Supporting Information

Differential Cellular Responses to FDA-Approved Nanomedicines: An Exploration of Albumin-Based Nanocarriers and Liposomes in Protein Corona Formation

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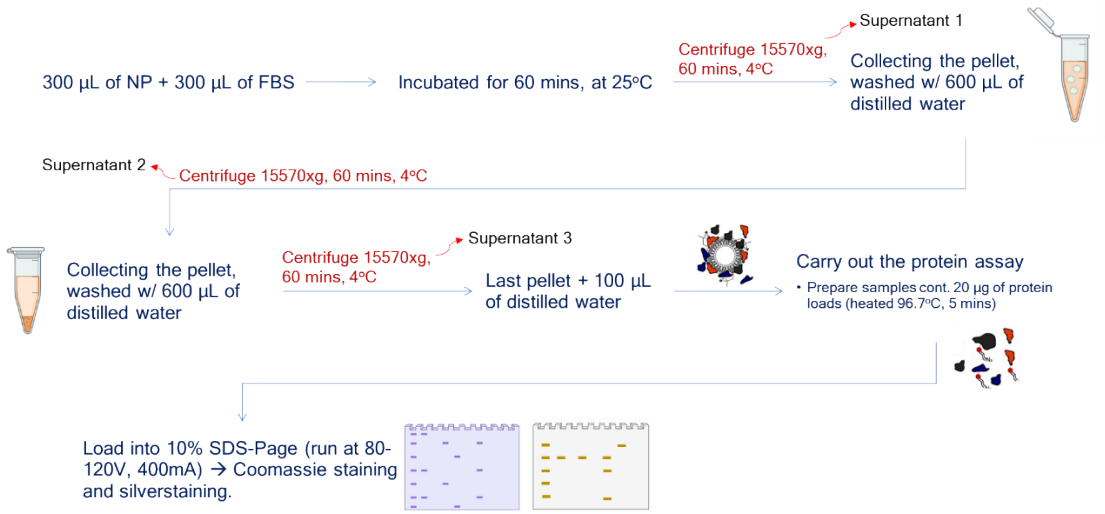
List of Materials

BSF (20%) human albumin solution (≥96.0%, CSL Behring, Melbourne, Australia), soybean lecithin (Lipoid S100) (Lipoid, Ludwigshafen am Rhein, Germany), 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) (MedChemExpress, USA), 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) (Avanti Lipids, USA), cholesterol (Sigma, Taiwan), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]ammonium salt) (DSPE-PEG) (Avanti Lipids, USA), 3,3-Dioctadecyloxacarbocyanine perchlorate (DiO perchlorate) (AATBioquest, USA), Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA), ethanol, chloroform, dimethyl sulfoxide (Echo, Miaoli, Taiwan). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), horse serum, penicillin-streptomycin solution, 0.25% Trypsin 2.21 mM EDTA 1× (Corning, Manassas, VA, USA), and were purchased commercially. All organic solvents, other chemicals, and solvents used herein were of fine analytical grade.



Scheme S1. Preparation of protein corona identification through gel electrophoresis starting with serum protein and nanoparticles incubation, followed by free-protein separation process, and characterization of hard protein corona.

