Electronic Supplementary Material (ESI) for Biomaterials Science.


# Supplementary Information

## Helper Lipid Structure Influences Protein Adsorption and Delivery of Lipid Nanoparticles to Spleen and Liver

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**Table S1. Lipid nanoparticle (LNP) formulation parameters.**

| Lipid Nanoparticle | Molar Percentage of Component (%) | | | | Mass Ratio |
|---|---|---|---|---|---|
| | Ionizable Lipid (C12-200) | Helper Lipid (DOPE or DSPC) | Lipid-PEG (PEG 1000, 2000, 3000, or 5000) | Cholesterol | Ionizable Lipid:b-DNA Ratio |
| 1 (DOPE and PEG1000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 2 (DOPE and PEG1000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 3 (DOPE and PEG2000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 4 (DOPE and PEG2000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 5 (DOPE and PEG3000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 6 (DOPE and PEG3000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 7 (DOPE and PEG5000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 8 (DOPE and PEG5000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 9 (DOPE and PEG1000) | 40 | 10 | 40 | 10 | 5:1 |
| 10 (DOPE and PEG1000) | 40 | 10 | 40 | 10 | 10:1 |
| 11 (DOPE and PEG2000) | 40 | 10 | 40 | 10 | 5:1 |
| 12 (DOPE and PEG2000) | 40 | 10 | 40 | 10 | 10:1 |
| 13 (DOPE and PEG3000) | 40 | 10 | 40 | 10 | 5:1 |
| 14 (DOPE and PEG3000) | 40 | 10 | 40 | 10 | 10:1 |
| 15 (DOPE and PEG5000) | 40 | 10 | 40 | 10 | 5:1 |
| 16 (DOPE and PEG5000) | 40 | 10 | 40 | 10 | 10:1 |
| 17 (DOPE and PEG1000) | 40 | 10 | 30 | 20 | 5:1 |
| 18 (DOPE and PEG1000) | 40 | 10 | 30 | 20 | 10:1 |
| 19 (DOPE and PEG2000) | 40 | 10 | 30 | 20 | 5:1 |
| 20 (DOPE and PEG2000) | 40 | 10 | 30 | 20 | 10:1 |
| 21 (DOPE and PEG3000) | 40 | 10 | 30 | 20 | 5:1 |
| 22 (DOPE and PEG3000) | 40 | 10 | 30 | 20 | 10:1 |
| 23 (DOPE and PEG5000) | 40 | 10 | 30 | 20 | 5:1 |
| 24 (DOPE and PEG5000) | 40 | 10 | 30 | 20 | 10:1 |
| 25 (DOPE and PEG1000) | 40 | 10 | 20 | 30 | 5:1 |
| 26 (DOPE and PEG1000) | 40 | 10 | 20 | 30 | 10:1 |
| 27 (DOPE and PEG2000) | 40 | 10 | 20 | 30 | 5:1 |
| 28 (DOPE and PEG2000) | 40 | 10 | 20 | 30 | 10:1 |
| 29 (DOPE and PEG3000) | 40 | 10 | 20 | 30 | 5:1 |
| 30 (DOPE and PEG3000) | 40 | 10 | 20 | 30 | 10:1 |
| 31 (DOPE and PEG5000) | 40 | 10 | 20 | 30 | 5:1 |
| 32 (DOPE and PEG5000) | 40 | 10 | 20 | 30 | 10:1 |
| 33 (DOPE and PEG1000) | 40 | 10 | 10 | 40 | 5:1 |
| 34 (DOPE and PEG1000) | 40 | 10 | 10 | 40 | 10:1 |
| 35 (DOPE and PEG2000) | 40 | 10 | 10 | 40 | 5:1 |
| 36 (DOPE and PEG2000) | 40 | 10 | 10 | 40 | 10:1 |
| 37 (DOPE and PEG3000) | 40 | 10 | 10 | 40 | 5:1 |
| 38 (DOPE and PEG3000) | 40 | 10 | 10 | 40 | 10:1 |

| | | | | | |
|---|---|---|---|---|---|
| 39 (DOPE and PEG5000) | 40 | 10 | 10 | 40 | 5:1 |
| 40 (DOPE and PEG5000) | 40 | 10 | 10 | 40 | 10:1 |
| 41 (DOPE and PEG1000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 42 (DOPE and PEG1000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 43 (DOPE and PEG2000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 44 (DOPE and PEG2000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 45 (DOPE and PEG3000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 46 (DOPE and PEG3000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 47 (DOPE and PEG5000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 48 (DOPE and PEG5000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 49 (DSPC and PEG1000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 50 (DSPC and PEG1000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 51 (DSPC and PEG2000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 52 (DSPC and PEG2000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 53 (DSPC and PEG3000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 54 (DSPC and PEG3000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 55 (DSPC and PEG5000) | 40 | 10 | 48.5 | 1.5 | 5:1 |
| 56 (DSPC and PEG5000) | 40 | 10 | 48.5 | 1.5 | 10:1 |
| 57 (DSPC and PEG1000) | 40 | 10 | 40 | 10 | 5:1 |
| 58 (DSPC and PEG1000) | 40 | 10 | 40 | 10 | 10:1 |
| 59 (DSPC and PEG2000) | 40 | 10 | 40 | 10 | 5:1 |
| 60 (DSPC and PEG2000) | 40 | 10 | 40 | 10 | 10:1 |
| 61 (DSPC and PEG3000) | 40 | 10 | 40 | 10 | 5:1 |
| 62 (DSPC and PEG3000) | 40 | 10 | 40 | 10 | 10:1 |
| 63 (DSPC and PEG5000) | 40 | 10 | 40 | 10 | 5:1 |
| 64 (DSPC and PEG5000) | 40 | 10 | 40 | 10 | 10:1 |
| 65 (DSPC and PEG1000) | 40 | 10 | 30 | 20 | 5:1 |
| 66 (DSPC and PEG1000) | 40 | 10 | 30 | 20 | 10:1 |
| 67 (DSPC and PEG2000) | 40 | 10 | 30 | 20 | 5:1 |
| 68 (DSPC and PEG2000) | 40 | 10 | 30 | 20 | 10:1 |
| 69 (DSPC and PEG3000) | 40 | 10 | 30 | 20 | 5:1 |
| 70 (DSPC and PEG3000) | 40 | 10 | 30 | 20 | 10:1 |
| 71 (DSPC and PEG5000) | 40 | 10 | 30 | 20 | 5:1 |
| 72 (DSPC and PEG5000) | 40 | 10 | 30 | 20 | 10:1 |
| 73 (DSPC and PEG1000) | 40 | 10 | 20 | 30 | 5:1 |
| 74 (DSPC and PEG1000) | 40 | 10 | 20 | 30 | 10:1 |
| 75 (DSPC and PEG2000) | 40 | 10 | 20 | 30 | 5:1 |
| 76 (DSPC and PEG2000) | 40 | 10 | 20 | 30 | 10:1 |
| 77 (DSPC and PEG3000) | 40 | 10 | 20 | 30 | 5:1 |
| 78 (DSPC and PEG3000) | 40 | 10 | 20 | 30 | 10:1 |
| 79 (DSPC and PEG5000) | 40 | 10 | 20 | 30 | 5:1 |
| 80 (DSPC and PEG5000) | 40 | 10 | 20 | 30 | 10:1 |
| 81 (DSPC and PEG1000) | 40 | 10 | 10 | 40 | 5:1 |
| 82 (DSPC and PEG1000) | 40 | 10 | 10 | 40 | 10:1 |
| 83 (DSPC and PEG2000) | 40 | 10 | 10 | 40 | 5:1 |
| 84 (DSPC and PEG2000) | 40 | 10 | 10 | 40 | 10:1 |

