Supporting Information

Supporting Information[1]

Design of an amphiphilic polymer for nanoparticle coating and functionalization

Cheng-An J. Lin[a,b,c,#], *Ralph A. Sperling*[a,d,#], *Jimmy K. Li*[c,&], *Ting-Ya Yang*[b], *Pei-Yun Li*[a], *Marco Zanella*[a,d], *Walter H. Chang*[b,c], *Wolfgang J. Parak*[*,a,d]

[a]Center for NanoScience, Ludwig-Maximilians-Universität München, Munich, Germany
[b]Department of Biomedical Engineering, Chung Yuan Christian University, Taiwan, R.O.C.
[c]R&D Center for Membrane Technology, Center for Nano Bioengineering, Chung Yuan Christian University, Taiwan, R.O.C.
[d]Fachbereich Physik, Philipps Universität Marburg, Marburg, Germany

[#]both authors contributed equally to this work
[&]present address: Institute of Biotechnology, National Cheng Kung University, Taiwan, R.O.C.
[*]corresponding author: Prof. Dr. Wolfgang Parak, Fachbereich Physik, Philipps Universität Marburg, Renthof 7, 35037 Marburg, Germany, email: Wolfgang.Parak@physik.uni-marburg.de

[1] The idea of this Supporting Information is to provide the interested reader with a detailed account of the raw data measured. It is by far not meant to be read from the beginning until the end. In contrast, due to the index the reader can access the piece of information that he/she is interested in. All important and relevant information is included in the main paper and this Supporting Information expands on further details to specific points. We have taken great care to describe here everything as detailed as possible, so that a documentation about all experimental and evaluation steps is given.

- Table of Content -

SI1: Synthesis of polymer with different modifications

Functional amphiphilic polymers can be used as a coating for various hydrophobic nanoparticles in order to transfer them to aqueous solution. The polymers developed for this purpose contain two main building blocks: hydrophobic sidechains which are compatible with the nanocrystals surface and a hydrophillic backbone which will be exposed to the aqueous solution.

Hydrophobic domains on the polymer coat intercalate with the hydrophobic tails of the surfactant molecules present around the nanoparticles and the hydrophilic backbone stabilizes the particles by electrostatic repulsion. The synthesis of these amphiphilic polymers is followed by conjugating amino-containing ligands (sidechains) to an anhydride chain (backbone), resulting in a tailored polymer with hydrophobic, hydrophilic, and (optional) functional properties as shown in ***Figure S1***. The illustration of each synthetic unit is described in S1.1 and their designs as well as examples are detailed in S1.2 and S1.3.

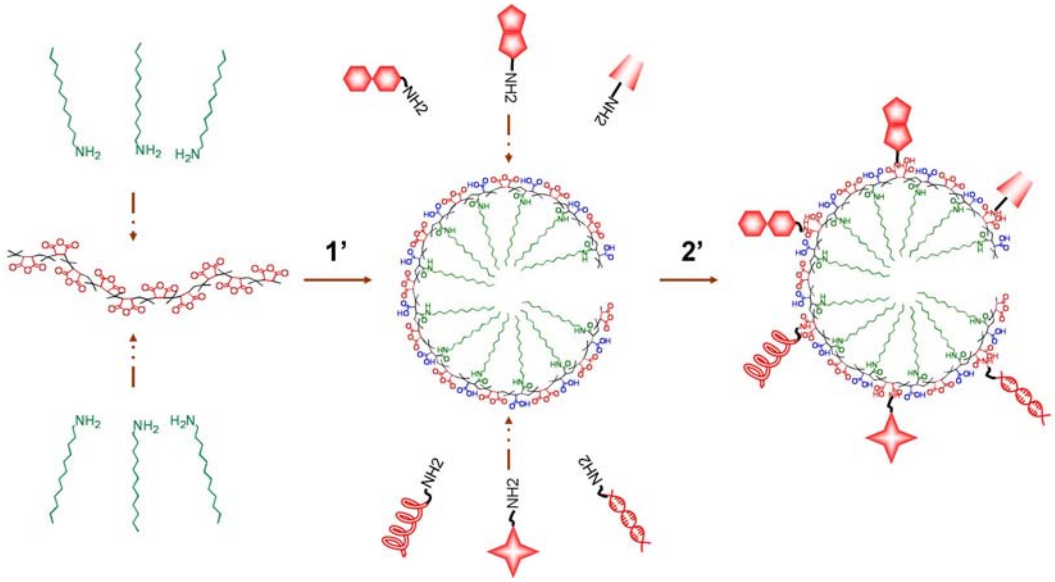
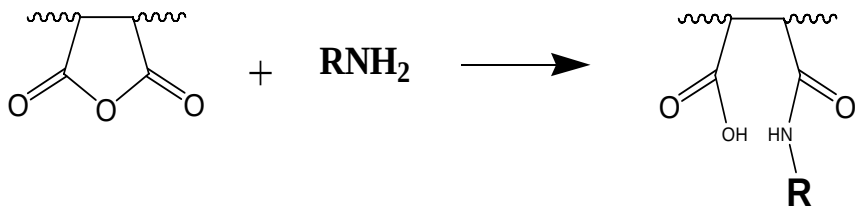


Figure S1. Conceptual scheme of polymer design. The first generation of polymer is created by conjugating alkyl amines to a maleic anhydride chain (1'), forming a basic amphiphilic polymer. The free anhydride groups on the polymer backbone can be covalently modified with functional ligands with amino terminal ($-NH_2$), e.g. fluorescent dyes, sugars, biotin, and polyethylene glycol, giving a multi-functional polymer (2') that can be used as a coating for various hydrophobic nanoparticles. When dispersed in an aqueous solution, the unconjugated maleic anhydride rings hydrolyze and yield two free carboxylic groups each, which stabilize the polymer-coated particles by electrostatic repulsion.

SI1.1 Illustration of synthetic units

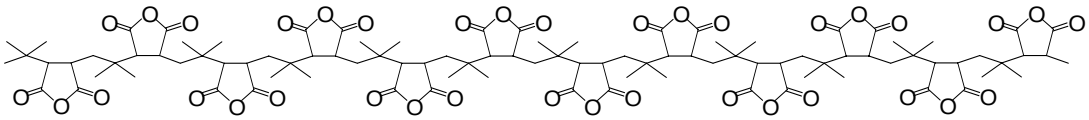
Spontaneous reaction

- **maleic anhydride + primary amine**



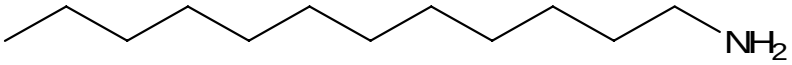
Hydrophilic backbone

- poly(maleic anhydride), e.g. poly(isobutylene-*alt*-maleic anhydride)



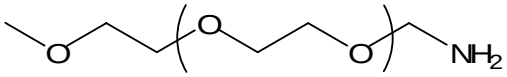
Hydrophobic sidechain

- **Primary alkyl amine** e.g. hexylamine, octylamine, decylamine, dodecylamine, tetradecylamine...

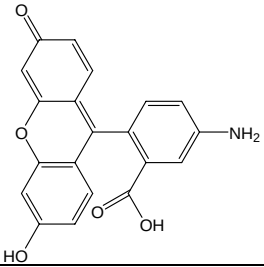


Functional molecules such as PEG, sugars, fluorescent dyes, biotin etc.

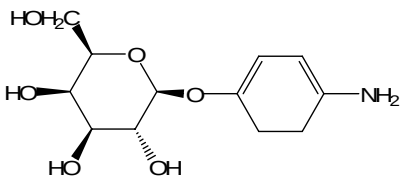
(e.g.) amino-PEG



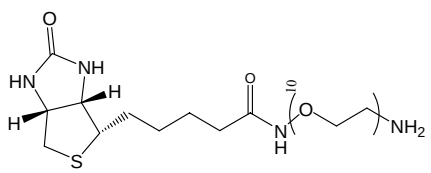
(e.g.) fluorescein-amine



(e.g.) amino-galactose

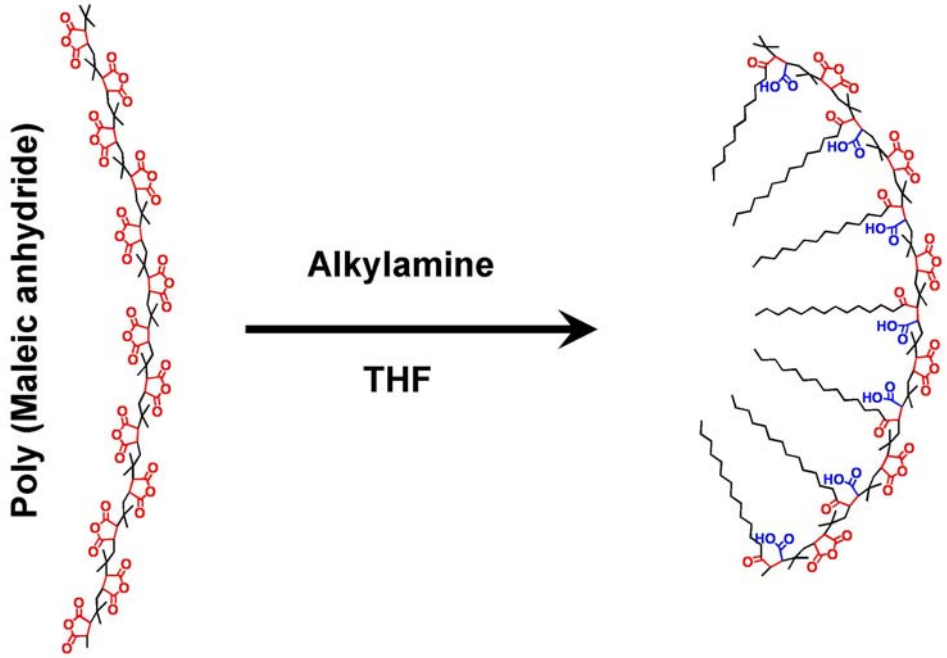


(e.g.) biotin-PEG-amine

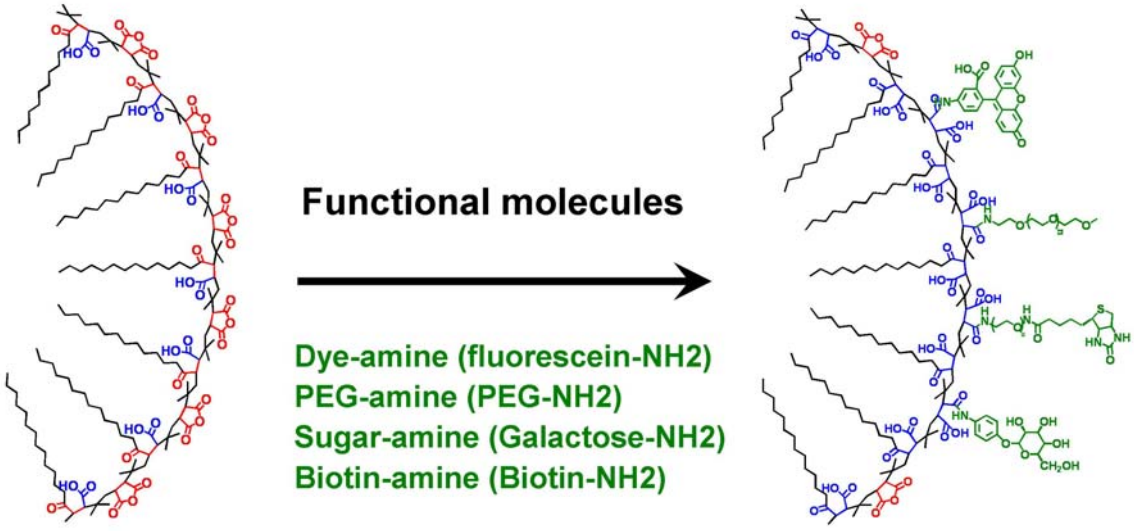


Amphiphilic polymer

- **poly(maleic anhydride) + alkylamine**



Amphiphilic/ functional polymer



SI1.2 Stoichiometry of polymer synthesis

The maleic anhydride groups of the poly(isobutylene-*alt*-maleic anhydride) backbone were assumed to be 100% reactive to primary amino-ligands through spontaneous amide linkage, which converts one maleic anhydride group into one corresponding amide and one free carboxylic acid group.

Each amino-containing ligand contributes to the final functionality of the amphiphilic polymer, e.g. the hydrophobicity, negative charge, optical properties and biological interaction. Briefly, the polymer contains three parts which include hydrophobic sidechains (from aliphatic modification), hydrophilic groups (from the backbone) and functional molecules[2].

SI1.3 Synthesis of amphiphilic polymer: [*25%Anhydride-75%C12COOH*]

"***25%Anhydride-75%C12COOH***" is defined as a polymer of which x = 75% of its maleic anhydride rings have been reacted with dodecylamine, leaving z = 25% of its anhydride rings intact. The amphiphilic polymer was synthesized by grafting the hydrophobic alkylamine onto the hydrophilic poly(maleic anhydride) backbone through spontaneous amide linkage, which converts one maleic anhydride into one corresponding amide and one free carboxylic acid. In general, 3.084 g (20 mmol monomer) of poly(isobutylene-alt-maleic anhydride) (Mw~6,000 Da, Sigma #531278) were placed in a round flask. Each polymer molecule contains 39 anhydride (= monomer) units on average[3]. It was assumed that the maleic anhydride was 100% reactive. When preparing a 75% hydrophobic modification of amphiphilic co-polymer, 15 mmol dodecylamine (98%, Sigma #D22,220-8) dissolved in 100 ml of anhydrous tetrahydrofurane (THF, ≥99.9%, Aldrich #186562) were quickly injected and vigorously mixed with the polymer powder. The cloudy mixture was sonicated for some seconds and then kept at 60 °C under vigorous stirring. During the spontaneous reaction the solution became transparent within 5 - 10 minutes. In order to quantitatively react the maleic anhydride with the primary amine, the reaction mixture was concentrated roughly up to one fifth of the original volume by a rotavapor system (Laborota 4000, Heidolph) under a reduced pressure (p = 200 - 120 mbar) after 3 hours of reaction. The concentrated solution was further incubated overnight at 60 °C under stirring. The solvent was then slowly evaporated until the polymer was completely dry (pale yellow solid). Finally, the resulting polymer was re-dissolved in anhydrous chloroform and adjusted to a final volume of 25 ml, yielding a calculated concentration of monomer units of 0.8 M[4]. The amphiphilic polymer containing 25% of unreacted maleic anhydride groups was ready for polymer coating or could be further modified with other functional molecules. After the modification of the backbone (6000 g/mol, 39 monomer units) with 75% dodecylamine (185.36 g/mol), the molecular weight of the total (anhydrous) amphiphilic polymer is approximately 11400 g/mol.

[2] If the hydrophobic modification with primary alkylamines consumes x% of the reactive anhydride rings of the backbone, there will be (100 - x)% of anhydride rings left for further amino-ligand modification. With another y% functional amine-containing molecules added for modification, there will be z = (100 – x – y) % of the original number of maleic anhydride rings left. After being dispersed in an aqueous solution, these z% residual anhydride rings will open to yield 2 z% free carboxylic groups, the total percentage of carboxylic groups will thus be (x + y + 2z).

[3] As the polymer has a molecular weight of Mw ~ 6000 g/mol the molecular weight of one polymer unit is Mw ~ 6000 g/mol / 39 ≈ 154 g/mol, as each polymer molecule comprises on average 39 monomer units.

[4] 20 mmol / 25 ml = 0.8 M.

SI1.4 Synthesis of poly(ethylene glycol)-grafted co-polymer: [*75%C12COOH-25%PEG-COOH*]

"***75%C12COOH-25%PEG-COOH***" is defined as a polymer of which x = 75% of its maleic anhydride rings have been reacted with dodecylamine and of which y = 25% of its maleic anhydride rings have been reacted with PEG-amine, leaving no remaining anhydrides unreacted (z = 0%). An appropriate amount of methoxypoly(ethylene glycol) amine (Fluka 07964, mPEG750-amine, Mw~ 750 g/mol) was dissolved in chloroform to make a stock solution of 0.08 M concentration. Then 5 ml mPEG-amine stock solution was dropped into 2 ml of ***25%Anhydride-75%C12COOH*** (0.8 M monomer concentration) under vigorous stirring. Thus, the addition of mPEG-amine was enough to react with 25% of the total amount of anhydride monomers of ***25%Anhydride-75%C12COOH***. In general, the chloroform was evaporated by the rotavapor system and then the dried polymer was re-dissolved in anhydrous chloroform with the final volume of 4 ml, i.e. 0.4 M final concentration. In order to allow complete reaction, the mixture was stirred at room temperature overnight.

