Supporting Information

In Vivo Protein Corona Patterns of Lipid Nanoparticles

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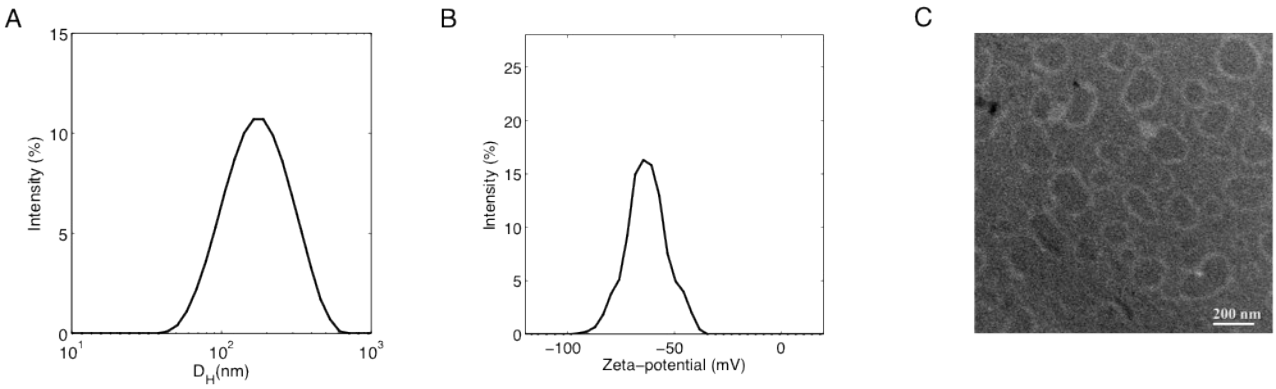

Figure S1. Representative size (A) and Zeta-potential (B) distributions of bare liposomes. (C) TEM images of liposomes before interaction with plasma proteins. Liposomes were made of hydrogenated soy phosphatidylcholine (HSPC), 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DSPG), and Cholesterol (Chol). Lipid composition and molar ratios of the lipid species were chosen to match the exact liposome composition of the clinically-used liposomal AmBisome®.

Table S1. The average size of liposomal AmBisome® before and after incubation with blood of FVB/N mice both *in vitro* (liposome-protein corona *in vitro*, liposome-PC) and *in vivo* (liposome-PC *in vivo*) was determined by TEM as well by DLS. PDI is the polydispersity index of size distributions. Zeta potentials were determined with a Zetasizer.

	TEM Diameter (nm)	DLS Diameter (nm)	PDI	Zeta-potential (mV)
Liposome	185±45	192±12	0.296±0.019	-61.2±3.5
Liposome-PC *in vitro*	310±30	334±60	0.312±0.112	-26.1±8.7
Liposome-PC *in vivo*	260±30	266±25	0.373±0.156	-20.5±2.8

