

Electronic Supplementary Material

Influence of Hydrophilic Polymers on the Accelerated Blood Clearance of mRNA Lipid Nanoparticles Upon Repeated Administration

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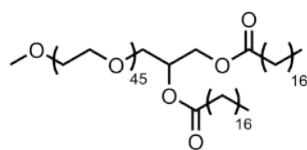
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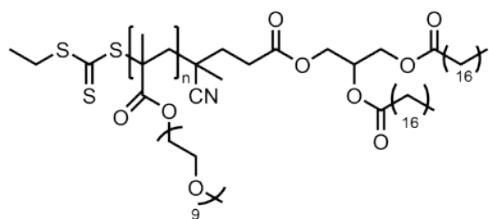
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Control PEG-lipid:

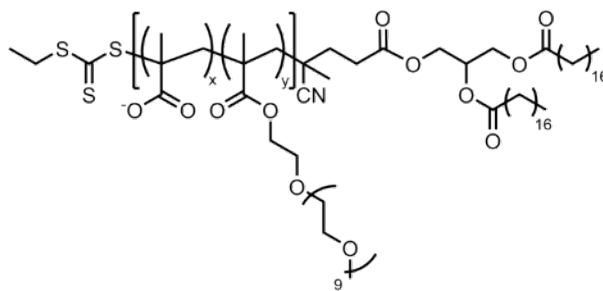


PEG-DSG

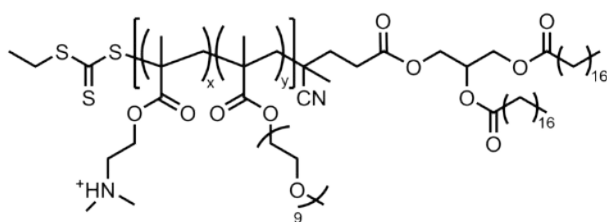
RAFT Polymer-lipids:



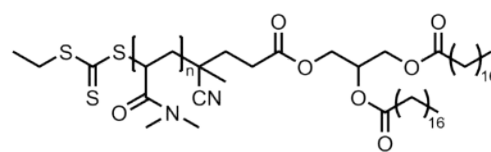
POEGMA-DSG
36 units



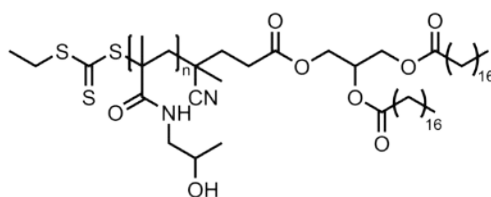
POEGMA-DSG (-)
31 units (P), 5 units (-)



POEGMA-DSG (+)
30 units (P), 4 units (+)



PDMA-DSG
53 units



PHPMA-DSG
71 units

Fig. S1 Chemical structures of polymer-lipids.

Table S1 Injection protocols for PEG-DSG LNPs from 0-4 h.

Dosing regimen (1 st injection → 2 nd injection)	First injection (day 1)	Second injection (day 7)	Blood collection post- 2 nd injection (<i>h</i>)
PBS Buffer → PEG LNP	PBS buffer control (No LNPs)	DiD-labelled PEG LNPs	0
			1
			2
			4
PEG LNP → PEG LNP	PEG LNPs		0
			1
			2
			4

Table S2 Size (number mean), polydispersity (PDI), zeta potential and encapsulation efficiency of prepared LNPs ($n = 2-3$).

Polymer	DiD	Size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
PEG	–	45.0 ± 0.7	0.196 ± 0.023	-14.6 ± 2.3	91.2 ± 1.1
POEGMA	–	65.2 ± 0.7	0.123 ± 0.014	-10.5 ± 0.9	72.8 ± 4.9
POEGMA (–)	–	65.2 ± 2.8	0.138 ± 0.023	-21.0 ± 2.2	65.6 ± 4.2
POEGMA (+)	–	70.7 ± 1.9	0.106 ± 0.019	-6.3 ± 3.2	74.0 ± 3.3
PDMA	–	57.5 ± 2.8	0.195 ± 0.019	-13.9 ± 1.2	87.6 ± 0.9
PHPMA	–	54.3 ± 1.2	0.152 ± 0.042	-14.0 ± 1.6	88.6 ± 0.5
PEG	✓	46.2 ± 2.4	0.148 ± 0.017	-10.2 ± 1.7	89.4 ± 2.7
POEGMA	✓	57.4 ± 2.2	0.157 ± 0.015	-11.8 ± 1.3	68.7 ± 3.4
POEGMA (–)	✓	58.3 ± 4.6	0.144 ± 0.029	-22.1 ± 4.7	66.6 ± 1.4
POEGMA (+)	✓	59.6 ± 0.4	0.133 ± 0.012	-4.5 ± 1.1	70.4 ± 4.4
PDMA	✓	58.6 ± 0.9	0.145 ± 0.033	-11.7 ± 4.6	92.7 ± 0.6
PHPMA	✓	43.6 ± 5.8	0.192 ± 0.015	-11.9 ± 2.4	91.9 ± 0.6

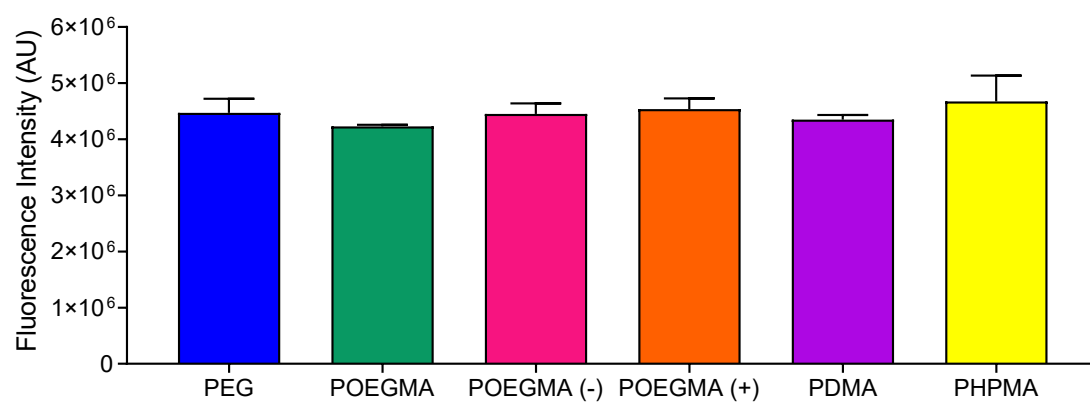


Fig. S2 Fluorescence intensity of DiD-labelled LNPs prior to second-dose injection. ($n = 2$).