237th WPI-IIIS Seminar
-Mini Symposium-

Gαs slow conformational transition upon GTP binding and a novel Gαs Regulator

G proteins are major signaling partners for G protein-coupled receptors (GPCRs). Although a number of high-resolution structures of GPCR-G protein complexes have been revealed by X-ray crystallography and cryo-electron microscopy (cryo-EM), the stepwise structural changes during GPCR–G protein coupling have not fully been understood fully. In this study, we analyzed step-wise conformational changes during GPCR-G protein coupling using beta2-adrenergic receptor (beta2AR) and Gs as a model GPCR and G protein. To understand the step-wise conformational changes, we used pulse-labeling hydrogen-deuterium exchange mass spectrometry (HDX-MS). HDX-MS can analyze the conformational dynamics of proteins and can tolerate conformational heterogeneity. To monitor the movement of alpha-helical domain (AHD) of Gα subunit, we developed an assay system using tryptophan-induced fluorescence quenching technique. The pulse HDX-MS showed that there are delayed structural changes even after GDP-release or GTP-binding. Specifically, the C-terminus of Gαs undergoes sustained conformational changes during beta2AR-Gs complex formation even after GDP is released. Likewise, AHD of Gαs undergoes sustained conformational changes after GTP is incorporated and Gαs is dissociated from the receptor and Gβγ. We further identified a novel AHD-binding protein, melanoma-associated antigen D2 (MAGE D2), which regulates the G proteins activation cycle by accelerating the GTP-induced closing of the Gαs AHD. Our data revealed the conformational changes during GPCR-Gs coupling that have not been observed by currently available high-resolution structures. The data suggest that the GPCR-G protein coupling specificity is determined by one or more transient intermediate states that serve as selectivity filters and precede the formation of the stable nucleotide-free GPCR-G protein complexes observed in crystal and cryo-EM structures. Furthermore, we observed that the GTP-binding mediated G protein activation kinetics can be regulated by proteins interacting at AHD.

Dr. Ka Young Chung

School of Pharmacy

Sungkyunkwan University

Date: **Thursday, November 6, 2025**

Time: **10:30 – 11:25**

Venue: **1F Auditorium, IIIS Building**

✻On-site participation only

INTERNATIONAL INSTITUTE FOR INTEGRATIVE SLEEP MEDICINE

Contact: International Institute for Integrative Sleep Medicine, University of Tsukuba
029-853-5857 (ext.5857) | wpi-iiis-alliance@ml.cc.tsukuba.ac.jp