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Assembly of citrate gold nanoparticles on hydrophilic monolayers

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Graphical Abstract

Highlights

The self-assembled layers were all hydrophilic with Lipa-pTHMMAA exhibiting close to full wetting. AFM reveals that the polyacrylamide layers smoothens the gold surface to a higher extent than the polyethylene glycol and lipoic acid terminated with an amino group.

SPR resonance curves were observed to shift to higher angles and become increasingly damped, while also the peaks strongly broaden when large nanoparticles assembled on the surface.

Topographical images confirmed that the highest number of particles were assembled on the polyethylene glycol monolayer.

By increasing the interaction time more particles could be assembled on the surface.

ABSTRACT

Self-assembled monolayers (SAMs) as model surfaces were linked onto planar gold films through lipoic acid or disulfide groups. The molecules used were polyethylene glycol (EG-S-S), *N*-[tris-(hydroxymethyl)methyl]acrylamide polymers with and without lipoic acid (Lipa-pTHMMAA and pTHMMAA) and a lipoic acid triazine derivative (Lipa-MF). All the layers, but Lipa-MF with a primary amino group were hydroxyl terminated. The layers were characterized by contact angle measurements and atomic force

microscopy, AFM. Citrate stabilized nanoparticles, AuNPs in water and phosphate buffer were allowed to assemble on the layers for 10 minutes and the binding was followed in real-time with surface plasmon resonance, SPR. The SPR resonance curves were observed to shift to higher angles and become increasingly damped, while also the peaks strongly broaden when large nanoparticles assembled on the surface. Both the angular shift and the damping of the curve was largest for nanoparticles assembling on the EG-S-S monolayer. High amounts of particles were also assembled on the pTHMMAA layer without the lipoic acid group, but the damping of the curve was considerably lower with a more even distribution of the particles. Topographical images confirmed that the highest number of particles were assembled on the polyethylene glycol monolayer. By increasing the interaction time more particles could be assembled on the surface.

Keywords

Hydrophilic monolayers, gold nanoparticles, surface plasmon resonance (SPR), atomic force microscopy (AFM), contact angle

1. Introduction

Gold nanoparticles (AuNPs) exhibit interesting optical and electronic properties that have found promising applications in the fields of sensors, catalysis, electronics, photonics, solar cells, cancer diagnosis and therapy, drug delivery, biomedical imaging, implants, and recently as antioxidants, and anti-coagulants [1-2,3,4,5,6,7,8,9,10]. Two ways exist for optical excitation of electrons in noble metals: through surface plasmons in thin layers of noble metals or through bulk plasmons in nanoparticles [11]. Nanoparticles of noble metals exhibit strong resonant scattering of light due to plasmonic oscillation of free electrons in the bulk metal. On the other hand, Surface Plasmon Resonance (SPR) occurs when monochromatic light is directed onto a planar gold surface, which causes a collective oscillation of surface electrons at the interface between a dielectric material and the gold surface. Combinations of nanoparticle and surface excitation of plasmons are obtained through the use of well-defined nanostructured metal layers on dielectric substrates, generally referred to as Plasmonics [12].

The SPR phenomenon is based on free electrons resonating at the metal surface, the surface plasmons, which are excited with visible or near infrared light. There are basically four parameters of the layer that can change when the nanoparticles adsorb to the surface: the thickness (d), the refractive index (n), the absorption coefficient (k) and the degree of scattering (S). The SPR curve will shift upwards along the angle axis when d and n increase, while the SPR phenomenon will become damped by increase of k with an increase in reflection coefficient. The fitting of SPR curves according to Fresnel theory is only valid when using isotropic layers absent of scattering effects and only concerns d , n and k . Scattering will give further broadening of the SPR spectra.

In nanoparticles, a strong absorption can be observed in the UV-Vis spectrum of a colloidal suspension. The absorbance spectra for Au nanoparticles have a maximum absorbance (λ_{\max}) at around 520 nm. This plasmon mode absorption band gives the colloidal gold its characteristic deep red-purple color. The location of the

absorbance peak is highly dependent upon the size and shape of the particles, but also their degree of aggregation. Controlled assemblies of AuNPs represent an area of growing interest. Nanoparticles can be assembled on the surface by physical and chemical assembly. Physical forces are involved in physical assemblies, which include spin coating, and various lithographic methods [13-14 15 16]. Often the physical methods combined with chemical ones utilizes coupling chemistries to direct and control the deposition of nanoparticles onto functionalized substrates [17-18 19,2021]. This allows tailoring of the nanostructures and formation of more complex nanostructured assemblies [22]. Most of the supported nanoparticle assemblies are based on small-sized (2-8 nm) nanoparticles [23,24], whereas less studies are found on large particles. Although many methods have been described to fabricate AuNP assemblies, simple and effective routes is still very much pursued when targeting commercial applications.

