Gold nanoparticles become stable to cyanide etch when coated with hybrid lipid bilayers†

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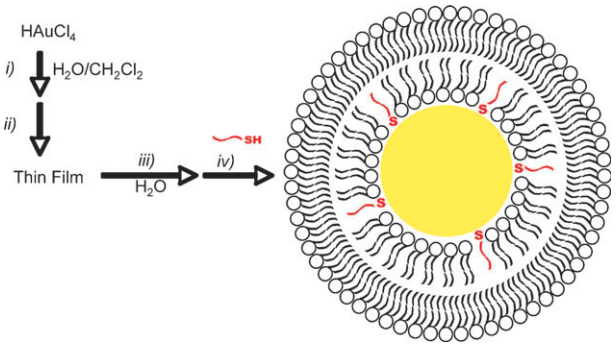
Hybrid bilayers composed of the lipid phosphatidylcholine (PC) and a submonolayer of 1-decanethiol bound to gold nanoparticles are very stable to potassium cyanide.

Naturally occurring lipids are ideal ligands for nanoparticle synthesis due to their diverse chemical structure, ability to form complex three-dimensional structures, and availability from inexpensive and renewable feedstocks.[1] Lipid-coated nanoparticles can function as biocompatible probes[2] and as model systems for understanding interactions that occur at cellular membranes.[3] Here we demonstrate a remarkable increase in the stability of lipid-coated gold nanoparticles when intermittent thiols are available to anchor the lipid layer.

Organic-soluble, lipid-coated gold nanoparticle starting materials were synthesized from $HAuCl_4$ (0.084 mmol) and Soy PC (0.084 mmol) in a biphasic mixture of water and CH_2Cl_2 using $NaBH_4$ (0.42 mmol) as a reducing agent as previously reported.[1] A thin film of these nanoparticles was formed by evaporation from CH_2Cl_2 and drying *in vacuo* for 12 h. The film was re-suspended in water to form PC-coated nanoparticles and then thiol-anchored, hybrid bilayer nanoparticles (HBNs) were prepared by partial phospholipid exchange (Scheme 1). 1-Decanethiol (10 μl of 1 mM in ethanol) was added to the re-suspended nanoparticles (OD at 526 nm = 1.2 a.u.) and the mixture (1 mL) was stirred for 5 min. The size of the nanoparticles (7 ± 2 nm) did not change after addition of thiol (see ESI†).

The stability of the resulting HBNs was examined by adding KCN to a final concentration of 6 mM (>14 fold excess relative to the gold atoms). Cyanide is commonly used to etch gold films[4] as well as nanoparticles[5] and resistance to cyanide (for minutes or hours) is one indication of a compact ligand shell.[6] The UV-Vis spectra of HBNs (red trace in Fig. 1) was recorded after 1 month of exposure to 6 mM KCN and it shows negligible signs of decomposition compared to the starting material (blue trace). Even at a total KCN concentration of 1 M, minimal decomposition was observed (35% drop in absorbance over 10 days). We are aware of no reports of comparable stability for any other gold nanoparticles. Stored in the dark, these HBNs are stable to KCN indefinitely. In contrast, gold nanoparticles stabilized with only 1-decanethiol



Scheme 1 Schematic depiction of hybrid bilayer nanoparticle (HBN) preparation. The synthesis involves (*i*) biphasic borohydride reduction of gold to form PC-coated nanoparticles, (*ii*) removal of solvent *in vacuo*, (*iii*) re-suspension in water, and (*iv*) addition of alkanethiol to form HBNs.

decompose completely in cyanide within hours.[5] Likewise, PC itself does not provide a substantial barrier to cyanide etch; using the PC-coated nanoparticle starting material as a control and omitting 1-decanethiol, the stability to 6 mM KCN is minimal (black trace, >90% drop in absorbance over 2 h of exposure). This indicates an essential role for both lipid and thiol in producing stable materials. At 10 μM 1-decanethiol, there is a ~42 fold excess of gold atoms relative to thiol and ~6 fold excess of surface gold atoms relative to thiol (see ESI†). This is a submonolayer of thiol coverage and the remaining gold surface is presumably occupied by lipid.

