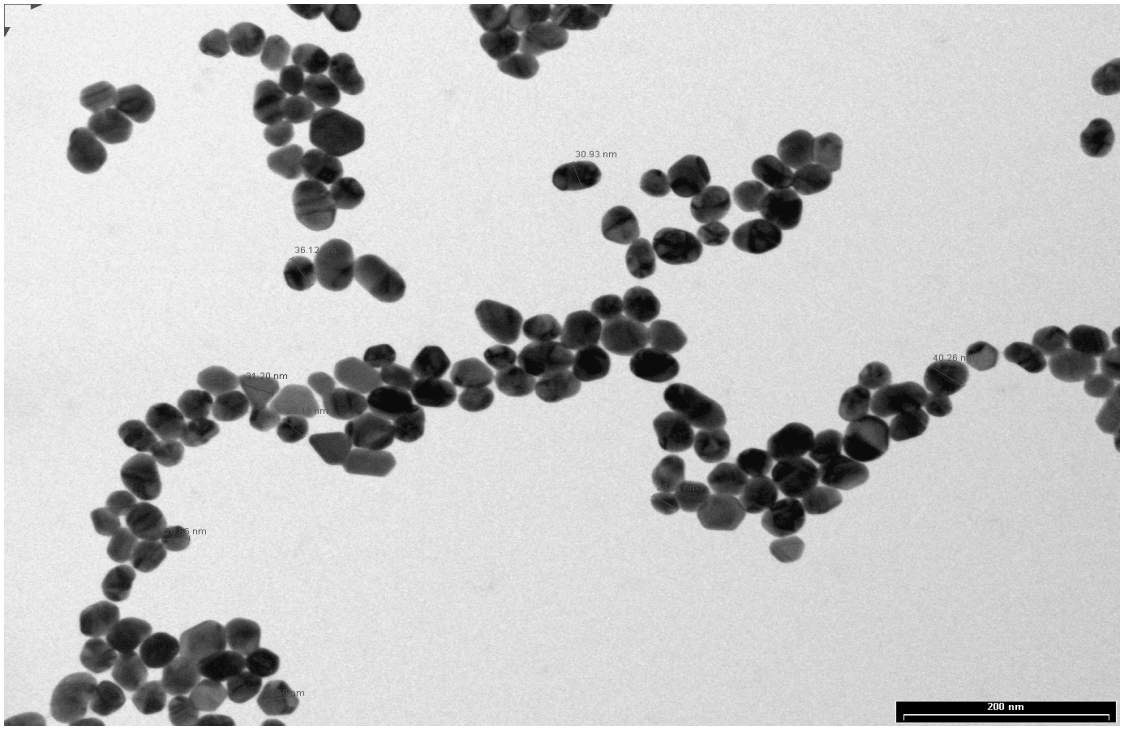
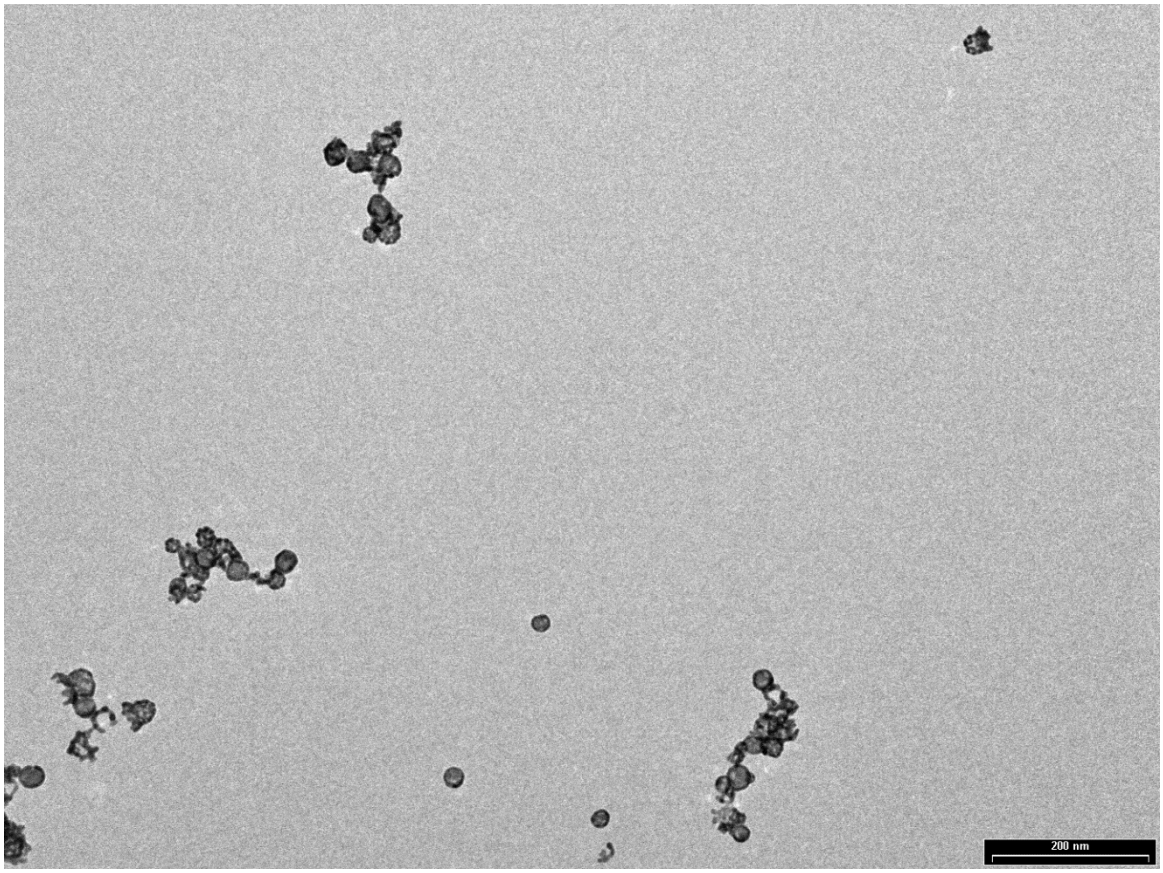
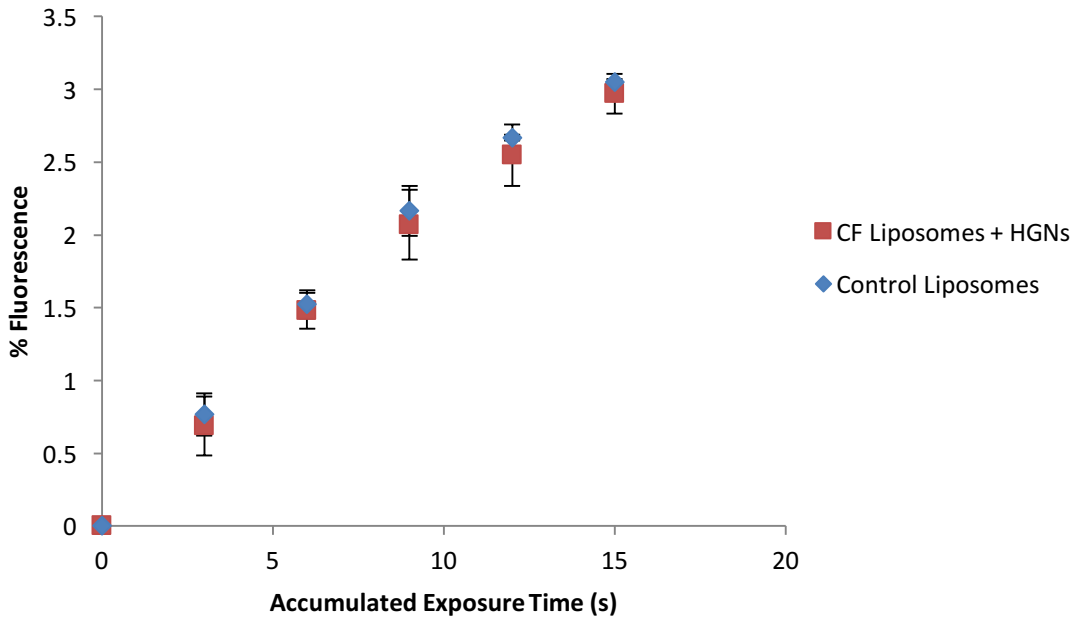


