

Integrated Electrochemical and Optical Methods for Studying TRPV Channel 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 Cardiovascular 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
- Screen for agonists, antagonists, allosteric modulators
 - Silicon-based, high-density biosensor arrays
- Evaluate protein-based sensing mechanisms
 - ion concentration, pressure, shear

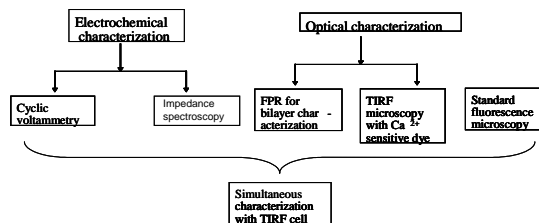
Vanilloid Receptor (VR1)

- The Transient response potential vanilloid (TRPV) family of channel proteins is important in detection of chemical and physical stimuli.
- VR1 (TRPV1) is expressed almost exclusively by primary neurons and is a nonselective cation channel that serves as a polymodal detector of stimuli such as protons, lipid metabolites, and heat
- Data obtained in Dr. Wang's lab indicate that in addition to its well known role in perception of visceral and somatic pain, VR1 may serve as a transducer for water and sodium balance
- It may also play a significant role in salt induced increases in blood pressure
- A protagonist (capsaicin) can open up this VR1 channel leading to decrease in blood pressure
- An antagonist (capsazepine) was found to close this channel leading to increase in blood pressure in rats fed a high salt diet
- VR1 shows preference for Ca²⁺ ions

Objective

- To measure changes in ions flow through the VR1 channel due to
 - **mechanostimulation** (effect of shear flow and pressure)
 - **noxious heat** (elevated temperatures)
 - **chemostimulation** (capsaicin, resiniferatoxin)
- To develop an experimental system that can precisely control the variables above, and measure their influence on VR1 activity
- The main aim of this project is to determine VR1's properties as a chemoreceptor and a mechanoreceptor. The technique will then be used to determine VR1's role in regulating blood pressure

Experimental Strategy



- Reconstitution of VR1 in the lipid bilayer in its functional form
- VR1 is a transmembrane protein; sufficient space is needed on either side of bilayer to allow the reconstituted protein to retain its functional form.
- The additional space can be created by using a PEG terminated lipid.
- Activation of VR1 channels results in flow of Ca²⁺.
- Ca²⁺ sensitive fluorescent dye located in ionic reservoir. In presence of Ca²⁺ ions, which pass through VR1 channel, dye (which is otherwise non-fluorescent) is excited by 488nm.
- Stimulation of Ca²⁺ dye causes increase in fluorescence
- Flow of ions across the channel causes changes in electrical properties of bilayer, which can be measured by impedance spectroscopy.