| | | | | | |
|---|---|---|---|---|---|
| 85 (DSPC and PEG3000) | 40 | 10 | 10 | 40 | 5:1 |
| 86 (DSPC and PEG3000) | 40 | 10 | 10 | 40 | 10:1 |
| 87 (DSPC and PEG5000) | 40 | 10 | 10 | 40 | 5:1 |
| 88 (DSPC and PEG5000) | 40 | 10 | 10 | 40 | 10:1 |
| 89 (DSPC and PEG1000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 90 (DSPC and PEG1000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 91 (DSPC and PEG2000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 92 (DSPC and PEG2000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 93 (DSPC and PEG3000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 94 (DSPC and PEG3000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| 95 (DSPC and PEG5000) | 40 | 10 | 1.5 | 48.5 | 5:1 |
| 96 (DSPC and PEG5000) | 40 | 10 | 1.5 | 48.5 | 10:1 |
| Naked Barcode | 0 | 0 | 0 | 0 | 0 |

**Table S2. LNPs were characterized for size and polydispersity (PDI) by dynamic light scattering (DLS).** LNPs denoted N/A were excluded from the injected pool based on analysis of DLS peaks and autocorrelation data.

| Lipid Nanoparticle | Z-Ave (d.nm) | PDI |
|---|---|---|
| 1 | 64.91 | 0.177 |
| 2 | 91.96 | 0.148 |
| 3 | 109.9 | 0.163 |
| 4 | 114.2 | 0.181 |
| 5 | 135.8 | 0.071 |
| 6 | 140.1 | 0.077 |
| 7 | 124.1 | 0.189 |
| 8 | 128.2 | 0.019 |
| 9 | 107 | 0.172 |
| 10 | 118.6 | 0.017 |
| 11 | 148.9 | 0.045 |
| 12 | 130.5 | 0.161 |
| 13 | 152.2 | 0.052 |
| 14 | 146.9 | 0.08 |
| 15 | 144.1 | 0.011 |
| 16 | 129.3 | 0.165 |
| 17 | 131.6 | 0.186 |
| 18 | 108.8 | 0.185 |
| 19 | 139.4 | 0.118 |
| 20 | 139 | 0.146 |
| 21 | 152.9 | 0.078 |
| 22 | 154.4 | 0.029 |
| 23 | 130.2 | 0.095 |
| 24 | 131.7 | 0.006 |
| 25 | 130.3 | 0.129 |
| 26 | 121.7 | 0.141 |
| 27 | 153.9 | 0.002 |
| 28 | 148 | 0.023 |
| 29 | 154.7 | 0.106 |
| 30 | 154.5 | 0.09 |
| 31 | 153.3 | 0.085 |
| 32 | 146.3 | 0.019 |
| 33 | 144.4 | 0.13 |
| 34 | 138 | 0.094 |
| 35 | 150.6 | 0.073 |
| 36 | 155.6 | 0.114 |
| 37 | 163.9 | 0.115 |
| 38 | 160.9 | 0.085 |
| 39 | 150.1 | 0.038 |
| 40 | 159 | 0.03 |
| 41 | 180.6 | 0.145 |

| | | |
|---|---|---|
| 42 | 170.5 | 0.074 |
| 43 | 162.8 | 0.043 |
| 44 | 166.1 | 0.094 |
| 45 | 170.7 | 0.063 |
| 46 | 166.1 | 0.111 |
| 47 | 171.4 | 0.061 |
| 48 | 173 | 0.104 |
| 49 | N/A | N/A |
| 50 | 88.87 | 0.106 |
| 51 | 90.47 | 0.261 |
| 52 | 91.93 | 0.19 |
| 53 | 117.7 | 0.016 |
| 54 | 128.5 | 0.035 |
| 55 | 115.1 | 0.079 |
| 56 | N/A | N/A |
| 57 | 94.74 | 0.191 |
| 58 | 122.8 | 0.013 |
| 59 | 118.6 | 0.043 |
| 60 | 125.4 | 0.03 |
| 61 | 120 | 0.093 |
| 62 | 148.4 | 0.037 |
| 63 | 123.1 | 0.059 |
| 64 | 118.6 | 0.114 |
| 65 | 107.9 | 0.208 |
| 66 | 73.25 | 0.216 |
| 67 | 131 | 0.039 |
| 68 | 126.8 | 0.067 |
| 69 | 138.9 | 0.104 |
| 70 | 134 | 0.078 |
| 71 | 122.5 | 0.077 |
| 72 | 132.8 | 0.007 |
| 73 | 120.9 | 0.153 |
| 74 | 111.6 | 0.13 |
| 75 | 132.2 | 0.118 |
| 76 | 134.9 | 0.097 |
| 77 | 144.6 | 0.064 |
| 78 | 136 | 0.074 |
| 79 | 143.6 | 0.103 |
| 80 | 148.2 | 0.042 |
| 81 | 141.3 | 0.103 |
| 82 | 124.2 | 0.087 |
| 83 | 134.8 | 0.116 |
| 84 | 131.4 | 0.113 |
| 85 | 154.9 | 0.076 |
| 86 | 157.8 | 0.091 |
| 87 | 156.1 | 0.061 |

| | | |
|---|---|---|
| 88 | 154.2 | 0.087 |
| 89 | 173.2 | 0.098 |
| 90 | 167.2 | 0.111 |
| 91 | 165.9 | 0.106 |
| 92 | 164.5 | 0.069 |
| 93 | 172 | 0.096 |
| 94 | 168.3 | 0.105 |
| 95 | 162.9 | 0.093 |
| 96 | 158.4 | 0.08 |

