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PEGylated Gold Nanoparticles Functionalized with β-Cyclodextrin Inclusion Complexes: Towards Metal Nanoparticle–Polymer–Carbohydrate Cluster Biohybrid Materials

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A cholesterol-functional trithiocarbonate reversible addition–fragmentation chain transfer (RAFT) agent was synthesized and employed to generate well-defined poly(polyethylene glycol) acrylate with cholesterol chain termini using RAFT polymerization. Subsequently, the polymers were grafted onto the surface of gold nanoparticles using the trithiocarbonate functionality to bind to the gold surface. The cholesterol moieties were then modified via complexation with β-cyclodextrin. The step-by-step modification of gold nanoparticles was characterized by dynamic light scattering, attenuated total reflection infrared spectroscopy and surface plasmon resonance analysis.

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Introduction

Over the past decade, metallic nanoparticles, especially gold nanoparticles (AuNPs) have been investigated extensively as they possess unique electronic, optical, and catalytic properties.[1] AuNPs can be used as multifunctional platforms for biomedical applications such as gene and drug delivery and contrast or imaging agents for diagnostic purposes.[2–6] Like most other noble-metallic nanoparticles, gold produces intense colours originating from surface plasmon resonance (SPR) phenomena appearing in the visible spectrum.[7] SPR describes the collective oscillation of the conduction band electrons on a particle surface induced by an interacting electromagnetic field. The size and shape of the particles, local environment such as the stabilizing ligand shell, surrounding solvent, and the charge state of the metal cores are all factors that affect the SPR.[8,9] Irregular gold nanoparticles such as nanorods, nanocaps, nanotriangles and nanocages usually exhibit red-shifted SPR to the near IR (NIR) region, leading to potential applications in the thermotreatment of tumours via laser illumination.[10–13] Applications of AuNPs in the biomedical field often necessitate their surface stabilization and modification using organic polymers.[14,15] The primary methods of nanoparticle and/or polymer brush preparation are 'grafting-to' and 'grafting-from'. The 'grafting-from' method usually involves initiation from the nanoparticle surface, followed by polymerization to afford a dense polymer. The 'grafting-to' method involves the attachment of preformed polymer chains to the nanoparticle surface, leading to less densely packed brush structures. Polyethylene glycol (PEG) and polyPEG acrylate are often known as 'stealth materials' as they are relatively inert in physiological media; therefore, they are often used to render surfaces resistant to biomolecule adsorption.[16,17] Reversible addition–fragmentation chain transfer (RAFT) polymerization has been employed to synthesize polyPEG acrylate and other polymers for modification of metal nanoparticles.[18–27] Gold–thiol chemistry has been used extensively to modify gold surface and nanoparticles for the fabrication of sensors and devices.[28–33] A few groups have found that the dithioester and trithiocarbonate groups can bind directly with gold surfaces, though the interaction was found to be less stable than the gold–thiol affinity.[34,35] Therefore, polymers prepared using RAFT polymerization have end-groups with di- and trithio-terminal functionalities that can bind to gold surfaces without further modification.[34–37]

Several targeting ligands such as monoclonal antibodies, sugars, hormones, peptides, vitamins, or other small organic molecules can be used for specific biological recognition. Specific recognition events are particularly useful for therapeutic and diagnostic applications. Targeting groups can be attached onto polymer-modified nanoparticle surfaces by covalent bonding, e.g. amine–carboxylic acid and thio–maleimide coupling chemistry.[38,39] An alternative (and novel) functionalization strategy is to use host–guest cyclodextrin (CD) inclusion complexes to assemble targeting functionality, as described in a recent review by van de Manakker et al.[40] The formation of CD-inclusion complexes with cholesterol has been described recently and can be exploited to functionalize nanoparticles or to build up hydrogel materials. The inclusion complexes thus formed retain their full complement of CD hydroxyl functionality, and so this can be seen as the first step in using polysaccharide clusters to functionalize nanoparticles (or surfaces in the broader sense). Stoddart and coworkers (and others) have published several papers describing the synthesis of polysaccharide clusters

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starting from cyclodextrin molecules.[41,42] Cyclodextrin can thus be used as a multifunctional unit for attachment to other groups through its multiple hydroxyl groups.[40,43,44] Cyclodextrin has previously been tailored with polymers to create hybrid materials for versatile applications.[45–48] Herein, we report the synthesis of cholesterol-terminated poly(polyethylene glycol) acrylate (polyPEG-A) using a cholesterol-modified RAFT agent for the modification of AuNPs to generate a β-CD functional surface as a precursor to creating nanoparticle–carbohydrate cluster materials.

