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Templated Spherical High Density Lipoprotein Nanoparticles

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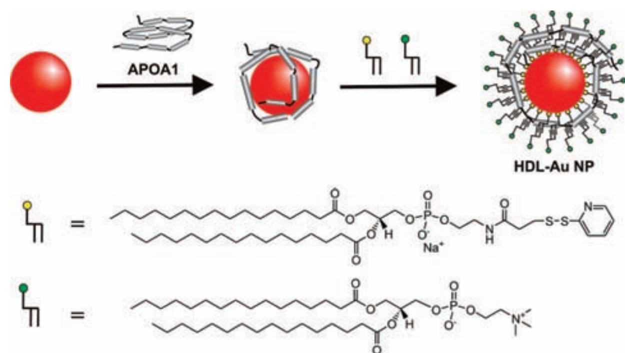
High density lipoprotein (HDL) is a dynamic serum nanostructure protective against the development of atherosclerosis and resultant illnesses such as heart disease and stroke.[1] Increasing circulating serum HDL levels provides a promising therapeutic approach to preventing and, potentially, reversing atherosclerosis by augmenting reverse cholesterol transport.[2−4] However, a facile route to synthetic HDL remains a challenge, as methods to control and mimic the size, surface chemistry, and activity of HDL have not yet been demonstrated. Due in part to their biocompatibility and tailorability, gold nanoparticle (Au NP) conjugates have shown promise as therapeutics,[5,6] intracellular gene regulation agents,[7,8] and *in vitro* diagnostic probes.[9−11] Herein, we report a new Au NP core−shell structure, where the Au NP core serves as a size- and shape-controllable scaffold[12] for constructing an HDL-like particle from phospholipids and apolipoprotein A-I (APOA1). Importantly, the proof-of-concept HDL-Au NP structure we report is designed to be within the size range of HDL and to mimic the general surface composition of HDL. Furthermore, we demonstrate that, like HDL, HDL-Au NPs are capable of binding cholesterol.

In a typical synthesis, an aqueous suspension of citrate-stabilized gold nanoparticles (5 ± 0.75 nm) is mixed with an aqueous solution of purified APOA1 and stirred overnight (Scheme 1).

Scheme 1. Synthesis of Templated Spherical HDL Nanoparticles



APOA1, comprised of 10 amphipathic alpha helices each with a hydrophobic domain and a negatively charged hydrophilic domain, is the main protein component of HDL and defines the structure and physiology of HDL *in vivo*.[13−16] Next, a 1:1 solution of disulfide-functionalized lipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[3-(2-pyridyldithio)propionate], and amine-functionalized lipid, 1−2-dipalmitoyl-*sn*-glycero-3-phosphocholine, was mixed in $CHCl_3$ and added to the aqueous suspension of particles in 100-fold excess with respect to the Au NPs. While several ratios were tried, these ratios were found to give loadings of lipid and protein similar to that of natural HDL (*vide infra*). The disulfide lipid was selected since the disulfide functionality allows for chemisorption to the surface of the Au NP. The amine-modified lipid is a naturally occurring phospholipid known to electrostatically and hydrophobically associate with APOA1.[13−16] This addition results in a two-phase mixture. Upon gradual heating, $CHCl_3$ is evaporated and the lipids are transferred to the aqueous phase containing the dispersed Au NPs with APOA1. Note that if the disulfide lipid is added alone and without prior addition of APOA1, the particles precipitate since they become hydrophobic upon lipid adsorption to the Au NP surface.

Purification of the HDL-Au NPs is accomplished via repeated centrifugation and resuspension in water or buffered saline solutions. UV−vis analysis of the purified HDL-Au NPs exhibits a band at 520 nm (Supporting Information), consistent with dispersed rather than aggregated HDL-Au NPs.[11,12] Dynamic light scattering experiments were used to follow the Au NP surface modification process. The results demonstrate sequential growth of the HDL structures (Table 1). An unmodified gold colloid (9.2 nm average hydrodynamic diameter) is first modified with APOA1 (11.0 nm) and subsequently the mixture of lipids (17.9 nm). The average size of the resulting HDL-Au NPs is similar to that for natural HDL.[13,17,18]

Table 1. Hydrodynamic Diameter of Conjugates

particles	hydrodynamic diameter (nm)
Au NP (5 nm diameter)	9.2 ± 2.1
Au NP + APOA1	11.0 ± 2.5
Au NP + APOA1 + phospholipids	17.9 ± 3.1

Investigations have shown that there are 2−5 copies of APOA1 and ∼80−160 phospholipids per natural mature spherical HDL.[15,16,19,20] To characterize the chemical composition of HDL-Au NPs, fluorophore labeled components (APOA1 and aminated phospholipids) were used to synthesize HDL-Au NPs as described. After dissolving the Au NPs using 0.04 M KCN, we quantitatively determined that the average number of proteins and aminated phospholipids per particle is 3 ± 1 and 83 ± 12, respectively (Supporting Information). Thus, these values correspond well to those reported for natural HDL.