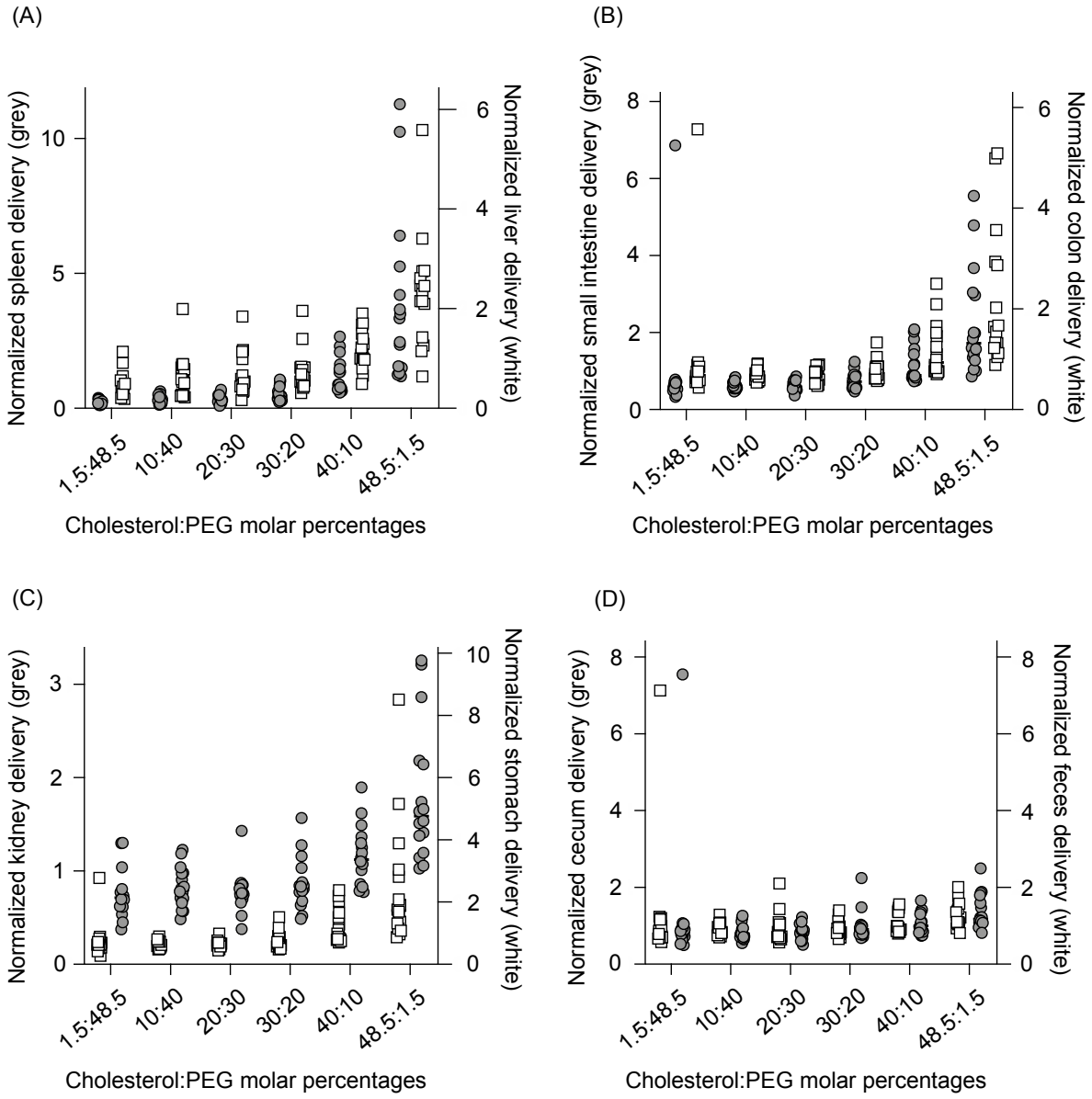


**Figure S1. LNPs formulated with higher ratios of cholesterol to lipid-PEG accumulated to a higher degree in most tissues analyzed.** LNPs were formulated with 1 ionizable lipid (C12-200), 6 different excipient molar ratios, 2 different ionizable lipid:b-DNA weight ratios, 2 different helper lipids, and 4 different lipid-anchored polyethylene glycol (PEG) conjugates. Details on specific excipient molar ratios for each LNP are provided in **Table S1**. 94 out of 96 formulations formed stable LNPs based on DLS data. LNP formulations were pooled together and injected to C57BL/6 mice via tail vein (N=4). Tissues were isolated six hours post injection and accumulation of b-DNA was quantified by deep sequencing. LNP accumulation in the (A) spleen and liver, (B) small intestine and colon, (C) kidney and stomach, and (D) cecum and feces was plotted based on the molar ratio of cholesterol to lipid-PEG.

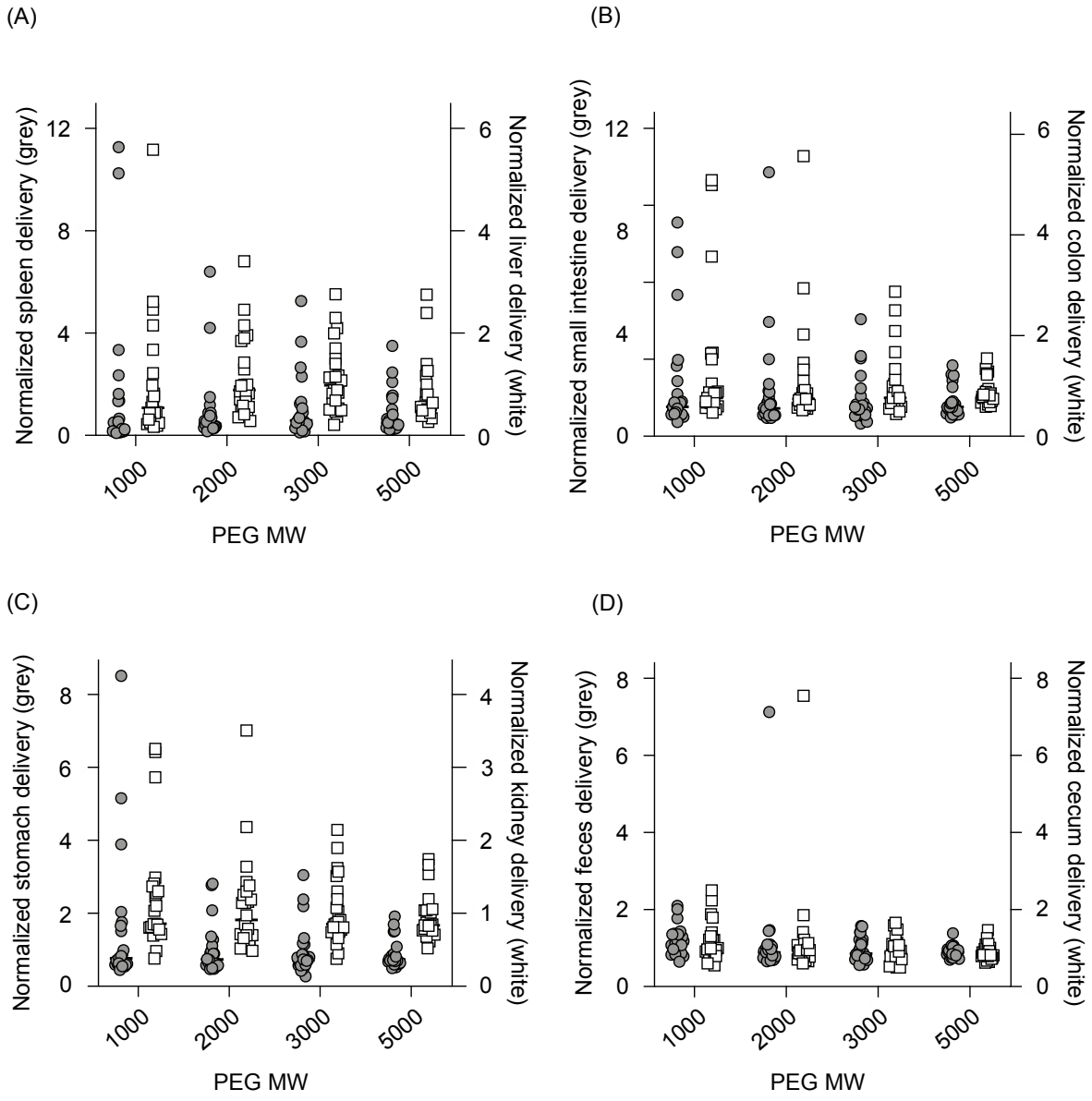


**Figure S2. LNPs formulated with lower molecular lipid-PEGs accumulated to a higher degree in most tissues analyzed.** LNPs were formulated with 1 ionizable lipid (C12-200), 6 different excipient molar ratios, 2 different ionizable lipid:b-DNA weight ratios, 2 different helper lipids, and 4 different lipid-anchored polyethylene glycol (PEG) conjugates. Details on specific excipient molar ratios for each LNP are provided in **Table S1**. 94 out of 96 formulations formed stable LNPs based on DLS data. LNP formulations were pooled together and injected to C57BL/6 mice via tail vein (N=4). Tissues were isolated six hours post injection and accumulation of b-DNA was quantified by deep sequencing. LNP accumulation in the (A) spleen and liver, (B) small intestine and colon, (C) stomach and kidney, and (D) feces and cecum was plotted based on the molecular weight of the lipid-PEG incorporated into the LNPs.