Experimental

Materials

Ethanethiol (Acros, >99%), potassium hydroxide (Analar), carbon disulfide (Analar, >99%), *p*-toluenesulfonyl chloride (Aldrich, 98%), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (Fluka, 99%), 4-dimethylaminopyridine (DMAP) (Aldrich, 99%), *p*-toluenesulfonic acid monohydrate (Sigma–Aldrich, 98.5%), acetone (Univar, >99.5%), poly(ethylene glycol) acrylate (PEG-A, average molecular weight 454, ethylene unit number $n = 8–9$) (Aldrich), *n*-hexane (Ajax, 95%), 2,2′-azobis(isobutyronitrile) (AIBN, 98%, Sigma–Aldrich), diethyl ether (Univar, >99%), *N*,*N*-dimethylacetamide (DMAc) (Aldrich, 99%), thiocholesterol (Sigma, 95%), β-cyclodextrin (β-CD) (Sigma, 98%), nitric acid (Ajax, 70%), hydrochloric acid (Ajax, 32%), hydrogentetrachloroaurate(III) hydrate ($HAuCl_4$, 99.9%, Aldrich); deionized water used for these experiments was purified by Milli-Q system with a resistivity of 17.9 mΩ cm^{-1}, sodium citrate dihydrate (Sigma–Aldrich 99%), 4,4′-azobis(4-cyanovaleric acid) (Fluka, 98%), tetrahydrofuran (THF, Honeywell, HPLC grade), dioxan (Tedia, 99%), [D]chloroform ($CDCl_3$, Cambridge Isotope Laboratories Inc., 99.8%).

Synthesis of Gold Nanoparticles (AuNPs)

AuNPs were prepared using a slight modification of a published method.[49] Initially, all the glass apparatus was washed with aqua regia solution (25% nitric acid, 75% hydrochloric acid) and then rinsed with Milli-Q water several times. Milli-Q water (100 mL) and hydrogentetrachloroaurate(III) ($HAuCl_4$) were mixed and heated to boiling point with vigorous stirring. Trisodium citrate dehydrate (5 mL, 1.053 g of trisodium citrate) was then added rapidly into the boiling solution. The resulting solution was boiled and stirred vigorously for another 30 min. The solution was then cooled down to room temperature, accompanied by a colour change from yellow to wine red. The particle sizes and distribution were characterized by dynamic light scattering (DLS) and examined using transmission electron microscopy (TEM).

Synthesis of RAFT Agent, 4-Cyano-4-ethyl-trithiopentanoic Acid (CETP)

CETP was synthesized using a precursor, bis(ethylsulfanylthiocarbonyl) disulfide (BETD), which was synthesized according to the method reported by Weber et al.[50] BETD (2.26 g, 82 mmol) was added to a solution of 4,4′-azobis(4-cyanovaleric acid) (2.77 g, 99 mmol) in ethyl acetate (40 mL). The resulting mixture was refluxed for 12 h under stirring. After removal of the solvent under vacuum, the residue was purified by silica gel chromatography using hexane/ethyl acetate (50:50) as the eluent to afford the expected product (3.78 g, 87%). δ_H ($CDCl_3$, 298 K, 300 MHz) 1.33–1.38 (t, 3H, S–CH_2–CH_3), 1.88 (s, 3H, R–C(–CH_3)–CN), 2.33–2.71 (m, 4H, CH_2–CH_2, CH_2–CH_2), 3.30–3.38 (q, 2H, S–CH_2–CH_3). δ_C (75 MHz, $CDCl_3$) 14.54 (CH_3–CH_2), 21.8 (CH_3–C), 29.5 (CH_2–COOH), 33.74 (C–CH_2–CH_2), 35.40 (–CH_2–CH_3), 38.5 (CN), 45.23 (CH_2S), 183.1 (COOH), 221.34 (C=S).