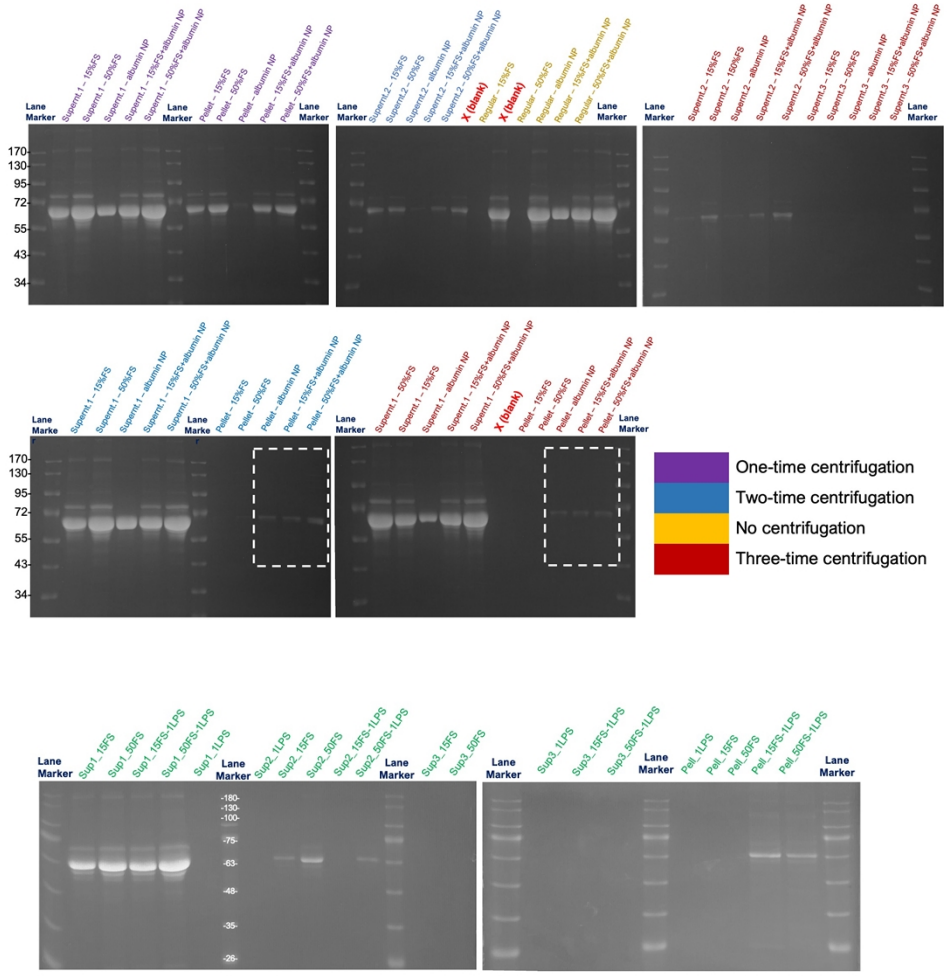


Figure S1. The electrophoresis gels results of the optimized centrifugation cycles (LPS=liposomes; FS=fetal bovine serum; Supernt.=supernatant; Sup=supernatant).

