

“Nail” and “Comb” effects of cholesterol modified NIPAm oligomers on cancer targeting liposomes†

Wengang Li^{a†}, Lin Deng^{a†}, Basem Moosa^a, Guangchao Wang^b, Afnan Mashat^a, Niveen Khashab^{a*}

^a Controlled Release and Delivery Lab (CRD), Physical Science and Engineering Division, Advanced Membranes and Porous Materials Center, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Kingdom of Saudi Arabia. Fax: +96628021172; Tel: +96628082410; E-mail: niveen.khashab@kaust.edu.sa

^b Advanced Nanofabrication, Imaging and Characterization Core Facilities, King Abdullah University of Science and Technology (KAUST), Thuwal, 23955-6900, Kingdom of Saudi Arabia

Preparation and characterization of NIPAm oligomers.

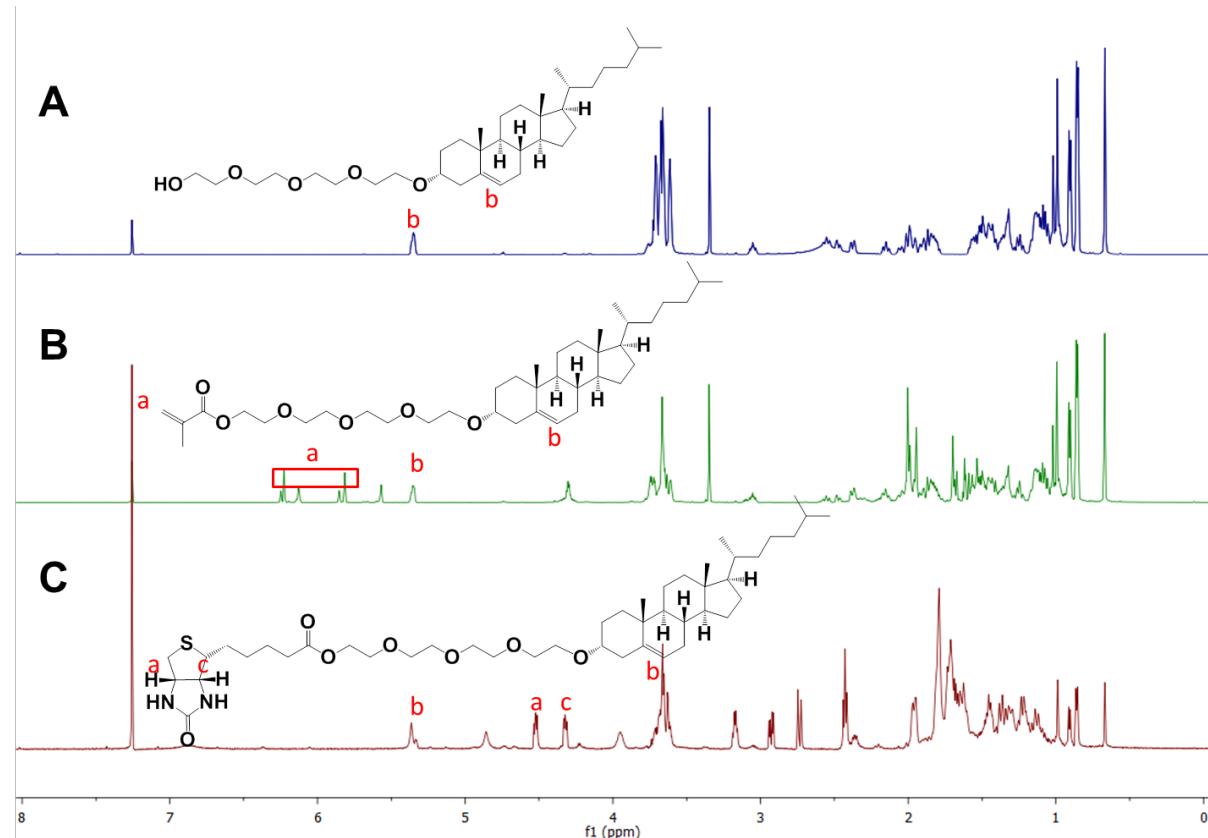


Fig. S1. ¹H NMR spectra of important molecules in the synthesis: A. TEG-cholesterol (**2**, Fig. 1), B. cholesterol monomer (**8**, Fig. 1) and C. biotinylated cholesterol (**3**, Fig. 1).