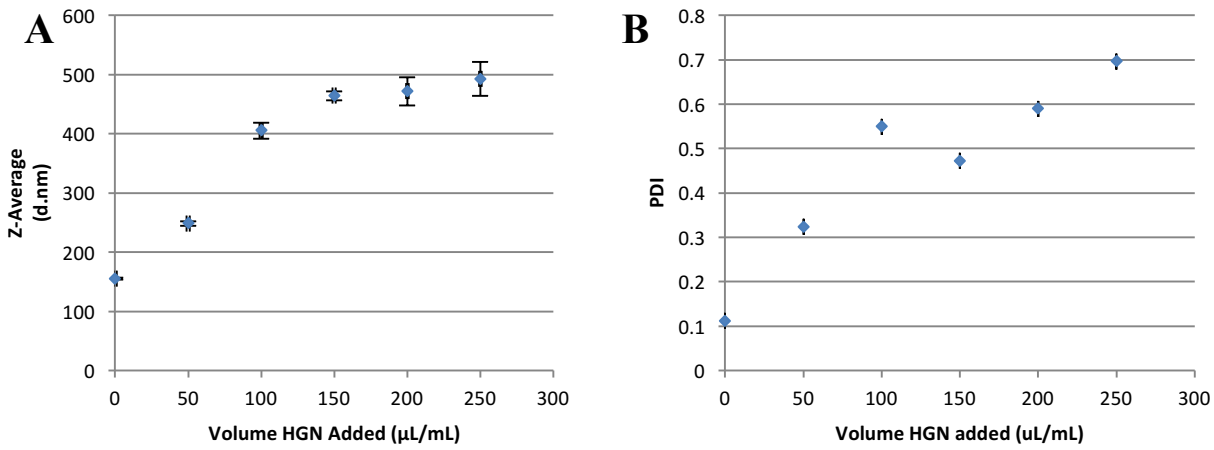
Supplementary Figure 1: Transmission electron microscopy of 25-30 nm solid gold nanoparticles (SNPs). Scale bar = 200 nm.



