Supplementary Materials

Poly(hydrophobic amino acids) and liposomes for delivery of vaccine against Group A Streptococcus

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Supporting Information

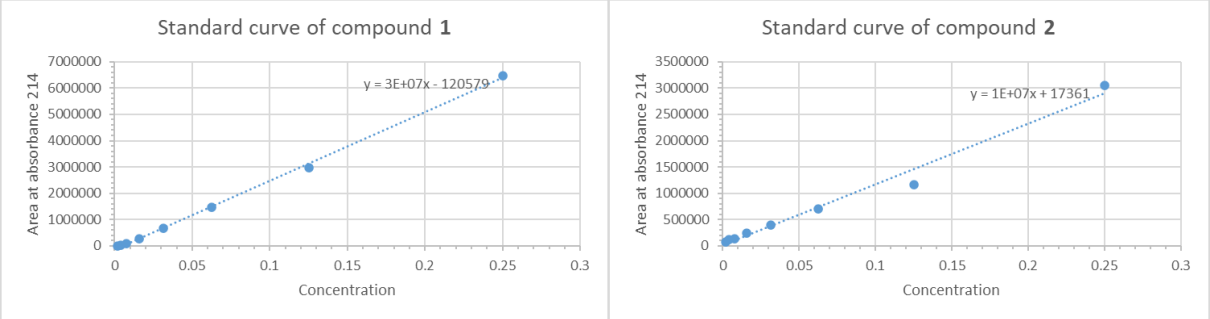


Figure S1. Standard curves of peptide **1** and polyleucine conjugate **2** used to calculate the concentrations of compound entrapped in the liposomes. These standard curves were plotted using area under compounds' peak against different compound concentrations (dissolved in PBS:methanol solution) that were injected (30 µL) into analytical RP-HPLC (on C18 or C4 Vydac column for compounds **1** and **2**, respectively) using 0-100% gradient of solvent B for 30 min with compound detection at 214 nm.

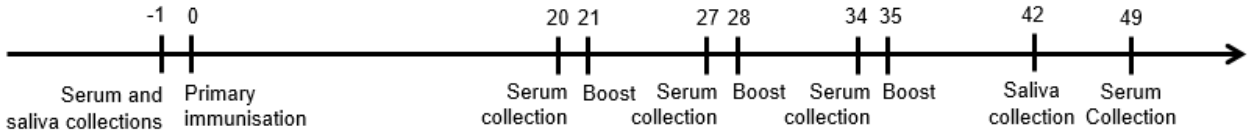


Figure S2. Immunization schedule with marked immunization, serum, and saliva collection days.