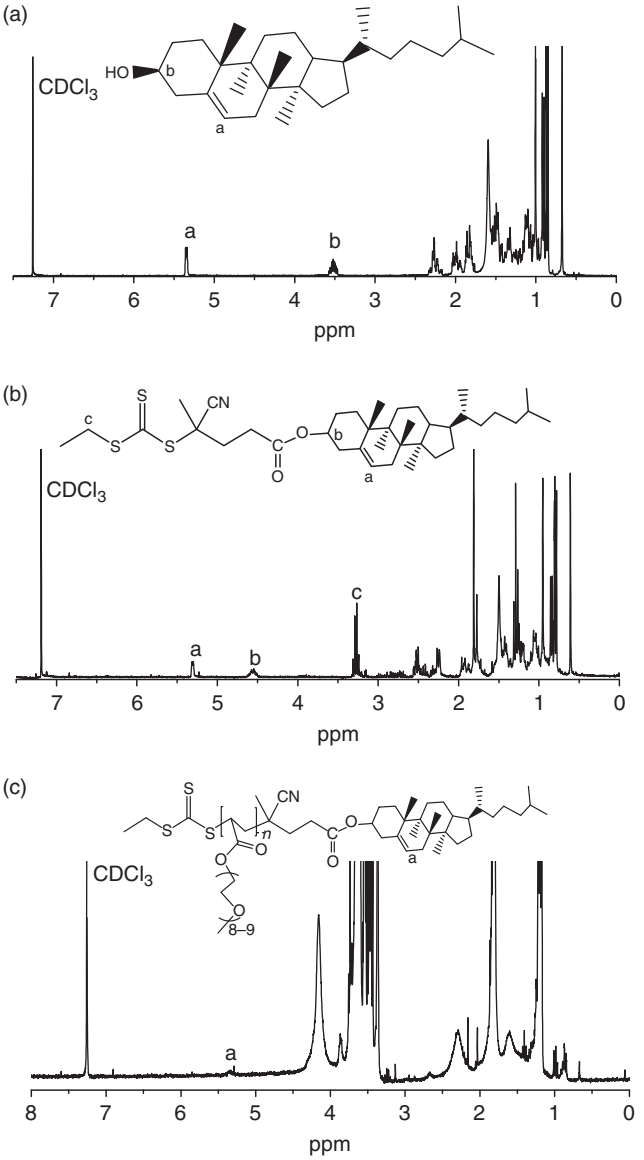
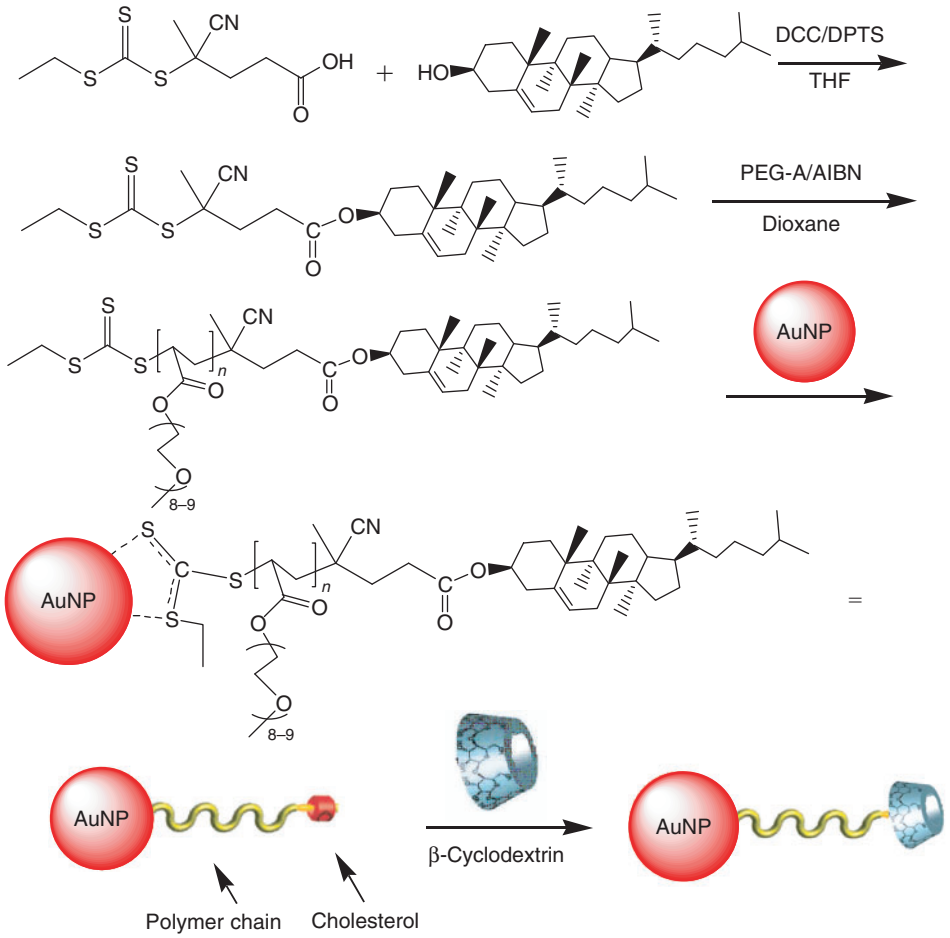


