

Figure 1: A comprehensive approach to improve mRNA LNP potency

Ionizable Lipids & LNPs	LNP Properties	In Vivo Properties	In Vivo Challenge
* Theoretical analyses of ionizable lipid candidates * Ionizable lipid synthesis, purification, characterization * NMR pKa * LNP design, assembly	* LNP Zeta Potential, TNS * Encapsulation/Size * Uptake, endosomal release * Translation, toxicity * CryoTEM, SAXS, SANS * LNP molecular model	* IVIS expression/localization * Distribution/trafficking * Immunogenicity/toxicity * Immune profile bias * Innate immune sensors	* Mouse-adapted Covid-19 in wild-type mice * Golden hamster * K18-hACE2 mouse * Ferret

FIGURE 1

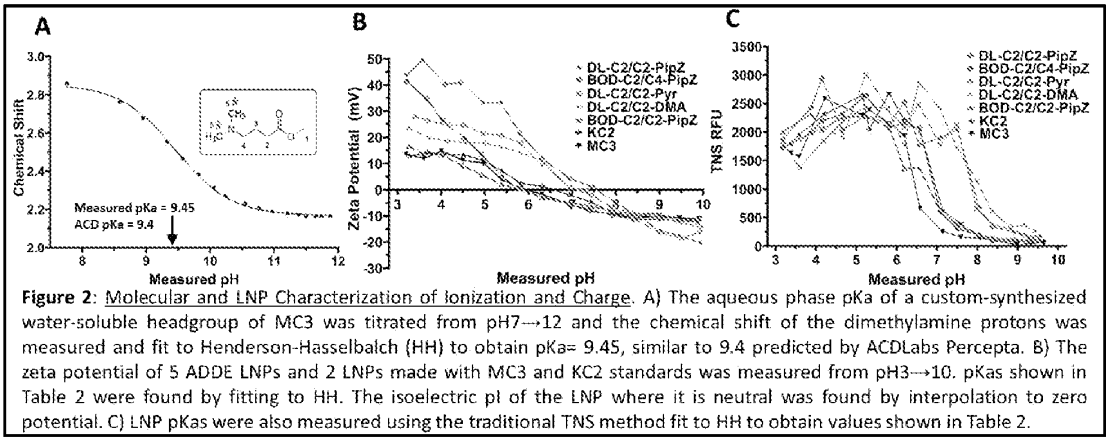


FIGURE 2

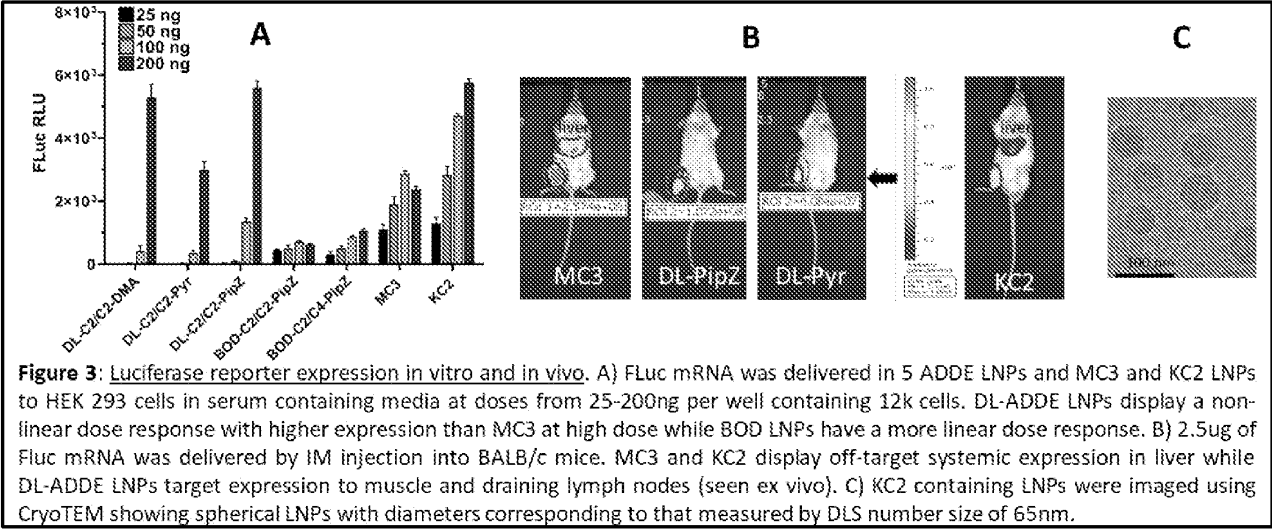


FIGURE 3

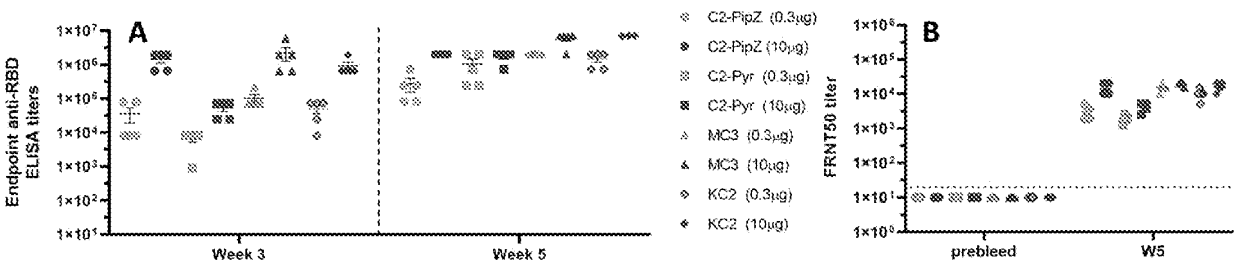


Figure 4 : First generation ADDE ionizable lipids elicits robust binding antibody responses similar to those of MC3 and KC2. BALB/c mice (n=5/group) were immunized at weeks 0 and 3 with 0.3 µg (green) and 10 µg (blue) of S-2P mRNA-encoded immunogen in 4 different LNPs and assessed for A) SARS-CoV-2 S-specific IgG by ELISA and B) neutralizing antibodies against VSVΔG-RFP SARS-CoV-2 pseudovirus.