Lipid bilayers are impermeable to ions and we interpret the high stability of the HBNs to be the result of an intact and

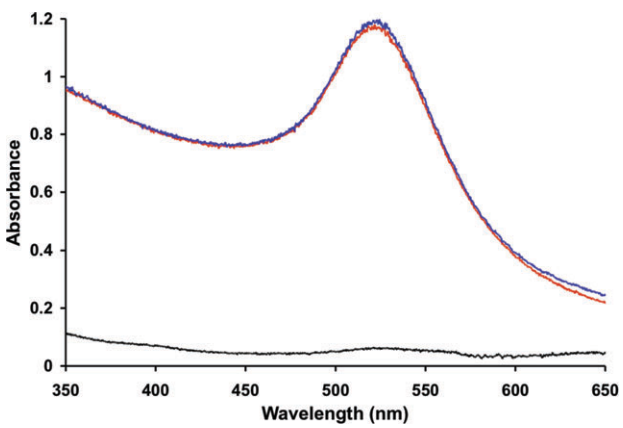


Fig. 1 UV-Vis spectra of nanoparticles. Prior to thiol addition (blue), 1-decanethiol stabilized hybrid bilayer nanoparticles (HBNs) one month after addition of 6 mM KCN (red), and nanoparticles with no thiol, 2 h after addition of 6 mM KCN (black) in water.

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† Electronic supplementary information (ESI) available: Synthetic details for both methods of HBN synthesis. TEM analysis of nanoparticles. Estimation of thiols available per nanoparticle. Stability studies in the presence of detergent Triton X-100. Stability of mixed thiol–PC nanoparticles in CH_2Cl_2. See DOI: 10.1039/b801525b

ion-impermeable lipid shell around the gold core. This hybrid bilayer shell forms only when thiol is present, as evidenced by the low KCN stability prior to thiol addition. On the surface of the nanoparticle, 1-decanethiol functions as an anchor for the lipids, similar to the role of alkanethiols in two-dimensional hybrid bilayers[7] or tethered bilayer lipid membranes (tBLMs).[8] Encapsulating a single nanoparticle of the diameter used here (7 ± 2 nm) would require a higher spontaneous curvature of the lipid than is predicted for PC.[9] Bilayers with a high curvature are known to exist at invaginated regions of biological membranes such as the caveola[10] and at clathrin-coated pits.[11] The strain energy associated with the high curvature of these structures is reduced by the proteins caveolin or clathrin, although the mechanism of action is not fully understood.[10] Here we think that the alkyl group of 1-decanethiol excludes a volume of lipid from the inner leaflet, thereby reducing the strain energy associated with a highly curved lipid layer.[12] In addition, we find that detergents do not disrupt the HBNs as seen by stability to KCN in the presence of Triton X-100 (see ESI†). This similarity to detergent-resistant caveolae could indicate that similar mechanisms of stabilization are in effect. This type of bilayer encapsulation, rather than embedding of nanoparticles between lipid leaflets is consistent with models of nanoparticles that interact strongly with lipid.[13]

The concentration dependence of this enhanced stability was measured by monitoring the time course of KCN mediated decomposition at various concentrations of 1-decanethiol (Fig. 2A). Spectra were collected every minute after KCN addition and the average intensity (at $\lambda_{max} \pm 2$ nm) was calculated. At low 1-decanethiol concentration (1 μM final concentration) or without thiol the nanoparticles decomposed rapidly in KCN with the absorbance dropping >60% within 1 h. At 1-decanethiol concentrations of 10 and 15 μM, no decomposition occurred. At intermediate concentrations of 3 or 5 μM a portion of the nanoparticles decompose, however, a portion remain stable indefinitely. Thiol-for-thiol exchange on gold nanoparticles is thought to occur fastest at areas with reduced steric hindrance, such as edges and vertices.[14] Nanoparticle edges are also the regions with highest curvature for ligands. If thiol-for-PC exchange occurs at these locations, this would be an effective means of reducing strain in a supported bilayer.

In addition to the above method, stable HBNs can also be prepared with the sequence of steps (ii) and (iv) reversed. In this second method 1-decanethiol ligand exchange was performed on PC-coated nanoparticles in an organic solvent and the resultant nanoparticles showed no enhanced stability to KCN (see ESI†). In a non-aqueous environment, these nanoparticles have a mixed ligand shell (1-decanethiol and PC) but do not form hybrid bilayers. At higher concentrations of 1-decanethiol, the nanoparticles cannot be re-solubilized in water and they are unstable to KCN. This is consistent with previous studies that showed 1-decanethiol itself provides a minimal barrier to KCN in organic solvents.[5] Since neither alkanethiol nor PC-stabilized nanoparticles show enhanced stability, it is not surprising that the mixed ligand shell nanoparticles in organic solvents do not either. However, after transferring nanoparticles prepared by this second route to water their stability was comparable to the HBNs that were synthesized in water (see ESI†). The ability to form HBNs by both these routes is an indication that the thiol binding sites are the same whether the thiol is added to an aqueous or non-aqueous sample.