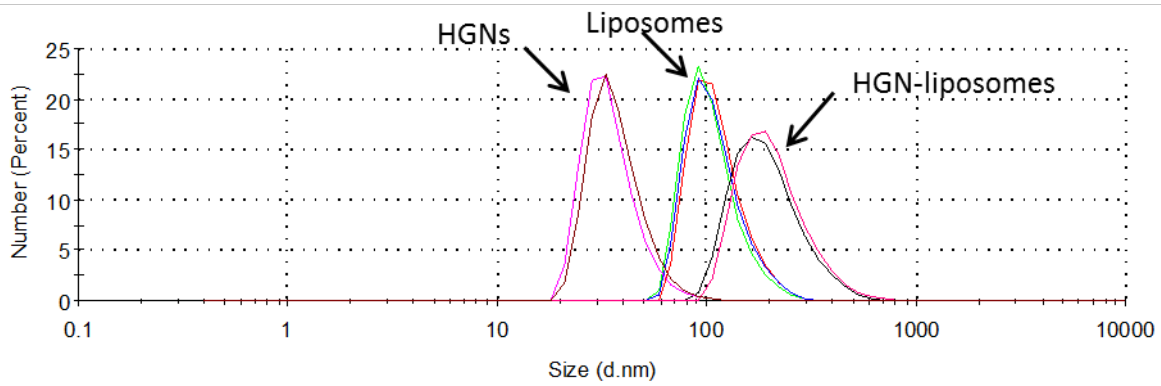
Supplementary Figure 2: Transmission electron microscopy of 25-35 nm hollow gold nanoshells (HGNs). Scale bar = 200 nm.