FIGURE 4

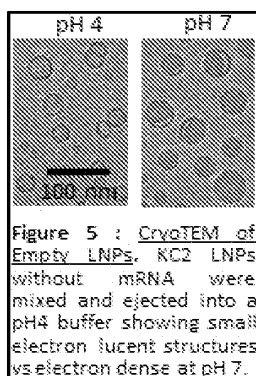


FIGURE 5

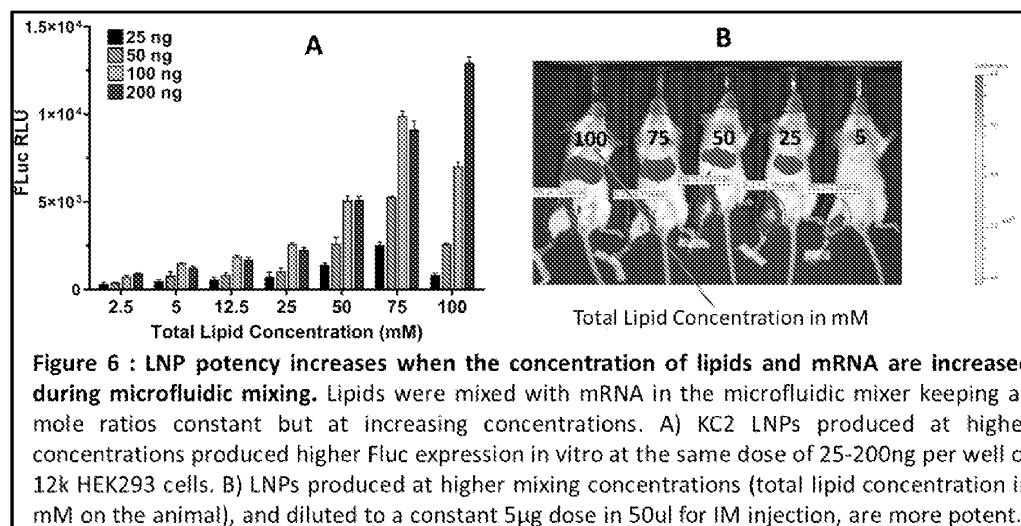


FIGURE 6

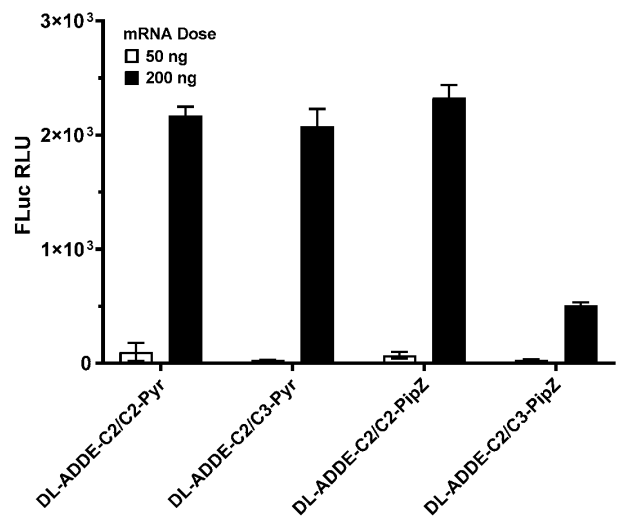


FIGURE 7

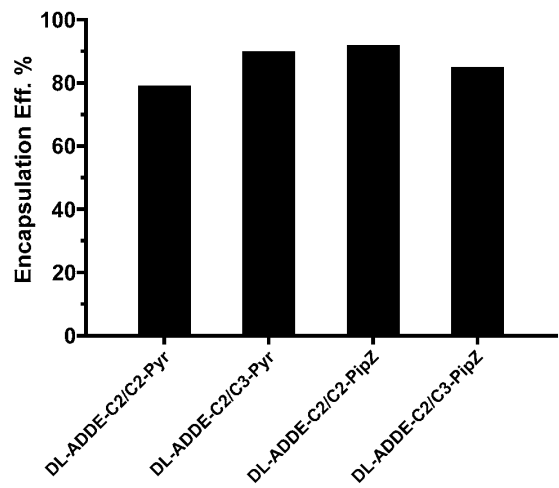


FIGURE 8

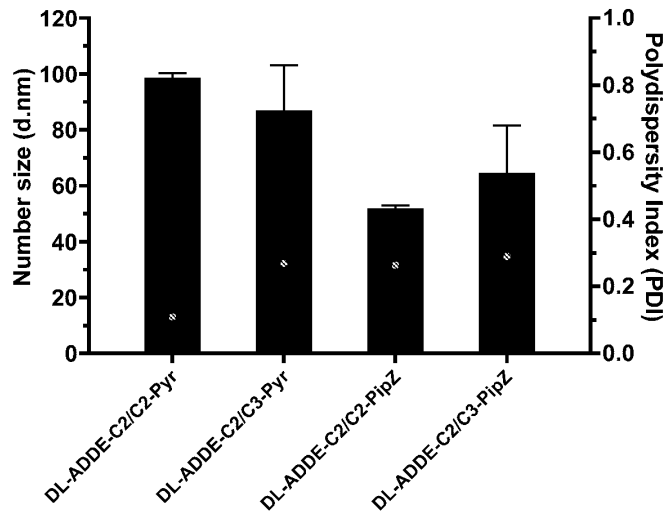


FIGURE 9

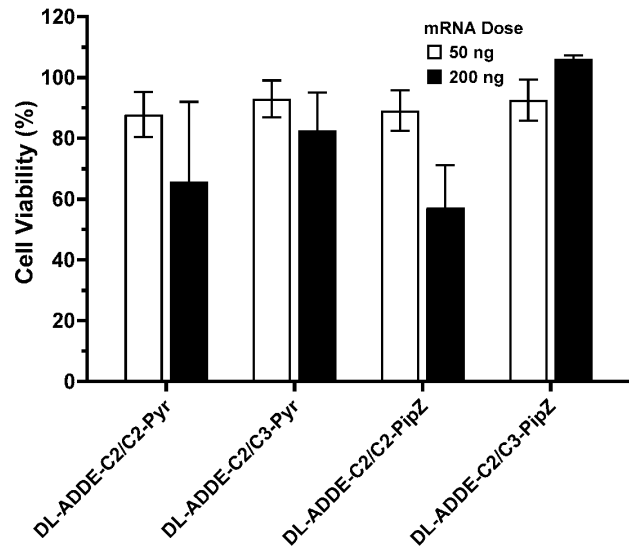


FIGURE 10

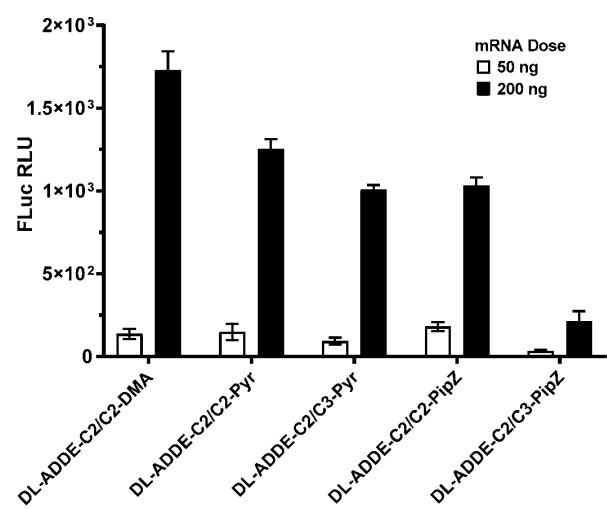


FIGURE 11

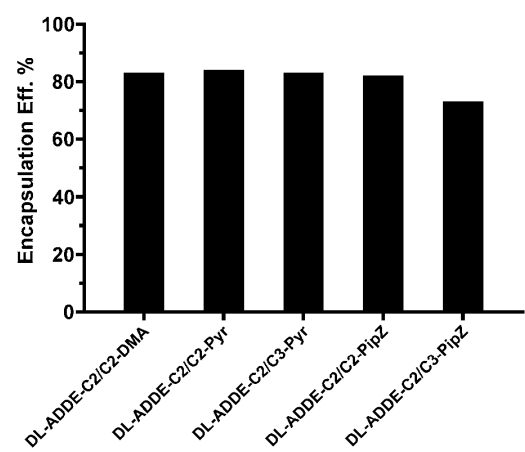


FIGURE 12

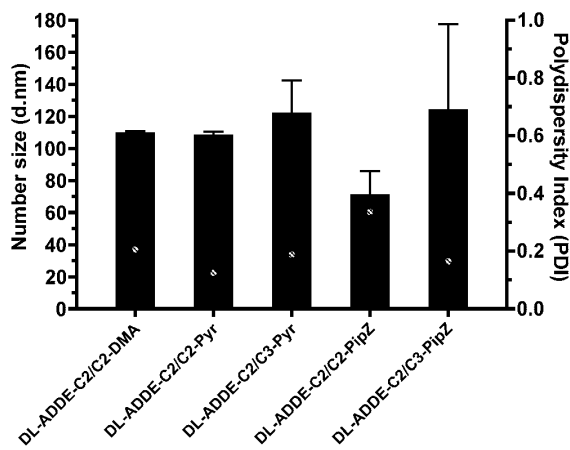


FIGURE 13

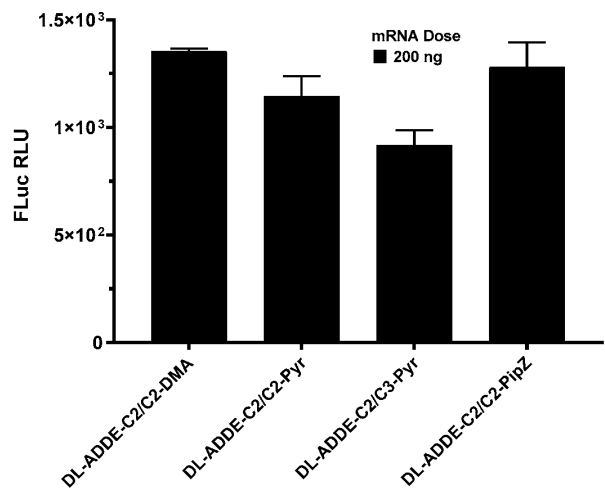


FIGURE 14

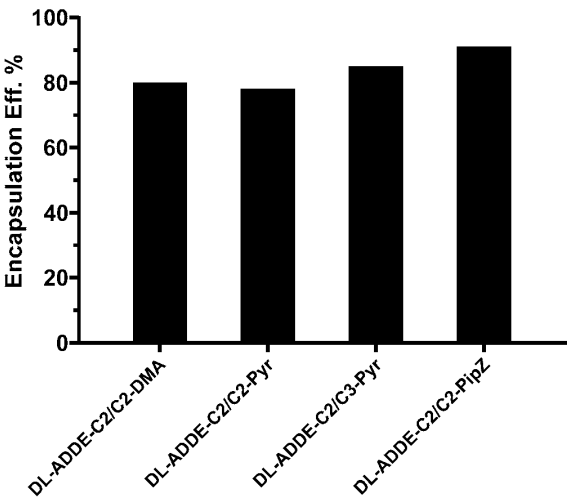


FIGURE 15

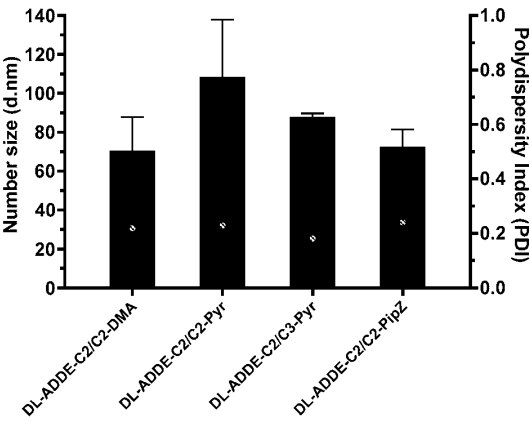


FIGURE 16

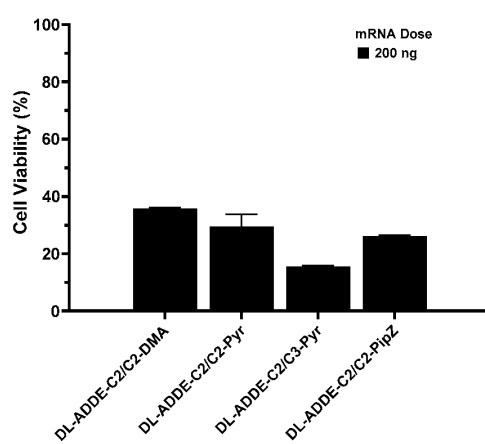


FIGURE 17

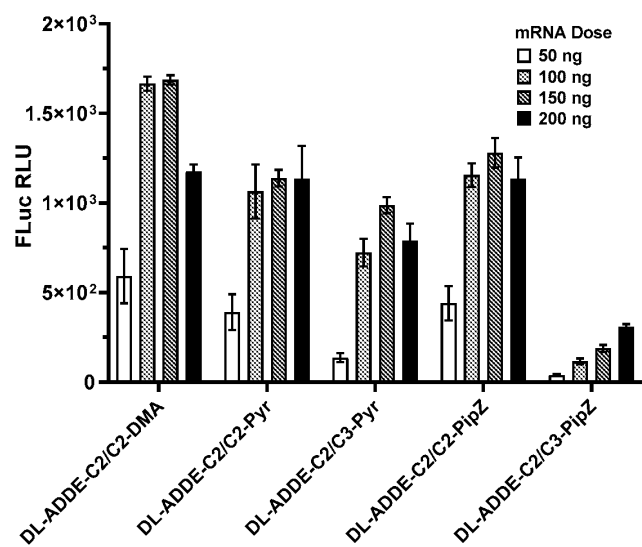


FIGURE 18

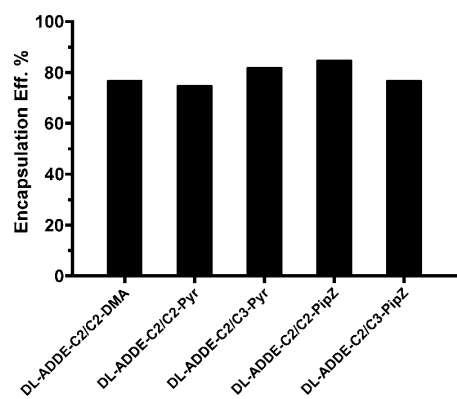


FIGURE 19

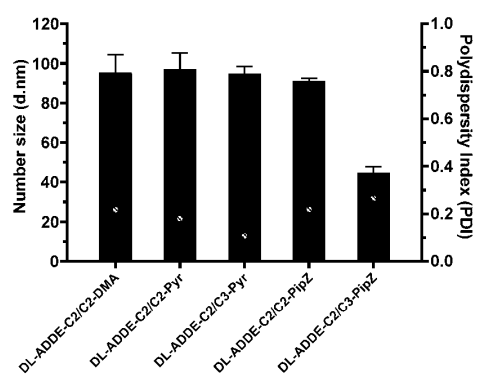


FIGURE 20

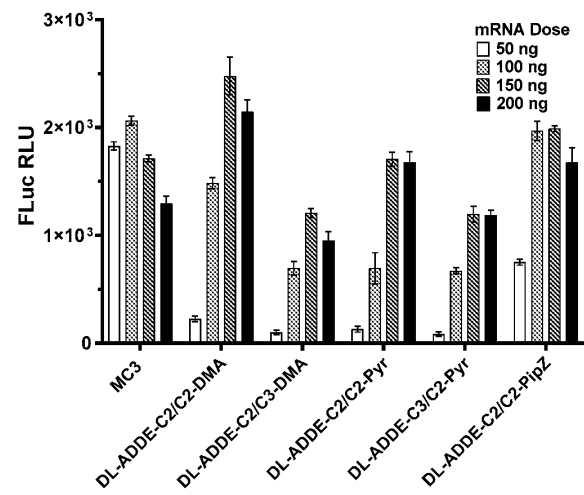


FIGURE 21

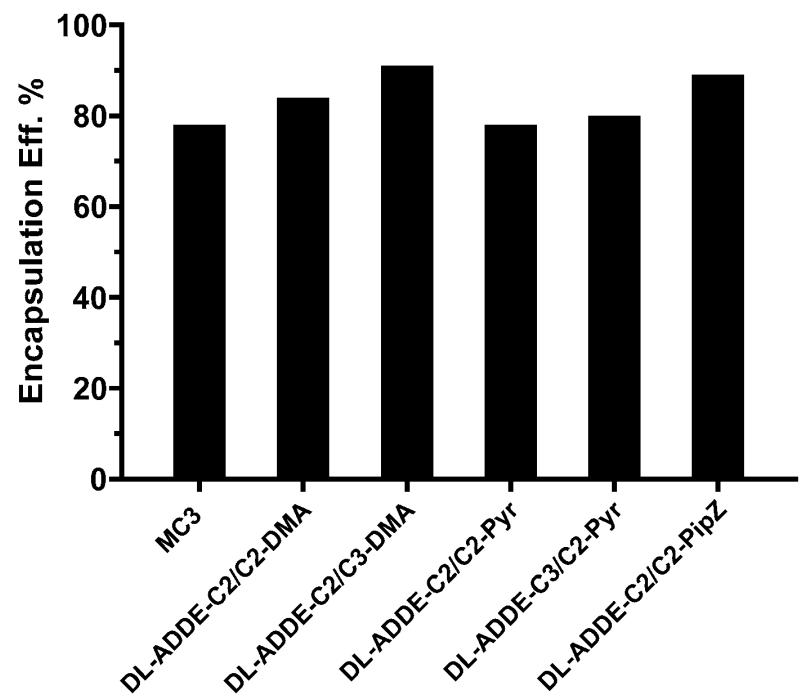


FIGURE 22

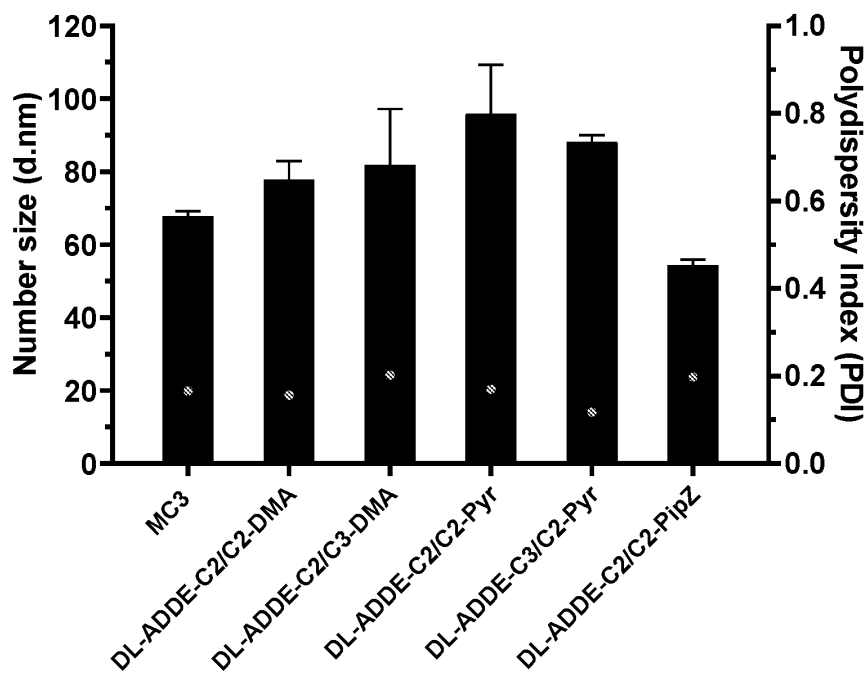


FIGURE 23

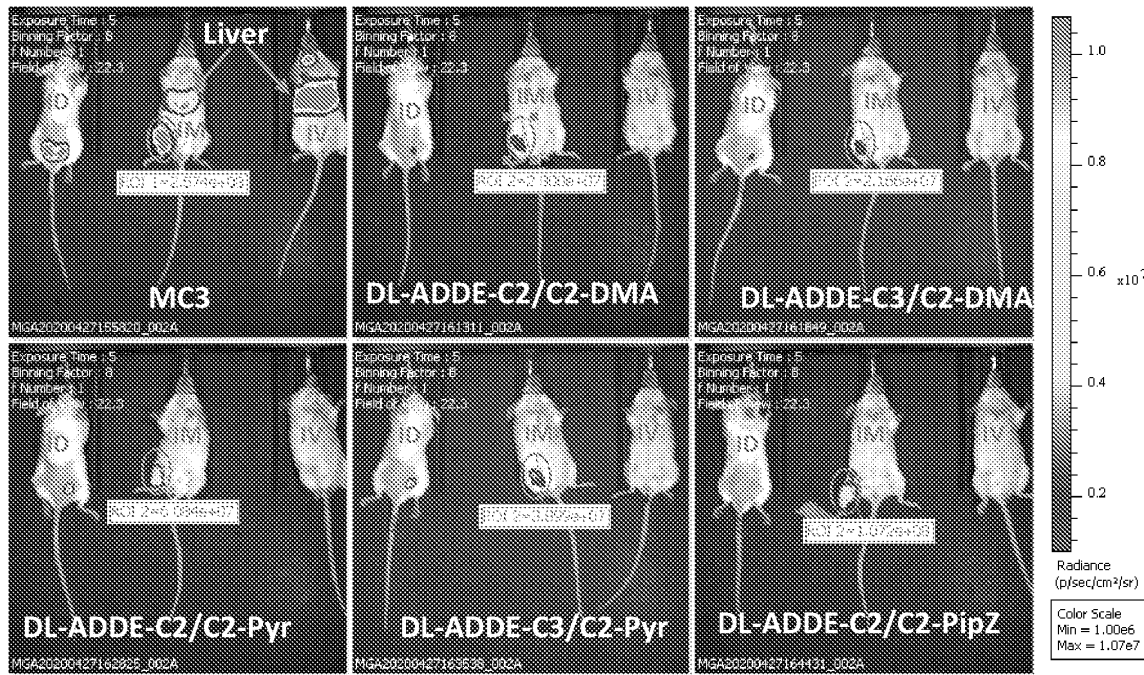


FIGURE 24

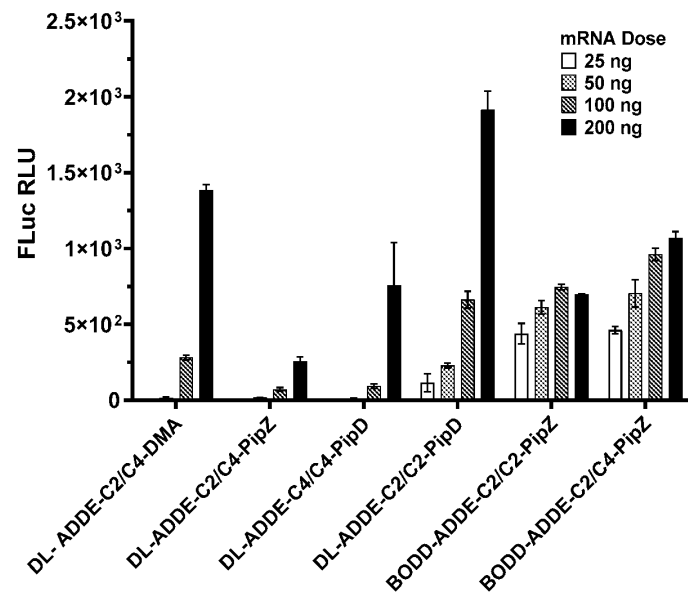


FIGURE 25

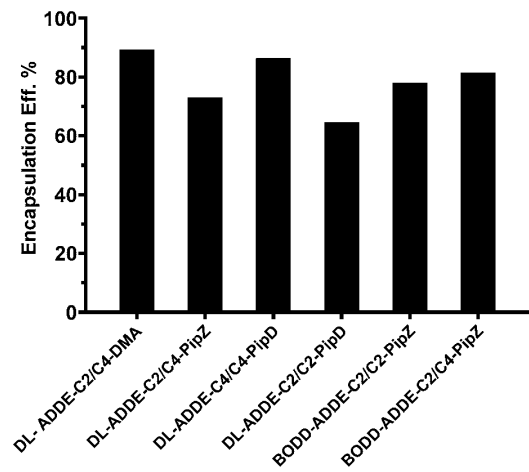


FIGURE 26

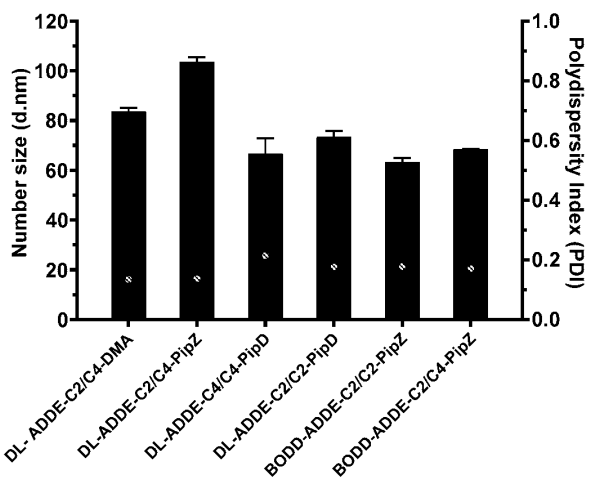


FIGURE 27

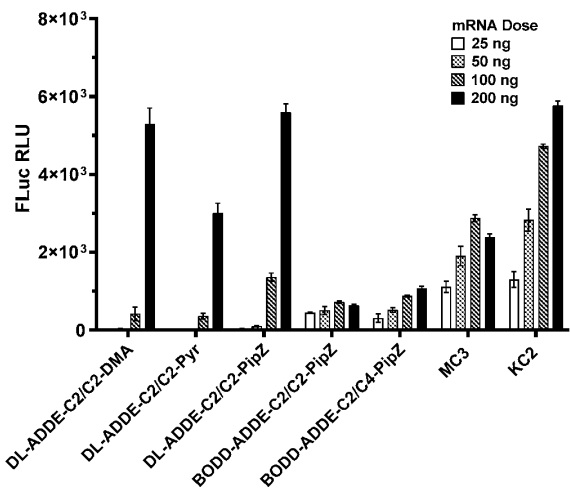


FIGURE 28

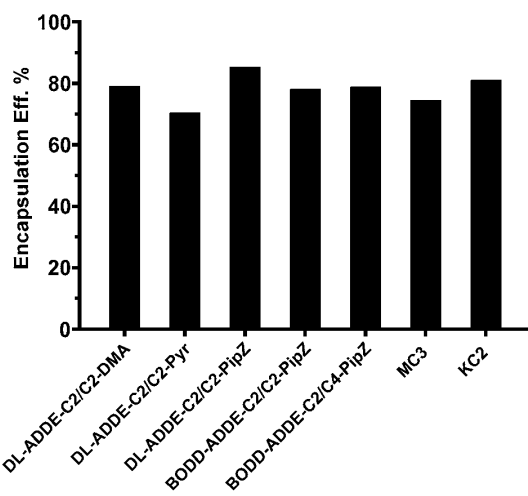


FIGURE 29

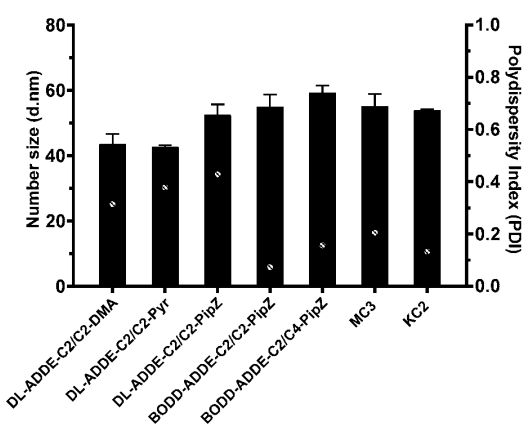


FIGURE 30

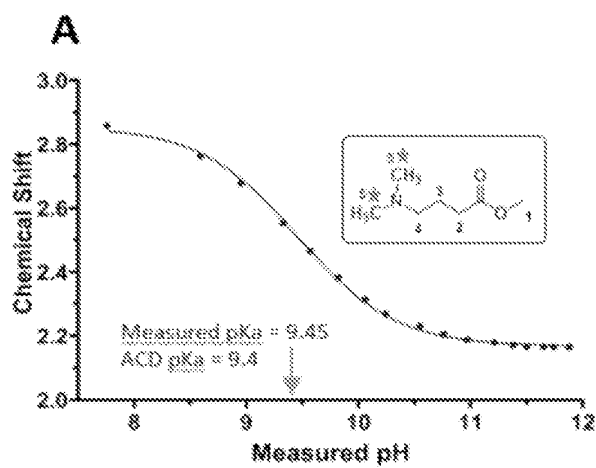


FIGURE 31A

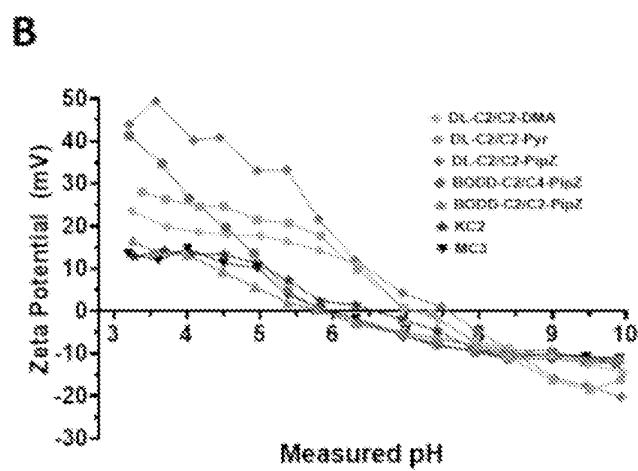


FIGURE 31B

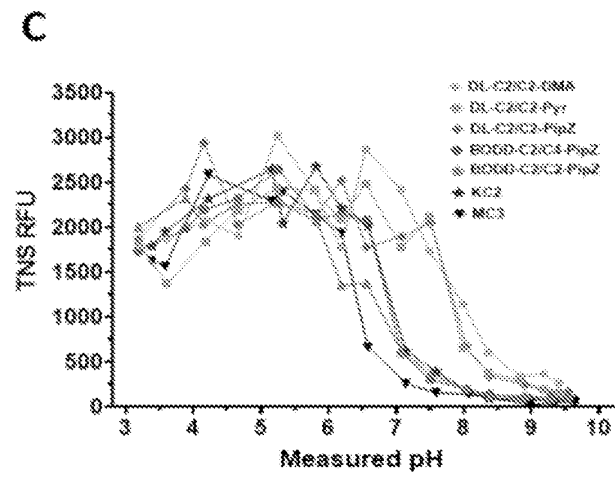


FIGURE 31C

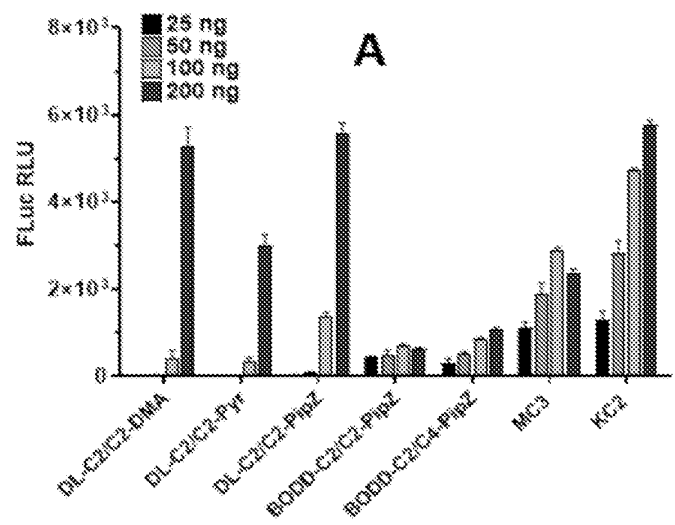


FIGURE 32A

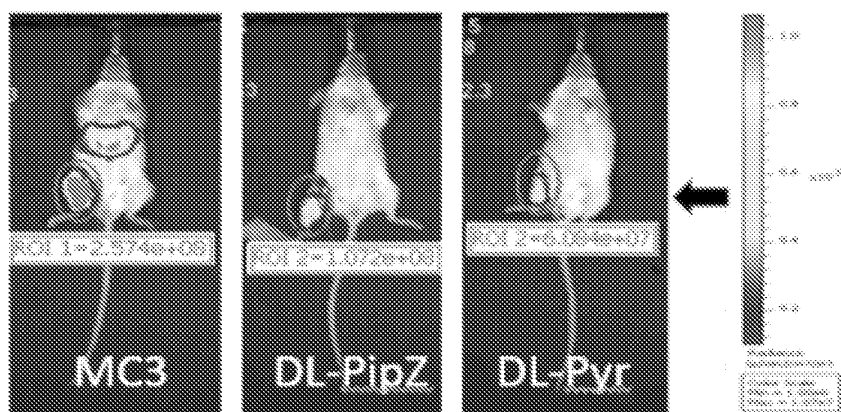
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FIGURE 32B

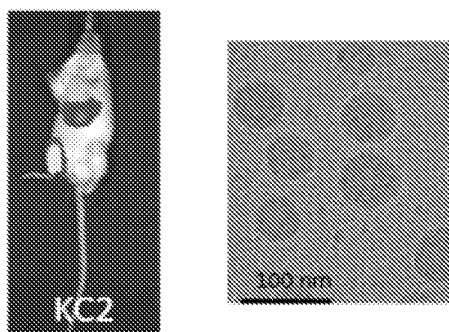
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FIGURE 32C

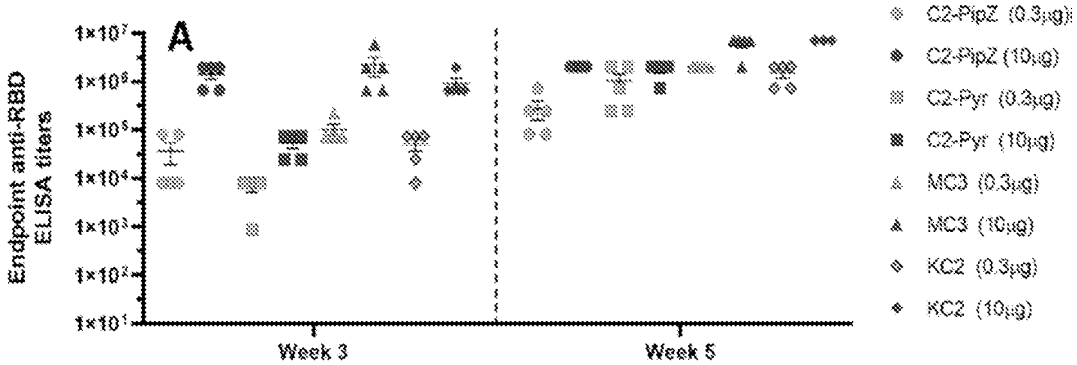


FIGURE 33A

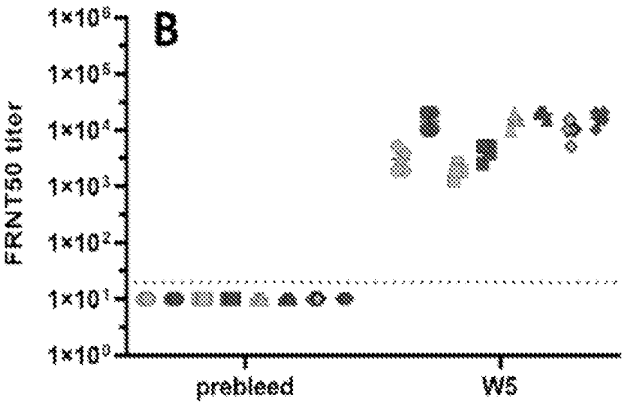


FIGURE 33A

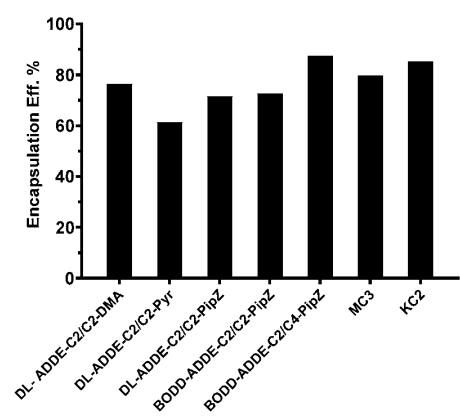


FIGURE 34

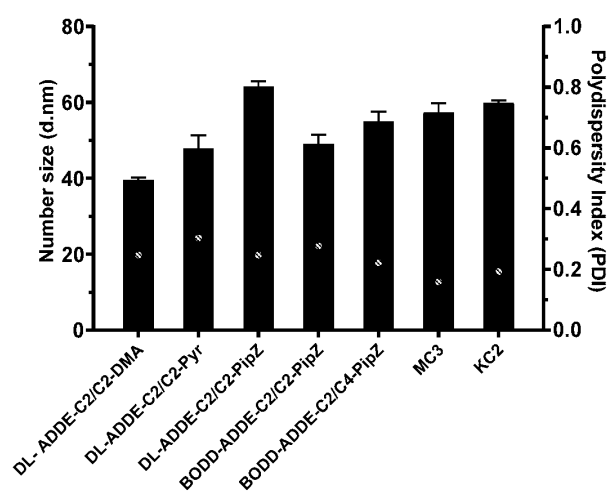


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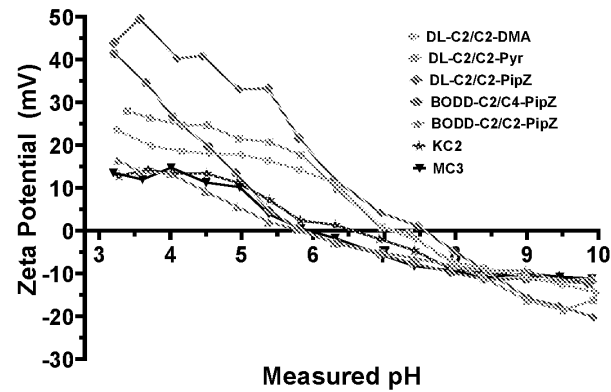


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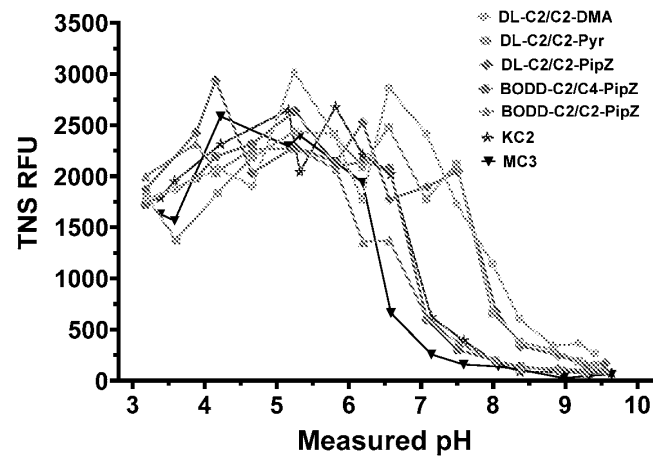


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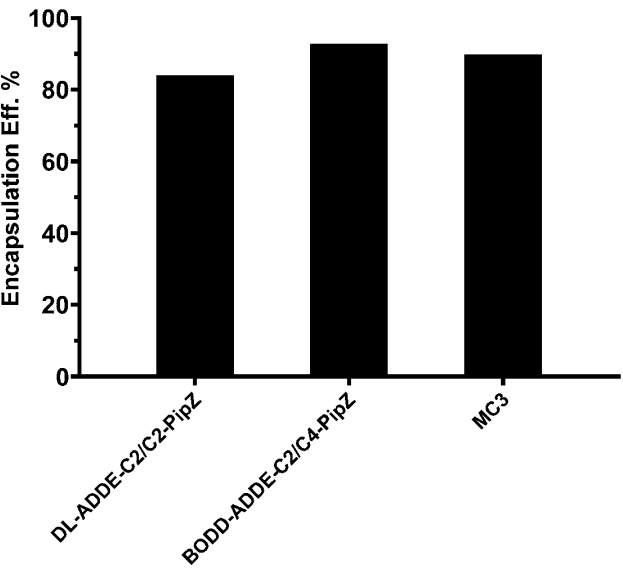


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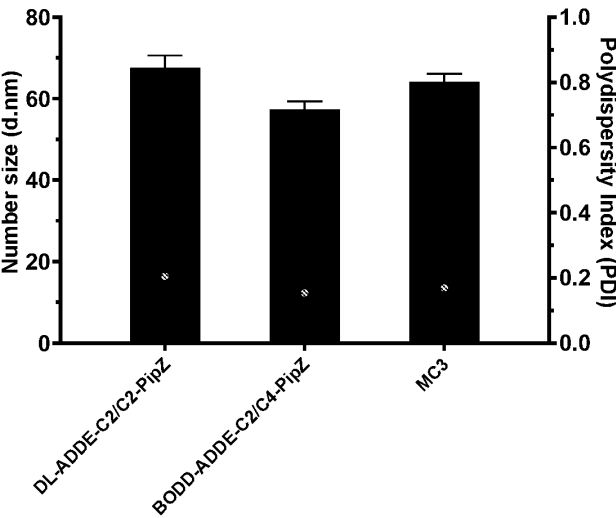
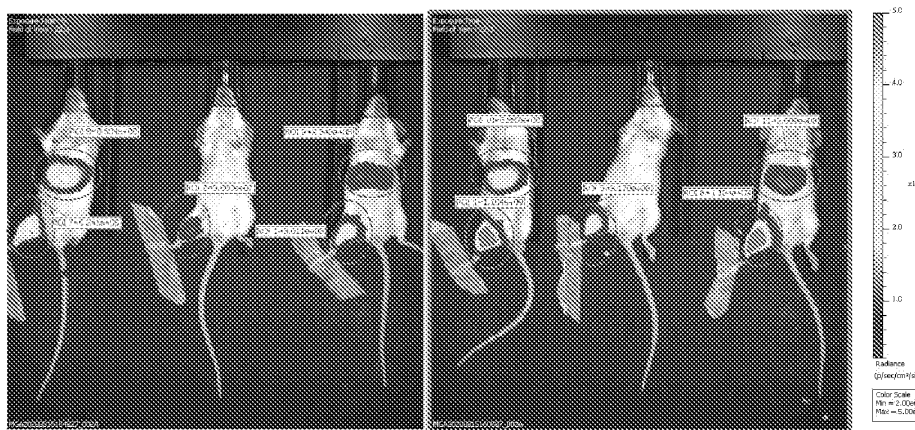


FIGURE 39

4 hour in-vivo imaging. IVIS

I.M. injections (left group 1, right group 2). From left to right (MC3 / DL-ADDE-



C2C2-4Me-PipZ / BODD-ADDE-C2C4-4Me-PipZ)

FIGURE 40A

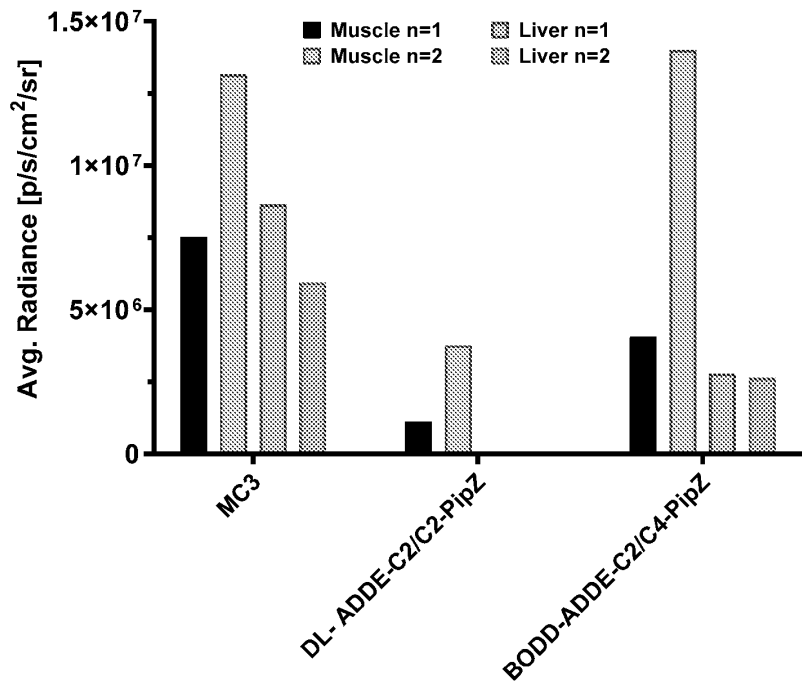


FIGURE 40B

24 hour in-vivo imaging
I.M. injections (left group 1, right group 2). From left to right (MC3 / DL-ADDE-C2C2-4Me-PipZ / BODD-ADDE-C2C4-4Me-PipZ)

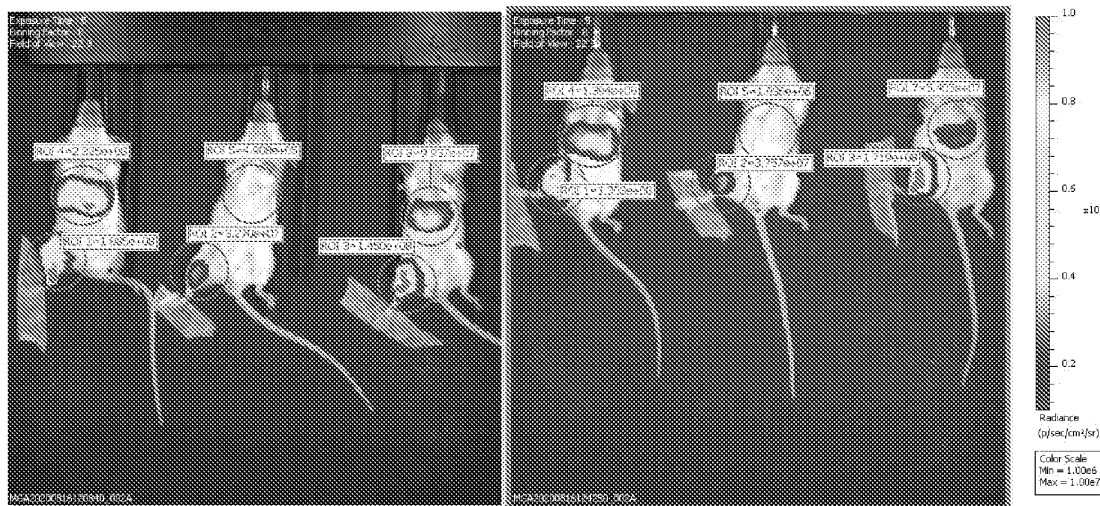


FIGURE 40C

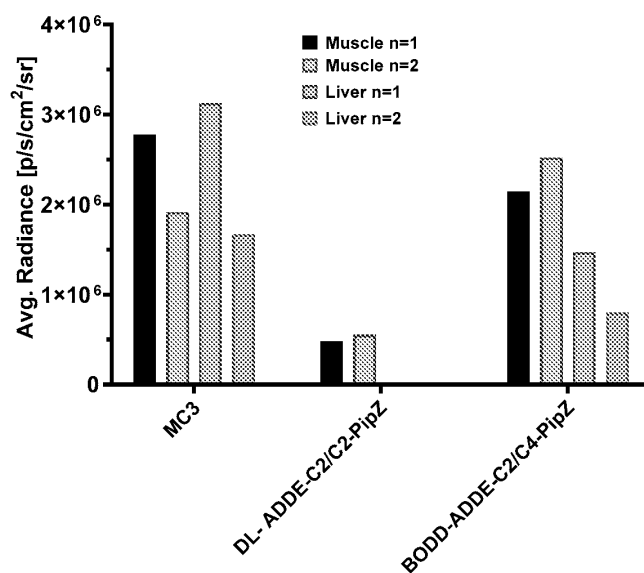


FIGURE 40D

24 hour ex-vivo imaging

I.M. injections (group 1). Samples top left to right (MC3 / BODD-ADDE-C2C4-4Me-PipZ), bottom left (DL-ADDE-C2C2-4Me-PipZ). Tissues/sample (top left = Inguinal lymph node, top right = Popliteal lymph node, middle left = spleen, middle right = liver, bottom left = injected muscle, bottom right = control muscle). MC3 has both muscles above lymph nodes.

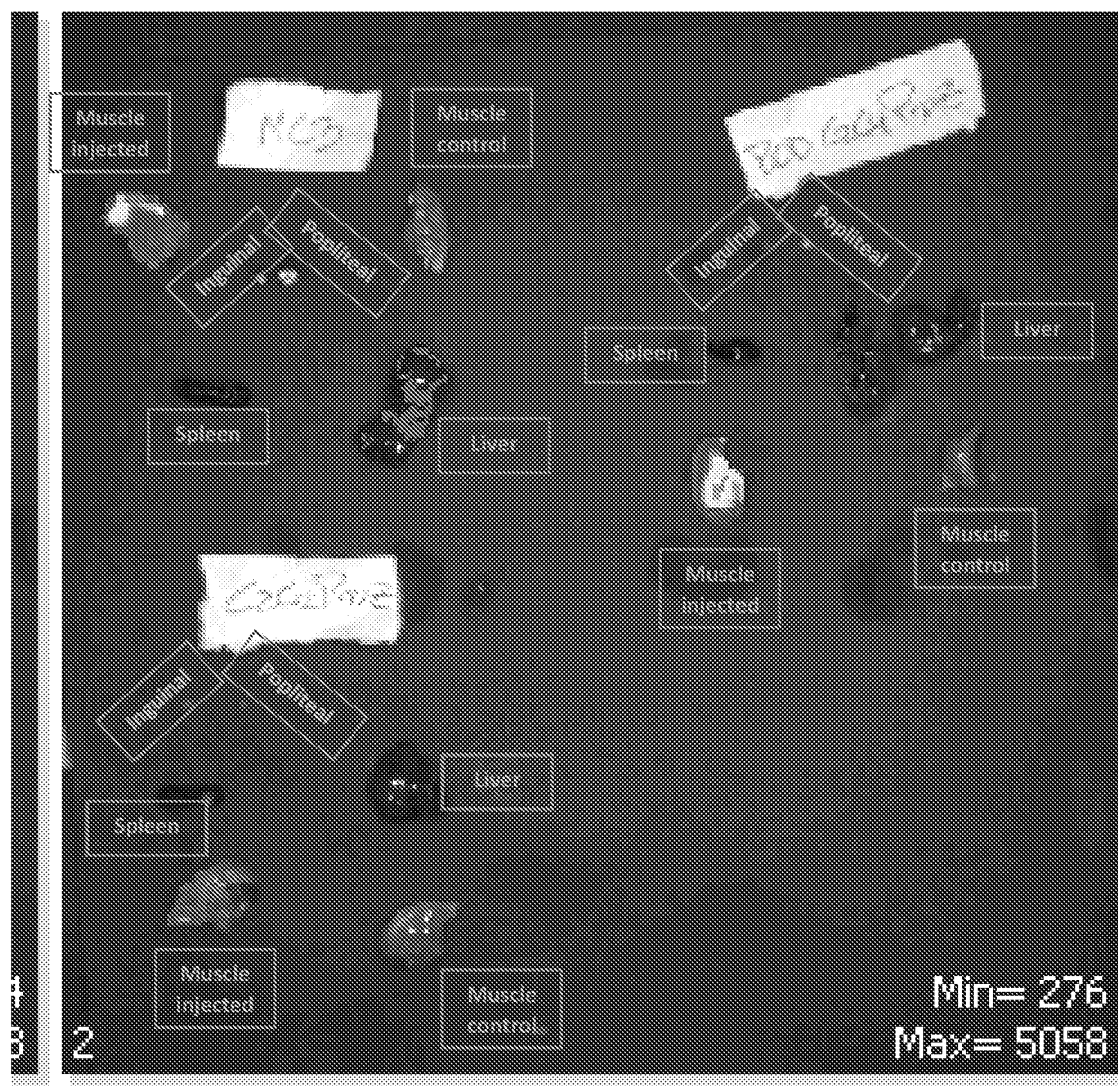
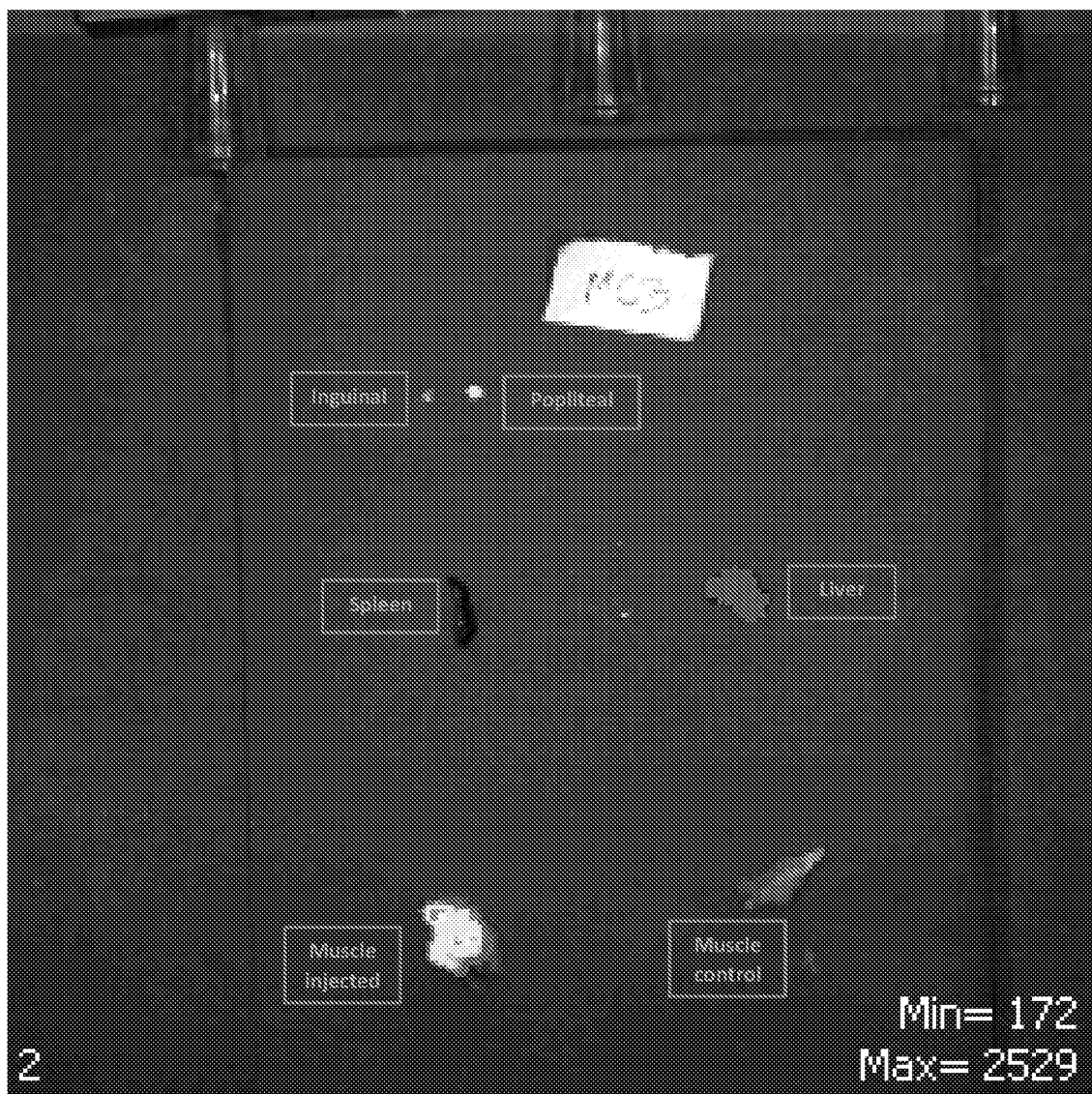
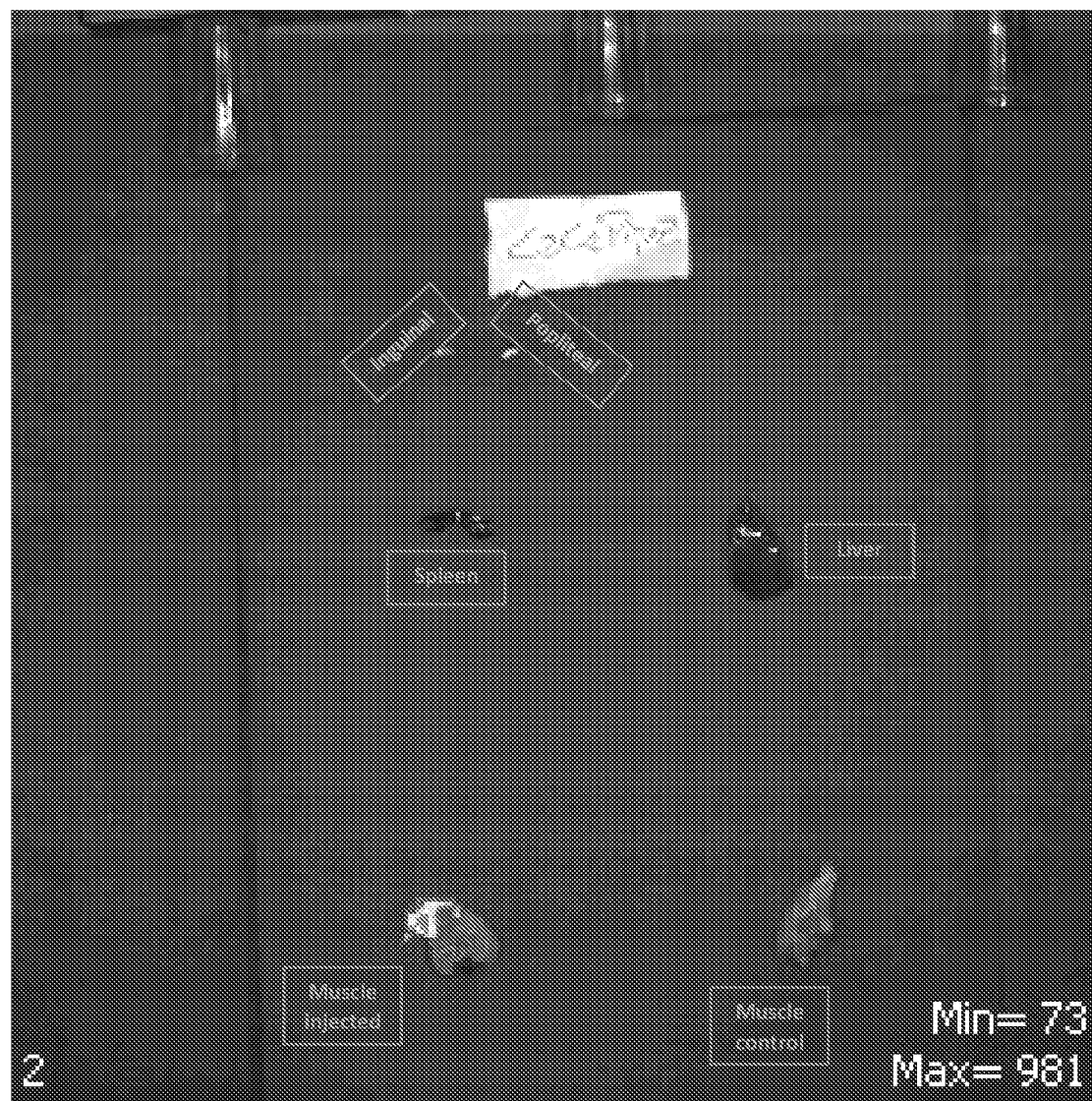


FIGURE 40E

I.M. injections (MC3 from group 2). Sample = MC3. Tissues/sample (top left = Inguinal lymph node, top right = Popliteal lymph node, middle left = spleen, middle right = liver, bottom left = injected muscle, bottom right = control muscle).