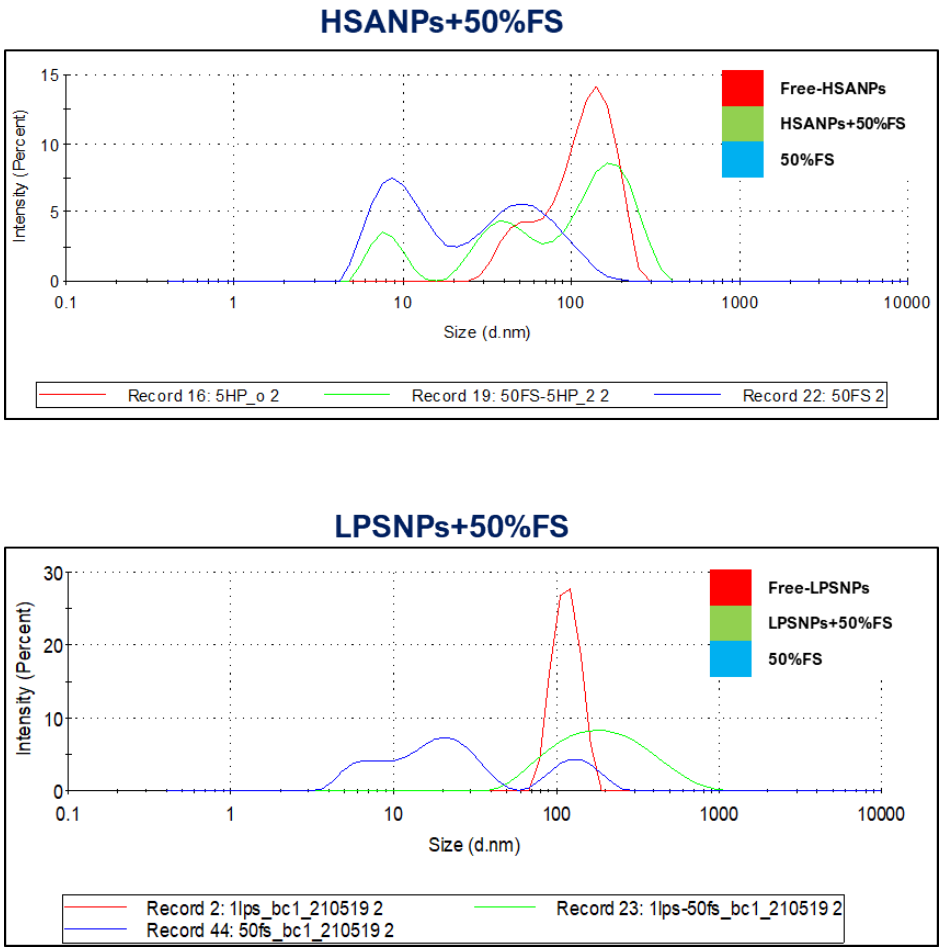


Figure S2. The size distribution of each nanoparticles type prior to and upon incubation with different concentrations of serum.

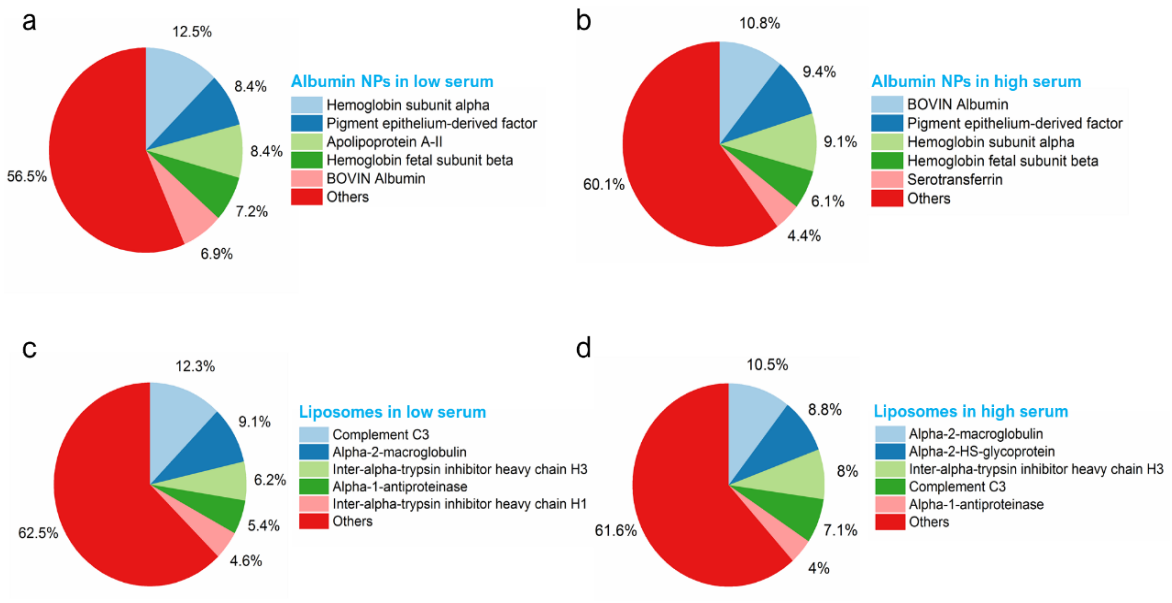


Figure S3. (a-b) Identification of hard-protein corona using LC-MS/MS on albumin NPs and (c-d) liposomes in different serum concentrations, according to the normalized spectra count values.