SI1.5 Synthesis of fluorescent dye-grafted co-polymer: [*24%Anhydride-75%C12COOH-1%Fluo*]

"***24%Anhydride-75%C12COOH-1%Fluo***" is defined as a polymer of which x = 75% of its maleic anhydride rings have been reacted with dodecylamine and of which y = 1% of its maleic anhydride rings have been reacted with fluorescein-amine, leaving z = 24% of the anhydrides unreacted. In order to anchor directly the fluorescent amino-dye on the polymer in organic phase, it was first prepared as stock solution in anhydrous chloroform. For example, 27.8 mg of fluorescein-amine (Aldrich 201626) were well dissolved in 10 ml of anhydrous THF to obtain a 8 mM fluorescein-amine stock solution (10x). This 10x solution was then further diluted 10 times with anhydrous THF before modification to a 1x stock solution. To obtain the 1% fluorescein-grafted co-polymer, i.e. addition of fluorescein-amine corresponding to 1% of the total amount of anhydride monomers, 0.5 ml of 0.8 M monomer units of ***25%Anhydride-75%C12COOH*** were mixed vigorously with 5 ml of 0.8 mM fluorescein-amine, which were then reacted overnight under stirring at room temperature. The resulting solution was then evaporated by the rotavapor system and the solid powder was re-dissolved in 10 ml of anhydrous chloroform, to yield a final monomer concentration of 40 mM fluorescein-polymer[5].

SI1.6 Synthesis of sugar-grafted co-polymer: [*21%Anhydride-75%C12COOH-4%Galactose*]

"***21%Anhydride-75%C12COOH-4%Galactose***" is defined as a polymer of which x = 75% of its maleic anhydride rings have been reacted with dodecylamine and of which y= 4% of its maleic anhydride rings have been reacted with galactose-amine, leaving z = 21% of anhydride rings intact. In order to anchor the sugar on the polymer, sugar-amine was first prepared in THF as a stock solution. For example, 43.4 mg of aminophenyl galactopyranoside (Sigma A9545) were dissolved in 20 ml of anhydrous THF by sonication to obtain an 8 mM galactose-amine stock solution (10x). The solution was further diluted 10 times with anhydrous THF before modification to yield a 0.8 mM stock solution (1x). To obtain 4% galactose-grafted co-polymer, i.e. addition of galactose-amine corresponding to 4%

[5] (0.5 ml • 0.8 mM) / 10 ml.

of the total amount of monomers (anhydride rings), 0.5 ml of 0.8M monomer of ***25%Anhydride-75%C12COOH*** were mixed vigorously with 20 ml of 0.8 mM galactose-amine, which were then reacted overnight under stirring at room temperature. The resulting solution was then evaporated by the rotavapor system and the solid powder was re-dissolved in 10 ml of anhydrous chloroform, to yield a final concentration of 40 mM monomer concentration of sugar-polymer or galactose-polymer.

SI1.7 Synthesis of biotin-grafted co-polymer: [*21%Anhydride-75%C12COOH-4%Biotin*]

"***21%Anhydride-75%C12COOH-4%Biotin***" is defined as a polymer of which x = 75% of its maleic anhydride rings have been reacted with dodecylamine and of which y = 4% of its maleic anhydride rings have been reacted with biotin-amine, leaving z = 21% of anhydrides unreacted. In order to anchor the biotin molecules on the synthetic polymer, 10 mg of biotin- poly(ethyleneglycol) amine (average Mw = 720 g/mol, Sigma B9931) were first prepared in 0.868 ml of THF (1.6 mM). To obtain a 4% biotin-grafted copolymer, i.e.addition of biotin-amine corresponding to 4% of the total amount of monomers, 0.25 ml of 0.8M monomer concentration of ***25%Anhydride-75%C12COOH*** were vigorously mixed with 10 ml of 0.8 mM biotin-amine (diluted with chloroform), which were reacted overnight under stirring at room temperature. The resulting solution was then evaporated by the rotavapor system and the solid powder was re-dissolved in 5 ml of anhydrous chloroform, to yield a final monomer concentration of 40 mM biotin-polymer.

In addition, the binding efficiency of the functional molecules (e.g. biotin, galactose etc.) to the polymer chain in principle depends on several factors such as the solubility, temperature, concentration and reaction time. As mentioned above, THF is a good solvent substitute for chloroform when the molecule of interest is not well soluble. The synthesis can be carried out in THF or chloroform, selected because of their low vapor point. Furthermore, the reaction efficiency could also be increased by reducing the volume of the polymer solution, i.e. several rounds of evaporation steps after adding THF or chloroform. The protocols above include examples for the synthesis of tailored amphiphilic polymers with several different classes of functional molecules.

SI2: Synthesis of different nanoparticles

SI2.1 Synthesis of colloidal gold nanoparticles (4 nm)

The 4-nm gold nanoparticles were synthesized according to the Brust two-phase method, with some modifications. Briefly, the organic phase containing 8.86 g of tetraoctylammonium bromide (TOAB, 98%, Sigma-Aldrich 29,413-6) was dissolved in 320 ml of toluene (Fluka 89682) and the aqueous phase was prepared by dissolving 1.2 g hydrogen tetrachloroaurate (III) (99.9%, Alfa Aesar #12325) in 100 ml of MilliQ water. The two phases were mixed in a 500-ml separation funnel and then the gold precursors (yellow part in water) was then gradually transferred into the oil phase (red color), indicating the formation of tetraoctylammonium-gold pairs. After discarding the aqueous solution (the lower part), the oil phase with gold precursors was transferred to a 500-ml round flask. In another beaker, a reduction agent was freshly prepared by dissolving 1.34 g of sodium borohydride (98%, Sigma-Aldrich #45.288-2) in 100 ml of MilliQ water with stirring, and was then added dropwise within one minute to the gold precursor solution in toluene. Upon vigorous stirring, the color changed from red to red-violet, indicating the nucleation of gold clusters mediated by sodium borohydride. Further growth was mediated by the residue of sodium borohydride, which reduced the remaining gold ions. The solution was transferred to a clean separation funnel after one-hour stirring and then 100 ml of 0.01 M HCl were added to remove the excess of sodium borohydride. The two-phases solution was shaken for minutes and the aqueous phase was discarded. Again, 100 ml of 0.01M NaOH were added in order to remove the excess acid by shaking for minutes and by discarding the aqueous phase. Finally, 100 ml of Milli-Q water were then added to remove the excess ions and the aqueous phase was removed after shaking for minutes. This last step was repeated four times. In order to enhance the stability of particles, 40 ml of 1-dodecanethiol (98%, Sigma-Aldrich #47,136-4) were added after one day stirring and the solution was then incubated at 65°C for 2 hours.

The high binding affinity of thiol to gold would displace the Br- ions and yield dodecanthiol-coated gold nanocrystals. The solution was cooled down to room temperature and larger agglomerates were first removed by centrifuging at 2500 r.p.m. To the collected supernatant, methanol was added until the solution turned cloudy, followed by centrifugation. After discarding the supernatant, the precipitate was dissolved in toluene upon vigorous shaking. To the nanoparticle solution again cold methanol was added until the solution turned cloudy, followed by centrifugation. The supernatant was removed and the precipitate was re-dissolved in 8 ml of toluene. In order to further improve the size distribution of the sample, a size-selective precipitation was carried out by adding 80 µl of cold methanol, followed by centrifugation and discarding of the pellet which comprises bigger particles or aggregates. The average of the inorganic core diameter of the gold nanocrystals was determined by TEM and the particle concentration was determined by UV-VIS absorption measurement (Cary 50, Varian). In this case, gold nanoparticles with an average diameter of 4 nm have the extinction coefficient of 8.7×10^6 $M^{-1}cm^{-1}$ at their plasmon peak around 518 nm. The concentration of the resulting gold nanoparticle solution was typically in the micromolar range.

SI2.2 Synthesis of colloidal gold nanoparticles (6 nm)

The synthesis of 6-nm gold nanoparticles (AuNP) was modified from Peng's protocol[1]. Appropriate amounts of didodecyldimethylammonium bromide (DDAB, Fluka 36785) and decanoic acid (Aldrich W236403) were dissolved separately in toluene to prepare a stock solution of 100 mM concentration. Then an appropriate amount of $AuCl_3$ (Aldrich 379948) was dissolved in DDAB solution to prepare a gold precursor solution of 25 mM concentration. Then, 25 mg of tetrabutylammonium borohydride (TBAB, Fluka 86855) dissolved in 1 ml of DDAB solution were mixed to 2.5 ml decanoic acid

solution. After nucleation by dropping 10 µl of $AuCl_3$ solution into this mixture, 0.8 ml gold precursor solution was then injected during vigorous stirring. The color of the solution changed to deep red within minutes, indicating formation of gold nanoparticles. After one-hour stirring, the solution was heated up to 60°C for 5 minutes in order to improve size distribution. After further adding 0.5 M trioctylphosphine (TOP, Aldrich 117854) which was prepared in toluene the plasmon peak of AuNP was shifted from 530 nm to 520 nm, indicating the successful capping of TOP surfactants on the AuNP, i.e., the phosphine heads were attached onto the gold surface and the alkyl tail protruded out. In most experiments the TOP: gold molar ratio was 1:1. After removing the larger particles by centrifugation (1500 rpm, 5 minutes), the 6-nm AuNP was purified by methanol precipitation and the dried agglomerates of gold nanoparticles were re-dissolved in anhydrous chloroform. The concentration of 6-nm AuNP having the extinction coefficient of 2.87×10^7 $M^{-1}cm^{-1}$ at its plasmon peak was determined by UV-VIS absorption measurement (Cary 50, Varian).

SI2.3 Synthesis of colloidal quantum dots

CdSe

Briefly, 3.86 g of trioctylphosphine oxide (TOPO, 99%, Sigma-Aldrich #22.330-1), 0.78 g of hexadecylamine (HDA, technical grade, Sigma-Aldrich #H7.40-8), 0.268 g of dodecylphosphonic acid (DDPA 98%, Polycarbon Inc.) and 51.4 mg of CdO (99.99+%, Sigma-Aldrich #20.289-4) were mixed in a 50-ml 3-necked flask equipped with a temperature controller stick, a rubber septum for injecting the stock solution, as well as a third neck connected vertically to the Schlenk line assembled in a glove box. First, the mixture was exposed to vacuum at 100 °C for 20 minutes under stirring in order to remove impurities, followed by switching the vacuum to argon flow. The temperature was elevated to 320°C until the color of the mixture turned to transparent pale yellow. At the same time, the Se stock solution was freshly prepared by dissolving 320 mg of Se powder (99.99%, Sigma-Aldrich #22.986-5) in 2 ml of trioctylphosphine (TOP, technical grade, Sigma-Aldrich #11.785-4) under vigorous stirring. After swiftly injecting the Se precursor solution into the hot TOPO solution (320 °C in Ar flow with vigorous stirring under reflux) followed by re-setting the temperature to 240 °C, the color of the solution turned from colorless to yellow-orange and the heating mantle was then removed. In order to synthesize quantum dots of other sizes, heating was continued without removing the heating mantle and the color gradually turned to red, dark red and finally to dark brown in 25-30 minutes after the injection (first exciton absorption peak at 620-630 nm). The growth of quantum dots was monitored by taking aliquots during reaction and by measuring their UV-visible absorption spectrum of the chloroform-diluted sample. The resulting reaction mixture was cooled to room temperature followed by methanol addition. The supernatant was then discarded after centrifugation; the precipitated nanocrystals were re-dissolved in chloroform without further purification. The growth of quantum dots varied slightly from batch to batch, but the synthesis could be stopped as soon as the first exciton peak reached a desired value, which could be easily fitted to an average size through calibration curves from Peng's group[2]. The concentration of quantum dots could also be easily estimated by the optical density in first exciton peak correlated with their extinction coefficient.

CdSe/ZnS

For the growth of ZnS shell on CdSe quantum dots, 4 g of TOPO and 1 g of HDA were weighted in a 50-ml 3-necked flask, pumped to vacuum at 120 °C for 20 minutes under stirring. After switching the vacuum to argon flow, an appropriate amount of CdSe solution (typically 4 ml with concentration of 10^{-5} M) was injected into the TOPO/HDA mixture and the chloroform was then distilled off under vacuum until there was no bubbling. Then the vacuum was switched to argon flow and the temperature was elevated to 140-220 °C depending on the size of nanocrystals according to the literature[3]. The

stock solution for ZnS shell growth was freshly prepared in the glove box by dissolving 0.31 g of diethylzinc solution ($C_4H_{10}Zn$, 1.0 M solution in heptane, Sigma-Aldrich #40.602-3) and 0.45 g of hexamethyldisilathiane ($C_6H_{18}Si_2S$, Aldrich #28.313-4) in 20.0 g TBP, which was then added dropwise to the CdSe/TOPO/HAD mixture. The amount of ZnS solution was calculated according to the size of the CdSe core, the desired number of ZnS monolayers and the concentration of core nanocrystals[3]. After dropping all the ZnS stock solution, the temperature was set to 100°C for the next 2 hours with stirring under argon flow. Then the reaction mixture was cooled down to room temperature followed by addition of methanol. The resulting cloudy solution was centrifuged to discard the supernatant with free surfactants; and the precipitated nanocrystals were re-dissolved in toluene. The concentration of core/shell quantum dots could also be easily estimated by the optical density in the first exciton peak correlated with their extinction coefficient. The prepared CdSe/ZnS quantum dots were nominally covered with two monolayers of ZnS. The solution was then transferred into a vial and stored for the polymer coating experiments.

SI2.4 Synthesis of colloidal iron oxide nanoparticles

Iron oxide nanoparticles were synthesized following Casula's protocol[4]. In a typical synthesis, a solution of tridecanoic acid in octyl ether mixed in a 50-ml 3-necked flask equipped with a temperature controller stick, a rubber septum for injecting the stock solution, as well as a third neck connected vertically to the Schlenk line assembled in a glove-box was out-gassed for 30 minutes and then heated under Ar flow at 293 ºC. Two solutions of iron pentacarbonyl and mCPBA in ether were then rapidly co-injected. The iron molar concentration was 0.1 M, and the iron: surfactant: oxidizer molar ratio was 1:3:1.5. The solution flask was heated at 293 °C for 5 minutes to allow particle growth, after which the solution was cooled to 40 °C, and then ethanol was then added to precipitate nanoparticles from the solution. Following two rounds of centrifugation, re-dispersion in toluene, and precipitation with ethanol, no further size selection procedure was carried out. The iron oxide nanoparticles thus obtained were stored in anhydrous hexane before use.

SI3: Polymer-coating of different systems

SI3.1 General introduction of polymer coating

The general concept of polymer coating has been described previously by Pellegrino et al.[5], in detail in the supporting information of this report. All kinds of colloidal nanoparticles, i.e. CdSe/ZnS quantum dots, gold nanoparticles, and iron oxide nanoparticles, were first cleaned by removing unbound surfactant before applying the polymer coating procedure. Adding an excess of anhydrous methanol to a 10-ml nanoparticle solution resulted in the reversible flocculation of nanoparticles. The flocculates were then separated from the supernatant by centrifugation and re-dispersed in 10 ml of anhydrous chloroform. Second, the amount of (functional) amphiphilic polymer was determined by the total surface area of hydrophobic nanocrystals. For each sample, the average diameter of inorganic nanocrystal core was determined by TEM analysis and the concentration (C) of the nanoparticle solutions were determined either by a gravimetric method[5] or by an absorption measurement[2].