**FIGURE 40F**

I.M. injections (DL-ADDE-C2C2-4Me-PipZ from group 2). Sample = DL-ADDE-C2C2-4Me-PipZ. Tissues/sample (top left = Inguinal lymph node, top right = Popliteal lymph node, middle left = spleen, middle right = liver, bottom left = injected muscle, bottom right = control muscle).

**FIGURE 40G**

I.M. injections (BODD-ADDE-C2C4-4Me-PipZ from group 2). Sample = BODD-ADDE-C2C4-4Me-PipZ. Tissues/sample (top left = Inguinal lymph node, top right = Popliteal lymph node, middle left = spleen, middle right = liver, bottom left = injected muscle, bottom right = control muscle).



FIGURE 40H

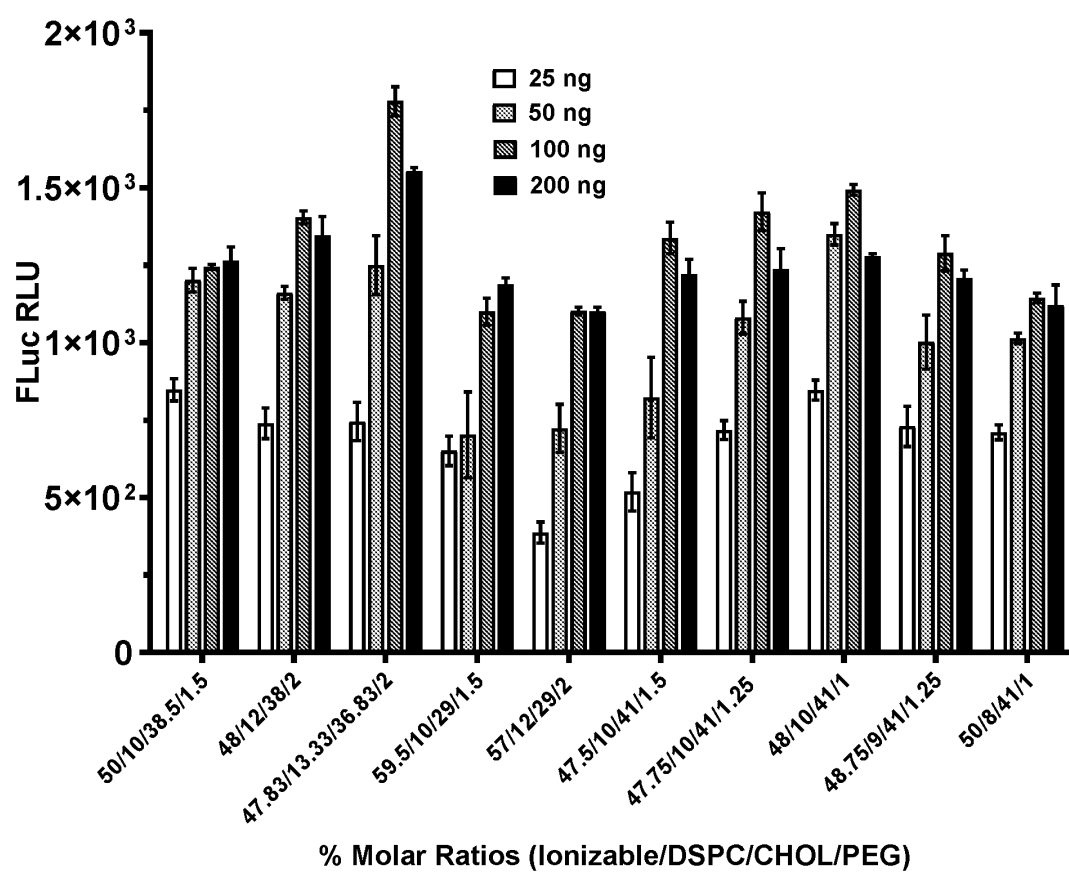


FIGURE 41

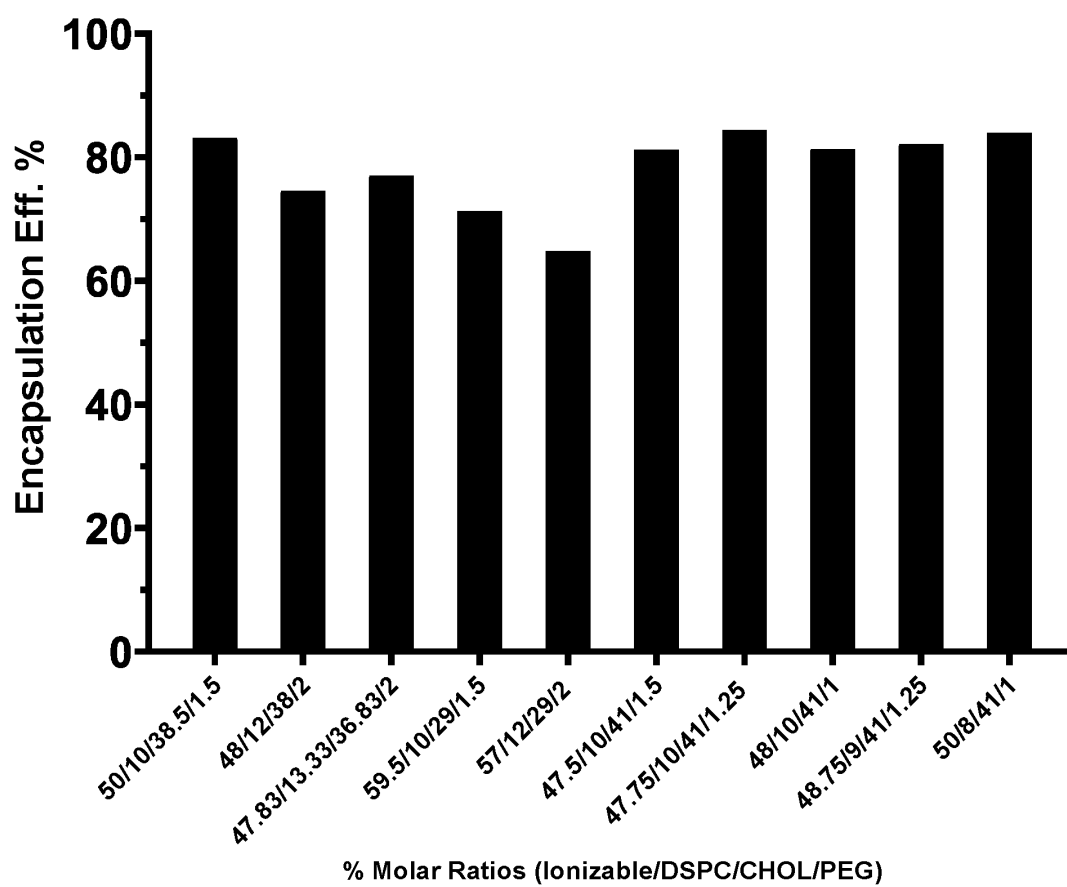


FIGURE 42

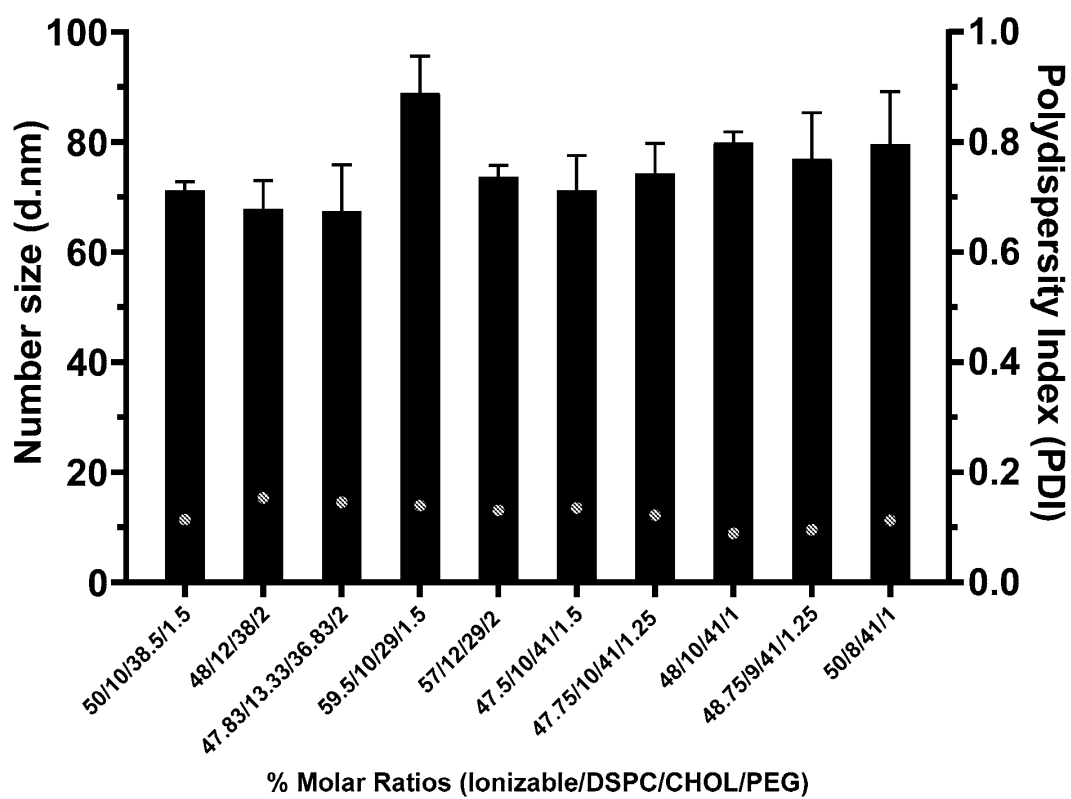


FIGURE 43

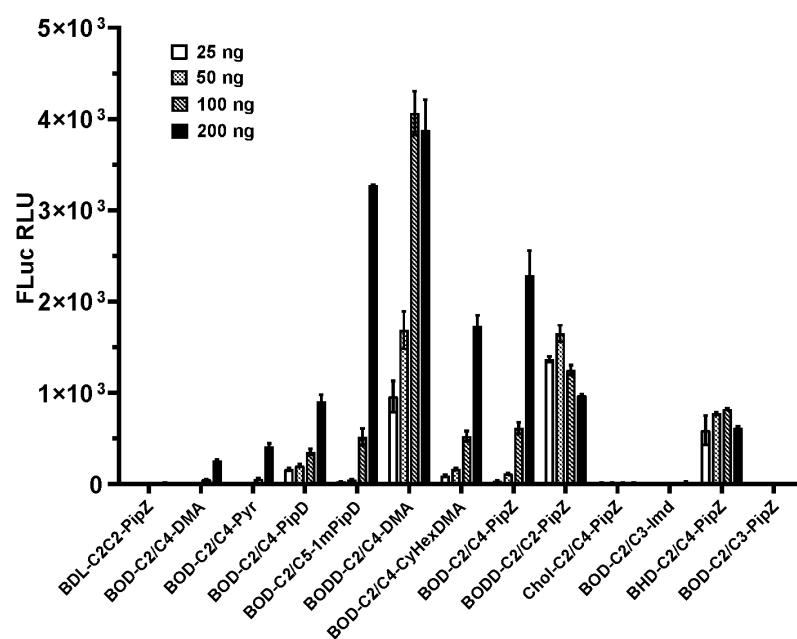


FIGURE 44

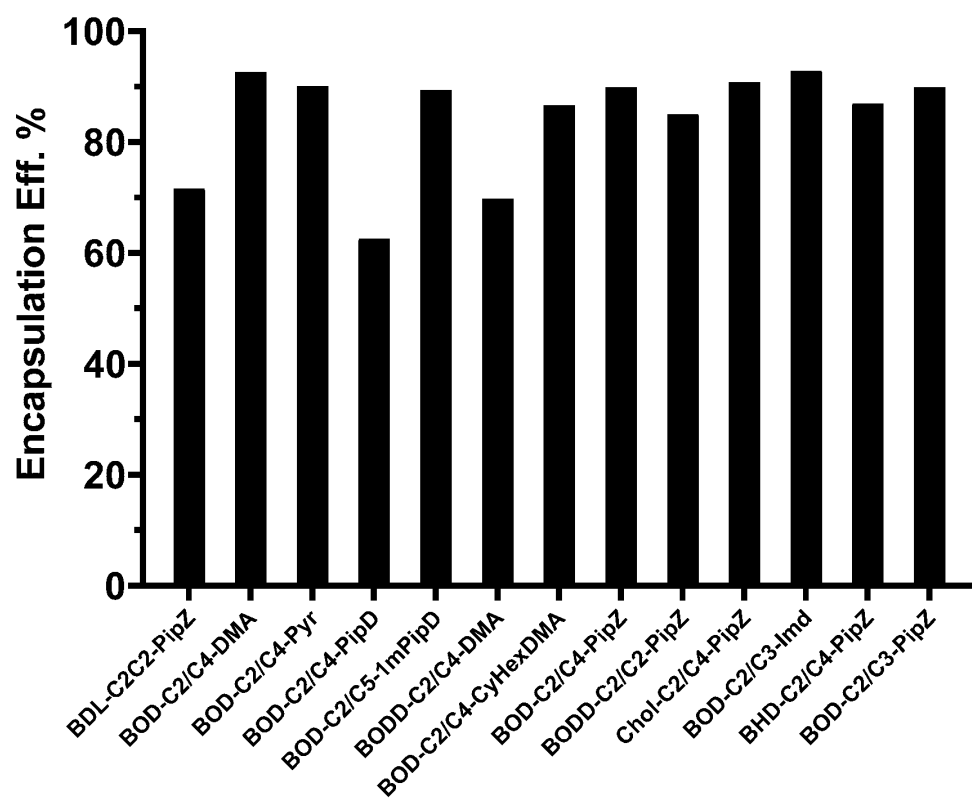


FIGURE 45

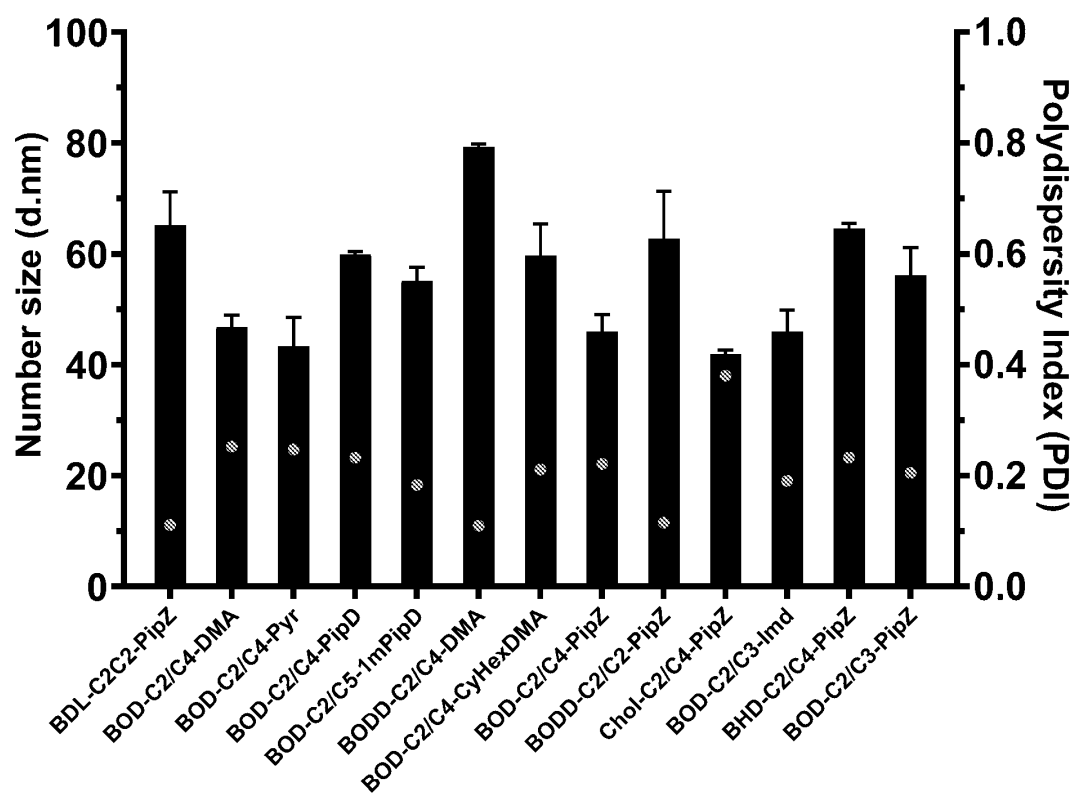
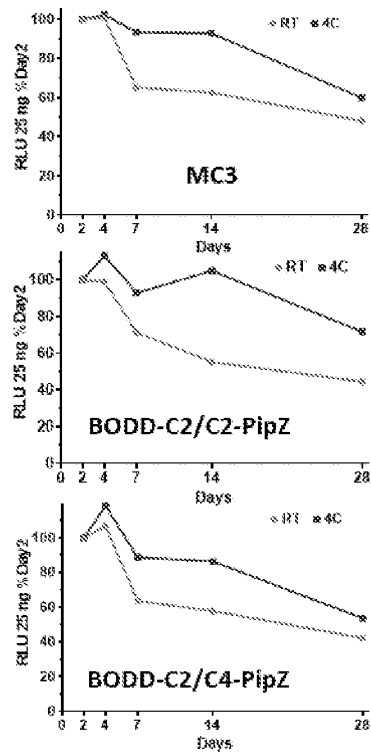


FIGURE 46

Relatively stable over 1 month *in PBS*



Unstable over 14 days *in PBS*

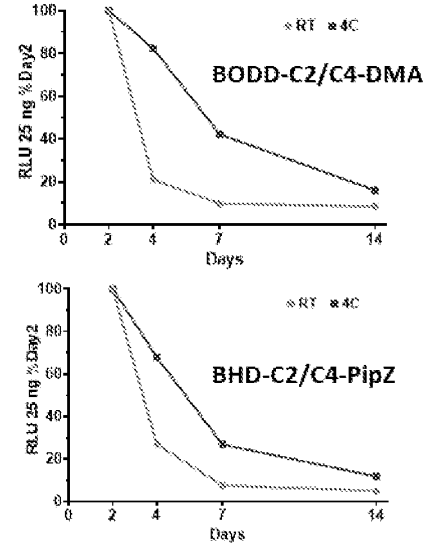
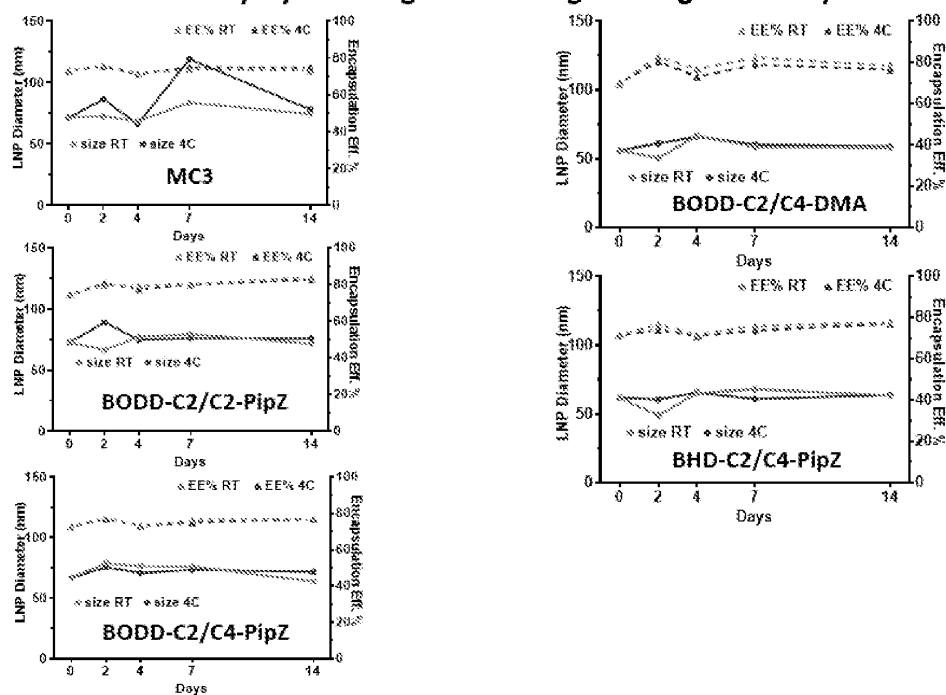


FIGURE 47

Size measurements by Dynamic Light Scattering & Ribogreen Assay to measure encapsulated mRNA**FIGURE 48**

