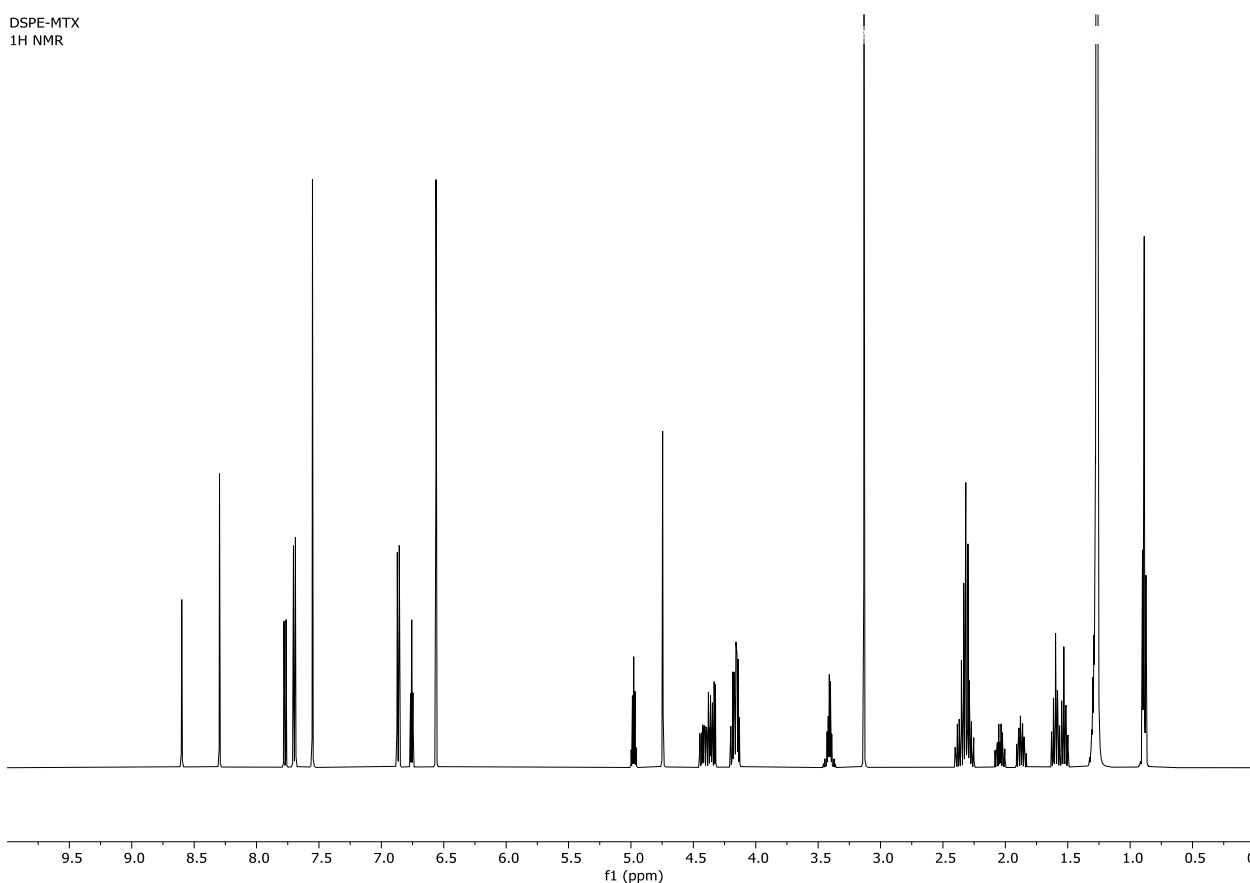


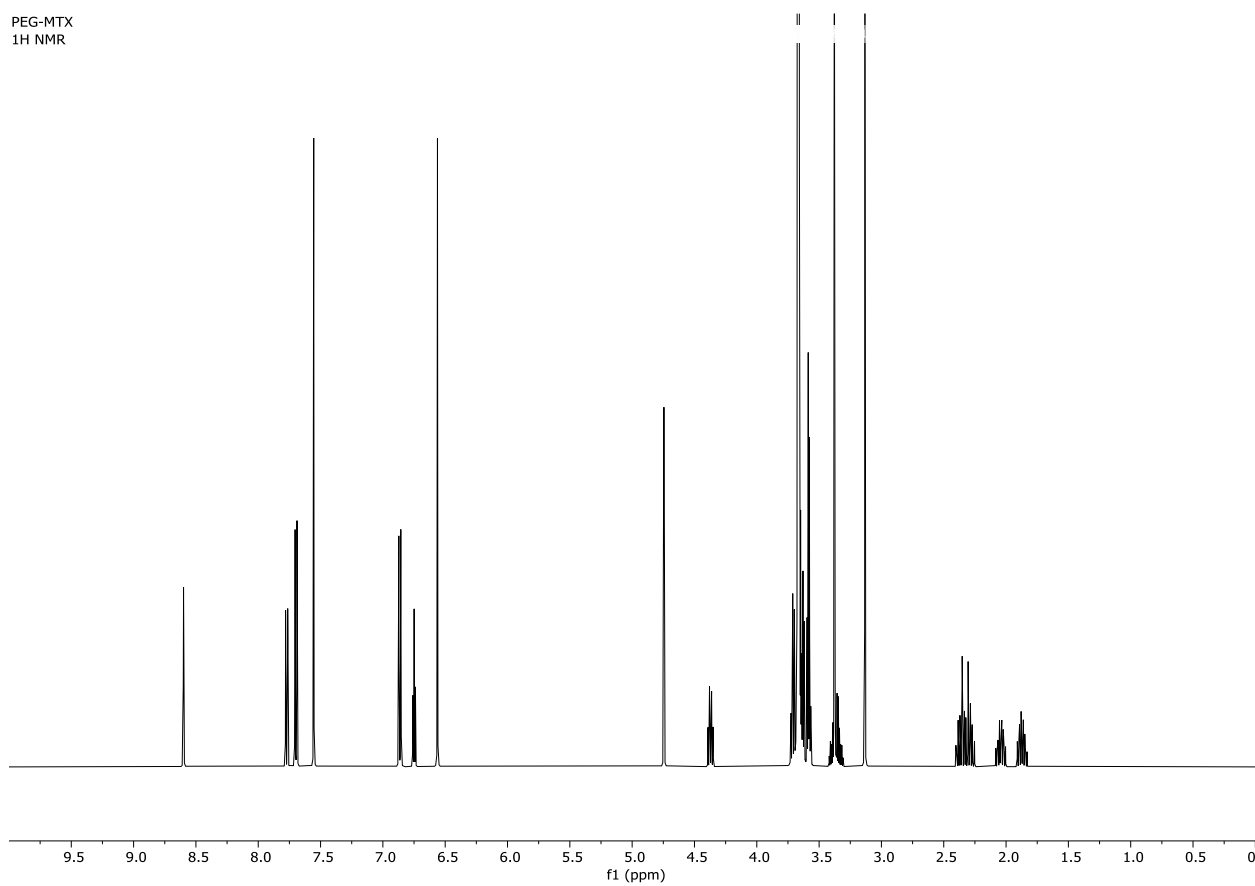
# Supplementary Materials: Synthesis of Two Methotrexate Prodrugs for Optimizing Drug Loading into Liposomes

Valentina Di Francesco, Martina Di Francesco, Paolo Decuzzi, Roberto Palomba and Miguel Ferreira