We hypothesize that, in addition to modifying the exposed gold surface, the disulfide functionalized lipid interacts with the hydrophobic portions of APOA1 forming a complex that is water soluble. In our working model of this structure, the aminated lipid forms a tail-to-tail hydrophobic complex with the adsorbed disulfide functionalized lipid and an electrostatic complex with the adsorbed APOA1. The basis for this model is the known lipid organization

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capabilities of APOA1,[13−16] the well precedented disulfide adsorption on gold chemistry,[21] the water solubility of the resulting complex, and fluorescence measurements aimed at removing and quantifying the number of proteins and aminated phospholipids that make up the final structure.

Transport of cholesterol to the liver by HDL is a key mechanism by which HDL protects against the development of atherosclerosis.[2] Thus, determining if HDL-Au NPs bind cholesterol is important for determining the potential of these structures as therapeutic agents. The binding of cholesterol to HDL-Au NPs was investigated with a fluorescent cholesterol analogue (25-{*N*-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-methyl]amino}-27-norcholesterol, NBD-cholesterol). NBD-cholesterol fluorescence is weak in polar environments such as water; however, in nonpolar matrices (such as a lipid membrane) NBD-cholesterol becomes fluorescent.[22,23] Quenching by the Au NP[24] causes the signal of the HDL-Au NP bound NBD-cholesterol to be partially dampened. However, titration of NBD-cholesterol into a solution of HDL-Au NPs provides a strong enough fluorescent signal to construct a binding isotherm (Figure 1). This isotherm was used to calculate a K_d of 3.8 ± 0.8 nM for NBD-cholesterol binding to HDL-Au NPs. Preliminary experiments indicate that NBD-cholesterol is irreversibly bound to HDL-Au NPs under the stated conditions; however, further experiments are underway to more thoroughly address this question. To the best of our knowledge, this is the first example of a K_d measured for a synthetic HDL analogue. Interestingly, there is little information regarding the K_d of natural HDL for comparison purposes.

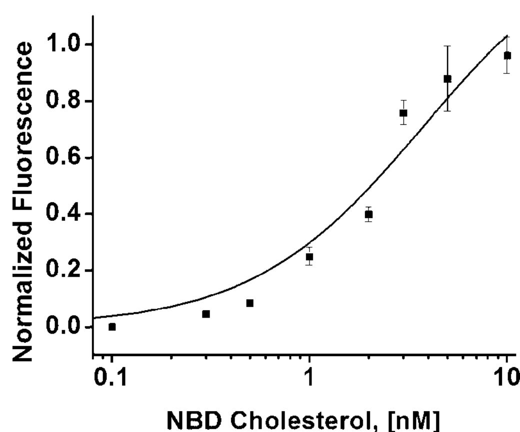

Figure 1. Binding isotherm of NBD-cholesterol to HDL-Au NPs. NBD-cholesterol was titrated into a 5 nM solution of HDL-Au NPs. The fluorescence versus NBD-cholesterol concentration was used to calculate the K_d of HDL-Au NPs.

In conclusion, we report the synthesis and characterization of a novel class of HDL-Au NPs, designed to mimic their biological HDL counterpart. Importantly, our data demonstrate that HDL-Au NPs can be used as biomimetic materials to bind cholesterol. Because it is being increasingly appreciated that the size, shape, and chemistry of HDL dictate *in vivo* physiology,[25,26] this reported general approach may provide a novel method to fabricate and rationally tailor HDL structures. We anticipate that this synthetic method will provide a new and general approach for the development of templated nanomaterials designed to mimic HDL, and with future study and characterization in biologically relevant conditions, these compounds may prove interesting for the development of therapeutic agents. Finally, the determination of the K_d for these particles, with respect to cholesterol complexation, provides a key starting and comparison point for measuring and evaluating the cholesterol binding properties of HDL.

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Note Added in Proof. During the review process, we discovered that a group independently was working on a synthetic form of HDL for imaging purposes. They did not show cholesterol binding and therefore determine a binding constant for these related structures.[27]

Supporting Information Available: Experimental conditions and HDL-Au NP characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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