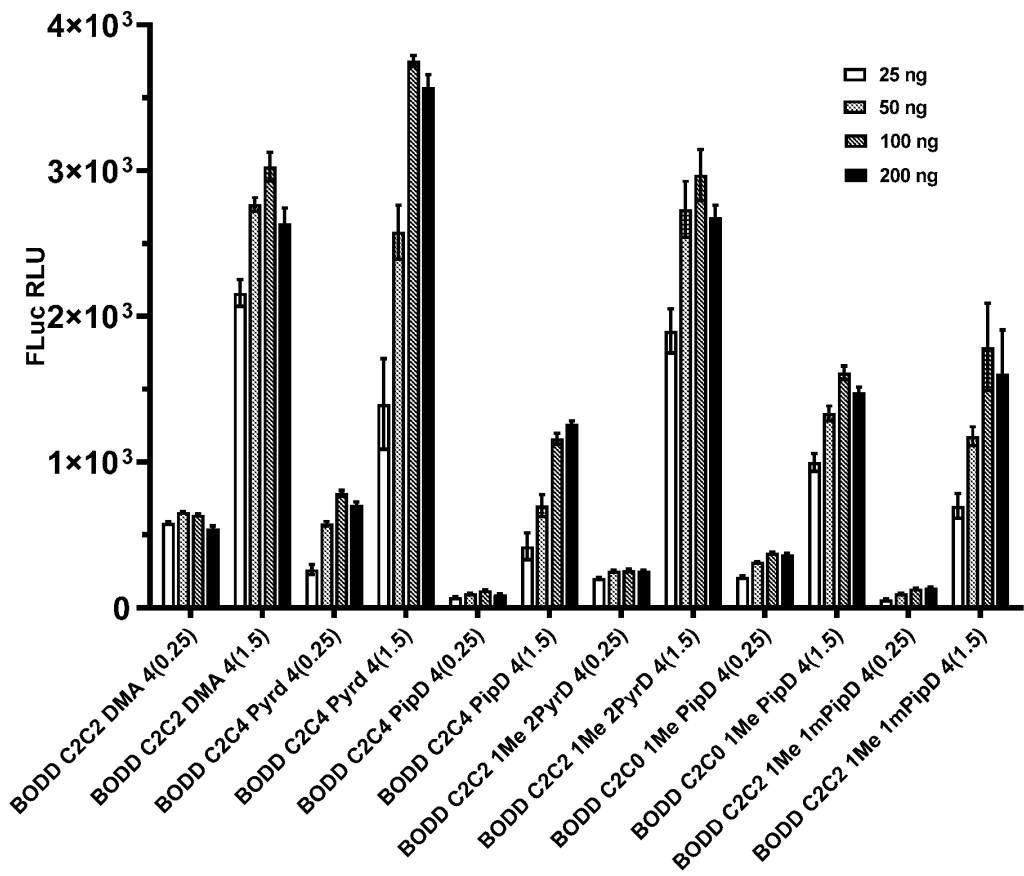


FIGURE 49

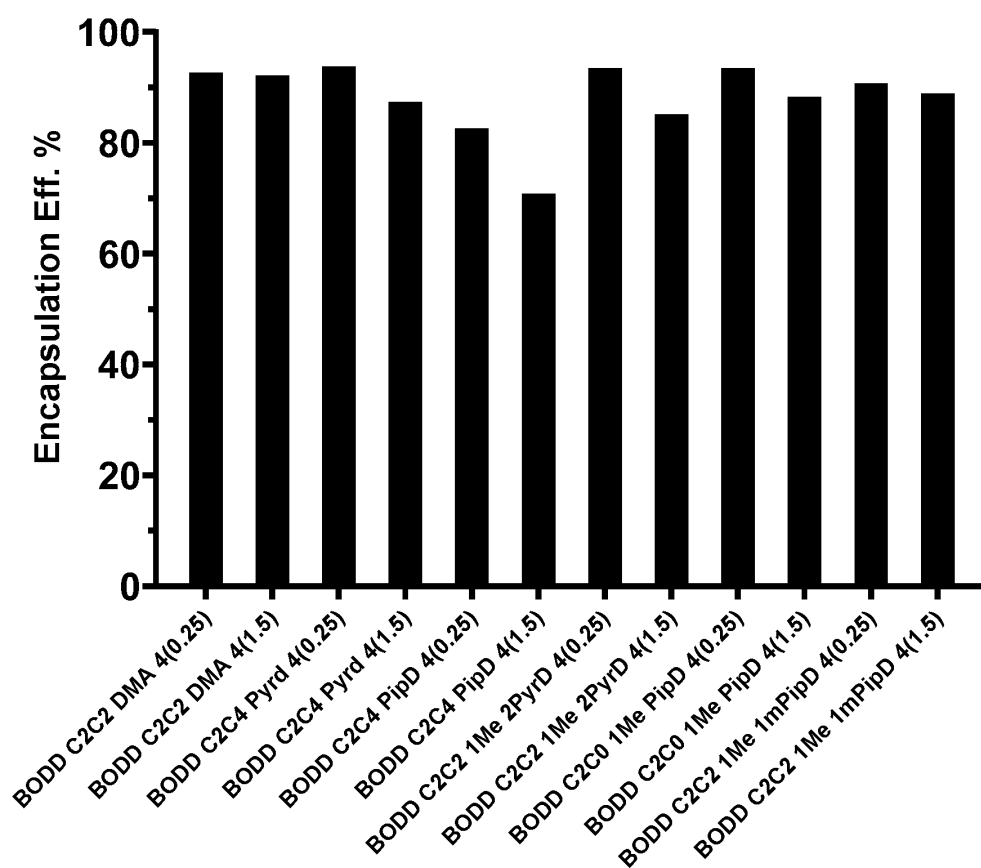


FIGURE 50

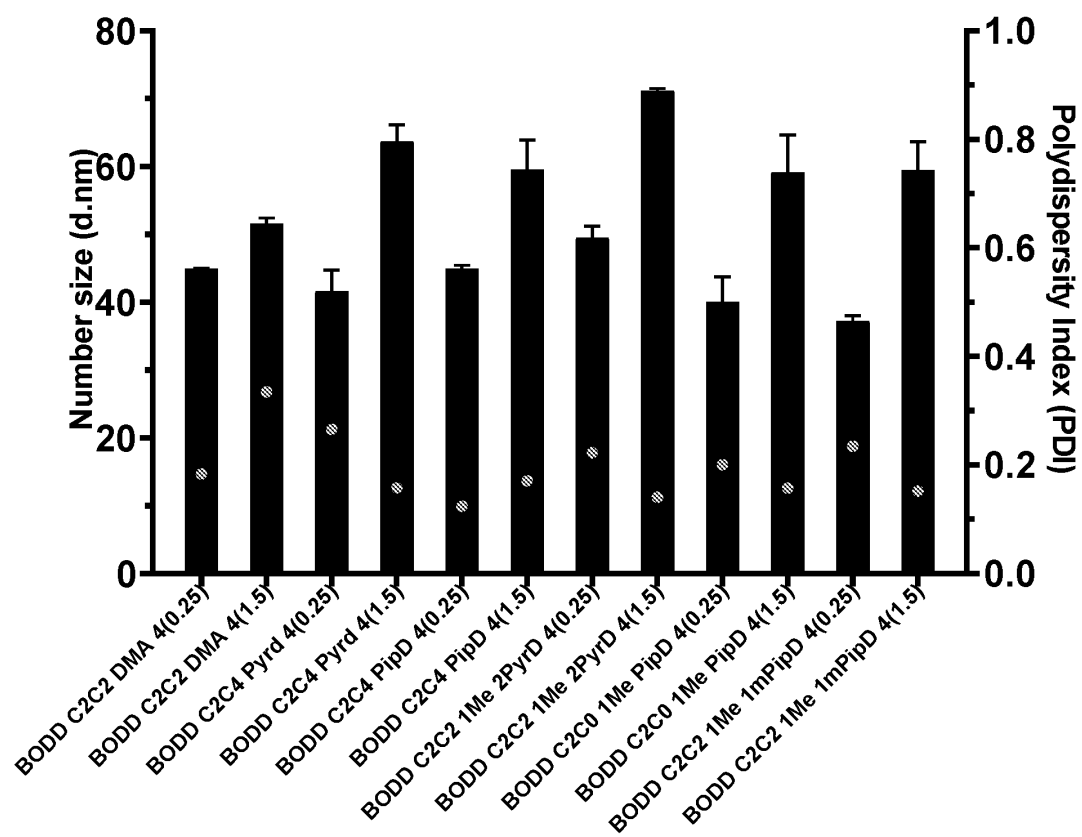


FIGURE 51

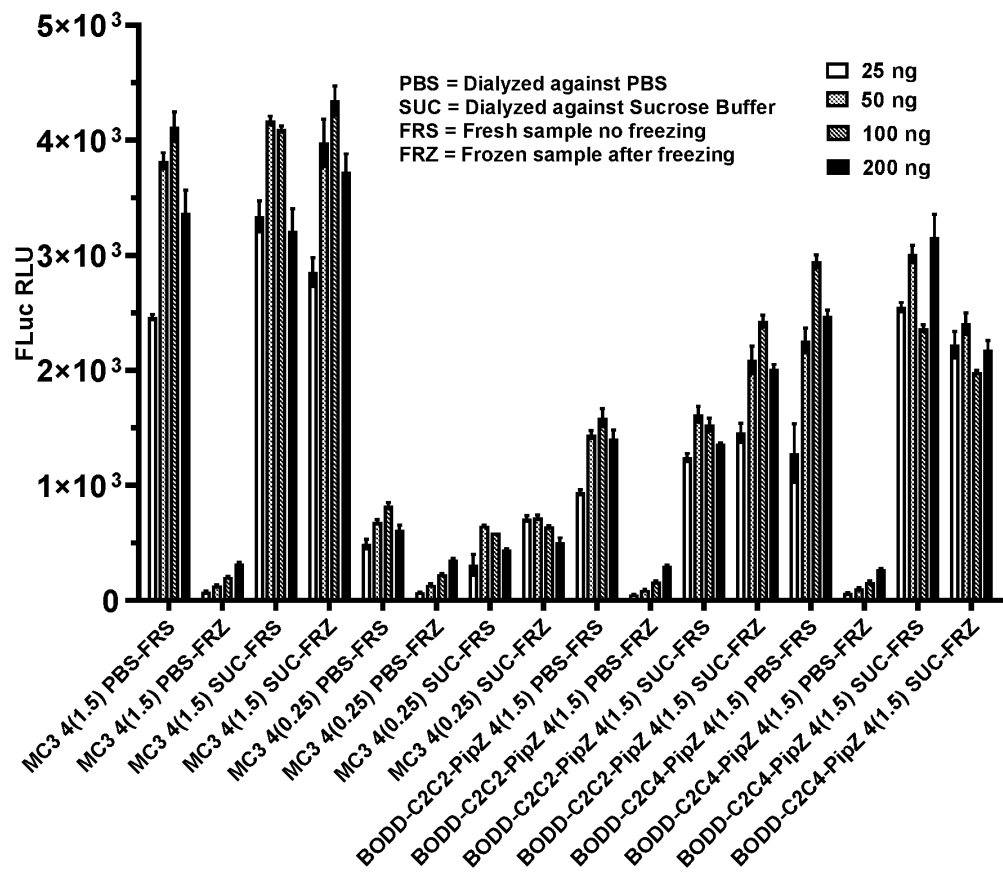


FIGURE 52

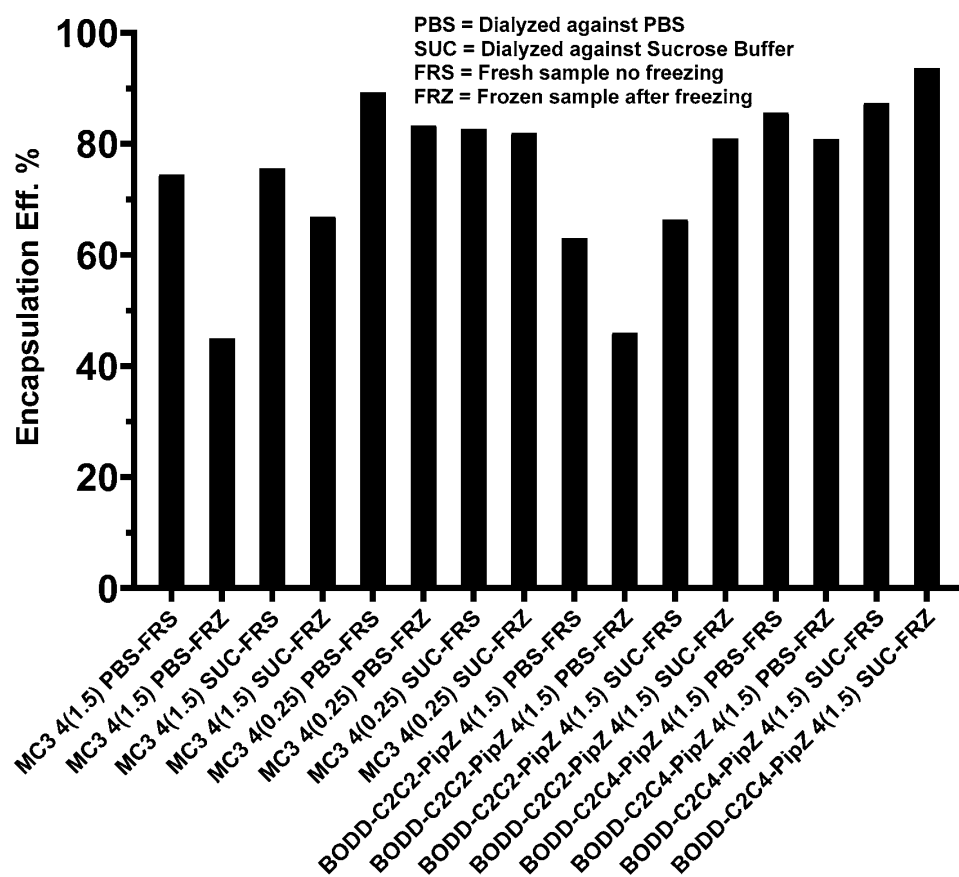


FIGURE 53

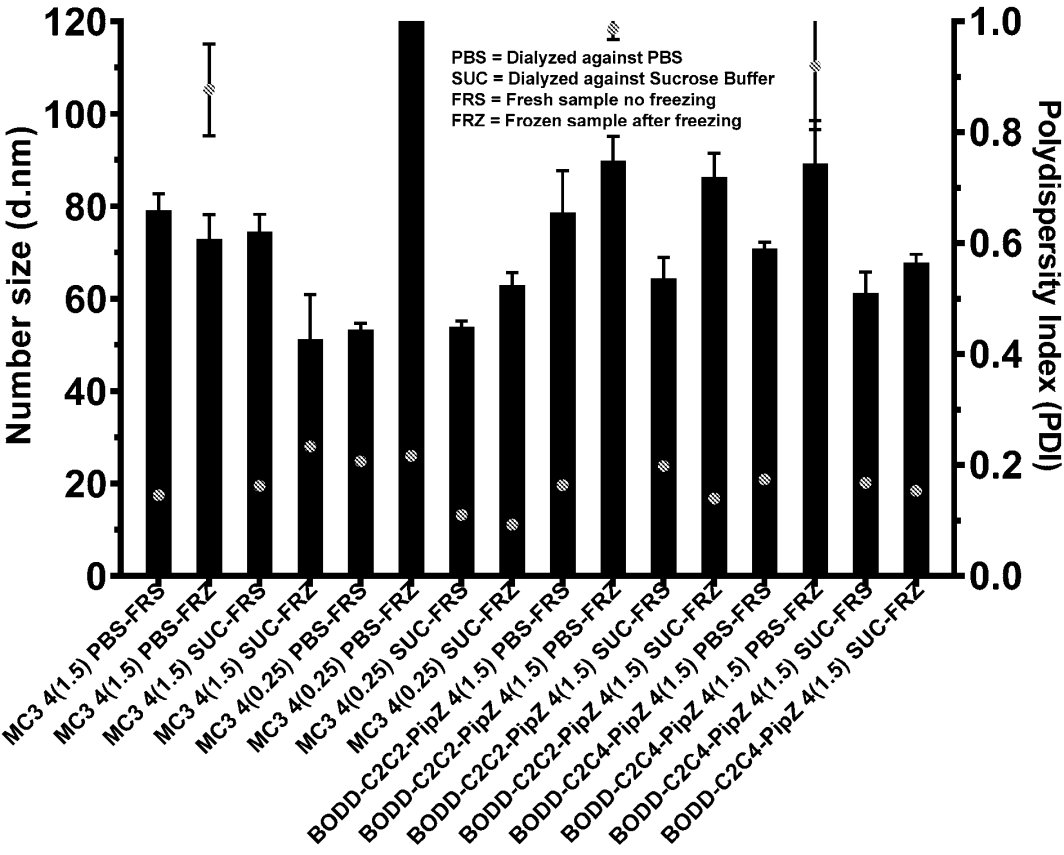


FIGURE 54

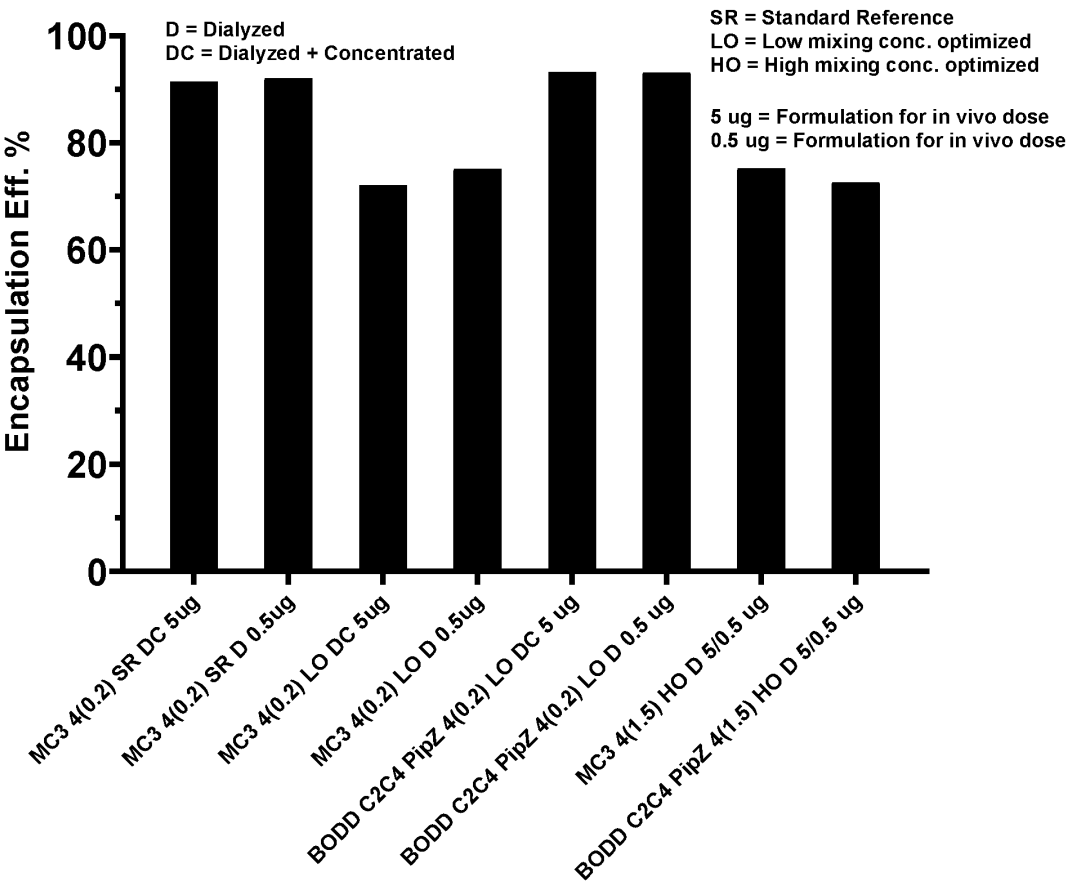


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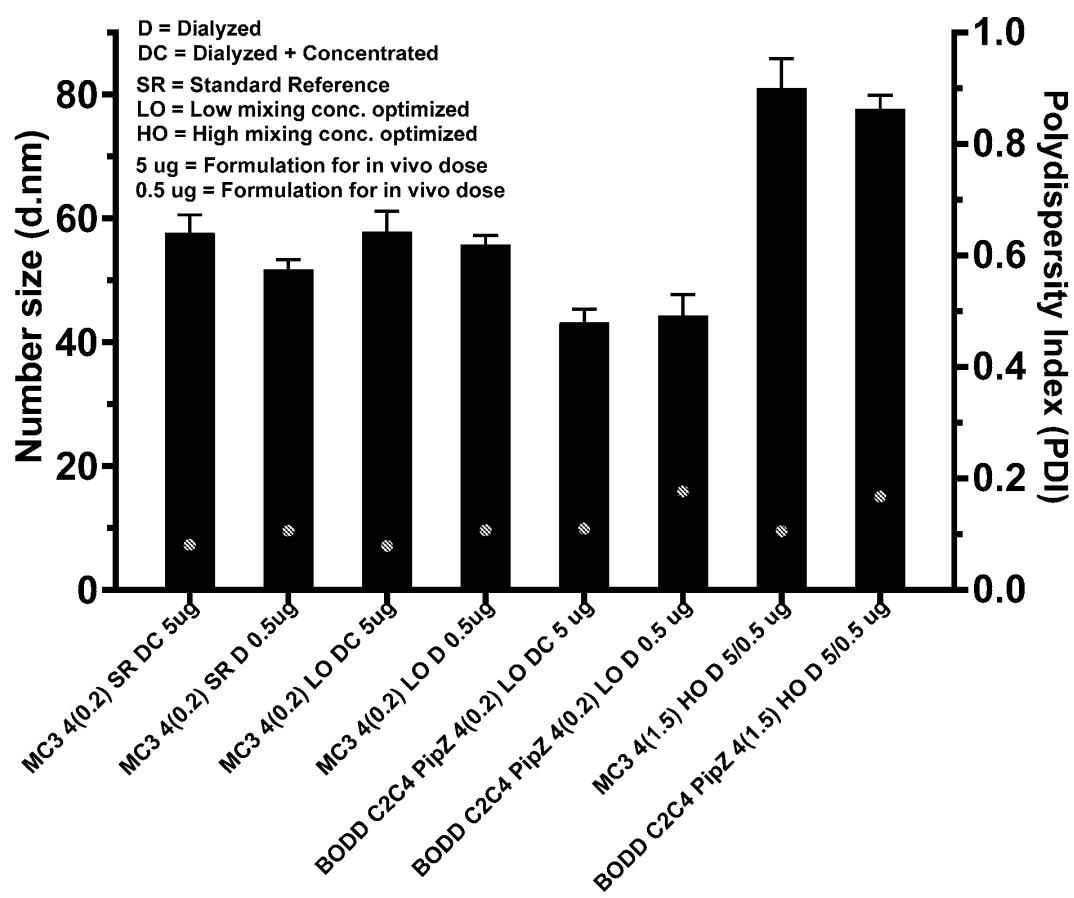


