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_a) phase without altering receptor oligomerization state. Here we expand our previous studies and report on the sequestering and oligomerization state of $\alpha_{\rm v}\beta_3$ and $\alpha_5\beta_1$ in asymmetric bilayer compositions containing coexisting l_a 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