Subproteome of Thromboxane A2 Receptor in Transfected HEK293T Cells

Thomas Huber.

www.sakmarlab.org, Rockefeller University, New York, NY, USA.

Single-molecule FRET (smFRET) has become a powerful tool for biophysical and biological research. Currently, progress is limited by the absence of general methods to incorporate two different fluorophores at specific sites in proteins. This is a major limiting factor in the development of labeling schemes that enable the fluorophores to be conjugated to one receptor (protein) in a well-defined manner. To prepare the dual-color labeled receptor two requirements need to be satisfied: 1) the generation of two different reactive handles at specific positions in the receptor and 2) the appropriate bioorthogonal labeling chemistries to distinguish between them. Here we show that the combination of maleimide reaction and azido/alkyne cycloaddition (‘click’ chemistry) meets these conditions. Maleimide chemistry specifically targets at the thiol group of cysteine, which can be easily generated or removed by mutagenesis. In contrast, the click chemistry requires the azido or alkyne functionality, which are not naturally present in proteins. We utilized the amber codon suppression technique to incorporate unnatural amino acids carrying these reactive groups. The first fluorophore is then attached using a maleimide handle and the second by a click reaction. In a series of proof-of-concept experiments we demonstrate the usefulness of the visual receptor rhodopsin. We have successfully demonstrated site-specific double labeling of a GPCR. Moreover, we have optimized the maleimide labeling protocol for rhodopsin and are conducting a comparative study on the copper-catalyzed click chemistry and copper-free click chemistry. We will discuss the results in terms of background level, material cost, and labeling stoichiometry. We envision a series of interesting applications for the dual-color labeled receptors, e.g., to probe the conformational change involved in GPCR activation. We propose to generalize this approach to other proteins, thereby permitting study of GPCRs signalsome.

The Potential Role of (-)-Epigallocatechin-3-Gallate in Protecting Cardiac Injury from Oxidation Stress through Lipid Rafts

Ying-Ming Liu, Chien-Sheng Hsu, Wei-Cheng Chen.

National Chung-Hsing University, Taichung, Taiwan.

Emerging evidence indicates that green tea consumption is inversely linked to cardiovascular diseases. The present study was planned to determine the potential mechanism for cardioprotection of (-)-epigallocatechin-3-gallate (EGCG) on hydrogen peroxide (H2O2)-induced oxidative stress in H9c2 rat cardiac myoblasts. H9c2 cells exposing to H2O2 suppress cell viability, while cells with EGCG pretreatment for 30 min prior to oxidative stress effectively improve cardiac cell survival. Measurement of intracellular reactive oxygen species (ROS) formation by dichlorofluorescein diacetate fluorescence showed that EGCG pre-treatment of H9c2 myoblasts cells decreased the intracellular ROS generation. The EGCG signaling pathways, EGFP (enhanced green fluorescence protein) was ectopically expressed in H9c2 cells. This allowed us to monitor the fluorescence changes as a means to distinguish the effects of Triton X-100 in dependence and independent compartments on the cell membrane. Results obtained indicated the involvement of Triton X-100 insoluble fraction on plasma membrane in transmission of the EGCG signals to modulate cardiac protection against oxidative stress. In addition, the cardiac proteomics study using a two-dimensional polyacrylamide gel electrophoresis was performed to identify the potential proteins for the EGCG-mediated cardioprotection. The adhesion dimensional polyacrylamide gel electrophoresis was performed to identify the potential proteins for the EGCg-mediated cardioprotection. The adhesion proteins include: cadherin, gap junction protein connexin 43, blood group A, glycolipid, lectin, complement factor 1, and the Golgi apparatus regulates protein secretion from the Golgi apparatus in response to proliferating signals. SAC1 might aid TP secretion and trafficking to the plasma membrane of cells undergoing proliferation. Since intracellular Ca2+ can regulate protein traffic it is possible that TP-ATP2A2 association serves as a feedback mechanism for TP expression. In summary, LC/MS/MS analysis identified ER membrane-spanning proteins that form multimeric complexes with TP, which may be involved in the receptor increased synthesis/traffic in diseased/proliferating vasculature. Supported by NIH.

Modular Mechanism of Wnt Signalling Inhibition by Wnt Inhibitory Factor 1

Tomas Malnauskas, Radu A. Aricescu, Lu Weixian, Christian Siebold, Yvonne E. Jones.

Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Wnt morphogens control embryonic development and adult tissue homeostasis. In vertebrates the N-terminal WIF domain (WIF-1) of six-domain Wnt inhibitory factor 1 (WIF-1) binds Wnts and inhibits signal transduction. Our human WIF-1 crystal structure reveals a novel binding site for phospholipid; two acyl chains extend deep into the domain while the head group is surface exposed. Biophysical and cellular assays, combined with structure-guided mutagenesis, indicate a WIF-1-Wnt binding surface proximal to the lipid head group, but also implicate the five epidermal growth factor (EGF)-like domains (EGFs I-V) in Wnt binding. The crystal structure of six-domain WIF-1 reveals EGFs I-V wrapped-back to interface with WIF-1 at EGF III. Binding studies locate a heparan sulfate proteoglycan (HSPG)-binding site in EGFs IV-V, consistent with highly conserved positively charged residues on EGF IV. This combination of HSPG- and Wnt-binding properties suggests a modular model for WIF-1 localization, and signal inhibition, within morphogen gradients.

Prolonged Stochastic Resonance in Single Ion Channel Recordings

Eric Stava1, Abhishek Bhat1, Siyong Choi2, Minrui Yu2, Hyunchel Shin2, Robert H. Blick2.

1Universität Hamburg, Hamburg, Germany, 2University of Wisconsin, Madison, WI, USA.

Stochastic resonance refers to the improved signal transduction through a system due to the addition of noise to that system. By prolonged stochastic resonance, we refer to the continued enhancement of signal transduction beyond the typical stochastic resonance maximum with increasing input noise. We present evidence of this phenomenon from single Alamethicin ion channels. Identification of the single channel in the presence of voltage noise was accomplished by extracting the conductance of the system by simultaneous voltage and current recording. We determined that, when applying low levels of voltage noise, no more than a single ion channel conducted at any given time. Further, we provide evidence that the prolongation of stochastic resonance in this system is due to the mechanotransductive effects of Alamethicin ion channels. It is well known that Alamethicin channels respond to mechanical tension in the lipid bilayer [1,2]. By extracting the capacitance of the system (again, via simultaneous voltage and current recordings), we record changes in membrane area with respect to applied voltage noise. We find that increasing voltage noise yields larger capacitances due to expansion of the lipid bilayer via the converse flexoelectric effect [3]. This area expansion induces tension on the ion channel, increasing its conductance and enhancing signal transduction through the...