FIGURE 56

1. Group 1 = 5 ug dose (4, 24 hours). Left to Right = MC3-SR/MC3-LO/MC3-HO/BODD-LO/BODD-HO

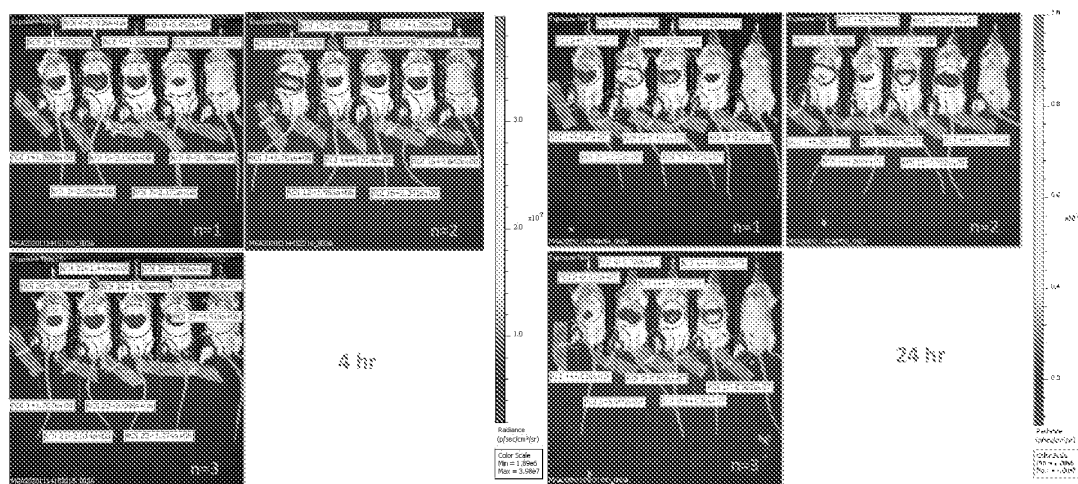


FIGURE 57A

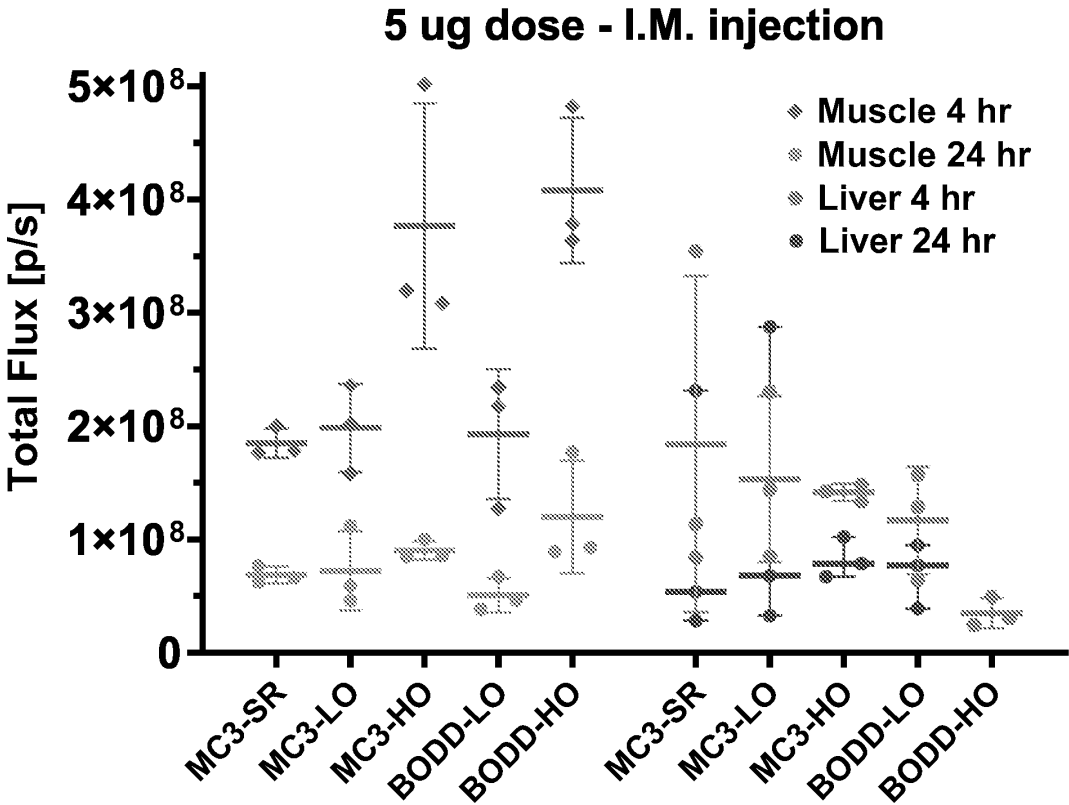


FIGURE 57B

2. Group 2 = 0.5 ug dose (4, 24 hours). Left to Right = MC3-SR/MC3-LO/MC3-HO/BODD-LO/BODD-HO

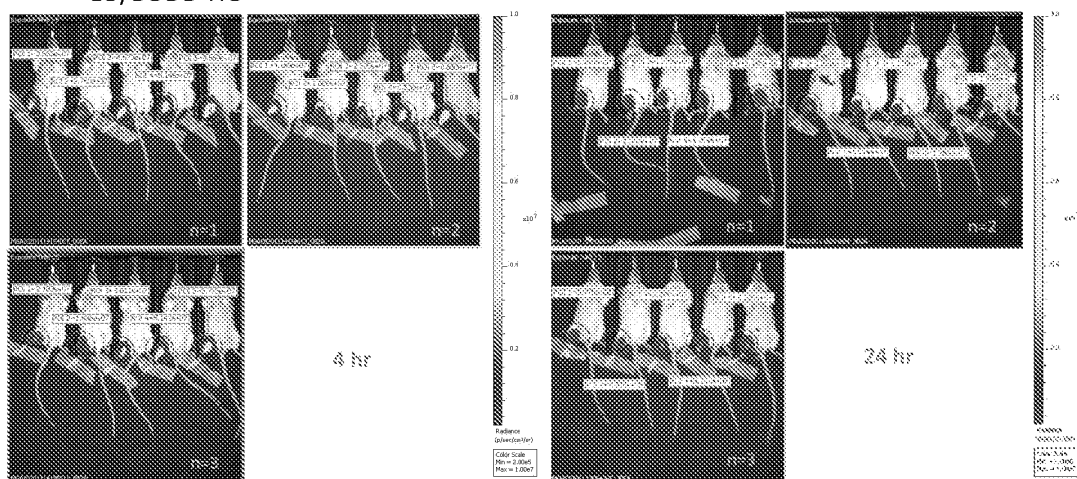


FIGURE 57C

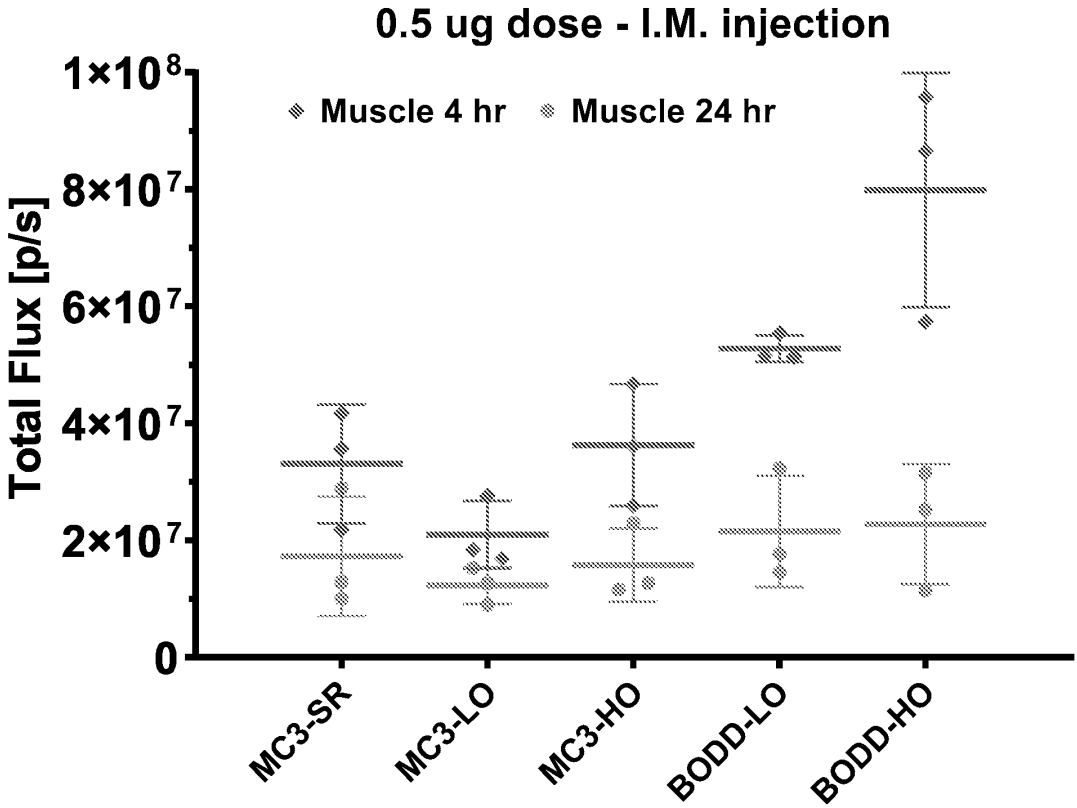


FIGURE 57D

3. Group 3 = 5 ug dose (4, 24, 48, 72, 120 hours) longevity. Left to Right = MC3-SR/MC3-HO/BODD-HO

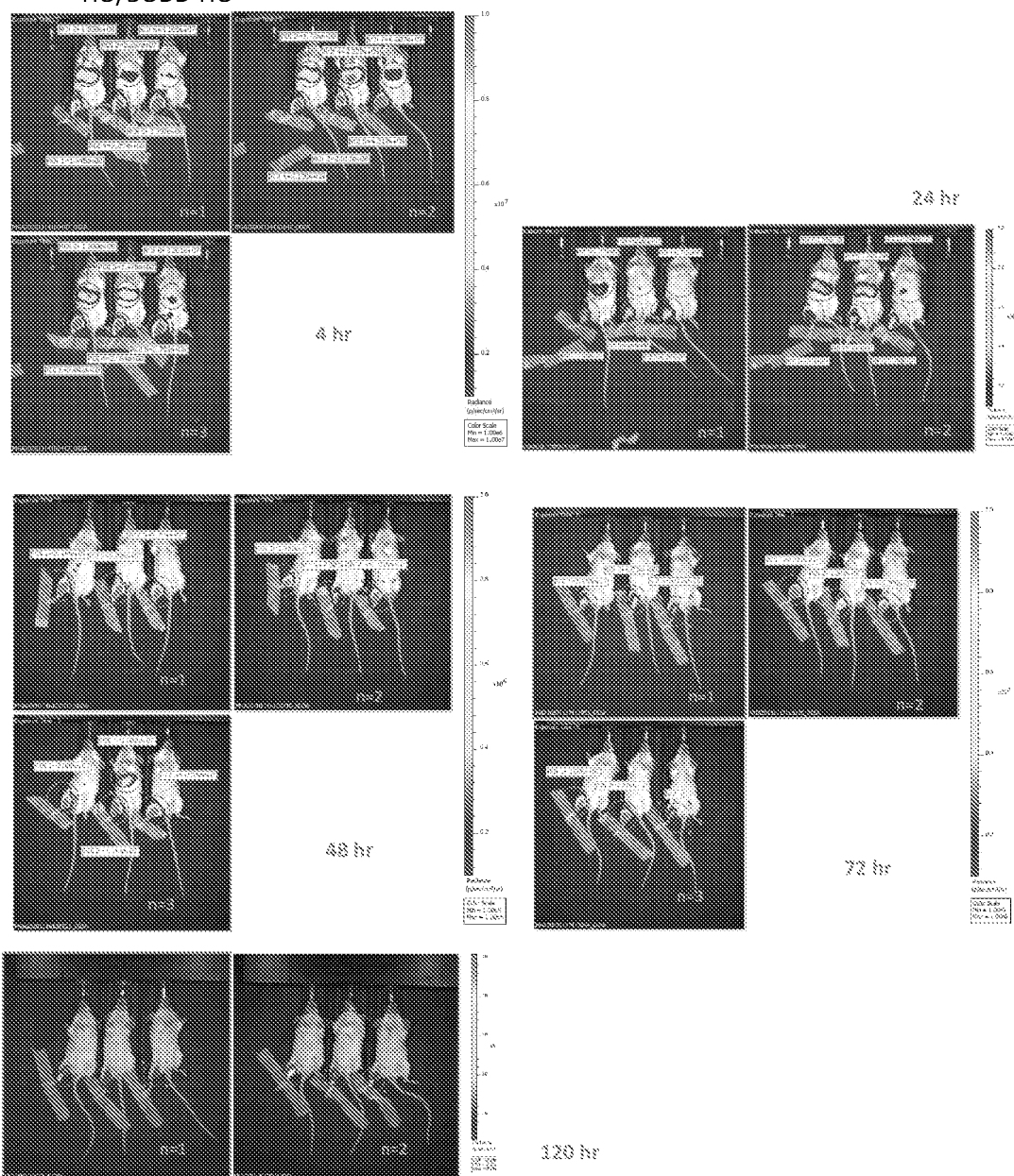


FIGURE 57E

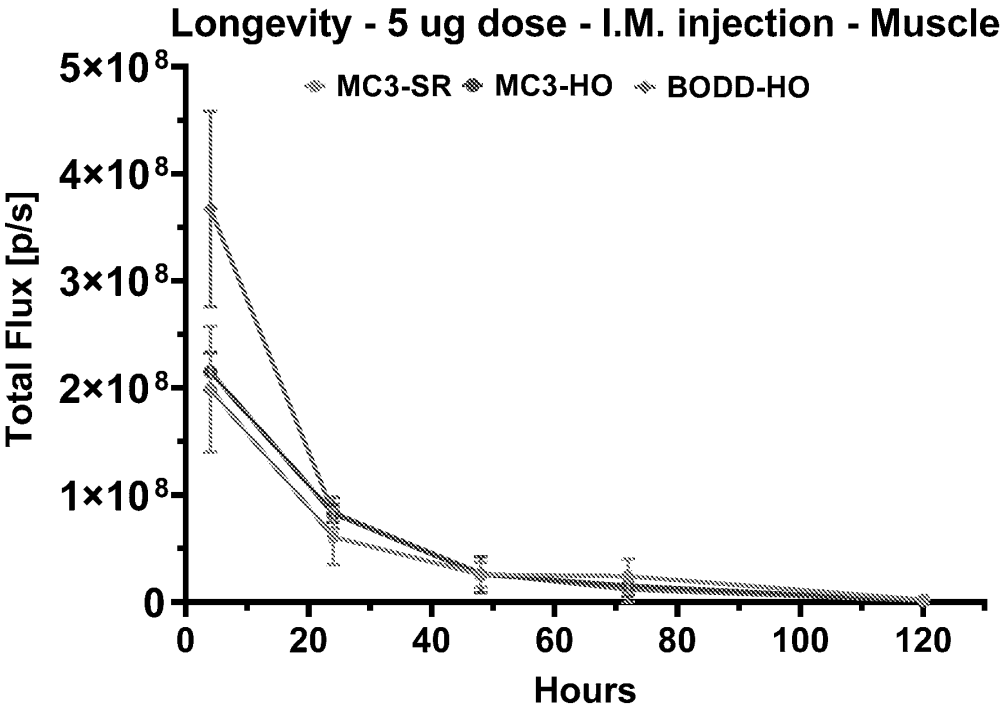


FIGURE 57F

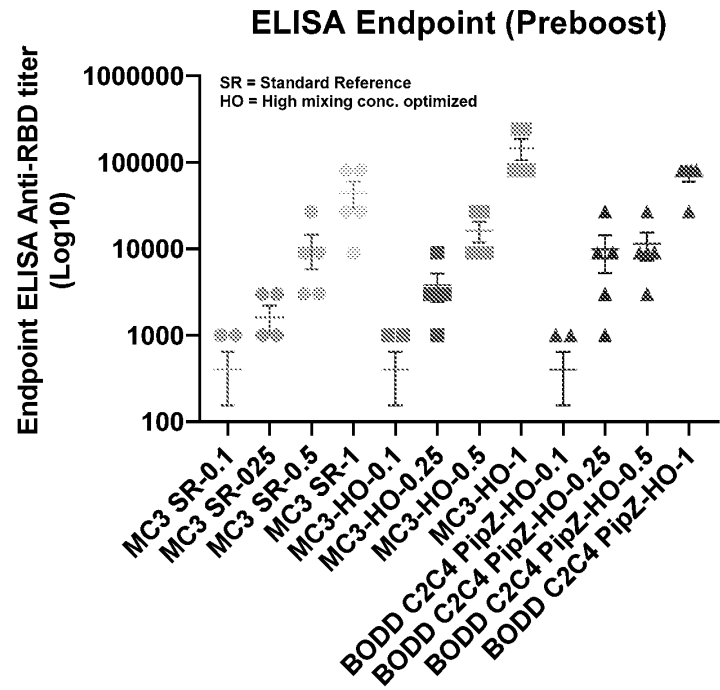


FIGURE 58A

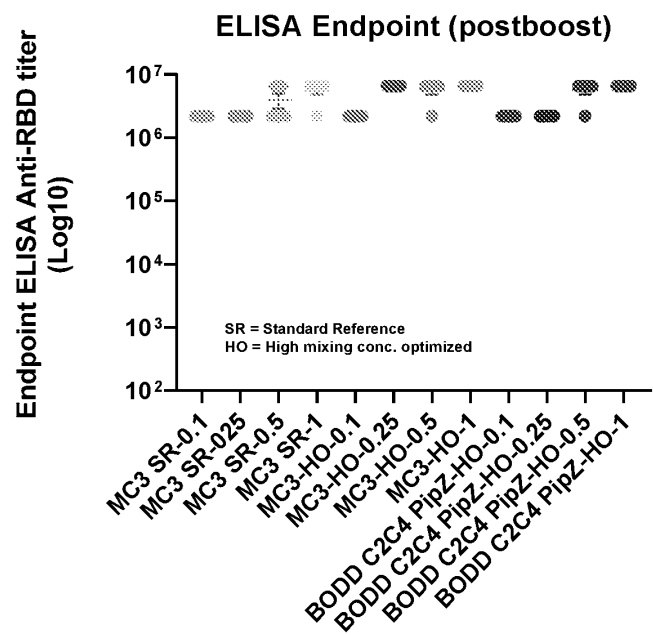


FIGURE 58B

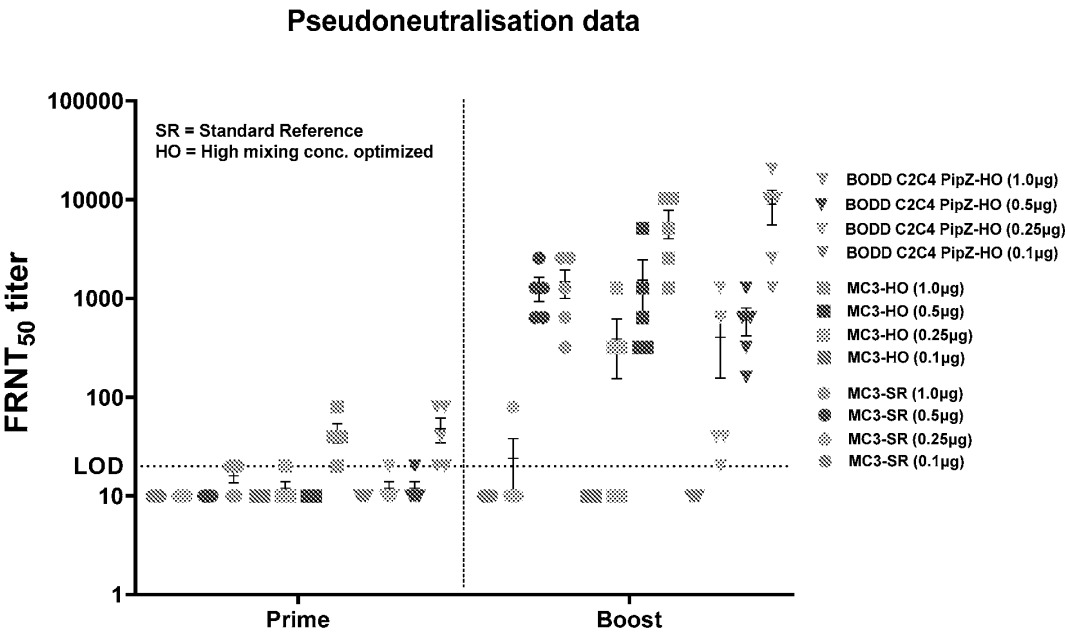


FIGURE 59

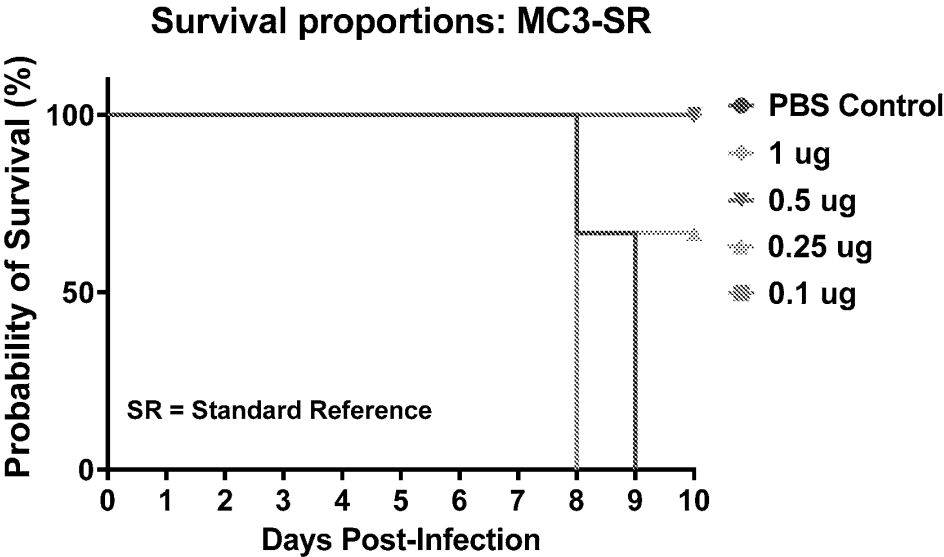


FIGURE 60A

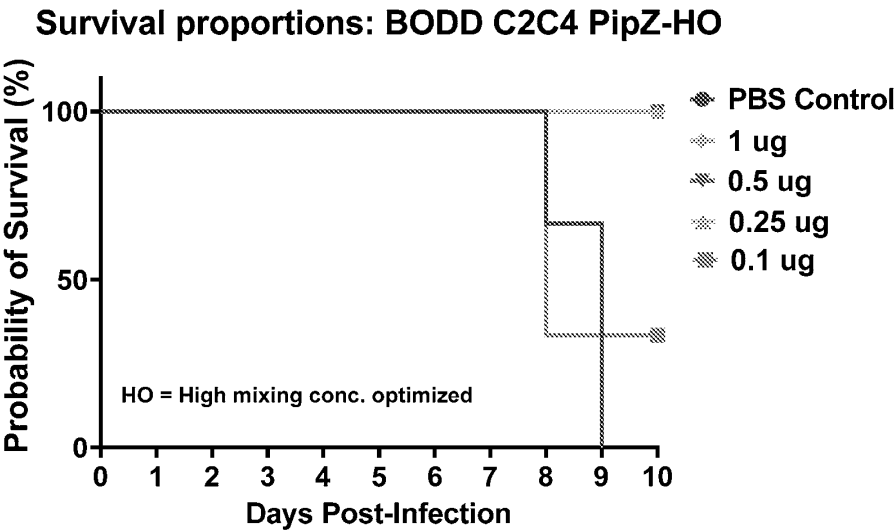


FIGURE 60B

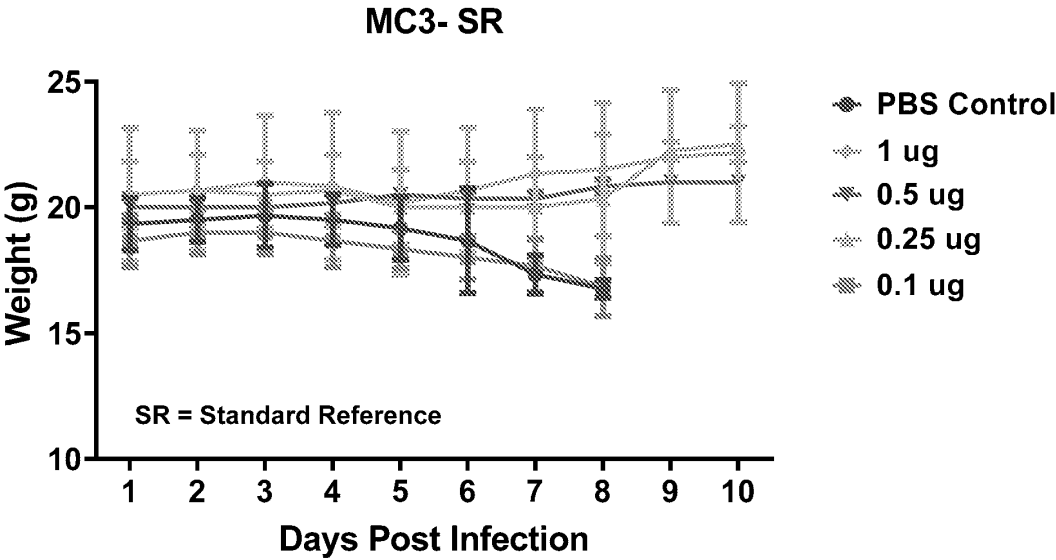


FIGURE 61A

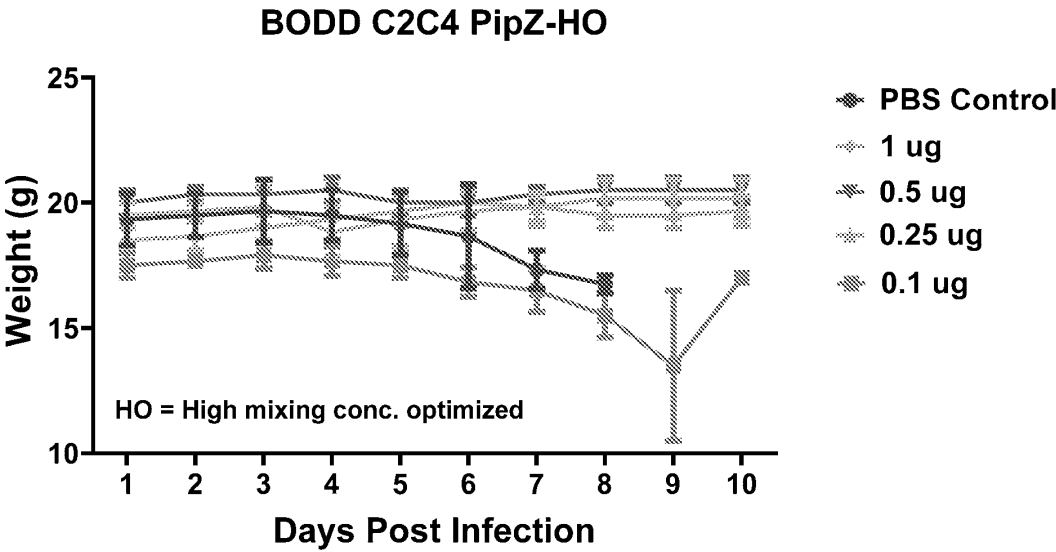


FIGURE 61B

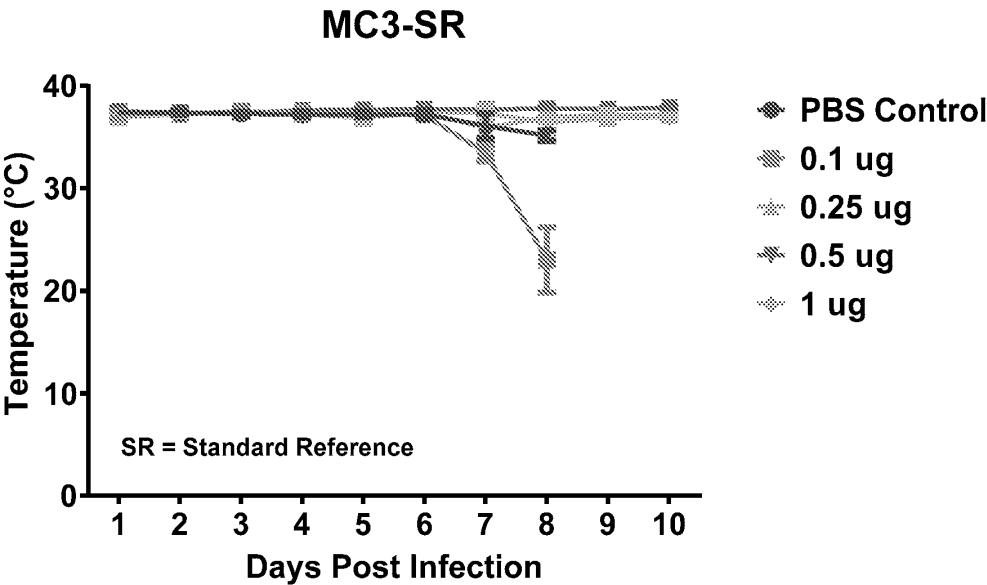


FIGURE 61C

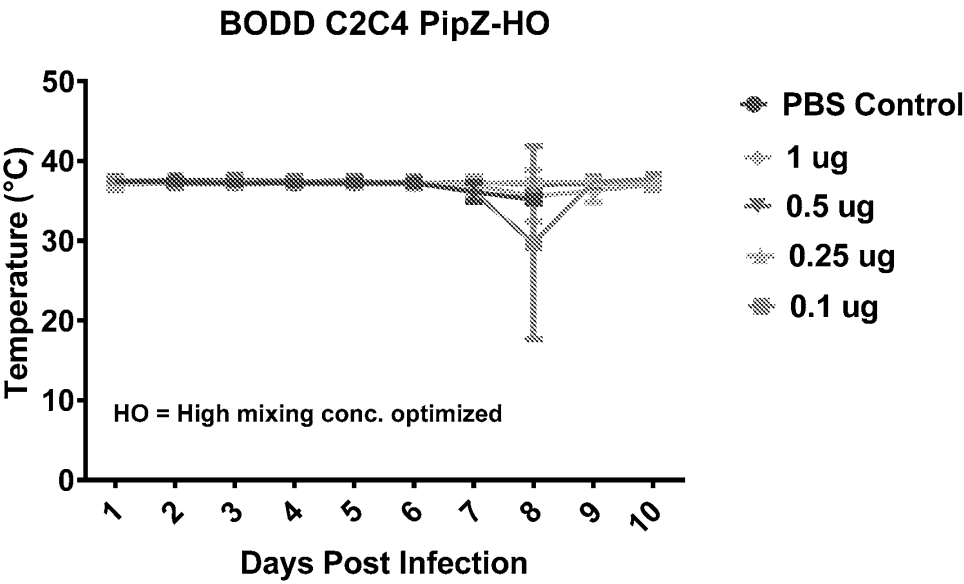


FIGURE 61D

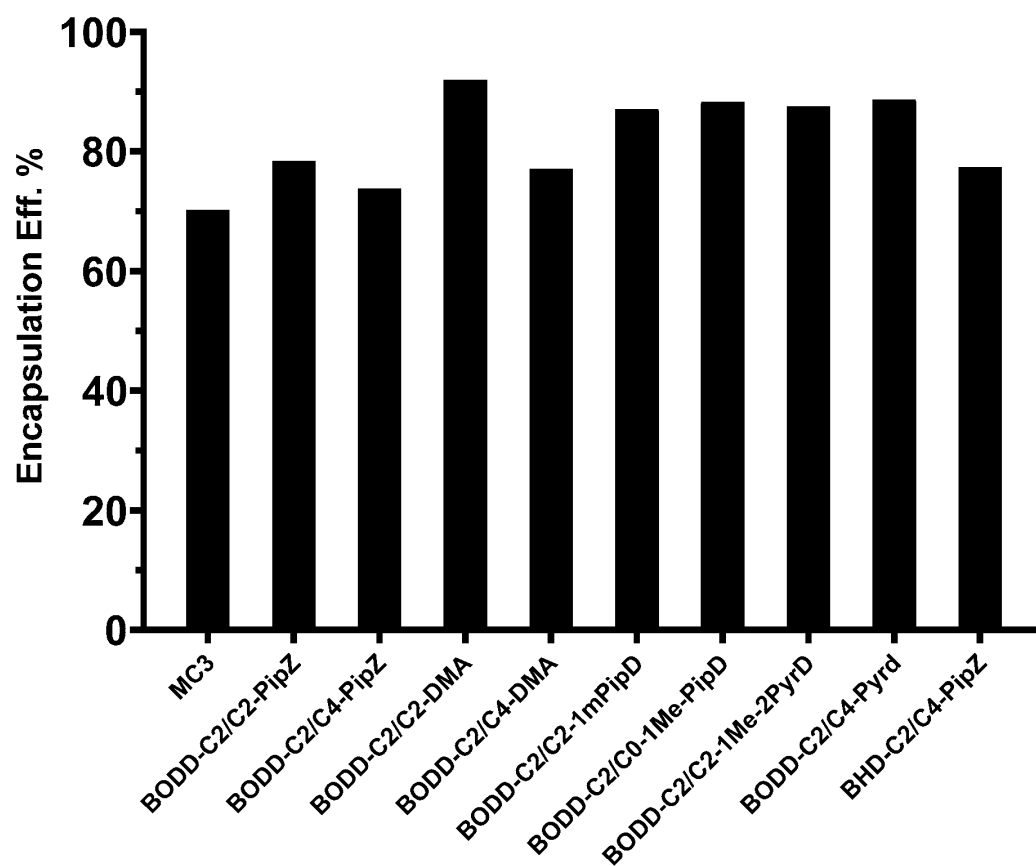


FIGURE 62

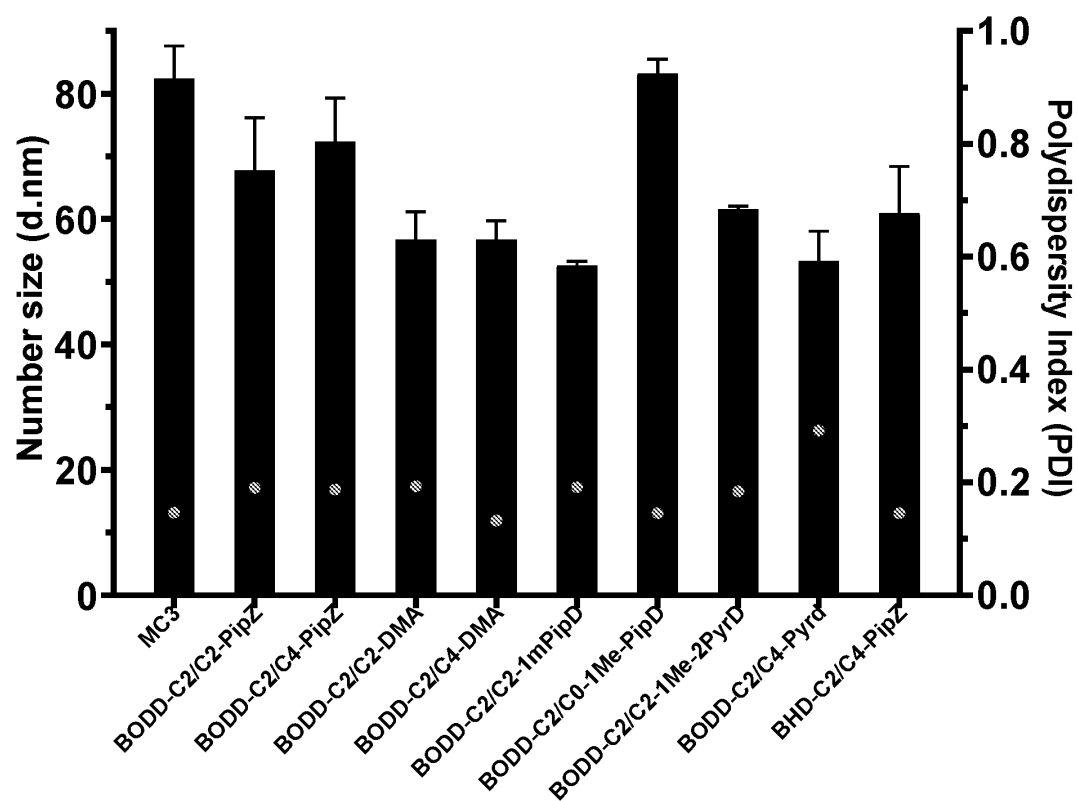


FIGURE 63

Group 1 IM - 1 ug dose (4, 24 hours) - N=1,2,3

Left to Right :

1.1 MC3

1.2 BODD-C2/C2-PipZ

1.3 BODD-C2/C4-PipZ

1.4 BODD-C2/C2-DMA

1.5 BODD-C2/C4-DMA

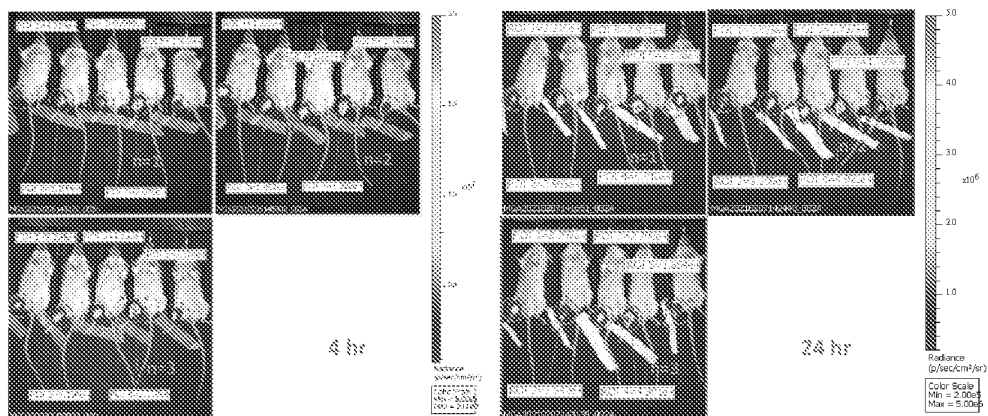


FIGURE 64A

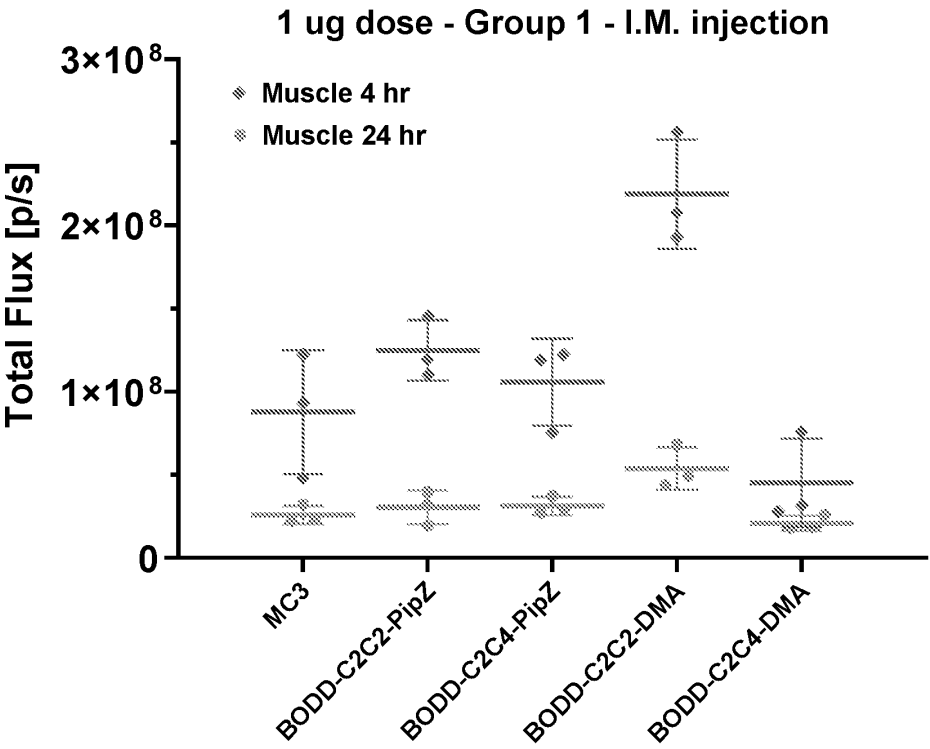


FIGURE 64B

Group 1 IV - 1 ug dose (4, 24 hours) - N=1,2,3

Left to Right :

2.1 MC3

2.2 BODD-C2/C2-PipZ

2.3 BODD-C2/C4-PipZ

2.4 BODD-C2/C2-DMA

2.5 BODD-C2/C4-DMA

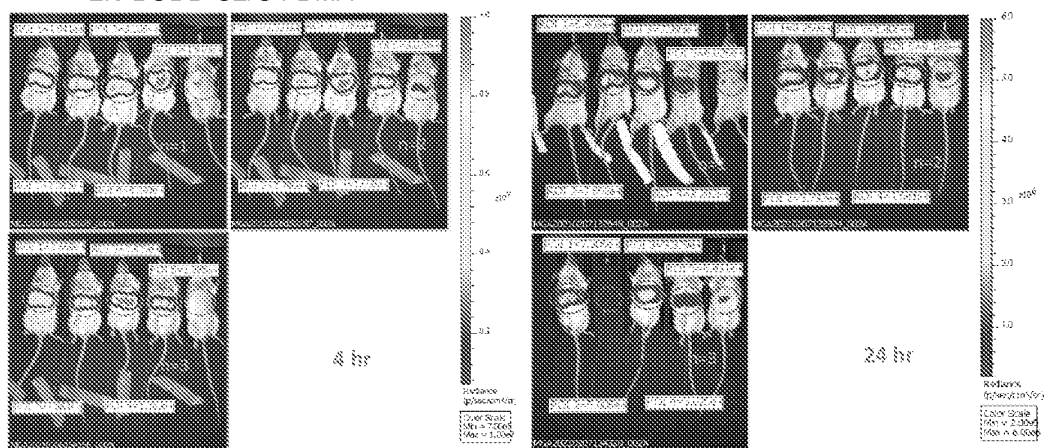


FIGURE 64C

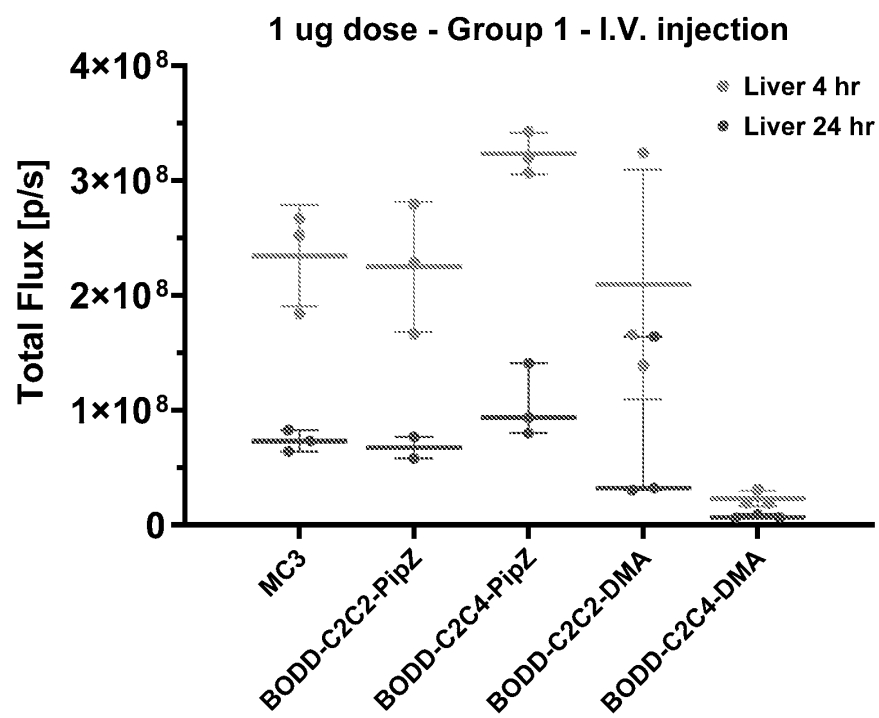


FIGURE 64D

Group 2 IM - 1 ug dose (4, 24 hours) - N=1,2,3

Left to Right :

- 3.1 BODD-C2/C2-1mPipD
- 3.2 BODD-C2/C0-1Me- PipD
- 3.3 BODD-C2/C2-1Me- 2PyrD
- 3.4 BODD-C2/C4-Pyrd
- 3.5 BHD-C2/C4-PipZ