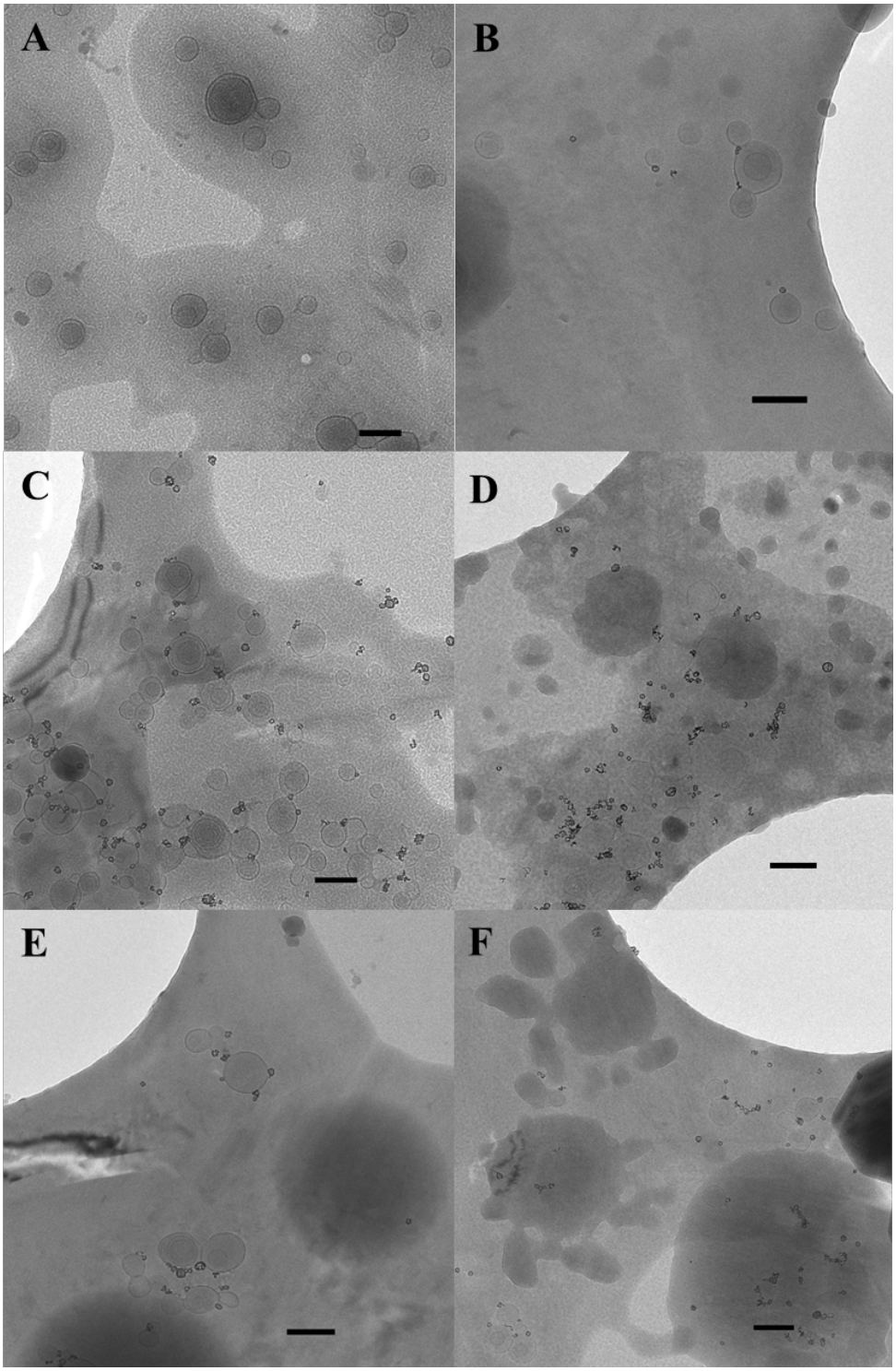
Supplementary Figure 3: Percentage fluorescence increase of CF released from liposomes in response to ultrasound at 1.5 W cm^{-2} (0.48 MPa) for control liposomes omitting the DSPE-PEG2000-SH tethering agent (blue diamonds) and liposomes co-suspended with HGNs (red squares). Error bars represent standard error of the mean (n=3).



Supplementary Figure 4: Hydrodynamic diameter (Z-average) (A) and polydispersity index (PDI) (B) of HGN-liposomes with an increasing HGN:lipid ratio. Error bars represent standard error of the mean (n=3).