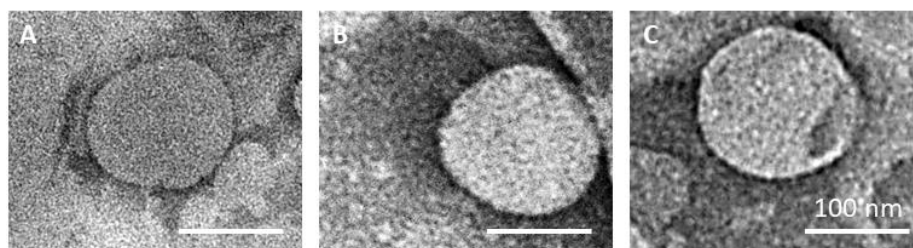
DSPE-MTX  
1H NMR



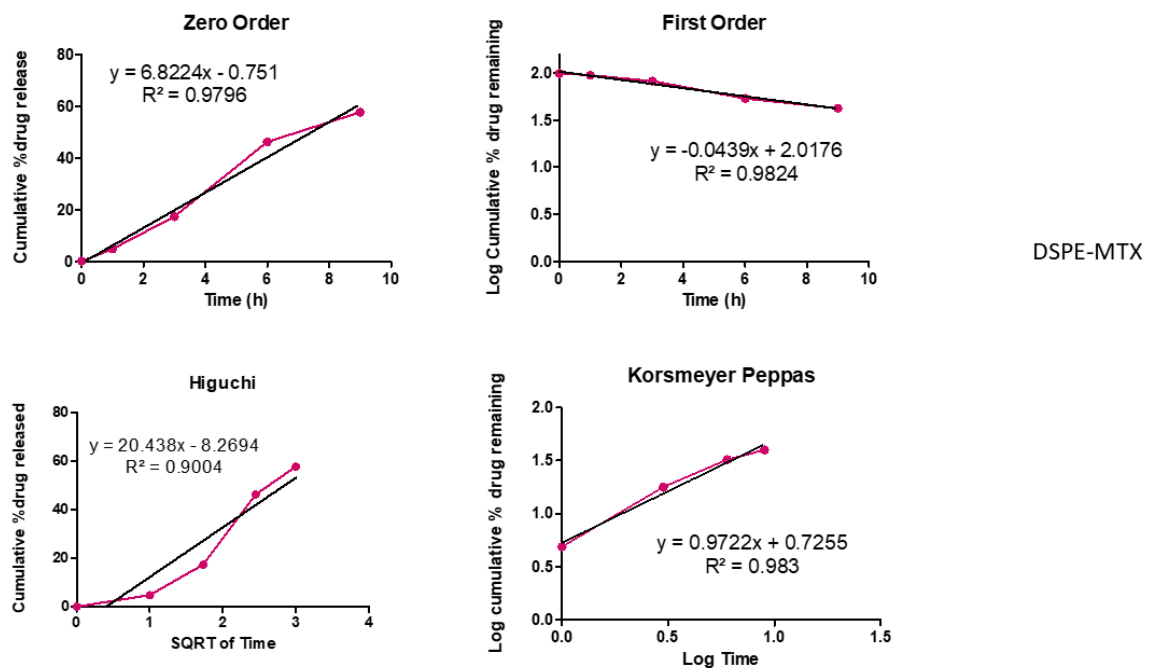
**Figure S1.**  $^1\text{H}$  NMR spectrum of DSPE-MTX in  $\text{CDCl}_3$ .



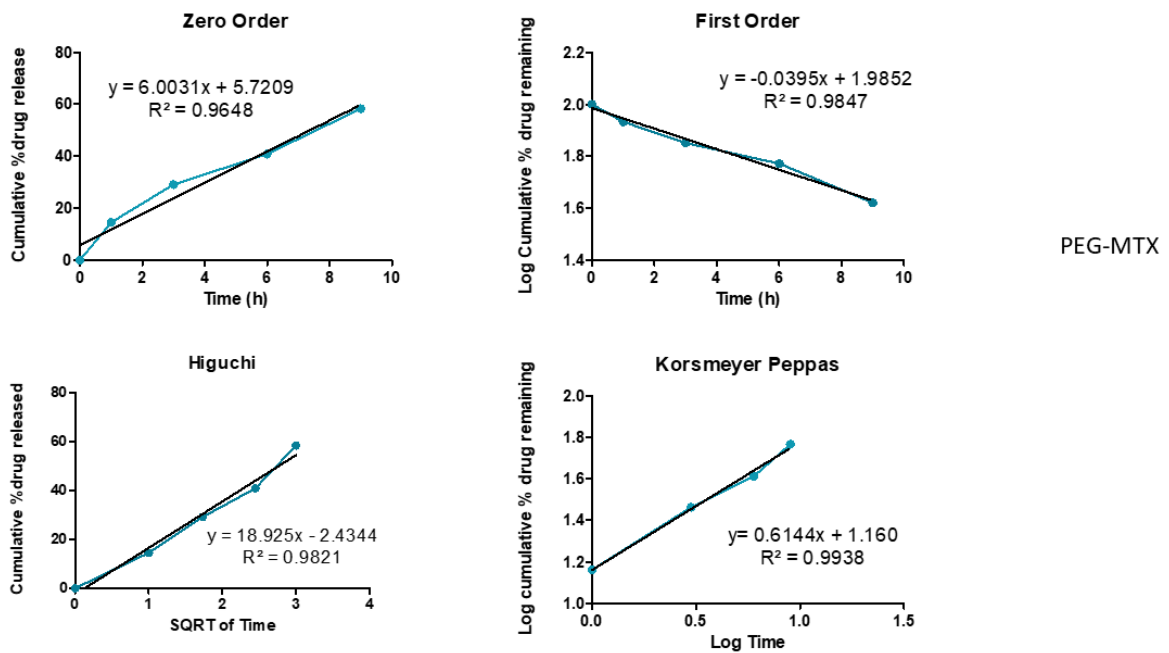
**Figure S2.**  $^1\text{H}$  NMR spectrum of PEG-MTX in  $\text{CDCl}_3$ .



