Ligand Mediated Sequestering of Integrins in Raft-Mimicking Lipid Mixtures: The Role of Bilayer Asymmetry and Cholesterol Content

Noor F. Hussain¹, Jiayun Gao¹, Amanda P. Siegel¹, Rainer Jordan², Christoph A. Naumann¹
¹ Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indiana; ² Makrolekulare Chemie, TU Dresden, Dresden, Germany

Lipid microdomains play an important functional role in plasma membranes. However, the small size and transient nature of lipid/membrane heterogeneities in the plasma membrane make characterization of microdomains and microdomain-related membrane processes quite challenging. To address this issue, we recently introduced a powerful model membrane system that allows the investigation of membrane protein sequestering and oligomerization in raft-mimicking lipid mixtures using combined confocal fluorescence spectroscopy, photon counting histogram (PCH), and epifluorescence microscopy. Our experiments on bilayer-spanning domains showed that \( \alpha_v\beta_3 \) and \( \alpha_5\beta_1 \) integrins predominantly exist as monomers and sequester preferentially to the liquid-disordered (\( l_d \)) phase in the absence of ligands. Notably, addition of vitronectin (\( \alpha_v\beta_3 \)) and fibronectin (\( \alpha_5\beta_1 \)) caused substantial translocations of integrins into the liquid-ordered (\( l_o \)) phase without altering receptor oligomerization state. Here we expand our previous studies and report on the sequestering and oligomerization state of \( \alpha_5\beta_3 \) and \( \alpha_5\beta_1 \) in asymmetric bilayer compositions containing coexisting \( l_o \) and \( l_d \) phases located exclusively in the top leaflet of the bilayer (bottom leaflet shows only \( l_d \) phase). Remarkably, in such a membrane environment, both integrins show a higher affinity for the top leaflet-restricted \( l_o \) domains in the absence of their respective ligands. A slight change in the integrin sequestration was observed after addition of their respective ligands. We also present experimental findings, which show that cholesterol content has a substantial influence on integrin sequestering and oligomerization in raft-mimicking lipid mixtures. The described experimental results highlight the potential importance of membrane asymmetry and lipid composition in the sequestering of membrane proteins in biological membranes.

Advisor: Christoph A. Naumann, Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indiana