The overall surface area (A_0) of each nanoparticle and the total amount of nanoparticles were calculated using the effective nanoparticle diameter d_{eff}=d_{core}+ 2 $l_{surfactant}$ (nm), i.e., the sum of the diameter of the inorganic core (d) plus the assumed thickness of the surfactant shell ($l_{surfactant}$ = 1 nm), as shown in *Formulas (1) and (2).*

$$A_0 = 4\pi \cdot (d_{eff}/2)^2 = \pi \cdot d_{eff}^{\ 2} \quad \text{..... (1)}$$

$$A = C \cdot V \cdot N_A \cdot A_0 = \pi \cdot C \cdot V \cdot N_A \cdot d_{eff}^{\ 2} \quad \text{..... (2)}$$

A_0 : *Surface area of one single spherical nanoparticle (nm^2)*

A : *Total surface area of all the colloidal nanoparticles in the aliquot solution (nm^2)*

d_{eff} : *Effective diameter of one nanoparticle (nm)*

C : *Concentration of colloidal nanoparticles in the aliquot solution (mol/l)*

V : *Volume of aliquot solution (l)*

N_A : *Avogadro constant, 6.02×10^{23}/mol*

The polymer solution synthesized from the previous section was added to the nanoparticle solution, in which the amount of polymer could be estimated by *formulas (3)* and *(4)*. After 10-20 minutes of stirring the nanoparticles with the polymer, the chloroform was slowly evaporated under reduced pressure by a rotavapor system (Laborota 4000, Heidolph). The cross-linker solution (bis(6-aminohexyl)amine (Sigma-Aldrich # 14506) in chloroform) was then (optionally) added to cross-link the polymer shell which was wrapped around the nanoparticles. The solution was again mixed well for 10 minutes followed by the slow evaporating procedure under reduced pressure to remove all the chloroform. The resulting solids were quickly dissolved in basic buffer solution (0.1 M NaOH or 50 mM sodium borate buffer (SBB, pH12) to yield nanoparticles in aqueous phase. A filtration through a syringe membrane filter (Roth # P818.1, 0.22 μm pore size) helped to remove any remaining aggregates. Then the buffer was changed to sodium borate buffer (SBB, pH 9) by two rounds of

dilution with SBB and reconcentration through 100 kDa MWCO Amicon centrifuge filters (Millipore, Amicon Ultra-15, #UFC9100). Small empty polymer micelles (MW << 100 kDa), which may form from excess polymer, could be removed by ultrafiltration. Most of the remaining polymer micelles could be removed using a size exclusion column (Sephacryl S-300, Pharmacia). Optionally, nanoparticles could also be further purified by gel electrophoresis (Sub-Cell GT electrophoresis cells, Bio-Rad)[6], which lead to a more efficient removal of empty polymer micelles than the purification by size exclusion columns. The concentrated nanoparticles were first loaded into wells of a 2% agarose gel (Invitrogen #15510027) in 0.5 x TBE solution and gel electrophoresis was performed for one hour under an applied electric field of 10 V/cm. The resulting bands which contained nanoparticles were cut out and transferred into a dialysis membrane bag (3500 Da MWCO, Roth) and run through gel electrophoresis again to expel the trapped nanoparticles. The nanoparticles solution was then filtrated with a syringe filter (0.22 μm pores) in order to remove small gel pieces and reconcentrated by ultrafiltration (100 kDa MWCO Amicon centrifuge filter).

$$V_P = \frac{N_P}{C_P} = \frac{A \times R_{P/Area}}{N_A \times C_P} \equiv \frac{\pi \cdot C \cdot V \cdot d_{eff}^{\ 2} \times R_{P/Area}}{C_P} \quad \text{.....(3)}$$

$$V_C = \frac{N_C}{C_C} = \frac{N_P \times r_{C/P}}{N_A \times C_P} = \frac{A \times R_{P/Area} \times r_{C/P}}{N_A \times C_P} \equiv \frac{\pi \cdot C \cdot V \cdot d_{eff}^{\ 2} \times R_{P/Area} \times r_{C/P}}{C_P} \quad \text{..... (4)}$$

V_P, V_C *: Volume of polymer (p) or cross-linker (c) solution added to nanoparticles (l)*

N_P, N_C *: Number of polymer monomers (p) or cross-linkers (c) needed in polymer coating (mol)*

C_P, C_C *: Concentration of polymer monomers (p) or cross-linkers (c) in stock solution (M)*

A *: Total surface area of all colloidal nanoparticles in the aliquot solution (nm^2)*

$R_{P/Area}$ *: Ratio of numbers of polymer monomers (p) added per surface area on nanoparticles (monomer units/nm^2)*

$r_{C/P}$ *: Ratio of added cross-linkers (c) to numbers of polymer monomers added*

Several experiments of polymer coating have been performed. All experiments always lead to a successful transfer of nanoparticles to the aqueous phase although the nanoparticles could vary in size, core material and/or surfactant ligands.

To optimize the parameters for a robust coating procedure, a series of tests were run in order to find the number of polymer monomers per surface area that give the best performance. In the case of gold nanoparticles for example, gold nanoparticles were coated with polymer of 25% anhydride-75% C12-CA with different ratios of added polymer per surface area on nanoparticles, i.e., $R_{P/Area}$ = 5, 10, 50, 100, 500, and 1000 monomer units/nm^2.

[6] In this electrophoresis apparatus, the buffer reservoirs around the electrodes have a much larger cross-section than the gel on the tray, therefore in analogy to a series of resistances the voltage is assumed to drop mainly at the edges of the agarose gel that has a length of 10 cm. With a constant applied voltage of 100 V, the electric field strength is then 10 V/cm.

After HPLC purification the particles were run through gel electrophoresis (10 V/cm, 1 hour). The result of this experiment is shown in Figure S2. As can be seen, there is a safe range ($R_{P/Area}$ = 50-500 units/ nm^2) where bands are sharp, indicating a good coating integrity as well as homogeneity of the polymer shell. Experiments with other nanoparticles led to similar results. Therefore a general protocol for the polymer coating of hydrophobic nanoparticles could be established, regardless of the nanocrystal material. In this protocol, 100 monomer units of polymer are added per nm^2 surface area of nanoparticles and the ratio of cross-linkers to the number of polymer added (in monomer units) is 0.05, i.e. the general values of $R_{P/Area}$ = 100 and $r_{C/P}$ = 0.05 were used in the following. Here we report in detail the protocol applied to three kinds of nanomaterials, namely semiconductor quantum dots (CdSe/ZnS), superparamagnetic nanoparticles (Fe_2O_3), and gold nanoparticles (Au).

The use of cross-linker during the polymer coating described in this study was optional. For our previously published polymer coating procedure (with poly(maleic anhydride alt-1-tetradecene) (Mw ~9000, discontinued product from Sigma-Aldrich, # 452513) and the cross-linker by bis(6-aminohexyl)amine (Fluka # 14506)), it was found that nanocrystals to which only the polymer solution was added, but without undergoing the cross-linking step, did not dissolve well water[5]. In contrast, nanoparticles with polymer coating procedure reported in this study could be quickly dissolved in basic solution (0.1 M NaOH) even without the cross-linker. When the polymer-coated particles were (post-) modified with PEG by means of EDC, it was difficult to reach complete saturation of the particles without precipitation. This was attributed to the smaller number of carboxylic groups on the polymer backbone compared with the commercial polymer used by Pellegrino[5].

The experimental results of the polymer coating of hydrophobic nanoparticles as well as their subsequent PEG modification are illustrated in ***Figure S3.***

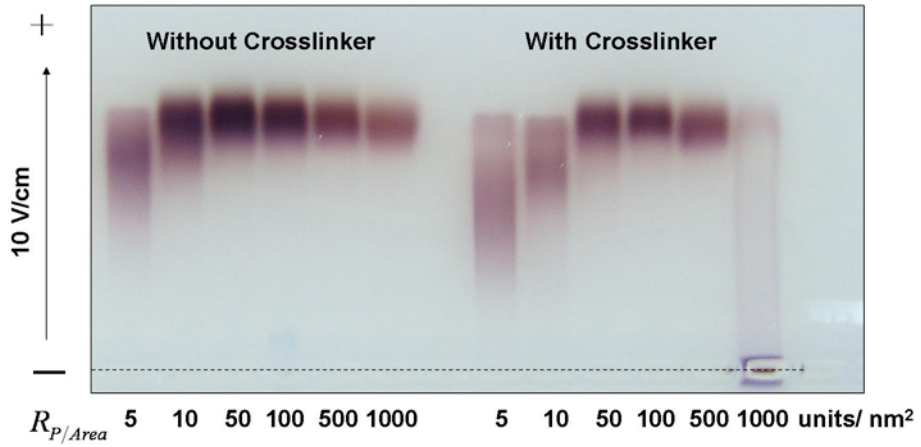


Figure S2. Polymer coating of Au nanoparticles (4-nm core diameter) with different amount of monomer added per surface area ($R_{P/Area}$) with or without crosslinker.

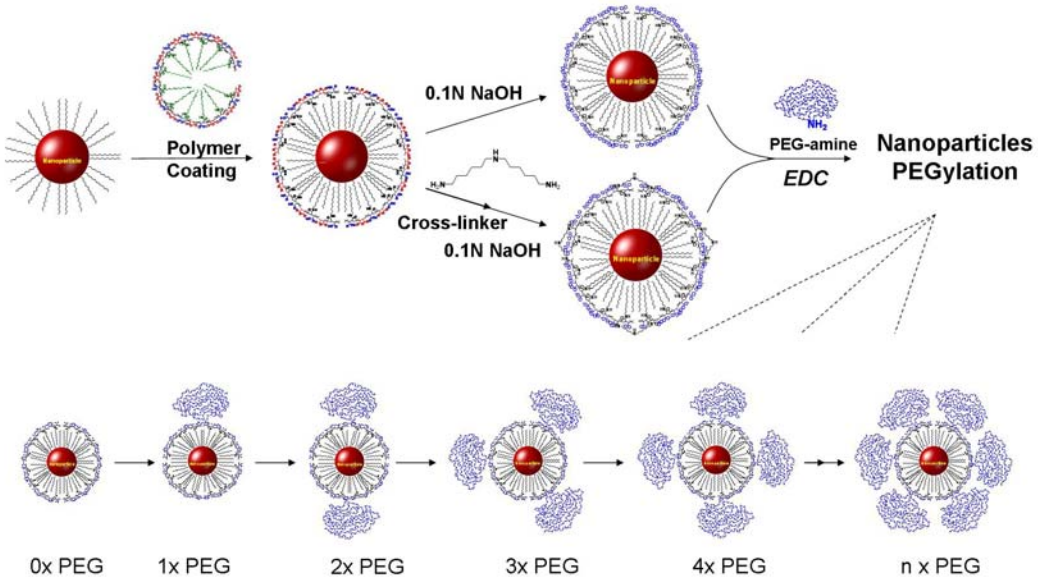


Figure S3. Scheme of polymer coating procedure. The hydrophobic alkyl chains of the polymer intercalate with the surfactant coating on the nanoparticles. The free carboxylic acid and anhydride rings are then located on the surface of these polymer-coated nanoparticles. The amino ends of the optionally added cross-linker open the anhydride ring and link the individual polymer chains for further stabilization. In both cases the nanoparticles become water soluble after 100 mM sodium hydroxide is added. The negative charges of the polymer shell stabilize the particles in water by electrostatic repulsion. Then the exposed carboxylic group on nanoparticles can be further conjugated with amine-containing molecules (e.g. PEG) through EDC chemistry.

SI3.2 Polymer coating of CdSe/ZnS quantum dots and test of PEGylation

The synthesis of CdSe/ZnS quantum dots has been described above. The excess surfactants of as-prepared quantum dots were cleaned by removing the supernatant of a methanol-mediated precipitation and the dried (still wet) agglomerates were then re-dissolved in anhydrous chloroform. The concentration of quantum dots was determined by UV-VIS absorption measurement (Cary 50, Varian). For quantum dots with an average diameter of 2.35 nm (first extinction peak at 500 nm from absorption spectrum) the extinction coefficient of 56047 $M^{-1}cm^{-1}$ was estimated from the literature[2]. The inorganic core diameter (d) was estimated to be 3.75 nm by assuming a thickness of two monolayers of the ZnS shell. Thus the effective diameter (d_{eff}) which comprises the surfactant layer was estimated to be 5.75 nm. In the aliquot solution of CdSe/ZnS nanocrystals (500 µl, 12 µM) the total surface area (A) of colloidal quantum dots was 3.75×10^{17} nm^2 calculated using *formula (1)*. Using a polymer to surface ratio of $R_{P/Area}$ = 100 units / nm^2, means that 62.3 µmol monomer of polymer has to be added to the nanocrystal solution[7]. Therefore 78 µl of polymer stock solution (0.8 M monomer concentration, ***25%Anhydride-75%C12COOH made in SI1.3***) and 2.42 ml of anhydrous chloroform were added to quantum dot solution, giving to a total volume of 3 ml[8]. After stirring for 15 minutes, the solvent was slowly evaporated by the rotavapor system, i.e. set to 200 mbar until no liquid was left and then set to 20 mbar for 10 minutes. For the optional crosslinking 1.56 ml of cross-linker solution (2 mM bis(6-

[7] $A = \pi \cdot 12\ \mu M \cdot 500\ \mu l \cdot 6.01 \cdot 10^{23}\ mol^{-1} \cdot (5.75\ nm)^2 = 3.75 \cdot 10^{17}\ nm^2$.
$N_P = A \cdot R_{P/Area} / N_A = 3.75 \cdot 10^{17}\ nm^2 \cdot 100\ nm^{-2} / 6.01 \cdot 10^{23}\ mol^{-1} = 62.3\ \mu mol$.
[8] 62.3 µmol / 0.8 M = 78 µl.

aminohexyl)amine in chloroform) was added to re-dissolve the resulting particle powder and stirred for 20 minutes. Afterward the solvent was again removed by evaporation, and the resulting powder of quantum dots with polymer shell was quickly dissolved in 2 ml of 0.1 M NaOH. The solution was further diluted to 15 ml by addition of water, filtered through a 0.22-µm syringe filter and finally gave a transparent solution. By two rounds of ultrafiltration with Amicon centrifuge filters (100kD MWCO), the buffer was changed to SBB (sodium borate buffer, 50 mM, pH 9) and the residues of polymer micelles were further removed by passing the solution through a size exclusion column (Sephacryl S-300, Pharmacia). The final concentration of purified quantum dots could be adjusted by concentration on a centrifuge filter. The surface chemistry of quantum dots was also characterized by the attachment of a discrete number of PEG molecules[6], i.e. a simple method that can evaluate the bio-conjugative capability of the nanoparticles. (***Figure S4***)

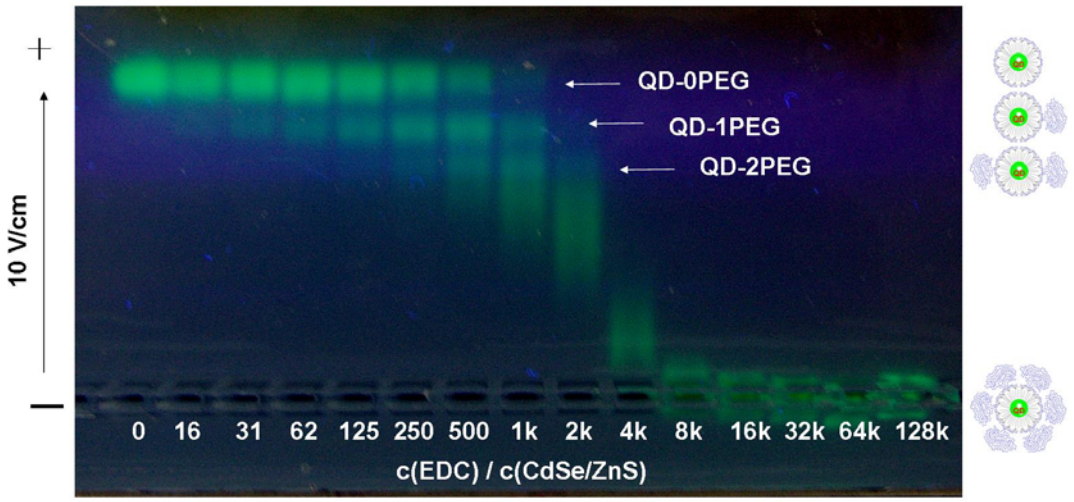


Figure S4. Discrete PEGylation of polymer-coated quantum dots (QD). First, 150 µl of 3 mM of methoxy-poly(ethylene glycol)amine (CH_3O-PEG-NH_2, Mw~5000 g/mol, Rapp Polymere) in SBB were mixed with an equal volume of polymer-coated quantum dots (6 µM, 2.35 nm core diameter) and split into aliquots of 20 µl each. To these samples, 10 µl of an EDC solution of appropriate concentration was added to yield different molar ratios of EDC molecules per nanoparticle, c(EDC)/c(CdSe/ZnS) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, the series was run by gel electrophoresis (10 V/cm, 1 hour) before the photo was taken under excitation by an UV lamp. The more EDC had been added, the more PEG molecules were bound to the quantum dots, and the more retarded the bands on the gel were. The lanes of the gel correspond to samples with increased EDC concentration from left to right. Discrete bands of particles with 0, 1, 2 ...attached PEG molecules per particles could be resolved in case of c(EDC)/c(CdSe/ZnS) < 2000, which demonstrates the possibility to prepare mono- and divalent quantum dots with the polymer coating procedure described in this report.

SI3.3 Polymer coating and PEGylation of iron oxide nanoparticles (FeNP)

Iron oxide nanoparticles were either synthesized or purchased (USPIO-C18, TAN bead, Taiwan). The concentration of the FeNP solution was determined by gravimetric measurements. Generally, after removing the free surfactants by methanol precipitation, all solvents were evaporated until the sample was completely dry. The weight concentration of FeNP solution was then adjusted to 10 mg/ml by chloroform after weighing the dried FeNP solid. With the bulk density of Fe_2O_3 of 5.24 g/cm^3, the mass of a single FeNP having a diameter of 13 nm (from TEM) was estimated to be 6.03×10^{-18} g. Finally, the molar particle concentration of 2.75 µM was calculated from the weight concentration. The calculation does not take into account the mass of the surfactant coating, which results in an over-estimation of the particle concentration. If we assume that the organic surfactant shell is 1 nm thick, its volume ratio V_{shell}/V_{core} is around 0.54 and the mass ratio M_{shell}/M_{core} is 0.09, the organic shell would contribute around 9 % to the total mass of one particle.