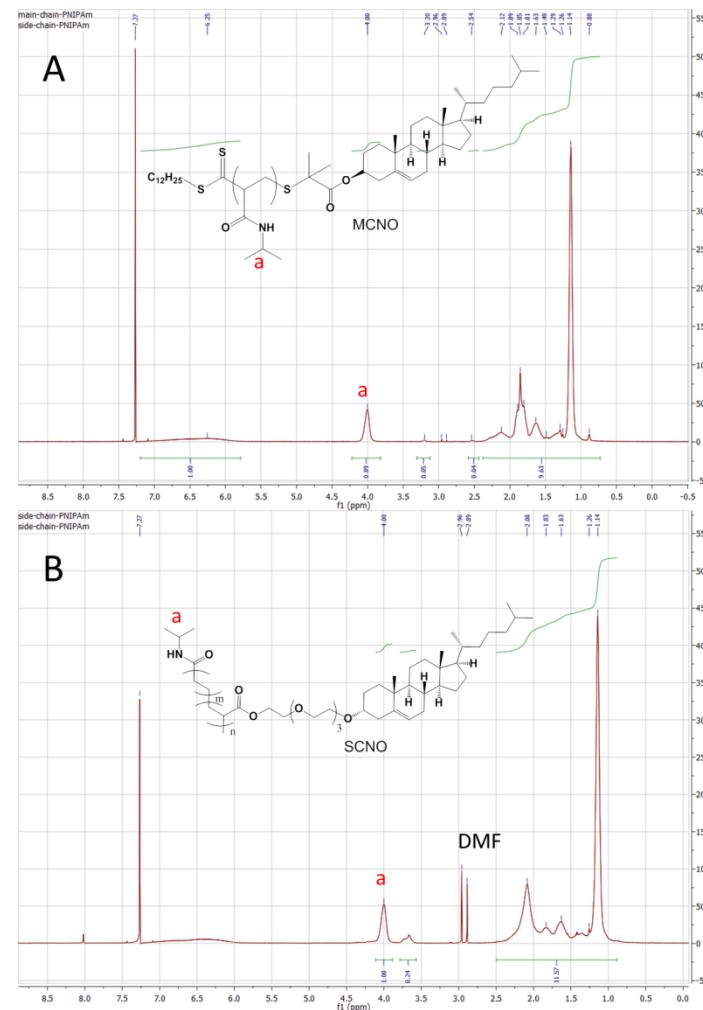


Fig. S2. ^1H NMR spectra of cholesterol modified oligomers: A. main-chain NIPAm oligomers (MCNOs), B. side-chain NIPAm oligomers (SCNOs).

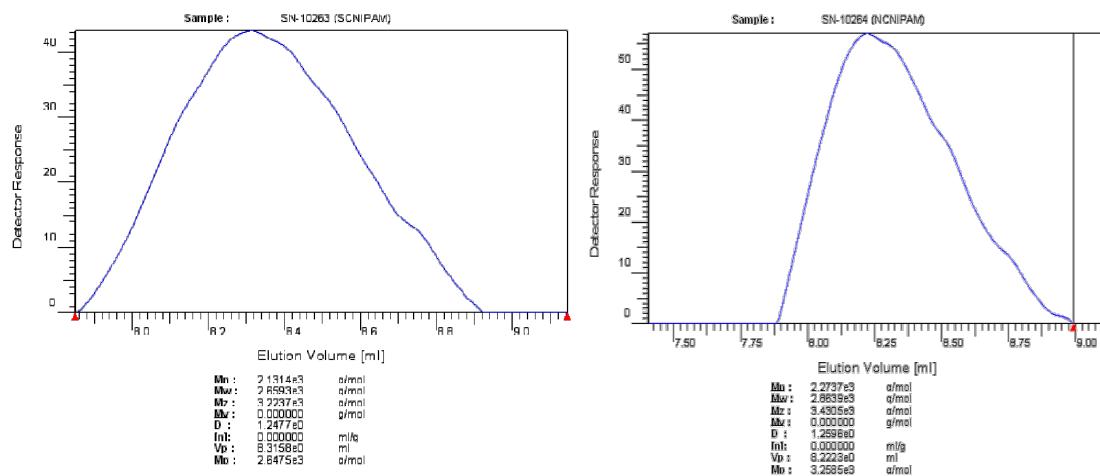


Fig. S3. GPC for SCNO (left) and MCNO (right). GPC data was obtained by Agilent 1200 GPC in THF, $M_n\text{-MCNO}=2270$, $\text{DPI}=1.26$; $M_n\text{-SCNO}=2130$, $\text{DPI}=1.24$.