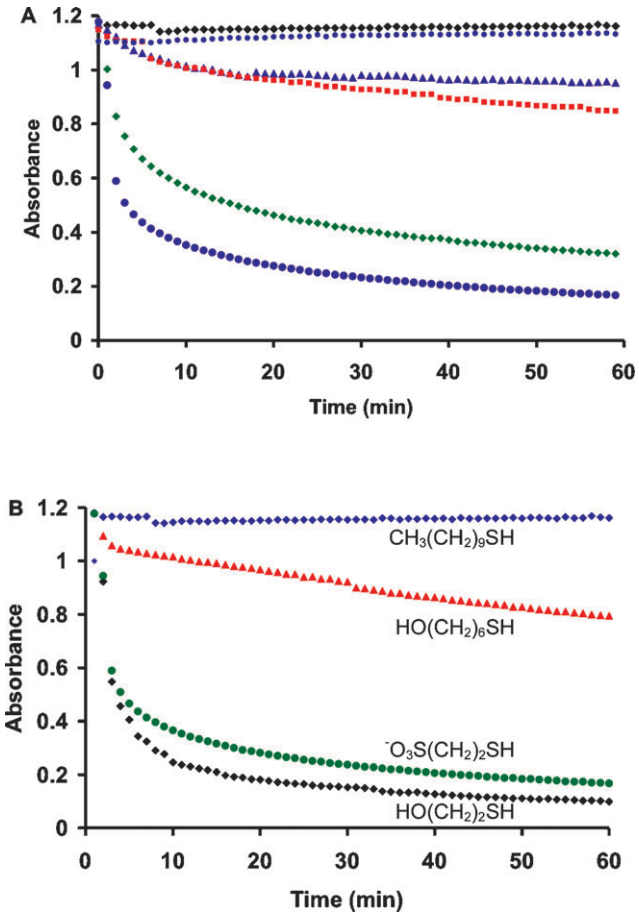

Fig. 2 Nanoparticle stability studies. (A) Stability of PC-coated nanoparticles without thiol (●) and with HBNs formed from 1-decanethiol at 15 μM (●), 10 μM (◆), 5 μM (▲), 3 μM (■), and 1 μM (◆) at a final KCN concentration of 6 mM. (B) Stability of nanoparticles formed with PC and various thiols at 10 μM; 1-decanethiol (◆), 6-mercaptohexanol (▲), 2-mercaptoethanesulfonate (●), and 2-mercaptoethanol (◆), in 6 mM KCN. Each point represents the average intensity of the $\lambda_{max} \pm 2$ nm.

To confirm the role of the alkyl group in anchoring the hydrophobic tails of PC, a series of substituted thiols were examined. The same synthetic procedure was repeated with 6-mercaptohexanol, 2-mercaptoethanol, or sodium 2-mercaptoethanesulfonate replacing 1-decanethiol. The stability of the resultant nanoparticles to KCN (6 mM final concentration) was low compared to nanoparticles prepared with 1-decanethiol (Fig. 2B). 2-Mercaptoethanol and sodium 2-mercaptoethanesulfonate exposed nanoparticles were very unstable to KCN. These shorter tail hydrophilic thiols were unable to form HBNs and the nanoparticles decomposed at a comparable rate to the PC-coated nanoparticles without thiol. For a thiol to function as an anchor for hydrophobic lipid tails it must expose a hydrophobic surface, similar to thiols used in analogous

two-dimensional supported bilayers.[7,8] Nanoparticles produced with 6-mercaptohexanol were of moderate stability. In contrast to 2-mercaptoethanol, the alkane chain of 6-mercaptohexanol exposes hydrophobic methylenes on the surface that can function as an anchor.

When added to a PC-coated nanoparticle, thiols rapidly exchange with phospholipids to form a hybrid bilayer on the nanoparticle surface. The bilayers that result are impermeable to ions, similar to biological membranes. This makes them remarkably stable to KCN compared with other thiol functionalized gold nanoparticles. These HBNs represent a new category of substrate-supported bilayer mimics that complement previously reported supported hybrid bilayers[7] and tethered bilayer membranes.[8] Similar to these other model systems, we anticipate that HBNs will be useful in the design of biosensors and in studying membrane–protein interactions. Understanding the system reported here will also result in improved etch resists for gold micropatterning.[7,11] Additionally, these materials may provide insight into how biological nanostructures such as caveolae are formed and this will contribute to our understanding of how synthetic nanosystems can be used for drug delivery. Efforts are underway to determine the effect of nanoparticle size on HBN formation and enhanced stability.

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