Fig. 1. (a) ^{1}H NMR spectra of cholesterol; (b) cholesterol-functional RAFT (reversible addition–fragmentation chain transfer) agent; and (c) the subsequent polyPEG-A (poly(polyethylene glycol) acrylate; M_n, 22400 g mol^{-1} from GPC (gel permeation chromatography); PDI (polydispersity index), 1.24).

Synthesis of Cholesterol-functionalized RAFT Agent

CETP (0.478 g, 181 mmol) was dissolved in THF (15 mL), followed by the addition of cholesterol (0.702 g, 181 mmol). The resulting mixture was stirred for 4 h in the presence of DMAP-*p*-toluenesulfonic acid salt (DPTS) (22 mg, 0.075 mmol) and DCC (0.450 g, 218 mmol). Solvent was removed under vacuum and the residue was purified by silica gel chromatography with dichloromethane/hexane (50:50) as eluent to afford the expected product (0.88 g, 75%). The ^{1}H NMR spectrum ($CDCl_3$, 298 K, 300 MHz) is shown in Fig. 1a.



Scheme 1. Synthesis of cholesterol-terminated RAFT (reversible addition–fragmentation chain transfer) agent, subsequent polymerization of poly(ethylene glycol) acrylate (PEG-A) and attachment onto gold nanoparticles (AuNPs) to tailor the AuNP surface with cholesterol molecules, and the subsequent complexation with β-cyclodextrin (β-CD).

Homopolymerization of PEG-A Controlled by the Cholesterol-functionalized RAFT

A solution of PEG-A (1.484 g, 3.27×10^{-3} mol), cholesterol-RAFT agent (21 mg, 3.27×10^{-5} mol), and AIBN (1.4 mg, 8×10^{-6} mol) in dioxan (5 mL) was prepared. The resulting mixture was deoxygenated with nitrogen for 30 min, followed by incubation in a water bath at 65°C. Six samples were taken from the incubating mixture using a degassed needle at 0.5, 1, 2, 3, 4, and 5 h polymerization times. The monomer conversion for each polymerization sample was determined by ^{1}H NMR in $CDCl_3$. The polymers were purified by precipitation in diethyl ether three times before drying under vacuum. The pure polymer was characterized by ^{1}H NMR and gel permeation chromatography (GPC).

