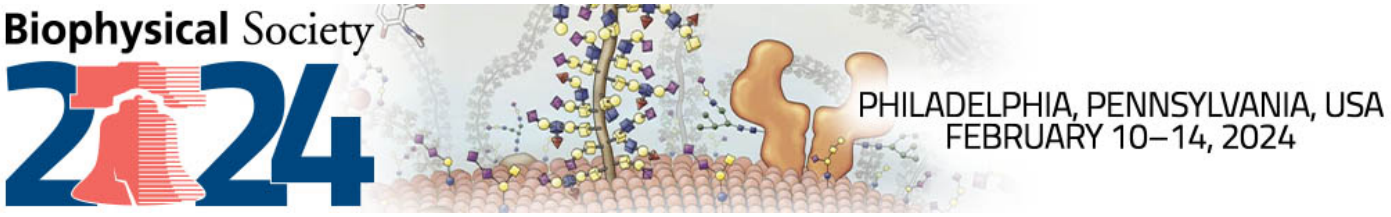

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MODULATING LIGAND DENSITY ON LIPOSOMES USING LIPID PHASE SEPARATION

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Abstract:
In biological membranes, lipid phase separation controls the spatial organization of membrane-associated molecules. Thus, it influences a vast array of crucial membrane processes. This phenomenon may be utilized to overcome the limitations of nanoscale liposomal delivery systems such as cell selectivity and uptake efficacy. This study aims to explore the use of lipid phase separation as a means to modulate presentation of targeting moieties on liposomal surfaces to enhance their cellular uptake. Folate-conjugated lipid, as the model targeting ligand, was incorporated into phase-separating liposomes on which it mainly partitioned into the liquid-ordered phase. Utilizing different ratios of DOPC:DPPC:Chol mixture, with distinct surface area fractions of ordered phase, we controlled folate's surface distribution and local density. Phase separation and area fraction of the ordered-disordered phases for each liposomal formulation was first investigated in giant unilamellar vesicles (GUVs) using fluorescence microscopy. Nanoliposomes of different phase-separating compositions, with equal folate concentrations, were then introduced to folate receptor (FR)-rich HeLa cells and their uptake was assessed using UV-spectroscopy. The results revealed that phase separated liposomes showed higher levels of uptake when compared to their homogenous counterparts, presumably due to the presentation of folate at higher local densities on these liposomes. Notably, tuning the ligand concentration within the literature-established range further altered the performance of each liposomal formulation in favorable or detrimental ways, suggesting the existence of an optimal ligand density for maximal uptake. Among the examined phase separating compositions, the one that amassed the highest ligand density exhibited the least improvement across multiple ligand concentrations. These findings show that phase separation can provide control over the spatial presentation of targeting moieties to modulate binding and uptake efficacy in nanoscale liposomal delivery systems and presents opportunities to address the shortcomings of delivery liposomes.

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