REVIEW

Reconstituted Lipoprotein: A Versatile Class of Biologically-Inspired Nanostructures

Daniel A. Bricarello,[†] Jennifer T. Smilowitz,[‡,§] Angela M. Zivkovic,[‡,§] J. Bruce German,[‡,§] and Atul N. Parikh[†,*]

[†]Department of Applied Science, [‡]Department of Food Science and Technology, and [§]Department of Foods for Health Institute, University of California-Davis, Davis, California 95616, United States

ABSTRACT One of biology's most pervasive nanostructures, the phospholipid membrane, represents an ideal scaffold for a host of nanotechnology applications. Whether engineering biomimetic technologies or designing therapies to interface with the cell, this adaptable membrane can provide the necessary molecular-level control of membrane-anchored proteins, glycopeptides, and glycolipids. If appropriately prepared, these components can replicate *in vitro* or influence *in vivo* essential living processes such as signal transduction, mass transport, and chemical or energy conversion. To satisfy these requirements, a lipid-based, synthetic nanoscale architecture with molecular-level tunability is needed. In this regard, discrete lipid particles, including reconstituted high density lipoprotein (HDL), have emerged as a versatile and elegant solution. Structurally diverse, native biological HDLs exist as discoidal lipid bilayers of 5—8 nm diameter and lipid monolayer-coated spheres 10—15 nm in diameter, all belted by a robust scaffolding protein. These supramolecular assemblies can be reconstituted using simple self-assembly methods to incorporate a broad range of amphipathic molecular constituents, natural or artificial, and provide a generic platform for stabilization and transport of amphipathic and hydrophobic elements capable of docking with targets at biological or inorganic surfaces. In conjunction with top-down or bottom-up engineering approaches, synthetic HDL can be designed, arrayed, and manipulated for a host of applications including biochemical analyses and fundamental studies of molecular structure. Also highly biocompatible, these assemblies are suitable for medical diagnostics and therapeutics. The collection of efforts reviewed here focuses on laboratory methods by which synthetic HDLs are produced, the advantages conferred by their nanoscopic dimension, and current and emerging applications.

KEYWORDS: HDL · lipoprotein · membrane proteins · bionanotechnology · nanoparticles · protein structure · infectious disease · drug delivery · energy harvesting

The earliest manifestations of nanotechnology in living systems are linked to the evolution of eukaryotic organisms themselves. Evolution of early unicellular organisms[1] found the self-assembling bilayer membrane an ideal scaffold not only for compartmentalization[2] but also for self-replication[3] and energy storage *via* simple charge separation and protein folding.[4] It is hard to imagine just how critical this simple membrane construction (and its ability to compartmentalize, replicate, and maintain charge separation) was to the molecular coevolution of energy-coupling membrane proteins,[5] but unquestionably all life forms are based upon it. This nanometer scale, pseudo-two-dimensional environment of the self-assembling lipid membranes persists in similar dimensions and provides similar functions to this day. A key necessity emerged as multicellular organisms proliferated and the roles of lipids expanded to include scaffolding, organization, energy, and regulation. Carriers that could efficiently transport these sometimes water-insoluble compounds through the aqueous milieu from the organs of absorption, synthesis, and storage to the sites of use, became vital. These carriers needed to meet several criteria: (1) encapsulation of a hydrophobic core; (2) presentation of a hydrophilic exterior surface; (3) robust yet dynamic organization for absorption of lipids *a posteriori;* (4) biospecific interactions with target cellular receptors; and (5) physical constraints (*e.g.,* size and density) for stability in circulation. Nature's solution to this general problem is the evolution of a class of multifunctional biological nanostructures, namely lipoprotein, which through a spontaneous co-operative self-assembly of lipid with a class of coat-protein termed apolipoprotein, form without dissipating energy.[6,7] These core—shell spherical or discoidal nanoparticles have the capacity to embed a substantial quantity of apolar lipids primarily as triacylglycerols and esterified cholesterol in their core. The outer coat is composed of a phospholipid-cholesterol monolayer (exposing polar head groups that facilitate water solubility) bounded by the coat apolipoproteins, which act concomitantly as receptor ligands, emulsion stabilizers, and scaffolds for the lipidic core. The common evolutionary origin of the coat apolipoproteins—established *via* sequence homology of these proteins across organisms—lend support to their primary and shared lipid-transport function *via* self-assembly of proteo-lipidic nanostructures.[8]

*Address correspondence to anparikh@ucdavis.edu.

Published online December 23, 2010.
10.1021/nn103098m

Native lipoproteins of the circulatory system are divided into four major categories (Figure 1) based on their size, density, and composition, each of which can be associated with highly differentiated, specific functions. The largest and the least dense chylomicrometers (>80 nm, $d = 0.95$ g/mL) are formed in enterocytes through the packaging of exogenous triacylglycerols (TAG) (assembled from absorbed free fatty acids and monoacylglycerols) with other lipids and apolipoproteinB-48 (ApoB-48). They have the highest TAG concentration (80−85% weight by weight, w/w) and the least protein content (1−2% w/w). The next smaller and correspondingly denser class of lipoproteins, termed very low density lipoproteins (VLDL, 30−80 nm, $0.95 < d < 1.006$) export endogenous triacylglycerols and cholesteryl esters from hepatocytes for use by peripheral tissues and are packaged with ApoB-100 prior to excretion from the liver. Like chylomicrometers, they are primarily composed of TAG (around 40−50% w/w) but also contain esterified cholesterol in addition to a number of lipid-soluble compounds including vitamins A and E. The removal of TAG by lipoprotein lipase converts VLDL particles into smaller, more stable, and somewhat denser, low density lipoproteins (LDL, 18−25 nm, $1.02 < d < 1.06$). LDL particles are cholesterol-rich (40−50% w/w) primary cargo vehicles for the delivery of cholesterol to peripheral tissues, accounting for 70−80% of the circulating cholesterol in humans hence an important biomarker in the progression of atherosclerosis. In contrast to chylomicrometers and VLDL particles, which have a substantial TAG component, LDL particles are composed primarily of cholesterol and cholesteryl esters, and remain characteristically associated with ApoB-100. High density lipoproteins (HDL, 7−16 nm, $1.063 < d < 1.200$) are the smallest of the lipoprotein particles, have the highest protein content (50−60% w/w)[9] and function to collect unesterified cholesterol from peripheral tissues and return it to the liver *via* reverse cholesterol transport.[10] It is primarily because of this cholesterol efflux property that renders HDL, in addition to LDL above, and its associated protein component (APOA-I) an important biomarker and key in the prevention or reversal of atherosclerosis,[11] a leading cause of death in industrialized nations.

The diverse structural protein components of lipoproteins (*e.g.*, ApoA through ApoH) have been extensively studied and are reviewed in detail elsewhere,[12] however it is worth noting the various lipid−protein associations to demonstrate the diversity and versatility of apolipoprotein. The typical combinations are summarized as follows; ApoA-I and ApoA-II (as well as ApoD) are the major protein constituents of and exclusively associated with HDL; ApoB-48 is associated with chylomicrometers and their remnants; ApoB-100 is associated with VLDL, LDL, and their remnants; ApoC-I, ApoC-II, and ApoC-III are components of both HDL and VLDL; and ApoE is found in all of the lipoprotein classes.

From the vantage of nanoscience and nanotechnology, these biological nanostructures—which evolved to provide multiple, well-differentiated functionalities while maintaining a single structural motif—are fascinating both *structurally*, because of their heterogeneous, dynamic, and adaptive character; and *functionally* because of their ability to engender multiple functionalities dependent upon subtle differences in their size, composition, and density. Recent efforts in biomimetic nanotechnologies have drawn inspiration from certain properties of individual biomolecules (*e.g.*, DNA) and modes of their organization (*e.g.*, protein folding and membrane assembly) to design new synthetic and hybrid materials.[13−15] In this vein, lipid-based nanostructures represent a distinct opportunity to exploit supramolecular nanoscale complexes and their emergent properties for both biological and nonbiological applications alike. Among them, liposomes formed by lamellar phases of lipids have been most extensively studied. Recent years have witnessed growing interest in other discrete nanoparticles formed from nonlamellar lipid phases[16] (*e.g.*, the so-called cubosomes and hexosomes)[17,18] primarily for their cargo (*e.g.*, drug) carrying potential[11] as well as discoidal and spheroidal lipoprotein, the subject of the current review. These efforts reveal fertile new areas of interactions between biology and nanoscale engineering, promising to not only recapitulate but surpass the efficiency of selected biological functions, to foster the development of new therapeutics and other functional materials for use outside the realm of biomedicine (*e.g.*, artificial photosynthesis). This review provides a physical-science-based description of the molecular-scale organization of these biological nanostructures, illustrates current, key areas of investigation, and projects their potential for future applications.

VOCABULARY: apolipoprotein – *in vivo*, these animal proteins package lipids for various nutritional and vascular functions. There are six main biological classifications (A, B, C, D, E, H) and a variety of artificially modified structures that find use in synthetic lipoprotein · **lipoprotein** – these assemblies of apolipoprotein and various amphipathic and hydrophobic lipid species provide a variety of functions *in vivo* Lipoproteins are distinguished by their mass density, apolipoprotein type, and protein-to-lipid ratio · **high density lipoprotein (HDL)** – as the name suggests, this class of lipoprotein possesses the highest density as well as the greatest protein-to-lipid ratio and smallest diameter (~10 nm). Apolipoprotein A-I, A-II, or E provides stabilization to the lipoprotein; encompassing either a discoidal lipid configuration or a spherical lipid shell with a hydrophobic, cholesterol ester interior · **reconstituted HDL (rHDL)** – this is the process by which naturally derived and artificial analogs of both apolipoprotein and lipid are combined to form high-density lipoproteins · **self-assembly** – HDL reconstitution technique where lipid-free apolipoprotein is added to phospholipid vesicles and allowed to incubate. The two reactants will spontaneously associate and form lipoprotein structures, given the appropriate reaction conditions · **detergent dialysis** – this technique is frequently used when incorporating membrane proteins into rHDL. A detergent solution, typically sodium cholate, is added to the mixture of apolipoprotein, phospholipid vesicles, and membrane proteins. As the detergent is dialyzed away, the cholate-solubilized lipid structures are stabilized by the apolipoprotein and form lipoprotein.

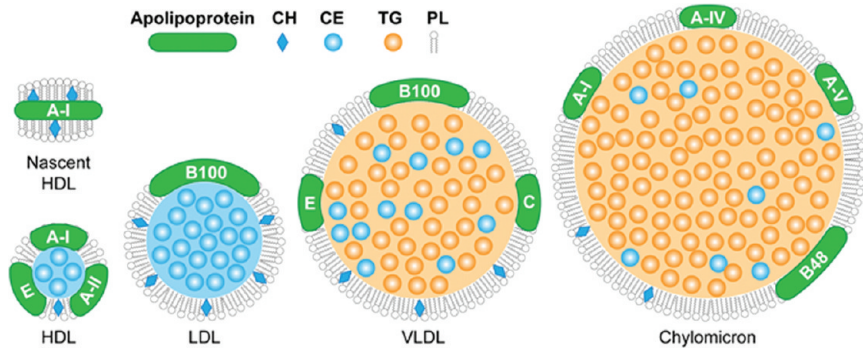

Figure 1. Illustration of the relative sizes and compositions (not to scale) of naturally produced lipoprotein particles, along with the different apolipoprotein associated with each. Abbreviations used: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; CH, cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid.