Grafting of Cholesterol-functionalized PEG-A Polymer to AuNPs

Cholesterol-terminated PEG-A polymer (10 mg, 5×10^{-7} mol) was dissolved in water (10 mL) before dropwise addition to the previously prepared AuNPs solution (10 mL, 0.05 mg mL^{-1}), followed by stirring for 3 h. The particles were purified by repetitive centrifugation and redispersion with water for three cycles. The particles were characterized by DLS, attenuated total reflection infrared spectroscopy (ATR-IR), UV-visible spectroscopy, and TEM.

β-Cyclodextrin Inclusion Complex with Cholesterol-functionalized AuNPs

The polymer-coated AuNPs were redispersed in water (10 mL) and β-CD (4 mg, 3.52×10^{-6} mol) was added under stirring. The mixture was stirred for another 5 h and the particles were purified by centrifugation at 20800 *g* for 20 min at 5°C. The same process was repeated three times. The particles were characterized by DLS, AT-IR, UV-visible spectroscopy, and TEM.

Analyses

Gel permeation chromatography was performed in DMAc (0.03% w/v LiBr, 0.05% butylated hydroxy toluene stabilizer) at 50°C (flow rate: 0.85 mL min^{-1}) using a Shimadzu modular system composed of a DGU-12A solvent degasser, an LC-10AT pump, a CTO-10A column oven, and an RID-10A refractive index detector. The system was equipped with a Polymer Laboratories (PL) 5.0-mm bead-size guard column (50×7.8 mm^2) followed by four 300×7.8 mm^2 linear PL columns (10^5, 10^4, 10^3, and 500 g mol^{-1}). Calibration was performed with narrowly polydisperse polystyrene standards ranging from 500 to 10^6 g mol^{-1}.

^{1}H NMR spectra were obtained using a Bruker AC300F (300 MHz) spectrometer or a Bruker DPX300 (300 MHz) spectrometer.

Electrospray ionization mass spectroscopy (ESI-MS) was carried out on a Finnigan LCQ Deca mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an atmospheric pressure ionization source operating in the nebulizer-assisted electrospray mode.

The AuNPs sizes before and after modification were analyzed at 25°C using DLS on a Malvern analyzer (Laser type: HeNe gas laser; beam wavelength 633 nm).

UV-visible spectra were recorded using a Cary 300 spectrophotometer (Bruker) equipped with a temperature controller.

ATR-IR spectra were obtained using a Bruker Spectrum BX ATR-IR system using diffuse reflectance sampling accessories.

Transmission electron microscopy images were obtained on JEOL1400 TEM at an accelerating voltage of 100 kV. Phosphotungsten acid was used as contrast agent for imaging of polymer-modified AuNPs.

Results and Discussion

The synthesis of cholesterol-terminated RAFT agent, polyPEG-A and the subsequent modification of AuNPs are illustrated in Scheme 1. The synthesis of cholesterol-functional RAFT agent was achieved by an esterification reaction in the presence of DCC and DPTS. The utilization of neutral DPTS instead of the weakly basic DMAP was to minimize any decomposition of the trithiocarbonate core of the RAFT agent. The successful synthesis of the cholesterol-terminated RAFT agent was evidenced by ^{1}H NMR spectra, as shown in Fig. 1a and 1b. The peak signals at 5.2–5.3 ppm (a), 4.5–4.6 ppm (b), and 3.2–3.3 ppm (c) correspond to the proton of the cycloalkene in cholesterol, the methane proton of cholesterol (linked to the RAFT functionality) and the two protons adjacent to the RAFT S atom respectively. The shift of the signal assigned to the methine proton (b) in cholesterol from 3.5 ppm (Fig. 1a) to 4.55 ppm (Fig. 1b) after esterification confirmed a successful coupling reaction. The successful synthesis of the cholesterol-functional RAFT agent was also supported by the presence of a parent sodium ion at *m*/*z* 654.72 (calc. *m*/*z* 654.65) measured by ESI-MS.

