Plasma membrane of eukaryotic cells are now described as a mosaic of micro or nanodomains that are highly dynamic and can also segregate to form functional platforms. Among them, raft microdomains have been largely characterized and are enriched in specific lipids such as gangliosides, especially GM1. In order to better understand molecular mechanisms associated to lipid lateral segregation within biological membranes, we investigated GM1 partition using artificial lipid bilayers presenting lipid phase separation. Using both standard and high-speed atomic force microscopy (HS-AFM), we demonstrated GM1 propensity to form stable nanodomains that partition into lipid-ordered phase. These domains were mainly mobile and we characterized their membrane behavior in terms of motion mode and diffusion coefficient using HS-AFM.