RECONSTITUTION OF LIPOPROTEINS

In the late sixties, Scanu isolated human apolipoprotein and phospholipid from donors using a two-step sequence of ultracentrifugal-density separation and extraction by organic solvents.[19] After extraction, the lipids were dried down and subsequently rehydrated with an aqueous saline solution. Co-incubation of lipids with the protein isolate, at physiological temperature, formed lipid−protein assemblies. Using a combination of size exclusion chromatography (SEC) to separate the various species, optical absorbance at 280 nm to quantify protein content, and ultracentrifugation to measure particle density; Scanu noted that the products of the lipid−protein incubation resulted in particles that exhibited a discrete set of stoichiometric ratios. This series of observations led to the notion that native lipoproteins could be reconstituted through self-assembly by simply incubating the constituents under appropriate conditions. Physical dimensions for these reconstituted HDL were determined a few years later using transmission electron microscopy (TEM).[20] Different density fractions contained particles with different diameters, the lowest density fraction ($d < 1.063$ g/mL) was 15−40 nm, the middle fraction ($1.063 < d < 1.21$) was 6.5−18 nm and the highest density fraction ($d > 1.21$) had the smallest diameter, 5−12 nm. This distribution of particle sizes obtained by reconstitution corresponds well with measurements of natural HDL isolated from human plasma published independently.[21]

Since this pioneering study, significant experimental efforts have confirmed that recombination of the total apolipoproteins with the total original lipids (including cholesterol and triglycerides) reforms the native HDL structure.[22] Moreover, these studies also establish that recombination of many apolipoprotein mutants and derivatives presenting the lipoprotein binding motif and a variety of natural or synthetic lipids also form reconstituted HDL or rHDL, which assume a discoidal shape. In living systems, the nascent, pre-β discoidal configuration of HDL is transient in its lifetime maturing into a esterified cholesterol-laden spherical particle *via* accumulation (and enzymatic esterification by Lecithin-cholesterol acyltransferase or LCAT) of cholesterol from peripheral tissues during circulation.[23] In contrast, discoidal rHDL lacking HDL-associating LCAT *in vivo* or prepared by reconstitution *in vitro* are indefinitely stable. It is in this configuration that rHDL offers the greatest potential for nanotechnology applications. (Figure 2)

The mechanism of co-operative proteolipidic self-assembly, which underlies lipoprotein reconstitution, remains incompletely understood. In a recent study of thermodynamic factors employing elution profiles obtained *via* gel filtration and fluorescence measurements of probe lipids, Fukuda and co-workers suggest that the association between lipid vesicles and ApoA-I protein is energetically downhill in an apparent enthalpy−entropy compensation. The enthalpic contribution (-620 ± 8 kJ mol^{-1}) out-competes the en-

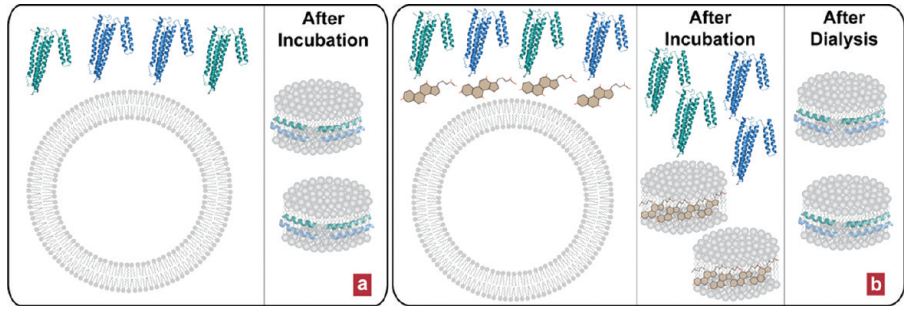

Figure 2. (a) Reconstitution of discoidal high density lipoprotein *via* self-assembly from lipid free apoA-I and phospholipid vesicles. (b) Putative formation process of high density lipoprotein from cholate dialysis. Apolipoproteins are shown in blue and green with phospholipids in gray and detergent in brown.

tropic gain (-570 ± 8 kJ mol^{-1}).[24] Enthalpic driving forces are attributed to the conformational transition in ApoA-I upon association with the vesicular lipids, during which the protein helical content increases markedly from ~60% in unlipidated state to more than 80% in the HDL-bound version.[25] The entropic gain diminishing the strength of the enthalpic contribution is thought to result from changes in the hydration of lipid head-groups and the confinement-induced tighter packing and increased ordering of the lipids[26] in rHDL configuration.[25] An alternative hypothesis suggests that the rHDL formation is prompted by defects in lipid packing at the vesicular surface, which allows apolipoproteins to access the hydrophobic−hydrophilic interface.[27,28] In this position, the N- and C-terminals of the protein can align such that their cylindrical cross-section is within the spherical plane of the vesicle. The secondary structure of apolipoproteinA-I in particular, is dominated by α-helical segments with opposing sides of hydrophobic and hydrophilic side chains. Thus, there exists an energetically favorable state with enough potential energy to induce globular apoA-I to adopt a new lipid-bound conformation. This hypothesis is consistent with the observations that rHDL formation occurs most readily under conditions that foster defect formation in the lipid phase such as in the vicinity of their phase transition temperature or when multicomponent vesicles comprising lipids with vastly different phase transition properties are employed. In these cases, transient fluctuations in lipid densities and domain formation provide mechanisms for defect formation in vesicular membranes.[29,30] This mechanism can also explain the observation reported by Fukuda and co-workers—that the spontaneous association of APOA-I with monocomponent, fluid phase POPC vesicles is exceedingly slow and remains incomplete even after several days—without supporting their conclusion of a high activation energy barrier for spontaneous formation of rHDL. To date however, these propositions remain unverified.

Experimentally, using direct proteo-lipidic self-assembly, incubation of purified apoA-I with small unilamellar vesicles (SUVs) consisting only of pure phospholipid(s) (*sans* cholesterol) readily forms the nascent or pre-β HDL. The phospholipids in these reconstituted HDL (rHDL, also referred to as nanodiscs or nanolipoprotein particles, NLPs) reorganize to adopt a disk-shaped planar bilayer configuration with two or more amphipathic protein molecules fencing the single bilayer disk in a looped-belt conformation.[31,32] The diameter of the lipid platform section of these HDL reconstitutions is tunable as the apolipoprotein belt has the ability to form tightly looped segments that limit the maximum possible length of the lipid-bound conformation. This usually occurs in response to variations in stoichiometry; an increased protein-to-lipid ratio typically results in a smaller diameter lipoprotein complex. Other factors such as the choice of apolipoprotein also influence the dimensions of the final complex.[33] Wild-type apolipoproteinA-I in particular, possesses a very adaptable structure that forms a looped-belt conformation upon binding with lipids.[25,32] The ability to fold over onto itself is required for *in vivo* lipid binding and allows apoA-I to create a remarkable variety of particles with different sizes, shapes, and accompanying functions.[34]

Other approaches to production of biomimetic HDL complexes require more sophisticated biochemical methodology. For instance, several groups have custom-manufactured different variants of apolipoproteins that offer more precise control over the final size of the particle. In the laboratory of S. G. Sligar, membrane-scaffolding proteins (MSPs) are expressed in the bacterium *Escherichia coli* using a synthetic gene.[35] The impetus to produce apolipoprotein-mimetics follows from efforts by structural biologists to crystallize apolipoprotein A-I for X-ray studies. The first alteration removed the first 43 residues to force the apolipoprotein into a confirmation thought to be similar to the lipid-bound state.[36] Subsequent investigations lead to the confirmation that in fact 22 residues located at the N-terminal are not involved in lipid binding.[37] By eliminating this segment from the protein structure, it became possible to more precisely control the size of the resulting lipoprotein particle or "nanodisc", at the expense of the apolipoprotein's conformational adaptability. The remaining structure consists of a histidine tag and cleavage site followed by 200 amino acids coiled into α-helices with amphipathic cross sections, designed to protect the hydrocarbon chains of phospholipids from the aqueous environment. Thus, the protein wraps around a section of lipid bilayer forming the water-soluble, discoidal pre-β HDL structure found in nature. Unlike the direct self-assembly approach, this protein requires incubation of MSP and phospholipid vesicles in a detergent solution containing either sodium cholate or octyl gluocoside, which reorganizes lipids from a vesicular to a micellar configuration.[38] Subsequent dialysis completes the assembly procedure; as the detergent is slowly removed, the MSP binds to the detergent-mediated lipid structures and adopts a stabilizing conformation. The final size of the nanodisc is very similar to its natural analog, approximately 10 nm in diameter and roughly 4−5 nm in height. Advantages of this technique include a compatibility with a variety of phospholipid structures (saturated and unsaturated) as well as tight control over the final diameter of the lipid platform.[37] Recent efforts at the Lawrence Livermore National Lab have explored several alternative lipid-scaffolding proteins including the 22 kDa mutant of apolipoproteinE-IV (apoE422K) and insect apolipophorin-III (apoLp-III). Of significant interest is the cell-free expression of apolipoproteins that allows for potential large-scale reconstitu-

TABLE 1. Factors Effecting Physical Structure of Reconstituted HDL[a]

scaffolding protein	lipid composition	preparation method	final stoichiometry (M_L/M_P)	shape	diameter (nm)
ApoA-I[d]	Egg PC	cholate dialysis	90 to 189	disc	10.4 to 26.4
ApoA-I[h]	DMPC	self-assembly	101	disc	9.5
ApoA-I[e]	DMPC−CH	cholate dialysis	104 to 263	disc	10.2 to 13.0
ApoA-I[f]	DPPC	self-assembly	115, 185, 233	disc	9.9, 14.3, 19.2
ApoA-I[f]	DPPC−CH	self-assembly	110, 160, 221	disc	9.7, 13.6, 18.6
ApoA-I[e]	DSPC−CH	cholate dialysis	82	disc	10.4
ApoA-I[e]	DOPC−CH	cholate dialysis	80	disc	10.3
ApoA-I[b]	POPC−CH	cholate dialysis	31 to 156	disc	8.8 to 10.2
ApoA-I[c]	POPC−CH-CE	cholate dialysis	68	sphere	9.3
ApoA-II[g]	DMPC	self-assembly	44 to 245	disc	11.4 to 24.2
ApoB-17[n]	DMPC	self-assembly	1125	disc	23.9
ApoC-I[k]	Egg PC−CH	cholate dialysis	18	disc	12.1
ApoC-II[k]	Egg PC−CH	cholate dialysis	22	disc	18.5
ApoC-III[j]	DMPC	self-assembly	51	ellipsoid	16
ApoE[i]	POPC	cholate dialysis	58	disc	11.5
MSP1[f]	DPPC	cholate dialysis	82	disc	9.8
MSP1[f]	POPC	cholate dialysis	61	disc	9.7
MSP1E3[f]	DPPC	cholate dialysis	167	disc	12.5
MSP1E3[f]	POPC	cholate dialysis	124	disc	12.5
ApoE422K [o]	DMPC	self-assembly	433/4, 783/5, 1270/6, 1780/7	disc	14.6, 18.8, 23.3, 28.7
ApoE422K[m]	DMPC	cholate dialysis	433/4	disc	14.6

[a]Abbreviations: M_L, moles of lipid; M_P, moles of protein; DMPC, dimyristoyl-phosphatidylcholine; DPPC, dipalmitoyl-phosphatidylcholine; POPC, palmitoyloleoyl-phosphatidylcholine; DOPC, dioleoyl-phosphatidylcholine; CH, cholesterol; CE, cholesterol esters. [b]Bergeron, J. *et al.*, *J. Biol. Chem.* **1995**, 270, 27429. [c]Jonas, A. *et al.*, *J. Biol. Chem.* **1990,** *265*, 22123. [d]Nichols, A. *et al.*, *(BBA)-Lipids and Lipid Metabolism* **1983,** *750*, 353−364. [e]Zorich, N. *et al.*, *(BBA)-Lipids and Lipid Metabolism* **1987,** *919*, 181−189. [f]Wald, J. *et al.*, *J. Biol. Chem.* **1990,** *265*, 20037. [g]Massey, J. *et al.*, *Biochemistry* **1981,** *20*, 1569−1574. [h]Hauser, H. *et al.*, *Eur. J. Biochem.* **1974**, 48, 583−594. [i]Narayanaswami, V. *et al.*, *J. Biol. Chem.* **2004**, 279, 14273. [j]Aune, K. *et al.*, *Biochemistry* **1977**, 16, 2151−2156. [k]Jonas, A. *et al.*, *J. Biol. Chem.* **1984**, 259, 6369. [l]Denisov, I. *et al.*, *J. Am. Chem. Soc* **2004**, 126, 3477−3487. [m]Chromy, B. *et al.*, *J. Am. Chem. Soc* **2007**, 129, 14348−14354. [n]Herscovitz, H. *et al.*, *Proc. Natl. Acad. Sci.* **1991**, 88, 7313. [o]Blanchette, C. *et al.*, *(BBA)-Biomembranes* **2009**, 1788, 724−731.

tion of lipoproteins. Furthermore, with this technique, it is possible to embed membrane proteins during the initial formation process.[39,40] Katzen *et al.* combined plasmids that code for the photoactivated proton transported bacteriorhodopsin (bR) and a truncated version of ApoA-I with a cell free expression system that relies on enzymes extracted from *E. coli*, to produce bR-laden HDL.[41] While the vast majority of reconstituted lipoprotein studies employ wild-type or modified human apolipoprotein, other animal sources have also been explored. Zebrafish apoA-I is similar to human-type but with subtle structural differences and reaction kinetics that suggest enhanced stability in the final lipoprotein assembly.[42]

The exact mechanisms of lipoprotein assembly from detergent dialysis are difficult to capture in physical experiments. However, recent molecular simulations have provided some insight into the self-assembly process, where lipid vesicles and lipid-free proteins are transformed into discoidal lipoprotein structures. With the use of coarse-grain molecular dynamics simulations efforts led by K. Schulten indicate that the cholate-stabilized discoidal lipid bilayers are rearranged into pseudomicelles when presented with uncoiled apoA-I proteins.[43] From these simulations, the final apolipoprotein arrangement is not the expected double belt but instead consists of two proteins positioned on opposite sides of the curved disk surface. The inability of the apoA-I molecules to interact and form salt bridges is attributed to the shortened time scales and simplicity of the coarse-grained model employed. Thus, this configuration is proposed to exist solely as an intermediate state of HDL formation. These initial efforts suggest that analysis of atomic interactions can be used to explain lipoprotein self-assembly while also demonstrating the complexity of the process.