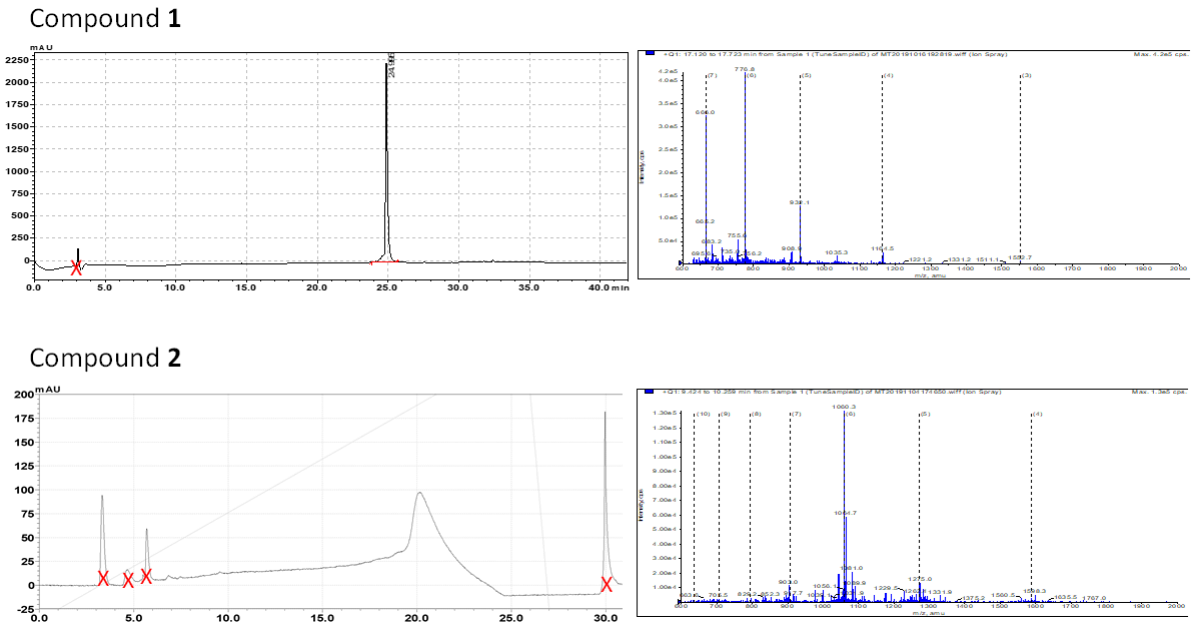


Figure S3. Analysis of purified compounds **1** and **2** by analytical RP-HPLC and ESI-MS. The compounds were purified using preparative RP-HPLC with solvent B concentration gradient 30-50% (**1**) and 65-85% (**2**) from Rt 5 min to 30 min. Analytical RP-HPLC graphs show pure compounds in single peak. The mass from these peaks, matched to the desired compounds in ESI-MS. "X" marks the background noise of the RP-HPLC column.

