Cubosomal self-assemblies, composed of monoolein (MO) and fatty acid, were prepared by the pH titration method. The assemblies including 25–50 mol% of MO showed gel-like high viscosity at pH 5.0. Various kinds of self-assemblies were systematically characterized by Raman spectroscopy and fluorescent probe analyses. The membrane property of cubosomal assembly was highly packed and less hydrated state. As application, cubosomal self-assembly could be prepared using functional lipids, e.g. linoleic acid (anti-oxidant), polymerizable lipid (precursor of polydiacethlene).