As alluded to earlier, physical dimensions of rHDL are tunable by adjusting a variety of parameters including lipid−protein stoichiometry, lipid type, and apolipoprotein choice. (Table 1) When allowing lipid and protein to self-assemble, changing the belt protein produces a visible trend in particle sizes. The average diameter shifts from 9−11 nm for MSP1T2 to 10−13 nm for ApoA-I to 12−18 nm for ApoE422K.[33] This size disparity is due to several key structural differences. While the final conformation of wild-type ApoA-I is variable giving a large degree of adaptability, artificial proteins (MSP1T2 and ApoE422K) can be engineered for specific sizes by altering the genetic sequence of the plasmid used in the *E. coli* expression system.[37] Depending upon the intended use, either a rigid or more flexible belt protein can be selected. Still further, customization of the molar ratio of lipid-to-protein provides additional tunability of lipoprotein size. At a ratio of 300/1, large discoidal complexes are formed and with a ratio near 100/1, the smallest possible discoidal HDL ap-

pear.[22] The larger lipoproteins contain either three or four apolipoproteins per particle, while the smallest have only one to two apolipoproteins. Between these two stoichiometries, the disk diameter varies from 9 to 35 nm. The structure of HDL can be further tuned with different lipid types. Saturated phospholipids of different lengths can provide specific hydrophobic thicknesses, the addition of cholesterol can alter the physical rigidity of the membrane portion, and unsaturated lipids can confer lateral molecular mobility over a wider temperature range. These adaptable structural features make rHDL a versatile platform for biocompatible applications.

As with any new technology, significance is determined by utility. In the case of reconstituted lipoprotein, the seminal feature is the packaging of membrane-bound components in a manner suitable for *in vitro* or *in vivo* use. In the past decade, dozens of published studies describe successful reconstitution of membrane proteins into natural, synthetic, or hybrid configurations of lipoprotein. Potential applications include scientific study of protein structure, investigation of receptor−ligand interactions at the cell surface, engineering of protein or enzyme function for analytical tools, medical therapies, and energy production. These initial publications portend many interesting opportunities to develop new technologies that incorporate the same molecular components and functions utilized by nature.

IN VITRO APPLICATIONS

Structural Studies of Membrane Proteins. Structural biologists strive to understand the relationship between the physical properties of biological assemblies and their attendant function. New techniques permit the extension of this investigation down to the submicroscopic level, where the organization and composition of large-scale molecular assemblies can be identified. Despite significant progress, high-resolution structural characterization of membrane proteins—a broad class of cell surface molecules performing a range of signaling, transport, and recognition functions—remains elusive. Technical difficulties related to isolation, purification, and organization continue to hamper efforts to ascertain their fundamental structural properties. Because of a strong amphipathic nature, membrane proteins are not readily soluble in aqueous solvents and are difficult to extract from their native lipid environments. To this end, the lipid scaffold provided by rHDL provides a stable and versatile platform to incorporate a host of membrane-associated proteins for structural investigations at the nanoscopic/molecular level. (Figure 3) Preparation of HDL-membrane protein suspensions typically begins with co-incubation of the target protein along with the appropriate apolipoproteins and phospholipids, in a sodium cholate solution. As the detergent is dialyzed away, the increasing polarity of the solvent presents a strong incentive for hydrophobic components to embed themselves in the amphipathic lipid platform. Next, affinity or size exclusion chromatography can be used to separate successfully assembled complexes from any unreacted reagents. This method is used in a number of structural and functional studies including: adsorbing catalytically active cytochrome P450 on mica surfaces,[44] assessing the binding properties of G-protein coupled receptors,[41,45] and to control the organization of oligomeric bacteriorhodopsin.[46]

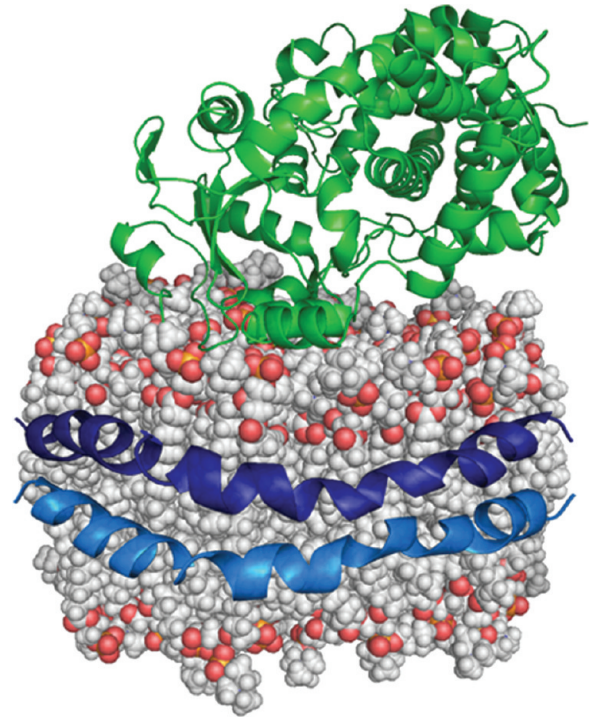

Figure 3. Schematic of a reconstituted HDL particle (lipids in red and gray, apolipoproteins in blue) with embedded cytochrome P450 (CYP3A4, shown in green). For lipid molecules, oxygen atoms are colored red and carbon and hydrogen are gray in order to distinguish between the primary hydrophilic and hydrophobic regions. Only the cytochrome protein domains exterior to the membrane are visible.

A detailed review recently published by J. L. Popot targets three nonconventional strategies for studying membrane proteins including nanodiscs, and discusses their relative strengths and weaknesses as well as their use in functional and structural assays.[47] In particular, the main advantage of synthetic lipoproteins lies in their ability to package membrane proteins in a lipid environment that closely mimics their native state and the larger/tunable size appropriate for larger proteins or polymeric complexes. By careful selection of scaffolding protein, it is possible to limit protein aggregation and insert monomeric versions of proteins for study.[45,46,48−51] This variability of disk diameter stems from deletion or addition of residues to the scaffolding protein, which is dictated by the gene used during protein expression. The primary disadvantage of synthetic lipoproteins is evident when working with proteins with

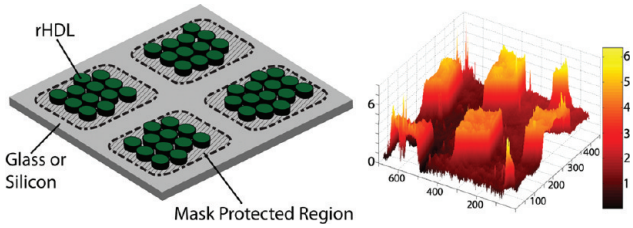

Figure 4. Schematic (left) and ellipsometric height map (right) depicting a single monolayer of reconstituted HDL composed of wild-type ApoA-I and DMPC adsorbed to a surface then patterned with UV photolithography. Lateral dimensions of the ellipsometric map are in micrometers and the height color bar is in nanometers. Copyright 2008 American Chemical Society.[61]

large transmembrane segments as the discoidal particles can become unstable.[47] Additional structural studies and potential membrane-protein targets for incorporation into HDL complexes are summed up in several recent topical reviews.[52,53]

The secondary and tertiary structure of proteins is typically probed with techniques such as X-ray crystallography and infrared spectroscopy. From the data gathered, a more general understanding of the relationship between protein structure and biological mechanisms can be achieved. However, thanks to synthetic lipoprotein, an old technique is finding new applications in the field of protein structure. Within the last year, biologists have begun performing structural studies using solution-state nuclear magnetic resonance (NMR) with membrane proteins embedded in reconstituted HDL.[54] Finding a suitable solvent to perform solution-state NMR spectroscopy is difficult as the amphipathic nature of membrane proteins requires detergents whose micelle configuration can distort protein conformation and is far too labile to prevent undesired thermal fluctuations. In contrast, rHDL offers a discoidal lipid environment with customizable rigidity and enhanced stability. *A priori* calculations indicate that rHDL particles undergo isotropic motion and have spectroscopic properties appropriate for NMR investigations. Indeed, initial attempts to combine the two technologies proved that binding between reconstituted lipoproteins and the antimicrobial peptide Antiameobin-I can be detected using high resolution NMR.[55] Furthermore, addition of a spin label to the lipid portion of the rHDL complex allows the conformation of the peptide upon binding to be indentified. Large integral membrane proteins that are typically difficult to solubilize and investigate with NMR can also be incorporated into synthetic lipoproteins. Examples include the membrane-spanning domain of CD4 (a glycoprotein involved in T cell activation),[56] the voltage-sensing domain of the archaeal potassium channel KvAP,[57] and a G-protein coupled receptor (GPCR) CC-chemokine receptor 5 involved in inflammatory response.[58] To date, Fourier transform infrared (FTIR) spectroscopy and X-ray crystallographic studies have only been conducted on synthetic HDL itself, however the potential for studies with transmembrane proteins remains.

Surface-Supported Analytical Systems. Because of their water-soluble nature and discoidal geometry, HDL are capable of adsorbing to hydrophilic surfaces such as glass and silicon oxides. (Figure 4) Simply incubating the substrate in an aqueous solution containing lipoproteins is sufficient to form a densely packed monolayer on the substrate surface.[59,60] Compatible with both bottom-up and top-down methodologies for microscale fabrication, HDLs represent an ideal platform for immobilizing membrane components in detailed spatial patterns over a surface for high-throughput binding assays. Patterning techniques including microcontact printing and UV photolithography are well-suited for use with thin organic films of lipid-based particles.[15,61] The result is a system with membrane components arranged over two discrete length scales; individual lipoproteins provide organization over a range of tens of nanometers while patterning produces isolated patches tens of micrometers in width. Hierarchical assembly is ideal for instruments and techniques using planar surface geometries for analysis of samples. These approaches are of strong interest to biochemists and engineers who seek to quantify interactions between soluble ligands and membrane-bound receptors. Arrays of UV-patterned HDLs are compatible with visualization techniques including imaging ellipsometry (IE) and fluorescence microscopy (FM),[61,62] for both fluorescently tagged and label-free ligand binding.