# Supplementary Discussion of QCM-D Experiments

Figure 4B in the main text presented the frequency shift of the third overtone ($^{\Delta F_3}/3$) versus time for LNPs formulated with the C12-200 ionizable lipid[1]. The time dependency has been removed in **Figure S3A** by plotting the measured dissipation $^{\Delta D_n}$ against $^{\Delta F_n}/n$, where *n* is the overtone. Plots of $^{\Delta D_n}$ vs $^{\Delta F_n}/n$ allow kinetic processes (e.g. adsorption or conformational changes) to be compared[2]. Data between values of $^{\Delta F_n}/n$ between 0 and ~−60 Hz correspond to the adsorption of ApoE on the Au coated sensor. The curves begin to diverge at larger negative frequency shifts as particle adsorption takes place. Here, for a given frequency shift the LNP formulated with DOPE (LNP 42) have a lower dissipation value than the LNP formulated with DSPC (LNP 90) for the same overtone. Control experiments for LNPs formulated with C12-200 interacting with a bare Au sensor show the opposite trend with a larger negative frequency shift for the DSPC formulation **(Figure S4)**, which is the reverse of the magnitudes observed in the presence of ApoE and is further evidence of specific interactions between the DOPE-containing LNPs and ApoE. Dissipation from heterogenous films of discrete, adsorbed particles arises from both hydrodynamic interactions with the particles and deformation of particle-surface contacts, with the majority of energy dissipated through the liquid.[3,4] One possible interpretation of the lower dissipation, is that ApoE forms wider, more rigid contacts with the DOPE-containing LNPs compared to DSPC-containing LNPs. However, the presence of the homogeneous, viscoelastic ApoE layer complicates the interpretation, and finite element methods are required for quantitative analysis[4]. Regardless, **Figure S3A** clearly demonstrates the nature of the adsorption depends on the choice of helper lipid.

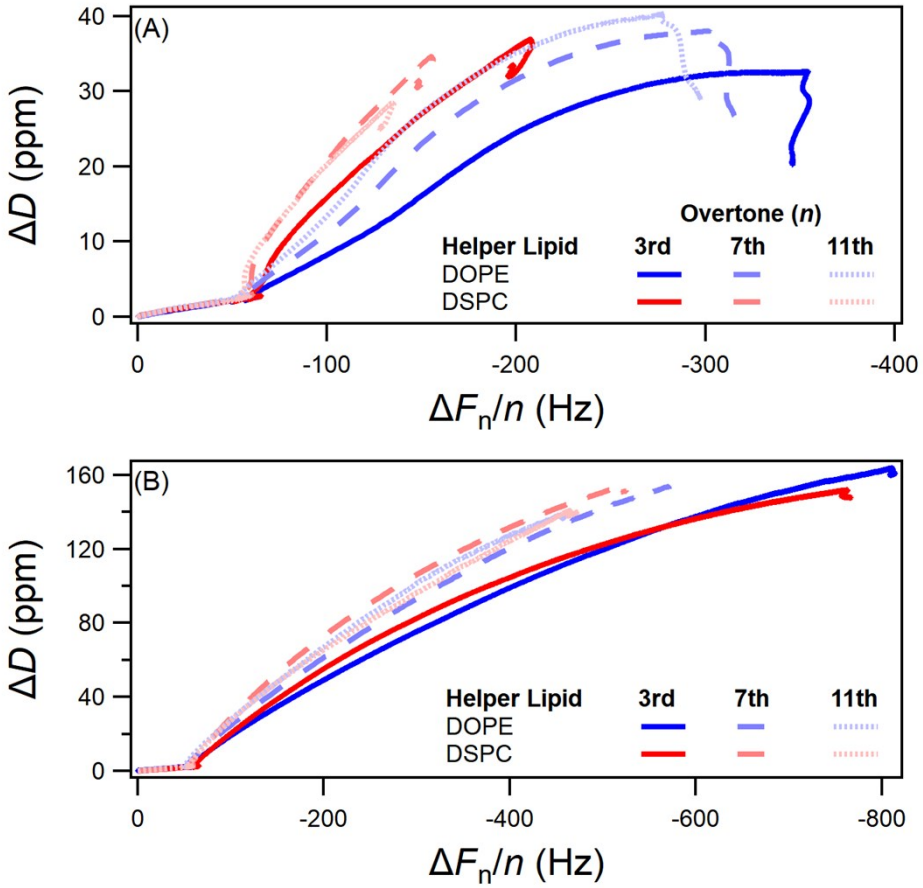


**Fig S3. Kinetic pathways of LNP adsorption.** (A) Dissipation values vs frequency shift ($^{\Delta F_n/n}$) follow different adsorption pathways at each overtone depending on choice of helper lipid for particles containing C12-200 (DOPE = LNP 42, DSPC = LNP 90). (B) Adsorption pathways are similar when C14-4 ionizable lipids are used. Note that the composition of the C14-4 containing LNPs is otherwise identical to the equivalent C12-200 LNPs.

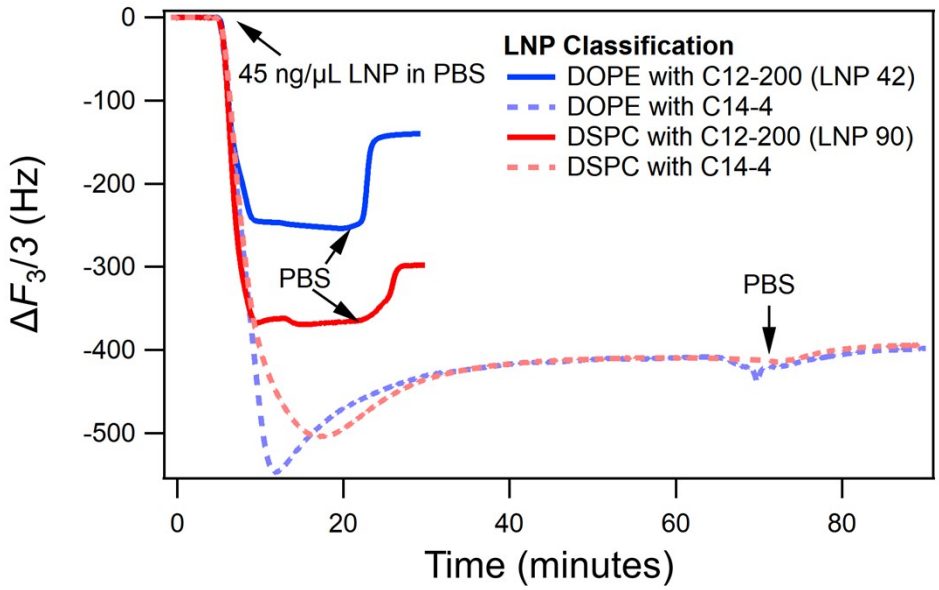


**Fig S4. Change in frequency of the third overtone versus time for LNPs adsorbing onto Au-coated QCM-D crystals.** LNPs vary by identity of the helper lipid (DOPE or DSPC) and the ionizable lipid (C12-200 or C14-4), but otherwise had identical compositions.

