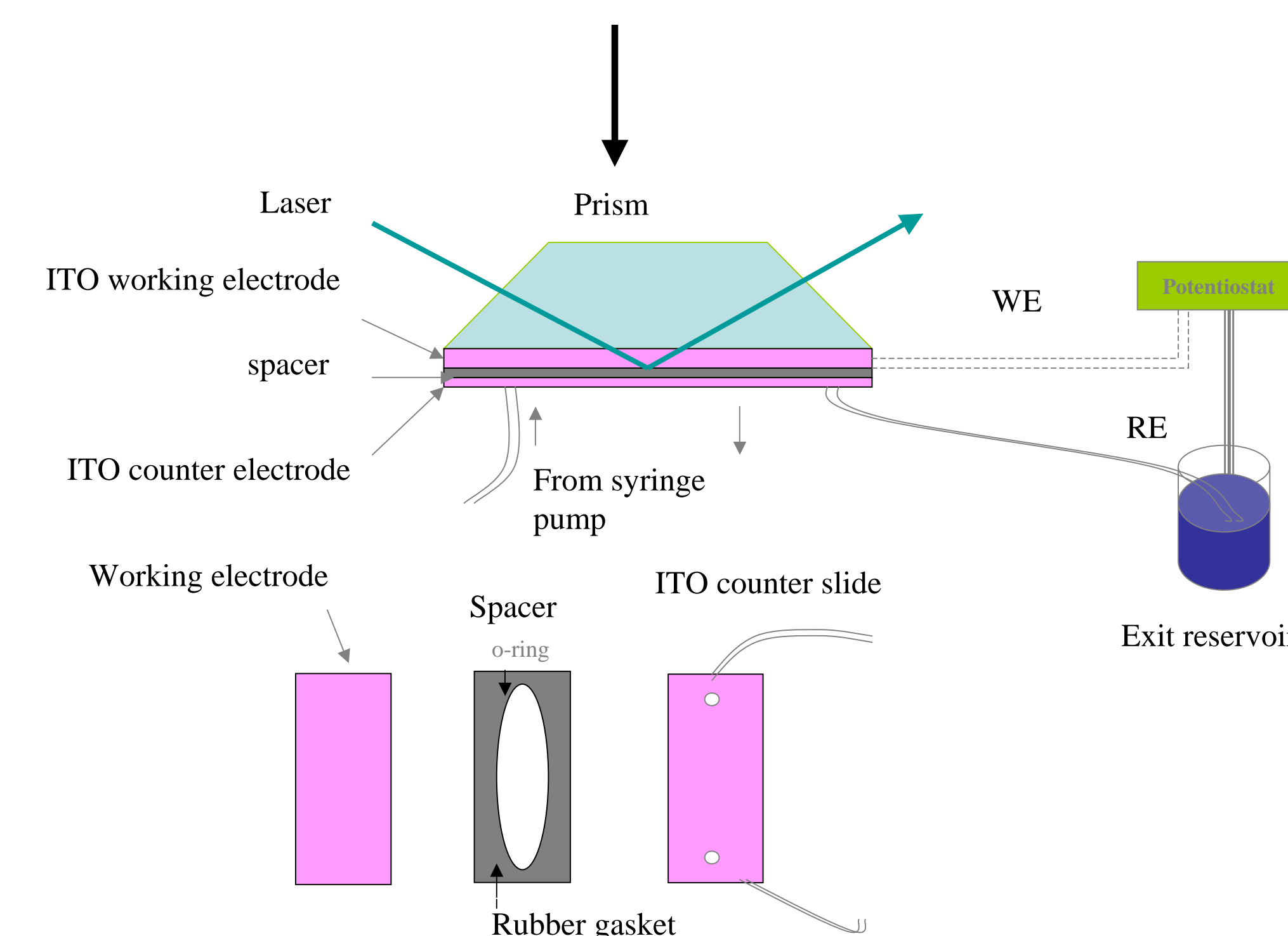
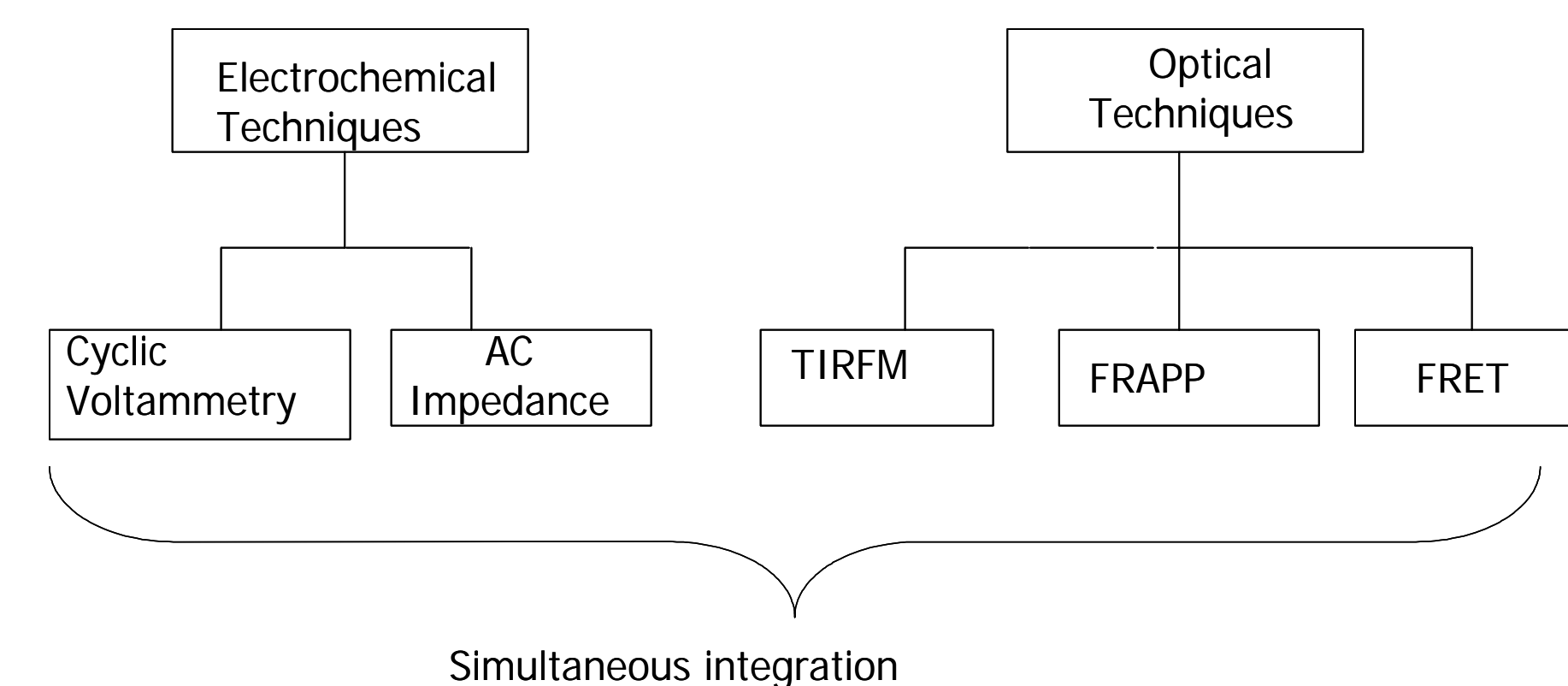
## Optical Techniques

- Total internal reflection fluorescence microscopy (TIRFM) is used to study the adsorption of biomolecules at interface
- Fluorescence resonance energy transfer (FRET) and TIRFM can be used to study the interactions of biomolecules on or within the BLM
- Fluorescence recovery after pattern photobleaching (FRAPP) can be used to measure the fluidity of BLM (see example recovery curves below).



## Objective

- Develop a system for the simultaneous integration of optical and electrochemical methods



## Acknowledgement

This work was funded by the Michigan Technology Tri-Corridor (MTTC), Michigan State University (MSU) Start-Up Funds, the MSU Intramural Research Grants Program, and the Seed Research Fund by the Center for Fundamental Materials Research at MSU.