The ability to array reconstituted lipoproteins over surfaces with incorporated membrane receptors immediately suggests their use in high-throughput, massively parallel biochemical assays. Potential combination with flow cytometry to investigate receptor−ligand interactions can create a unique set of analytical tools with the capability to isolate individual parameters such as receptor aggregation and conformation, and rigidity of the local environment. Additional advantages include the ability to attach proteins to surfaces without concern for detrimental substrate interactions and providing analyte access to all external domains of transmembrane proteins.[63,64] The most popular scheme makes use of the 6x His tag added to the synthesized apolipoprotein. This protein sequence binds with high affinity to nitrilotriacetic acid with an attached nickel ion. The Ni-NTA chelate complex is covalently attached to thiol or silane molecules, which self-assemble over planar substrates to functionalize surfaces such as silicon, glass, or gold. A MALDI-TOF mass spectrometry investigation employing this strategy along with nanodisc-embedded rhodopsin was able to successfully demonstrate the binding of light-activated rhodopsin to transducin as well as detecting the inhibition of binding with the nucleotide GTPγS.[65] Also, synthetic lipoprotein stabilized by ze-

brafish apolipoproteinA-I and bearing the same G-coupled protein receptor, rhodopsin, was immobilized over a silicon crystal for attenuated total reflection Fourier tranform infrared spectroscopy (ATR-FTIR) using the same His-tag Ni-NTA linkage. This configuration allowed probing of the functionality of the rhodopsin photoreceptor by surface enhanced infrared absorption (SEIRA) difference spectroscopy.[66]

Several preliminary investigations indicate that HDL is capable of regulating the molecular dynamics of membrane-bound receptors, thus allowing investigation of various biophysical parameters and their influence on ligand binding.[52,62,67] The interaction between the membrane-associated glycolipid GM1 and the cholera toxin protein subunit B is a well-studied system and frequently employed for assessing the capabilities of any new analytical technique. Since binding is pentavalent, a variety of different physical parameters can be analyzed, for instance, membrane rigidity, membrane fluidity, receptor organization and receptor mobility.[68,69] By careful selection of the constituent lipids, rHDL can isolate these individual factors and analyze the effect of each on the receptor−ligand interaction. Published results indicate that cholera toxin association with glycolipids (contained in surface-supported synthetic HDL) agrees well with expectations; there is a detectable enhancement of binding in systems that limit GM1 receptor aggregation.

Energy and Chemical Conversion. Many photoactive and chemically active proteins are membrane associated. Examples of photoactive proteins include photosynthetic reaction centers from purple bacteria, photosystem PSII, and bacteriorhodopsin. Efforts by researchers in the field of X-ray crystallography have revealed the physical structure of these proteins and their amenability to incorporation in synthetic lipoprotein.[70−72] Potential artificial photosynthetic devices may combine rHDL with rhodobacteria photosynthetic reaction centers (that can be produced in abundance)[73] and charge transporting substrates, or researchers may pursue enhancement of current solar cell technology. Graff *et al.* have developed a technique to mate single-walled carbon nanotubes with poly-His tagged proteins.[74] As described earlier, when apolipoproteins are produced by *E. Coli* injected with a DNA plasmid, this genetic material often codes for several His tags at the N terminus for later isolation with affinity chromatography. This modification does not effect function[61] and is readily compatible with the nanotube attachment strategy. A collaborative effort spearheaded by M. S. Strano has very recently demonstrated a functional light-harvesting device, integrating photosynthetic reaction centers embedded in nanodiscs with highly conductive single-walled carbon nanotubes.[75]

Chemical converters and other enzymes capable of breaking down or building specific molecular materials have also found incorporation into synthetic lipoprotein platforms. Functional hydrogenases embedded in NLPs are capable of producing hydrogen from starches and simple sugars.[39] This process could find use as a hydrogen generator for hydrogen-fueled devices or perhaps where biofriendly production is necessary. The cytochrome P450 enzymes are membrane-bound proteins responsible for catalyzing the oxidation of organic substances *in vivo*, a role which includes metabolism and bioactivation of therapeutic drugs in humans. Nanodisc technology is enabling new explorations into the structure and kinetic properties of these critical cell surface components.[76] Because of their importance in drug metabolism, it is not difficult to envision this system finding use in the pharmaceutical industry where simple and inexpensive tests could be conducted to determine how a potential drug molecule may be received in the body.[77]

IN VIVO APPLICATIONS

Infectious Disease. In addition to use in a laboratory or industrial setting, reconstituted lipoproteins are also being explored as a direct therapy for a variety of diseases. *In vivo*, HDL particles can be remodeled to perform innate immune functions by addition of antimicrobial agents to HDL. A pathogenic protozoa *Trypanosoma brucei brucei* that causes a potentially lethal sleeping sickness is 500-fold less active in the presence of HDL that contain haptoglobin-related protein and apolipoproteinL-1. The apoA-I-stabilized HDL molecule supplies a platform for the assembly and protective function of these synergistic proteins.[78]

From the robust, nanosized lipid scaffold, rHDLs are capable of exogenously presenting a variety of pathogen targets that may serve as decoys to prevent infection of healthy cells. (Figure 5) One example is the glycolipid GM1, a signaling molecule typically found at the cell surface but whose presence is exploited by infectious agents such as cholera toxin.[79] In fact, recent research shows that rHDL-supported GM1 do bind cholera and are able to protect populations of mammalian cells once exposed to the toxin.[62] Other membrane-anchored glycoconjugates similar to GM1 play a role in a variety of viral infections including HIV,[80] influenza,[81] sendai,[82] rotavirus,[83] rabies,[84] and polyoma viruses.[85] These examples span a broad range of classes, from lipid-enveloped to nonenveloped and both DNA and RNA types. By introducing these attachment molecules to the body, embedded in reconstituted HDL, pathogens may bind with these decoys instead of the receptors at the cell surface. And by reducing or eliminating this targeted attachment of pathogens to cells, these infections can be effectively treated without a lengthy and expensive drug development process that must be tailored to each individual disease.

While this pathogen-decoy strategy is not a recent development, rHDL has greater potential to be successful than many of the proposed alternative approaches.

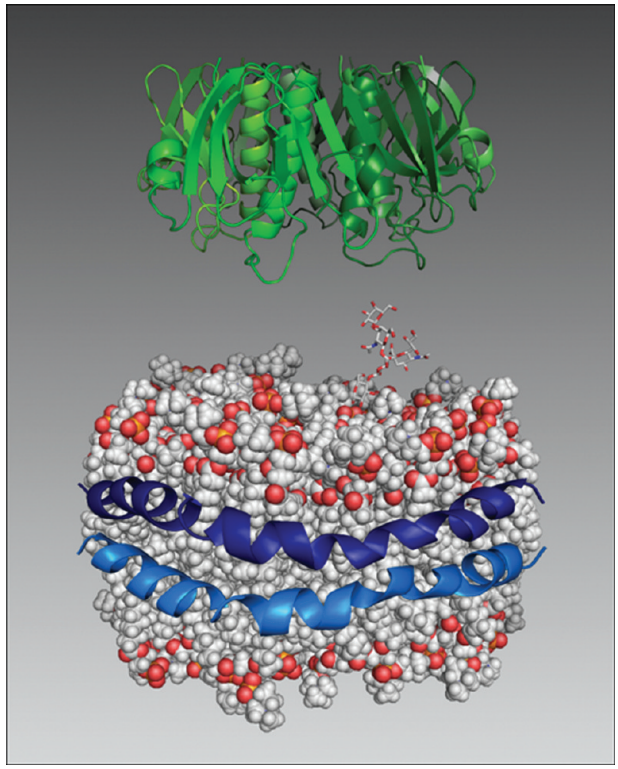

Figure 5. Schematic of a reconstituted HDL bearing a single GM1 molecule (red, gray and blue), capable of binding pentameric cholera toxin B (green).

Prior efforts have focused on polymer or liposome-based approaches. Liposomes prepared from native lipid compositions exhibit poor stability *in vivo*, and any incorporation of stabilizing agents (*e.g.,* PEGylated derivatives) can adversely impact efficacy with unknown side-effects.[86,87] Furthermore, the two-dimensional vesicular environment is not particularly well-suited to controlling the lateral organization of membrane-bound receptors.[68] In the case of multivalent toxins (*e.g.*, shiga and cholera), control of receptor aggregation is necessary to selectively promote monovalent interactions and maximize the amount of toxin-decoy binding. While the physical dimensions of liposomes lie on the nanometer scale, they typically contain thousands of receptors in a fluid environment, rendering them ill-suited for these applications. Another frequently studied approach makes use of a polymer scaffold to present toxin-binding domains. These synthetic structures allow for some limited control over lateral organization *via* conformational flexibility of the polymer backbone.[88,89] Their practical applications, however, are hampered by several unresolved issues: (1) the need for an extensive synthetic effort; (2) the difficulties in designing polymers with a useful range of conformational dynamics; and (3) a lack of control over orientation of receptor binding motifs relative to the scaffold. Moreover, *in vivo* implementation of many of these treatments has proven ineffective or even toxic.[90] Finally, soluble analogs of typical membrane-bound receptors have also been proposed as potential binding decoys.[91,92] However, without the lipid environment, control over their conformation and aggregation is difficult. In addition, their binding affinity can be reduced from native levels by a full order of magnitude,[93,94] presumably because the lipid structure performs some necessary role in the binding process.[95,96]

Even without additional modifiers, rHDLs have proven effective against serious communicable disease. Infections of the circulatory system are frequently lethal; the danger stemming not only from bacterial pathogenesis (called endotoxemia) but also the stimulated immune response that can lead to whole body inflammation (a condition known as septic shock). As seen in clinical studies with human and animal subjects, rHDLs can reduce the production of tumor necrosis factor (TNF) in response to challenges from gram-negative bacteria.[97−100] TNF is a signaling protein that serves as an indicator of the severity of the immune response. Gram-negative bacteria (examples include *E.Coli* and *Salmonella)* are distinguished by the endotoxin known as lipopolysaccharide (LPS), which is anchored at the bacterial cell surface to provide structural integrity and resistance against chemical attack. LPS is released during host defense by human lipopolysaccharide-binding protein (LBP) and soluble CD14. Although this mechanism is necessary to protect against infection, there is a strong risk of an overaggressive immune response resulting in whole body inflammation (septic shock) and furthermore, the endotoxins are not actually cleared from the bloodstream. However, HDLs are able to bind and neutralize the LPS released during normal immune function, without prompting further inflammation. Even more significantly, HDLs are effective against both smooth and rough LPS types, which are characterized by the presence or absence (respectively) of an O-antigen. Each O-antigen (or its absence) requires a specific antibody in order to be recognized by the host as a pathogen, if this antibody is not present then the infection may be lethal. In contrast, the lipid platform of HDL can bind endotoxin through a nonspecific interaction with the LPS Lipid A domain. This lack of O-antigen specificity for LPS neutralization suggests HDLs can mitigate the dangers of sepsis by serving as a quick and universal treatment.

Reconstituted HDLs can also be engineered to serve as vaccines. Membrane components isolated from pathogens can stimulate an immune response, induce the body to produce antibodies, and provide immunity in case of infection. One example where this strategy has proved effective is influenza. When administered subcutaneously or *via* the nasal passages, nanodiscs housing recombinant viral hemagglutin stimulate the immune system of mice, as evidenced by the expression of IgA, IgG, and IgM antibodies.[101] The build-up of these antibodies is necessary to provide protection against future infections. Although this study tests only

influenza envelope proteins, rHDLs are appropriate for any membrane-associated viral fusion protein. Another example combines synthetic lipoprotein technology with West Nile virus. By embedding nickel-chelating lipids in a nanolipoprotein particle, water-soluble hexahistidine-tagged versions of viral lipid envelope proteins can be attached. The nanoscale dimensions of the HDL platform hold the viral proteins in close proximity and amplify polymerization, which is thought to lead to the enhancement of immunogenicity and protection against infection in mice models.[102] With these techniques, HDL technology can be used as a general platform to produce a broad spectrum of vaccines, with the added benefit of superior shelf life due to their robust structure and water-soluble nature.