From the inorganic core diameter d = 13 nm the effective diameter d_{eff} = 15 nm was estimated. In the aliquot of FeNP solution (500 µl, 2.75 µM), the total surface area (A) of FeNP was 5.85×10^{17} nm^2 calculated using *formula (2),* $R_{P/Area}$ = 100 nm^{-2}, meaning that 97.2 µM monomer units of polymer had to be added to the nanocrystal solution. Then, 122 µl of polymer stock solution (0.8 M, ***25%Anhydride-75%C12COOH made in SI1.3***) and 2.38 ml of anhydrous chloroform were added to the FeNP solution, giving a total volume of 3 ml. After stirring for 15 minutes, the solvent was slowly evaporated by the rotavapor system, i.e., by setting the pressure to 200 mbar until the solvent was evaporated, and finally to 20 mbar for 10 min until the sample was completely dry. Optionally the polymer shell could be cross-linked. For this 2.43 ml of cross-linker solution (2 mM bis(6-aminohexyl)amine in chloroform) were added to re-dissolve the solid and allowed to react under stirring for 20 minutes. Afterward all solvent was again removed by evaporation, and the solid complex of FeNP with polymer coat was quickly dissolved in 2 ml of basic SBB buffer (SBB, pH12 adjusted by NaOH). The solution was further diluted to 15 ml by the addition of water, filtered through a 0.22-µm syringe filter and finally gave a transparent solution. The SBB buffer exchange was carried out by two rounds of ultrafiltration using Amicon centrifuge filters (100kD MWCO) and residues of polymer micelles were further removed by running the sample through a size exclusion column (Sephacryl S-300, Pharmacia) or the particles were further purified by gel electrophoresis. The final concentration of purified FeNP could be adjusted by ultrafiltration. The surface chemistry of FeNP was also characterized by discrete PEGylation[6] (***Figure S5***).

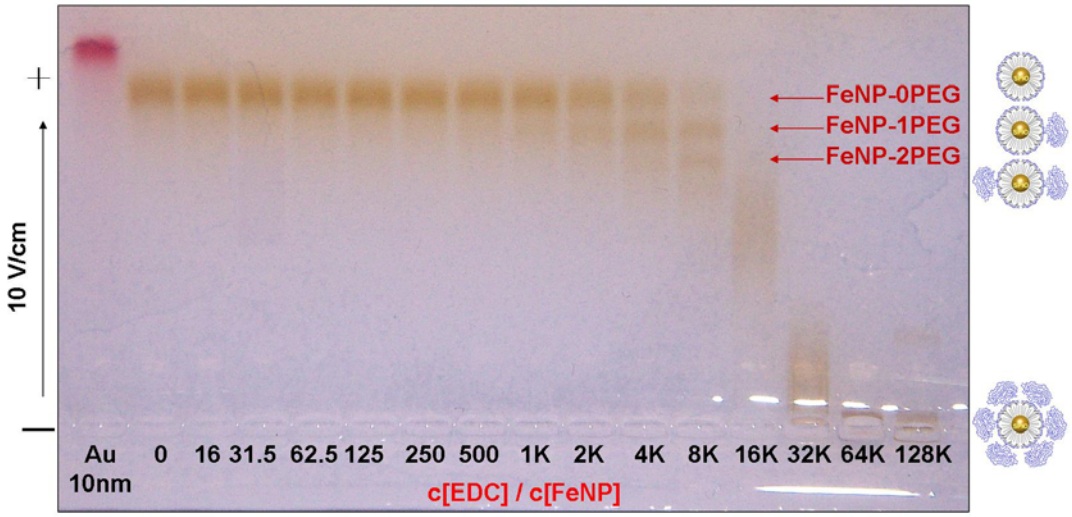


Figure S5. Discrete PEGylation test of polymer-coated iron oxide nanoparticles (FeNP). First, 150 µl of 3mM methoxy-poly(ethylene glycol)amine (CH_3O-PEG-NH_2, Mw~5000 g/mol, Rapp Polymere) in SBB were mixed with an equal volume of polymer-coated FeNP (3 µM, 13 nm core diameter) and split into aliquots of 20 µl each. To these samples, 10 µl of an EDC solution of appropriate concentration were added to yield different molar ratios of EDC molecules per nanoparticle, c(EDC)/c(FeNP) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, the series was run by gel electrophoresis (10 V/cm, 1 hour, reference sample with phosphine-coated 10-nm Au particles (BBI)). The more EDC had been added, the more PEG molecules were bound to the FeNP, and the more retarded were the bands on the gel. The lanes of the gel correspond to samples with increased EDC concentration from left to right. The discrete bands of particles with 0, 1, 2 ...attached PEG molecules could be resolved in case of 500 < c(EDC)/c(FeNP) < 8k, which demonstrates the possibility to prepare mono- and divalent iron oxide nanoparticles with the polymer coating procedure described in this study.

SI3.4 Polymer coating of gold nanoparticles and test of PEGylation

The synthesis of 4-nm of hydrophobic gold nanoparticles (AuNP) has been described previously by Brust et al.. The excess surfactants of as-prepared AuNP were cleaned by removing the supernatant of methanol-mediated precipitation and the dried agglomerates were then re-dissolved in anhydrous chloroform. The concentration of AuNP was determined by UV/Vis absorption measurements (Cary 50, Varian). In the case of AuNP with an average diameter of 4 nm, the gold nanoparticles are assumed to have an extinction coefficient of 8.7×10^6 $M^{-1}cm^{-1}$ at their plasmon peak. The inorganic core diameter (d) was determined to be 4 nm (from TEM) and the effective diameter (d_{eff}) was assumed to be 6 nm (1 nm thickness of the surfactant shell). Of an aliquot of AuNP (500 µl, 7.5 µM) the total surface area (A) of colloidal AuNP was calculated to be 2.55×10^{17} nm^2 using *formula (2),*($R_{P/Area} = 100$ nm^{-2}), meaning that 42.4 µM monomer units of polymer had to be added to the nanocrystal solution. Then, 53 µl of polymer stock solution (0.8 M, ***25%Anhydride-75%C12COOH as described in SI1.3***) and 2.45 ml of anhydrous chloroform were added to the colloidal gold solution, giving a total volume of 3 ml. After stirring for 15 min, the solvent was slowly evaporated by the rotavapor system by setting the vacuum to 200 mbar until the solvent had evaporated, then to 20 mbar for 10 min until the sample was completely dry. In the optional case the polymer shell was cross-linked, 1 ml of cross-linker solution (2 mM bis(6-aminohexyl)amine in chloroform) was then added to re-dissolve the solid particle powder and the sample was stirred for 20 minutes. After this the solvent was again removed by evaporation, and the solid complex of AuNP particle powder with polymer coat was quickly dissolved in 2 ml of 0.1 M NaOH. The solution was further diluted with water, filtered through a 0.22-µm syringe filter, leading to a transparent solution. The buffer was changed to SBB by two rounds of ultrafiltration using Amicon centrifuge filters (100kD MWCO). Residual polymer micelles were further removed by running the sample through a size exclusion column (Sephacryl S-300, Pharmacia) or the particles were further purified by gel electrophoresis. The final concentration of AuNP could be adjusted by ultrafiltration. The surface chemistry of AuNP was also characterized by discrete PEGylation[6] (***Figure S6***).

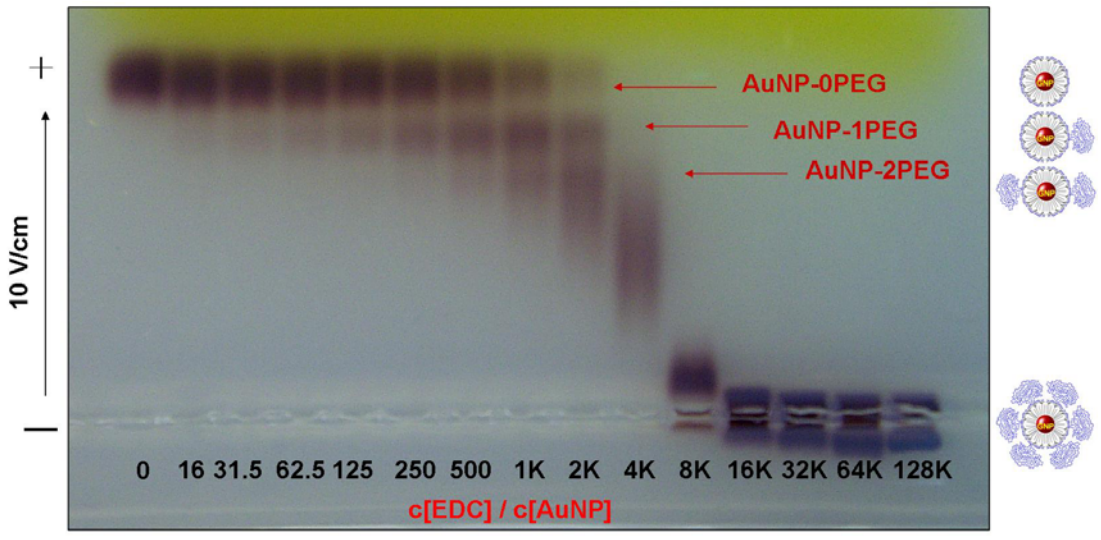


Figure S6. Discrete PEGylation test of polymer-coated gold nanoparticles (AuNP). First, 150 µl of 3 mM of methoxy-poly(ethylene glycol)amine molecules (CH_3O-PEG-NH_2, Mw~5000 g/mol, Rapp Polymere, Germany) in SBB were mixed with equal amounts of polymer-coated AuNP (6 µM, 4 nm core diameter). Then, 10 µl of each fraction was sequentially activated using different concentrations of EDC, i.e. c(EDC)/c(AuNP) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, all fractions were run with gel electrophoresis (10 V/cm, 1 hour). The more PEG molecules were attached to the gold nanoparticles, the more retardation the gold nanoparticles were run through gel electrophoresis. The lanes in the gel correspond to samples with decreasing EDC concentrations from right to left. Discrete bands of different PEG molecules attached could be resolved in the case of 125 < c(EDC)/c(AuNP) < 4k, which also demonstrates the possibility to make AuNP with single functionality.

SI3.5 Characterization of empty polymer micelles and PEGylation test

It is expected that free polymer molecules will form micelles in an aqueous solution, behaving similar to the polymer-coated nanoparticles, but without nanoparticle inside the polymer shell. To get visible polymer micelles the polymer was modified with fluorescein-amine (***24%Anhydride-75%C12COOH-1%Fluo as described in SI1.5***) to yield fluorescent-labeled micelles. First, 400 µl of fluorescein-polymer stock solution (40 mM monomer units in chloroform) were transferred to a round flask and the solvent was slowly evaporated by the rotavapor system. After drying, the solid film formed by the fluorescein-polymer powder was quickly dissolved in 2 ml of 0.1 M NaOH. The solution was further diluted to 15 ml by the addition of water, filtered through a 0.22-µm syringe filter, and finally gave a yellow transparent solution. The solution was washed by two rounds of ultrafiltration on Amicon centrifuge filters (100kD MWCO) and the polymer micelles in the retentate were collected[9]. Instead of the polymer micelle concentration which could not be directly determined by UV/Vis measurements, the fluorescein concentration of the dye-modified polymer was calculated from the absorption peak of fluorescein (extinction coefficient 72,000 $M^{-1}cm^{-1}$ at 495 nm). With 1% fluorescein modification, the micelle concentration was estimated to be on the order of one µM. The surface chemistry of fluorescein-polymer micelles was also characterized by the discrete PEGylation test[6] (***Figure S7***), however the stoichiometry compared with the series of polymer-coated AuNP might be different due to the uncertainty of the micelle concentration.

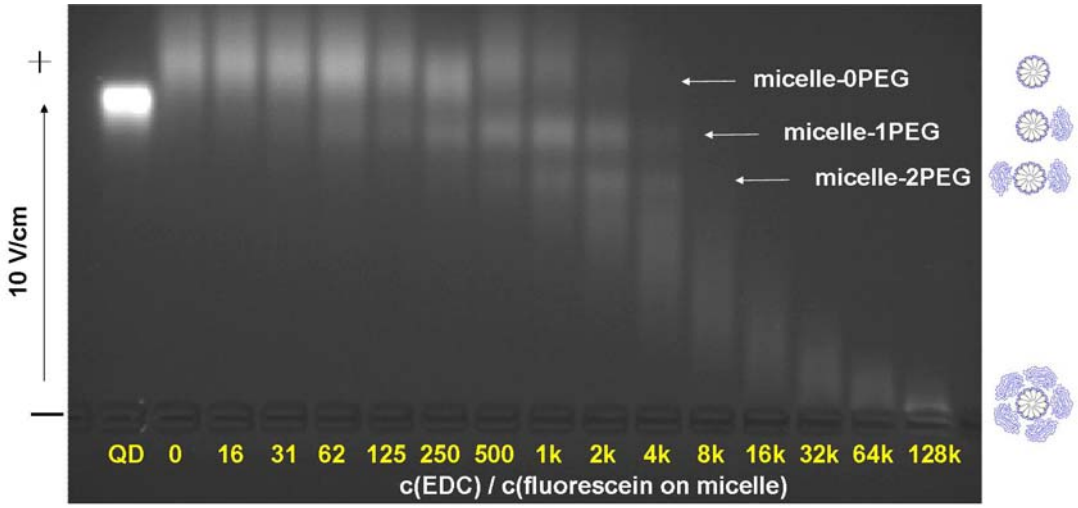


Figure S7. Discrete PEGylation test of fluorescein-modified polymer micelles. First, 150 µl of 3 mM methoxy-poly(ethylene glycol)amine molecules (CH_3O-PEG-NH_2, Mw~5000 g/mol, Rapp Polymere) in SBB were mixed with an equal amount of fluorescein-polymer micelles, i.e., 6 µM of fluorescein concentration. Then, 20 µl of each fraction were sequentially activated with 10 µl of EDC of different concentrations, such thatc(EDC)/c(fluorescein) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, all samples were run by gel electrophoresis. (10 V/cm, 1 hour). The more PEG molecules were attached per polymer micelle, the more retarded the samples were on the gel. The lanes in the gel correspond to samples with increasing EDC concentration from left to right. The bands of the polymer micelles are running faster and are not as sharp as those from polymer-coated quantum dots (first lane), indicating a smaller size but broader size distribution.

[9] Interestingly, the permeate was also colored, presumably part of the fluorescein-modified polymer formed small micelles that could pass the 100 kDa MWCO membrane.

In the same way, also micelles of plain polymer without fluorescein modification were prepared. The absorption and emission spectra of polymer micelles with and without fluorescein modification as well as fluorescein-amine were compared as shown in ***Figure S8***. For the fluorescence spectra, all samples were excited at 455 nm. There was a significant difference in the fluorescence intensity between fluorescein-polymer micelles and free fluorescein-amine. The fluorescence of free fluorescein-amine was quenched in aqueous solution (50 mM SBB pH 9) but was highly fluorescent when conjugated to the polymer[10].

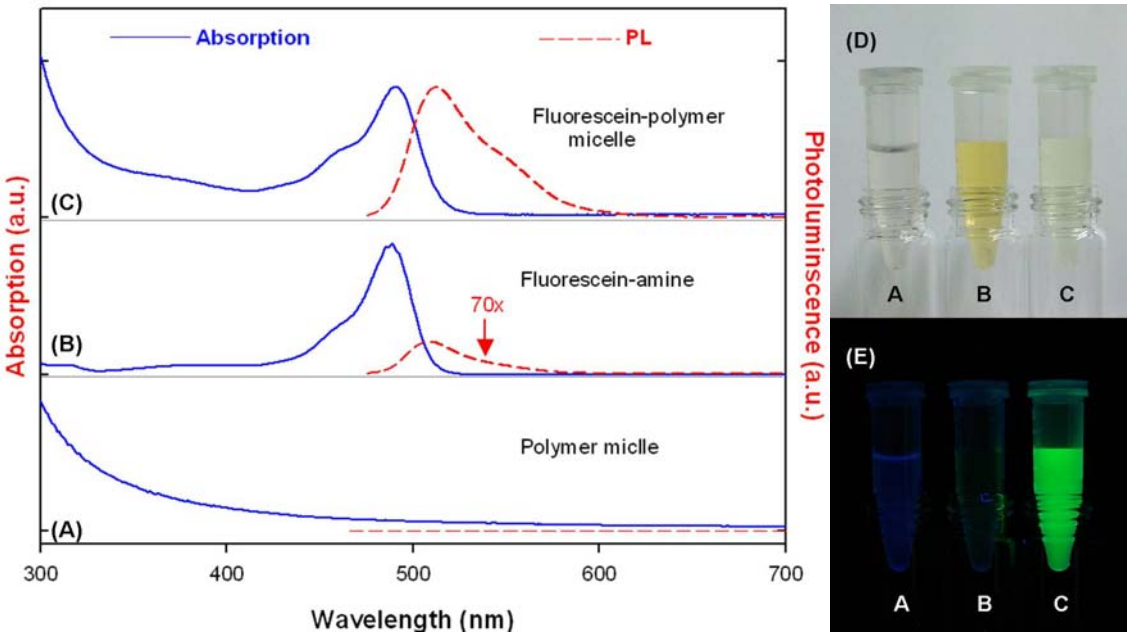


Figure S8. Absorption and photoluminescence (PL) of fluorescein-polymer micelles. First, 1% fluorescein-modified polymer micelles were prepared without inorganic nanoparticles inside and were collected in an (100 kDa MWCO) Amicon centrifuge. (A) plain polymer micelles (Mw > 100 kDa) in SBB (50 mM, pH 9) solution; (B) fluorescein-amine in SBB solution; (C) fluorescein-polymer micelles in SBB solution; white-light image (D) and UV lamp-excited image (E) of the three samples.