In this study, bi-functional *N*-[tris-(hydroxymethyl)methyl]acrylamide polymers (Lipa-pTHMMAA and pTHMMAA), polyethylene glycol (EG-S-S) and Lipa-metformin (Lipa-MF) were used for linking gold nanoparticles to solid surfaces with a thin sputtered layer of gold (Scheme 1). The molecules were self-assembled on the gold surface by covalent linkage through lipoic acid or disulfide groups. The pTHMMAA polymer without the lipoic acid group was used as references. This polymer was also used to coat polystyrene. The coupling chemistries often use amino groups for nanoparticle immobilization. We chose Lipa-MF that possesses an amino group and the other molecules with hydroxyl groups for assembling nanoparticles on the surface. Atomic force microscopy (AFM) and contact angle was used for characterization of the layers and SPR for measuring the interaction of the AuNPs in real-time to the self-assembled layers.

Lipa-pTHMMAA is nonionic, very hydrophilic, biocompatible, and inert to biological fouling [25]. We have previously used Lipa-pTHMMAA conjugates to increase the stability of antibodies and avidin on gold surfaces and suppress non-specific binding in immunoassays [26-27,282930]. Surface-bound poly(ethylene glycol) has been used for retarding the non-specific adsorption of proteins and other biological species [31,32]. During our immunoassay studies we had noted that uncoated AuNPs adhered to surfaces treated with Lipa-pTHMMAA. We wanted to investigate this further as we found no studies on the interaction of uncoated AuNPs with hydroxyl terminated layers.

2. Experimental

2.1 Chemicals and Synthesis

Chloroauric acid (HAuCl₄ aq) 99.999% grade was purchased from Aldrich. Biograde sodium citrate was from Sigma-Aldrich. 10 mM phosphate buffer (PB) pH 7.4 was used. Poly(ethylene glycol) with a disulphide linkage (P8735-EG-disulfide, Mw 6500 g/mol), hereafter named EG-S-S, was from Polymer Source Inc. Quebec, Canada (Scheme 1c). The polymers pTHMMAA and Lipa-pTHMMAA were prepared according to the method by Albers *et al.* (Mw in the range of 4500 and 6000 g/mol) [25,28]. For the preparation of the pTHMMAA polymer, 4,4'-azobis(cyanovaleric acid) from Sigma-Aldrich was used as the azo-initiator. α -Lipoic acid *N*-succinimidyl ester was prepared as described by Tappura *et al.* [33]. Metformin (1,1dimethylbiguanide) was

prepared from the hydrochloride salt by the method of Huttunen *et al.* [34]. The compound Lipa-MF was synthesized according to the following procedure: To a mixture of 1,1-dimethylbiguanide (1.15 g, 8.87 mmol) in acetonitrile (50 mL) was added a mixture of lipoic acid NHS ester (1.0 g, 3.3 mmol) in acetonitrile (50 mL). A white precipitate was formed and the mixture was stirred for 1 h. The reaction was judged completed by TLC analysis (eluent: ethyl acetate). The mixture was filtered and the clear yellow filtrate was concentrated by rotary evaporation to about 15 mL. The mixture was purified by chromatography (silica gel, eluent dichloromethane/methanol, 20/1, v/v). The fractions containing the pure product were combined and the solvent removed to give a yellow solid, 6-[4-(1,2-dithiolan-3-yl)butyl]-*N,N*-dimethyl-1,3,5-triazine-2,4-diamine, **Lipa-MF** (0.74 g, 75%). ^1H NMR (CDCl_3 , 300 MHz): δ 5.06 (2H, br, s, NH_2), 3.57 (1H, m, H-3), 3.23-3.01 (8H, m, H-1, H-11, H-12), 2.49 (2H, t, $J = 7.4$ Hz, H-7), 2.45 (1H, m, H-2'), 1.90 (1H, m, H-2''), 1.73 (4H, m, H-4, H-5), 1.51 (2H, m, H-6). ESI-MS: accurate mass, MH^+ 300.1324, calcd. 300.1317; MNa^+ 322.1122, calcd. 322.1136.