Drug Delivery. Delivery and targeting of therapeutic drugs to specific tissues or cells is important for maximizing efficacy and limiting adverse reactions. The inherent toxic nature of many drugs (*e.g.*, traditional chemotherapy) can lead to dangerous side effects when administered in the large dosages needed to elicit therapeutic action. To satisfy these conditions, rHDLs have been tested for their efficacy and specificity in the targeted delivery of cancer drugs,[103,104] cardiovascular disease drugs,[105] and hepatitis B drugs.[106] One promising study exploited the overexpression of folate receptors in tumor cells; folate receptor FR-α is a membrane-bound protein that exists in limited quantities in the body, appearing only in the kidney.[107] Thus by conjugating folic acid to the apolipoprotein, it was possible to specifically target lipoprotein-carried drugs (*e.g.*, paclitaxel) to tumor cells.[108] In this example, LDL was selected as the drug carrier; however, there is still ample free volume in the hydrophobic core of spherical HDL as well.[109] An additional advantage of rHDLs for drug delivery is their structural similarity to native analogs. HDLs effectively avoid recognition by macrophages that clear foreign substances and do not trigger immune responses like other synthetic materials such as liposomes.[105]

A significant portion, nearly 40%, of new drug candidates are lipophilic, leading to poor bioavailability, high intra- and intersubject variability and lack of dose proportionality.[110] One approach to offset these challenges is to encapsulate lipophilic drugs inside solid lipid nanoparticles that contain a gel-phase hydrophobic core consisting of mono- and triglycerides and waxes.[111] Unfortunately, due to their crystalline lipid matrices, these particles are susceptible to coagulation and premature expulsion of loaded drugs.[112] However nature produces its own variant of a hydrophobic molecule transporter, HDL, which contains a lipophilic core, composed of cholesterol esters and triacylglycerol. In addition, scavenger receptors exist in many cell types that can be harnessed to actively internalize synthetic lipoproteins.[62] Encapsulating lipophilic drugs in the core of rHDL has great potential for safe and effective delivery to target tissues of interest. For example, nosiheptide, a lipophilic peptide with antihepatitis B viral activity, has poor distribution in the liver. Yet when complexed with rHDL the drug distribution in rat liver was 7 times higher than in plasma. It is not surprising that the dose needed to achieve 50% viral inhibition in hepatocytes was 200 times lower when the drug was complexed to rHDL *versus* free nosiheptide.[113] Similar results were found with rHDL doped with the antiviral drug acyclovir. Chemically modifying the drug to include a lipid palmitate moiety helped foster incorporation into the HDL lipid platform. The resulting complex exhibited improved liver targeting and antiviral activity.[114] Along these lines, targeting of antifungal agents to specific macrophages can improve drug efficacy. After administration of amphotericin B complexed with lipid nanodiscs at regular intervals over several days, mice infected with the parasitic protozoa *Leishmania* demonstrated visible improvement in symptoms without a detectable change in immune response. This cutaneous infection is potentially lethal for immune-compromised patients and current therapies are hampered by poor drug solubility and toxic side effects, thus the need for a targeting delivery vector is critical.[115] Other studies have shown that spherical HDL is appropriate for targeting and delivery of the lipophilic drug aclacinomycin, even leading to elevated cellular uptake.[116] And finally, HDL reconstituted with sphingosine-1-phosphate (a signaling lipid involved in immune and vascular function) administered in conjunction with doxorubicin can reduce cytotoxic side effects and protect cardiomyocytes.[117] While strategies for controlled release have yet to be resolved, reconstituted lipoprotein remains a viable option for selective targeting and delivery of a wide variety of nonsoluble or amphipathic therapeutics.

Noncommunicable Disease. Careful study of the nanoscale structure and behavior of lipoprotein components has influenced the design of new and improved therapies for a variety of noncommunicable diseases. Two recent reviews address the rationale for the therapeutic use of HDL in cardiovascular disease and other associated metabolic diseases such as type 2 diabetes. These reviews also delineate the current progress of lipoprotein-related therapies including, rHDL, ApoA-I, mimetic ApoA-I, and the various pharmaceutical approaches to directly increasing total HDL cholesterol.[118,119] In summary, specific structural and compositional properties of HDLs determine their efficacy in performing key functions, including reverse cholesterol transport (the removal of cholesterol from macrophages and arterial plaque) endothelial repair, and protection *via* stimulation of nitric oxide production, as well as antioxidant, anti-inflammatory, and antithrombotic functions.[119] These properties may be derived from specific proteins loaded into HDL (similar in composition to the assemblies produced for *in vitro* studies) by the body in response to the onset of cardiovascular

disease. The loaded proteins are known to be involved in lipid metabolism as well as immune-related protease inhibition, complement activation, and acute-phase response; auxiliary functions thought to facilitate antiatherosclerotic activity.[120]

The concept of "dysfunctional HDL" or HDL with aberrant composition and structure that render them ineffective at reverse cholesterol transport and other functions are increasingly being documented in samples from patients with cardiovascular disease.[118] As a result, clinical studies where reconstituted HDLs of the correct structure and composition are introduced to the patient show significant potential. A recent crossover, placebo-controlled clinical trial in 13 male type 2 diabetes patients showed that infusion of rHDL increased the anti-inflammatory properties of isolated HDL, reduced neutrophil adhesion, and increased the efflux of cholesterol from macrophages by 40−60%.[121] Another recent 20-patient clinical trial showed a significant reduction in expression of compounds responsible for cell adhesion, a reduction in arterial plaque lipid content and a 20% increase in HDL cholesterol concentration with rHDL infusion.[122] Another placebo-controlled intervention in a larger group of patients ($n = 60$) using intravascular ultrasound to assess change in plaque burden found no significant reductions in plaque volume but did result in improvements in plaque characterization score and coronary score with rHDL treatment.[123] However, patients receiving the rHDL infusions experienced increases in liver enzymes, which were self-limiting and mild in the lower dose group (40 mg/kg) but in the higher dose group (80 mg/kg) these changes were indicative of liver dysfunction and treatment was discontinued early in the study. Clearly further study is still needed.

Reconstituted HDLs have also been found to reduce pancreatic β-cell dysfunction and can improve β-cell function and reduce islet inflammation in type 2 diabetes.[124] In the body, β-cells are responsible for producing insulin and amylin, proteins that help regulate blood glucose levels. The dysfunction of these pancreatic cells is directly responsible for the development of diabetes mellitus. Typical lipoprotein receptors such as ABCA1 and ABCG1 can be found at the membrane surface of these cells, allowing HDLs to dock and remove excess cholesterol that may otherwise cause detrimental inflammation. By tuning HDLs with the appropriate lipid/protein composition for stability in the body and docking compatibility, it may be possible to directly mitigate the causes of type 2 diabetes. This approach offers significant improvement over many current treatments which simply alleviate symptoms. Other studies detail additional mechanisms by which HDL-based therapies can combat diabetes. For example, rHDL infusion is capable of decreasing platelet aggregation, in parallel with improved cholesterol efflux and reduced lipid raft assembly in platelets;[125] improving plasma glucose by increasing plasma insulin and muscle glucose uptake;[126] and improving endothelial function[127,128] in patients with type 2 diabetes.

ApoA-I and apoA-I-containing rHDL also show promise as biologically effective therapeutic solutions for treating symptoms of Alzheimer's Disease (AD). The pathophysiology of AD includes accumulation of amyloid-β peptide (Aβ) aggregates in brain tissue leading to oxidative damage, inflammation, synaptic injury, and ultimately neuronal dysfunction.[129] Phage peptide screening led to the identification of a peptide that was homologous to an amino acid sequence comprising the N-terminal domain of apoA-I that could bind to aggregated Aβ. In addition, binding experiments of purified human apoA-I and rHDL containing apoA-I bound to amyloid-β led to hippocampus neuronal protection from amyloid-β-induced oxidative stress and neurodegeneration in rats.[130] Interestingly, an apoA-I mimetic, D-4F, which mimics the anti-inflammatory, antioxidant, and cardioprotective functions of HDL,[131] was found to inhibit Aβ deposition and improved the cognitive performance in a mouse model of Alzheimer's disease.[132] In terms of therapy, HDLs are capable of delivering small molecules such as vitamin E across the blood-brain barrier, as shown in an *in vitro* model. Also, apolipoprotein A-I found in human cerebrospinal fluid is effective against forming plaques associated with Alzheimer's disease and was thought to have originated in the blood. These results suggest that not only are HDLs capable of initiating mass transport across the blood-brain barrier but they themselves may enter the brain through endothelial cell caveolae, providing a physiological pathway for treatment.[133] Thus rHDL and apoA-I mimetics not only offer cardiovascular protection but may be potential therapeutics for Alzheimer's disease where no current treatment exists.

Medical Imaging. Medical researchers are exploring both biological and synthetic molecular structures for use as *in vivo* diagnostic tools. Currently, fully artificial systems are composed of heavy metals such as colloidal gold nanoparticles for imaging tumors[134] or potentially toxic polymers used to encapsulate magnetic contrast agents in magnetic resonance imaging (MRI).[135] The safety to human health and occupational hazards that may manifest during construction, implementation, and disposal of synthetic nanoparticles is cause for concern. Also, the long-term toxicity of new classes of synthetic nanosubstances and their ecological impact has yet to be assessed. As a result, some researchers are turning toward more biocompatible systems centered around reconstituted lipoproteins. While these approaches can include metals such as gadolinium or iron oxide, these materials are widely considered to be safe for use as MRI contrast agents, and complexing with HDLs enables *in vivo* biodegradation *via* customary bodily clearance mechanisms.[136,137] Once again size plays a crucial role as the nanoscale dimensions of

HDL allow it to pass through the endothelial barrier and access specific tissues for imaging.[138]

OUTLOOK AND CONCLUSIONS

The rapidly growing appreciation for the enabling nature of reconstituted lipoprotein, inspired by nature's own nanotechnology, is fueling interest in both fundamental studies and exploration of their use in technological applications. Both research scientists and engineers have put rHDL-based nanostructures to use for many purposes: to investigate receptor−ligand interactions, for structural studies of difficult-to-isolate membrane proteins, to pursue potential therapeutic applications, and to build functional devices that are capable of efficient conversion of energy and molecular materials. The adaptability and robustness of HDL both *in vitro* and *in vivo* make this biomimetic nanotechnology attractive for a diverse group of applications.

Of course there are issues and concerns with synthetic lipoprotein that need addressing. While rHDLs permit isolation of membrane proteins in their *in vivo* conformation, their presence can complicate physical measurements performed with NMR, X-ray scattering, and infrared spectroscopy. For therapeutic or diagnostic applications, many cell types contain receptors such as ABCA-I or SR-BI that bind HDL and may result in the delivery of therapeutic agents to undesirable locations. While these concerns are not trivial, the advantages provided by reconstituted HDL over alternative approaches are multifold; robust stability, long storage life, high biocompatibility, limited toxicity, adaptable or rigid structure, and of course the nanoscopic dimensions of the assembled complex.

In the immediate future, researchers are likely to focus on broadening the catalog of membrane components compatible with synthetic lipoproteins. In the slightly more distant future, it is reasonable to expect demonstration of new types of functional lipoproteins, incorporated in artificial constructs yet compatible with biological systems. For instance, in therapeutic applications the development of targeting agents that can be added to rHDL is highly critical. The ability to identify and limit drug interactions to pathogens, tumors, or other disease-causing factors would drastically enhance current treatments by improving efficacy and limiting adverse drug interactions. In other applications, limited research has shown considerable promise in the deployment of biomimetic technologies in fields such as chemical synthesis and energy harvesting, yielded insights into the structure of cellular membrane components and demonstrated the ability to successfully incorporate and replicate the function of biomolecules in inorganic devices. And finally, reconstituted lipoprotein has also shown promise as a diagnostic and therapeutic in cultured cell and animal models and early phase clinical trials. The pharmaceutical industry will undoubtedly continue to pursue, develop, and patent these new approaches. With a lipid platform of tunable composition, a size-adaptable scaffolding protein and proven versatility, these biomimetic nanoparticles can influence and replicate biological functions in a wide variety of technological applications.

Acknowledgment. We would like to thank J. Petrlova and J. C. Voss for their assistance. The work here is supported by a grant from the U.S. Department of Energy (Biomolecular Materials, Grant DE-FG02-04ER46173) and a grant from the California Dairy Research Foundation (Grant 10 GEB-01 NH).