In contrast to the data in **Figure S3A**, control experiments for LNPs formulated with the C14-4 ionizable lipid[5] displayed no significant difference in the nature of their adsorption (**Figure S3B)**. However, **Figure S5** demonstrates that the nature of the helper lipid has a strong influence on the initial rate of adsorption of these control particles. In particular, **Figure S5B** shows that the peak rate of change of the frequency shift of the 3$^{rd}$ overtone was approximately 3 times larger for the DOPE containing formulation than the DSPC containing formulation. Control experiments that looked at adsorption of these particles on bare Au sensors showed no significant difference in the rate of initial particle adsorption (**Figure S4**). This suggests that adsorption is aided by specific interactions between DOPE and ApoE.

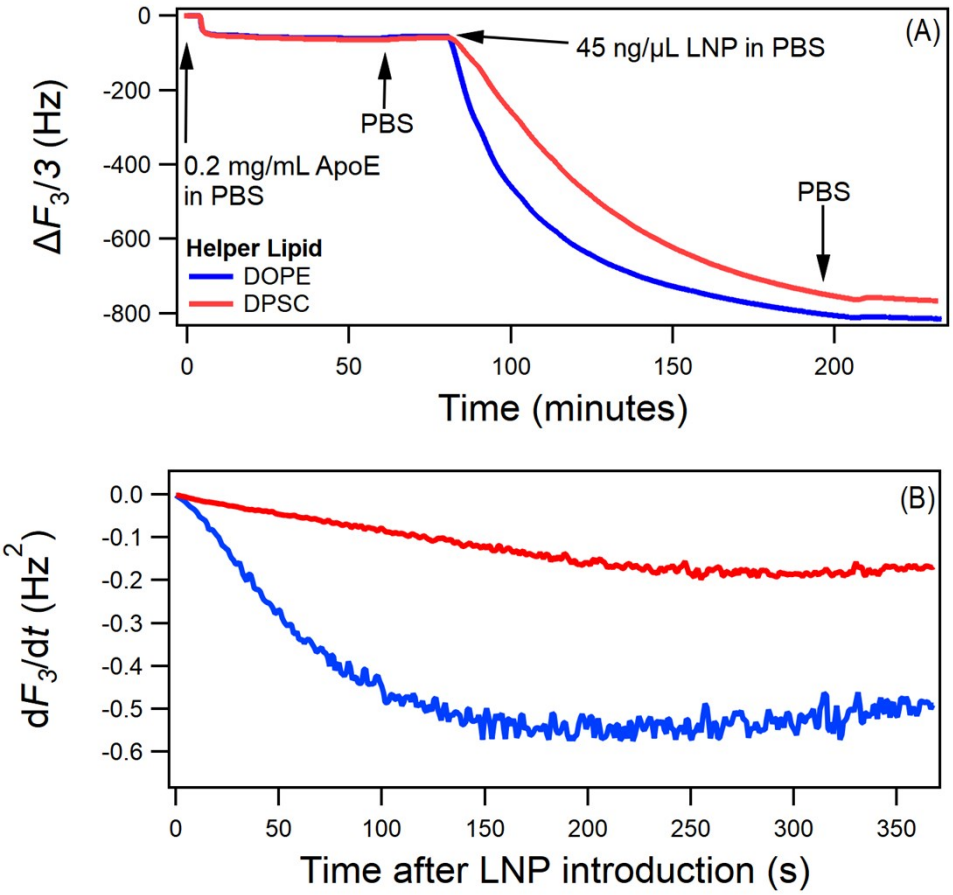


**Fig S5. (A) Change in frequency of the third overtone versus time for LNPs containing C14-4 ionizable lipid adsorbing onto ApoE-coated QCM-D crystals.** Aside from the ionizable lipids, the composition of these LNPs is identical to the equivalent DOPE (LNP 42) and DSPC (LNP 90) containing particles. (B) Rate of change of frequency relative to time after the introduction of the LNPs.

Further control experiments were performed investigating LNP interactions with bare Au sensors. Measured frequency shifts of the third overtone are shown in **Figure S4.** Here, all formulations showed strong interactions with the Au surface and are stable to rinsing with PBS. LNPs formulated with the ionizable lipid C14-4 resulted in a larger frequency shift when compared to the C12-200 LNPs, which may result from stronger adsorption and/or larger LNPs. For the C14-4 containing LNPs, a peak in the frequency is also observed during adsorption. This peak may result from rupture and/or fusion of the nanoparticles, as often seen for phospholipid assemblies adsorbing on solid supports.[6] However, the large magnitude of the plateau frequency shifts suggests that the LNPs did not rupture completely, if at all. It is possible that stronger interactions with the underlying Au substrate and/or that hydrodynamic contributions from the larger LNP size mask the contribution of ApoE-LNP contact results in the similar nature of adsorption on ApoE observed in **Figure S3B**.

