Membrane Lipids Affect the Interplay between the Transmembrane Domain of the EGF Receptor and Ganglioside GM3 – Thermodynamic Quantification of the Lateral Interaction using FRET

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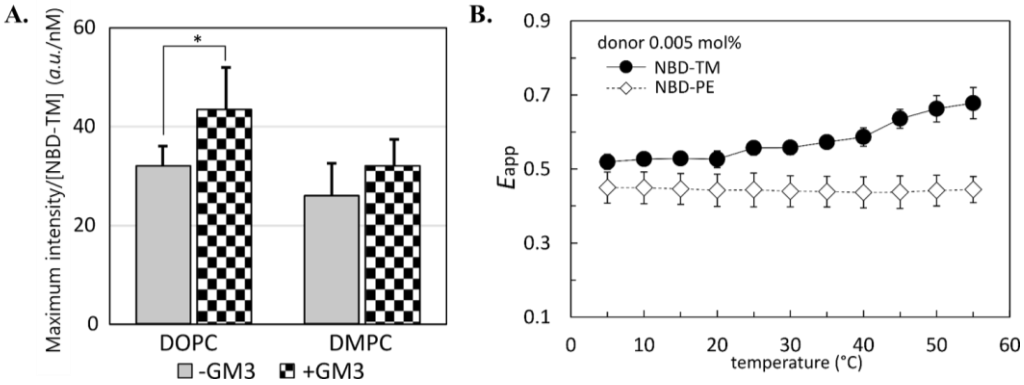
Ganglioside GM3 in the plasma membranes has been reported to suppress cell growth by preventing the autophosphorylation of the epidermal growth factor (EGF) receptor.[1] In previous studies, it was supposed that dimerization of the transmembrane (TM) domain of the receptor is one of the most crucial steps of the activation.[2] Thus, surrounding lipids such as GM3 possibly regulate the topology of the TM domain. However, the direct interaction between GM3 and the TM domain of the EGF receptor has not been proven yet.

To elucidate the mechanism by which ganglioside GM3 regulates the dimerization states of the EGF receptor, we performed fluorescence analysis using fluorescently labeled probes of GM3 and the receptor transmembrane peptide (NBD-TM). NBD-TM was prone to form dimer in DOPC bilayer by observing NBD self-quenching, while the addition of GM3 increased the NBD fluorescence intensity due to recovery from self-quenching. The data indicated that GM3 promotes peptide disassociation depending on lipid bilayer thickness. The apparent FRET efficiency (E_{app}) between NBD-TM and ATTO594-GM3 in DOPC bilayer was significantly higher than that of using NBD-PE as a control, indicating a specific TM-GM3 interaction. Furthermore, E_{app} between NBD-TM and labeled GM3 increased significantly in response to temperature increases, suggesting that the quantitative analysis was achieved with the lipid bilayers conditions where the monomer peptide was stable.



1) E. Bremer, *et al. J. Biol. Chem.*, **1984**, *259*, 6818–6825.
2) N. Endres, *et al. Cell*, **2013**, *152*, 543–556.