Supporting Information for Templated Spherical High Density Lipoprotein Nanoparticles

C. Shad Thaxton[¶], Weston L. Daniel*, David A. Giljohann*, Audrey Thomas[¶], Chad A. Mirkin*

[¶]Northwestern University, Feinberg School of Medicine, Department of Urology, 303 E. Chicago Ave., Tarry 16-703, Chicago, IL 60611 and *Northwestern University, International Institute for Nanotechnology and Department of Chemistry, 2145 Sheridan Road, Evanston, IL 60208
cthaxton003@md.northwestern.edu, chadnano@northwestern.edu

Experimental Section

HDL-Au NP Synthesis. In a typical HDL-Au NP synthesis, citrate-stabilized gold nanoparticles (80 nM, 5 nanometer diameter, Ted Pella, Inc.) in aqueous solution are mixed with 5-fold excess of purified APOA1 (400 nM, Biodesign International) in a glass vial. This solution is allowed to mix overnight at room temperature while stirring. Next, a 1:1 ratio of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine- N-[3-(2-pyridyldithio)propionate] : 1-2-dipalmitoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids) each in 100-fold excess with respect to the concentration of Au NPs was prepared in chloroform. The phospholipid mixture is then added to the Au NP/APOA1 solution which results in a layered mixture. The mixture is vortexed, and heated gradually to ~ 65 °C in order to evaporate the chloroform. After allowing the solution to cool, purification of the HDL-Au NPs is accomplished via repeated (2X) centrifugation (21,000 *g*) and re-suspension in Nanopure^{TM} water or phosphate buffered saline with 0.05% (w/v) bovine serum albumin (PBS, 137 nM NaCl, 10 mM phosphate, 2.7 nM KCl, pH 7.4, Hyclone).

APOA1 and Phospholipid Binding. To measure the protein loading on the Au NPs, APOA1 was fluorescently labeled using an Alexa Fluor 488 protein labeling kit (Invitrogen). HDL-Au NPs were synthesized using the procedure described above, and their concentration was determined by UV-Vis ($\varepsilon = 1.2 \times 10^7$ L / mol cm). Gold nanoparticles were oxidized with KCN in order to liberate fluorescently bound APOA1, and the fluorescence of the solution was measured. The number of proteins per particle was determined by comparing the obtained fluorescence measurements to that of a standard curve prepared with known concentrations of labeled APOA1. Phospholipid loading on the HDL-Au NPs was determined with similar experiments. The fluorescently modified phospholipid 1-palmitoyl-2{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoly}-*sn*-glycero-phosphoethanolamine (Avanti Polar Lipids) was used in place of the aminated lipid to determine aminated lipid loading.

Conjugate Characterization. HDL-Au NPs were diluted to 1 nM concentration in water. Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano ZS (Malvern). Stability of HDL-Au NPs to aggregation in water and buffered saline solutions was measured using a Cary 5000 UV-Vis spectrophotometer (Varian) (Figure S1).

Cholesterol Binding Experiments. Cholesterol binding to HDL-Au NPs was determined by adding 5 μL of varying concentrations of 25-{N-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-methyl]amino}-27-norcholesterol (NBD-cholesterol) in DMF to 995 μL of 5 nM HDL-Au NPs in water. The solutions were vortexed and incubated for at least 20 min. Fluorescence spectra of the solutions were measured on a Jobin Yvon Fluorolog 3. The solutions were excited at 473 nm and scanned from 500 to 600 nm in 1 nm increments with 1 sec integration times. The binding of NBD-cholesterol to HDL-Au NPs leads to an increase in fluorescence intensity. The

fluorescence intensity of control solutions of NBD-cholesterol without HDL-Au NPs were measured in order to subtract background signal from the samples. The fluorescence intensity increase at 520 nm upon NBD-cholesterol binding was used to construct a binding isotherm. K_d was determined by analyzing the binding curves with the "one site total binding" function in GraphPad Prism 5.0 software using the equation: Fluorescence = (B_{max} * [NBD-cholesterol]) / (K_d + [NBD-cholesterol]).

Supporting Figure 1

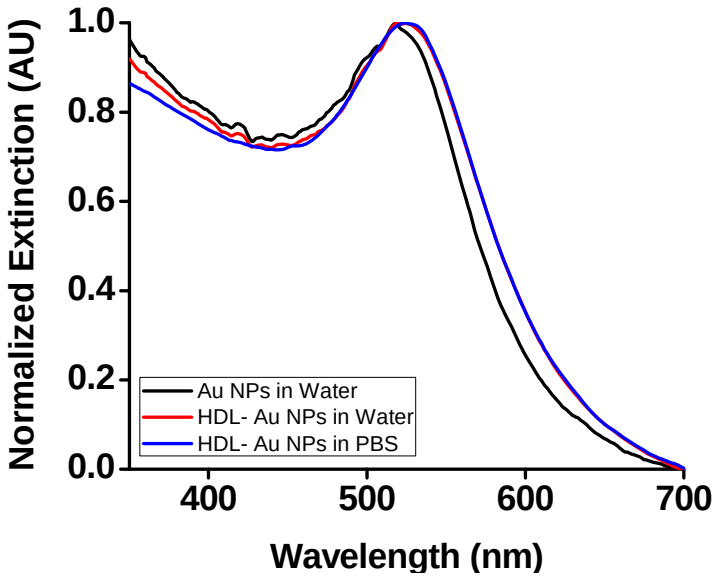

Figure S1. UV-Vis spectra of unconjugated Au NPs in water and HDL-Au NPs in water and PBS.