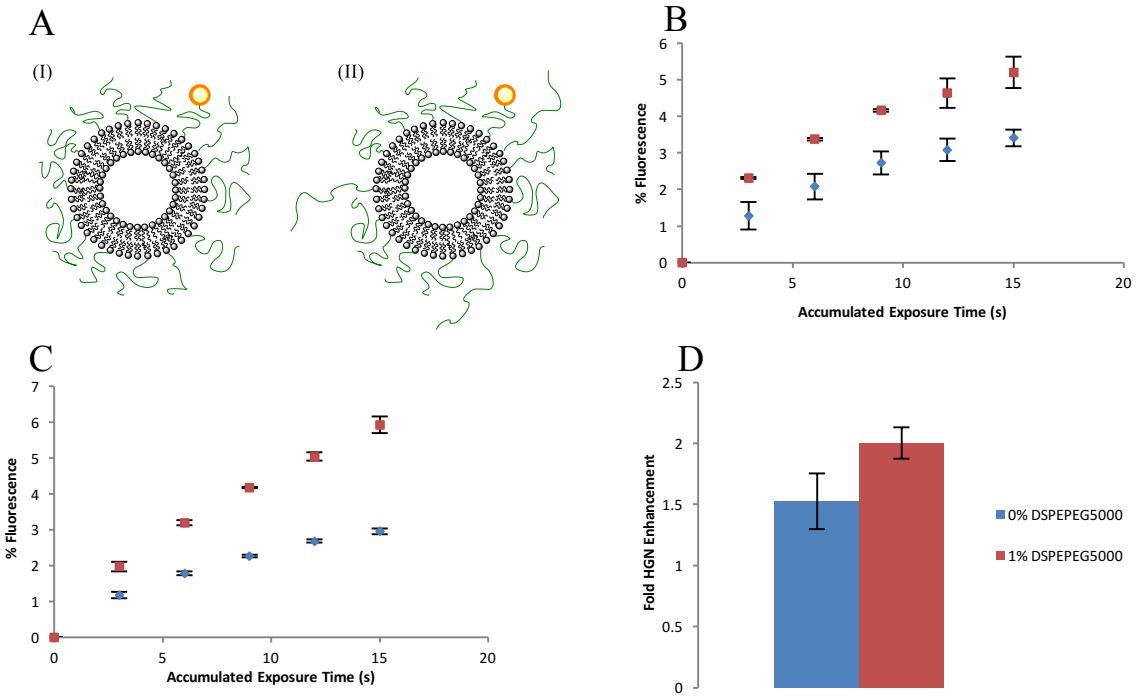
Supplementary Figure 5: DLS indicating the hydrodynamic diameter of HGNs, tether-ready liposomes, and HGN-liposome suspensions. Error bars represent standard error of the mean (n=3).



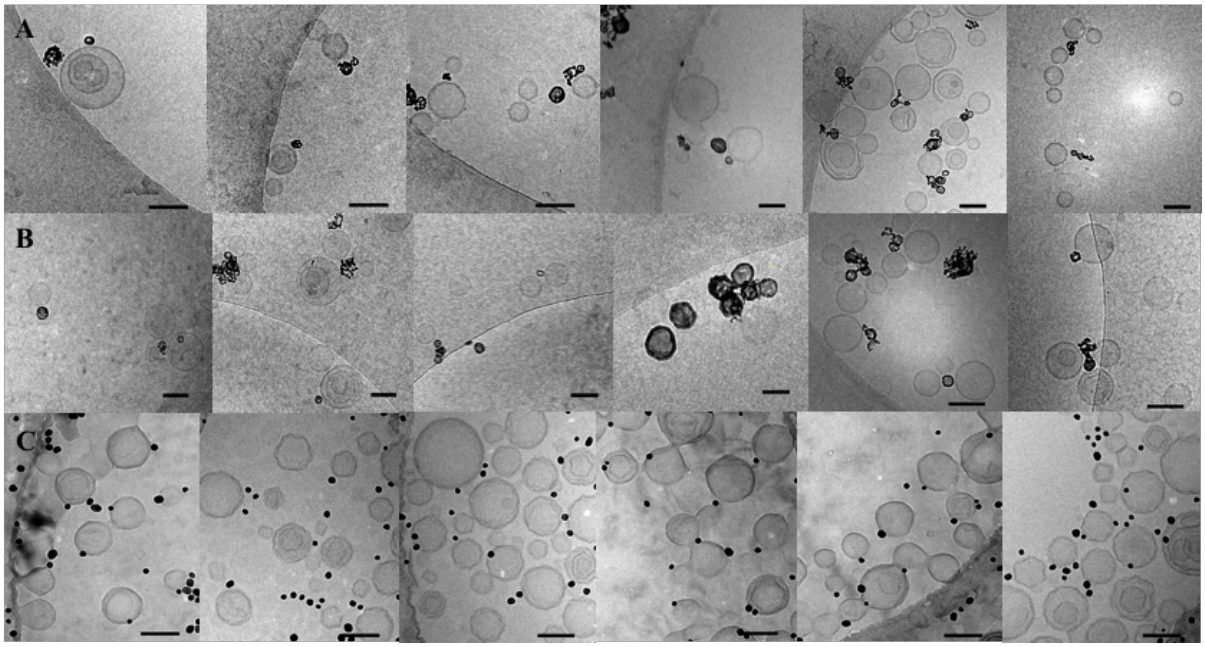
Supplementary Figure 6: Cryo-TEM of HGN-Liposomes prepared with increasing volumes of an 8 mg mL^{-1} HGN suspension. (A) control liposomes, 0 µL HGN; (B) 50 µL; (C) 100 µL; (D) 150 µL; (E) 200 µL; (F) 250 µL. Scale bar = 200 nm.