2.2 Preparation of gold nanoparticles

Gold nanoparticles were prepared according to the method by Frens [35]. Briefly, a 50 mL aqueous solution of HAuCl_4 (0.25 mM) was heated to boiling and trisodium citrate (138 mM) was added. The solution was allowed to boil for another 20 minutes, and cooled down during continuous stirring. The resultant gold colloids were stored in 4 °C. The pH of the AuNP solution was adjusted to a pH of 6.4. These particles are termed "AuNPs citrate". A set of AuNPs was centrifuged at 4°C at 4000 rpm for 30 minutes. The supernatant was discarded, phosphate buffer, PB (10 mM sodium phosphate at pH 7.4) was added and the tube was centrifuged once more. This procedure was repeated twice to change to PB buffer. These nanoparticles are termed "AuNPs PB".

2.3 Instrumentation

The ^1H NMR spectra were recorded with a 300 MHz Varian Mercury NMR spectrometer. High-resolution MS spectra were recorded with a Waters LCT premier XE ESI-TOF mass spectrometer in the positive ion mode. UV-vis absorption spectra were recorded using a Perkin-Elmer UV-vis Envision Spectrophotometer at room temperature. A Zetasizer Nano ZS (Malvern Instruments) was used for dynamic light scattering and zeta potential measurements.

Butwar-coated copper grids were made hydrophilic by glow discharging with an EMS/SC7620 Mini sputter coater according to the instructions by the manufacturer. Gold nanoparticle samples (5 μL) were added on the grids and incubated for about 1 min after which the excess sample was blotted with Whatman 3 mm paper. The samples were dried overnight and imaged with a JEM-1400 (JEOL) transmission electron microscope.

SPR measurements were performed with SPR NaviTM 210A-L instrument (BioNavis, Tampere, Finland), using two different wavelengths. A flow-rate of 10 $\mu\text{L}/\text{min}$ was used throughout the experiments. SPR curves were recorded every 11th second, in total about 60 curves were recorded for the interaction with AuNPs. The slides

were rinsed with water and stored in room temperature until further measurements were performed when removed from the SPR instrument.

An NTEGRA Prima (NT-MDT, Russia) atomic force microscope (AFM) was used to analyze the topography of the samples in intermittent-contact mode. The images (1024×1024 pixels) were recorded in ambient conditions in air ($RH = 35 \pm 3\%$, $T = 25 \pm 1$ °C) using rectangular cantilevers (Model: NSG01, NT-MDT, Russia) with a nominal tip radius of 10 nm. A scan speed was set to 0.39 Hz. The obtained images were processed and analyzed with commercial image analysis software (SPIP 5.1.3, Image Metrology, Denmark).

The morphology and roughness of the samples were quantified using following set of parameters: number of particles per area, particle height, the root-mean-square (RMS) surface roughness (S_q), the surface area ratio (Sdr) expressing the increment of the interfacial surface area relative to the area of the projected flat plane and autocorrelation length (Scl) which is defined as the length for which the autocorrelation coefficient has decayed to e^{-1} (~37%) [36]. The S_q parameter describes the square root of the sum of the squares of the individual heights and depths from the mean line. It is valid for an $M \times N$ rectangular sampling area with the lateral directions x and y and vertical direction z and it is defined as follows:

$$S_q = \left(\frac{1}{MN} \sum_k^{M-1} \sum_l^{N-1} [z(x_k, y_l)]^2 \right)^{\frac{1}{2}}$$

The Scl value represents the main wavelength for a given length scale and can be considered as a length for which individual height readings for a random surface become statistically independent of one another. The S_q and Scl parameters can be used to describe the vertical and lateral distribution of height [37]. The values are average of 2-5 measurements.

A CAM 200 contact angle goniometer (KSV Instruments Ltd., Finland) was used for the determination of the static contact angle of purified water (MilliQ) of the samples. Bare glass and Au substrates were plasma (air) cleaned, washed with ethanol and water and dried under N_2 -flow prior analysis. Contact angles were measured in air in ambient conditions ($RH = 30 \pm 3\%$, $T = 24 \pm 3$ °C) using drop volume of 2 μ l. Droplets were gently deposited on the samples and the contact angles were recorded as a function of time using the software supplied with the instrument. Apparent contact angle values ($\theta_{a,w}$) were obtained at a point of time where the drop diameter, contact angle and volume had stabilized to a constant level utilizing a Laplace fit to the projected drop curvature. The values are average of 2-9 measurements.

