Electronic Supplementary Material (ESI) for Nanoscale Advances.

Materials and Methods

Lipids for the preparation of GUVs and liposomes including 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (NBD PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Liss Rhod PE), 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl] (nickel salt) (DGS-NTA-Ni), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)-2000] (DSPE-PEG) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Recombinant human TRAIL with his-tag was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Human prostate cancer PC3 and DU145 cells and acute T-cell leukemia Jurkat cells were obtained from American Type Culture Collection (ATCC) (Rockville, MD, USA) and cultured in F-12k, EMEM and RPMI media, respectively, with 10% FBS.

Preparation of Giant Unilamellar Vesicles (GUVs)

Preparation of GUVs was initiated by formation of a lipid/sugar film, followed by hydration with PBS. To form the film, a solution of mixed lipids prepared in a 10 mL glass test tube following the formulation in the table below:

Lipids (500 nmol in total)	DSPC	DOPC	Cholesterol	DGS-NTA(Ni)	NBD PE	Liss Rhod PE	DSPE-PEG
Stock Solution in $CHCl_3$, mg/mL	10.0	10.0	25.0	10.0	1.0	1.0	25.0
Molar Percentage	50.25 %	16.75%	30%	2%	0.5%	0.1%	1%
Volume, µL	20	6.5	1.6	1.1	2.2	0.7	0.6

To the mixed solution, 200 µL MeOH was added, containing glucose that is 10-molar fold of the total lipids. The organic solvent was removed with a nitrogen stream. To remove residual solvent, the test tube was placed for at least 2 h in a vacuum desiccator. The film was hydrated at 65°C by gently adding 1 mL PBS. GUVs were sampled from the hydration solution after 30 min of hydration.

Preparation of nanoscale liposomes

A lipid film was prepared using the same lipid formulation as the GUVs with no glucose included. The film was hydrated at 65°C using 1 mL PBS and the hydration was allowed to proceed for one hour before the mixture was extruded through a 100 nm polycarbonate membrane 10 times at 65°C. The liposome suspension was then cooled down to room temperature. The number concentration of liposomes was estimated using an equation from www.liposomes.org. A calculated amount of his tag TRAIL was added to a liposome suspension form liposomes with 100, 200 and 400 TRAIL in average on an individual liposome assuming the conjugation yield is nearly 100%. The number of liposomes was estimated using an equation on www.liposomes.org. Size distribution before and after TRAIL conjugation was measured using a ZetaSizer instrument (Malvern Panalytical, UK). For long term storage, trehalose was added at 10X molar of the total lipids and the

liposomes were flash frozen with dry ice/ethanol. The liposomes were stored at -80°C before use.

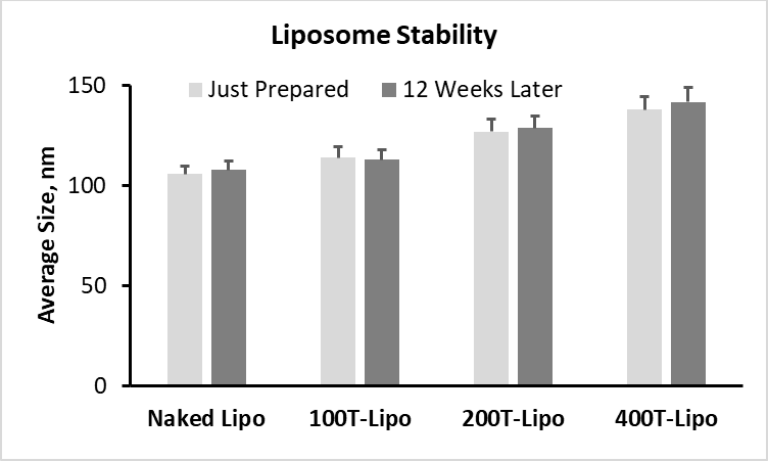

Figure 1. Long-term liposome stability. Size distribution was measured immediately after preparation and thawed after storage at -80°C. 100T-, 200T- and 400T-Lipo refers to liposomes conjugated with 100, 200 and 400 TRAIL molecules on average on an individual liposome.

Confocal imaging of GUVs and live cells

30-50 μL suspension of GUVs or cells in PBS was placed on a glass slide and covered with a cover slip that was spaced with the glass slides using small pieces of broken cover slip. The samples were sealed before imaging with a Zeiss LSM 900 confocal microscope with a 63x objective (Munster, Germany).

Apoptosis Assay

PC3 or DU145 cells were seeded in 12-well plates at an initial density of 10^5 cells per well, 24 h before treatment. Media was changed immediately before treatment. Cells were incubated with soluble TRAIL and liposomal TRAIL at 100 ng/mL for PC3 cells, and 250 ng/mL for DU145 cells. Jurkat cells were seeded at the same cell density and treated immediately with the same formulations at 50 ng/mL TRAIL for 24 h. TRAIL concentration for the binding of TRAIL liposomes to Jurkat cells was 10 μg/mL. Cell apoptosis was analyzed at 24 h by Annexin V/Propidium Iodide (PI) apoptosis assay using a Guava® easyCyte™ 11HT flow cytometer (MilliporeSigma, Burlington, MA, USA).