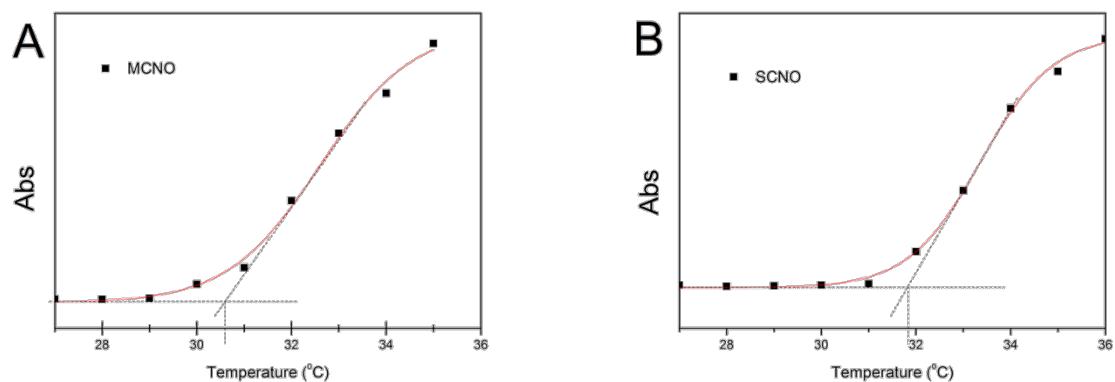


Fig. S4 LCST data determined with turbidity method of A. MCNOs ($30.8\text{ }^{\circ}\text{C}$) and B. SCNOs ($31.8\text{ }^{\circ}\text{C}$).

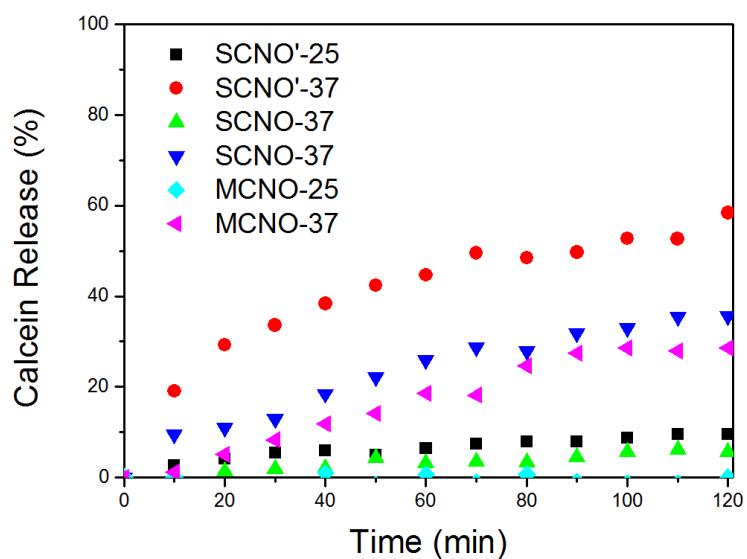


Fig. S5. Release profile of MCNO, SCNO (Mw 2200) and SCNO' (Mw 4500) liposomes at 25 and $37\text{ }^{\circ}\text{C}$.