REFERENCES AND NOTES

1. Segré, D.; Ben-Eli, D.; Deamer, D.; Lancet, D. The Lipid World. *Origins Life Evol. Biospheres* **2001**, *31*, 119–145.
2. Szostak, J.; Bartel, D.; Luisi, P. Synthesizing Life. *Nature* **2001**, *409*, 387–390.
3. Bachmann, P.; Luisi, P.; Lang, J. Autocatalytic Self-Replicating Micelles as Models for Prebiotic Structures. *Nature* **1992**, *357*, 57–59.
4. Deamer, D. The First Living Systems: A Bioenergetic Perspective. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 239–261.
5. Mulkidjanian, A.; Galperin, M.; Koonin, E. Co-evolution of Primordial Membranes and Membrane Proteins. *Trends Biochem. Sci.* **2009**, *34*, 206–215.
6. Barker, W.; Dayhoff, M. Evolution of Lipoproteins Deduced from Protein Sequence Data. *Comp. Biochem. Physiol., Part B* **1977**, *57*, 309–315.
7. Mahley, R.; Innerarity, T.; Rall Jr, S.; Weisgraber, K. Plasma Lipoproteins: Apolipoprotein Structure and Function. *J. Lipid Res.* **1984**, *25*, 1277–1294.
8. Karathanasis, S. Apolipoprotein Multigene Family: Tandem Organization of Human Apolipoprotein AI, CIII, and AIV Genes. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6374–6378.
9. Kaess, B.; Fischer, M.; Baessler, A.; Stark, K.; Huber, F.; Kremer, W.; Kalbitzer, H.; Schunkert, H.; Riegger, G.; Hengstenberg, C. The Lipoprotein Subfraction Profile: Heritability and Identification of Quantitative Trait Loci. *J. Lipid Res.* **2008**, *49*, 715–723.
10. Ikonen, E. Cellular Cholesterol Trafficking and Compartmentalization. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 125–138.
11. Linsel-Nitschke, P.; Tall, A. HDL as a Target in the Treatment of Atherosclerotic Cardiovascular Disease. *Nat. Rev. Drug Discovery* **2005**, *4*, 193–205.
12. German, J.; Smilowitz, J.; Zivkovic, A. Lipoproteins: When Size Really Matters. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 171–183.
13. Katz, E.; Willner, I. Integrated Nanoparticle-Biomolecule Hybrid Systems: Synthesis, Properties, and Applications. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042–6108.
14. Sarikaya, M.; Tamerler, C.; Alex, K.; Schulten, K.; Baneyx, F. Molecular Biomimetics: Nanotechnology through Biology. *Nat. Mater.* **2003**, *2*, 577–585.
15. Zhang, S. Fabrication of Novel Biomaterials through Molecular Self-Assembly. *Nat. Biotechnol.* **2003**, *21*, 1171–1178.
16. Luzzati, V.; Tardieu, A.; Gulik-Krzywicki, T. Polymorphism of Lipids. *Nature* **1968**, *217*, 1028–1030.
17. Barauskas, J.; Johnsson, M.; Tiberg, F. Self-Assembled Lipid Superstructures: Beyond Vesicles and Liposomes. *Nano Lett.* **2005**, *5*, 1615–1619.
18. Drummond, C.; Fong, C. Surfactant Self-Assembly Objects as Novel Drug Delivery Vehicles. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 449–456.
19. Scanu, A. Binding of Human Serum High Density Lipoprotein Apoprotein with Aqueous Dispersions of Phospholipids. *J. Biol. Chem.* **1967**, *242*, 711–719.
20. Scanu, A.; Cump, E.; Toth, J.; Koga, S.; Stiller, E.; Albers, L. Degradation and Reassembly of a Human Serum High-Density Lipoprotein. Evidence for Differences in Lipid Affinity among Three Classes of Polypeptide Chains. *Biochemistry* **1970**, *9*, 1327–1335.

21. Forte, T.; Norum, K.; Glomset, J.; Nichols, A. Plasma Lipoproteins in Familial Lecithin: Cholesterol Acyltransferase Deficiency: Structure of Low and High Density Lipoproteins as Revealed by Electron Microscopy. *J. Clin. Invest.* **1971**, *50*, 1141–1148.
22. Jonas, A. Reconstitution of High-Density Lipoproteins. *Methods Enzymol.* **1986**, *128*, 553–582.
23. Wu, Z.; Wagner, M.; Zheng, L.; Parks, J.; Shy, J.; Smith, J.; Gogonea, V.; Hazen, S. The Refined Structure of Nascent HDL Reveals a Key Functional Domain for Particle Maturation and Dysfunction. *Nat. Struct. Mol. Biol.* **2007**, *14*, 861–868.
24. Fukuda, M.; Nakano, M.; Miyazaki, M.; Handa, T. Thermodynamic and Kinetic Stability of Discoidal High-Density Lipoprotein Formation from Phosphatidylcholine/Apolipoprotein AI Mixture. *J. Phys. Chem. B* **2010**, *114*, 8228–8234.
25. Thomas, M.; Bhat, S.; Sorci-Thomas, M. Three-Dimensional Models of HDL APOA-I: Implications for Its Assembly and Function. *J. Lipid Res.* **2008**, *49*, 1875–1883.
26. Shaw, A.; McLean, M.; Sligar, S. Phospholipid Phase Transitions in Homogeneous Nanometer Scale Bilayer Discs. *FEBS Lett.* **2004**, *556*, 260–264.
27. Fukuda, M.; Nakano, M.; Sriwongsitanont, S.; Ueno, M.; Kuroda, Y.; Handa, T. Spontaneous Reconstitution of Discoidal HDL from Sphingomyelin-Containing Model Membranes by Apolipoprotein AI. *J. Lipid Res.* **2007**, *48*, 882–889.
28. Tricerri, M.; Toledo, J.; Sanchez, S.; Hazlett, T.; Gratton, E.; Jonas, A.; Garda, H. Visualization and Analysis of Apolipoprotein AI Interaction with Binary Phospholipid Bilayers. *J. Lipid Res.* **2005**, *46*, 669–678.
29. Mouritsen, O. Theoretical Models of Phospholipid Phase Transitions. *Chem. Phys. Lipids* **1991**, *57*, 179–194.
30. Mouritsen, O.; Jørgensen, K. Dynamical Order and Disorder in Lipid Bilayers. *Chem. Phys. Lipids* **1994**, *73*, 3–25.
31. Segrest, J.; Jones, M.; Klon, A.; Sheldahl, C.; Hellinger, M.; De Loof, H.; Harvey, S. A Detailed Molecular Belt Model for Apolipoprotein AI in Discoidal High Density Lipoprotein. *J. Biol. Chem.* **1999**, *274*, 31755–31758.
32. Martin, D.; Budamagunta, M.; Ryan, R.; Voss, J.; Oda, M. Apolipoprotein AI Assumes a Ìlooped Beltî Conformation on Reconstituted High Density Lipoprotein. *J. Biol. Chem.* **2006**, *281*, 20418–20426.
33. Chromy, B.; Arroyo, E.; Blanchette, C.; Bench, G.; Benner, H.; Cappuccio, J.; Coleman, M.; Henderson, P.; Hinz, A.; Kuhn, E. Different Apolipoproteins Impact Nanolipoprotein Particle Formation. *J. Am. Chem. Soc.* **2007**, *129*, 14348–14354.
34. Bolanos-Garcia, V.; Miguel, R. On the Structure and Function of Apolipoproteins: More Than a Family of Lipid-Binding Proteins. *Prog. Biophys. Mol. Biol.* **2003**, *83*, 47–68.
35. Bayburt, T.; Grinkova, Y.; Sligar, S. Self-Assembly of Discoidal Phospholipid Bilayer Nanoparticles with Membrane Scaffold Proteins. *Nano Lett.* **2002**, *2*, 853–856.
36. Borhani, D.; Rogers, D.; Engler, J.; Brouillette, C. Crystal Structure of Truncated Human Apolipoprotein AI Suggests a Lipid-Bound Conformation. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12291–12296.
37. Denisov, I.; Grinkova, Y.; Lazarides, A.; Sligar, S. Directed Self-Assembly of Monodisperse Phospholipid Bilayer Nanodiscs with Controlled Size. *J. Am. Chem. Soc.* **2004**, *126*, 3477–3487.
38. Walter, A.; Vinson, P.; Kaplun, A.; Talmon, Y. Intermediate Structures in the Cholate−Phosphatidylcholine Vesicle−Micelle Transition. *Biophys. J.* **1991**, *60*, 1315–1325.
39. Baker, S.; Hopkins, R.; Blanchette, C.; Walsworth, V.; Sumbad, R.; Fischer, N.; Kuhn, E.; Coleman, M.; Chromy, B.; Le tant, S. Hydrogen Production by a Hyperthermophilic Membrane-Bound Hydrogenase in Water-Soluble Nanolipoprotein Particles. *J. Am. Chem. Soc.* **2009**, *131*, 7508–7509.
40. Cappuccio, J.; Hinz, A.; Kuhn, E.; Fletcher, J.; Arroyo, E.; Henderson, P.; Blanchette, C.; Walsworth, V.; Corzett, M.; Law, R. Cell-Free Expression for Nanolipoprotein Particles: Building a High-Throughput Membrane Protein Solubility Platform. *Methods Mol. Biol.* **2009**, *498*, 273–296.
41. Katzen, F.; Fletcher, J.; Yang, J.; Kang, D.; Peterson, T.; Cappuccio, J.; Blanchette, C.; Sulchek, T.; Chromy, B.; Hoeprich, P. Insertion of Membrane Proteins into Discoidal Membranes Using a Cell-Free Protein Expression Approach. *J. Proteome Res.* **2008**, *7*, 3535–3542.
42. Banerjee, S.; Huber, T.; Sakmar, T. Rapid Incorporation of Functional Rhodopsin into Nanoscale Apolipoprotein Bound Bilayer (Nabb) Particles. *J. Mol. Biol.* **2008**, *377*, 1067–1081.
43. Shih, A.; Arkhipov, A.; Freddolino, P.; Schulten, K. Coarse Grained Protein−Lipid Model with Application to Lipoprotein Particles. *J. Phys. Chem. B* **2006**, *110*, 3674–3684.
44. Bayburt, T.; Carlson, J.; Sligar, S. Reconstitution and Imaging of a Membrane Protein in a Nanometer-Size Phospholipid Bilayer. *J. Struct. Biol.* **1998**, *123*, 37–44.
45. Leitz, A.; Bayburt, T.; Barnakov, A.; Springer, B.; Sligar, S. Functional Reconstitution of β_2-Adrenergic Receptors Utilizing Self-Assembling Nanodisc Technology. *BioTechniques* **2006**, *40*, 601–610.
46. Bayburt, T.; Grinkova, Y.; Sligar, S. Assembly of Single Bacteriorhodopsin Trimers in Bilayer Nanodiscs. *Archives of Biochem. Biophys.* **2006**, *450*, 215–222.
47. Popot, J. Amphipols, Nanodiscs, and Fluorinated Surfactants: Three Nonconventional Approaches to Studying Membrane Proteins in Aqueous Solutions. *Annu. Rev. Biochem.* **2010**, *79*, 737–775.
48. Baas, B.; Denisov, I.; Sligar, S. Homotropic Cooperativity of Monomeric Cytochrome P450 3A4 in a Nanoscale Native Bilayer Environment. *Arch. Biochem. Biophys.* **2004**, *430*, 218–228.
49. Bayburt, T.; Leitz, A.; Xie, G.; Oprian, D.; Sligar, S. Transducin Activation by Nanoscale Lipid Bilayers Containing One and Two Rhodopsins. *J. Biol. Chem.* **2007**, *282*, 14875–14881.
50. Tsukamoto, H.; Sinha, A.; DeWitt, M.; Farrens, D. Monomeric Rhodopsin Is the Minimal Functional Unit Required for Arrestin Binding. *J. Mol. Biol.* **2010**, *399*, 501–511.
51. Wang, H.; Na, B.; Yang, H.; Tai, P. Additional *in Vitro* and *in Vivo* Evidence for SecA Functioning as Dimers in the Membrane: Dissociation into Monomers Is Not Essential for Protein Translocation in *Escherichia Coli*. *J. Bacteriol.* **2008**, *190*, 1413–1418.
52. Borch, J.; Hamann, T. The Nanodisc: A Novel Tool for Membrane Protein Studies. *Biol. Chem.* **2009**, *390*, 805–814.
53. Nath, A.; Atkins, W.; Sligar, S. Applications of Phospholipid Bilayer Nanodiscs in the Study of Membranes and Membrane Proteins. *Biochemistry* **2007**, *46*, 2059–2069.
54. Raschle, T.; Hiller, S.; Etzkorn, M.; Wagner, G. Nonmicellar Systems for Solution NMR Spectroscopy of Membrane Proteins. *Curr. Opin. Struct. Biol.* **2010**, *20*, 471–479.
55. Lyukmanova, E.; Shenkarev, Z.; Paramonov, A.; Sobol, A.; Ovchinnikova, T.; Chupin, V.; Kirpichnikov, M.; Blommers, M.; Arseniev, A. Lipid−Protein Nanoscale Bilayers: A Versatile Medium for NMR Investigations of Membrane Proteins and Membrane-Active Peptides. *J. Am. Chem. Soc.* **2008**, *130*, 2140–2141.
56. Gluck, J.; Wittlich, M.; Feuerstein, S.; Hoffmann, S.; Willbold, D.; Koenig, B. Integral Membrane Proteins in Nanodiscs Can Be Studied by Solution NMR Spectroscopy. *J. Am. Chem. Soc.* **2009**, *131*, 12060–12061.
57. Shenkarev, Z.; Lyukmanova, E.; Paramonov, A.; Shingarova, L.; Chupin, V.; Kirpichnikov, M.; Blommers, M.; Arseniev, A. Lipid−Protein Nanodiscs as Reference Medium in Detergent Screening for High-Resolution NMR Studies of Integral Membrane Proteins. *J. Am. Chem. Soc.* **2010**, *132*, 5628–5629.
58. Yoshiura, C.; Kofuku, Y.; Ueda, T.; Mase, Y.; Yokogawa, M.; Osawa, M.; Terashima, Y.; Matsushima, K.; Shimada, I. NMR Analyses of the Interaction between CCR5 and Its Ligand