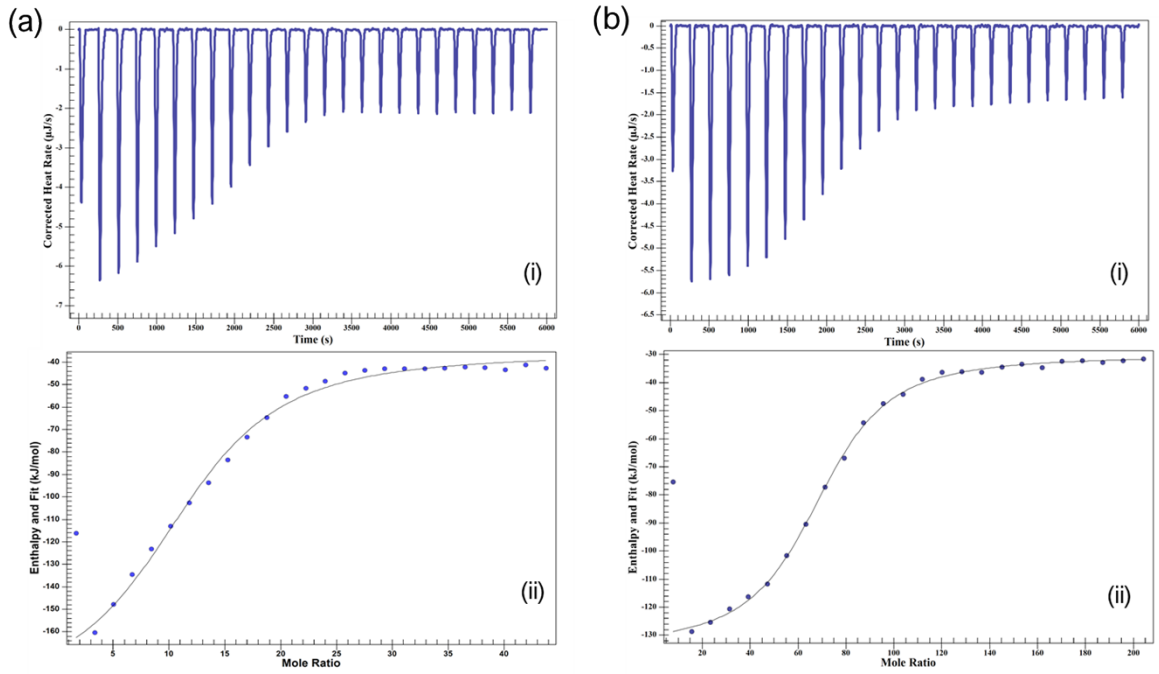


Figure S4. The difference of heat transfer rate and enthalpy of interaction upon injection of serum (0.3 mM) to (a) albumin NPs (0.7 μM) and (b) liposomes (0.15 μM). The (i) and (ii) depict the heat transfer rate data and the calculated enthalpies per injection for the binding isotherms, respectively.