Liposomal fusion test

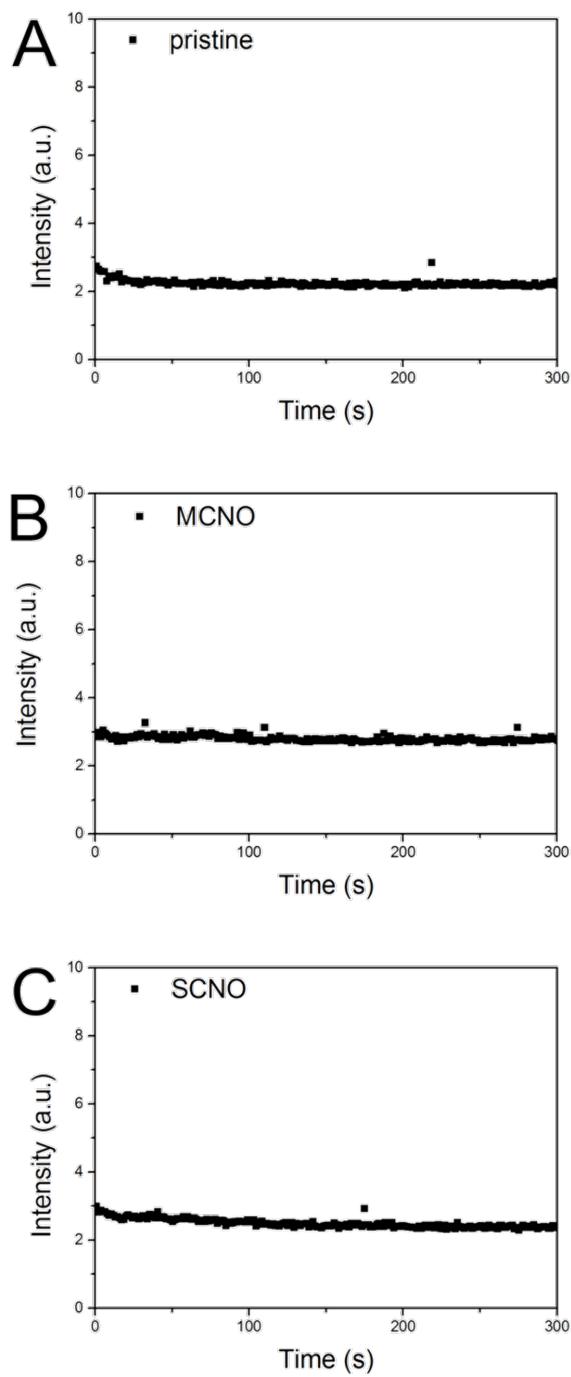


Fig. S6. Tb^{3+} /DPA assays (Ext: 276 nm; Ems:545 nm) of liposomes at 37 °C for 300 s: A. pristine, B. SCNO and C. MCNO.

