Gangliosides, glycosphingolipids located in the outer leaflet of cellular membranes, play essential roles in cell communication, signaling, and serving as receptors for membrane-binding proteins. Their functionality is linked to their ability to self-assemble into nanoscopic lipid domains, often referred to as lipid rafts. However, the molecular mechanisms driving this nanoscopic segregation remain unclear. This thesis investigates the role of ganglioside sugar headgroups in ganglioside nanodomain formation and characterizes their properties using novel microscopy techniques. By combining MC-FRET (Förster resonance energy transfer analyzed with Monte Carlo simulations) and molecular dynamics (MD) simulations, we explored the tendency of gangliosides to self-assemble into nanodomains, identifying key molecular groups involved in this process. To analyze domain dynamics, we employed a super-resolution microscopy technique, STED FCS (Stimulated Emission Depletion Fluorescence Correlation Spectroscopy), and developed a new quantitative method for interpreting STED-FCS diffusion law plots. This approach allows for extraction of detailed, dynamic diffusion characteristics of lipids both inside and outside nanodomains, providing a more physiologically accurate representation of membrane heterogeneity. Overall, this work identifies key sugar moieties driving segregation of gangliosides into nanodomains and introduces a new quantitative analysis of STED-FCS diffusion law plots, enhancing the level of detail and information obtainable from acquired data.