# Additional Supplementary Information

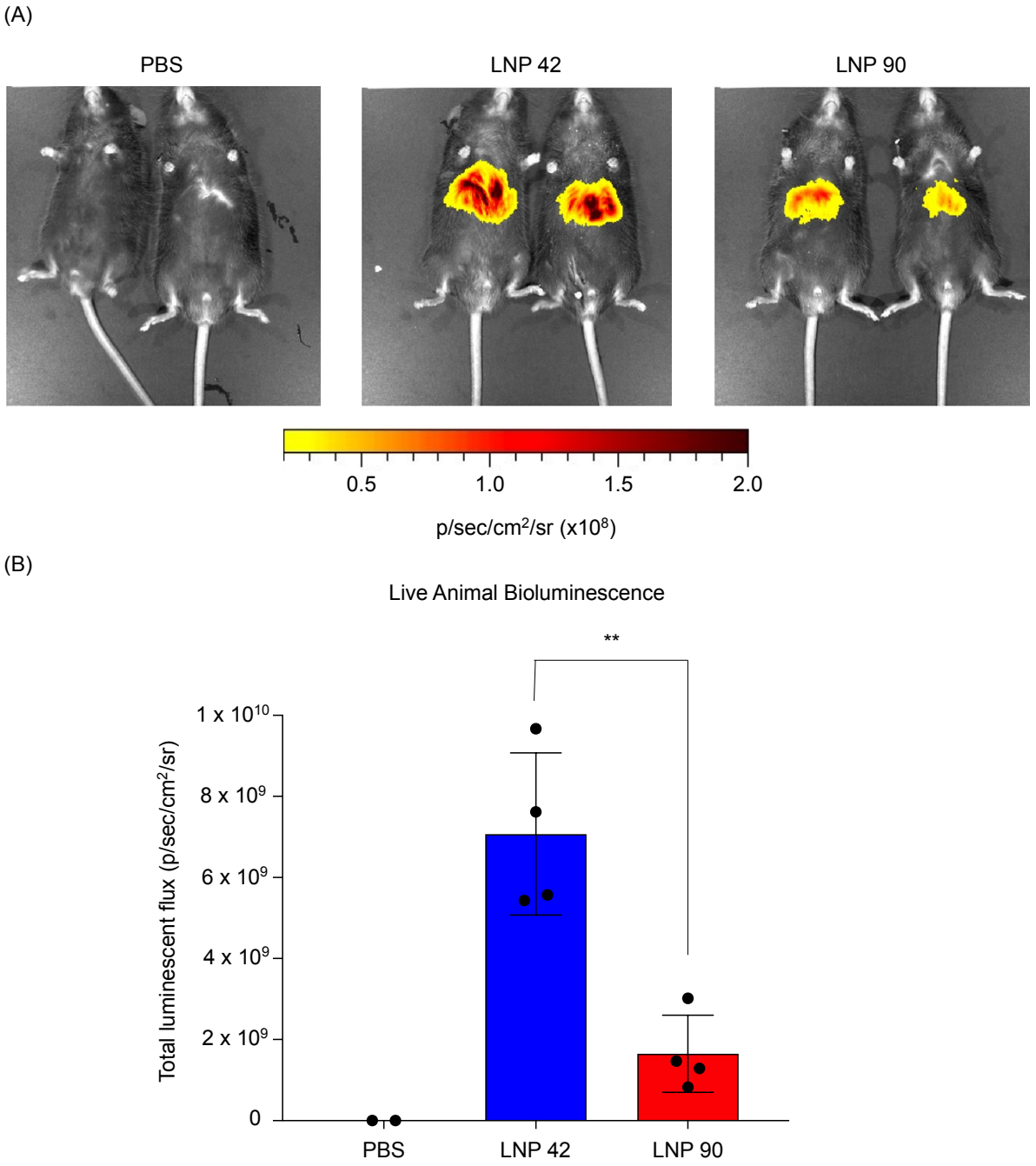


**Fig S6. Firefly luciferase mRNA-LNPs were administered via tail vein injection.** LNP 42 and LNP 90 were formulated by microfluidic mixing to encapsulate mRNA encoding for firefly luciferase. C57BL/6 mice were intravenously injected with either LNP 42 or LNP 90 at 0.2 mg/kg mRNA via tail vein. Total luminescent flux was quantified 6 hours post-injection (**P=0.0027). N=4 mice per experimental group. N=2 mice per PBS control group. Representative images for N=2 mice are shown. Data was plotted as mean ± SD. **P<0.005 by t-test.

**Table S3. Library of b-DNA sequences.** NNNNNNNNNN represents unique molecular identifiers (UMI). *denotes phosphorothioate bond locations.

| Barcode | b-DNA Sequence |
|---|---|
| b-DNA 1 | A*G*A* C*G*TGTGCTCTTCCGATCTGAGGGTACTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 2 | A*G*A* C*G*TGTGCTCTTCCGATCTGACAATTGCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 3 | A*G*A* C*G*TGTGCTCTTCCGATCTTAACGCACCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 4 | A*G*A* C*G*TGTGCTCTTCCGATCTATGATCGTCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 5 | A*G*A* C*G*TGTGCTCTTCCGATCTTGTCTCCCATNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 6 | A*G*A* C*G*TGTGCTCTTCCGATCTGGAGAAACAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 7 | A*G*A* C*G*TGTGCTCTTCCGATCTCGTACAAACGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 8 | A*G*A* C*G*TGTGCTCTTCCGATCTGATTTGTGGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 9 | A*G*A* C*G*TGTGCTCTTCCGATCTTTGCAGCCTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 10 | A*G*A* C*G*TGTGCTCTTCCGATCTGAATGCTGACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 11 | A*G*A* C*G*TGTGCTCTTCCGATCTATCCATGAGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 12 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCCACGATGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 13 | A*G*A* C*G*TGTGCTCTTCCGATCTGCTGGGAATTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 14 | A*G*A* C*G*TGTGCTCTTCCGATCTCAAAACGACGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 15 | A*G*A* C*G*TGTGCTCTTCCGATCTTCTCGCCTTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 16 | A*G*A* C*G*TGTGCTCTTCCGATCTCAGATCAGAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 17 | A*G*A* C*G*TGTGCTCTTCCGATCTGACACGTTCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 18 | A*G*A* C*G*TGTGCTCTTCCGATCTGCTAAGGTCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 19 | A*G*A* C*G*TGTGCTCTTCCGATCTTGTTCGACCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 20 | A*G*A* C*G*TGTGCTCTTCCGATCTCCCAAAGACANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 21 | A*G*A* C*G*TGTGCTCTTCCGATCTCAGGTAGGAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 22 | A*G*A* C*G*TGTGCTCTTCCGATCTCATTATCGCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 23 | A*G*A* C*G*TGTGCTCTTCCGATCTCAGAGACTGANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 24 | A*G*A* C*G*TGTGCTCTTCCGATCTTGACATGCACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 25 | A*G*A* C*G*TGTGCTCTTCCGATCTTGTTGGTTCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 26 | A*G*A* C*G*TGTGCTCTTCCGATCTCTCTCTGAACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 27 | A*G*A* C*G*TGTGCTCTTCCGATCTCACTAGCCAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 28 | A*G*A* C*G*TGTGCTCTTCCGATCTTGACTTTGCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 29 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCAGCGAAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 30 | A*G*A* C*G*TGTGCTCTTCCGATCTACAGGCATACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 31 | A*G*A* C*G*TGTGCTCTTCCGATCTCGACTCCTAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 32 | A*G*A* C*G*TGTGCTCTTCCGATCTCACGCTATCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 33 | A*G*A* C*G*TGTGCTCTTCCGATCTAAGTCCGCTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 34 | A*G*A* C*G*TGTGCTCTTCCGATCTATCTAGACCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 35 | A*G*A* C*G*TGTGCTCTTCCGATCTAATCCCCCTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 36 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCGCATCTGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 37 | A*G*A* C*G*TGTGCTCTTCCGATCTTTGGAGTTCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |