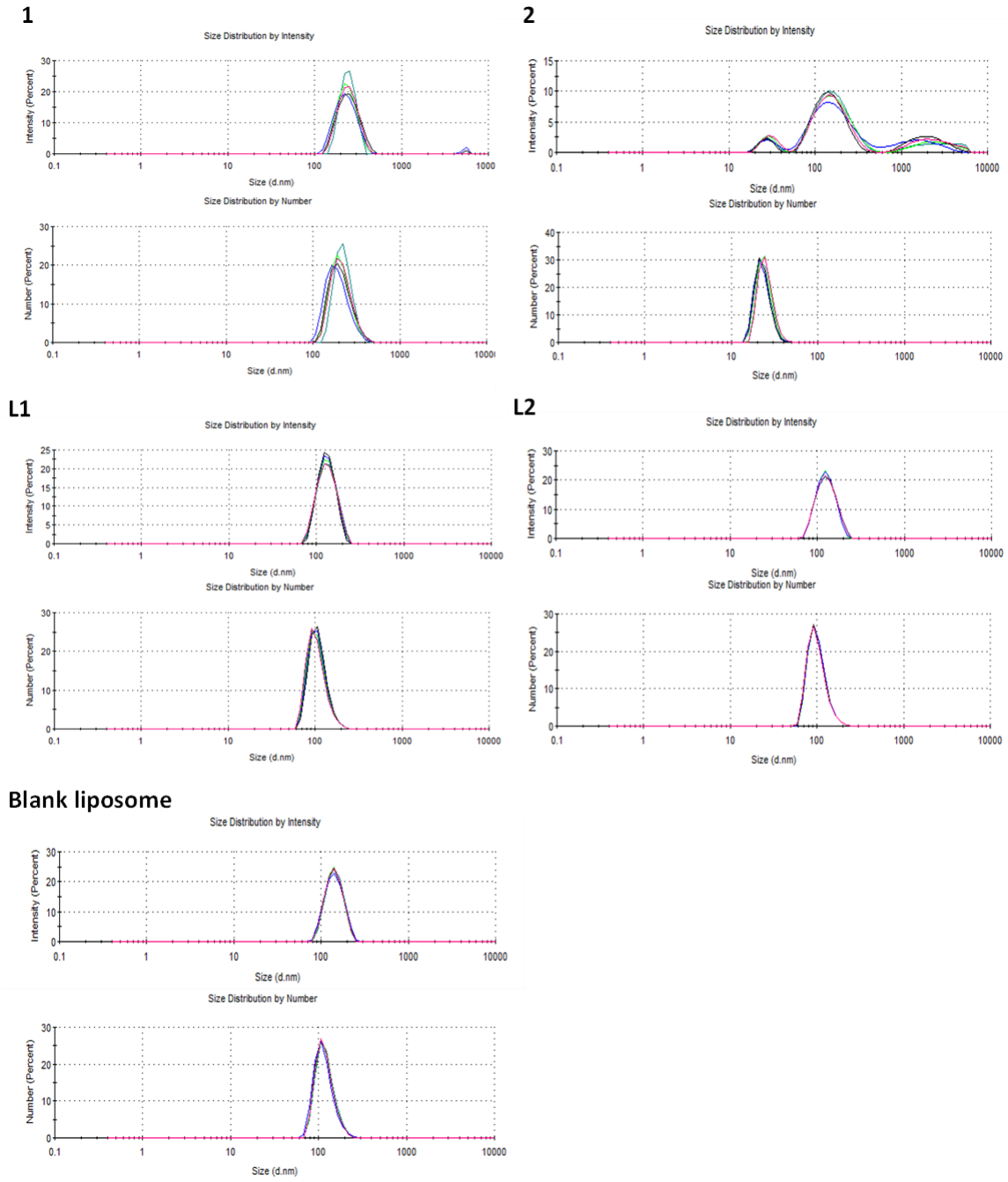
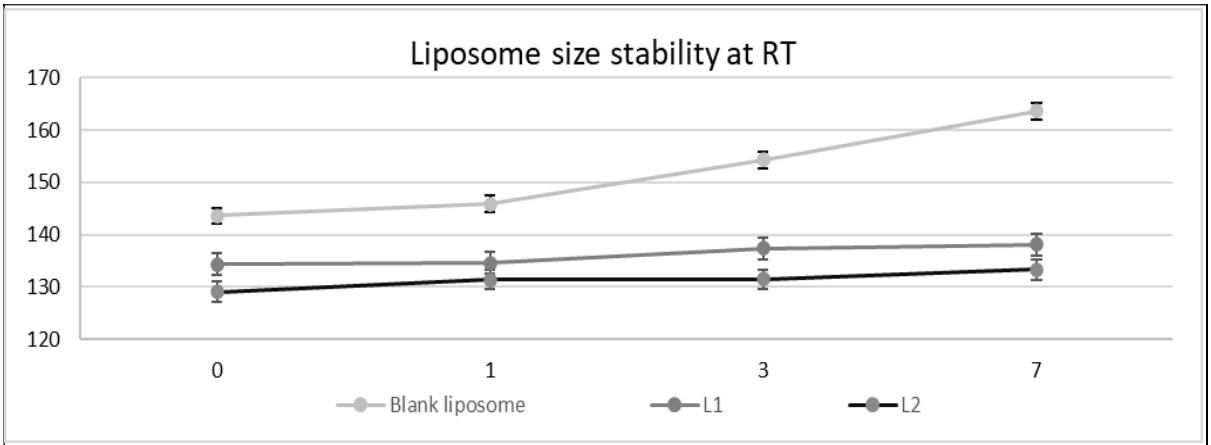
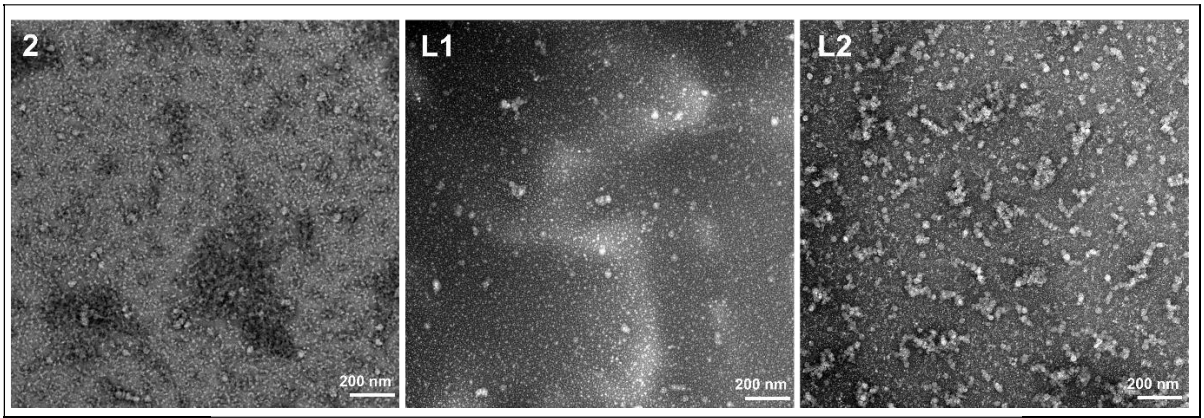


