## Purification and AFM Analysis of P2X<sub>4</sub> Receptor and Bacteriorhodopsin for Nanobiological Devices

Tiffany Yeh, Youichi Shinuzaki, Koji Sumitomo, Keiichi Torimitsu NTT Basic Research Laboratories, Atsugi-shi, Japan

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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> proteins were seen to correspond to the previously described P2X<sub>2</sub> structure, a related P2 purinergic receptor. In addition to P2X<sub>4</sub>, 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<sub>4</sub> and bR have potential uses in future nanobiological applications.

## Purification and AFM Analysis of P2X4 Receptor and Bacteriorhodopsin for Nanobiological Devices

<u>Tiffany Yeh</u>, Youichi Shinozaki, Koji Sumitomo, Keiichi Torimitsu NTT Basic Research Laboratories, Atsugi-shi, Japan

## Abstract Materials & Methodology 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 Protein Purification components in nanobiology, specifically, the protein bacteriorhodopsin (bR) and P2X4 purinergic receptors. Protein purification plays a key role in obtaining available Basic Preparation proteins from cultured cells. Multiple methods such as membrane fractionation, ion Plasma Membrane Fractionation Methodoloav Analysis Results Images exchange chromatography, and immunoprecipitation were conducted in order to purify the P2X4 protein. Atomic force microscopy (AFM) was then used to observe the 1321N1 P2X4-expressed cells 1321N1 P2X4-expressed cells Basic prep -> Purified protein around 80kDa Native P2X<sub>4</sub> A structure of the protein in native conditions. In the analysis, although the ion channel TBS buffer with detergent HEPES buffer with EDTA, EGTA, structure could not be resolved, single P2X4 proteins were seen to correspond to the Immunoprecip (single subunit) via Ag staining, (1% CHAPS, 0.5% Triton, or and protease inhibitors previously described P2X2 structure, a related P2 purinergic receptor. In addition to 5mM DDM) and protease inhibitors lipid bilayer visualized, purified P2X4, the possibility of utilizing bR as a proton pump in future optoelectronic devices protein presence is not was studied. Using AFM, the repeating bR trimer structure surrounded by a lipid membrane was visualized. bR embedded in membrane was then suspende over Si confirmed nanoholes. Though research is still at an early stage, membrane proteins such as Immunoprecipitation P2X4 and bR have potential uses in future nanobiological applications Ion Exchange Chromatography Membrane Unable to visualize, could not fraction -> IEC obtain concentration reading Rabbit anti-P2X4 antibody IgG DEAE Sepharose Fast Flow gel column Antibody binding Protein A -> Immunoprecip Introduction Elution by pH gradient of: coupled to sepharose beads 8.0/7.75/7.5/7.25/7.0/6.75/6.5/6.25/6.0 Goals of Nanobiology 0.1M glycine-HCl pH 2.7 (dissociate P2X4 P2X4 isoelectric point at 7.41 Membrane Dense protein band obtained Native P2X<sub>4</sub> from antibody-Protein A bead) 1/10 vol 1M Tris-HCl pH 8.6 to neutralize pH Fusion of molecular biology, neuroscience, and (80kDa) via detergent-based fraction -> B-D nanotechnology can lead to novel technologies in Immunoprecip purification, visualized as mmunoprecipitation electronics, engineering, and medicine particles (images); w/o Protein A detergent, 250kDa trimer Anti-P2X4 antibodi structure conserved -Protein-based devices Analysis Increased and larger sized Crosslinked Utilizing biological systems Membrane to create molecular scale devices fraction -> globular aggregations P2X₄ E-H Polyacrylamide Gel Electrophoresis visualized (PAGE) with Ag staining Immunoprecip -> **Bio-Rad Protein Assay** cross linking (possibly forming trimer) -Guided neuron arowth Atomic Force Microscopy Create synaptically active neuron network on rosslinking patterned surfaces Glutaraldehyde -Neuron activity measurements AFM Results on multi-electrode array sensor. Understand signal transduction through the brain Native P2X4 Crosslinked P2X 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<sub>1</sub> to P2X<sub>7</sub>) + glutaraldehyde Bacteriorhodopsin Light-driven proton pump (photoreceptor) found in Halobacterium salinarum Trimers arranged to form hexagonal lattice Resembles vertebrate rhodopsin found in the retina Bacteriorhodopsin Specifically, this study aims to further understand the biological components of nanobio Purify P2X<sub>4</sub> purinergic receptor protein Analyze structure of P2X<sub>4</sub> protein Analyze bR surrounded by lipid membrane and then suspended over nanoholes 1----心 NANOTECHNOLOGY RICE Rice University NanoJapan Program Generously Supported by a Grant from the National Science Foundatior

NTT BASIC RESEARCH LABORATORIES