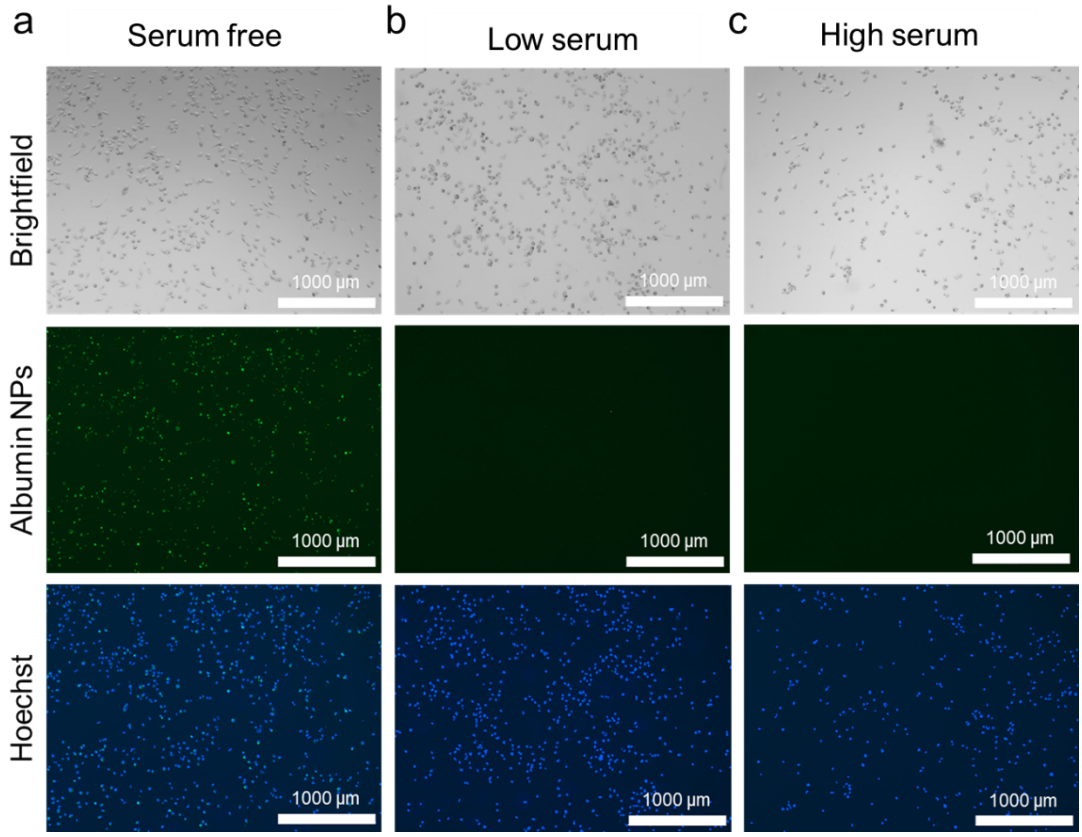


Figure S5. Internalized albumin NPs (green) in Mia Paca-2 cells in directly incubated with free-serum, low-incubated, and high-incubated serums containing media. Nuclei were stained with Hoechst 3342 (blue). The merged images are in the third rows.

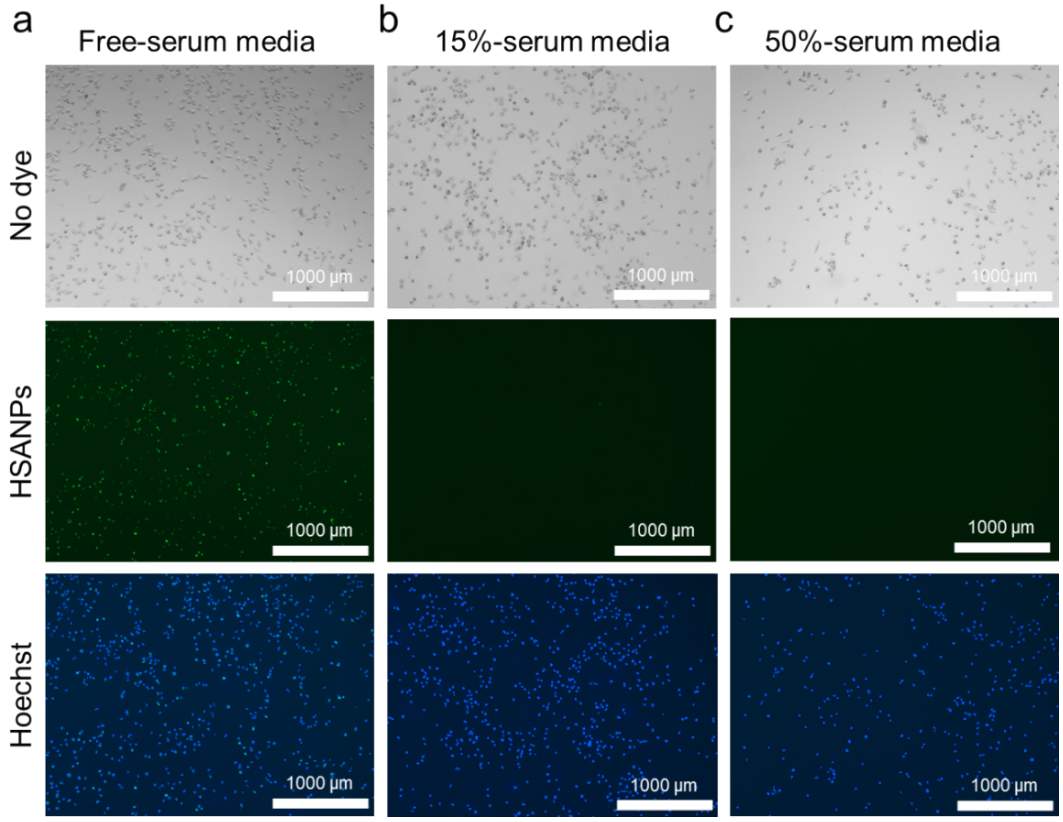


Figure S6. Internalized albumin NPs in Mia Paca-2 cells upon the 1 h incubation of nanoparticles with serum-containing media. In this experiment, the incubation effect of the nanoparticles with the serum-containing media before being introduced into the cells for 24 h was investigated. The results demonstrated that there is almost no difference for the nanoparticles uptake into the cells either with or without prior incubation treatment with the serum-containing media as it might still have the unseparated soft-corona protein within the incubated system (**Supplementary Figure S4 and S5**).