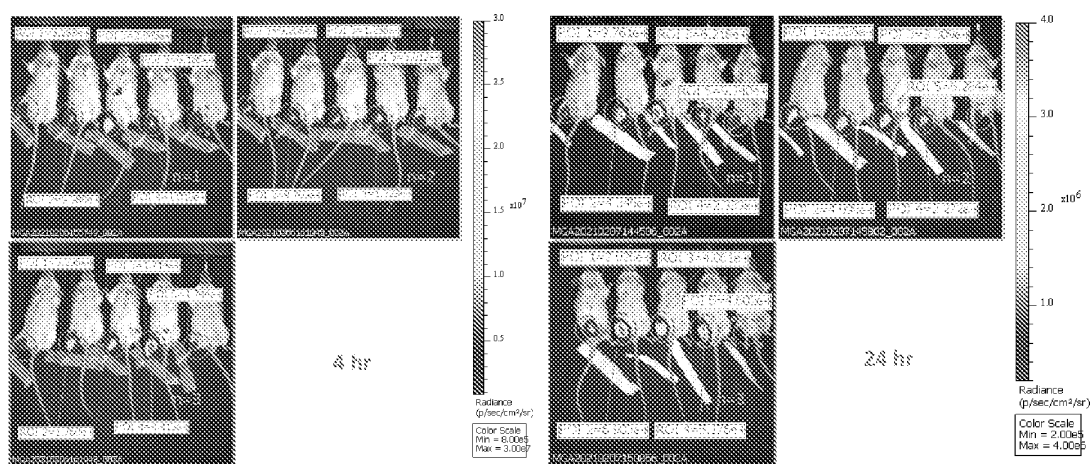


FIGURE 64E

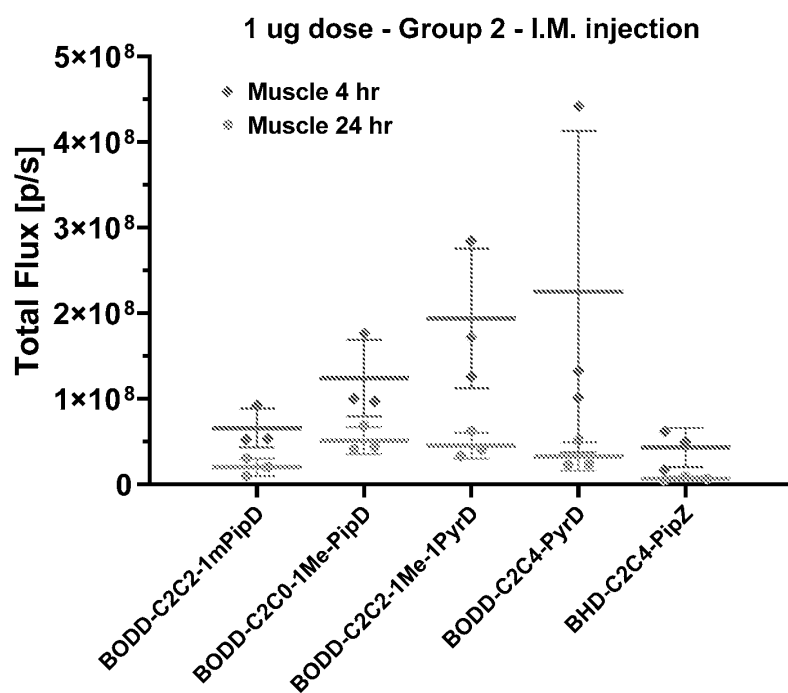


FIGURE 64F

Group 2 IV - 1 ug dose (4, 24 hours) - N=1,2,3

Left to Right :

