Supplementary Information (SI) for Nanoscale.

PEGylated Lipid Screening, Composition Optimization, and Structure-Activity Relationship Determination for Lipid Nanoparticle-Mediated mRNA Delivery

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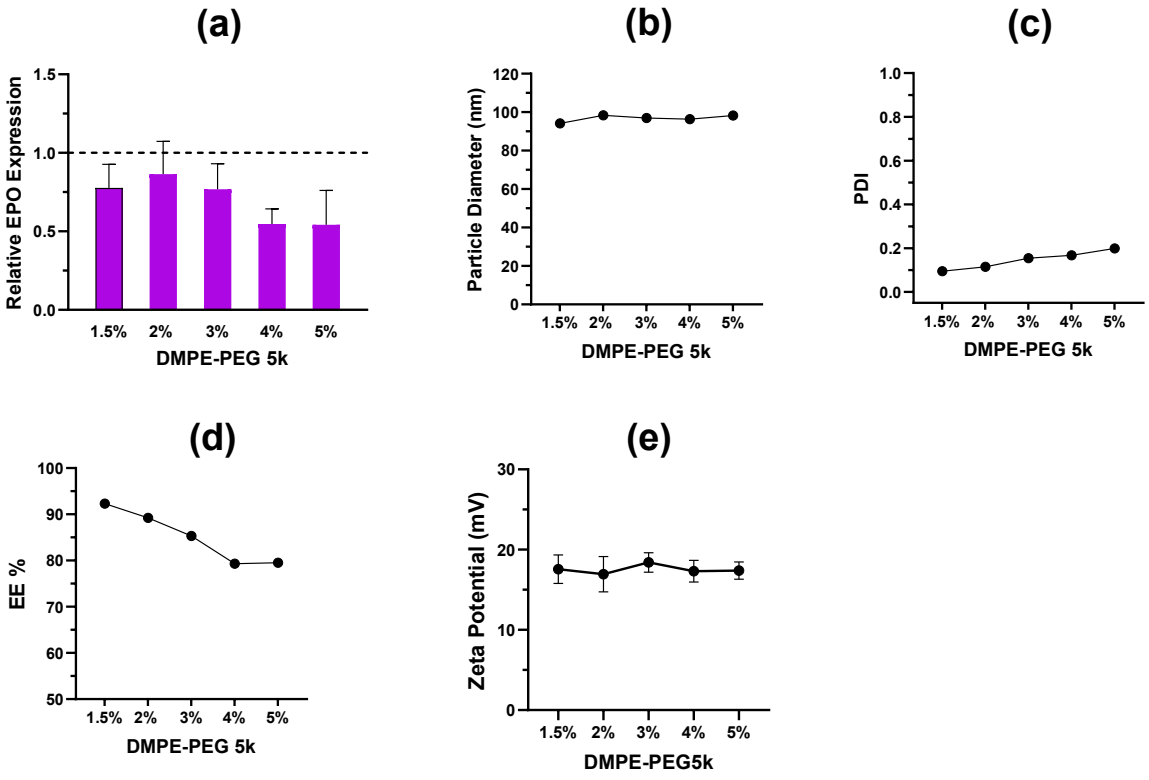

Figure S1. Characterization of LNPs formulated with different percentages of DMPE-PEG5k lipid, including protein expression, particle diameter, size distribution (PDI), mRNA encapsulation efficiency, and zeta potential.