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|---|---|
| b-DNA 38 | A*G*A* C*G*TGTGCTCTTCCGATCTTTGGCCAATCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 39 | A*G*A* C*G*TGTGCTCTTCCGATCTAAAGGCGACANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 40 | A*G*A* C*G*TGTGCTCTTCCGATCTAATGTCGCTGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 41 | A*G*A* C*G*TGTGCTCTTCCGATCTCAACGTATGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 42 | A*G*A* C*G*TGTGCTCTTCCGATCTCTACCTAACCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 43 | A*G*A* C*G*TGTGCTCTTCCGATCTTCACGGTGTANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 44 | A*G*A* C*G*TGTGCTCTTCCGATCTCCCAGAAGTANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 45 | A*G*A* C*G*TGTGCTCTTCCGATCTACAAGCGCTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 46 | A*G*A* C*G*TGTGCTCTTCCGATCTGGATTAACCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 47 | A*G*A* C*G*TGTGCTCTTCCGATCTCCTCGTGATANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 48 | A*G*A* C*G*TGTGCTCTTCCGATCTGACTATTCGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 49 | A*G*A* C*G*TGTGCTCTTCCGATCTATCGTCTCAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 50 | A*G*A* C*G*TGTGCTCTTCCGATCTGCGTTATGACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 51 | A*G*A* C*G*TGTGCTCTTCCGATCTGTTTACGTGTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 52 | A*G*A* C*G*TGTGCTCTTCCGATCTGTACAATGGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 53 | A*G*A* C*G*TGTGCTCTTCCGATCTCTAGGCGGATNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 54 | A*G*A* C*G*TGTGCTCTTCCGATCTCATTGGTTGANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 55 | A*G*A* C*G*TGTGCTCTTCCGATCTCAGAAGTGGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 56 | A*G*A* C*G*TGTGCTCTTCCGATCTCAGGAACACCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 57 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCGTGACCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 58 | A*G*A* C*G*TGTGCTCTTCCGATCTTCGGGAAAGANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 59 | A*G*A* C*G*TGTGCTCTTCCGATCTAGTGTGAGAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 60 | A*G*A* C*G*TGTGCTCTTCCGATCTCTGAACACATNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 61 | A*G*A* C*G*TGTGCTCTTCCGATCTAGGGTGTAAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 62 | A*G*A* C*G*TGTGCTCTTCCGATCTTGGGGTTAGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 63 | A*G*A* C*G*TGTGCTCTTCCGATCTGGCAACGTACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 64 | A*G*A* C*G*TGTGCTCTTCCGATCTCACTATAGGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 65 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCACCAGCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 66 | A*G*A* C*G*TGTGCTCTTCCGATCTAATCGGTGAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 67 | A*G*A* C*G*TGTGCTCTTCCGATCTTCTATGCACTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 68 | A*G*A* C*G*TGTGCTCTTCCGATCTGAGAACGGGTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 69 | A*G*A* C*G*TGTGCTCTTCCGATCTTATGTGTACGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 70 | A*G*A* C*G*TGTGCTCTTCCGATCTCTCTCTTGCANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 71 | A*G*A* C*G*TGTGCTCTTCCGATCTTCGCAACGAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 72 | A*G*A* C*G*TGTGCTCTTCCGATCTGGTGAGCAATNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 73 | A*G*A* C*G*TGTGCTCTTCCGATCTTTCATTGCGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 74 | A*G*A* C*G*TGTGCTCTTCCGATCTTGATGCTTGANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 75 | A*G*A* C*G*TGTGCTCTTCCGATCTTAACGCCAAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 76 | A*G*A* C*G*TGTGCTCTTCCGATCTGGGTTGCGATNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 77 | A*G*A* C*G*TGTGCTCTTCCGATCTGAGACAACCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 78 | A*G*A* C*G*TGTGCTCTTCCGATCTGGATGACCTCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 79 | A*G*A* C*G*TGTGCTCTTCCGATCTGTGGCATGTTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 80 | A*G*A* C*G*TGTGCTCTTCCGATCTAAGGATGTGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |

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|---|---|
| b-DNA 81 | A*G*A* C*G*TGTGCTCTTCCGATCTTCGCAGGACANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 82 | A*G*A* C*G*TGTGCTCTTCCGATCTAACTTCTCCGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 83 | A*G*A* C*G*TGTGCTCTTCCGATCTCAAGACTGCANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 84 | A*G*A* C*G*TGTGCTCTTCCGATCTGAGATTTCCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 85 | A*G*A* C*G*TGTGCTCTTCCGATCTACTAGAAGGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 86 | A*G*A* C*G*TGTGCTCTTCCGATCTGCAATGGGAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 87 | A*G*A* C*G*TGTGCTCTTCCGATCTGACTCTCCAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 88 | A*G*A* C*G*TGTGCTCTTCCGATCTTGTGCTCGTANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 89 | A*G*A* C*G*TGTGCTCTTCCGATCTAGCATTATCCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 90 | A*G*A* C*G*TGTGCTCTTCCGATCTGATTACCAACNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 91 | A*G*A* C*G*TGTGCTCTTCCGATCTGCCTTGATTGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 92 | A*G*A* C*G*TGTGCTCTTCCGATCTGTCCTCCATCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 93 | A*G*A* C*G*TGTGCTCTTCCGATCTTACGCCACCANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 94 | A*G*A* C*G*TGTGCTCTTCCGATCTGGAACGAGGTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 95 | A*G*A* C*G*TGTGCTCTTCCGATCTCGGTCGAATCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 96 | A*G*A* C*G*TGTGCTCTTCCGATCTAACCTTTGGGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 97 | A*G*A* C*G*TGTGCTCTTCCGATCTGTAGAAGGCTNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 98 | A*G*A* C*G*TGTGCTCTTCCGATCTCCCATCATGCNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 99 | A*G*A* C*G*TGTGCTCTTCCGATCTTGGATGGCAANNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |
| b-DNA 100 | A*G*A* C*G*TGTGCTCTTCCGATCTGCTCATGGAGNNNNNNNNNN AGATCGGAAGAGCGT*C*G *T*G*T |

## Table S4. Library of full-length reverse primers used in preparing the b-DNA library for deep sequencing.

| Primers | Sequence |
|---|---|
| Miseq_primer 1 | CAAGCAGAAGACGGCATACGAGATCCTGGTAGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 2 | CAAGCAGAAGACGGCATACGAGATTAAGCATGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 3 | CAAGCAGAAGACGGCATACGAGATAGATGTGCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 4 | CAAGCAGAAGACGGCATACGAGATGTCGAGCAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 5 | CAAGCAGAAGACGGCATACGAGATGAATTGCTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 6 | CAAGCAGAAGACGGCATACGAGATAAGCAACTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 7 | CAAGCAGAAGACGGCATACGAGATCTAACTGGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 8 | CAAGCAGAAGACGGCATACGAGATAGGCTCAAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 9 | CAAGCAGAAGACGGCATACGAGATCAGTTGGTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 10 | CAAGCAGAAGACGGCATACGAGATTCTGGACCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 11 | CAAGCAGAAGACGGCATACGAGATTGTTATACGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 12 | CAAGCAGAAGACGGCATACGAGATTCAGCGAAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 13 | CAAGCAGAAGACGGCATACGAGATGTCAAGTTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 14 | CAAGCAGAAGACGGCATACGAGATAGGATGTGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 15 | CAAGCAGAAGACGGCATACGAGATCATTCCGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 16 | CAAGCAGAAGACGGCATACGAGATACATCCTTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 17 | CAAGCAGAAGACGGCATACGAGATTCGTGTGCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 18 | CAAGCAGAAGACGGCATACGAGATTCGCCAGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 19 | CAAGCAGAAGACGGCATACGAGATTCGCTATGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 20 | CAAGCAGAAGACGGCATACGAGATGGCTCCTGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 21 | CAAGCAGAAGACGGCATACGAGATATCCGACAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 22 | CAAGCAGAAGACGGCATACGAGATAACATAATGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 23 | CAAGCAGAAGACGGCATACGAGATATGGTAGGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 24 | CAAGCAGAAGACGGCATACGAGATGCTAAGTAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 25 | CAAGCAGAAGACGGCATACGAGATACTTCTTCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 26 | CAAGCAGAAGACGGCATACGAGATTAGATCCTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 27 | CAAGCAGAAGACGGCATACGAGATTTACTGTCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 28 | CAAGCAGAAGACGGCATACGAGATGGCATAGGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 29 | CAAGCAGAAGACGGCATACGAGATCAAGGCGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 30 | CAAGCAGAAGACGGCATACGAGATGACGCTATGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 31 | CAAGCAGAAGACGGCATACGAGATAAGGCGACGTGACTGGAGTTCAGACGTGTG |