Using Functional Reconstitution of CCR5 in Lipid Bilayers. *J. Am. Chem. Soc.* **2010**, *132*, 6768–6777.
59. Carlson, J.; Jonas, A.; Sligar, S. Imaging and Manipulation of High-Density Lipoproteins. *Biophys. J.* **1997**, *73*, 1184–1189.
60. Malmsten, M.; Siegel, G.; Wood, W. Ellipsometry Studies of Lipoprotein Adsorption. *J. Colloid Interface Sci.* **2000**, *224*, 338–346.
61. Vinchurkar, M.; Bricarello, D.; Lagerstedt, J.; Buban, J.; Stahlberg, H.; Oda, M.; Voss, J.; Parikh, A. Bridging across Length Scales: Multiscale Ordering of Supported Lipid Bilayers *via* Lipoprotein Self-Assembly and Surface Patterning. *J. Am. Chem. Soc.* **2008**, *130*, 11164–11169.
62. Bricarello, D.; Mills, E.; Petrlova, J.; Voss, J.; Parikh, A. Ganglioside Embedded in Reconstituted Lipoprotein Binds Cholera Toxin with Elevated Affinity. *J. Lipid Res.* **2010**, *51*, 2731–2738.
63. Borch, J.; Torta, F.; Sligar, S.; Roepstorff, P. Nanodiscs for Immobilization of Lipid Bilayers and Membrane Receptors: Kinetic Analysis of Cholera Toxin Binding to a Glycolipid Receptor. *Anal. Chem.* **2008**, *80*, 6245–6252.
64. Fischer, N.; Blanchette, C.; Chromy, B.; Kuhn, E.; Segelke, B.; Corzett, M.; Bench, G.; Mason, P.; Hoeprich, P. Immobilization of His-Tagged Proteins on Nickel-Chelating Nanolipoprotein Particles. *Bioconjugate Chem.* **2009**, *20*, 460–465.
65. Marin, V.; Bayburt, T.; Sligar, S.; Mrksich, M. Functional Assays of Membrane-Bound Proteins with Samdi-TOF Mass Spectrometry. *Angew. Chem., Int. Ed.* **2007**, *46*, 8796–8798.
66. Zaitseva, E.; Saavedra, M.; Banerjee, S.; Sakmar, T.; Vogel, R. Seira Spectroscopy on a Membrane Receptor Monolayer Using Lipoprotein Particles as Carriers. *Biophys. J.* **2010**, *99*, 2327–2335.
67. Morrissey, J.; Pureza, V.; Davis-Harrison, R.; Sligar, S.; Ohkubo, Y.; Tajkhorshid, E. Blood Clotting Reactions on Nanoscale Phospholipid Bilayers. *Thromb. Res.* **2008**, *122*, S23–S26.
68. Shi, J.; Yang, T.; Kataoka, S.; Zhang, Y.; Diaz, A.; Cremer, P. GM_1 Clustering Inhibits Cholera Toxin Binding in Supported Phospholipid Membranes. *J. Am. Chem. Soc.* **2007**, *129*, 5954–5961.
69. Wang, R.; Shi, J.; Parikh, A.; Shreve, A.; Chen, L.; Swanson, B. Evidence for Cholera Aggregation on GM_1-Decorated Lipid Bilayers. *Colloids Surf., B* **2004**, *33*, 45–51.
70. Ermler, U.; Fritzsch, G.; Buchanan, S.; Michel, H. Structure of the Photosynthetic Reaction Centre from Rhodobacter Sphaeroides at 2.65 Å Resolution: Cofactors and Protein-Cofactor Interactions. *Structure* **1994**, *2*, 925–936.
71. Pebay-Peyroula, E.; Rummel, G.; Rosenbusch, J.; Landau, E. X-Ray Structure of Bacteriorhodopsin at 2.5 Angstroms from Microcrystals Grown in Lipidic Cubic Phases. *Science* **1997**, *277*, 1676–1681.
72. Zouni, A.; Witt, H.; Kern, J.; Fromme, P.; Krauss, N.; Saenger, W.; Orth, P. Crystal Structure of Photosystem II from Synechococcus Elongatus at 3.8 Å Resolution. *Nature* **2001**, *409*, 739–743.
73. Laible, P.; Scott, H.; Henry, L.; Hanson, D. Towards Higher-Throughput Membrane Protein Production for Structural Genomics Initiatives. *J. Struct. Funct. Genom.* **2004**, *5*, 167–172.
74. Graff, R.; Swanson, T.; Strano, M. Synthesis of Nickel−Nitrilotriacetic Acid Coupled Single-Walled Carbon Nanotubes for Directed Self-Assembly with Polyhistidine-Tagged Proteins. *Chem. Mater.* **2008**, *20*, 1824–1829.
75. Ham, M.; Choi, J.; Boghossian, A.; Jeng, E.; Graff, R.; Heller, D.; Chang, A.; Mattis, A.; Bayburt, T.; Grinkova, Y.; *et al.* Photoelectrochemical Complexes for Solar Energy Conversion That Chemically and Autonomously Regenerate. *Nat. Chem.* **2010**, *2*, 929–936.
76. Denisov, I.; Sligar, S. Cytochromes P450 in Nanodiscs. *Biochim. Biophys. Acta* **2010**, *1814*, 223–229.
77. Wacher, V.; Wu, C.; Benet, L. Overlapping Substrate Specificities and Tissue Distribution of Cytochrome P450 3A and P-Glycoprotein: Implications for Drug Delivery and Activity in Cancer Chemotherapy. *Mol. Carcinog.* **1995**, *13*, 129–134.
78. Shiflett, A. M.; Bishop, J. R.; Pahwa, A.; Hajduk, S. L. Human High Density Lipoproteins Are Platforms for the Assembly of Multicomponent Innate Immune Complexes. *J. Biol. Chem.* **2005**, *280*, 32578–32585.
79. Holmgren, J.; Lonnroth, I.; Svennerholm, L. Tissue Receptor for Cholera Exotoxin: Postulated Structure from Studies with GM_1 Ganglioside and Related Glycolipids. *Infect. Immun.* **1973**, *8*, 208–214.
80. Harouse, J.; Bhat, S.; Spitalnik, S.; Laughlin, M.; Stefano, K.; Silberberg, D.; Gonzalez-Scarano, F. Inhibition of Entry of HIV-1 in Neural Cell Lines by Antibodies against Galactosyl Ceramide. *Science* **1991**, *253*, 320–323.
81. Suzuki, Y.; Nagao, Y.; Kato, H.; Matsumoto, M.; Nerome, K.; Nakajima, K.; Nobusawa, E. Human Influenza A Virus Hemagglutinin Distinguishes Sialyloligosaccharides in Membrane-Associated Gangliosides as Its Receptor Which Mediates the Adsorption and Fusion Processes of Virus Infection. Specificity for Oligosaccharides and Sialic Acids and the Sequence to Which Sialic Acid Is Attached. *J. Biol. Chem.* **1986**, *261*, 17057–17061.
82. Markwell, M.; Svennerholm, L.; Paulson, J. Specific Gangliosides Function as Host Cell Receptors for Sendai Virus. *Proc. Natl. Acad. Sci.* **1981**, *78*, 5406–5410.
83. Rolsma, M.; Kuhlenschmidt, T.; Gelberg, H.; Kuhlenschmidt, M. Structure and Function of a Ganglioside Receptor for Porcine Rotavirus. *J. Virol.* **1998**, *72*, 9079–9091.
84. Superti, F.; Hauttecoeur, B.; Morelec, M.; Goldoni, P.; Bizzini, B.; Tsiang, H. Involvement of Gangliosides in Rabies Virus Infection. *J. Gen. Virol.* **1986**, *67*, 47–56.
85. Tsai, B.; Gilbert, J.; Stehle, T.; Lencer, W.; Benjamin, T.; Rapoport, T. Gangliosides Are Receptors for Murine Polyoma Virus and SV40. *EMBO J.* **2003**, *22*, 4346–4355.
86. Dewailly, P.; Decoopman, E.; Desreumaux, C.; Fruchart, J. Plasma Removal of Intravenous Essential Phospholipids in Man. *Phosphatidylcholine: Biochem. Clin. Aspects Essent. Phospholipids* **1976**, 80–92.
87. Ulrich, A. Biophysical Aspects of Using Liposomes as Delivery Vehicles. *Biosci. Rep.* **2002**, *22*, 129–150.
88. Mourez, M.; Kane, R.; Mogridge, J.; Metallo, S.; Deschatelets, P.; Sellman, B.; Whitesides, G.; Collier, R. Designing a Polyvalent Inhibitor of Anthrax Toxin. *Nat. Biotechnol.* **2001**, *19*, 958–961.
89. Pan, J.; Charych, D. Molecular Recognition and Colorimetric Detection of Cholera Toxin by Poly(diacetylene) Liposomes Incorporating GM_1 Ganglioside. *Langmuir* **1997**, *13*, 1365–1367.
90. Dhal, P.; Huval, C.; Holmes-Farley, S. Functional Polymers as Human Therapeutic Agents. *Ind. Eng. Chem. Res.* **2005**, *44*, 8593–8604.
91. Brekke, O.; Sandlie, I. Therapeutic Antibodies for Human Diseases at the Dawn of the 21st Century. *Nat. Rev. Drug Discovery* **2003**, *2*, 52–62.
92. Kitov, P.; Sadowska, J.; Mulvey, G.; Armstrong, G.; Ling, H.; Pannu, N.; Read, R.; Bundle, D. Shiga-like Toxins Are Neutralized by Tailored Multivalent Carbohydrate Ligands. *Nature* **2000**, *403*, 669–672.
93. Lauer, S.; Goldstein, B.; Nolan, R. L.; Nolan, J. P. Analysis of Cholera Toxin−Ganglioside Interactions by Flow Cytometry. *Biochemistry* **2002**, *41*, 1742–1751.
94. Nakashima, T.; Kobayashi, Y.; Yamasaki, S.; Kawakami, A.; Eguchi, K.; Sasaki, H.; Sakai, H. Protein Expression and Functional Difference of Membrane-Bound and Soluble Receptor Activator of NF-B Ligand: Modulation of the Expression by Osteotropic Factors and Cytokines. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 768–775.
95. Reig, N.; van der Goot, F. About Lipids and Toxins. *FEBS Lett.* **2006**, *580*, 5572–5579.
96. Wenk, M. Lipidomics of Host−Pathogen Interactions. *FEBS Lett.* **2006**, *580*, 5541–5551.
97. Casas, A.; Hubsch, A.; Doran, J. Effects of Reconstituted High-Density Lipoprotein in Persistent Gram-Negative Bacteremia. *Am. Surgeon* **1996**, *62*, 350–355.