3.1 BODD-C2/C2-1mPipD

3.2 BODD-C2/C0-1Me- PipD

3.3 BODD-C2/C2-1Me- 2PyrD

3.4 BODD-C2/C4-PyrdBHD-C2/C4-PipZ

3.5 BHD-C2/C4-PipZ

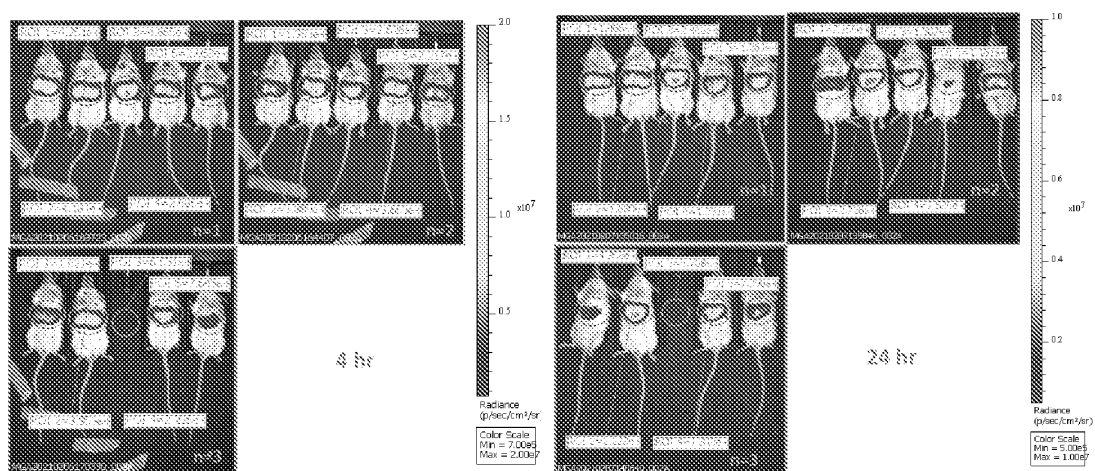


FIGURE 64G

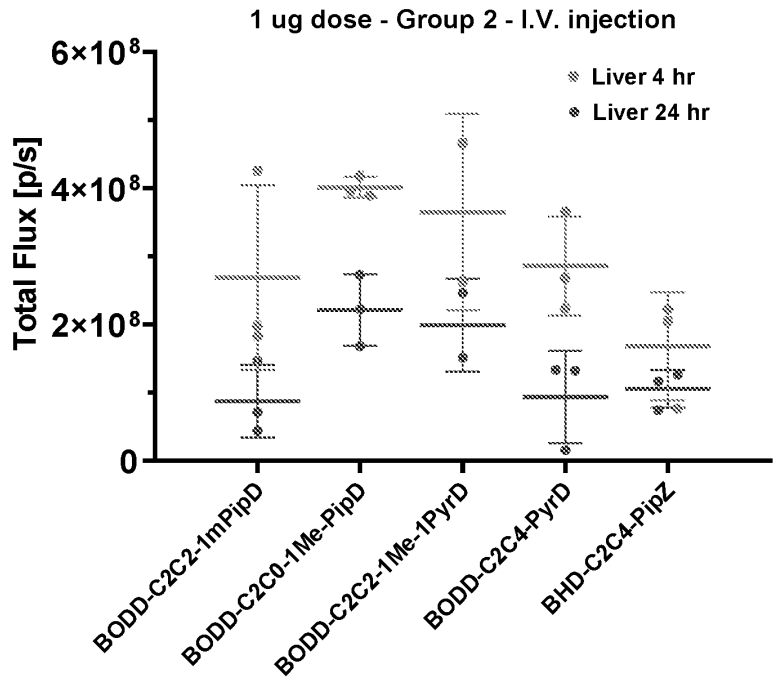


FIGURE 64H

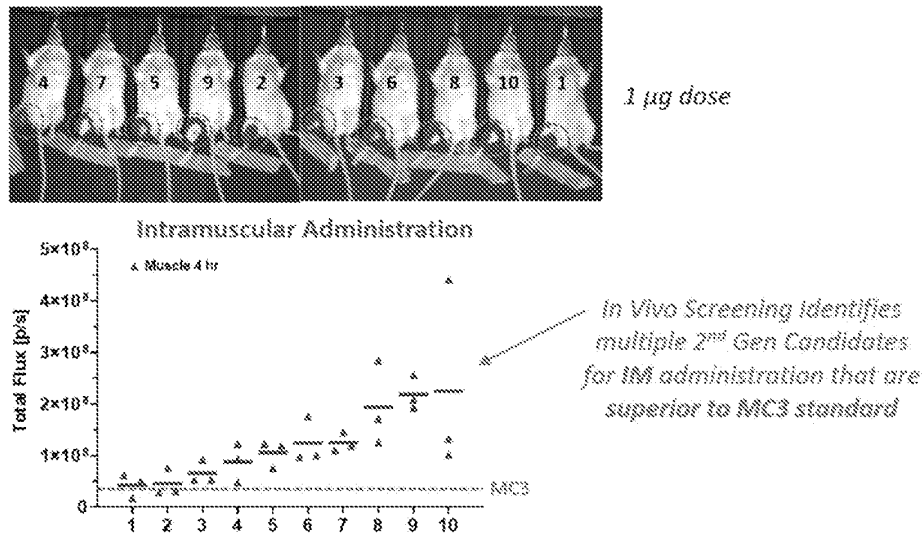


FIGURE 64I

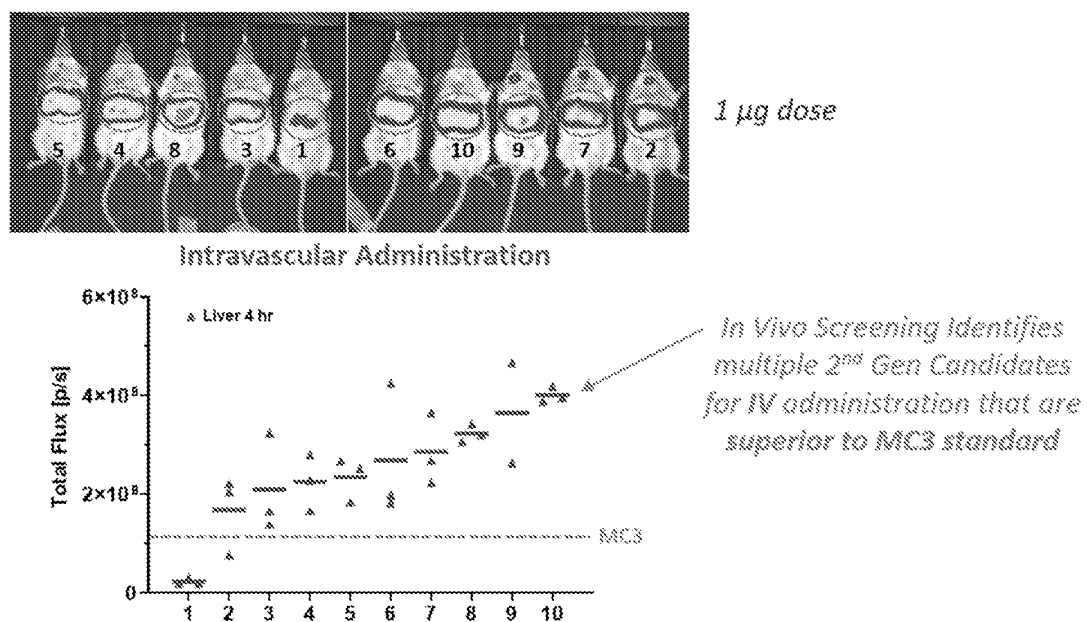


FIGURE 64J

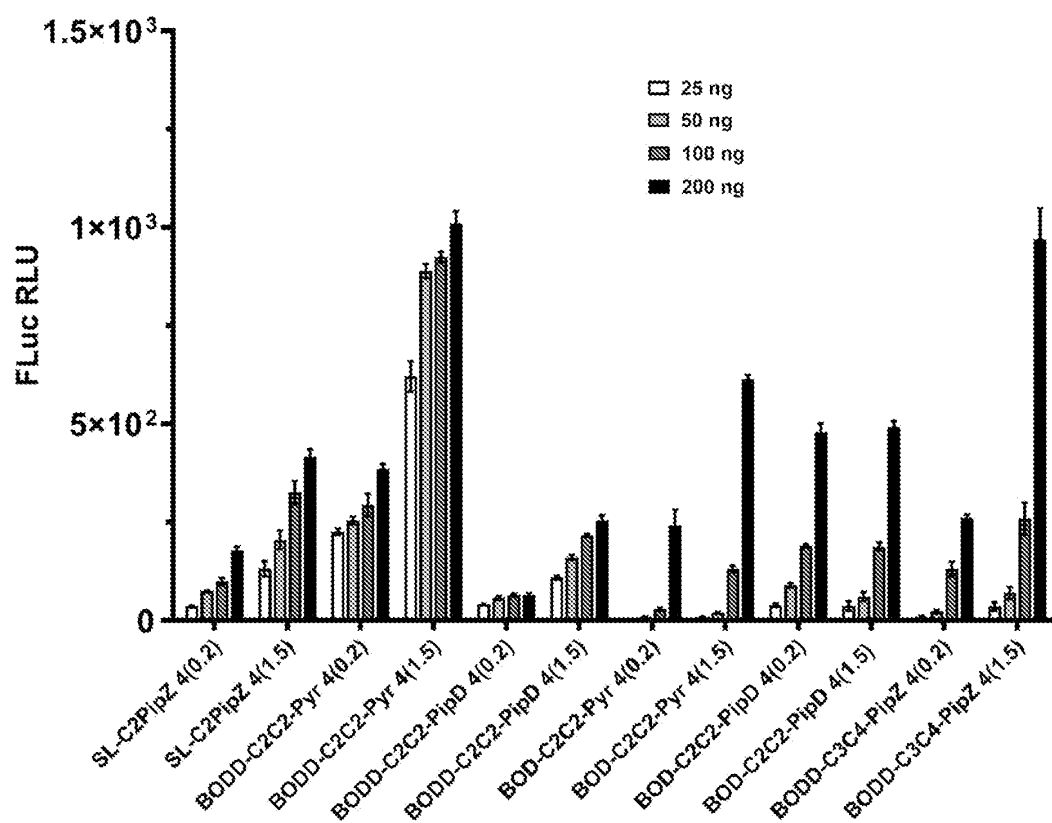


FIGURE 65

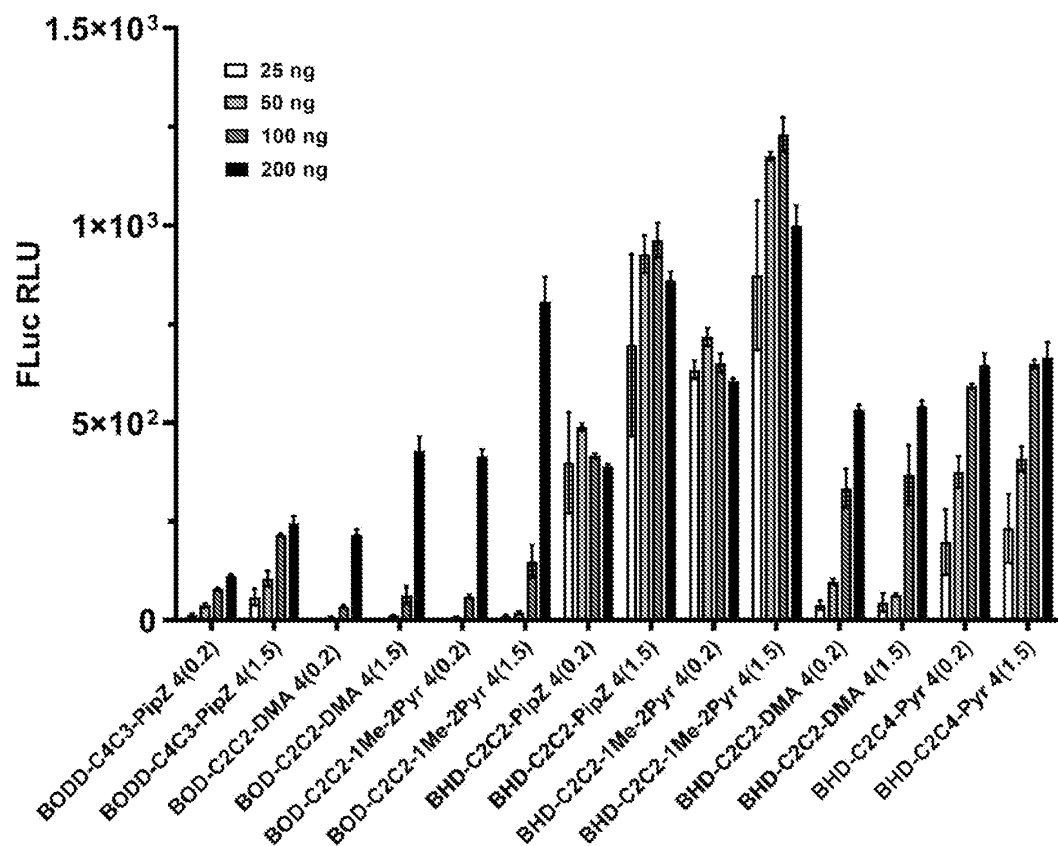


FIGURE 66

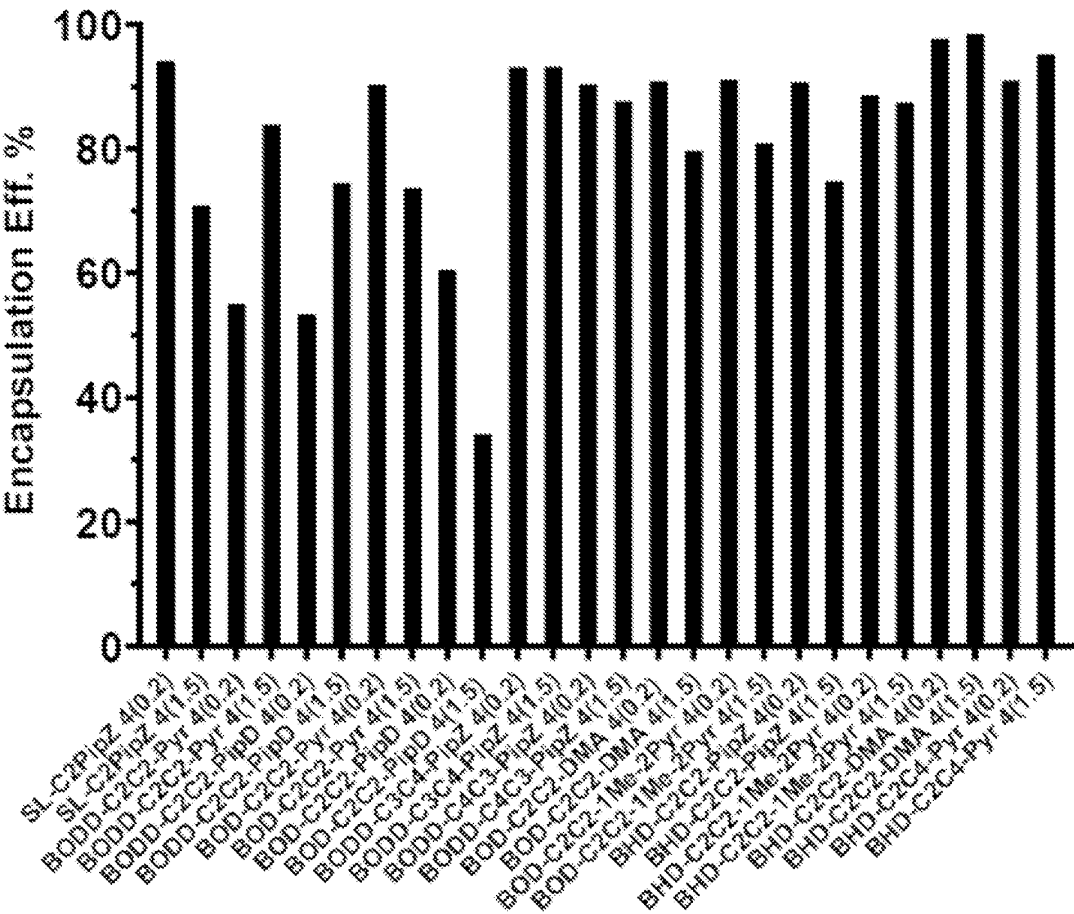


FIGURE 67

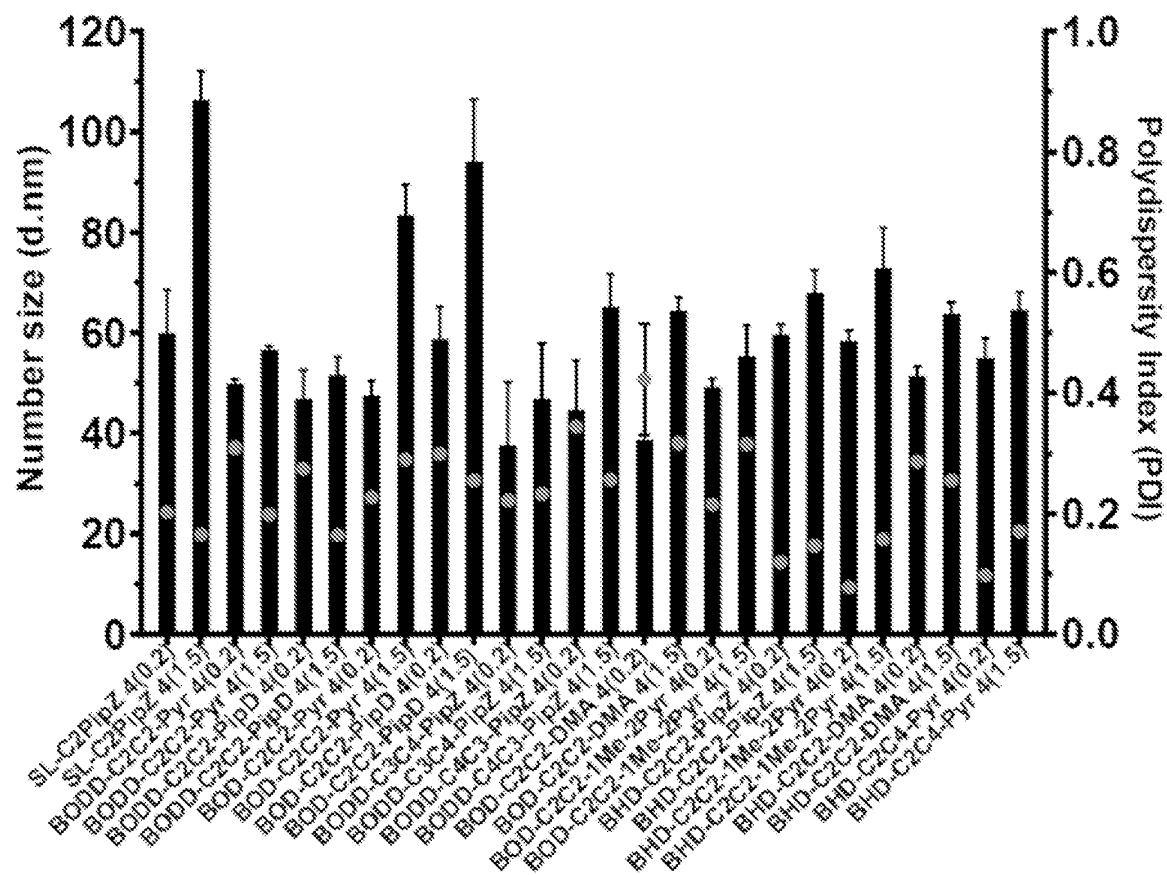


FIGURE 68

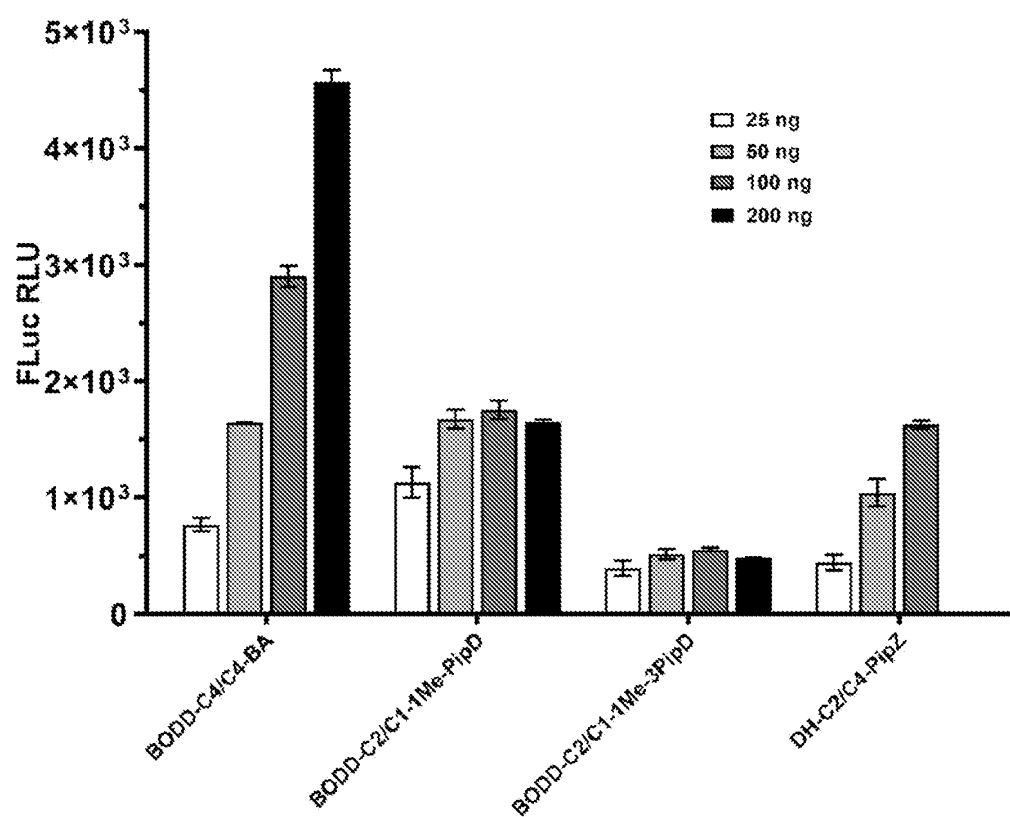


FIGURE 69

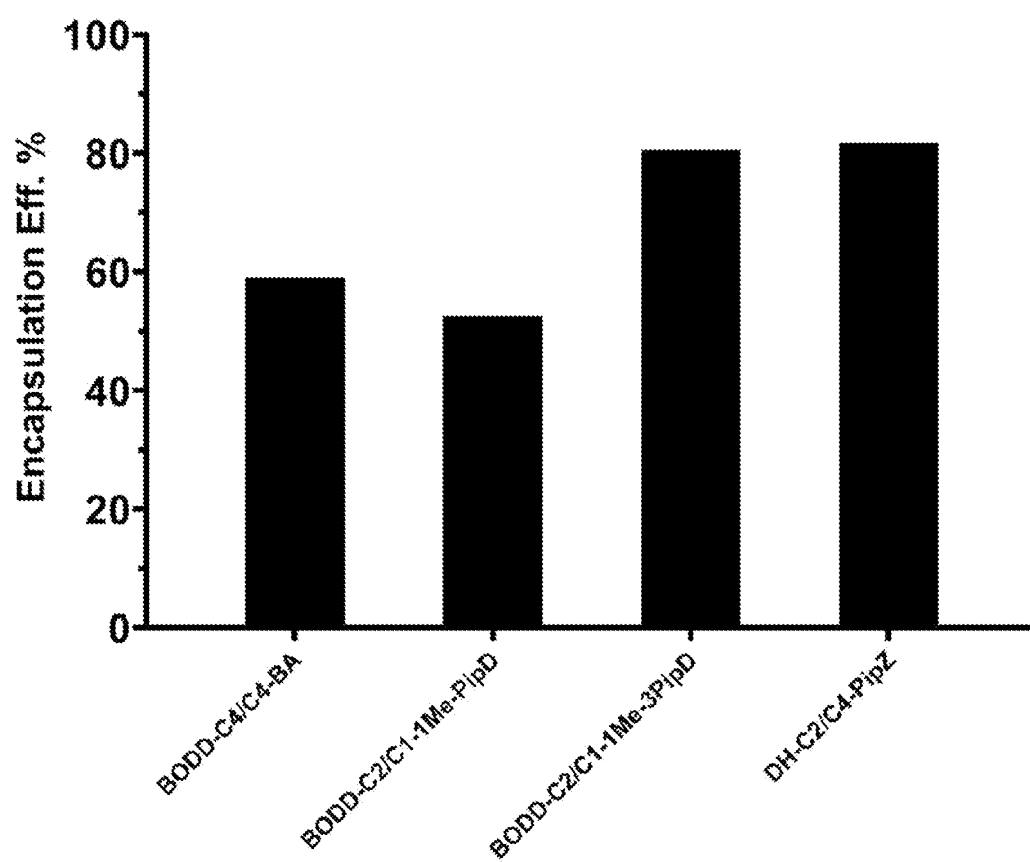


FIGURE 70

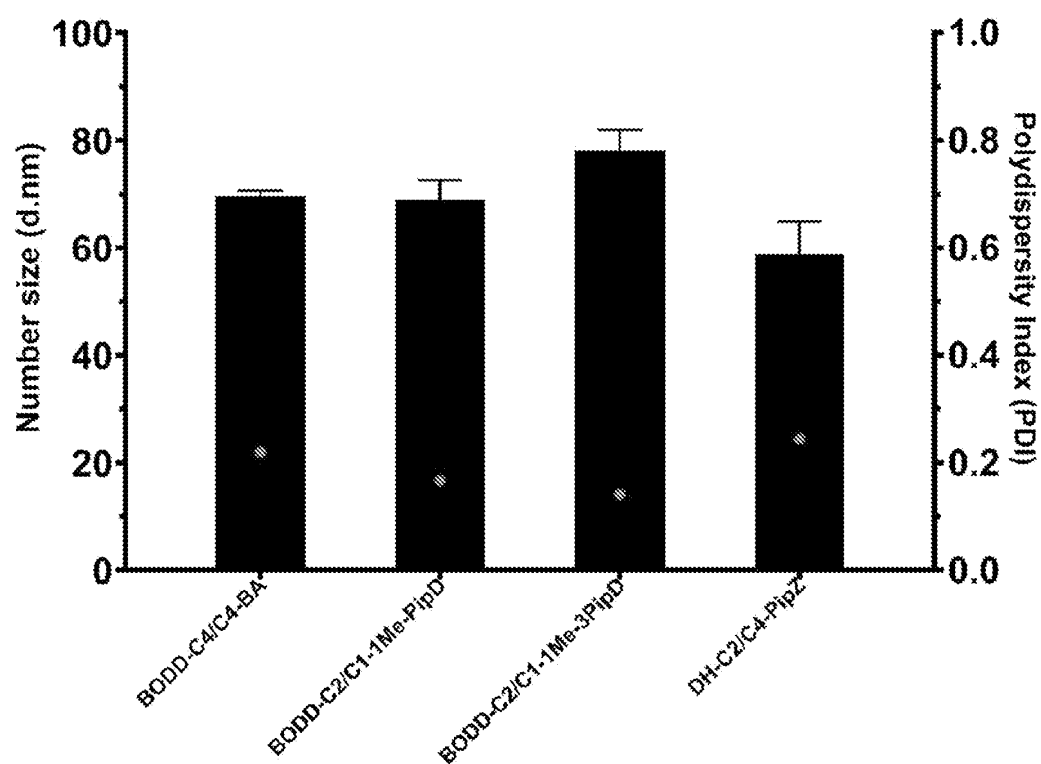


FIGURE 71

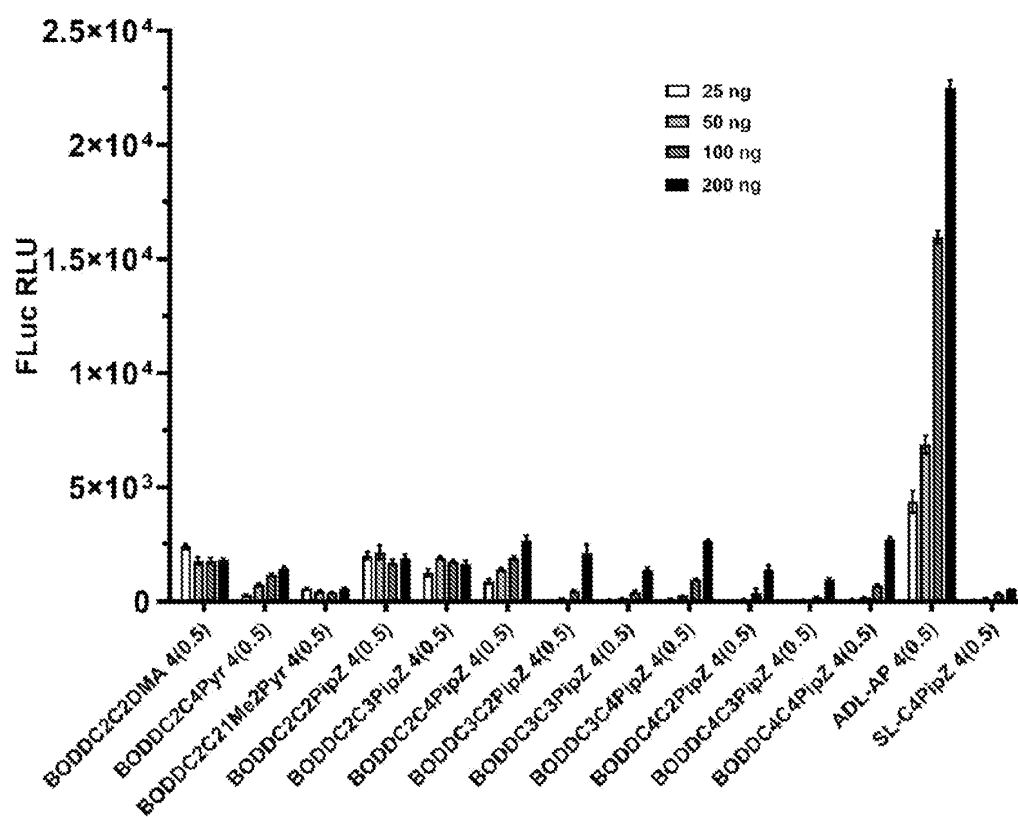


FIGURE 72

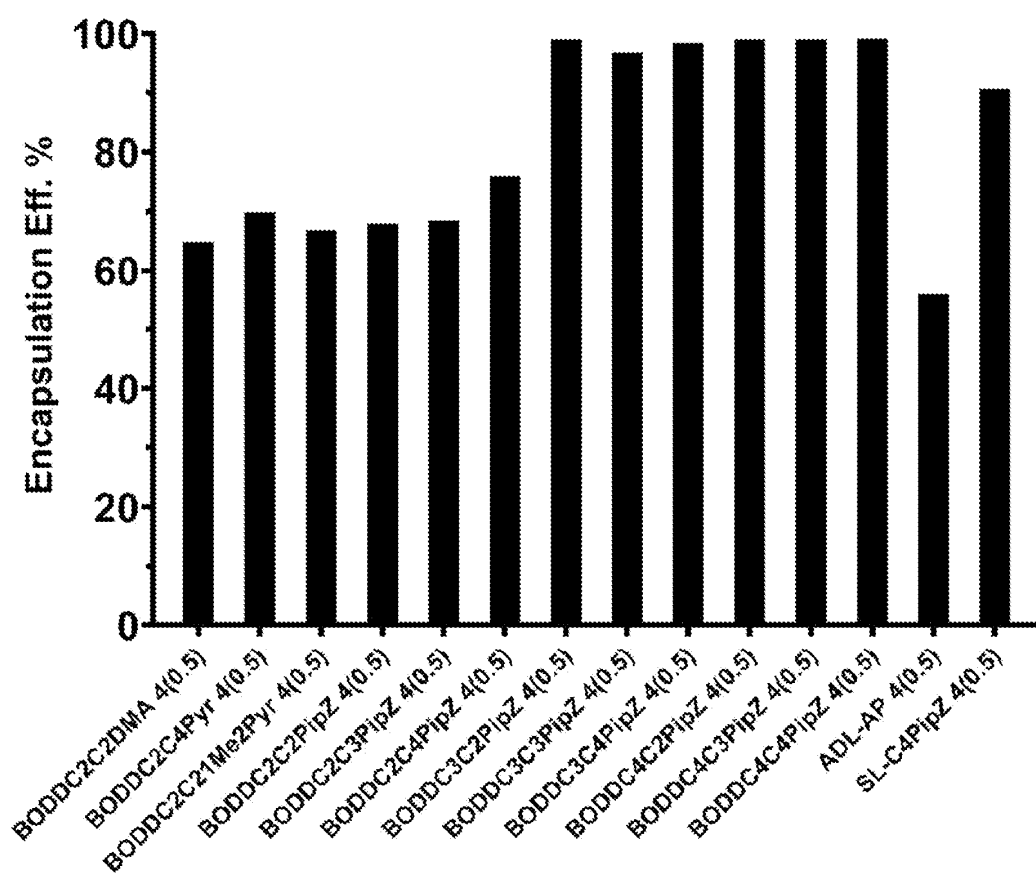


FIGURE 73

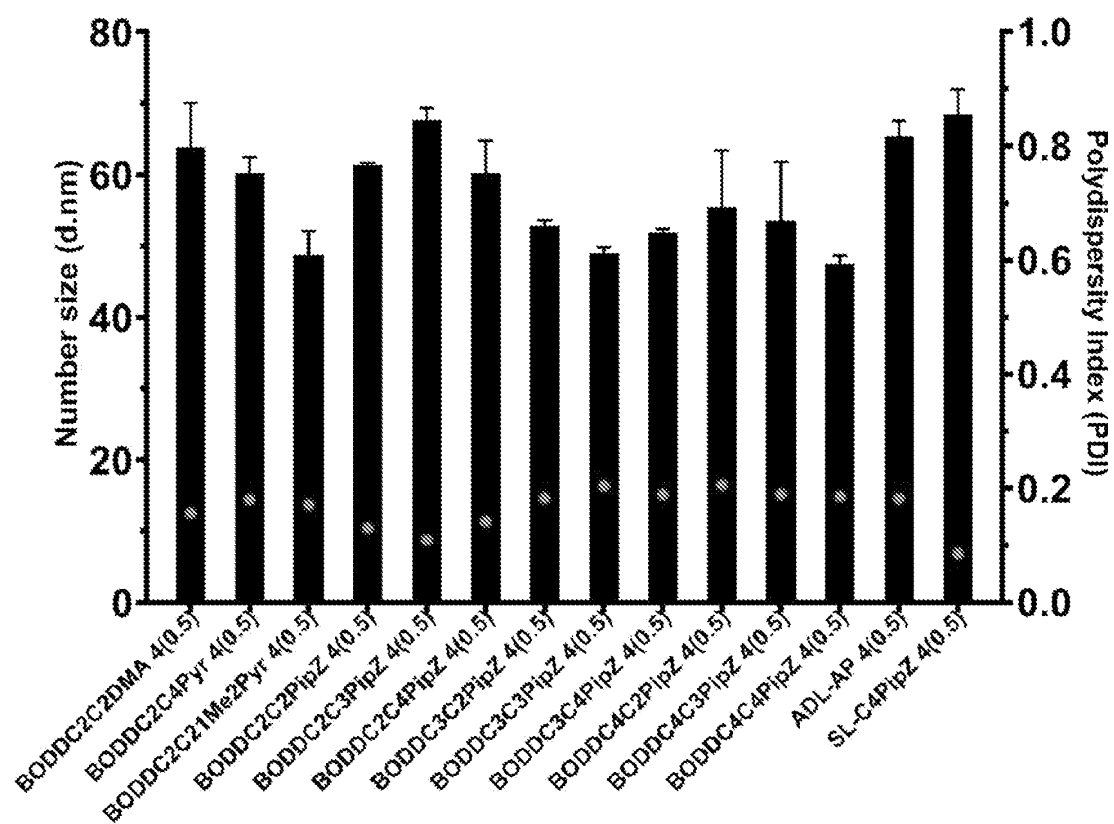


FIGURE 74

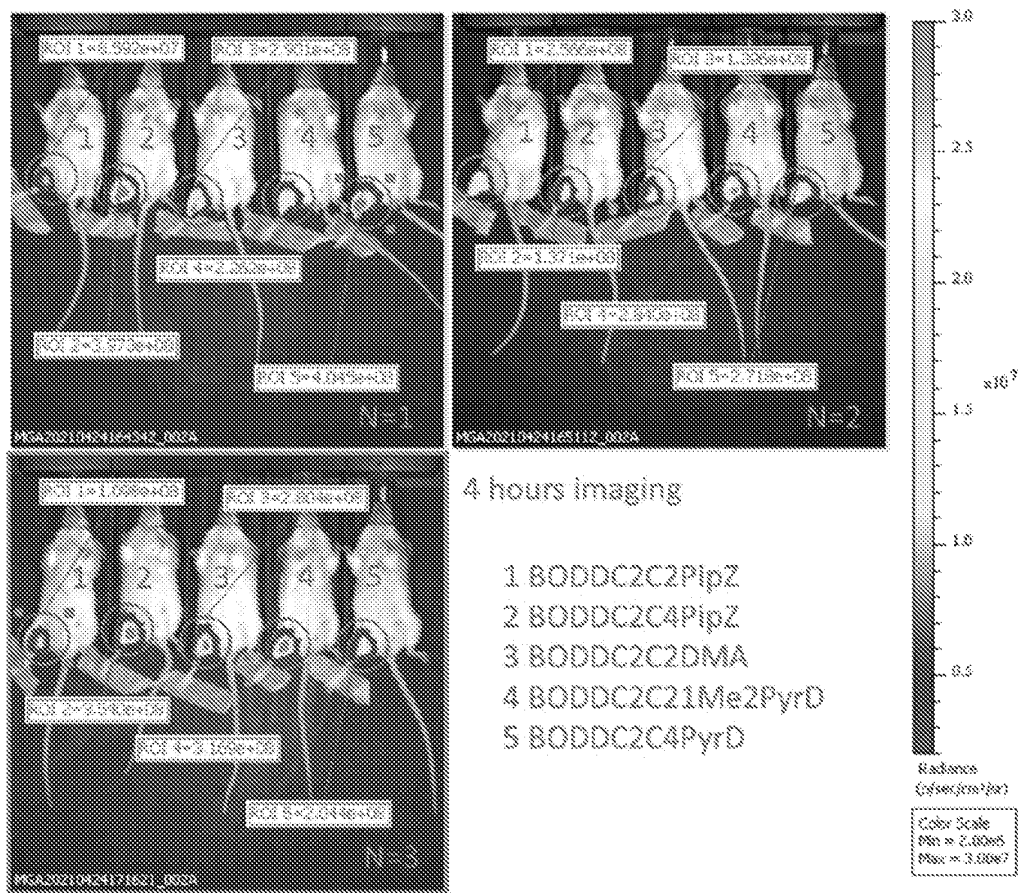


FIGURE 75

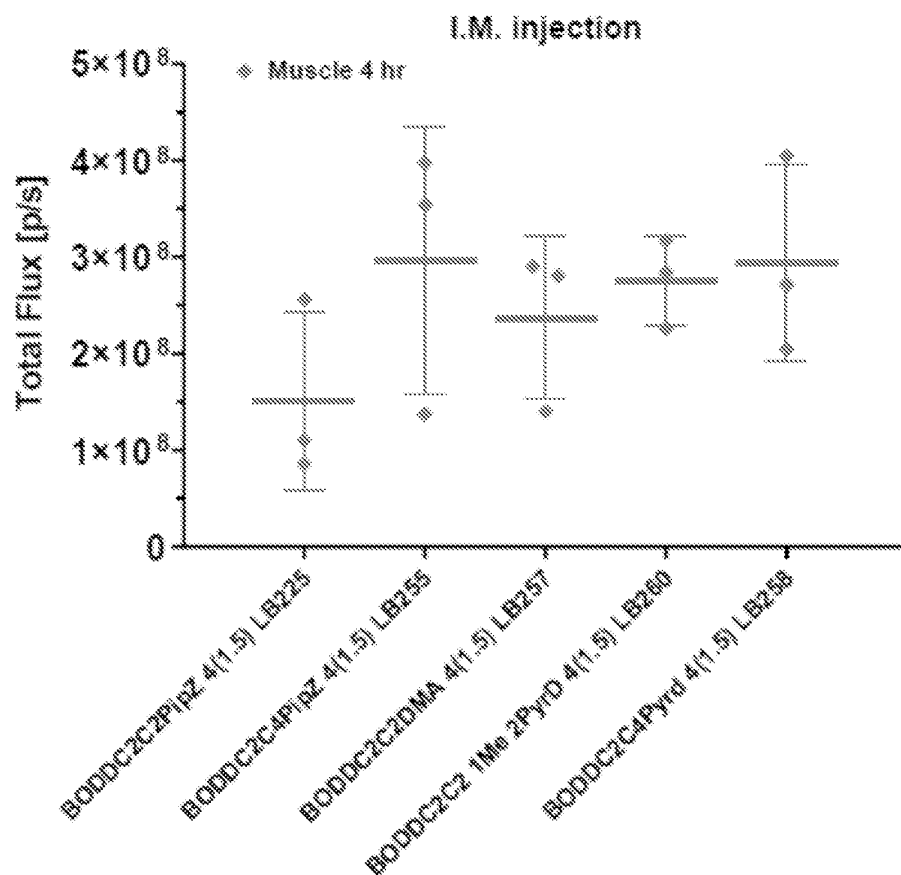


FIGURE 76

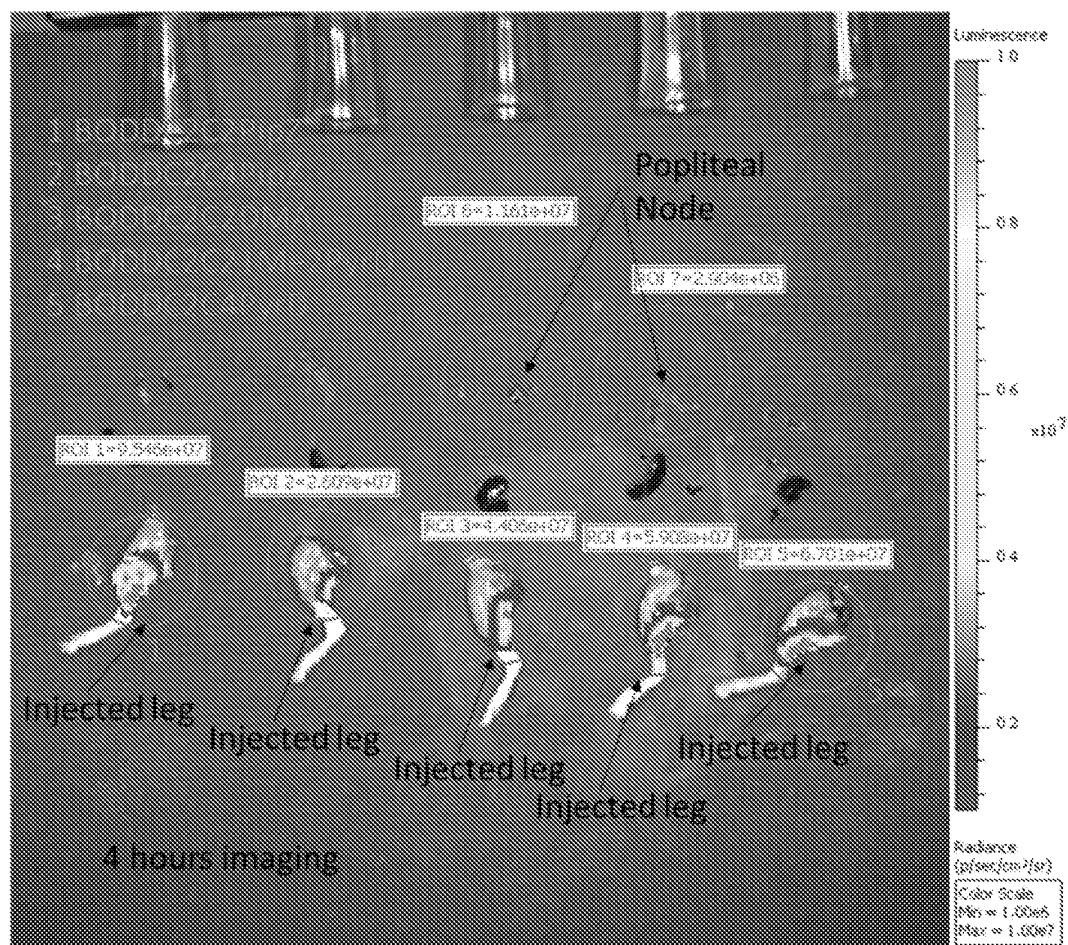


FIGURE 77

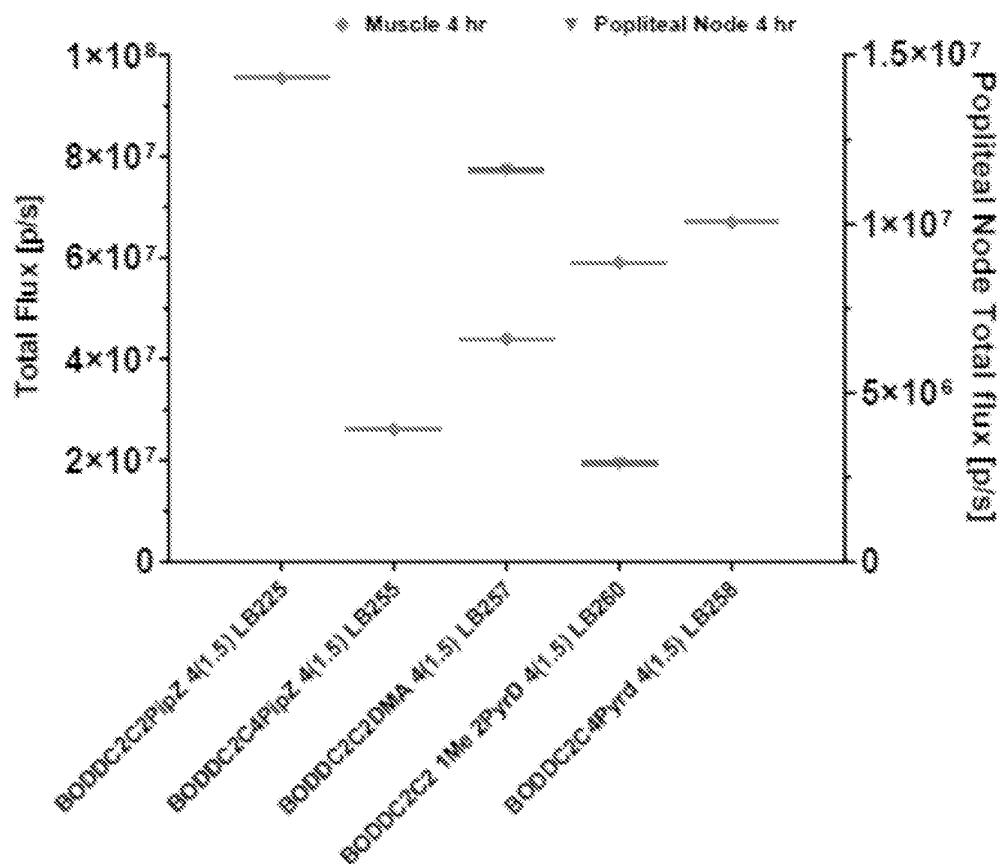


FIGURE 78

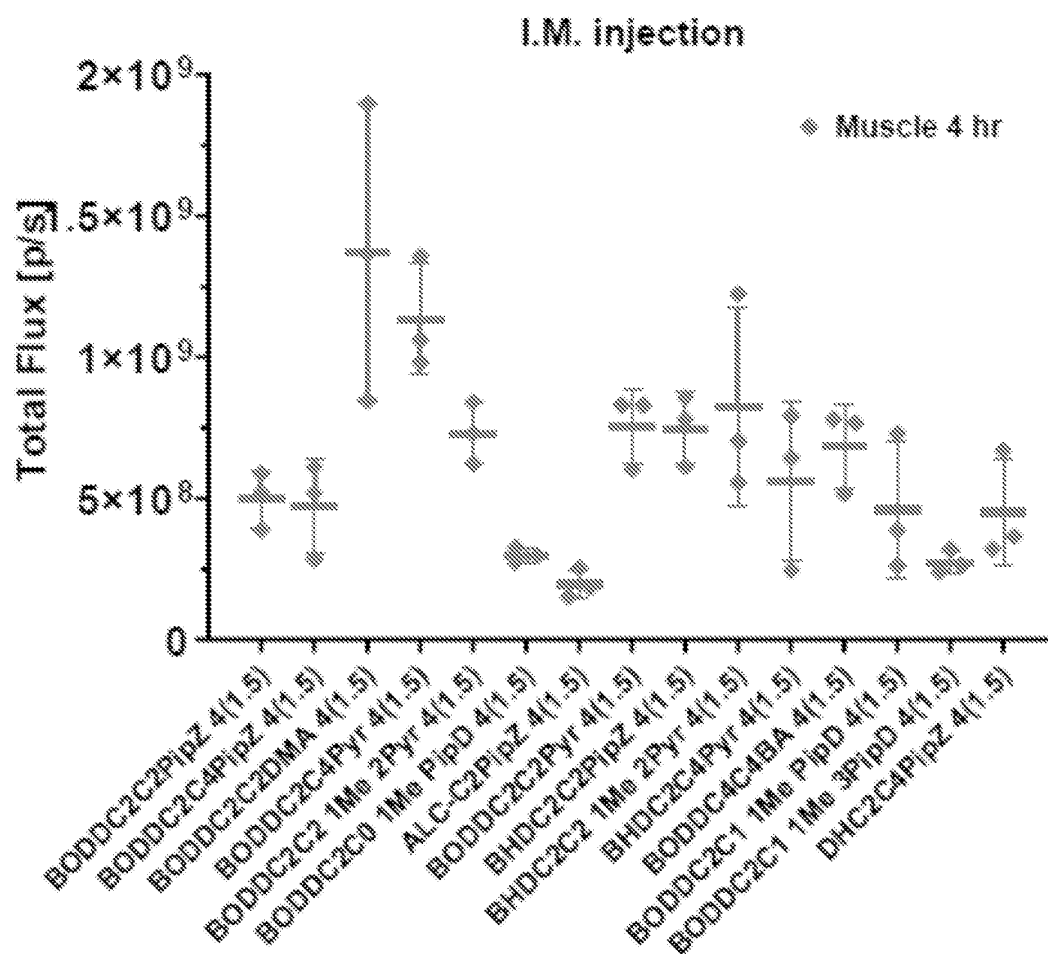


FIGURE 79

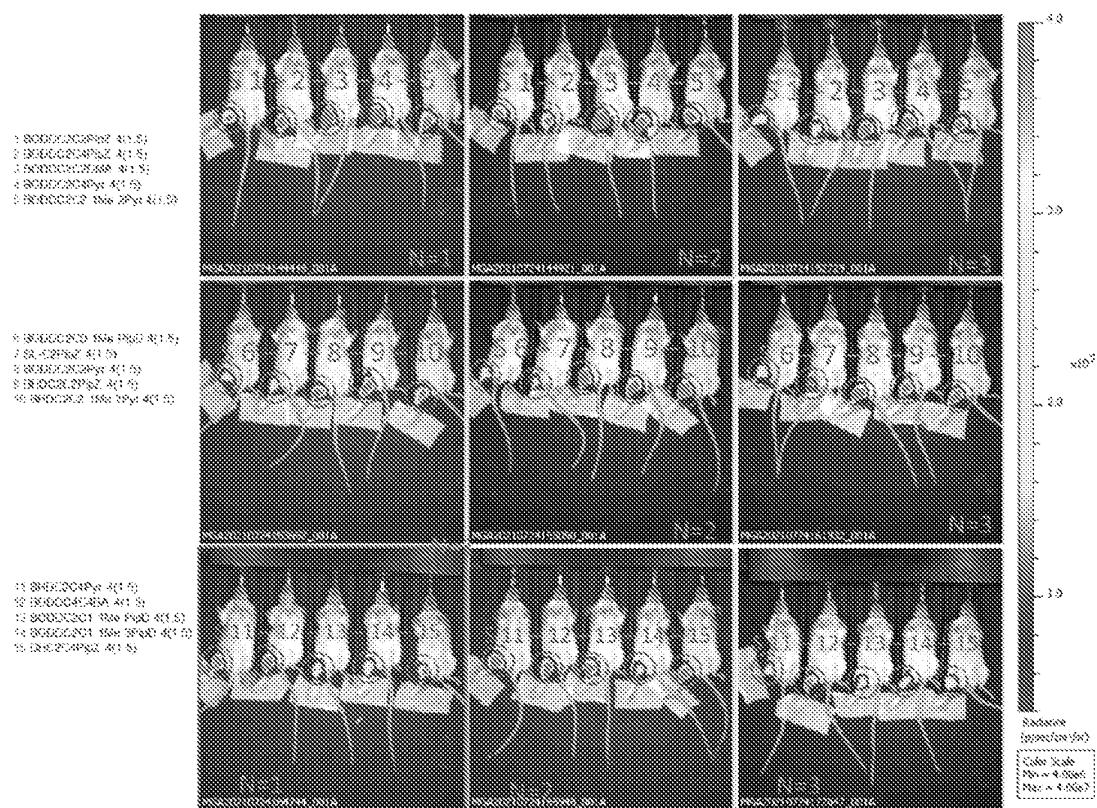


FIGURE 80

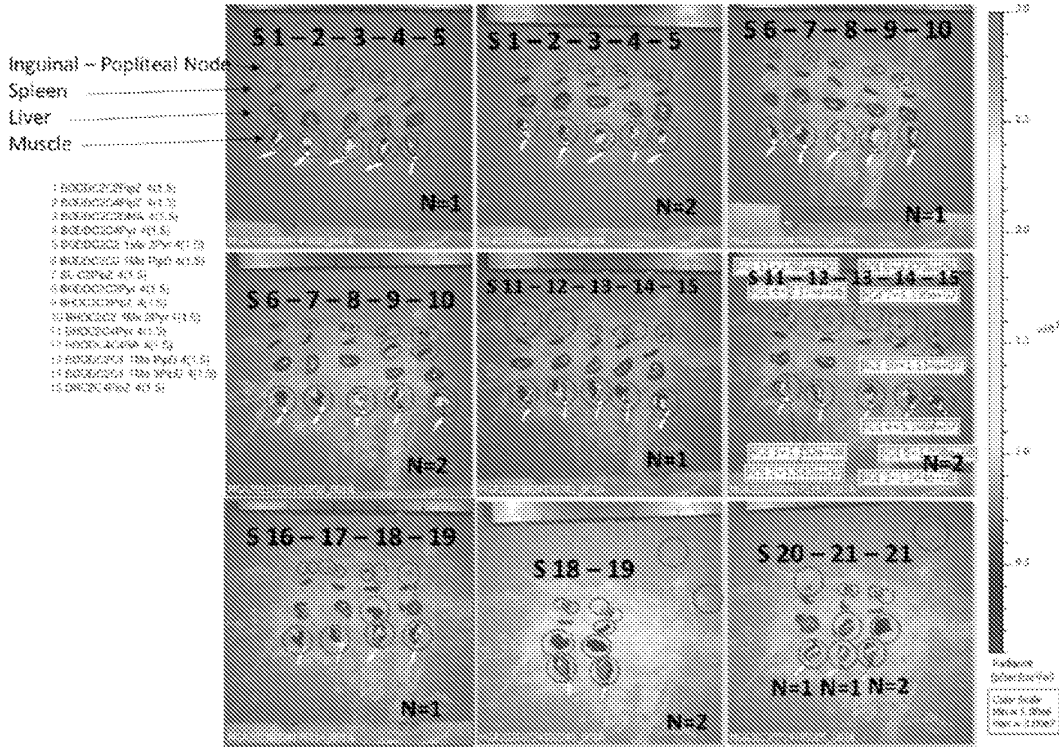


FIGURE 81

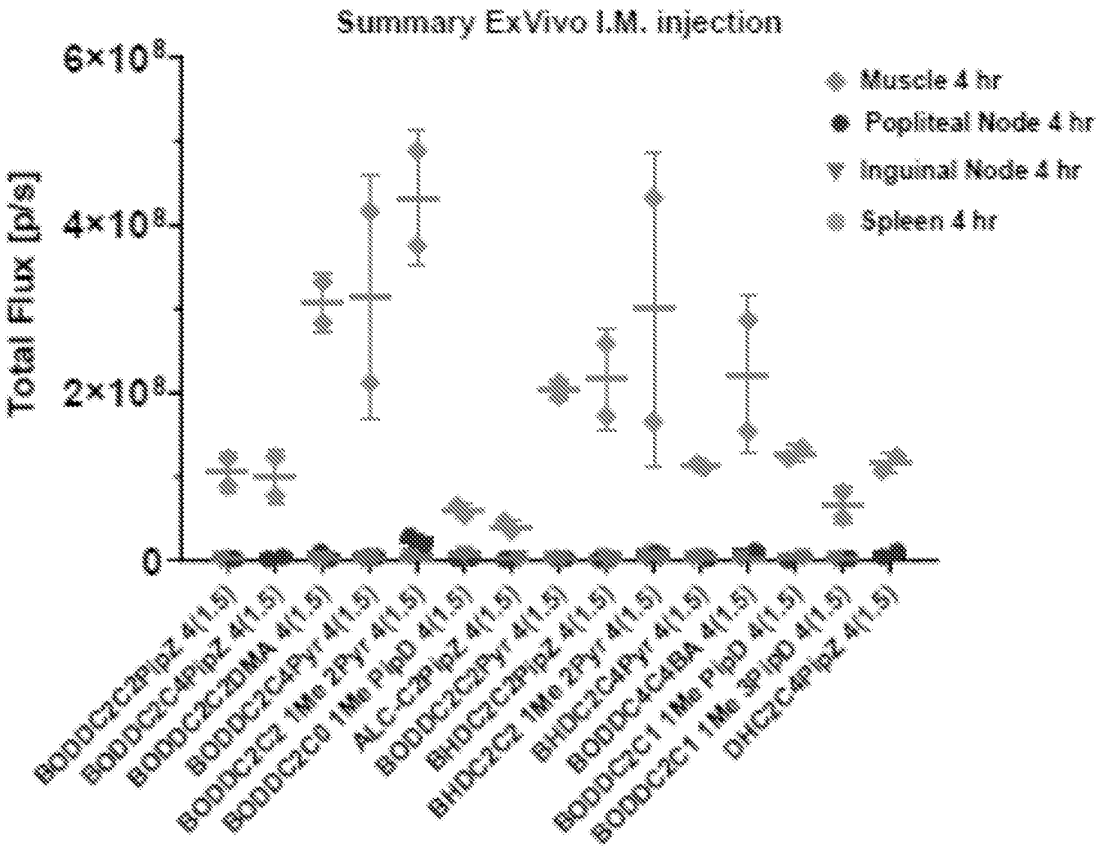


FIGURE 82

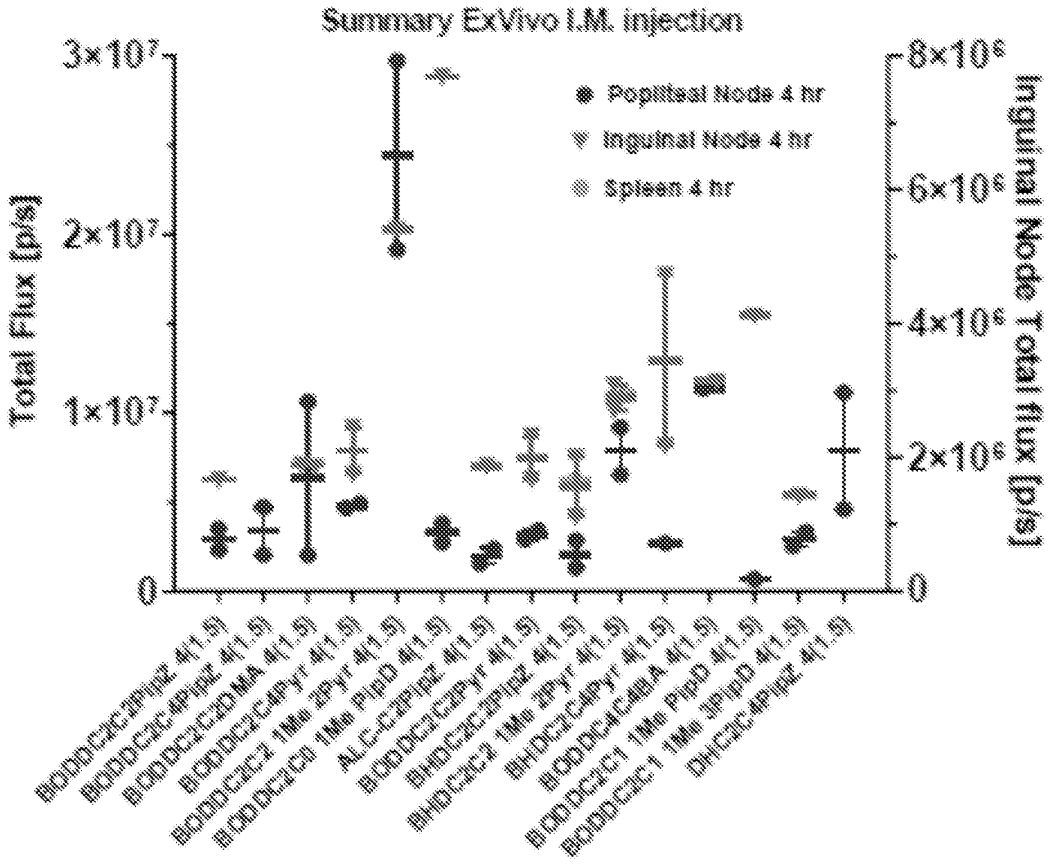


FIGURE 83

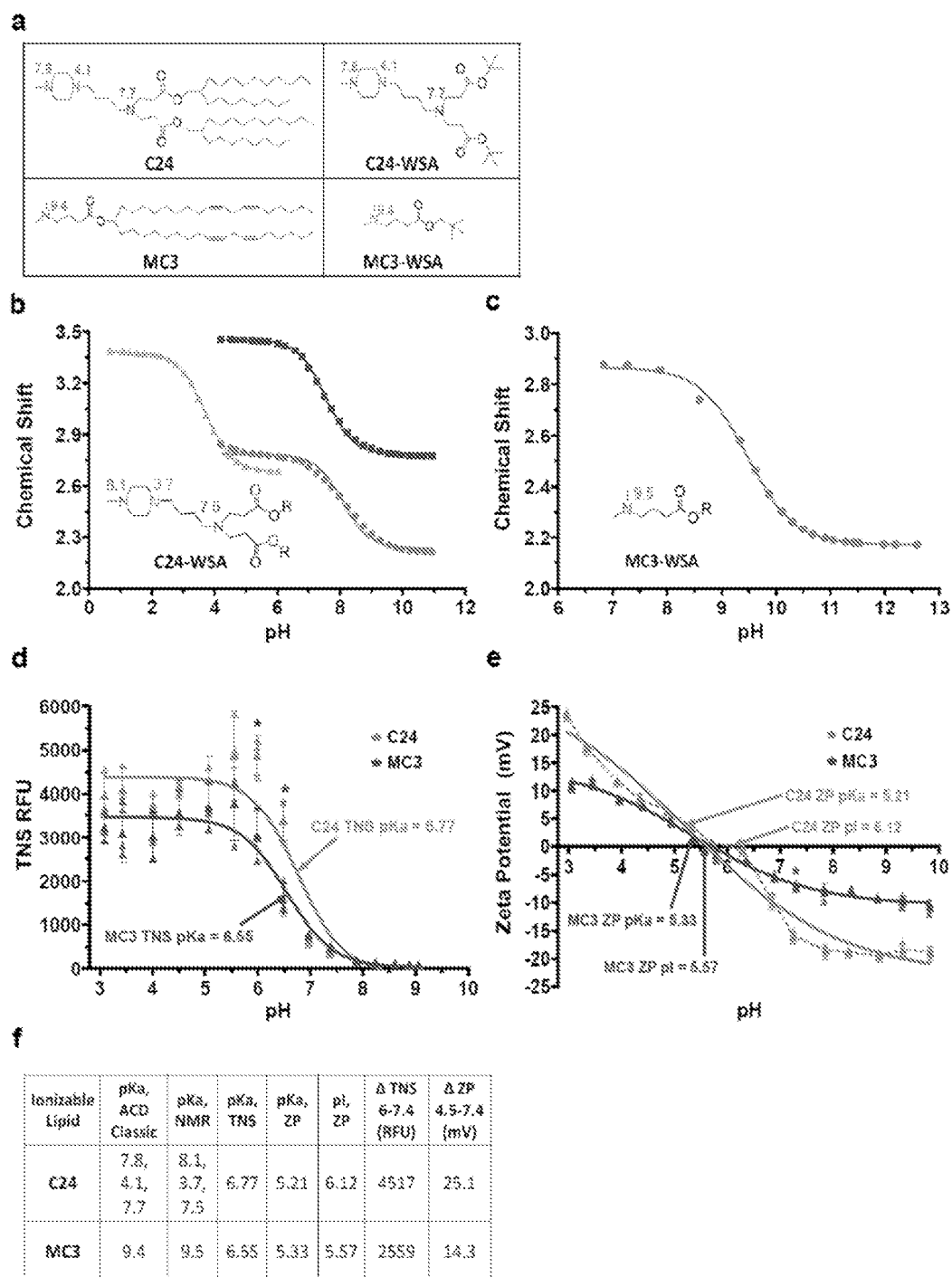


FIGURE 84

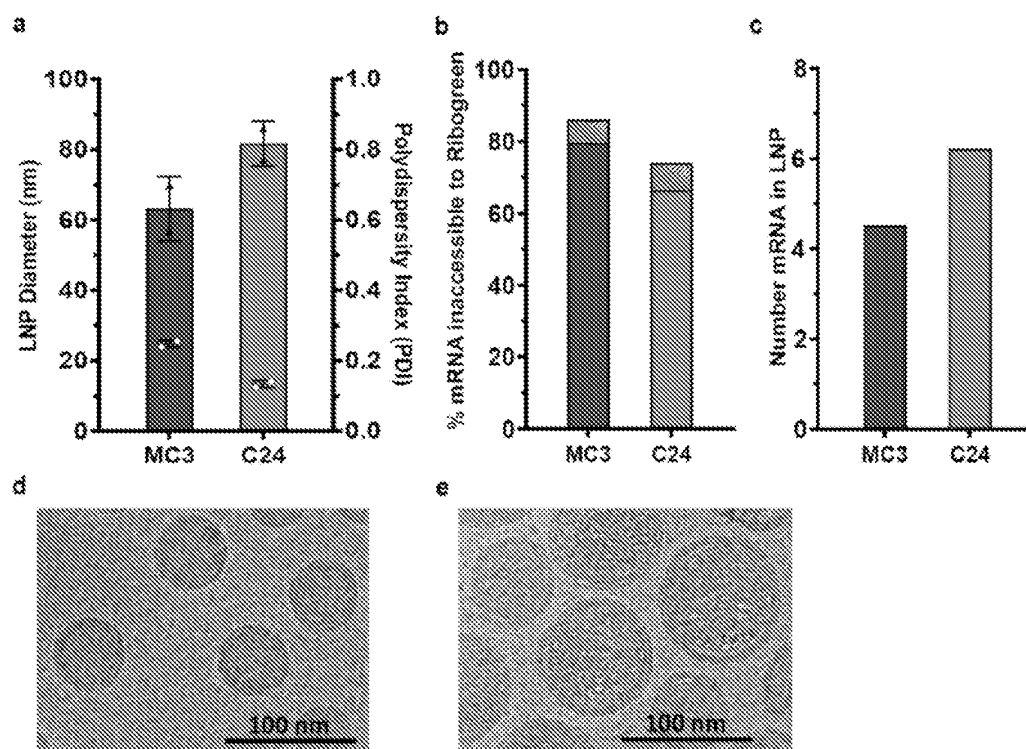


FIGURE 85

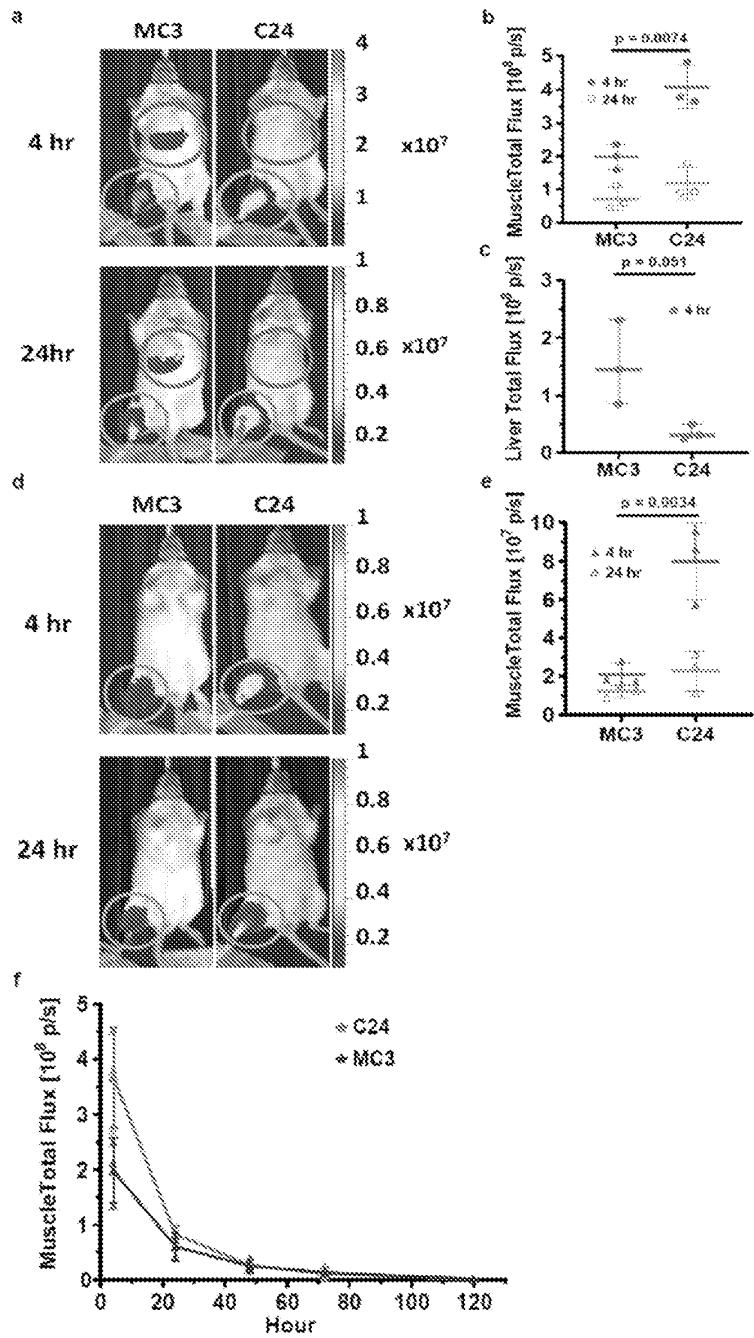


FIGURE 86

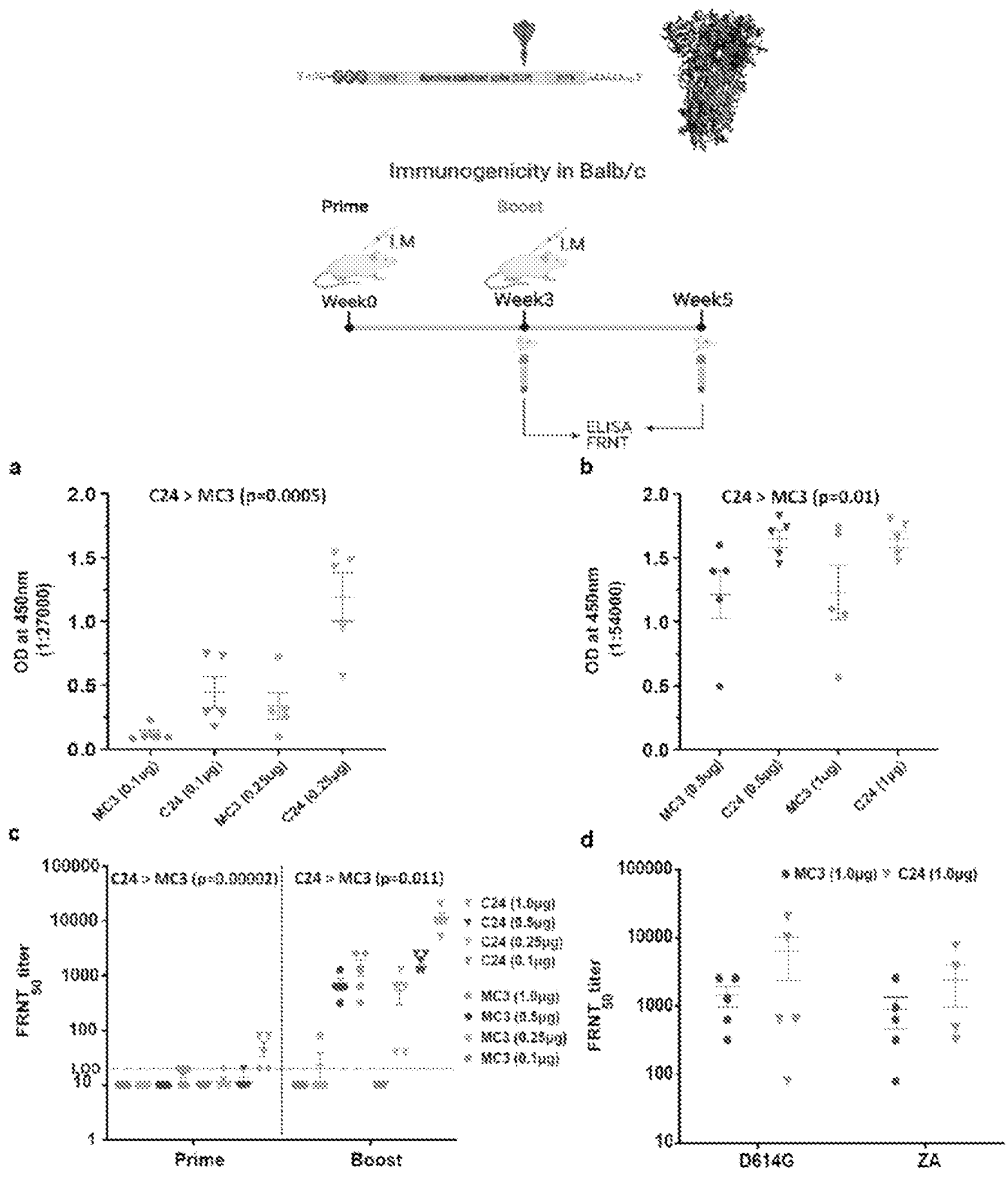


FIGURE 87

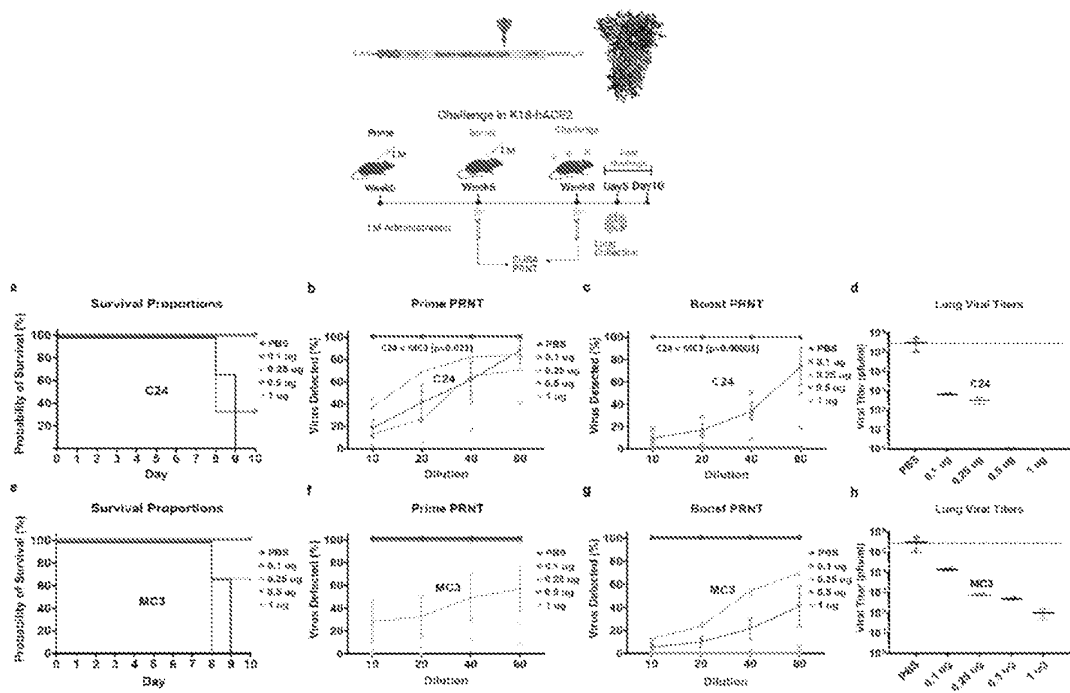


FIGURE 88

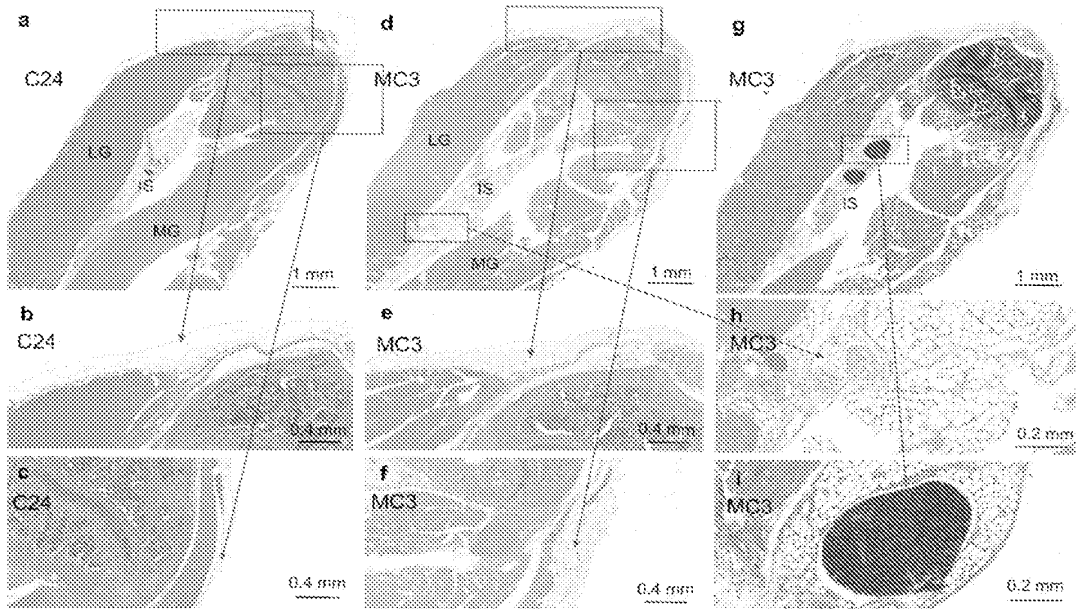


FIGURE 89

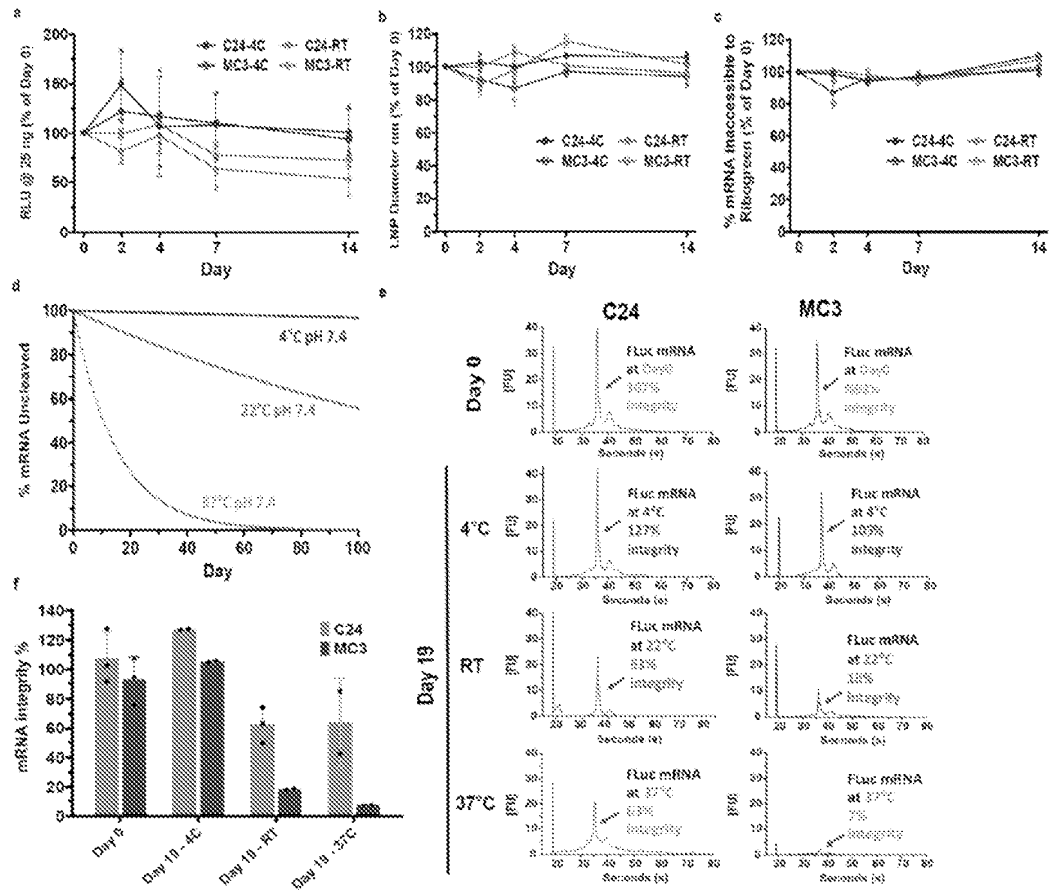


FIGURE 90

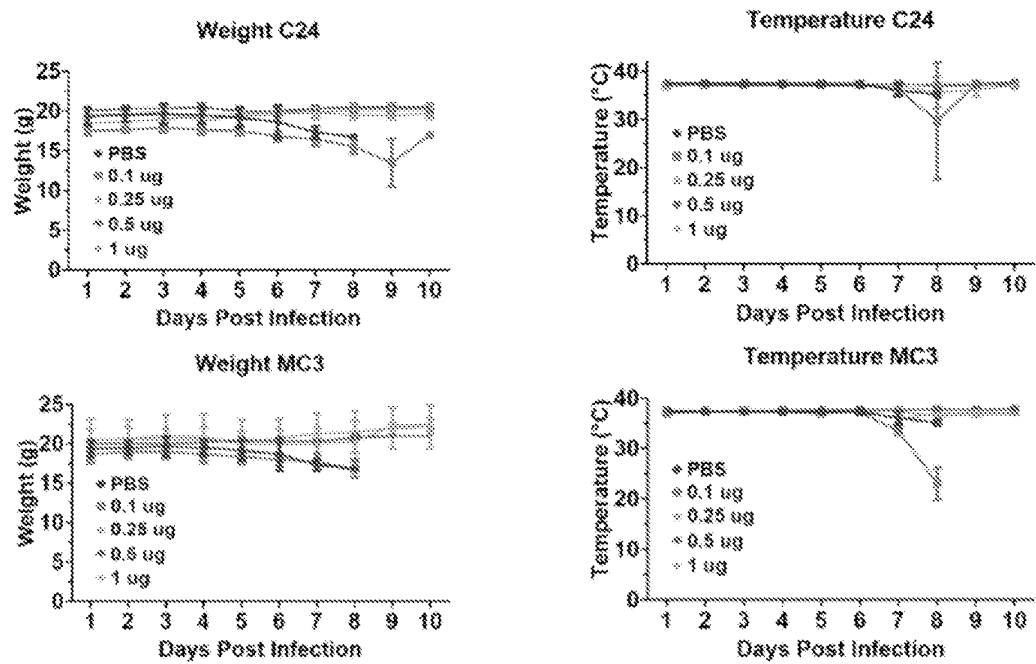


FIGURE 91

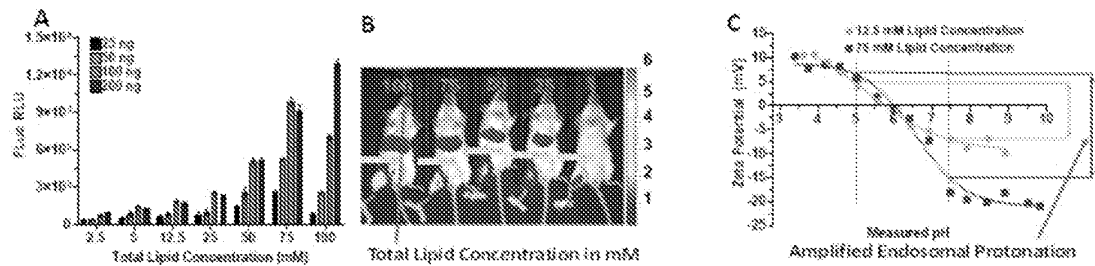


FIGURE 92



$C_{n=1-3}/C2$	$C_{n=1-3}/C3$	$C_{n=1-3}/C4$