[10] In this experiment, the fluorescein concentrations of the different samples were not adjusted to equal values.

SI3.6 Direct PEG modification of the amphiphilic polymer

The amphiphilic polymer described in the previous section consists of a hydrophilic backbone and hydrophobic side-chains. We have demonstrated the possibility to use this amphiphilic polymer to transfer hydrophobic nanoparticles, e.g. quantum dots, iron oxide and gold nanoparticles, into an aqueous solution. The colloidal nanoparticles of different core materials were stable in buffer solution for months, had the same surface chemistry and could be modified with PEG in a post-modification by means of EDC. Alternatively, the PEG can also be attached directly to the polymer in organic solution, before the nanoparticle coating.

Solutions of methoxy-PEG5k-amine (mPEG5k-amine, Rapp Polymere) in anhydrous chloroform were prepared with a concentration of 8, 4, 2, and 1 mM. Then, 2 ml of each mPEG5k-amine solution were dropped into 1 ml of polymer (0.8 M monomer concentration, ***25%Anhydride-75%C12COOH as described in SI1.3***) under vigorous stirring, giving 0.25 - 2 % PEG5k-modified polymer[11]. After stirring overnight at room temperature, the resulting solutions were then evaporated by the rotavapor system and the solid films of dried particle powder were re-dissolved in anhydrous chloroform with a final concentration of 40 mM of monomer units.

Then, 4-nm gold nanoparticles in chloroform were coated with 0.25-2 % mPEG5k-modified polymer by the standard protocol described before and analyzed by gel electrophoresis (10 V/cm, 1 hour), which showed the direct attachment of PEG to the polymer similar to particles that had undergone a post-modification after the polymer-coating and phase transfer to aqueous solution.

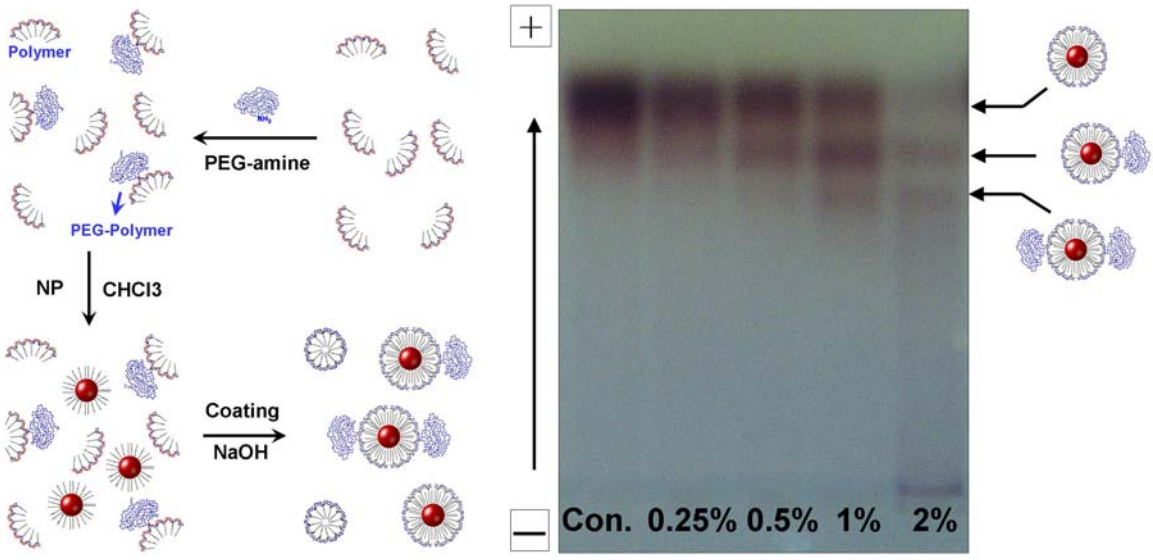


Figure S9. Different mPEG5k-polymer coatings of gold nanoparticles. The polymer was directly modified in organic solution with a different percentage of methoxy-PEG5k-amine, prior to the polymer-coating of gold nanoparticles. In gel electrophoresis, discrete bands from nanoparticles with exactly 0, 1 or 2 covalently attached PEG molecules per particle are observed.

[11] I.e. 0.25 - 2% of the anhydride rings of the polymer were used to attach PEG.

SI4: Characterization of polymer-coated particles with different functionalities

In the following section we select three functional molecules, fluorescein-amine, galactose-amine, and biotin-amine, to demonstrate the possibility of a direct modification of the polymer in order to add functionality before the actual coating process.

SI4.1 Gold nanoparticles coated with sugar-modified polymer

The preparation of 4% galactose-grafted co-polymer has been described in section 1. For the polymer coating of 6-nm gold nanoparticles the inorganic core diameter (d) was determined to be 6 nm (from TEM) and the effective diameter (d_{eff}) was then assumed to be 8 nm. In the sample of gold nanoparticles (500 µl, 1.5 µM) the total surface area (A) of the nanoparticles was 9.08×10^{16} nm^2 calculated using *formula (2)*,($R_{P/Area} = 100$ nm^{-2}), meaning that 15.1 µM monomer units of polymer had to be added to the nanocrystal solution. Then, 377 µl of 4% galactose-grafted co-polymer stock solution (40 mM monomer concentration, ***21%Anhydride-75%C12COOH -4%Galactose as described in SI1.6***) and 2.13 ml of anhydrous chloroform were added to the colloidal gold solution giving a total volume of 3 ml. After stirring for 15 minutes, the solvent was slowly evaporated by the rotavapor system until the sample was completely dry. Then, the solid powder of gold nanoparticles with galactose-copolymer shell was quickly dissolved in 2 ml of 0.1M NaOH. The solution was further diluted to 15 ml by the addition of water, filtered through a 0.22-µm syringe filter, and finally gave a transparent solution. The buffer was changed to PBS by two rounds of ultrafiltration with Amicon centrifuge filters (100 kDa MWCO) and the residual empty polymer micelles were further removed by a size exclusion column (Sephacryl S-300, Pharmacia) and again purified by agarose gel electrophoresis. The final concentration of purified gold nanoparticles was adjusted by concentrating the sample with ultrafiltration.

The surface density of galactose on the gold nanoparticles could be controlled by varying the percentage of galactose-modification of the polymer. To examine whether the galactose moieties on the particles were accessible to bind large proteins; the particles were exposed to lectin from *Ricinus communis* (castor bean) Agglutinin (RCA_{120}). RCA_{120} lectin is a 120 kDa lectin biomolecule with two identical and independent galactose-binding sites[7]. When appropriate amounts of galactose-copolymer-coated nanoparticles are mixed with RCA_{120} lectin, agglutination is expected to occur. Such agglutination should also be rapidly reversed when free galactose is added in excess. For the experiment, 10 µl of galactose-coated gold nanoparticles (1 µM in PBS buffer) were mixed with the same volume of RCA_{120} (Sigma L7886), giving a final concentration of 0.25, 0.5, and 1 mg/ml lectin solution. As negative control, plain polymer-coated gold nanoparticles (without galactose) were also treated with 1 mg/ml lectin in PBS buffer but were not found to aggregate.

The results are shown in ***Figure S10***, where the lectin was found to lead to agglutination of the galactose-coated nanoparticles within a few minutes. The particles precipitated completely after 30 minutes of static condition. The cross-linked network scattered light, and the increasing agglutination could be detected by increased absorbance at high wavelengths (in the red / near-IR)[12]. Our data demonstrate that the spectra of the aggregated, galactose-modified particles recovered to the initial values after adding galactose solution (Sigma-Aldrich G0625, 600 mM in PBS), but not in the case

[12] *Note:* all samples (including the precipitated samples) were re-dispersed and diluted to a final volume of 500 µl with PBS buffer before measuring the absorbance.

when excess glucose solution (Sigma-Aldrich G5767, 600 mM in PBS) was added. This demonstrates that the galactose molecules can disassemble the cross-linked network formed by gold nanoparticles and lectin (***Figure S11***).

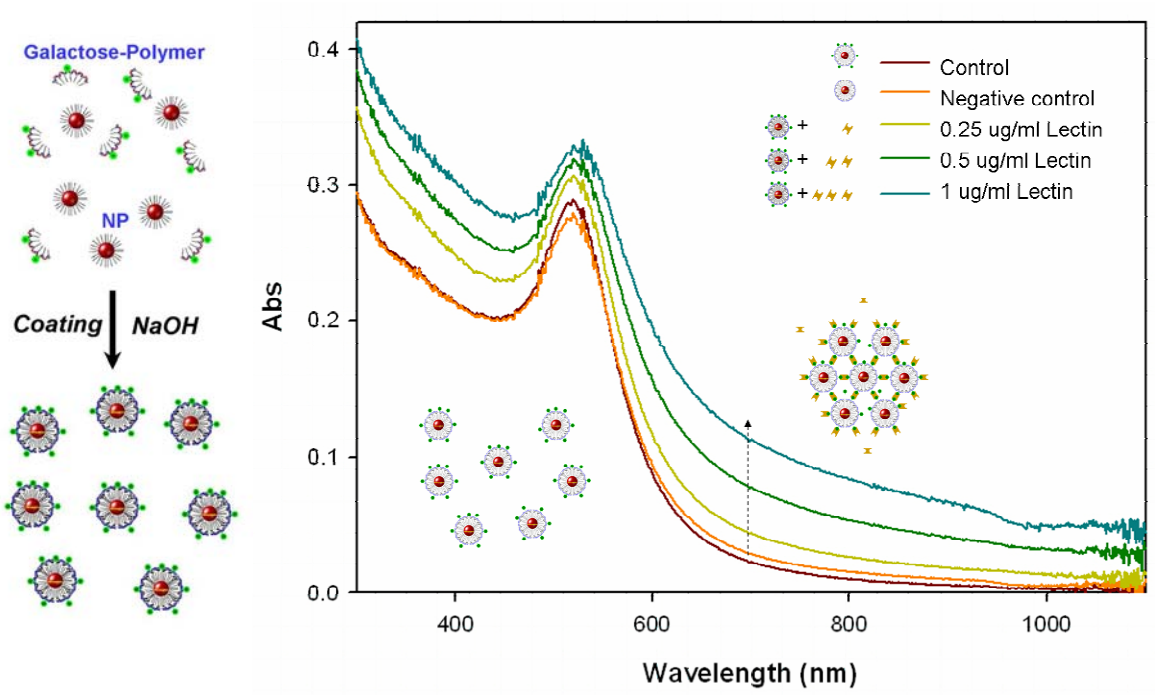


Figure S10. Agglutination effect of galactosylated gold nanoparticles. First, 10 µl of galactose-modified gold nanoparticles (1 µM in PBS) were mixed with equal volumes of RCA_{120} lectin solution with a final concentration of 0.25, 0.5, and 1 mg/ml of RCA_{120} lectin. A negative control of polymer-coated particles without galactose was also treated with 1 mg/ml lectin to examine non-specific binding. All samples were diluted to a final volume of 500 µl before measuring the absorbance. The cross-linked networks of particles scattered light, and the agglutination could be detected by the increased absorbance in the red/near-infrared spectrum. The more lectin was added, the more the galactosylated gold nanoparticles were agglutinated.

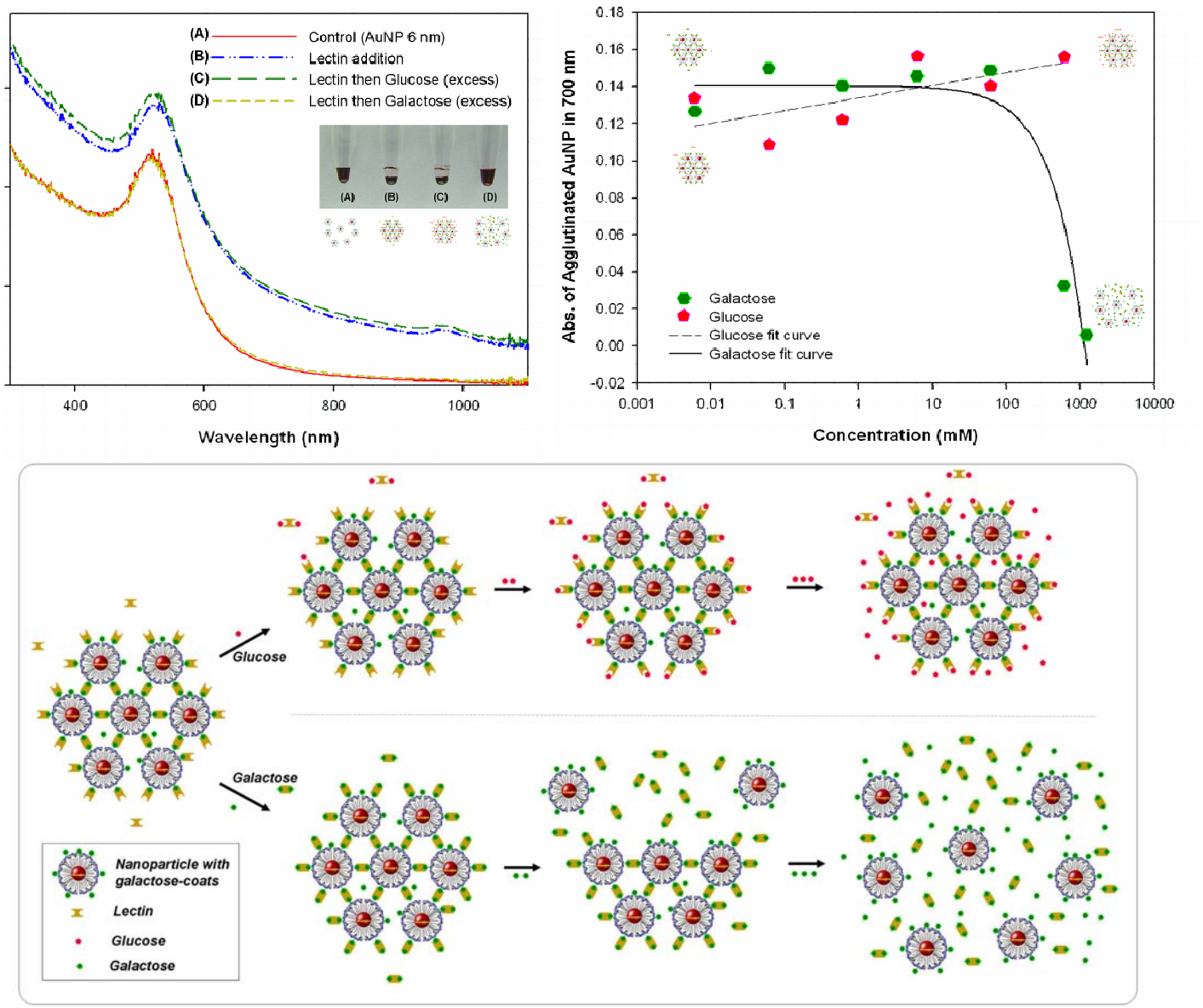


Figure S11. Sugar effects on agglutinated gold nanoparticles (AuNP). ***(Upper-Left figure)*** The agglutinated gold nanoparticles resulting from the lectin cross-linked complex could be selectively disassembled by the addition of excess galactose, but not with excess glucose (the inset picture). The effect could be seen in the absorption spectra of the diluted samples with (A) the galactose-modified AuNP (1 μM); (B) after the addition of RCA_{120} lectin (2 μg/ml) to the AuNP with galactose; (C) after the addition of glucose (600 mM) to sample B; and (D) after the addition of galactose (600 mM) to sample B. The lectin-induced agglutination of gold nanoparticles was fully reversible after adding excess galactose (downward shift). The ***upper-right figure*** shows the optical density of agglutinated gold nanoparticles after the addition of various amounts of sugar solution (with reference to the optical density of sample A at 700 nm). Agglutinated samples were tested with galactose (green) and glucose (red), the lines are curves fitted to the data points. It was found that the agglutinated gold nanoparticles start to disassemble above a concentration of 100 mM galactose, as derived from the decreased optical absorption at 700 nm. This effect is illustrated in the *lower figure* which shows the difference before and after the addition of galactose and glucose to the lectin-agglutinated nanoparticles.