Homopolymerization of PEG-A Controlled by the Cholesterol-functionalized RAFT Agent

The results of the synthesis of polyPEG-A using the cholesterol-functional RAFT agent are summarized in Table 1 and Fig. 2. It is evident that the monomer conversion increased concomitantly with polymerization time, and the radical concentration remained constant with conversion as indicated by the pseudo-first-order plot (Fig. 2a). Fig. 2b shows that both the experimental (measured by GPC) and the theoretical molecular weights (MW) were found to be proportional to the monomer conversion. The theoretical MW values were slightly higher than the experimental ones and the polydispersity indices (PDI) of the purified polyPEG-A were less than 1.25, indicating a well-controlled polymerization, consistent with the known traits of living radical polymerization.[51–53] The MWs obtained from GPC and ^{1}H NMR are quite consistent, indicative of reliable molecular weight characterization (Table 1). A purified polyPEG-A (M_n 22400 g mol^{-1} from GPC; PDI 1.24) was analyzed by ^{1}H NMR using $CDCl_3$ as the deuterated solvent (Fig. 1b). The signal at 5.3 ppm can be assigned to the proton of cycloalkene in cholesterol, showing that the integrity of the cholesterol structure is maintained after polymerization.

Table 1. Results for the homopolymerization of poly(ethylene glycol) acrylate (PEG-A) using cholesterol-functional RAFT (reversible addition–fragmentation chain transfer) agent

Time [h]	Conversion [%]	M_n [A]	ln[M_o/M_t]	M_n [B]	PDI
0.5	11.2	3600	0.12	3100	1.23
1	30.9	8900	0.37	8000	1.19
2	56.1	15700	0.82	12800	1.24
3	69.8	19300	1.16	17500	1.21
4	76.2	21000	1.44	18600	1.25
5	81.2	22400	1.68	20200	1.24

[A]Measured by gel permeation chromatography (GPC); [B]measured from ^{1}H NMR. PDI, polydispersity index.

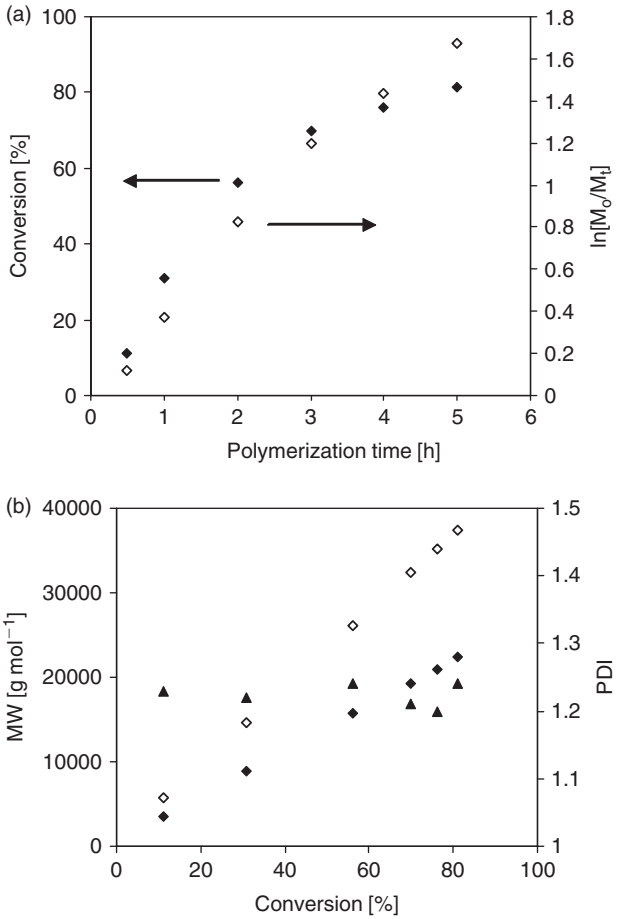


Fig. 2. Polymerization of poly(ethylene glycol) acrylate (PEG-A) using the cholesterol-functional RAFT (reversible addition–fragmentation chain transfer) agent in dioxan at 65°C ([M]/[RAFT]/[AIBN] = 100:1:0.25 (AIBN, 2,2′-azobis(isobutyronitrile)). (a) Monomer conversion at varying polymerization times. (b) Molecular weight (MW) and polydispersity indices (PDI) of the polyPEG-A (poly(polyethylene glycol) acrylate) against monomer conversion (filled and empty diamonds represent the experimental (obtained by GPC (gel permeation chromatography)) and theoretical MW values, respectively, whereas the filled triangles represent PDIs).