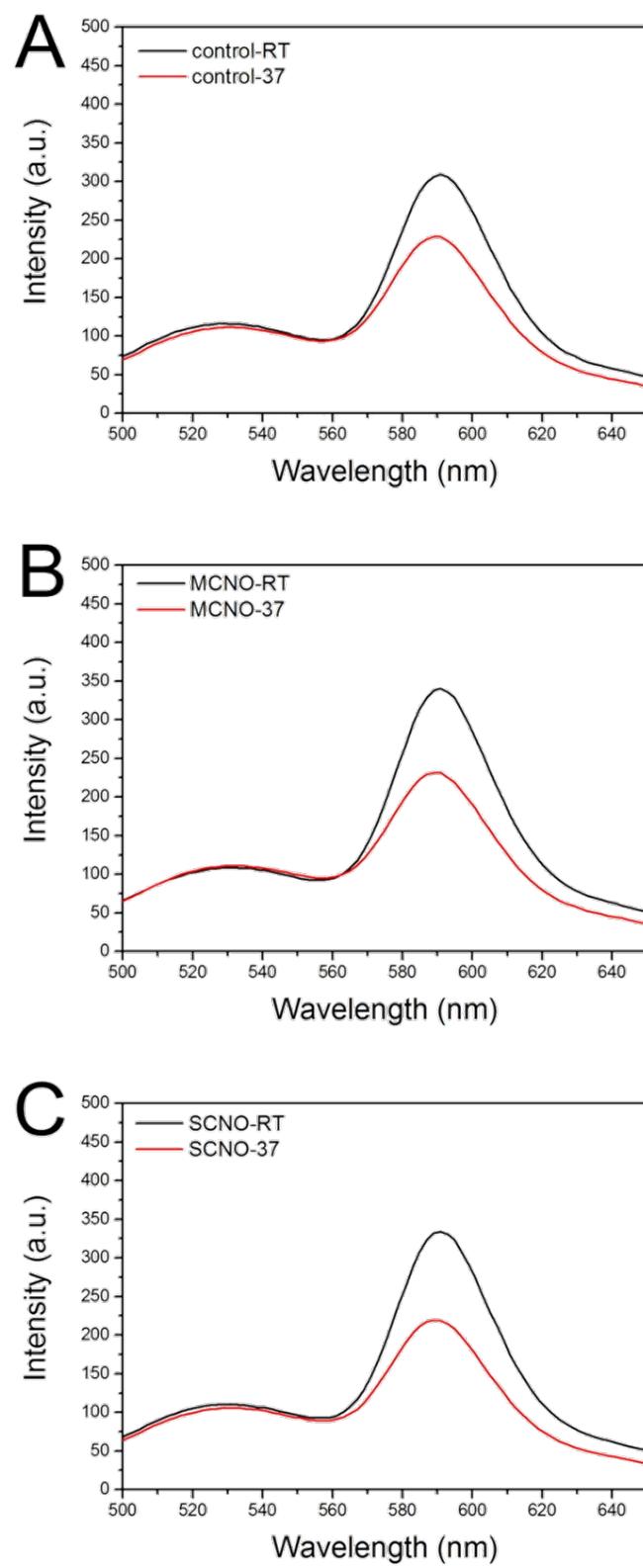


Fig. S7. NBD/RhB assays of liposomes at RT and heated at 37 °C for 5 min (NBD/RhB-labeled liposomes: pure liposomes=1:9):
A. pristine, B. MCNO and C. SCNO.

***In vitro* characterizations for pristine, MCNO and SCNO liposomes.**

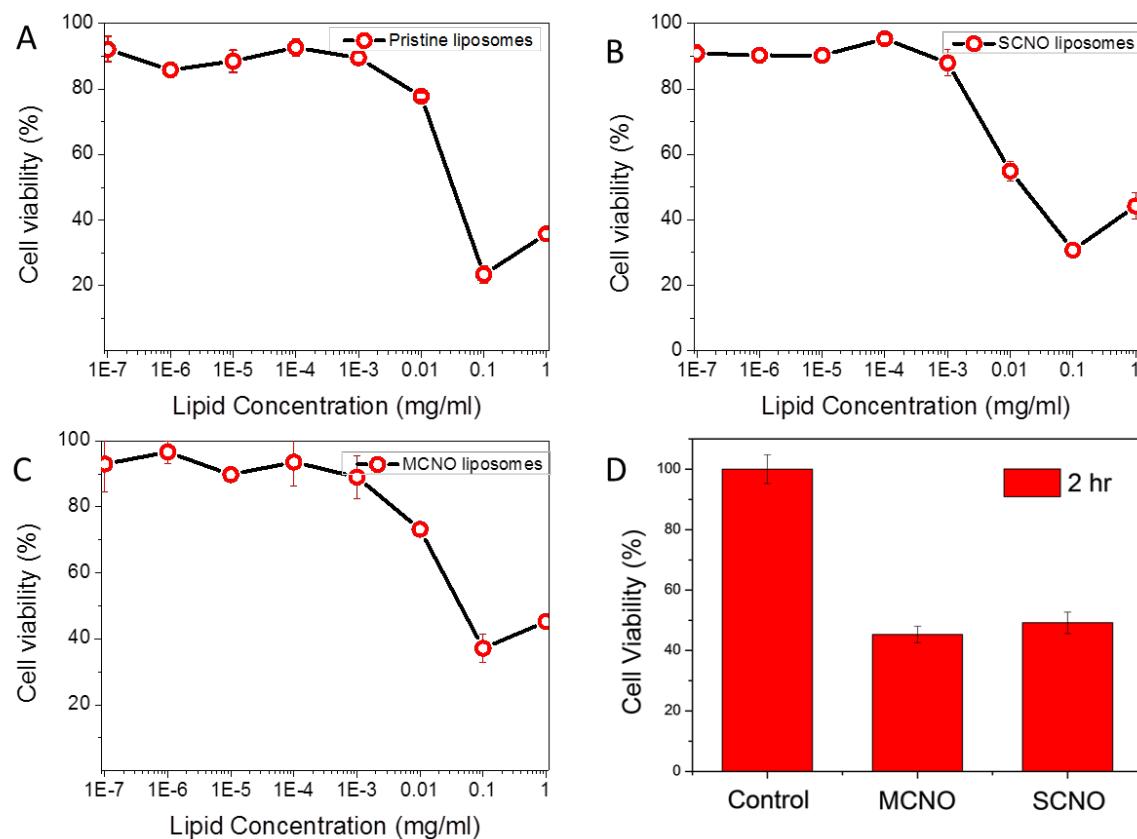


Fig. S8. *In vitro* cytotoxicity of pristine and modified liposomes (A. pristine liposomes, B. SCNO liposomes, C. MCNO liposomes) incubated with HeLa cells for 24 hrs (37 °C) and (D) MTT assays for dox loaded SCNO and MCNO liposomes incubated with HeLa cells for 2 hrs (37 °C).

