

Recombinant proteins produced
in different kind of cells

Cell Structure and Function

Cell

- Like atom is a unit of matter
- Cell is a basic unit of life
- Smallest living unit of an organism
- Grow, reproduce, use energy, adapt, respond to their environment.
- Many cannot be seen with the naked eye:

Membrane Structure S Openwetware Btn Btn Success

Laura D. Hughes



Membrane Structure S Openwetware Btn Btn Success:

Advanced Biotechnology R C Dubey, 2014 The book embodies 22 chapters covering various important disciplines of biotechnology such as cell biology molecular biology molecular genetics biophysical methods genomics and proteomics metagenomics enzyme technology immune technology transgenic plants and animals industrial microbiology and environmental biotechnology The book is illustrative It is written in a simple language

Model Membrane Architectures for the Study of Membrane Proteins Laura D. Hughes, 2013 Ion channels are ubiquitous proteins in the cellular membrane which contain pores that open or close in response to chemical or electrical changes When the pore opens ions flow down their electrochemical gradient Thus by selectively allowing ions to enter or leave the cytoplasm cell membranes are electrically excitable This capability allows for a multitude of processes including homeostasis neuronal signaling and muscle contraction Despite the importance of ion channels to understanding physiology membrane associated proteins are difficult to study and many questions remain A central question that has motivated this work is how precisely voltage gated potassium channels respond to electrical changes in the lipid membrane Though it is well understood that a portion of the channel known as a voltage sensor induces a protein motion which causes the pore to open and ions to flow exactly how the voltage sensor moves is still debated Experiments to directly link the structure of the protein with its function are challenging Traditional biochemical assays are difficult with membrane proteins since these proteins denature outside the confines of a lipid bilayer unless they are solubilized by detergent Cell based work has provided essential information on the function of the proteins but the cellular milieu is a complex environment Efforts to simplify the system using model supported lipid bilayers can be used with surface sensitive techniques and allow greater control over the lipid composition number of membrane proteins and interactions between components However these systems are often incompatible with membrane proteins due to interactions between the protein and the surface which have been found to limit the lateral mobility and sometimes the functionality of the proteins Our laboratory has developed a membrane architecture called a DNA tethered bilayer which is a promising avenue to enable surface sensitive measurements in a format compatible with membrane proteins In this system a planar lipid bilayer is formed through DNA hybridization between a giant unilamellar vesicle (GUV) displaying DNA on its surface and its complement covalently tethered to a glass slide The bilayer is therefore located near a surface but held apart by the length of the DNA tether Recently our laboratory has used this system to study membrane fusion and in preliminary work to study an integral membrane protein for which the lateral mobility was similar to that in a native environment To implement the DNA tethered bilayer as a general platform for studying membrane proteins however two significant challenges among others remain First the placement of the lipid bilayer needs to be controllable which would enable interfacing with electrodes and eventual multiplexing of measurements Second a robust and general method for delivering and incorporating membrane proteins must be developed This thesis tackles these tasks First we develop a

method for controlling the location of a DNA tethered lipid bilayer using microarray printing Chapter 2 DNA sequences are covalently attached to a glass slide in a pattern dictated by microarray printing GUVs which display the DNA complement to the patterned sequence are incubated with the surfaces and DNA tethered lipid bilayers form selectively over the patterned regions This method therefore produces arrays of isolated tethered membrane patches Moreover the composition of these bilayers can be altered to produce arrays of different lipid bilayers This technique offers promise in positioning bilayers near electrodes as well as producing arrays of tethered bilayers containing different membrane proteins Towards the second goal of incorporating membrane proteins into these tethered bilayers we have expressed and purified a functional voltage gated potassium channel Chapter 3 The channel was labeled with fluorescent dyes reconstituted into synthetic lipid vesicles and its functionality was measured in planar lipid bilayers Next we incorporated these channels into DNA tethered lipid bilayers by two different methods Chapter 4 In the first DNA mediated fusion was used to transfer proteoliposomes into tethered patches In the second GUVs were formed from proteoliposomes incubated with DNA lipid molecules and ruptured on a surface containing DNA Both of these methods successfully formed DNA tethered lipid bilayers containing potassium channels However no lateral mobility was observed for these channels Possible reasons for this immobility are discussed and future experiments are proposed Lastly we report on a common problem in fluorescence microscopy the tendency of fluorescent dyes to interact with lipid bilayers Chapter 5 This association can lead to experimental artifacts either from false fluorescence signals or from altered interactions between the labeled object and the bilayer Despite all the dyes being highly water soluble many of the dyes showed substantial interaction with the bilayer and the interaction spanned four orders of magnitude between the dyes tested

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