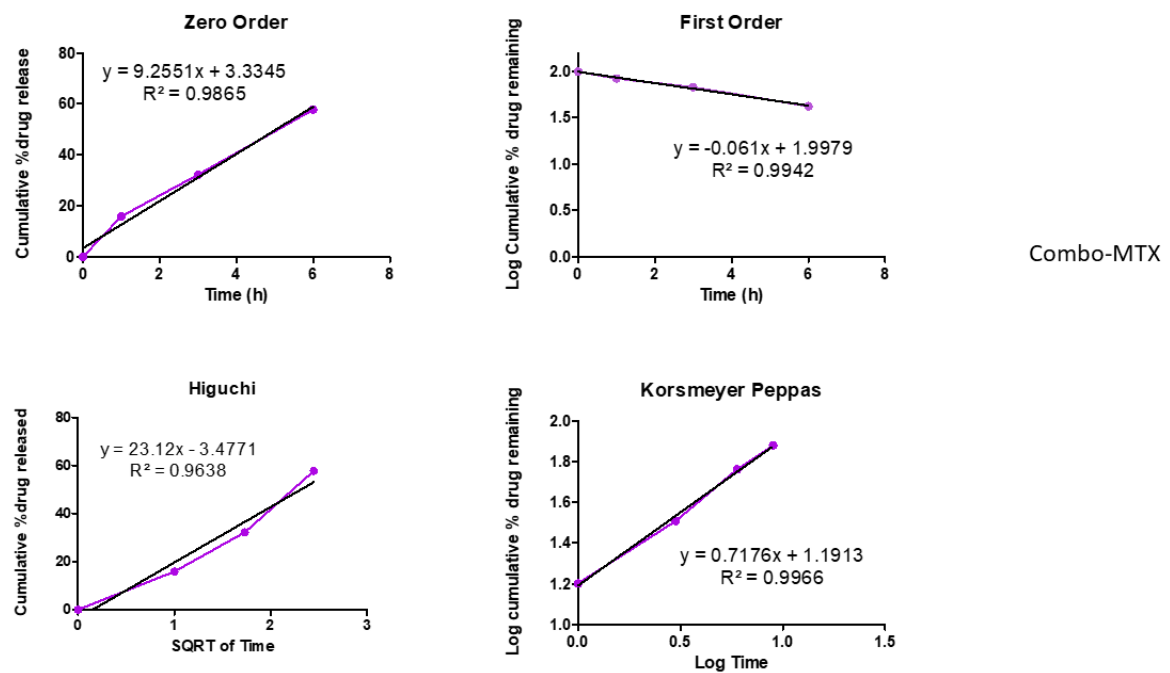
**Figure S3.** Transmission electron microscopy images of PEG-MTX-LIP, DSPE-MTX-LIP and Combo-LIP, respectively (scale bar: 100 nm).



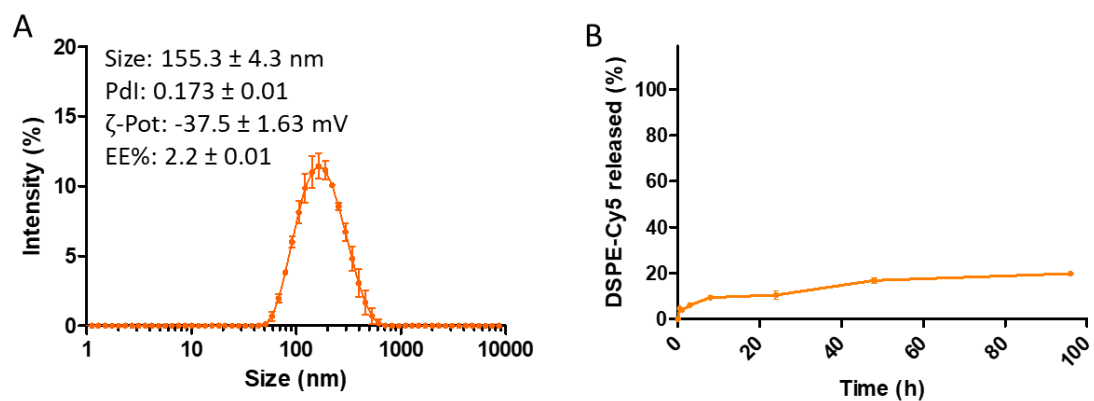
**Figure 4.** Mathematical models showing the fitting of DSPE-MTX-LIP. Results are representative of three independent experiments  $\pm$  S.D. ( $n = 3$ ).



**Figure S5.** Mathematical models showing the fitting of PEG-MTX-LIP. Results are representative of three independent experiments  $\pm$  S.D. ( $n = 3$ ).



**Figure S6.** Mathematical models showing the fitting of Combo-LIP. Results are representative of three independent experiments  $\pm$  S.D. ( $n = 3$ ).



**Figure S7.** Physico-chemical characterization of Cy5-LIP (A) and DSPE-CY5 release profile (B).



**Figure S8.** Image reporting a maximum intensity profile of a z-stack of BMDM treated with liposome reporting in blue the nuclei, in green the plasma membrane and in red the liposomes, split channel visualization.