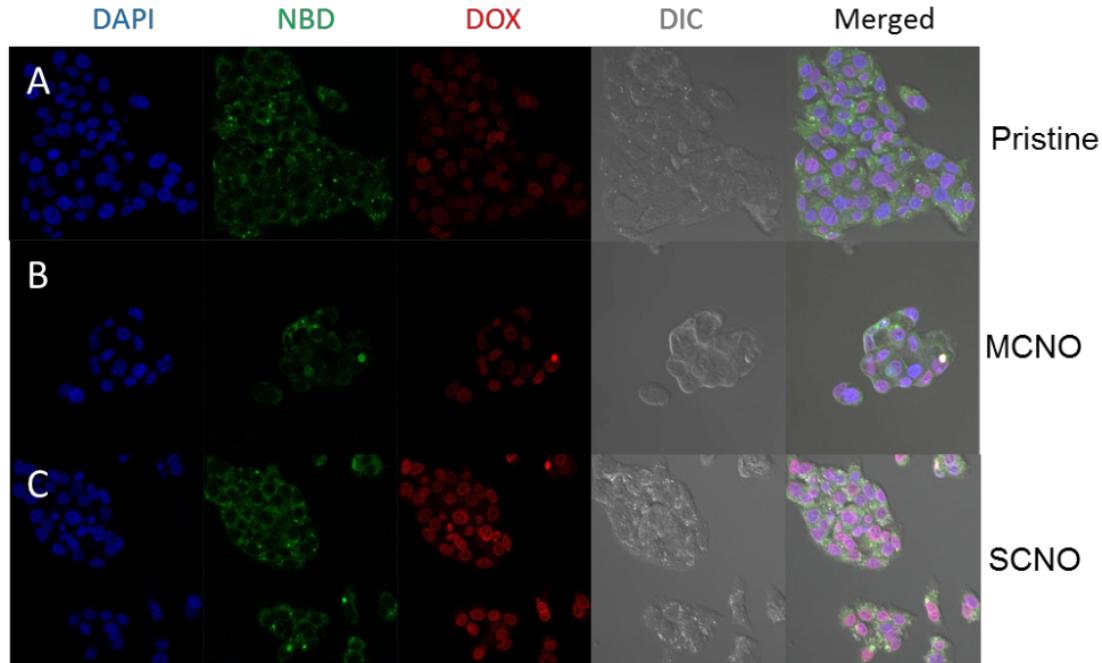


Fig. S9 CLSM images of dox loaded liposomes incubated with HepG2 cells at 37 °C for 1 hr (from left to right, DAPI, NBD, Dox, bright field, merged): A. pristine liposomes, B. MCNO liposomes, C. SCNO liposomes.

Localization and cellular uptake of biotinylated liposomes.

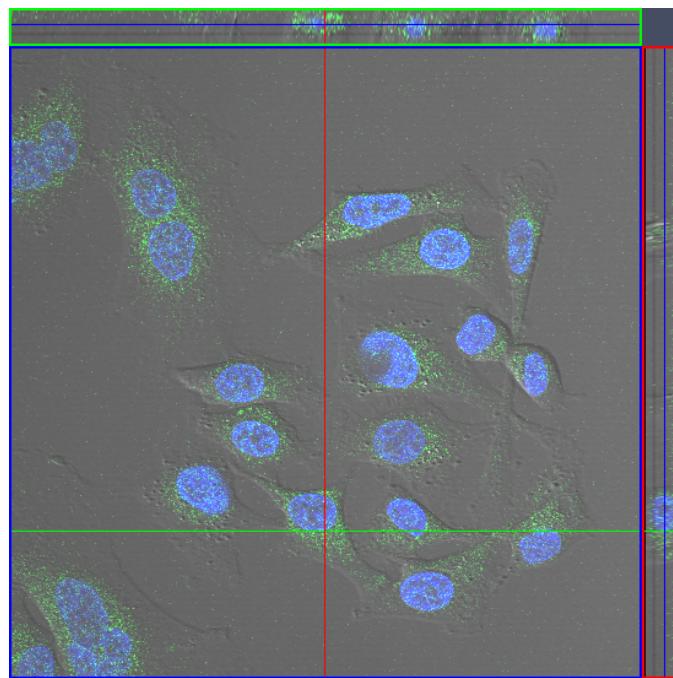


Fig. S10. Z-stack CLSM image for Biotinylated liposomes incubated with HeLa cells for 30 min.