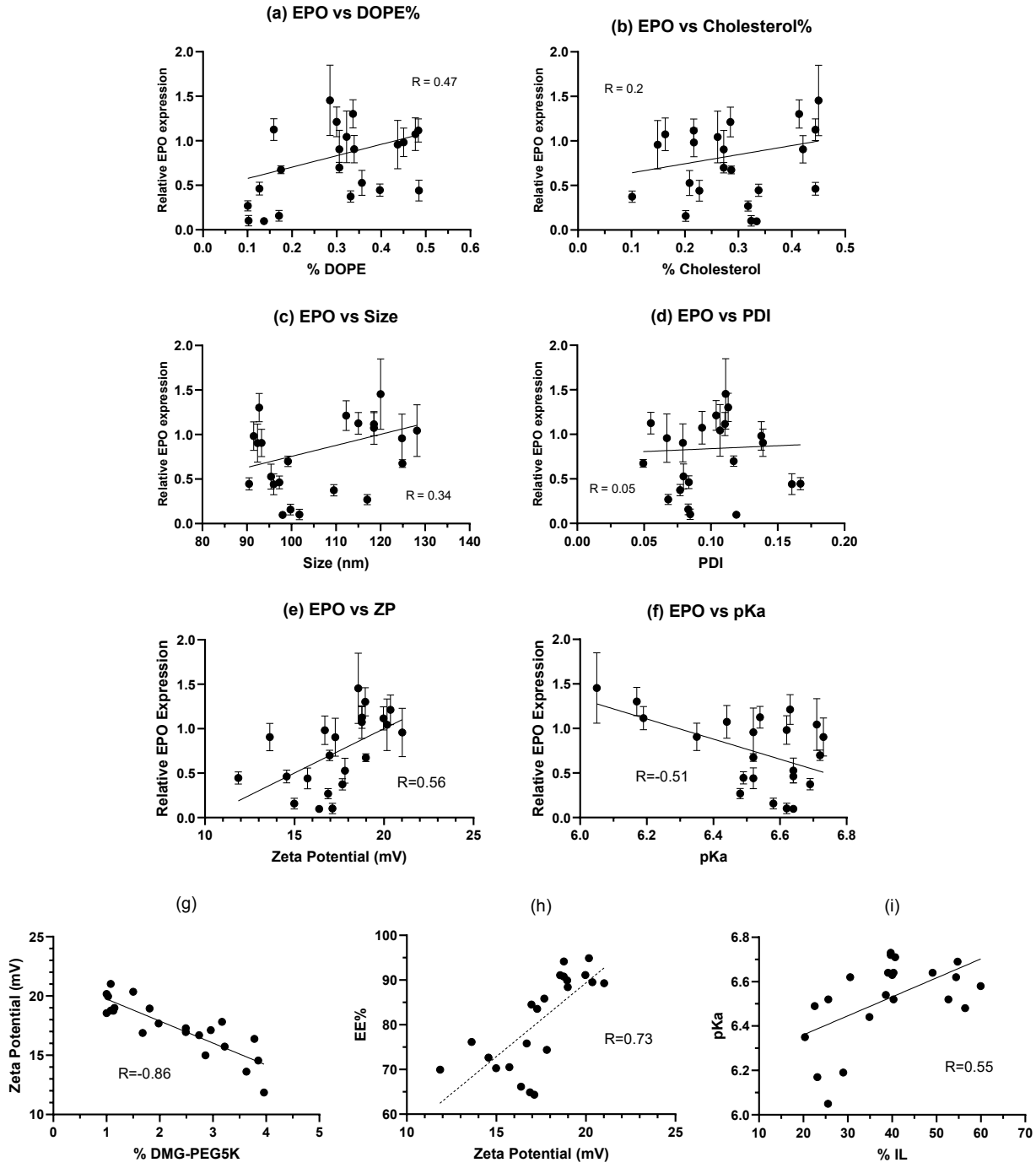

Figure S2. Correlations between protein expression and various formulation parameters, including the fraction of DOPE (a), fraction of cholesterol (b), particle size (c), PDI (d), zeta potential (e), and pKa (f). Additionally, correlations are shown between zeta potential and the percentage of DMG-PEG5k (g), encapsulation efficiency and zeta potential (h), and pKa and the percentage of ionizable lipid (i) for DMG-PEG5k DoE, OPT and Std2 formulations.