Chemical structure showing the repeating unit for $C_{n=1-3}/C2$ with $n=1$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 7.8 Å (N-C), 9.6 Å (C-C), 9.2 Å (C-N), and 9.0 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C3$ with $n=1$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.9 Å (N-C), 7.8 Å (C-C), 4.9 Å (C-N), and 4.8 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C4$ with $n=1$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.4 Å (N-C), 8.7 Å (C-C), 6.3 Å (C-N), and 6.2 Å (C-C).
Chemical structure showing the repeating unit for $C_{n=1-3}/C2$ with $n=2$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.9 Å (N-C), 8.8 Å (C-C), 9.4 Å (C-N), and 9.0 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C3$ with $n=2$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.1 Å (N-C), 8.7 Å (C-C), 8.3 Å (C-N), and 8.2 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C4$ with $n=2$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.2 Å (N-C), 8.7 Å (C-C), 8.8 Å (C-N), and 8.7 Å (C-C).
Chemical structure showing the repeating unit for $C_{n=1-3}/C2$ with $n=3$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.2 Å (N-C), 8.8 Å (C-C), 7.8 Å (C-N), and 7.7 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C3$ with $n=3$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.4 Å (N-C), 8.7 Å (C-C), 7.3 Å (C-N), and 7.2 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C4$ with $n=3$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.8 Å (N-C), 8.7 Å (C-C), 7.7 Å (C-N), and 7.6 Å (C-C).
Chemical structure showing the repeating unit for $C_{n=1-3}/C2$ with $n=4$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.4 Å (N-C), 8.8 Å (C-C), 7.3 Å (C-N), and 7.2 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C3$ with $n=4$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.8 Å (N-C), 8.7 Å (C-C), 7.8 Å (C-N), and 7.7 Å (C-C).	Chemical structure showing the repeating unit for $C_{n=1-3}/C4$ with $n=4$. The structure includes a piperazine ring connected via a methylene group to a carbonyl-containing chain. Bond lengths are indicated: 8.8 Å (N-C), 8.7 Å (C-C), 7.8 Å (C-N), and 7.7 Å (C-C).

FIGURE 94

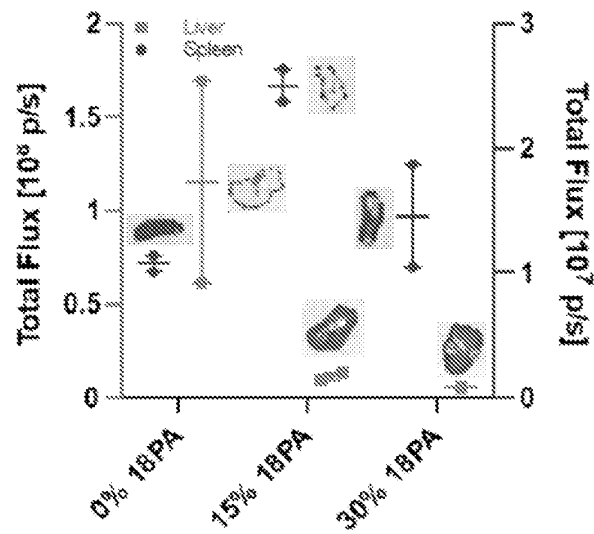


FIGURE 95

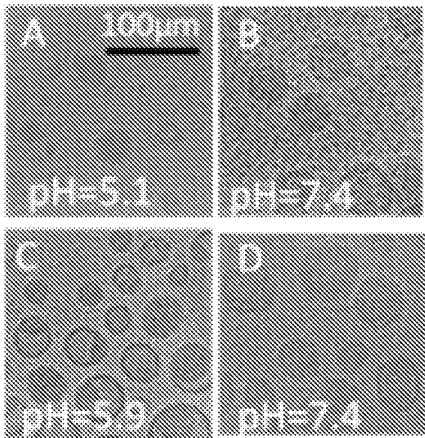


FIGURE 96