SI4.2 Gold nanoparticles coated with biotin-modified polymer

The preparation of biotin-modified polymer has been described in section 1. To prepare biotin-polymer-coated gold nanoparticles, the inorganic core diameter (d) of gold nanoparticles was determined to be 6 nm (from TEM) and the effective diameter (d_{eff}) was then assumed to be 8 nm. In the sample of gold nanoparticles (500 µl, 1.5 µM) the total surface area (A) of colloidal gold nanoparticles was 9.08×10^{16} nm^2 calculated using *formula (2)*,($R_{P/Area}$ = 100 nm^{-2}). Therefore, 15.1 µmol monomer units of polymer were added to the nanocrystal solution. For this, 377 µl of biotin-modified polymer stock solution (40 mM monomer concentration, ***21%Anhydride-75%C12COOH-4%Biotin as described in SI1.7***) and 2.13 ml of anhydrous chloroform were added to the colloidal gold solution, giving a total volume of 3 ml. After stirring for 15 min, the solvent was slowly evaporated by the rotavapor system (200 mbar until all solvent was evaporated, then the pressure was set to 20 mbar for 10 minutes until the sample was completely dry). After all solvent had been removed by evaporation, the solid complex of the powder of gold nanoparticles with the biotin-modified polymer was quickly dissolved in 2 ml of 0.1 M NaOH. The solution was further diluted to 15 ml by the addition of water, filtered through a 0.22-µm syringe filter to give finally a transparent solution. The buffer was changed to SBB by two rounds of ultrafiltration on Amicon centrifuge filters (100 kDa MWCO) and residual polymer micelles were further removed by a size exclusion column (Sephacryl S-300, Pharmacia) or optionally purified by gel electrophoresis. The final concentration of purified gold nanoparticles coated with 4%- biotin-polymer could be adjusted by concentration with ultrafiltration.

To examine whether the biotin-groups on the particles were accessible to bind avidin molecules, the particles were exposed to 2 types of biotin-binding proteins: avidin and streptavidin. Avidin is a 66 kDa glycoprotein from egg white with four identical and independent biotin binding sites[7]. Streptavidin is a derivative of avidin produced by the bacterium *Streptomyces avidinii* and has like avidin four binding sites for biotin. Both have been extensively used in biotechnology, such as immunochemical systems, conjugation to antibodies, enzymes or fluorochromes. However, avidin (pI~10.5) has higher isoelectric point than streptavidin (pI~5-6). When streptavidin (or avidin) is added in excess to biotinylated gold nanoparticles, the protein is expected to bind to the nanoparticles and thus to increase the diameter of nanoparticles by the protein layer.

For the experiment, aliquots of 10 µl of streptavidin (Sigma S4762) or avidin (Sigma A9275) with different concentrations were mixed each with 10 µl (1 µM) of 4 % biotinylated gold nanoparticles solution and incubated for 30 min at room temperature. The electrophoretic mobility of each sample was analyzed through gel electrophoresis (10 V/cm, 1 hour) as shown in ***Figure S12***.

Non-biotinylated gold nanoparticles without ("control") and with streptavidin added ("negative") were tested for their non-specific binding. Both samples had the same mobility on the gel, indicating that no streptavidin is bound to the particles without biotin (under the here-used conditions).

The samples coated with biotin-modified polymer (without streptavidin added, lane "0") were slightly retarded on the gel when compared with the plain polymer-coated nanoparticles without biotin. This indicates a slight increase in particle size due to the additional biotin molecules attached to a short PEG spacer (MW 720 g/mol).

When the streptavidin concentration of the solution added to biotin-modified particles was increased, the nanoparticles were more and more retarded and discrete bands became visible, indicating the binding of streptavidin molecules to the particles. At high concentrations, a molar excess of streptavidin was added to the particles, so every particle could bind several streptavidin molecules. Their number could not be resolved anymore as discrete bands on the gel. At low streptavidin concentration, more nanoparticles than streptavidin were in the reaction mixture, but the nanoparticles

did not precipitate, indicating the absence of large cross-linked nanoparticle-streptavidin networks.

In case of avidin, the particles were much more retarded on the gel, even the control sample of plain polymer-coated gold nanoparticles (data not shown). This is probably due to the high isoelectric point of avidin. The electrostatic attraction between the gold nanoparticles (negative charge) and avidin (positive charge) in the SBB solution might have been responsible for the observed non-specific binding (data not shown).

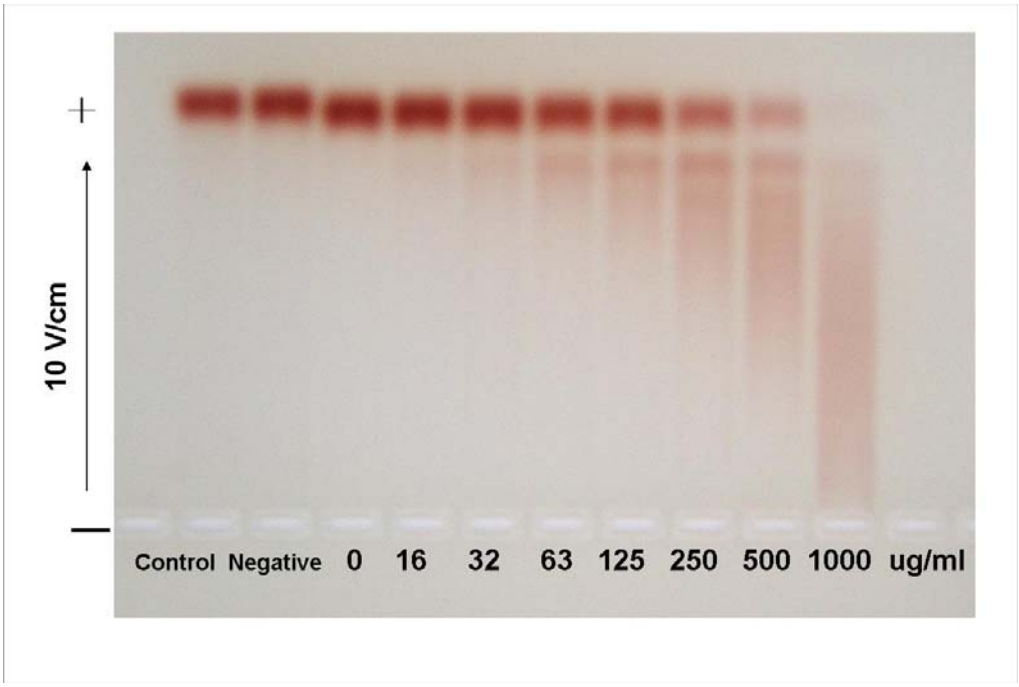


Figure S12 4% biotinylated gold nanoparticles (AuNP). First, 10 µl of each fraction from 4 % biotinylated gold nanoparticles (1 µM) were sequentially mixed using different concentrations of streptavidin solution, i.e. the resulting concentrations of streptavidin were equal to 0, 16, 31, 63, 125, 250, 500, and 1000 µg/ml. Non-biotinylated gold nanoparticles (as control) and their mixture with streptavidin (as negative control) were also tested for their non-specific binding. After 2 hours of reaction, all fractions were run through gel electrophoresis. (10 V/cm, 1 hour) The more streptavidin molecules were added, the more the AuNP-streptavidin complexes were retarded on the gel. The lanes in the gel correspond to samples with decreasing streptavidin concentrations from right to left. Discrete bands appeared for 63 µg/ml $<$ c(streptavidin) $<$ 500 µg/ml, which indicates the binding of streptavidin to the gold nanoparticles coated with biotin-modified polymer. There was no non-specific binding between plain gold nanoparticles and streptavidin observed. The slight shift between particles coated with the plain polymer (without biotin) and the biotin-modified polymer is due to the increase in size with biotin-PEG molecules used for the modification of the polymer.

SI4.3 Gold nanoparticles coated with fluorescein-modified polymer

The preparation of fluorescein-modified polymer has been described in Section 1. Of a sample of Au NP, the inorganic core diameter (d) of gold nanoparticles was 6 nm (determined with TEM) and the effective diameter (d_{eff}) was assumed to be 8 nm. In the aliquot of gold nanoparticles (0.5 ml, 1.5 µM) the total surface area (A) of colloidal gold nanoparticles was 9.08×10^{16} nm^2 calculated using *formula (2)*,($R_{P/Area}$ = 100 nm^{-2}), meaning that 15.1 µM monomer units of polymer were required for the polymer coating. For that, 377 µl of fluorescein-grafted co-polymer stock solution (40 mM monomer concentration, ***24%Anhydride-75%C12COOH-1%Fluo as described in SI1.5***) and 2.13 ml of anhydrous chloroform were added to the colloidal gold solution, giving a total volume of 3 ml. The concentration of gold nanoparticles in this solution, i.e. ~0.25 µM, was sufficiently low to avoid cross-linking effects among nanocrystals and the polymer could efficiently coat the gold nanoparticles, forming the polymer shell. After stirring for 15 minutes, the solvent was slowly evaporated with a rotavapor system. After all the solvent had been removed by evaporation, the completely dry solid of gold nanoparticles with fluorescein-modified polymer shell was quickly dissolved in 2 ml of 0.1 M NaOH. The solution was further diluted to 15 ml by the addition of water, filtered through a 0.22-µm syringe filter, and finally gave a transparent solution. The sample was washed with PBS buffer by two rounds of ultrafiltration on Amicon centrifuge filters (100 kDa MWCO) and residual polymer micelles were further removed by a size exclusion column (Sephacryl S-300, Pharmacia) and the particles could be optionally further purified by gel electrophoresis. The final concentration of purified gold nanoparticles coated with 1% fluorescein-polymer could be adjusted by ultrafiltration. For comparison plain polymer-coated nanoparticles (without fluorescein) were prepared and purified by the same procedures. The high fluorescence of the fluorescein-polymer was affected by quenching after coating the gold nanoparticles (***Figure S13***), which could be due to the spectral overlap between the gold plasmon absorption and the emission of the fluorescein. The extinction coefficient of gold nanoparticles (6 nm) was also two orders higher than that of fluorescein, resulting in a non-detectable fluorescein absorption for the sample of Au NP coated with the fluorescein-modified polymer.

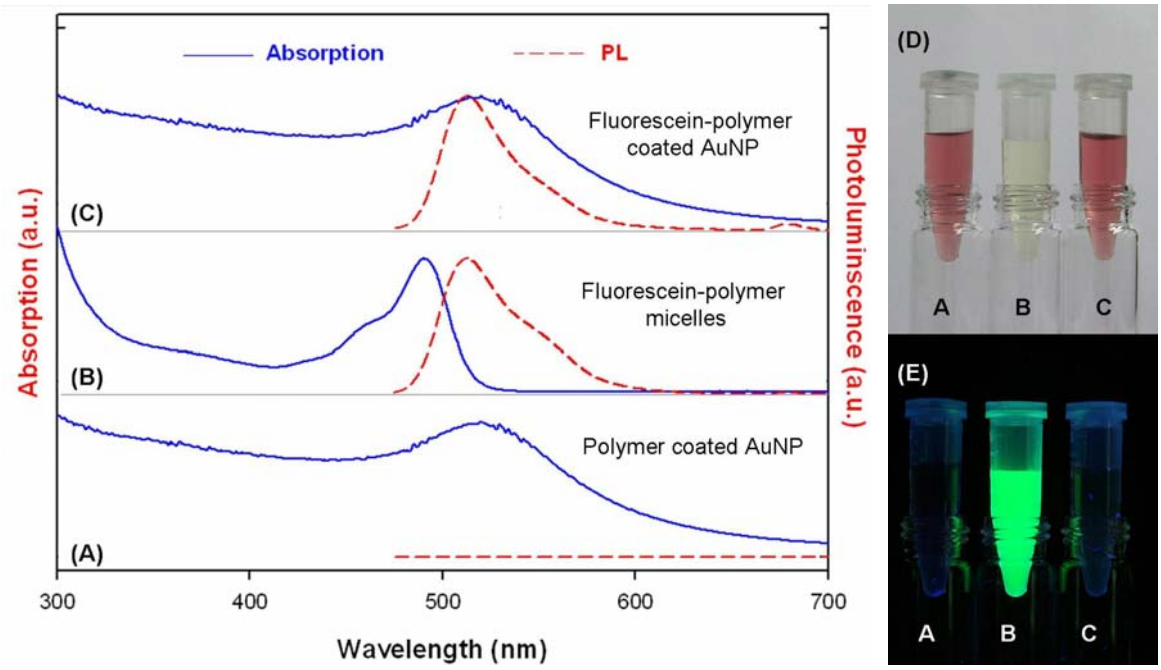


Figure S13. Absorption and photoluminescence (PL) of fluorescein-polymer gold nanoparticles (AuNP). Gold nanoparticles (6 nm core diameter) were coated with 1 % fluorescein-modified polymer, for comparison also empty micelles of the fluorescein-polymer and Au particles coated with the plain polymer (without fluorescein) were prepared. (The excitation wavelength for the PL measurements was 455 nm.) (A) The plain-polymer coated AuNP; (B) the fluorescein-modified polymer micelles; (C) the fluorescein-polymer coated AuNP. On the right: samples under white-light (D) and UV lamp excitation (E).

SI5: Protein-mediated Assembly of 1- or 2-Biotin Functionalized Nanoparticles

SI5.1 Description of nanoparticle assembly

After polymer coating and phase-transfer to aqueous solution, the originally hydrophobic nanoparticles are negatively charged. If there is a large number of (bio-) functional groups on the nanoparticles (e.g. biotin or galactose), the nanoparticles start to assemble when a binding partner (e.g. avidin or lectin, respectively) is added. Generally, the aggregation depends on the valency of both nanoparticles and biomolecules and their stoichiometry. Thus, it is hard to predict size and shape of each assembled nanostructure when the number of bio-functional groups on the particles is unknown.

Poly(ethylene glycol) is a biocompatible and linear polymer which can have functional groups at its two ends. For the polymer-coated nanoparticles described here, it has been demonstrated that by means of PEG molecules with a MW $\geq$ 5000 g/mol it is possible to separate particles with exactly no, one or two covalently bound PEG molecules per particle. In the case of bifunctional PEG this means particles with exactly no, one or two free (bio-) functional groups per particle. In ***Figure S14***, polymer-coated gold and CdSe/ZnS nanoparticles were reacted with PEG of different molecular weight, and discrete bands indicating the attachment of a discrete number of PEG molecules per particle can be resolved by gel electrophoresis with PEG of a molecular weight of 5000, 10000 or 20000 g/mol. In the following experiments, gold nanoparticles have been modified with biotin-PEG-amine (5000 g/mol), by binding the amine terminal covalently to the carboxylic groups of the polymer-coated particles by means of EDC chemistry. By the separation of the particle fractions with 0, 1 or 2 PEG molecules per particle, particles with exactly 0, 1 or 2 biotin groups were prepared.

One single (strept)avidin molecule offers four binding sites for biotin, allowing for a relatively simple model system to study the assembly of predictable nanostructure through the biotin-avidin systems.

SI5.2 Isolation of 1- or 2-biotin functionalized nanoparticles

The PEG modification (***Figure S14***) of polymer-coated gold particles (or quantum dots) was examined again with biotin-PEG5k-amine and by varying the amount of EDC in order to find the optimum stoichiometry to prepare particles with 0, 1, or 2 PEG molecules per particle. For that, 150 µl of 0.3 mM biotin-poly(ethylene glycol)amine molecules (biotin-PEG5k-amine, Mw~5000 g/mol, Rapp Polymere) in SBB were mixed with an equal volume of polymer-coated gold nanoparticles (AuNP, 6 µM, 4 nm core diameter). Here we used a smaller ratio of PEG per particle in order to reduce the needed amount of biotin-PEG5k-amine, i.e. PEG:AuNP = 50:1. Then, 20 µl of each fraction were sequentially activated by adding 10 µl of EDC solution of different concentrations, whereby in the final reaction mixtures the molar ratios were c(EDC)/c(AuNP) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, all aliquots were run by gel electrophoresis (10 V/cm, 1 hour). Usually discrete bands appeared clearly in the range of 500 < c(EDC)/c(AuNP) < 8000. Based on these data we selected the ratio of c(EDC)/c(AuNP) = 2000 as best condition for a the production of larger amounts of sample. For that, 2 ml of gold nanoparticle solution (6 µM) were mixed with 2 ml of biotin-PEG5k-amine solution (0.3 mM) in a 20-ml glass vial, followed by the addition of 2 ml of freshly prepared EDC solution. The reaction mixture was shaken overnight and then concentrated by ultrafiltration on Amicon centrifuge filters (100 kDa). The resulting samples were loaded into the wells of 2 % agarose gels, followed by gel eletrophoresis (10 V/cm, 1 - 2 hours). The discrete bands of 0-, 1-, 2-biotin gold nanoparticles (i.e. gold particles with exactly 0, 1, 2 biotin molecules per particle) were cut out from the gel, transferred into a dialysis membrane bag (3500 Da

MWCO, Roth), and run again with gel electrophoresis to elute the nanoparticles from the gel. The resulting nanoparticle solution was taken out, filtered with a 0.22 µm syringe filter and concentrated on a 100 kDa MWCO Amicon centrifuge filter. Then, the samples were purified by a second round of gel electrophoresis in order to remove residual particles of other fractions (Figure S15 (D)). The final nanoparticle samples were adjusted to a concentration of 1.5 µM for further experiments (***Figure S15***).