2.4 Self-assembled layers

Glass slides and glass slides coated with a thin film of gold were cleaned in a hot solution of $H_2O_2:NH_4OH:H_2O$ (1:1:5) and rinsed with water. Thermo Scientific™ 96-well microtiter microplates (polystyrene) were only rinsed with water. Suspensions of the molecules at concentrations of 1 mg/mL were applied to the cleaned surfaces for 15 minutes. All molecular preparations were usually dissolved in PB, but Lipa-MF was dissolved in

DMSO. The Lipa-MF layer was rinsed with DMSO and water and the other layers were rinsed only with water. AuNPs were allowed to interact with the surface layers generally for 10 minutes, after this the layer was rinsed with buffer. For EG-S-S this cycle was repeated three times. For UV-vis studies the nanoparticles were also allowed to assemble for 12h.

3. Results and Discussion

3.1 Monolayer formation of polyacrylamide and polyethylene glycol

The Lipa-pTHMMAA and polyethylene glycol polymers used for coupling of AuNPs to the surface possess groups for covalent attachment to gold surfaces (lipoic acid and disulfide), and on the other end the molecules were terminated by hydroxyl groups. The lipoic acid derivatives and EG-S-S are expected to attach to gold through the disulfide groups that are known to undergo S–S bond reductive cleavage and adsorb as thiolates on gold [38,39]. Disulfide forms a similar self-assembled monolayer to a thiol analogue [40].

The Lipa-pTHMMAA polymer forms a monolayer with a surface coverage of 460 ± 20 ng/cm² and the height of the layer is about 4 nm as measured by SPR [28]. The surface coverage of the polymer without lipoic acid is much lower: in the range of 200 ng/cm² with a height about half of the Lipa-pTHMMAA polymer. This is probably due to the lower molecular weight and the circumstance that the polymer without lipoic acid groups is not being covalently linked, but physically adsorbed to the surface. The surface density and thickness correspond to that of a grafted polyethylene glycol with a molecular weight in the same range (190 ng/cm² and 1.7nm) [41]. The hydrodynamic radius of polymers in solution corresponding to pTHMMAA and Lipa-pTHMMAA is 2.2 ± 0.4 and 2.7 ± 0.5 nm, respectively [25]. Polymers without lipoic acid group adopt flatter conformations on gold colloid surfaces than polymers with a lipoic group (0.9 and 3.1 nm, respectively) [25]. The surface coverage of EG-S-S was even lower than that of pTHMMAA and corresponds to 100 ng/cm² as measured with SPR (data not shown). The Lipa-pTHMMAA layer is thicker and the chains are more densely packed and stretched than in the pTHMMAA and EG-S-S layer. The monolayer forming of Lipa-MF could not be studied with SPR, because the molecule was not soluble in water, but DMSO was used. However, taking the molecular weight of Lipa-MF into account the monolayer can be expected to be quite thin.

Contact angle measurements are highly effective for investigating surface modifications, since the wetting behavior of a liquid (usually water) changes strongly already with a structural change in the top 2–3 Å layer of the surface [42]. Table 1 lists the $\theta_{a,w}$ values for the different monolayers deposited on glass and gold. As expected, each of the studied monolayers were clearly hydrophilic and the wetting of water was increased compared to the bare Au surface. The wetting properties of the pTHMMAA polymer film on the Au surface was in the same range as that of EG-S-S and of that reported for thiol terminated EG (advancing (34–38°) and receding (22–25°) static contact angles of water, respectively) [43]. The pTHMMAA polymer formed a less wettable film on glass compared to the Au surface with approximately equal $\theta_{a,w}$ value to that of the bare glass substrate. An asymmetric wetting was in general observed for pTHMMAA. As expected the highest contact

angle was observed for Lipa-MF due to the amino head group and lack of hydroxyl groups. The Lipa-pTHMMAA layer exhibited close to full wetting.

Figure 1 shows AFM topographs of bare and coated glass and gold surfaces. Values of selected roughness parameters used to quantify the topography are listed in Table 1. Relatively low Sdr values indicate that wetting is not significantly affected by the roughness induced increase in surface area. Normalizing the Sq value by Scl (Sq/Scl, the surface slope), both the vertical and lateral distribution of height can be considered in the evaluation of the roughness. The Sq/Scl values show that EG-S-S and Lipa-MF had equal roughness, whereas pTHMMAA developed a clearly less rough (by almost an order of magnitude) surface compared to the bare Au substrate. The higher roughness the EG-S-S and Lipa-MF layers compared to the polyacrylamide layers can be explained by the much higher surface coverage and thickness of the layers as observed by SPR. The deposition of pTHMMAA induced a smoothening of the surface also on the glass substrate.