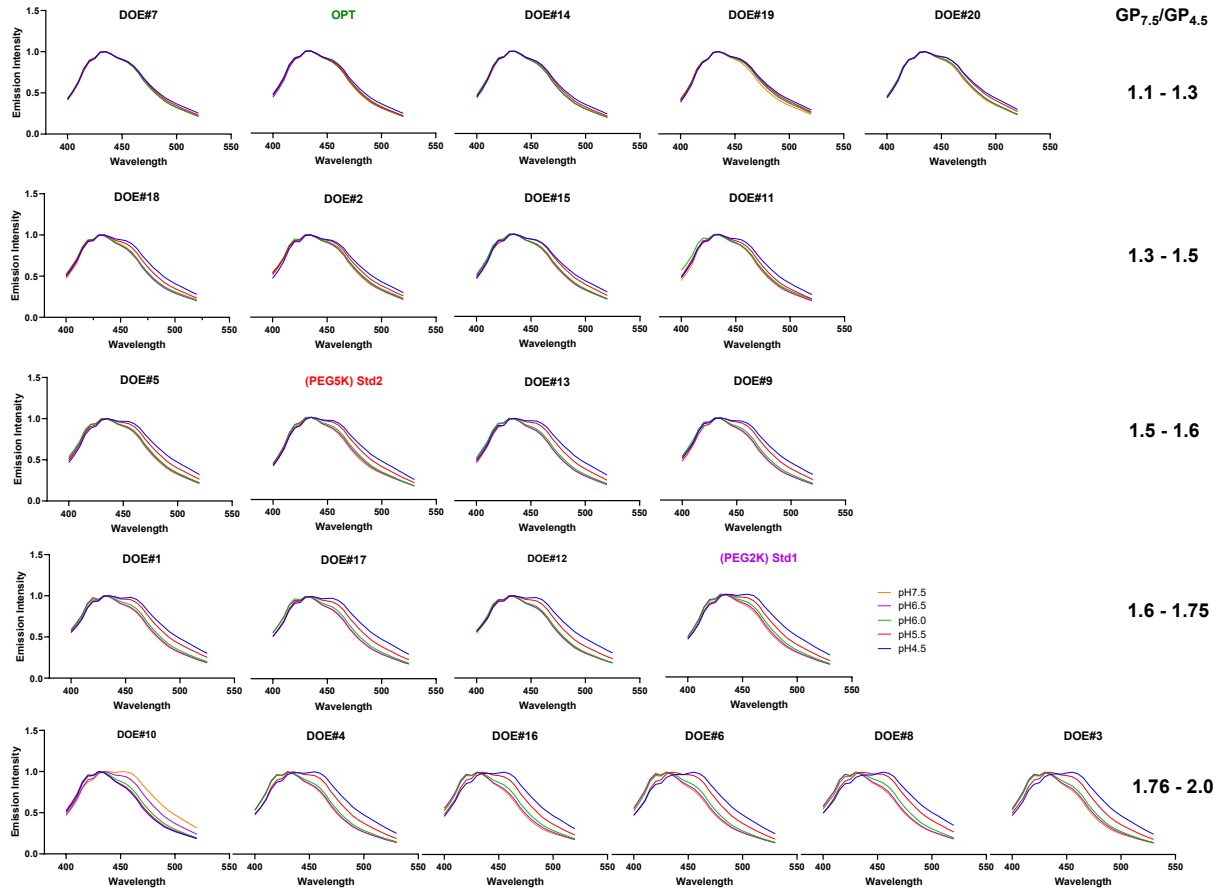

Figure S3. Overlay of Laurdan spectra for DMG-PEG5k DoE, OPT, Std2, and Std1 formulations, measured across various pH levels.

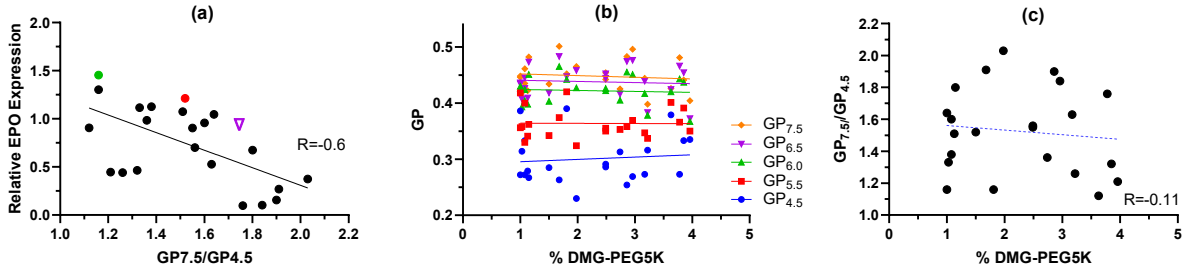

Figure S4. (a) Negative correlation between protein expression and the ratio of GP values at pH 7.5 to pH 4.5 ($GP_{7.5}/GP_{4.5}$). The green dot (●), red dot (●), and purple triangle (∇) represent the OPT, Std2, and Std1 compositions, respectively. (b) and (c) show no correlation of GP values or $GP_{7.5}/GP_{4.5}$ with the PEG percentage in the DMG-PEG5k DoE formulations, measured at different pH values using the Laurdan assay.