Figure S4. DLS spectra of particle size, by intensity (top) and number (bottom) for compounds **1** and **2**, encapsulated liposomes **L1** and **L2**, and blank liposome.



Liposome	Particle size (nm)		PDI	
	Day 0	Day 7	Day 0	Day 7
Extrusion				
L1	134 ± 1	138 ± 4	0.03 ± 0.02	0.02 ± 0.01
L2	129 ± 1	133 ± 3	0.05 ± 0.02	0.02 ± 0.01
Blank Liposome	144 ± 1	164 ± 2	0.02 ± 0.01	0.02 ± 0.01
Sonication				
L1	81 ± 1 4199 ± 536	88 ± 5 4514 ± 271	0.23 ± 0.02	1.00 ± 0.01
L2	121 ± 6 4737 ± 260	127 ± 5 4778 ± 163	0.19 ± 0.01	0.21 ± 0.02
Blank Liposome	79 ± 2 4469 ± 199	2690 ± 180	0.16 ± 0.01	0.26 ± 0.03

Figure S5. Liposome size stability over 7 days period at RT.



Compound	Particle size (nm)		PDI
	TEM	DLS[a)]	
FBS	Particles not visible (data not shown)	14 ± 1 59 ± 1	0.29 ± 0.01
1	Particles not visible (data not shown)	48 ± 1 4949 ± 282	0.31 ± 0.04
2	10-20 nm NP and CLANs	27 ± 1 153 ± 1 4393 ± 578	0.97 ± 0.05
L1	Liposomes and particles not visible	23 ± 5 164 ± 46 2244 ± 130	0.97 ± 0.05
L2	10-20 nm NP, CLANs, and 50-70 nm spherical aggregates	16 ± 2 47 ± 4 537 ± 24 4304 ± 530	0.99 ± 0.01

[a)]*size as measured by intensity.*
NP = nanoparticles; CLANs = chain like aggregates of nanoparticles

Figure S6. Stability of the vaccine candidates after incubation in FBS at 37°C for 1 hour.