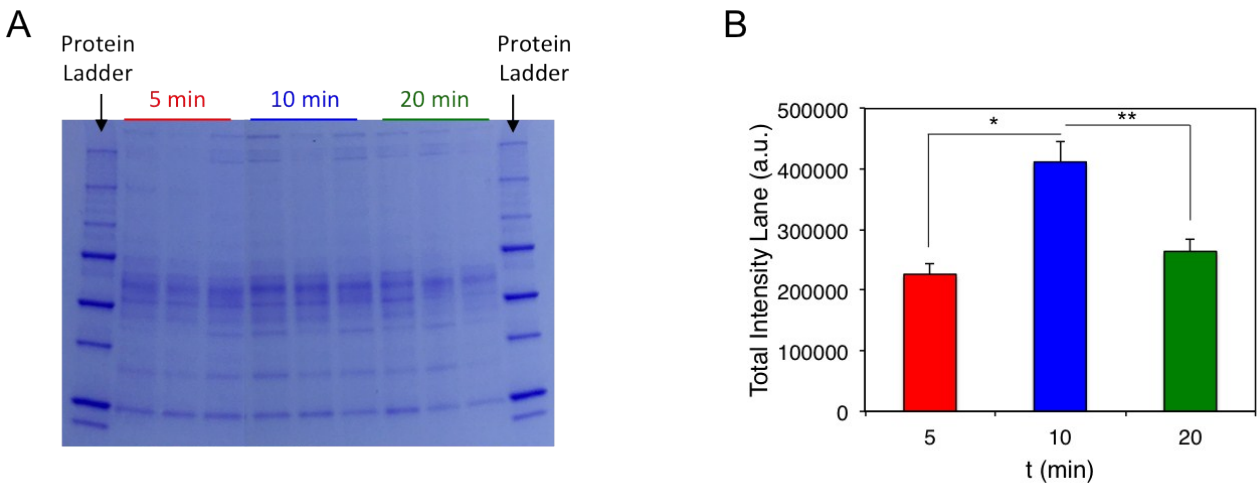

Figure S2. (A) Proteins associated with AmBisome® liposomes recovered from FVB/N mice by cardiac puncture 5 min, 10 min and 20 min post-injection were separated by sodium dodecyl sulfate polyacrilamide gel electrophoresis (SDS-PAGE) and visualized with Coomassie PhastGel Blue R-350 (GE Healthcare, Milan, Italy). For each time point, three mice were used (i.e. each lane is a biological replicate, i.e. it reflects the protein corona pattern formed around lipid nanoparticles recovered from a single mouse) (**B**). Histograms represent the total lane intensity of proteins recovered from AmBisome® liposomes that can be assumed as an estimation of the amount of protein bound to lipid surface. Statistical significance was evaluated by Student's t-test: * indicates $p < 0.005$ and ** indicates $p<0.01$.

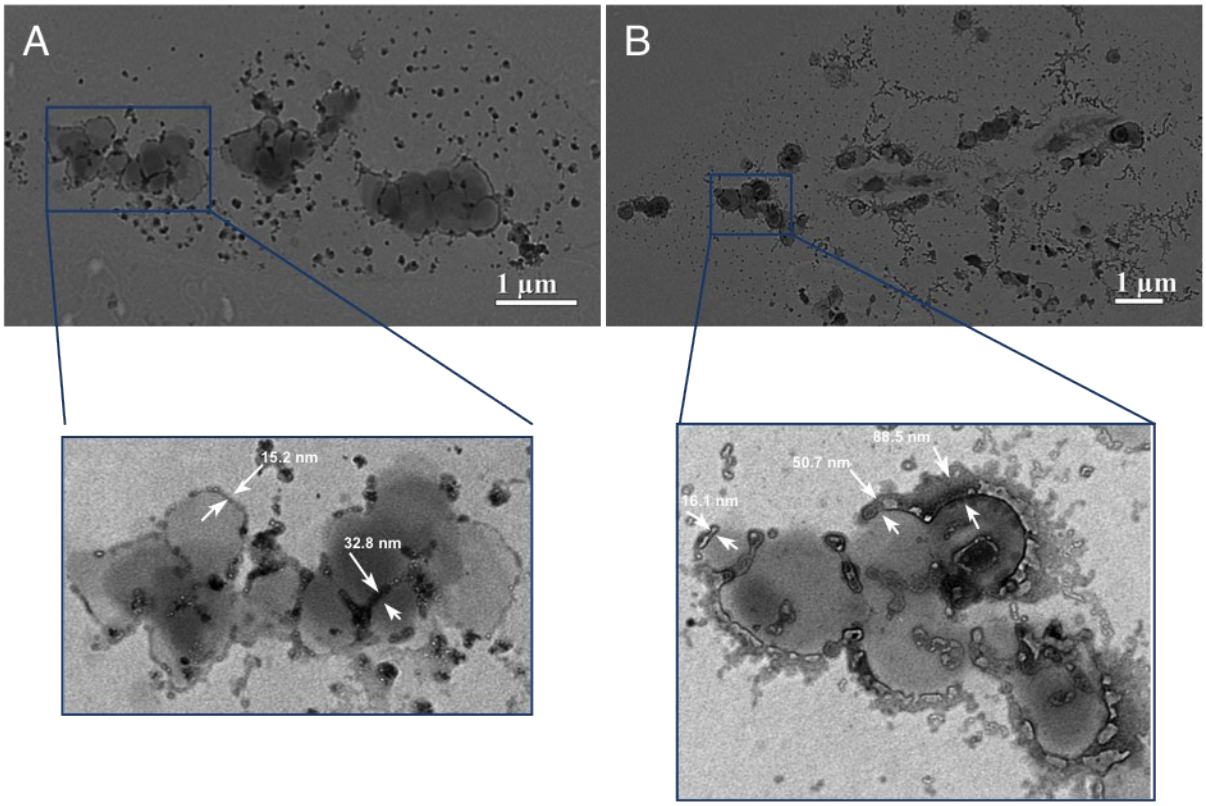

Figure S3. Morphology and morphometric features of AmBisome® liposomes following *in vitro* (A) and *in vivo* (B) incubation have been observed by transmission electron microscopy (TEM). Mean diameter of aggregates is reported in Table S1 and is in very good agreement with particle size as determined by dynamic light scattering measurements. At relative high magnification, aggregates show appearance of dark thick rims indicated by white arrows. Since sample preparation was free of staining agents, these edges are likely due to proteins adsorbed at the lipid surface. Dark rims are also made of filaments with branched shape.