Electronic Supplementary Information

Article Title: Killing cancer cells with nanotechnology: novel poly(I:C) loaded liposome-silica hybrid nanoparticles

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Fitting procedure of synchrotron small angle X-ray scattering data

The scattered SAXS intensity collected from both unpegylated and pegylated MC liposomes was fitted with the simplest of the realistic bilayer models[21] in which the EDP is given by a negative Gaussian at the center of the bilayer accounting for the hydrocarbon chains and two positive Gaussians representing the headgroups that were always assumed to be symmetric to the bilayer center of weakly ordered membrane stacks

$$I(q) = 2\pi \frac{[2\sigma_H \exp(-\sigma_H^2 q^2/2)\cos(qz_H) - \sigma_C \rho \exp(-\sigma_C^2 q^2/2)]^2}{q^2}$$

where σ_H is the half width at half maximum of the Gaussian representing the polar region, z_H is the distance of the headgroup to the bilayer center, σ_C is the standard deviation of the Gaussian at the center of the bilayer accounting for the hydrocarbon chains, ρ is the ratio between the methyl terminus electron density amplitude and the headgroup and the $1/q^2$ term is the usual Lorentz correction factor for isotropic scatterers. The primary parameters determined by the fit are σ_H, σ_C and z_H, from which the bilayer thickness, d_B, and the extension of the hydrophobic region, d_C, are calculated with results shown in Table S1.

Table S1. Structural parameters of unpegylated and pegylated MC cationic liposomes.

	Unpegylated MC liposomes	Pegylated MC liposomes
d_B (nm)	4.42 ± 0.13	4.40 ± 0.15
d_C (nm)	0.87 ± 0.07	0.91 ± 0.09

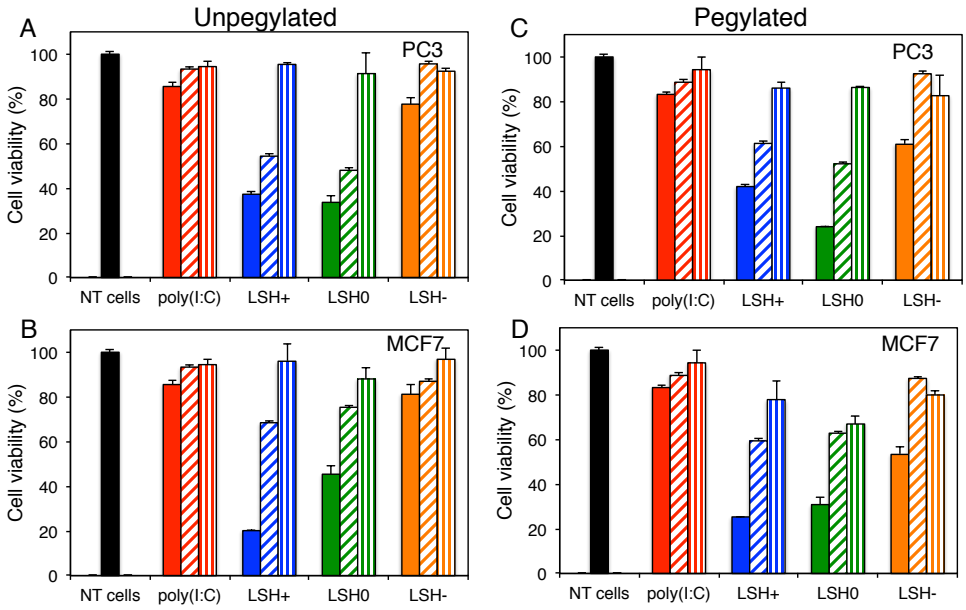

Figure S1. Counterpart of Figure 5 (for clarity reported below) for bare NPs (i.e. not loaded with poly(I:C)).

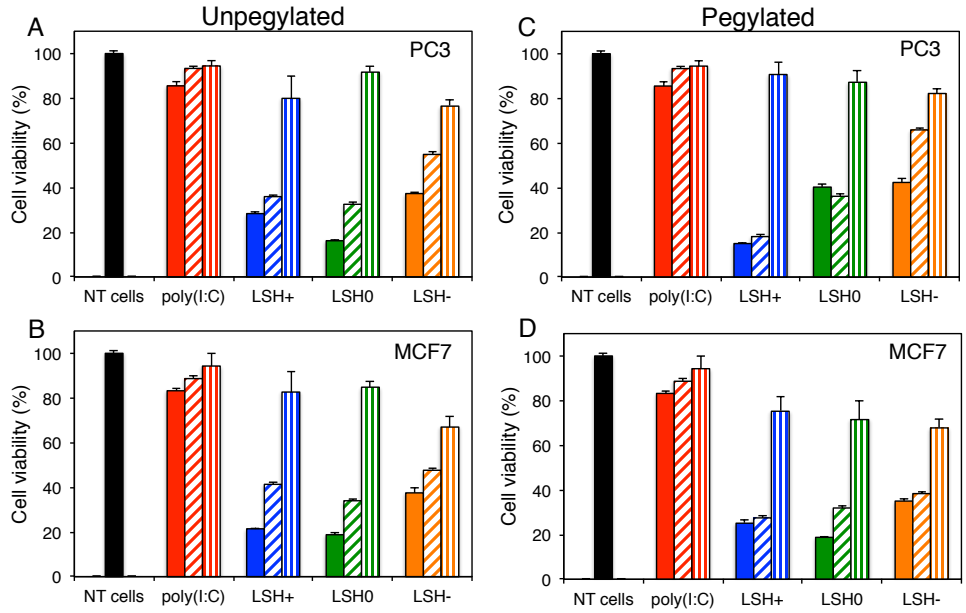

Figure 5. Cell viability of cancer cells after administration of positive, neutral and negative liposome-silica hybrid nanoparticles (LSH NPs) (LSH+, LSH0 and LSH- respectively) loaded with poly(I:C) at three different poly(I:C) concentrations spanning one order of magnitude: 2 (solid bars), 1 (diagonal dashed bars) and 0.2 (vertical dashed bars) μg/ml per well. Cell viability of unpegylated LSH NPs at 24 h after administration in PC3 and MCF7 cell lines (panels A and B). Cell viability of pegylated LSH NPs at 24 h after administration in PC3 and MCF7 cancer cell lines (panels C and D).

Encapsulation efficiency of poly(I:C)

The absorption spectra were recorded on a Jasco Spectrophotometer V-630 by using quartz cuvettes of 1 cm optical path, scan speed 200 nm/min. poly(I:C) concentration was determined by a dedicated Jasco software (*Protein/Nucleic Acid Quantitative Analysis*). 200 µl of LSH NPs were centrifuged at 14000 rpm for 20 min. The pellet was resuspended in 50 µl ultrapure water. Poly(I:C) in the supernatant was assumed to be free, i.e. not bound to LSH NPs. Loading ability was quantified according to the formula:

$$\text{Encapsulation (\%)} = 1 - \frac{\text{free poly(I:C)(µg)}}{\text{total poly(I:C)(µg)}} \text{x}100$$

Table S2. Encapsulation efficiency of poly(I:C) of unpegylated and pegylated positive, neutral and negative liposome-silica hybrid nanoparticles (LSH NPs) (LSH+, LSH0 and LSH- respectively).

	Encapsulation (%)	
	Unpegylated	**Pegylated**
LSH+	80	64
LSH0	95.5	74
LSH-	83	66