

**Crosslinking-Induced Nanodomains in Model Membranes Have 8nm in Radius**

Štefl, M.<sup>1</sup>, Šachl, R.<sup>1,2</sup>, Humpolíčková, J.<sup>1</sup>, Cebecauer, M.<sup>1</sup>, Macháň, R.<sup>1</sup>, Kolářová, M.<sup>1</sup>, Johansson, L.B.-A.<sup>2</sup>, Hof, M.<sup>1</sup>

<sup>1</sup>Department of Biophysical Chemistry, J. Heyrovsky Institute of Physical Chemistry of the Academy of Sciences of the Czech Republic, Dolejškova 2155/3, Prague 8, Cz-182 23, Czech Republic

<sup>2</sup>Department of Chemistry: Biophysical Chemistry, Umea University, Umea, S-901 87, Sweden

Cell membrane organization has been subject of intensive research for last few decades. It was even more actual when revealed membrane rafts and their contribution to cell functioning. Crosslinking of raft components using various proteins resulting in microscopic phase separation in model membranes has been already confirmed. Crosslinking of raft gangliosides GM1 with cholera toxin (CTxB) forming micrometer-sized structures is example of such induced phase separation, however, the CTxB-GM1 complexes forming a minimal lipid units are still subject of ongoing cell membrane research and characterization of such subdiffraction sized structures in terms of dynamics and size has never been successfully realized.

Using two-color z-scan fluorescence correlation spectroscopy we show strong evidence of existence of nano-sized domains in model membranes containing lower amount of sphingomyelin (Sph) than needed for micro-sized domain formation. We also show two types of Sph dependent membrane nanostructures which we characterized by means of fluorescence resonance energy transfer in combination with Monte Carlo simulations. Modeling of donor decay we calculated the domain radius of approximately 8 nm, which increases with higher Sph content. Observed two types of differently behaving domains suggest a dual role of the crosslinker: first, local transient condensation of the GM1 molecules compensating lack of sphingomyelin and second, coalescence of existing nanodomains ending in large scale phase separation.