98. McDonald, M.; Dhadly, P.; Cockerill, G.; Cuzzocrea, S.; Mota-Filipe, H.; Hinds, C.; Miller, N.; Thiemermann, C. Reconstituted High-Density Lipoprotein Attenuates Organ Injury and Adhesion Molecule Expression in a Rodent Model of Endotoxic Shock. *Shock* **2003**, *20*, 551–557.
99. Pajkrt, D.; Doran, J.; Koster, F.; Lerch, P.; Arnet, B.; Van der Poll, T.; Ten Cate, J.; Van Deventer, S. Antiinflammatory Effects of Reconstituted High-Density Lipoprotein During Human Endotoxemia. *J. Exp. Med.* **1996**, *184*, 1601–1608.
100. Parker, T.; Levine, D.; Chang, J.; Laxer, J.; Coffin, C.; Rubin, A. Reconstituted High-Density Lipoprotein Neutralizes Gram-Negative Bacterial Lipopolysaccharides in Human Whole Blood. *Infect. Immun.* **1995**, *63*, 253–258.
101. Bhattacharya, P.; Grimme, S.; Ganesh, B.; Gopisetty, A.; Sheng, J.; Martinez, O.; Jayarama, S.; Artinger, M.; Meriggioli, M.; Prabhakar, B. Nanodisc-Incorporated Hemagglutinin Provides Protective Immunity against Influenza Virus Infection. *Jo. Virol.* **2010**, *84*, 361–371.
102. Fischer, N.; Infante, E.; Ishikawa, T.; Blanchette, C.; Bourne, N.; Hoeprich, P.; Mason, P. Conjugation to Nickel-Chelating Nanolipoprotein Particles Increases the Potency and Efficacy of Subunit Vaccines To Prevent West Nile Encephalitis. *Bioconjugate Chem.* **2010**, *21*, 1018–1022.
103. McConathy, W.; Nair, M.; Paranjape, S.; Mooberry, L.; Lacko, A. Evaluation of Synthetic/Reconstituted High-Density Lipoproteins as Delivery Vehicles for Paclitaxel. *Anti-cancer Drugs* **2008**, *19*, 183–188.
104. Zhang, X.; Chen, B. Recombinant High Density Lipoprotein Reconstituted with Apolipoprotein AI Cysteine Mutants as Delivery Vehicles for 10-Hydroxycamptothecin. *Cancer Lett.* **2010**, *298*, 26–33.
105. Zhang, W. L.; Gu, X.; Bai, H.; Yang, R. H.; Dong, C. D.; Liu, J. P. Nanostructured Lipid Carriers Constituted from High-Density Lipoprotein Components for Delivery of a Lipophilic Cardiovascular Drug. *Int. J. Pharm.* **2010**, *391*, 313–321.
106. de Vrueh, R. L.; Rump, E. T.; van De Bilt, E.; van Veghel, R.; Balzarini, J.; Biessen, E. A.; van Berkel, T. J.; Bijsterbosch, M. K. Carrier-Mediated Delivery of 9-(2-Phosphonylmethoxyethyl) Adenine to Parenchymal Liver Cells: A Novel Therapeutic Approach for Hepatitis B. *Antimicrob. Agents Chemother.* **2000**, *44*, 477–483.
107. Parker, N.; Turk, M.; Westrick, E.; Lewis, J.; Low, P.; Leamon, C. Folate Receptor Expression in Carcinomas and Normal Tissues Determined by a Quantitative Radioligand Binding Assay. *Anal. Biochem.* **2005**, *338*, 284–293.
108. Zheng, G.; Chen, J.; Li, H.; Glickson, J. Rerouting Lipoprotein Nanoparticles to Selected Alternate Receptors for the Targeted Delivery of Cancer Diagnostic and Therapeutic Agents. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17757–17762.
109. Kader, A.; Pater, A. Loading Anticancer Drugs into HDL as Well as LDL Has Little Affect on Properties of Complexes and Enhances Cytotoxicity to Human Carcinoma Cells. *J. Controlled Release* **2002**, *80*, 29–44.
110. Neslihan Gursoy, R.; Benita, S. Self-Emulsifying Drug Delivery Systems (SEDDS) for Improved Oral Delivery of Lipophilic Drugs. *Biomed. Pharmacother.* **2004**, *58*, 173–182.
111. Martins, S.; Sarmento, B.; Ferreira, D.; Souto, E. Lipid-Based Colloidal Carriers for Peptide and Protein Delivery-Liposomes *versus* Lipid Nanoparticles. *Int. J. Nanomed.* **2007**, *2*, 595–607.
112. Puri, A.; Loomis, K.; Smith, B.; Lee, J.; Yavlovich, A.; Heldman, E.; Blumenthal, R. Lipid-Based Nanoparticles as Pharmaceutical Drug Carriers: From Concepts to Clinic. *Crit. Rev. Ther. Drug Carrier Systems* **2009**, *26*, 523–580.
113. Feng, M.; Cai, Q.; Shi, X.; Huang, H.; Zhou, P.; Guo, X. Recombinant High-Density Lipoprotein Complex as a Targeting System of Nosiheptide to Liver Cells. *J. Drug Target.* **2008**, *16*, 502–508.
114. Feng, M.; Cai, Q.; Huang, H.; Zhou, P. Liver Targeting and Anti-HBV Activity of Reconstituted HDL-Acyclovir Palmitate Complex. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 688–693.
115. Nelson, K.; Bishop, J.; Ryan, R.; Titus, R. Nanodisk-Associated Amphotericin B Clears Leishmania Major Cutaneous Infection in Susceptible Balb/C Mice. *Antimicrob. Agents Chemother.* **2006**, *50*, 1238–1244.
116. Lou, B.; Liao, X.; Wu, M.; Cheng, P.; Yin, C.; Fei, Z. High-Density Lipoprotein as a Potential Carrier for Delivery of a Lipophilic Antitumoral Drug into Hepatoma Cells. *World J. Gastroenterol.* **2005**, *11*, 954–959.
117. Frias, M.; Lang, U.; Gerber-Wicht, C.; James, R. Native and Reconstituted HDL Protect Cardiomyocytes from Doxorubicin-Induced Apoptosis. *Cardiovasc. Res.* **2009**, *2009*, 1–9.
118. Besler, C.; Heinrich, K.; Riwanto, M.; Luscher, T. F.; Landmesser, U. High-Density Lipoprotein-Mediated Anti-atherosclerotic and Endothelial-Protective Effects: A Potential Novel Therapeutic Target in Cardiovascular Disease. *Curr. Pharm. Des.* **2010**, *16*, 1480–1493.
119. Spillmann, F.; Schultheiss, H. P.; Tschope, C.; Van Linthout, S. High-Density Lipoprotein-Raising Strategies: Update 2010. *Curr. Pharm. Des.* **2010**, *16*, 1517–1530.
120. Vaisar, T.; Mayer, P.; Nilsson, E.; Zhao, X.-Q.; Knopp, R.; Prazen, B. J. HDL in Humans with Cardiovascular Disease Exhibits a Proteomic Signature. *Clin. Chim. Acta* **2010**, *411*, 972–979.
121. Patel, S.; Drew, B.; Nakhla, S.; Duffy, S.; Murphy, A.; Barter, P.; Rye, K.; Chin-Dusting, J.; Hoang, A.; Sviridov, D. Reconstituted High-Density Lipoprotein Increases Plasma High-Density Lipoprotein Anti-inflammatory Properties and Cholesterol Efflux Capacity in Patients with Type 2 Diabetes. *J. Am. Coll. Cardiol.* **2009**, *53*, 962–971.
122. Shaw, J. A.; Bobik, A.; Murphy, A.; Kanellakis, P.; Blombery, P.; Mukhamedova, N.; Woollard, K.; Lyon, S.; Sviridov, D.; Dart, A. M. Infusion of Reconstituted High-Density Lipoprotein Leads to Acute Changes in Human Atherosclerotic Plaque. *Circ. Res.* **2008**, *103*, 1084–1091.
123. Tardif, J.; Heinonen, T.; Noble, S. High-Density Lipoprotein/Apolipoprotein AI Infusion Therapy. *Curr. Atheroscler. Rep.* **2009**, *11*, 58–63.
124. Kruit, J.; Brunham, L.; Verchere, C.; Hayden, M. HDL and LDL Cholesterol Significantly Influence [β]-Cell Function in Type 2 Diabetes Mellitus. *Curr. Opin. Lipidol.* **2010**, *21*, 178–185.
125. Calkin, A. C.; Drew, B. G.; Ono, A.; Duffy, S. J.; Gordon, M. V.; Schoenwaelder, S. M.; Sviridov, D.; Cooper, M. E.; Kingwell, B. A.; Jackson, S. P. Reconstituted High-Density Lipoprotein Attenuates Platelet Function in Individuals with Type 2 Diabetes Mellitus by Promoting Cholesterol Efflux. *Circulation* **2009**, *120*, 2095–2104.
126. Drew, B. G.; Duffy, S. J.; Formosa, M. F.; Natoli, A. K.; Henstridge, D. C.; Penfold, S. A.; Thomas, W. G.; Mukhamedova, N.; de Courten, B.; Forbes, J. M. High-Density Lipoprotein Modulates Glucose Metabolism in Patients with Type 2 Diabetes Mellitus. *Circulation* **2009**, *119*, 2103–2111.
127. Nieuwdorp, M.; Vergeer, M.; Bisoendial, R. J.; op't Roodt, J.; Levels, H.; Birjmohun, R. S.; Kuivenhoven, J. A.; Basser, R.; Rabelink, T. J.; Kastelein, J. J.; *et al.* Reconstituted Hdl Infusion Restores Endothelial Function in Patients with Type 2 Diabetes Mellitus. *Diabetologia* **2008**, *51*, 1081–1084.
128. van Oostrom, O.; Nieuwdorp, M.; Westerweel, P. E.; Hoefer, I. E.; Basser, R.; Stroes, E. S.; Verhaar, M. C. Reconstituted HDL Increases Circulating Endothelial Progenitor Cells in Patients with Type 2 Diabetes. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1864–1865.
129. Hardy, J.; Selkoe, D. J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science* **2002**, *297*, 353–356.
130. Paula-Lima, A. C.; Tricerri, M. A.; Brito-Moreira, J.; Bomfim, T. R.; Oliveira, F. F.; Magdesian, M. H.; Grinberg, L. T.; Panizzutti, R.; Ferreira, S. T. Human Apolipoprotein A-I Binds Amyloid-[β] and Prevents a[β]-Induced Neurotoxicity. *Int. J. Biochem. Cell Biol.* **2009**, *41*, 1361–1370.
131. Navab, M.; Shechter, I.; Anantharamaiah, G.; Reddy, S.; Van

Lenten, B.; Fogelman, A. Structure and Function of HDL Mimetics. *Arterioscler., Thromb., Vasc. Biol.* **2010**, *30*, 164–168.
132. Handattu, S.; Garber, D.; Monroe, C.; van Groen, T.; Kadish, I.; Nayyar, G.; Cao, D.; Palgunachari, M.; Li, L.; Anantharamaiah, G. Oral Apolipoprotein AI Mimetic Peptide Improves Cognitive Function and Reduces Amyloid Burden in a Mouse Model of Alzheimer's Disease. *Neurobiol. Dis.* **2009**, *34*, 525–534.
133. Balazs, Z.; Panzenboeck, U.; Hammer, A.; Sovic, A.; Quehenberger, O.; Malle, E.; Sattler, W. Uptake and Transport of High Density Lipoprotein (HDL) and HDL Associated Tocopherol by an *in Vitro* Blood−Brain Barrier Model. *J. Neurochem.* **2004**, *89*, 939–950.
134. Perrault, S. D.; Chan, W. C. W. *In Vivo* Assembly of Nanoparticle Components to Improve Targeted Cancer Imaging. *Proc. Natl. Acad. Sci.* **2010**, *107*, 11194–11199.
135. Liu, H.-L.; Hua, M.-Y.; Yang, H.-W.; Huang, C.-Y.; Chu, P.-C.; Wu, J.-S.; Tseng, I.-C.; Wang, J.-J.; Yen, T.-C.; Chen, P.-Y.; *et al*. Magnetic Resonance Monitoring of Focused Ultrasound/Magnetic Nanoparticle Targeting Delivery of Therapeutic Agents to the Brain. *Proc. Natl. Acad. Sci.* **2010**, *107*, 15205–15210.
136. Chen, W.; Jarzyna, P.; van Tilborg, G.; Nguyen, V.; Cormode, D.; Klink, A.; Griffioen, A.; Randolph, G.; Fisher, E.; Mulder, W. RGD Peptide Functionalized and Reconstituted High-Density Lipoprotein Nanoparticles as a Versatile and Multimodal Tumor Targeting Molecular Imaging Probe. *FASEB J.* **2010**, *24*, 1689.
137. Skajaa, T.; Cormode, D.; Falk, E.; Mulder, W.; Fisher, E.; Fayad, Z. High-Density Lipoprotein-Based Contrast Agents for Multimodal Imaging of Atherosclerosis. *Arterioscler., Thromb., Vasc. Biol.* **2010**, *30*, 169–176.
138. Corbin, I.; Chen, J.; Cao, W.; Li, H.; Lund-Katz, S.; Zheng, G. Enhanced Cancer-Targeted Delivery Using Engineered High-Density Lipoprotein-Based Nanocarriers. *J. Biomed. Nanotechnol.* **2007**, *3*, 367–376.