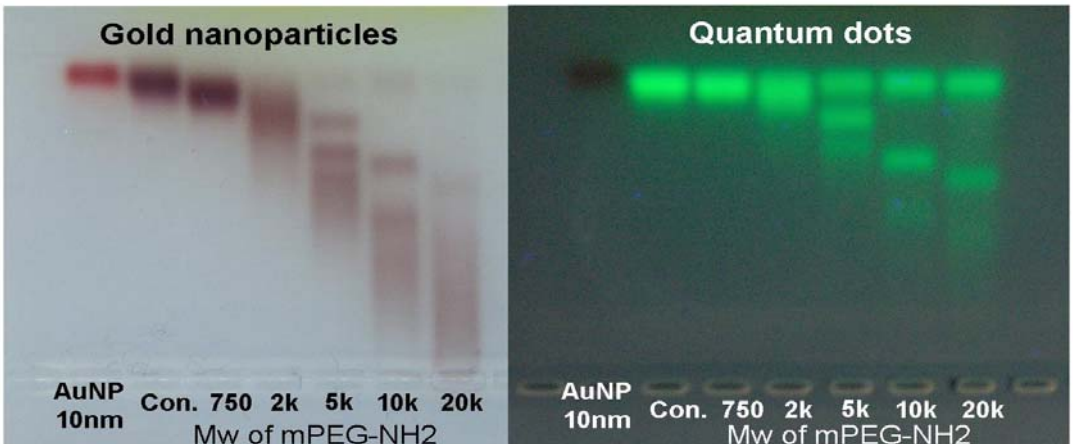


Figure S14. Polymer-coated Au and CdSe/ZnS nanoparticles conjugated with PEG molecules of different molecular weight. Each sample of 10 µl of 4-nm gold nanoparticles (left) or quantum dots (right) was individually mixed with the equal volume of methoxy-PEG-amine solution, i.e. 3 mM mPEG-amine with 0, 750, 2k, 5k, 10k, and 20 kDa in SBB solution. Then 10 µl of 6 mM EDC solution prepared freshly in SBB were added to each AuNP/PEG (or QD/PEG) aliquot and reacted for 2 hours. The resulting reaction mixtures were run on 1% agarose gel (Invitrogen #15510027) by gel electrophoresis (10 V/cm, 1 hour). As reference, 10 nm gold nanoparticles (BBInternational) stabilized with bis(*p*-sulphonatophenyl) phenylphosphine (Strem Chemicals) were run in the first lane of both gels. The discrete bands appeared only in case of PEG with molecular weight ≥ 5000 g/mol.

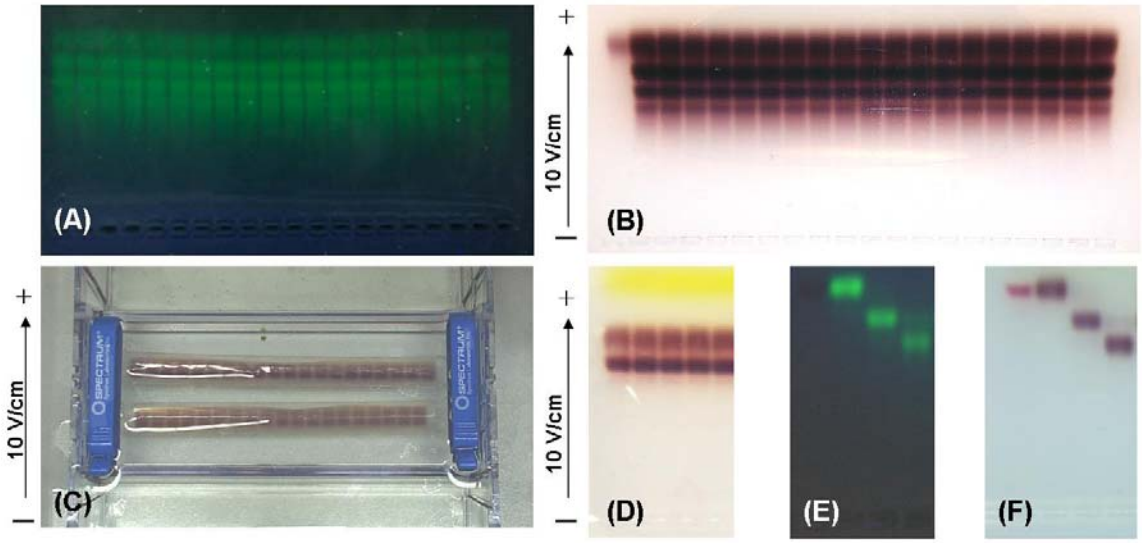


Figure S15. Isolation of 1- or 2-biotin functionalized nanoparticles. First, 2 ml of gold nanoparticles (Au) or quantum dots (QD) solution (6 µM) were mixed with 2 ml of biotin-PEG5k-amine solution (0.3 mM) in a 20-ml glass vial, followed by the addition of 2 ml of fresh EDC solution. The molar ratio was c(EDC)/c(NP) = 2000. The reaction mixture was shaken overnight and then concentrated by ultrafiltration (100 kDa). The resulting sample was loaded into the wells of a 2% agarose gel, followed by gel eletrophoresis (10 V/cm, 1 hour). The results are shown in (A) for the QDs and in (B) for Au. The discrete bands of 0-, 1-, 2-biotin Au were cut out separately, then transferred to a dialysis membrane bag (3500 Da MWCO, Roth) and run again with gel electrophoresis to elute the nanoparticles (C). The obtained nanoparticle solutions were purified using a syringe filter (0.22 µm), and were re-concentrated through 100 kDa MWCO Amicon centrifuge filters. Then the samples were purified in a second round of gel electrophoresis in order to remove residual particles of the other fractions (D). Finally, a part of the purified nanoparticles with 0-, 1-, 2-biotin molecules per particle was run again for control purpose on a 1 % agarose gel, giving a clear band for each fraction of the QDs (E) and Au particles (F).

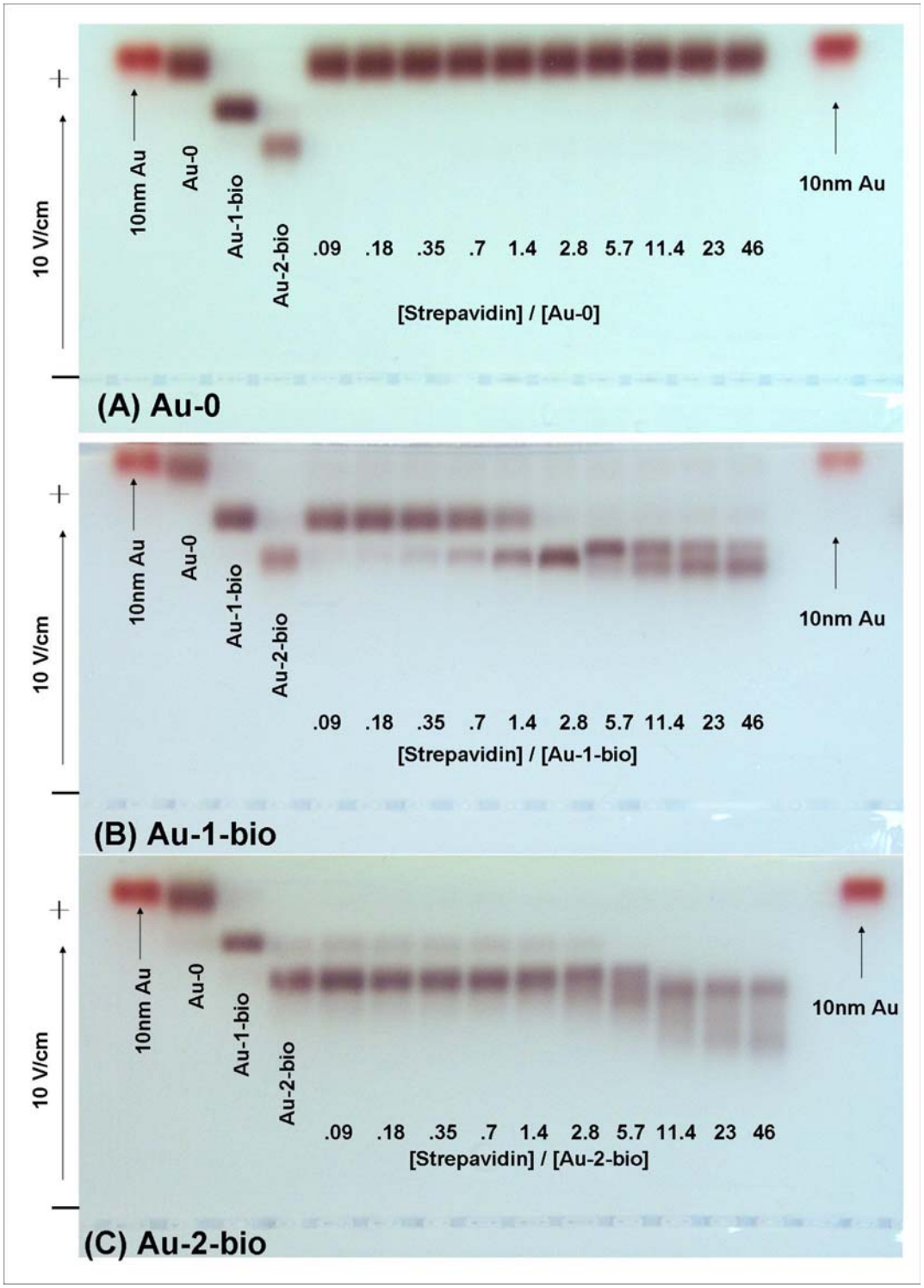


Figure S16. Assembly of 0-, 1- or 2-biotin gold nanoparticles (Au-x-bio) with streptavidin (SA). The lanes of each gel from left to right comprise four kinds of references (10-nm phosphine stabilized Au nanoparticles, Au-0, Au-1-bio, and Au-2-bio, i.e. gold particles with 0, 1, and 2 biotin molecules attached per particle), and SA-Au-x-bio conjugates with increasing molar ratios of c(streptavidin)/ c(Au-x-bio). For these conjugates the amount of c(Au-x-bio) =1.5 µM x 10 µl was kept constant, whereas the amount of streptavidin was varied. (A) gradient of c(SA)/ c(Au-0); (B) gradient of c(SA)/ c(Au-1-bio); and (C) gradient of c(SA)/ c(Au-2-bio). All samples were run through 1% agarose gel at 10 V/cm for 1 hour.

SI5.3 Assembly of 1- or 2-biotin nanoparticles

The purified gold nanoparticles (Au) with x-biotin (x = 0, 1, 2) were adjusted to a concentration of 1.5 μM by ultrafiltration. Then different proteins from the avidin family, such as streptavidin (Sigma S4762), avidin (Sigma A 9275), NeutrAvidin (Pierce #31000), streptavidin-FITC (Sigma S3762), neutravidin-FITC (Pierce #31006), were prepared with 4 mg/ml in double destilled water and stored at 4°C before use. Au-x-bio solutions were incubated at room temperature with a concentration series of the protein solutions and then analyzed by gel electrophoresis on 1% agarose gels. For the concentration series, each 10 μl of streptavidin solution were prepared by diluting the 4 mg/ml stock solution, i.e. with a concentration of 4×2^{-n} mg/ml (n = 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9) or the corresponding molar concentration of 66×2^{-n} μM (n = 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9). Each aliquot of streptavidin solution was mixed with an equal volume of Au-x-bio solution (x = 0, 1, or 2), the resulting molar ratios are shown in the figures. After overnight reaction, each sample of Au-streptavidin mixture was loaded into the wells of 1% gels and run by gel electrophoresis. (***Figure S16***). Then, 10-nm gold nanoparticles (BBInternational) stabilized with bis(*p*-sulphonatophenyl) phenylphosphine (Strem Chemicals) in the first lane were run as reference. The following three lanes were nanoparticles without proteins, i.e. Au-0, Au-1-bio, Au-2-bio. The Au-0-bio sample was first tested to see the effect of non-specific binding between streptavidin and polymer-coated gold nanoparticles (***Figure S16A***). If there were proteins attached to the nanoparticles, their increased size would retard the particles on the gel. Clearly, streptavidin showed a negligible non-specific binding from the gel results under the here used conditions. However, Au-1-bio particles with streptavidin resulted in one retarded band. When the streptavidin concentration was further increased, this band seemed to split into two bands.

In the same way, the interaction of the biotinylated AuNP was also studied with avidin and neutravidin, resulting in similar retarded bands on the gel. However, gold nanoparticles showed now a serious retardation when avidin was added at high concentration to Au-2-bio as well as for Au-1-bio and Au-0-bio, indicating the formation of larger particle-protein networks that lead to aggregation (***Figure S17***). This indicates unspecific (electrostatic) interactions stronger for avidin than for the other analogues due its higher isoelectric point (10 – 10.5, streptavidin: ~ 5, neutravidin: ~ 6.3).

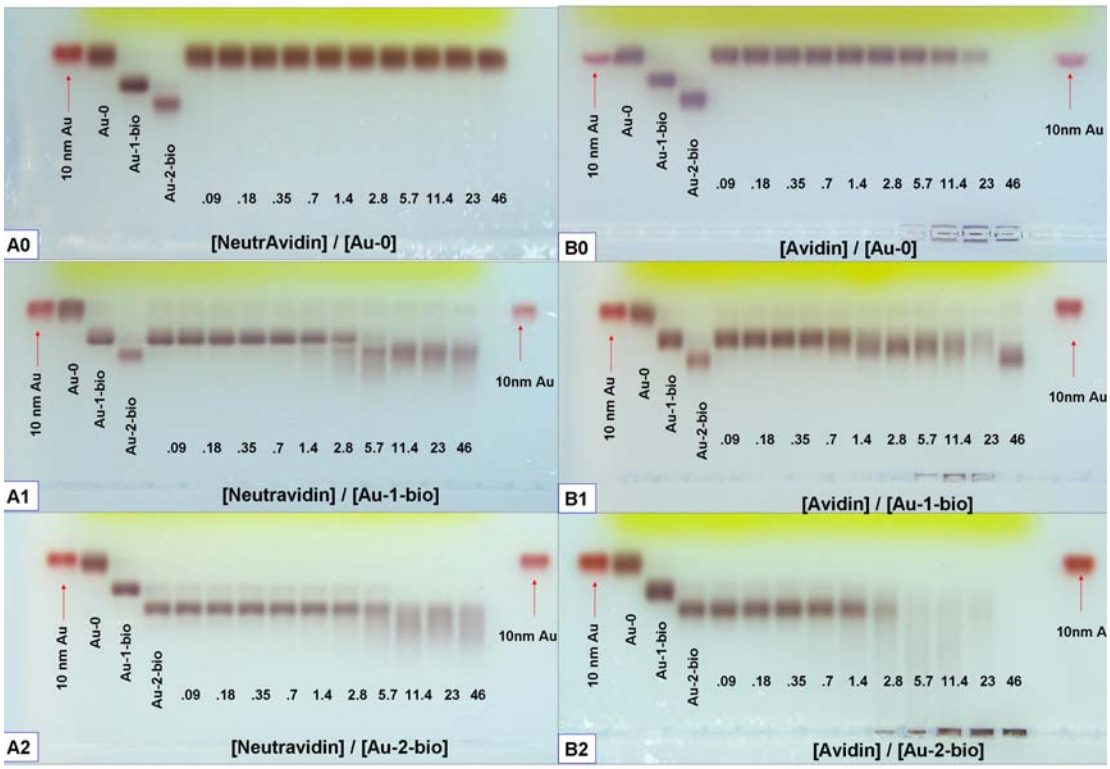


Figure S17. Assembly of 0-, 1- or 2-biotin gold nanoparticles (Au-x-bio) with neutravidin and avidin. The lanes of each gel from left to right comprise four kinds of references (10-nm Au nanoparticles, Au-0, Au-1-bio, and Au-2-bio), increasing molar ratios of c(Avidin or Neutravidin)/ c(Au-x-bio) with a constant amount of c(Au-x-bio) =1.5 µM x 10 µl, blank, and again 10-nm Au; (*Abbreviation: Neutravidin, NA; Avidin, AV*); (A0) gradient of c(NA)/ c(Au-0); (A1) gradient of c(NA)/ (Au-1-bio); (A2) gradient of c(NA)/ (Au-2-bio); (B0) gradient of c(AV)/ c(Au-0); (B1) gradient of c(AV)/ c(Au-1-bio); (B2) gradient of c(AV)/ (Au-2-bio). All samples were run through 1% agarose gels @10 V/cm for 1 hour (the yellow color is from Orange G that has been added to the gel-loading buffer).