Figure S5. The IC_{50} values of MPT0B291-loaded albumin NPs in different serum conditions after 24 h incubation with Mia Paca-2 cells.

	IC50 (μg/mL)		
	Serum-free	**Low-serum**	**High-serum**
MPT0B291-loaded Albumin NPs	0.6179 ± 0.0447	0.7354 ± 0.3466	3.2700 ± 0.5428

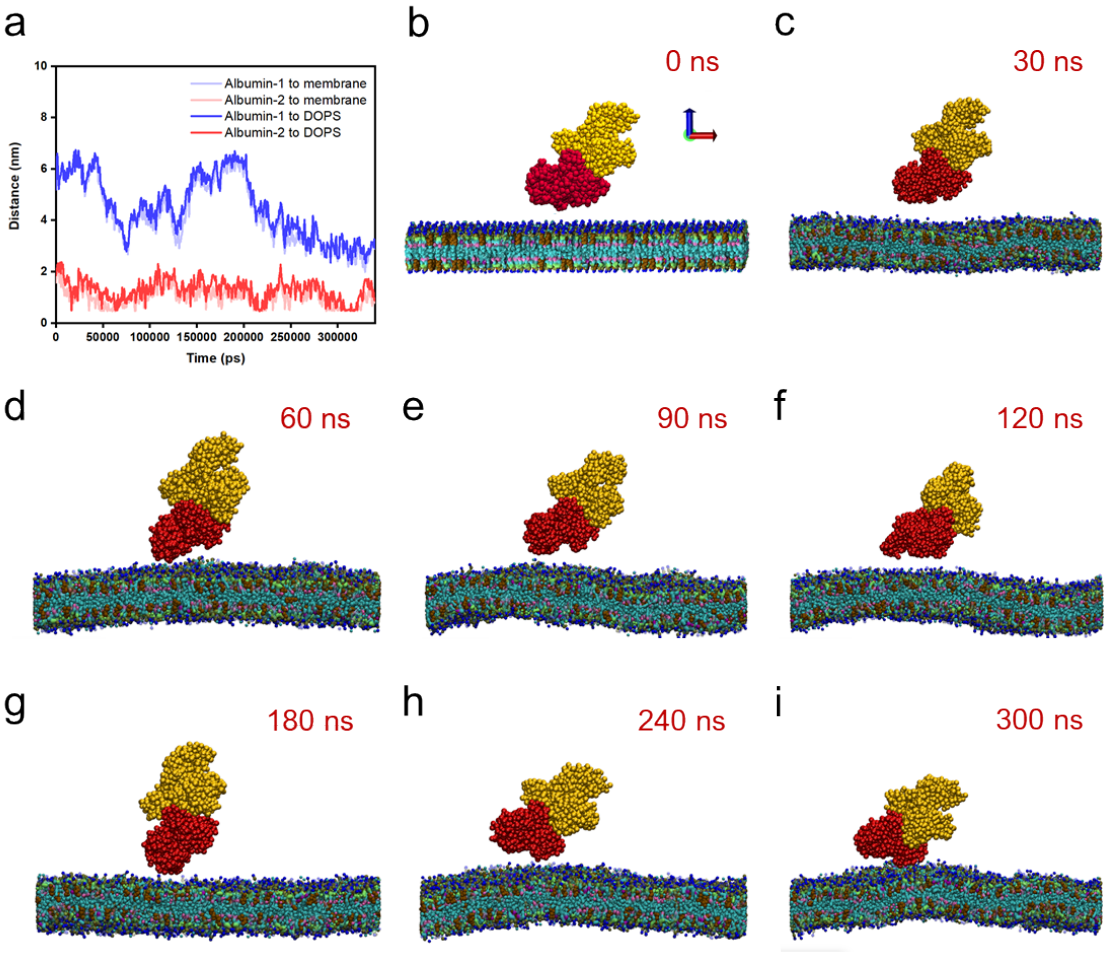


Figure S7. (a) Distance obtained from center of mass calculation between two entities. (b-i) Snapshot of molecular dynamics simulation of albumin NPs with albumin toward the membrane bilayer in certain time points.