3.2 Assembling of AuNPs on the surface layers

When “AuNPs citrate” and “AuNPs PB” were injected over gold surfaces coated with pTHMMAA, EG-S-S and Lipa-MF there was a significant shift in the SPR curve with time (Figure 2-3, Table 2). The angular shift was also significant for “AuNPs citrate” on Lipa-pTHMMAA, but only minor for “AuNPs PB”. The surface of nanoparticles prepared by citrate reduction was negatively charged with zeta potentials of -42.8 ± 9.5 and -29.0 ± 10.6 mV for “AuNPs citrate” and “AuNPs PB”, respectively. The particles can be expected to assemble on positively charged surfaces like Lipa-MF due to an electrostatic interaction as has been shown for citrate-stabilized AuNPs on amine-terminated self-assembled layers [44,45]. Assembling on the hydroxyl-terminated layers is more complex.

When nanoparticles assembled in time, the SPR curves were observed to shift to higher angles and become increasingly damped, while also the peaks strongly broaden. For all the layers the minimum angle also shifted upwards, although the shift was only minor for “AuNPs PB” on Lipa-pTHMMAA. The damping of the resonance peak took place in less than 10 minutes, the time normally used to follow the assembling of the nanoparticles. The angular shift could not be reliably obtained for the kinetic curves in Figure 3. The automatic calculation of the resonance angle will fail if the SPR curves are broadening too much or have a reflection coefficient > 0.5 . The values in Table 2 were in these cases manually calculated.

The angular shift at 670 nm caused by “AuNPs citrate” on EG-S-S was twice that on pTHMMAA and three-fold that on Lipa-MF (Table 2A). The resonance peak was almost completely damped for EG-S-S (Figure 2A). 70% of the curve disappeared. The broadening of the curve was less pronounced at 785nm and the damping of the resonance was in the same range for all the layers.

The angular shift caused by “AuNPs PB” was similar to that of “AuNPs citrate”, except for Lipa-pTHMMAA. The damping of the resonance curves was only minor on pTHMMAA and Lipa-pTHMMAA, but half of the resonance curve disappeared for Lipa-MF and 32% for EG-S-S. AuNPs continued to adsorb on the surface

layers till the SPR curve was completely damped when the particles were allowed to interact with the surface layer for a prolonged time (30 minutes; Figure 2C & D c, Figure 3B b, d insets). The number of particles adhering to the surface increased with time. The adhesion of the particles is dependent on the time allowed for adhesion, the surface monolayer and the particle concentration. Highly clustered aggregates with a complete damping of the SPR curve has been observed, when assembling AuNPs on a dithiol self-assembled layer for 30 minutes [46]. Tarnawski and Ulbricht obtained a shift in SPR resonance corresponding to 150 mdegree when dodecanethiol modified gold colloids (diameter 10nm) adsorbed to a self-assembled layer terminated with methyl groups [47]. This interaction is similar to that observed for “AuNPs PB” on the Lipa-pTHMMAA layer, but considerably lower than for the other layers.

AFM topographs of substrates after the AuNP deposition are shown in Figure 4 and 5. Generally, the average height of AuNPs on different substrates (Table 3) was very comparable to the average particle size obtained from TEM ($35,5 \pm 0,7$ nm) (Figure 6a) and DLS measurements (35 ± 20 nm). The clear exception was “AuNPs PB” on Lipa-pTHMMAA-Au with a size of only 4nm. The much smaller particles on Lipa-pTHMMAA-Au most probably originated from the AuNP suspensions. DLS measurements indicated in addition to larger particles, the presence of smaller particles with the average size of only 4.9 ± 1.2 nm. This suggests very poor adhesion of large AuNP particles on Lipa-pTHMMAA - Au substrate. Only a minor shift in minimum resonance and no broadening of the SPR spectra was observed for “AuNPs PB” on Lipa-pTHMMAA layers. The low SPR angular shift in intensity is here related to the particle size with only a low number of particles assembled on the surface and no additional resonance caused by inter-particle interaction.

The number of AuNPs (both citrate and PB) is largest on the EG-S-S monolayer (Table 3). The SPR measurements also showed the highest shift in SPR peak angular position and peak minimum intensity for “AuNPs citrate” on EG-S-S. The lowest amount of AuNPs was detected on Lipa-MF layers and there was no difference between “AuNPs citrate” and “AuNPs PB”. For reference, negligible amount of AuNPs were detected on the bare Au substrate, which was confirmed by no shift in the SPR curve. Nor was there any change when the AuNPs were coated with a mixed layer of antibodies and Lipa-pTHMMAA. Coated gold colloids has been shown to adsorb from the aqueous solution to polyethylene glycol polymer surfaces in a similar fashion as proteins [48]. We have previously used Lipa-pTHMMAA as a non-fouling layer in immunoassays [26-27,28,29,30].