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|---|---|
| Miseq_primer 32 | CAAGCAGAAGACGGCATACGAGATCCTAGAATGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 33 | CAAGCAGAAGACGGCATACGAGATTGGTAACGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 34 | CAAGCAGAAGACGGCATACGAGATCATCAGACGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 35 | CAAGCAGAAGACGGCATACGAGATGTGCGTAAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 36 | CAAGCAGAAGACGGCATACGAGATCTATTCAAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 37 | CAAGCAGAAGACGGCATACGAGATAGTGTCTTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 38 | CAAGCAGAAGACGGCATACGAGATCCTTGCTGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 39 | CAAGCAGAAGACGGCATACGAGATTTGCTGGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 40 | CAAGCAGAAGACGGCATACGAGATAGCTCTGGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 41 | CAAGCAGAAGACGGCATACGAGATACCAAGGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 42 | CAAGCAGAAGACGGCATACGAGATGATAACCTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 43 | CAAGCAGAAGACGGCATACGAGATTAGATGACGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 44 | CAAGCAGAAGACGGCATACGAGATTGCGAAGGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 45 | CAAGCAGAAGACGGCATACGAGATGACCGAGAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 46 | CAAGCAGAAGACGGCATACGAGATCAGACAATGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 47 | CAAGCAGAAGACGGCATACGAGATCTAGGTTCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 48 | CAAGCAGAAGACGGCATACGAGATGTTCATTAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 49 | CAAGCAGAAGACGGCATACGAGATAATGCGTTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 50 | CAAGCAGAAGACGGCATACGAGATGAGAGTTGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 51 | CAAGCAGAAGACGGCATACGAGATGATTACAGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 52 | CAAGCAGAAGACGGCATACGAGATTGTGCTTAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 53 | CAAGCAGAAGACGGCATACGAGATAGAACATTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 54 | CAAGCAGAAGACGGCATACGAGATTACCGCTGGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 55 | CAAGCAGAAGACGGCATACGAGATTCCTGGTCGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 56 | CAAGCAGAAGACGGCATACGAGATCCTGGATAGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 57 | CAAGCAGAAGACGGCATACGAGATATACCTGTGTGACTGGAGTTCAGACGTGTG |
| Miseq_primer 58 | CAAGCAGAAGACGGCATACGAGATAATGTTGGGTGACTGGAGTTCAGACGTGTG |

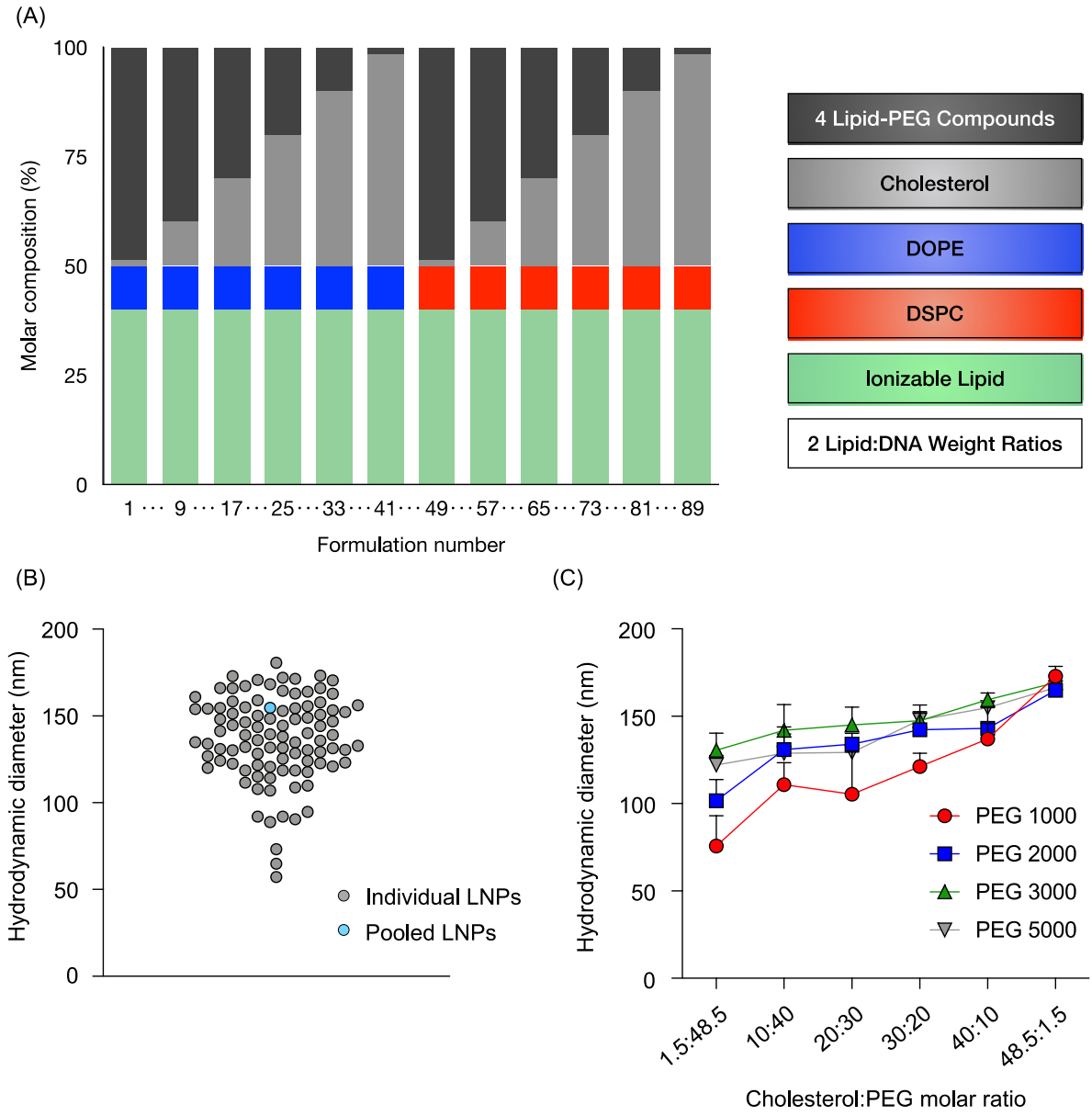


**Figure S7. LNPs formulated by pipette mixing varied in size from 50 nm to 200 nm.** (A) LNPs were formulated with 1 ionizable lipid (C12-200), 6 different excipient molar ratios, 2 different ionizable lipid:b-DNA weight ratios, 2 different helper lipids, and 4 different lipid-anchored polyethylene glycol (PEG) conjugates. Details on specific excipient molar ratios for each LNP are provided in **Table S1**. 94 out of 96 formulations formed stable LNPs based on DLS data. (B-C) LNP formulations were measured for hydrodynamic diameter by dynamic light scattering (DLS). (B) Individual LNPs and the uninjected pool were analyzed. (C) LNPs were analyzed based on their cholesterol to lipid-PEG molar ratio and their PEG molecular weight.

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