

High Performance Liquid Chromatography (HPLC) elution profile of BSA-A647 and BSA. Elution from the column was monitored with UV 250 nm (red) to detect protein and fluorescence to detect A647 (FL)(blue). BSA had only a UV peak from the amide group of the protein (lower chart), while FL peak associated with UV peak was detected for BSA-A647 (upper chart). This result indicates Alexa647 dye was successfully conjugated to BSA via NHS chemistry.



Characterization of BSA-GNP by dynamic light scattering (DLS) and zetapotential analysis of the molecules shown in schematic form on the right (A-C). DLS analysis of BSA (A) demonstrated a zeta potential (Z.P.) or charge = -12.1 mV and a mean hydrodynamic diameter or size of 2.3 nm. (B) GNP had a mean size of 5.2 nm and a Z.P. = -33.0 mV. (C) BSA-GNP had a mean size of 12.1 nm and a Z.P. = -18.0 mV.



BSA-GNP was characterization by transmission electron microscopy (TEM). (A) TEM analysis of GNP shows no corona around the GNP while BSA-GNP (B) has a corona of BSA molecules. TEM demonstrates that this coating with BSA did not cause aggregation. (Scale bar = 100 nm and scale bar for each inset = 20 nm)



Ultrastructural identification of BSA-GNP in mouse choroid. (A) TEM image of choriocapillaris in a 14-month-old WT mouse injected with BSA labeled gold. Arrows indicate gold within the lumen at the surface of CC endothelial cells as well as in a TEC and caveolae and in the EC cytoplasm. (B) Same image as A but the background and ultrastructure have been thresholded away in Adobe Photoshop, leaving only the extremely electron dense GNP visible. Then the image was inverted to make the GNP white and background black. (White arrows, BSA-GNP)



Ultrastructural distribution of GNP in the CC/BM/RPE complex of the left eyes of 3-month-old WT mice when animals were sacrificed at 30 min post-injection. (A,B) The non-conjugated or naked GNP did not bind to the surface receptors of EC or at the fenestration pores, nor were they present in caveolae or TECs. (Scale bar, 500 nm)



Uptake of Fluorescently tagged albumen (BSA-A647; red) (A, B) and free Alexa647 dye (C, D) by human umbilical vein endothelial cells (HUVECs) after 60 minutes incubation. Fixed cells were imaged under the identical conditions. Nuclei were labeled with Hoechst 33342 (blue). BSA-A647 was internalized in HUVECs (A, B) whereas A647 dye was not found in HUVEC (C, D).