For monolayers assembled on gold topographical images show that the citrate nanoparticles adhere in the order of EG-S-S > pTHMMAA > Lipa-pTHMMAA > Lipa-MF. When taking the size of the particles into account the order is slightly different and agree with that of the SPR angular shift at 670nm (EG-S-S > Lipa-pTHMMAA > pTHMMAA > Lipa-MF). This is also the case for the “AuNPs PB” except for Lipa-pTHMMAA as already discussed. Waters molecules on the completely wetted and smooth Lipa-pTHMMAA monolayer (with a high surface energy) seems to shield the interactions between the surface and large AuNPs. Only a low number of small molecules are assembled. Physisorbed citrate anions may not be sufficiently stable to remain bound to the AuNPs in phosphate buffer during adsorption onto the surface.

The Lipa-MF layer was the least hydrophilic of the layers and amino groups are mainly responsible for assembly of the nanoparticles. The damping of the SPR curve was in the same order as that for EG-S-S at both wavelengths. The wetting properties of the surface coated with gold nanoparticles (contact angle of 86°) on the physisorbed pTHMMAA layer were in the same order as that of an uncoated thin gold film (Table 1). The contact angle of the AuNps layer attached onto the lipoic acid and EG-S-S layers were somewhat lower 74° .

Qualitatively speaking, combined surface characteristics of higher roughness (i.e. high S_q/S_{cl} ratio) and good water wetting property (low $\theta_{a,w}$) seemed to enhance the adhesion of AuNPs under the current experimental conditions. However, several attractive or repulsive forces could have contributed to the adhesion of colloidal AuNPs on the substrate in aqueous liquid, including van der Waals, electrostatic, solvation and structural, hydration and hydrophobic and polymer-mediated forces [49]. For example, the interactions between a colloidal Au and a flat Au surface in an aqueous medium only includes van der Waals forces which are not strong enough to induced good gold-to-gold adhesion [50]. From that respective, roughness being equal for both the bare Au and the EG-S-S film surfaces suggests that this property was not the dominating factor for enhancing adhesion of AuNPs on the EG-S-S film. This is not surprising, as the amplitude of roughness (i.e. S_q) was generally more than an order of magnitude lower than the average size of AuNPs.

3.3. UV-vis characterization of surface assembled AuNPs

A maximum in the UV-Vis spectrum was observed at the same wavelength as that of the aqueous AuNPs solution when “AuNPs citrate” were allowed to interact for 10 minutes with the monolayers physisorbed onto microtiter plates (535 nm, Figure 6). There was no increase in absorbance for the other layers after 10 minutes nor when assembling “AuNPs PB”. The number of nanoparticles assembled on pTHMMAA physisorbed on glass and gold was 20 and $61/\mu\text{m}^2$, respectively, whereas half the amount were assembled from an “AuNPs PB” solution (Table 3). The damping of the SPR resonance curve was also much lower for “AuNPs PB” than for “AuNPs citrate” (13 and 35%, respectively). The roughness of the pTHMMAA layers on glass and gold was the same and the contact angle in the same range. The pTHMMAA can be expected to physisorb differently on polystyrene that have advancing (93.4°) and receding (70.6°) contact angles of water, respectively than on glass and gold [51]. No nanoparticles were assembled on the other layers during 10 minutes.

After an assembling period of 12 hours the absorbance increased tenfold for nanoparticles on the pTHMMAA monolayer, but only a minor increase in absorbance was observed on the layers with a lipoate and disulfide group. The absorbance maximum shifted to 565 nm for both citrate and PB nanoparticles. The distance between the nanoparticles becomes shorter and near-field inter-particle coupling on the particle plasmon resonance increases. This leads to significantly shifted absorption peak to higher wavelengths [52,53]. The time thus influences the number of particles assembled on the surface as observed both with SPR and AFM. A golden layer was visible by eye on the bottom of the microtiter plate for AuNPs assembled on pTHMMAA. The SPR cuvette being exposed to several injections of gold nanoparticles also exhibited a thick golden layer visualized by eye.

