Toll-like receptor 4 (TLR4) of macrophages triggers pro-inflammatory signaling pathways upon activation by bacterial lipopolysaccharide (LPS). The LPS-induced signaling leads to production of pro-inflammatory cytokines which launch immune responses facilitating eradication of bacteria. The mechanism of LPS recognition in macrophages is complex and involves initial binding of LPS to CD14 protein which next transfers the LPS to TLR4. CD14 is localized on the surface of plasma membrane nanodomains, named rafts. One of the most important mechanisms which control association of intracellular proteins with rafts is their reversible modification - S-palmitoylation. Hence, protein S-palmitoylation can control the onset of LPS-induced signalling affecting the CD14-TLR4 interaction. Our proteomic studies on proteins palmitoylated in Raw264 macrophage-like cells followed by detailed analysis of selected proteins revealed that LPS induced palmitoylation of flotillin-1, a submembraneous raft protein. These studies were based on metabolic labeling of cells with 17ODYA, palmitic acid analogue functionalized with alkyne group, and its subsequent detection via click reaction with biotin-azide. Owing to the propensity of flotillins for clustering we hypothesize that flotillins can play an essential role during co-clustering of CD14 and TLR4 in LPS-stimulated cells and also can be involved in vesicular trafficking of those proteins. Indeed, we found that an exposure of cells to antibodies which force clustering of CD14, induced palmitoylation of flotillin-1 and to higher extent of flotillin-2. Furthermore, silencing of flotillins reduced LPS-induced activation of NFkappaB and IRF3 transcription factors and following production of TNFalpha and RANTES cytokines. Taken together the data indicate that contribution of flotillins is required for maximal pro-inflammatory signaling in LPS-stimulated cells.