Synthesis of Gold Nanoparticles and the Subsequent Modification with Cholesterol-terminal Polymer and β-CD

The synthesis of citrate-stabilized AuNPs was carried out following a literature method (see Experimental section),[49] resulting in relatively uniform AuNPs with an average size of 18 nm

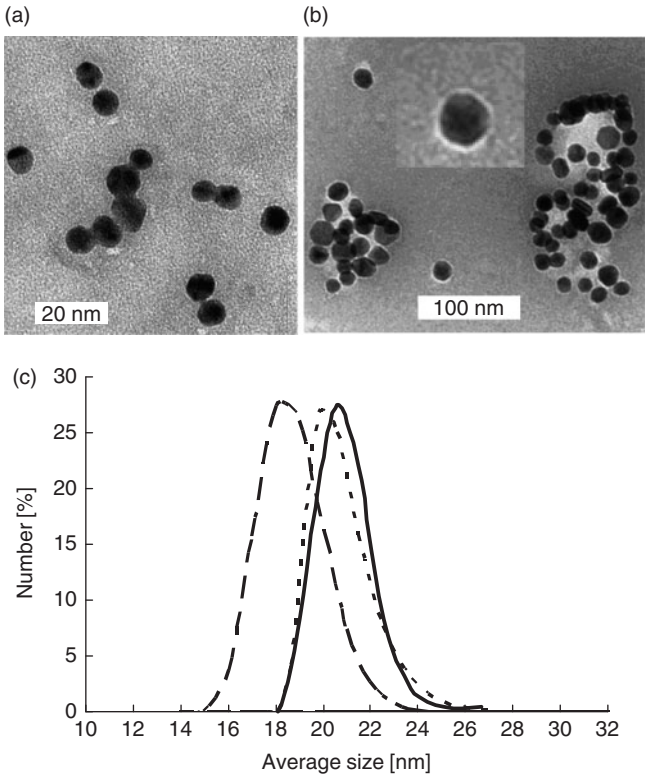


Fig. 3. Transmission electron microscopy (TEM) image of gold nanoparticles (AuNPs) before (a), and after (b) modification with cholesterol-terminal polyPEG-A (poly(polyethylene glycol) acrylate) (phosphotungsten acid was used as contrast agent for imaging of polymer-modified AuNPs); and (c) dynamic light scattering (DLS) analysis of AuNPs before (dashed curve) and after (dotted curve) polymer grafting and subsequent β-cyclodextrin (β-CD) complexation (solid curve).