5 Summary

Lipoic acid and disulfide terminated polyacrylamide and polyethylene glycol polymers with hydroxyl groups on the other end and a lipoic acid triazine derivative terminated with an amino group (Lipa-MF) were used to produce self-assembled monolayers. The monolayers were all hydrophilic and the polyacrylamide covalently linked to gold exhibited close to full wetting. The polyacrylamide layers were clearly less rough than their polyethylene analogue and Lipa-MF. This was due to the height and to densely packed chains of the polyacrylamide layers. Real-time Surface Plasmon Resonance, SPR can be used to study the assembly of gold nanoparticles on surfaces in real time. SPR curves shift to higher angles and become increasingly damped, while also the peaks broaden when large nanoparticles assemble on the surface. The angular shift was considerably smaller for small particles and no broadening and damping of the curve was observed. High amounts of particles were also assembled on the polyacrylamide layer without the lipoic acid group, but damping of the curve was considerably lower than that on polyethylene glycol layers. The area of citrate terminated gold particles assembled from water was highest on the polyethylene glycol layer as evaluated by atomic force microscopy and agree with that observed by SPR. High amounts of particles were also assembled on the pTHMMAA layer without the lipoic acid group. The damping of the curve was considerably lower than that on EG-S-S. This is in accordance with the roughness of the layer. However, the roughness of the polymer films did not alone influence the assembling of nanoparticles on the surface. The damping of the SPR curve assembled with nanoparticle in phosphate buffer was much lower for the EG-S-S and polyacrylamide layers, whereas only minor difference was observed for Lipa-MF.

The surface does not become densely packed with AuNPs during an assembling time of 10 minutes. The number of particles assembled, however, increase with time. A physisorbed layer of pTHMMAA gives a more densely packed layer of nanoparticles on polystyrene than the other polymer layers or the lipoic acid triazine derivative. The pTHMMAA layer is more favorable assembled on polystyrene than the layers possessing lipoic acid or disulfide groups.

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Scheme 1 Structure of a) pTHMMAA, b) Lipa-pTHMMAA, c) EG-S-S and d) Lipa-MF.

Figure 1 AFM topographs of an uncoated a) glass and b) gold substrate. AFM topographs of pTHMMAA on c) glass and d) gold. AFM topographs of e) Lipa-pTHMMAA, f) EG-S-S and g) Lipa-MF on gold. Image size 1 μm x 1 μm .

Figure 2 SPR resonance curves at A, C) 670nm and B, D) 785nm for binding of A, B) “AuNPs-citrate” and C, D) “AuNPs PB” to monolayers of a) pTHMMAA, b) Lipa-pTHMMAA, c) EG-S-S and d) Lipa-MF assembled on a gold surface. Curves were recorded every 11th second as shown from left to

right as AuNPs interacts with the surface layer. The insets in C, D c) show three injections of “AuNPs PB”.

Figure 3 Shift in A) SPR peak minimum and B) minimum intensity during binding of “AuNPs citrate” (upper curves) “AuNPs PB” (lower curves”) to monolayers of pTHMMAA, Lipa-pTHMMAA, EG-S-S and Lipa-MF. The insets in B) shows three “AuNPs PB” injections on EG-S-S with rinsing in between. Curves that not agree with the SPR resonance curves are marked in grey.

Figure 4 AFM images of “AuNPs citrate assembled on A) pTHMMAA, B) Lipa-pTHMMAA, C) EG-S-S and D) Lipa-MF. *Left*: Large scale (5 μm x 5 μm) AFM topograph. *Right*: Small scale (1 μm x 1 μm). The height scale is 50 nm all images.

Figure 5 AFM images of “AuNPs PB” assembled on A) pTHMMAA, B) Lipa-pTHMMAA, C) EG-S-S and D) Lipa-MF. *Left*: Large scale (5 μm x 5 μm) AFM topograph. *Right*: Small scale (1 μm x 1 μm). The height scale is (A, C and D) 50 nm and (B) 10 nm.

Figure 6a) TEM image of AuNPs. An average particles size of $35,5 \pm 0,7$ nm was obtained by imaging 20 nanoparticles. UV-vis absorbance spectra of b) “AuNPs citrate” and c) “AuNPs PB” assembled on microtiter plates (PS) coated with pTHMMAA (for 10 minutes and 12h), Lipa-pTHMMAA, EG-S-S and Lipa-MF for 12h. The insets show the absorption spectra of the aqueous AuNPs.

Table 1 Apparent water contact angles ($\theta_{a,w}$) and selected roughness parameters for monolayers on gold and glass substrates.