In addition, the previous experiments were repeated with fluorescence-labeled streptavidin-FITC (Sigma S3762) and neutravidin-FITC (Pierce #31006). The fluorescence-labeled proteins allowed for localizing the protein on the gel. The experimental results were basically the same as those with the unlabeled streptavidin and neutravidin, but now the proteins could be found co-located with the gold particles, a clear indication for the formation of NP-protein structures. The visible and UV images were taken by a gel documentation system (BioRad GelDoc). The color images were taken by Pentax Optio MX4 digital camera. (***Figure S18, Figure S19***)

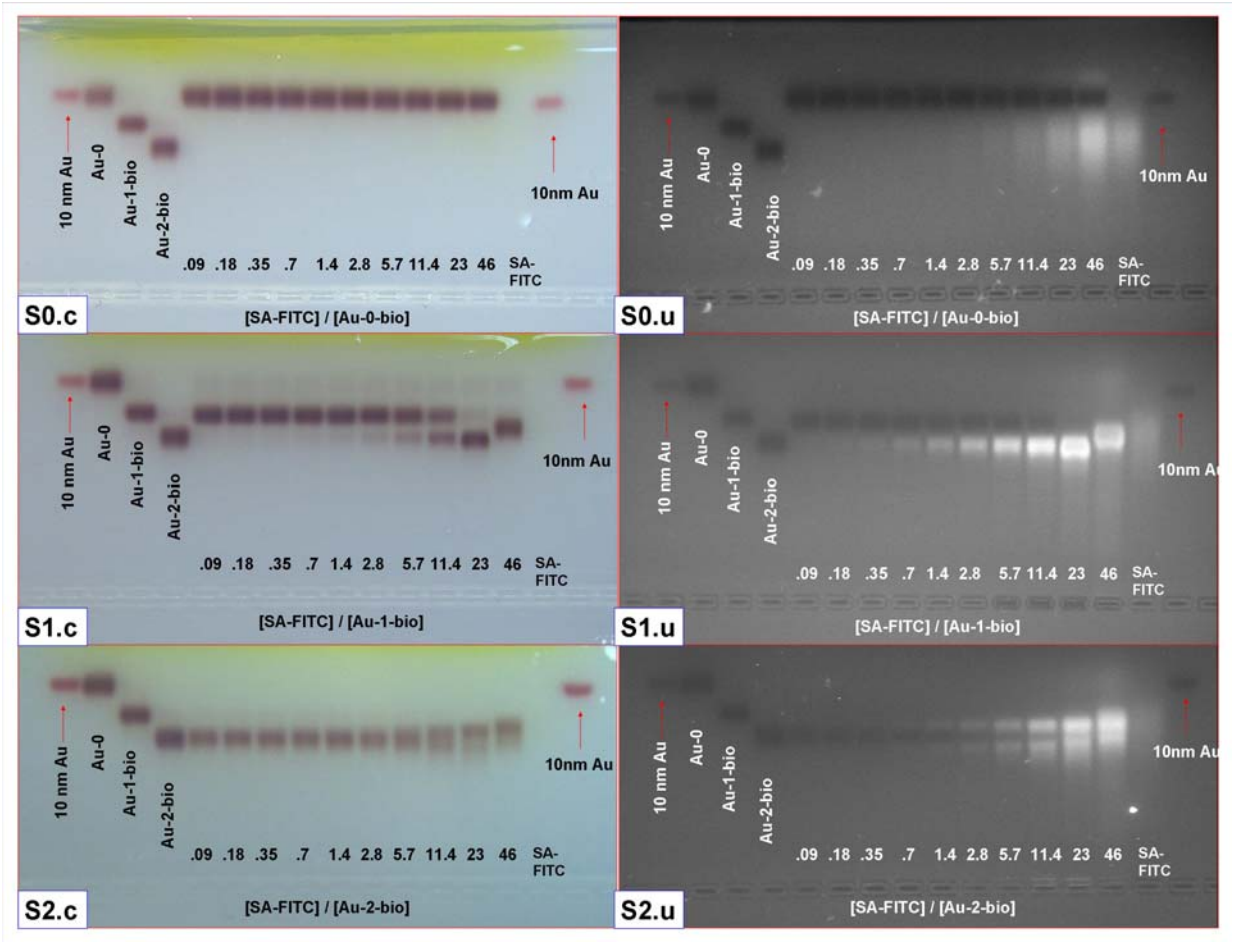


Figure S18. Assembly of 0-, 1- or 2-biotin gold nanoparticles (Au-x-bio) with streptavidin-FITC (SA-FITC). The lanes of each gel from left to right comprise four kinds of references (10 nm phosphine stabilized Au nanoparticles, Au-0, Au-1-bio, and Au-2-bio), increasing molar ratios of c(SA-FITC)/ c(Au-x-bio) with a constant amount of c(Au-x-bio) =1.5 µM x 10 µl, SA-FITC, and again 10-nm Au; in the left column is color picture of the gradient of c(SA-FITC)/ c(Au-0) *(S0.c)*, c(SA-FITC)/ c(Au-1-bio) *(S1.c)*, or c(SA-FITC)/ c(Au-2-bio) *(S2.c)*; and the right column shows UV light images of gradients of c(SA-FITC)/ c(Au-0) *(S0.u)*, c(SA-FITC)/ c(Au-1-bio) *(S1.u)*, and c(SA-FITC)/ c(Au-2-bio) *(S2.u)*; All samples were run through 1% agarose gel, 10 V/cm for 1 hour.

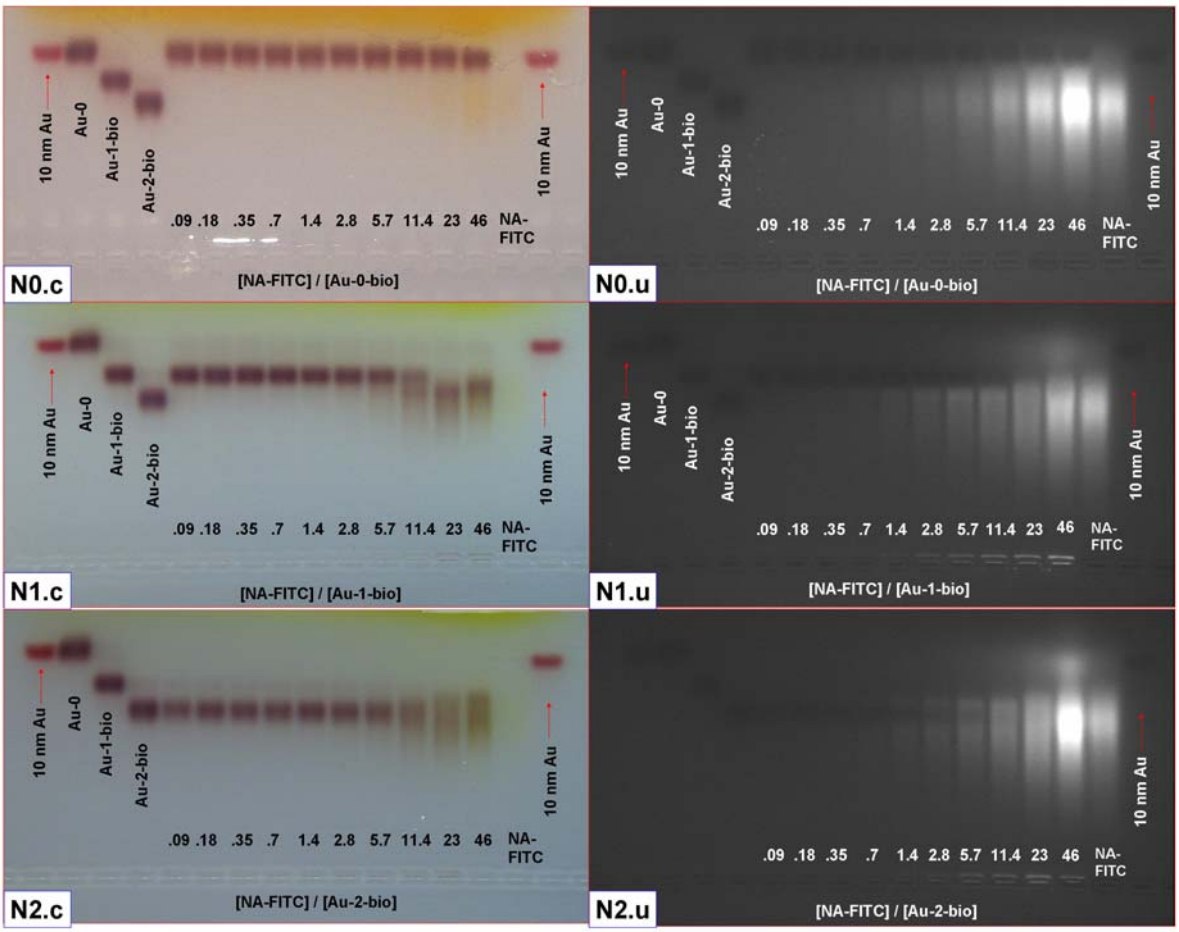


Figure S19. Assembly of 0-, 1- or 2-biotin gold nanoparticles (Au-x-bio) with neutrAvidin-FITC (NA-FITC). The lanes of each gel from left to right comprise four kinds of references (10-nm Au nanoparticles, Au-0, Au-1-bio, and Au-2-bio), increasing molar ratios of c(NA-FITC)/ c(Au-x-bio) with a constant amount of c(Au-x-bio) =1.5 µM x 10 µl, NA-FITC, and again 10 nm AuNP; The left columns show white light color pictures of gradients of c(NA-FITC)/ c(Au-0) *(N0.c)*, c(NA-FITC)/ c(Au-1-bio) *(N1.c)*, or c(NA-FITC)/ c(Au-2-bio) *(N2.c)*; and the right columns show UV light images of gradients of c(NA-FITC)/ c(Au-0) *(N0.u)*, c(NA-FITC)/ c(Au-1-bio) *(N1.u)*, and c(NA-FITC)/ c(Au-2-bio) *(N2.u)*; All samples were run through 1% agarose gels, 10 V/cm for 1 hour.

SI5.4 Assembly of 1- biotin quantum dots with streptavidin

Quantum dots (QD) with a defined number of biotin molecules, i.e. the QD-0, QD-1-bio and QD-2-bio, were prepared in the same way as gold nanoparticles (see details in SI5.2). Briefly, the discrete PEGylation test of polymer-coated quantum dots (previously prepared) was examined again with biotin-PEG5k-amine in order to find the optimal conditions for the attachment of single biotin-PEG molecules. For that, 150 µl of 0.3 mM biotin-poly(ethylene glycol)amine (biotin-PEG5k-amine, Mw 5000 g/mol, Rapp Polymere) in SBB were mixed with an equal volume of polymer-coated CdSe/ZnS QDs (6 µM, 3.75 nm core/shell diameter). Here a smaller molar ratio of PEG molecules per particle was used in order to use as little biotin-PEG5k-amine as possible, i.e. PEG:QD=50:1. Then each 20 µl of the mixture were sequentially activated by EDC of different concentration, i.e., c(EDC)/c(QD) = 0, 16, 31, 63, 125, 250, 500, 1k, 2k, 4k, 8k, 16k, 32k, 64k, and 128k. After 2 hours of reaction, all aliquots were run by gel electrophoresis (10 V/cm, 1 hour). Usually the discrete band appeared clearly in the range of 500 < c(EDC)/c(QD) < 8000. From this small-scale screening experiment we selected a molar

ratio of c(EDC)/c(QD) = 2000 as the condition for a scaled-up reaction. For this, 2 ml of QD solution (6 μM) were mixed with 2 ml of biotin-PEG5k-amine solution (0.3 mM) in a 20-ml glass vial, followed by the addition of 2 ml of freshly prepared EDC solution. The reaction mixture was shaken overnight and then concentrated by ultrafiltration (Amicon centrifuge filters, 100 kDa MWCO). The resulting sample was loaded into the wells of a 2% agarose gel, followed by gel electrophoresis (10 V/cm, 1 - 2 hours) as shown in ***Figure S15A***. The discrete bands of 0-, 1-, 2-biotin QD were then cut out separately and transferred to dialysis membrane bags (3500 Da MWCO, Roth) and run through gel electrophoresis again to elute the trapped nanoparticles. The resulting nanoparticle samples were filtered using a syringe filter (0.22 μm) and reconcentrated on a 100 kDa MWCO Amicon centrifuge filter. Then the samples were purified again by a second round of gel electrophoresis in order to remove residual neighbor fractions. The final nanoparticle samples were adjusted to a concentration of 1.5 μM by ultrafiltration for further experiments.

Aliquots of 10 μl of streptavidin solution with concentrations of 4 mg/ml were prepared by a dilution series, i.e. 4 x 2^{-n} mg/ml (n=0, 1, 2, 3, 4, 5, 6, 7, 8, and 9) or the corresponding molar concentrations of 66 x 2^{-n} μM (n=0, 1, 2, 3, 4, 5, 6, 7, 8, and 9). Each streptavidin solution was quickly mixed with an equal volume of QD-1-bio solution. After overnight reaction, each sample of QD-streptavidin mixture was loaded into the wells of 1% agarose gels and run through gel electrophoresis under an applied electrical field in order to resolve the nanoparticle-streptavidin groupings (***Figure S20***). Similarly, QD-1-bio also yields three different retarded bands when increasing the streptavidin concentration, as compared with the experiments on Au-1-bio.

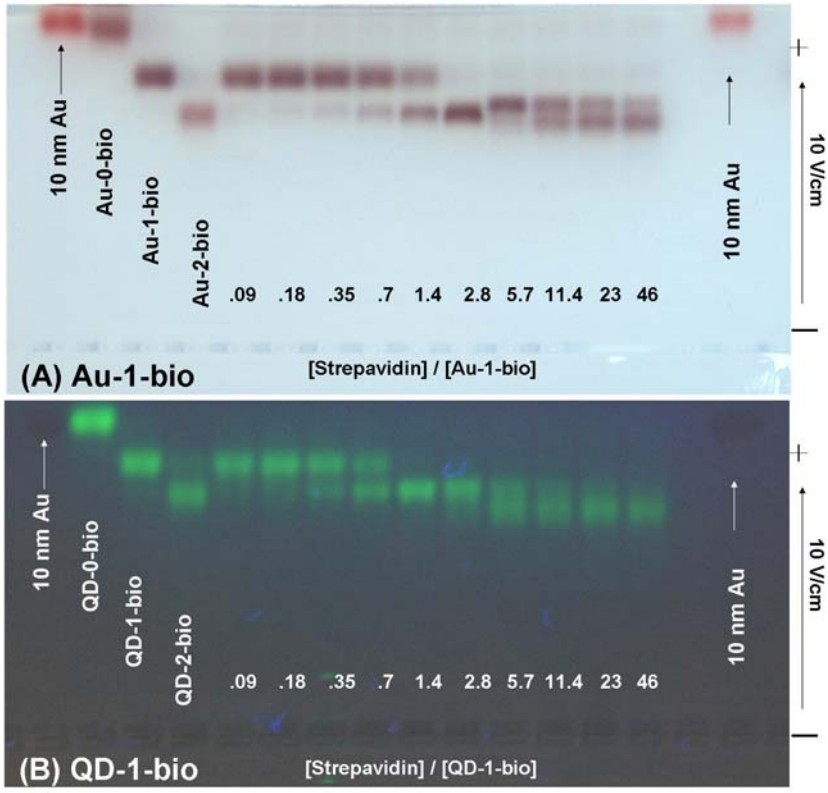


Figure S20. Assembly of 1-biotin nanoparticles with streptavidin (SA). The single biotin on gold nanoparticles (Au) or quantum dots (QD) that were assembled with different concentrations of streptavidin was tested. (A): the lanes of each gel from left to right consist four kinds of standards (10 nm Au nanoparticles, Au-0, Au-1-bio, and Au-2-bio), the increasing ratio of c(Streptavidin)/ c(Au-1-bio) with a constant amount of c(Au-1-bio) = 1.5 μM, blank, and again 10 nm Au; (B): the lanes of each gel from left to right comprise four kinds of references (10-nm Au nanoparticles, QD-0, QD-1-bio, and QD-2-bio), increasing ratios of c(Streptavidin)/ c(QD-1-bio) with a constant amount of c(QD-1-bio) =1.5 μM, blank, and again 10 nm phosphine stabilized Au.

SI6: References

[1] N. R. Jana, X. G. Peng, *Journal of the American Chemical Society* **2003**, *125*, 14280.

[2] W. W. Yu, L. Qu, W. Guo, X. Peng, *Chemistry of Materials* **2003**, *15*, 2854.

[3] B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, M. G. Bawendi, *Journal of Physical Chemistry B* **1997**, *101*, 9463.

[4] M. F. Casula, Y. W. Jun, D. J. Zaziski, E. M. Chan, A. Corrias, A. P. Alivisatos, *Journal of the American Chemical Society* **2006**, *128*, 1675.

[5] T. Pellegrino, L. Manna, S. Kudera, T. Liedl, D. Koktysh, A. L. Rogach, S. Keller, J. Rädler, G. Natile, W. J. Parak, *Nanoletters* **2004**, *4*, 703.

[6] R. A. Sperling, T. Pellegrino, J. K. Li, W. H. Chang, W. J. Parak, *Advanced Functional Materials* **2006**, *16*, 943.

[7] J. P. Van Wauwe, F. G. Loontiens, C. K. De Bruyne, *Biochim Biophys Acta* **1973**, *313*, 99.