Supplementary Table 1: Hydrodynamic diameter (Z-average) and polydispersity index of HGNs and liposomes.

Sample	Z-Average (nm)	Polydispersity Index
HGN16	52	0.247
PEG2000 liposomes	180	0.189
PEG2000 liposomes + HGN	459	0.495
PEG5000 liposomes	178	0.248
PEG5000 liposomes + HGN	223	0.313



Supplementary Figure 7: (A) Schematic illustration of HGN-liposomes containing (i) only DSPE-PEG2000 or (ii) DSPE-PEG2000 and DSPE-PEG5000 stabilising agents. (B) CF release from control (blue diamonds) and HGN-tethered liposomes (red squares) containing only DSPE-PEG2000. (C) CF release from control (blue diamonds) and HGN-liposomes (red squares) containing 1 mol% DSPE-PEG5000. (D) Comparison of the fold-enhancement in the release of CF from HGN-conjugated liposomes over control liposomes containing only DSPE-PEG2000 (Blue) or containing 1 mol% DSPE-PEG5000 (Red). Error bars represent standard error of the mean (n=3).



Supplementary Figure 8: Cryo-transmission electron microscopy of liposome-nanoparticle conjugates. (A) HGN-tethered liposomes 18 hours after combining the HGN and tether-ready liposome suspensions. (B) HGN-tethered liposomes 96 hours after combining the HGN and tether-ready liposome suspensions. (C) Liposomes tethered to solid gold nanospheres (SNPs) 96 hours after combining the SNP and tether-ready liposome suspension. Scale bars = 200 nm.

Supplementary Table 2: Experimental parameters used for liposome manufacture and cryo-TEM image analysis to assess number and type of species present in HGN-liposome suspensions.

Volume of HGN suspension (μL) (8 mg mL^{-1} Au)	**Volume of liposome suspension] (mL) (8.05 mg mL^{-1} total phospholipid)**	**[Au] : [Phospholipid] ratio. (mg/mg)**	**Number of images**	**Total species counted**
0 μL	1.0	0	39	-
50 μL	1.0	0.05	97	709
100 μL	1.0	0.1	85	798
150 μL	1.0	0.15	54	480
200 μL	1.0	0.2	86	617
250 μL	1.0	0.25	79	753

Supplementary Table 3: Raw data from image analysis assessing number and type of species present in HGN-liposome suspensions.

0 μL HGN			**50 μL HGN**		
Species	*Number*	*Percentage*	*Species*	*Number*	*Percentage*
Untethered HGNs	-	0	*Untethered HGNs*	32	5
Untethered liposomes	-	100	*Untethered liposomes*	379	53
I HGN/Liposome	-	0	*1 HGN/Liposome*	270	38
Multiple HGN/liposome	-	0	*Multiple HGN/liposome*	12	2
Clusters	-	0	*Clusters*	16	2