| Layer | $\theta_{a,w}$ [°] | Sq [nm] | Sdr [%] | Scl [nm] | Sq/Scl |
|-------------------|--------------------|---------------|-----------------|-------------|--------|
| Glass | 39.2 ± 1.1 | 0.4 ± 0.1 | 0.04 ± 0.01 | 34 ± 4 | 0.012 |
| Au | 84 ± 5.1 | 1.5 ± 0.1 | 0.9 ± 0.1 | 29 ± 4 | 0.052 |
| pTHMMAA - Glass | 36.3 ± 6.4 | 0.5 ± 0.3 | 0.2 ± 0.1 | 80 ± 20 | 0.006 |
| pTHMMAA - Au | 29.4 ± 8.8 | 0.4 ± 0.1 | 0.05 ± 0.01 | 69 ± 14 | 0.006 |
| Lipa-pTHMMAA - Au | < 15 | 1.1 ± 0.2 | 0.2 ± 0.1 | 73 ± 11 | 0.015 |
| EG-S-S - Au | 25.4 ± 8.1 | 1.4 ± 0.1 | 0.8 ± 0.1 | 24 ± 3 | 0.058 |
| Lipa-MF -Au | 58.3 ± 1.9 | 3.7 ± 1.5 | 2.6 ± 0.1 | 64 ± 10 | 0.058 |

Table 2 Summary of the total shift in SPR peak angular position and peak minimum intensity during binding of A) “AuNPs citrate” B) “AuNPs PB” to monolayers of pTHMMAA, Lipa-pTHMMAA, EG-S-S and Lipa-MF at 670 and 785nm. #Values obtained from SPR curves manually.

| A. “AuNPs citrate” Monolayer | 670nm | | | 785nm | | |
|---------------------------------|----------------------|----------------------|-------------|----------------------|----------------------|-------------|
| | Angle shift [degree] | Min Intensity [a.u.] | Damping [%] | Angle shift [degree] | Min Intensity [a.u.] | Damping [%] |
| | | | | | | |

| | | | | | | |
|--------------|------------------------|------|----|------------------|------|----|
| pTHMMAA | 2.2 | 0.26 | 35 | 1.6 | 0.31 | 40 |
| Lipa-pTHMMAA | (5.4) 2.7 [#] | 0.36 | 48 | 3.3 | 0.27 | 31 |
| EG-S-S | 4.5 | 0.54 | 69 | 6.4 [#] | 0.31 | 40 |
| Lipa-MF | (5.4) 1.5 [#] | 0.40 | 62 | 3.0 [#] | 0.26 | 41 |

| B. “AuNPs PB” | 670nm | | | 785nm | | |
|---------------|----------------------|----------------------|-------------|----------------------|----------------------|-------------|
| | Angle shift [degree] | Min Intensity [a.u.] | Damping [%] | Angle shift [degree] | Min Intensity [a.u.] | Damping [%] |
| Monolayer | | | | | | |
| pTHMMAA | 2.6 | 0.09 | 13 | 1.3 | 0.04 | 5 |
| Lipa-pTHMMAA | 0.1 | 0.02 | 3 | 0.1 | 0.010 | 1 |
| EG-S-S | 4.25 [#] | 0.24 | 32 | 3.1 | 0.13 | 17 |
| Lipa-MF | 2.4 [#] | 0.42 | 54 | 3.1 | 0.23 | 26 |

Table 3 Topographical characteristics of “AuNPs citrate” and “AuNPs PB” on the monolayers.

| A. “AuNPs citrate” | | | | |
|--------------------|-------------|-------------|--|---|
| Monolayer | Height [nm] | Width [nm] | Number of particles [1/μm ²] | Area [#] [10 ⁻⁴ nm ²] |
| pTHMMAA - Glass | 34.0 ± 4.8 | 77.2 ± 9.8 | 20 | 1.8 |
| pTHMMAA | 26.2 ± 3.9 | 96.4 ± 17.7 | 61 | 3.3 |
| Lipa-pTHMMAA | 36.7 ± 5.7 | 87.5 ± 8.3 | 46 | 4.9 |
| EG-S-S | 33.1 ± 8.8 | 93.6 ± 17.0 | 213* 71 | 6.1 |
| Lipa-MF | 39.7 ± 5.6 | 75.2 ± 8.8 | 28 | 3.5 |

| B. “AuNPs PB” | | | | |
|---------------|-------------|-------------|--|---|
| Monolayer | Height [nm] | Width [nm] | Number of particles [1/μm ²] | Area [#] [10 ⁻⁴ nm ²] |
| pTHMMAA | 35.4 ± 4.8 | 71.6 ± 5.6 | 33 | 3.2 |
| Lipa-pTHMMAA | 4.4 ± 1.1 | 93.5 ± 12.9 | 63 | 1.0 |
| EG-S-S | 32.9 ± 10.3 | 80.4 ± 4.1 | 247* 82 | 7.0 |
| Lipa-MF | 35.0 ± 4.0 | 82.6 ± 6.9 | 29 | 2.8 |

*three times longer adsorption time; [#] Area = $(\pi/4) \times H^2 \times$ number of particles, where H is the height of the particles.

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