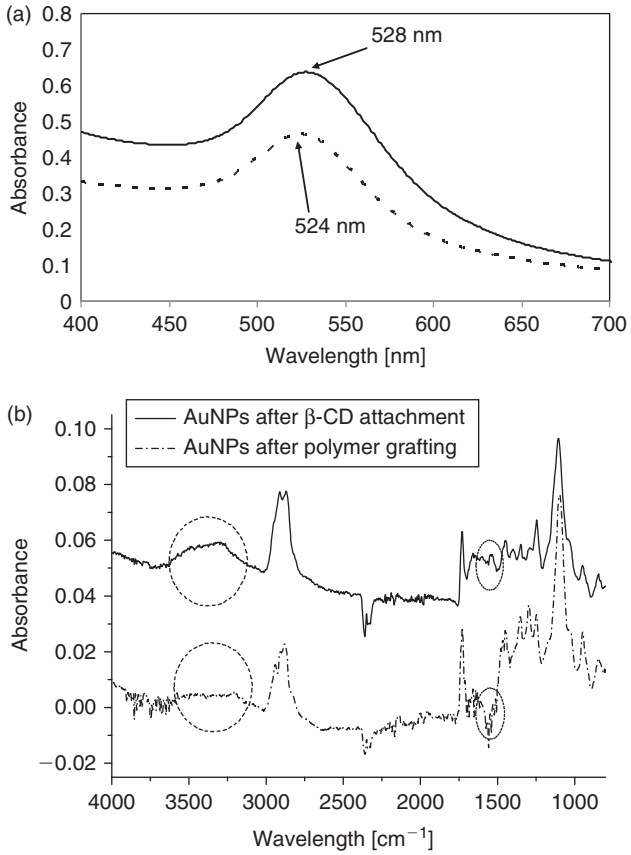


Fig. 4. (a) UV-visible spectra of unmodified gold nanoparticles (AuNPs) (dashed curve) and after attachment of polymers (solid curve). (b) Attenuated total reflection infrared (ATR-IR) spectra of polymer modified AuNPs (dashed curve) and after further complexation with β-cyclodextrin (β-CD) (solid curve).

as characterized by TEM, as shown in Fig. 3a. A few groups have found that the dithioester and trithiocarbonate groups can bind directly with gold surfaces, although the interaction was found to be less stable than the gold–thiol affinity.[24] No colour change was observed when the AuNPs were modified with cholesterol-functionalized PEG-A polymer in aqueous solution, indicating minimal agglomeration. The TEM image of the polymer-modified AuNPs revealed a light polymer corona as shown in the inset of Fig. 3b; this light corona might be an artefact caused by Fresnel fringes, and therefore cannot confirm the presence of polymer. However, DLS measurements confirmed an increase in the hydrodynamic diameter of the AuNPs after polymer grafting from 18 to 20 nm. Further β-CD complexation onto the cholesterol groups was hard to detect by light scattering as the size change was too small to detect reliably (Fig. 3b). However, ATR-IR analysis (described below) furnished some evidence of successful β-CD attachment. Zeta potential measurements were also performed along with the DLS analysis to monitor the step-by-step modification of the AuNPs. The zeta potential of the unmodified AuNPs was measured to be ∼−40 mV, corresponding to the presence of negatively charged citrate ions stabilizing the GNPs. After polymer grafting, the zeta potential dropped to ∼0 mV, indicating the successful attachment of polymers on the AuNPs surface. Further attachment of β-CD did not cause further zeta potential change, as expected.

It is well known that when AuNPs are small enough, their colour is ruby red as they strongly absorb green light at ∼520 nm, corresponding to the frequency at which a plasmon resonance occurs within the gold.[7] It has been reported that plasmon adsorption for alkanethiol-coated spherical silver nanoparticles is hardly influenced by modification with alkanethiol and only slightly red-shifted for gold nanoparticles.[54] In our work, grafting the AuNPs with cholesterol-functionalized polyPEG caused a 4-nm red shift compared with that of unmodified AuNP precursors at 524 nm (Fig. 4a). This slight absorption shift can be attributed to a refractive index variation caused by the surface-grafted polymer layer on AuNPs, in accord with previously reported results.[8,9,55]

The presence of a grafted polyPEG-A layer on the AuNPs was confirmed by ATR-IR analyses. As shown in Fig. 4b, the ATR-IR analysis revealed characteristic polyPEG-A absorption signals: 2900–2850 cm^{-1} (CH_2 stretching), 1730 cm^{-1} (C=O bond), 1480 cm^{-1} (C–O ether bond), 1041 cm^{-1} (C–O–C stretching). The subsequent complexation with β-CD on cholesterol bound to the gold surface was clearly evidenced by the ATR-IR analysis (Fig. 4b). The AuNPs modified with β-CD exhibited a significant increase in the signals at 3200–3600 cm^{-1}, originating from the O–H stretching of the β-CD, and signals observed at 1550–1600 cm^{-1}, attributed to typical O–H bending from β-CD.[56]

In conclusion, we have demonstrated a facile method for the synthesis of cholesterol-terminal polyPEG-A using a cholesterol-functionalized RAFT agent. These polymers were

grafted onto gold surfaces using the trithiocarbonate end-groups. Further facile functionalization was achieved using host–guest chemistry with β-CD. This work points towards further complex material syntheses exploiting the retained OH functionality on the CD to build up carbohydrate clusters applicable to multiligand recognition events and binding.

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