100 μL HGN			**150 μL HGN**		
Species	*Number*	*Percentage*	*Species*	*Number*	*Percentage*
Untethered HGNs	220	28	*Untethered HGNs*	129	27
Untethered liposomes	168	21	*Untethered liposomes*	89	19
I HGN/Liposome	284	36	*I HGN/Liposome*	145	30
Multiple HGN/liposome	87	11	*Multiple HGN/liposome*	88	18
Clusters	39	5	*Clusters*	30	6
200 μL HGN			**250 μL HGN**		
Species	*Number*	*Percentage*	*Species*	*Number*	*Percentage*
Untethered HGNs	166	27	*Untethered HGNs*	382	51
Untethered liposomes	108	18	*Untethered liposomes*	50	7
I HGN/Liposome	125	20	*I HGN/Liposome*	92	12
Multiple HGN/liposome	153	25	*Multiple HGN/liposome*	178	24
Clusters	65	11	*Clusters*	51	7

Supplementary Note 1: Procedure for quantifying liposomal free-thiols available for nanoparticle conjugation.

The number of active free thiols available for conjugation at the liposome surface was quantified by spectrophotometry using Ellman's reagent. A standard curve was prepared in order to ascertain thiol activity by reacting a fixed concentration of DTNB with increasing concentrations of cysteine (0 – 0.1 mM) under alkaline conditions (Tris buffer, 10 mM, pH 8.01) (Table 1). The activity of the DSPE-PEG2000-SH tethering could then be characterised by preparing a 0.05 mM solution of the linker agent and incubating it with DTNB, and measuring the concentration by spectrophotometry. The number of accessible thiol moieties per liposome can also be quantified. Tether-ready liposomes were prepared via the aforementioned procedure, alongside control liposomes which were prepared by replacing the DSPE-PEG2000-SH constituent with an equivalent amount of additional DSPE-PEG2000. Control or tether-ready liposomes (280 μL of liposome suspension) were incubated with the appropriate quantity of DTNB and Tris buffer, and the thiol activity ascertained by absorbance at 408 nm. The thiol concentration is then calculated from the difference in absorption between the DTNB + tether-ready liposome and tether-ready liposomes alone to account for the liposomes scattering effect. DTNB was incubated with control liposomes (no DSPE-PEG2000-SH) to ensure no colorimetric reaction occurs with the other phospholipids present in the liposome suspension.

Supplementary Table 4: Concentrations and volumes of reagents used for the spectrophotometric quantification of thiols in suspension.

DTNB (10 mM stock) (μL)	**Tris buffer (10 mM, pH 8) (mL)**	**Cysteine volume (10 mM stock) (μL)**	**Cysteine concentration (mM)**
50	2.7	0	0
50	2.725	28	0.01
50	2.694	56	0.02
50	2.638	112	0.04
50	2.582	168	0.06
50	2.526	224	0.08
50	2.470	280	0.1

Supplementary Note 2: Peak ultrasound sensitivity occurs with one tethered nanoparticle (Electron microscopy investigation)

A cyro-transmission electron microscopy investigation was undertaken to assess the predominant species present in HGN-liposome suspensions. An incrementally increasing volume of hollow gold nanoshells (0 – 250 μL; 8 mg mL^{-1} Au concentration) was added to a fixed volume of tether-ready liposomes encapsulating carboxyfluorescein (1 mL; 8.05 mg mL^{-1} total phospholipid concentration; 100 mM carboxyfluorescein, pH 7.4. 20 mM $NaHPO_4$). Samples were flash-frozen in liquid ethane using a Leica KF80 plunge freezing device with a metal mirror (MM80) attachment (Leica Microsystems, Vienna Austria). The samples were subsequently stored in an aluminum 8-specimen sample holder under liquid nitrogen until use. Images were collected using a JEOL 2200FS transmission electron microscope (Materials and Methods) (Figure S6; representative examples).

Images were analysed to identify the different species present and to assess their relative populations manually. A total of 361 images were collected across the 5 samples, with a total of 3357 species counted (Table S2). Species were identified by 5 unique descriptors: bare liposomes; untethered HGNs, liposomes containing one tethered HGN; liposomes containing multiple tethered HGNs; and HGN-liposome clusters. Species were counted when only clearly demarcated. For example: liposomes containing one (or multiple) tethered particles are defined as single liposomes contacting one (or multiple) hollow gold nanoshells; and clusters are defined multiple liposomes and nanoparticles grouped together with continuous contact visible throughout the structure. It was noted that an average HGN suspension prior to liposome conjugation contains a majority of single nanoshells, and a minority population of dimers, trimers and larger aggregates, consistent with the results from DLS analysis.