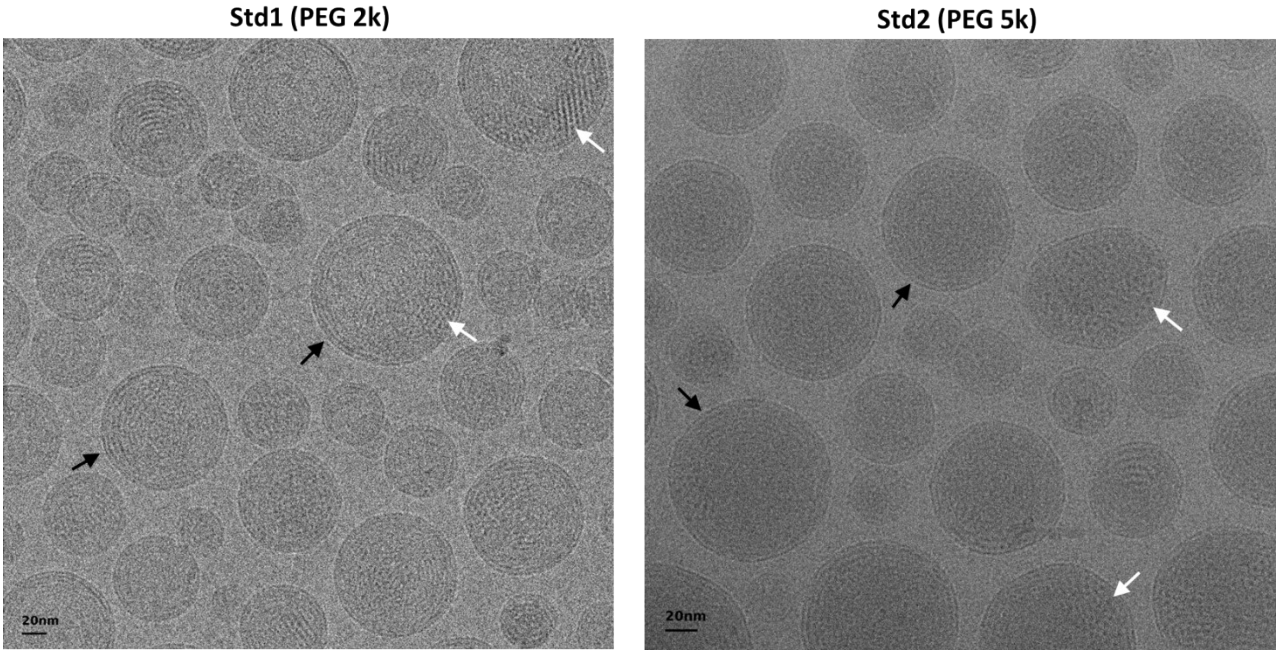

Figure S5. Cryo-TEM images of DMG-PEG2k (Std1) and DMG-PEG5k (Std2) formulations. Black arrows indicate multilamellar structures, while white arrows show inverted hexagonal structures.

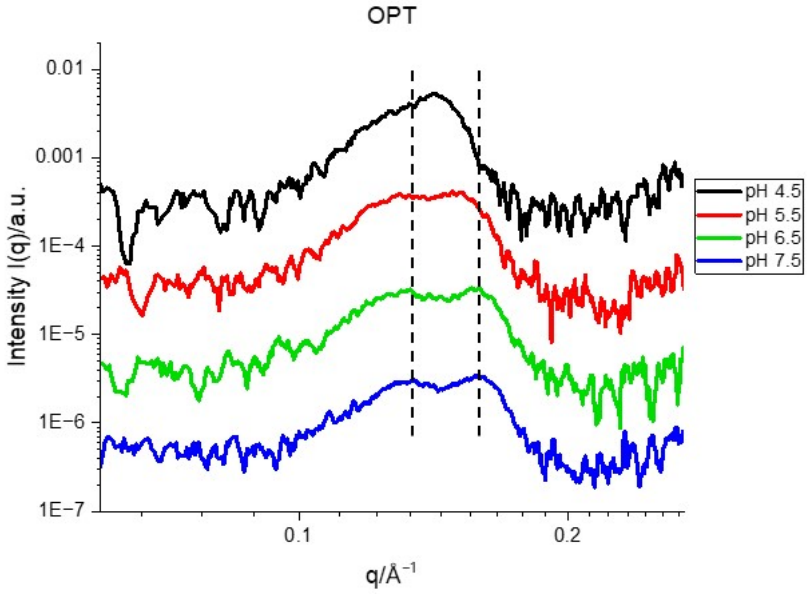

Figure S6. SAXS diffractograms of the OPT mRNA-LNP formulation mixed with citrate-phosphate buffers at different pH values.