Notes for Figure S7 and S8:

After 60 ns of simulation period, the nanoparticles-albumin system reached the membrane bilayer and bound after 300 ns (**Supplementary Figure S6**). On the other hand, the nanoparticle-apolipoprotein E complex seems to attract backward against the membrane bilayer started from 30 ns. This interaction was further evaluated by calculating the center of mass of the protein complex to the bilayer system. It showed that during the simulation period, the positions of all the residues' beads of the nanoparticles lowered

toward the membrane bilayer. It suggests that typical interaction was triggered by the present of albumin (as the corona protein) in which induced albumin-nanoparticle complex and was favorable to interact with the membrane bilayer through greater hydrophobic interaction. Meanwhile, the nanoparticles-apolipoprotein E corona complex increase the center of mass distance due to the discharge of the apolipoprotein E pointing outward the membrane after 150 ns dynamic period (**Supplementary Figure S7**).

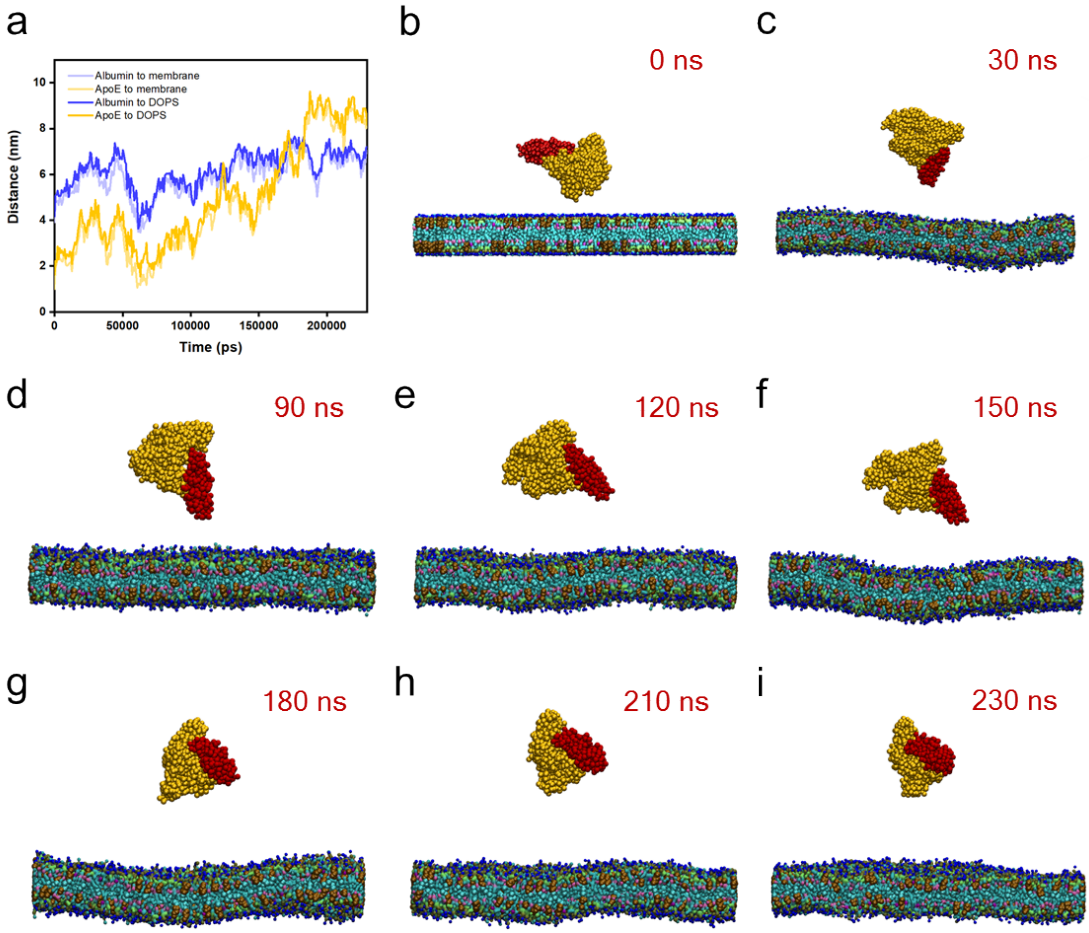


Figure S8. (a) Distance obtained from center of mass calculation between two entities. (b-i) Snapshot of molecular dynamics simulation of albumin NPs (represented by subset of an albumin protein) with apolipoprotein E toward the membrane bilayer in certain time points.