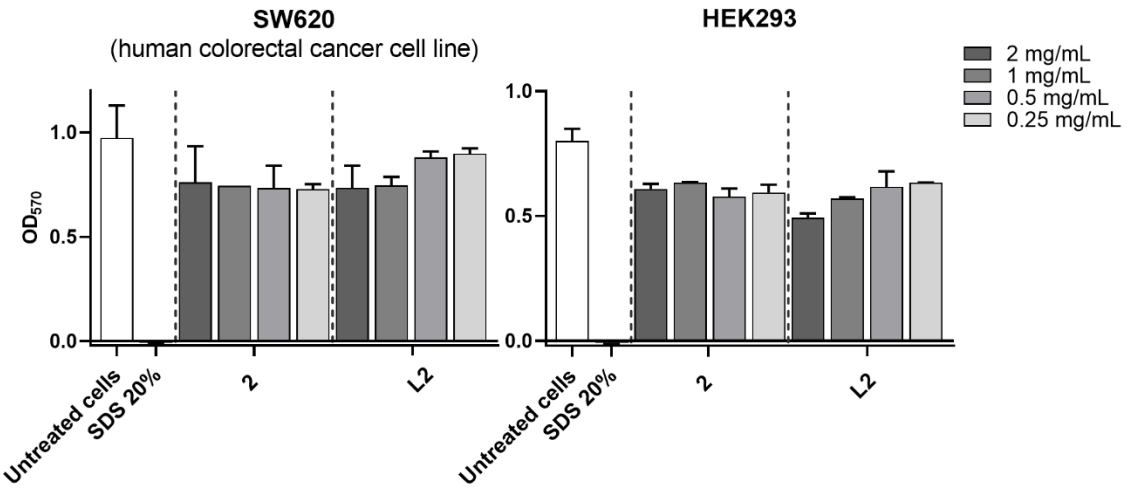


Figure S7. MTT cytotoxicity assay of polyleucine conjugate **2** and liposome **L2**.

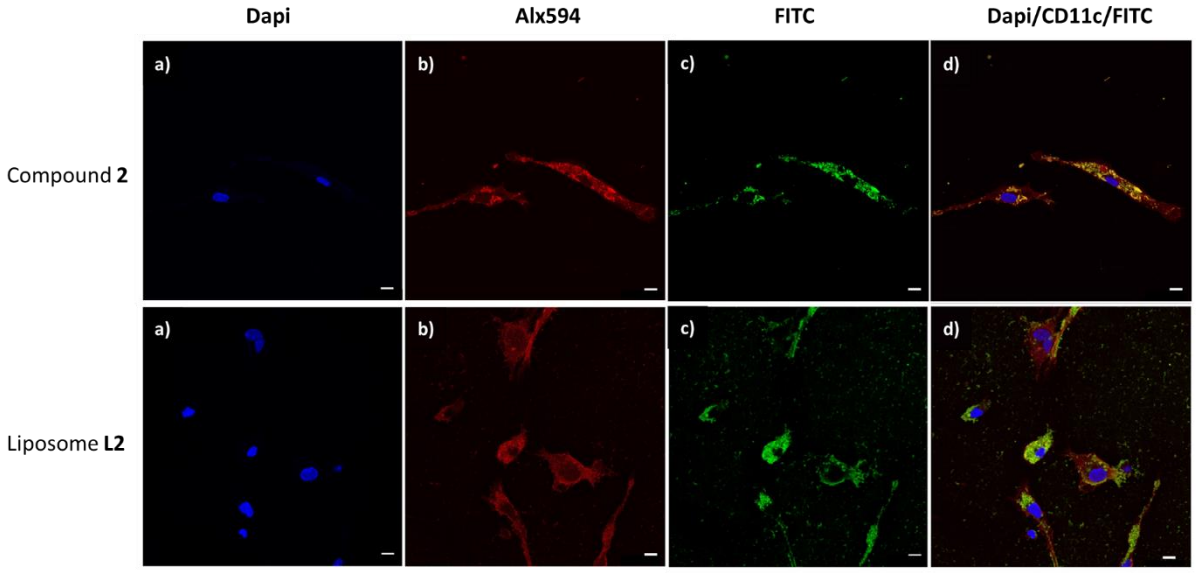


Figure S8. Microscopy of dendritic cells showing nanoparticle **2** and liposome **L2** uptake. Confocal 60x in oil immersion showing nanoparticle and liposome uptake. Cell channels were split to (**a**) Dapi channel showing cell nuclei (blue staining), (**b**) Alx594 channel showing CD11c cytoplasm (red staining; CD11c membrane marker was chosen to show nanoparticle/liposome uptake, where particles are surrounded by red staining in the membrane), (**c**) FITC channel showing nanoparticle or liposome (green staining) and (**d**) merged images showing nanoparticles or liposomes were uptaken into the cells.