Purification and AFM Analysis of $P2X_4$ Receptor and Bacteriorhodopsin for Nanobiological Devices

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Nanobiology seeks to combine recent developments in nanotechnology with biological mechanisms to create novel electronic and biosensing molecular-scale devices. This overview identifies properties of the biological components in nanobiology, specifically, the protein bacteriorhodopsin (bR) and $P2X_4$ purinergic receptors. Protein purification plays a key role in obtaining available proteins from cultured cells. Multiple methods such as membrane fractionation, ion exchange chromatography, and immunoprecipitation were conducted in order to purify the $P2X_4$ protein. Atomic force microscopy (AFM) was then used to observe the structure of the protein in native conditions. In the analysis, although the ion channel structure could not be resolved, single $P2X_4$ proteins were seen to correspond to the previously described $P2X_2$ structure, a related P2 purinergic receptor. In addition to $P2X_4$, the possibility of utilizing bR as a proton pump in future optoelectronic devices was studied. Using AFM, the repeating bR trimer structure surrounded by a lipid membrane was visualized. bR embedded in membrane was then suspended over Si nanoholes. Though research is still at an early stage, membrane proteins such as $P2X_4$ and bR have potential uses in future nanobiological applications.

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Abstract

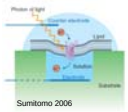
Nanobiology seeks to combine recent developments in nanotechnology with biological molecules to create novel electronic and biosensing molecular-scale devices. This overview identifies properties of the biological components in nanobiology, specifically, the protein bacteriorhodopsin (bR) and P2X4 purinergic receptors. Protein purification plays a key role in obtaining available proteins from cultured cells. Multiple methods such as membrane fractionation, ion exchange chromatography, and immunoprecipitation were conducted in order to purify the P2X4 protein. Atomic force microscopy (AFM) was then used to observe the structure of the protein in native conditions. In the analysis, although the ion channel structure could not be resolved, single P2X4 proteins were seen to correspond to the previously described P2X2 structure, a related P2 purinergic receptor. In addition to P2X4, the possibility of utilizing bR as a proton pump in future optoelectronic devices was studied. Using AFM, the repeating bR trimer structure surrounded by a lipid membrane was visualized. bR embedded in membrane was then suspended over Si nanoholes. Though research is still at an early stage, membrane proteins such as P2X4 and bR have potential uses in future nanobiological applications.

Introduction

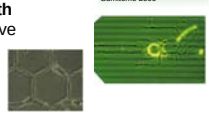
Goals of Nanobiology

Fusion of molecular biology, neuroscience, and nanotechnology can lead to novel technologies in electronics, engineering, and medicine

-**Protein-based devices**
Utilizing biological systems to create molecular scale devices



-**Guided neuron growth**
Create synaptically active neuron network on patterned surfaces



-**Neuron activity measurements**
on multi-electrode array sensor. Understand signal transduction through the brain

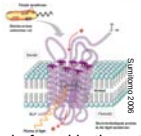
Proteins

P2X proteins

Form ion channels (via homo/heterotrimerization)
Approximately 3-5nm height, 10-20nm width
Activated by extracellular ATP
Found in various tissue including CNS
7 subclasses ($P2X_1$ to $P2X_7$)

Bacteriorhodopsin

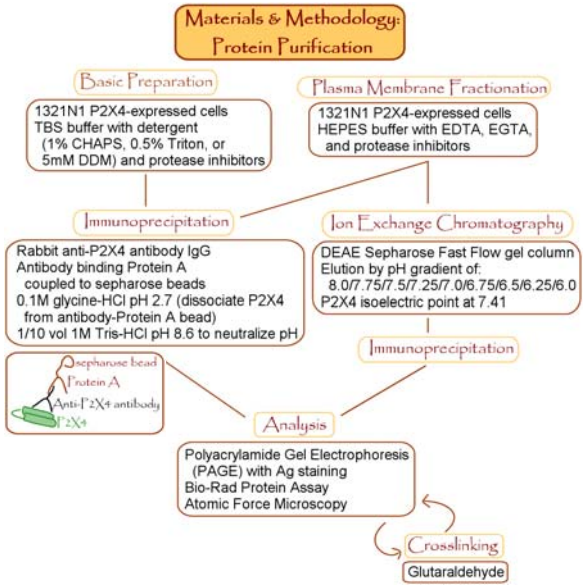
Light-driven proton pump (photoreceptor) found in *Halobacterium salinarum*
Trimers arranged to form hexagonal lattice
Resembles vertebrate rhodopsin found in the retina



