

Table S1. Conjugation efficiency and size measurements of liposomes conjugated with click chemistry

Incorporation of more than 2% DSPE-PEG(2000)-DBCO lipid causes aggregation of the liposomes, also when no peptide is conjugated at all. With lower percentages of DBCO lipid, formulations are stable in size, also when peptide is conjugated. Coupling efficiency is measured by UPLC and no free peptide could be detected in all but the 1:1 sample. The detection limit was >0.01% of the lowest amount added. All liposomes contained 6% of PEG lipids.

DBCO content	DBCO:azide ratio	Size	PDI	Zeta potential	Coupling efficiency
0%	-	151.4±2.3	0.08±0.05	-11.4±0.8	-
1%	-	152.8±1.5	0.13±0.02	-11.2±1.2	-
1%	4:1	152.1±0.5	0.12±0.01	-3.8±0.9	>99.9%
1%	2:1	155.3±3.0	0.09±0.05	-3.1±1.8	>99.9%
1%	1:1	343.7±23.1	0.86±0.07	-2.1±1.5	>95.0%
2%	-	165.2±1.9	0.15±0.01	-13.4±0.6	-
2%	8:1	162.4±2.3	0.14±0.01	-4.6±1.4	>99.9%
2%	4:1	165.2±0.8	0.12±0.01	-2.7±1.7	>99.9%
2%	2:1	164.7±0.4	0.13±0.04	-3.5±1.1	>99.9%
3%	-	234.0±11.4	0.52±0.06	-12.6±2.3	-
3%	12:1	222.2±9.6	0.44±0.04	-4.5±1.5	>99.9%
3%	6:1	222.0±4.5	0.45±0.05	-4.6±1.2	>99.9%
3%	3:1	231.8±5.1	0.45±0.01	-3.9±0.9	>99.9%

Table S2. Physicochemical properties of peptides

Peptide	HPLC T _R (min)	Molecular Weight	
		Calculated	Found [M] ²⁺
Melittin 1	14.09 ^a	2846.52	1422.34
Azido-Melittin 2	18.90 ^a	2929.55	1464.44
Acid-Melittin-cysteine 3	18.42 ^a	2898.34	1449.46
Azido-Acid-Melittin 4	19.57 ^b	2878.24	1438.71
Hydrazide-Acid-Melittin-Cysteine 5	23.21 ^a	3040.50	1520.20

^a Linear gradient from 40 to 70% of (B) for 30 min at a flow rate 1 mL/min, Dr. Maisch C₁₈ column

^b Linear gradient from 50 to 80% of (B) for 30 min at a flow rate 1 mL/min, Dr. Maisch C₁₈ column

The following solvent system was used: (A) acetonitrile in 0.1% aqueous TFA (5:95, v/v) and (B) acetonitrile in 0.1% aqueous TFA (95:5, v/v).

Table S3. Calcein Leakage measurements with DMSO (RAW DATA)

pH 7.4	pH 6.8	pH 6.2	pH 5.6	
149,2937	148,0347	145,9575	115,0819	0.5% Triton
149,9865	149,2962	145,9884	116,8047	0.5% Triton
149,5735	149,3956	145,32	107,4901	0.5% Triton
10,1344	9,6981	9,9809	9,8657	2%DMSO
10,1373	9,8895	10,0158	9,9043	2%DMSO
10,1193	9,9806	9,8925	9,8261	2%DMSO
10,0848	9,8243	9,9757	9,8877	2%DMSO
9,8163	9,4492	9,6059	9,4855	no DMSO
9,7979	9,6174	9,5789	9,4699	no DMSO
9,8081	9,6103	9,6509	9,3782	no DMSO
9,5069	9,5829	9,525	9,4588	no DMSO