Specifically, this study aims to further understand the biological components of nanobio

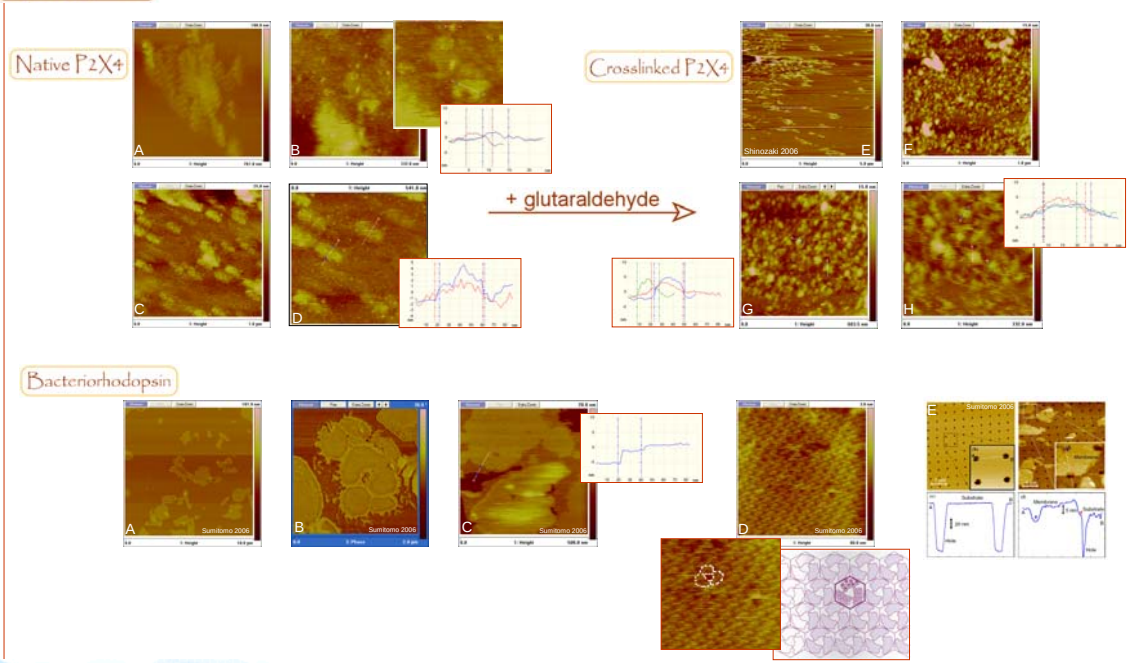
Purify $P2X_4$ purinergic receptor protein
Analyze structure of $P2X_4$ protein
Analyze bR surrounded by lipid membrane and then suspended over nanoholes

Materials & Methodology: Protein Purification



Methodology	Analysis Results	Images
Basic prep -> Immunoprecip	Purified protein around 80kDa (single subunit) via Ag staining, lipid bilayer visualized, purified protein presence is not confirmed	Native $P2X_4$ A
Membrane fraction -> IEC -> Immunoprecip	Unable to visualize, could not obtain concentration reading	
Membrane fraction -> Immunoprecip	Dense protein band obtained (80kDa) via detergent-based purification, visualized as particles (images); w/o detergent, 250kDa trimer structure conserved	Native $P2X_4$ B-D
Membrane fraction -> Immunoprecip -> cross linking	Increased and larger sized globular aggregations visualized (possibly forming trimer)	Crosslinked $P2X_4$ E-H

AFM Results



Summer Nanotechnology Study Program in Japan
NSF
Rice
Rice University NanoJapan Program
Generously Supported by a Grant from the National Science Foundation

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