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Structure and stability of PEG- and mixed PEG-layer coated nanoparticles at high particle concentrations studied in situ by small-angle X-ray scattering

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Ligand layer structure and stability of gold nanoparticles (AuNP) coated with α -methoxypoly(ethylene glycol)- ω -(11-mercaptoundecanoate)- (PEGMUA-) layers and mixed layers of PEGMUA and 11-mercaptoundecanoic acid (MUA) at high AuNP concentrations are studied in situ by small angle X-ray scattering (SAXS). The thickness of the ligand layer is modified by the molecular weight of the PEG-ligands (2 and 5 kDa) and the PEG grafting density is decreased by coadsorption of MUA. The response of the conjugates to a pressure of up to 4 kbar is probed. The results indicate strongly hydrated PEG-layers at high grafting densities. The stability of the mixed ligand layer conjugates is lower. This is most probably due to enhanced interparticle PEG-PEG interactions at lower grafting densities. The presented study demonstrates that a detailed structural characterization of polymer ligand layers in situ and in response to external stimuli is possible with SAXS.

1. Introduction

Highly concentrated nanoparticle (NP) solutions with increasingly complex surface chemistry become more and more important for applications, for example in nanomedicine, inkjet printing or self-assembly-based fabrication strategies.^{[1]–[3]} Introducing complexity into the ligand layer to achieve functionality of nanomaterials is often not only challenging a thorough characterization^[4] but also conflicting with the desired stability. Additional functional groups,

binding motifs or molecular structures in general usually come along with unwanted interactions such as agglomeration or adhesion to vessel walls or unwanted biological interactions compared to a passive, stabilizing coating.^[5] Furthermore, the changed structure of mixed ligand layers can increase their susceptibility to competitive ligand displacements or other unwanted reactions at the particle surface. The stabilization of ligand-coated nanoparticles in solution cannot be described by just adding an attractive Van der Waals-potential of the cores to the repulsive potential of the ligand layer.^[6] More complex interactions of the ligands become increasingly important at high concentrations and fundamental studies are needed in order to gain an understanding of these effects.

In this work we study the correlation of ligand layer structure and NP stability at high concentrations with a clear model system recently established.^[7] We address the role of grafting density and ligand layer thickness for the AuNP stability with well-defined gold nanoparticles (AuNP) coated with either a tailored PEG-ligand (PEGMUA) or with mixtures of this PEG-ligand and MUA. In order to avoid problems of standard-characterization techniques such as UV/vis-spectroscopy and dynamic light scattering (DLS) with large volume fractions ($> 1\%$), we employed small-angle X-ray scattering (SAXS) for an in situ structure determination. In a recent work we demonstrated that the thickness of the ligand layer cannot be determined from form factor measurements, because it does not contribute sufficiently to the scattering signal (scattering contrast $\Delta\rho$ of PEG in aqueous solution: $\Delta\rho_{\text{PEG}} = 40 \text{ nm}^{-3}$; $\Delta\rho_{\text{Au}} = 4000 \text{ nm}^{-3}$). Instead it can be obtained by refining the SAXS data of the concentrated solutions with a sticky hard sphere (SHS) model.^[8] This way, the structure of the ligand layer and its response to external stimuli, like pressure and temperature, as well as the presence of aggregates can be addressed. We observe a general and good agreement of the structural data for AuNP-conjugates with different ligand shell thicknesses and grafting densities obtained by SAXS with values derived from complementary characterization experiments (thermogravimetric analysis (TGA), DLS). Based on this confidence, we develop

a simple, accessible geometrical model, indicating strongly hydrated PEG-layers at high grafting densities. The model system in combination with the experiment allow studying the structure of polymer brushes and their responses to external stimuli detailed and in situ.

2. Results and Discussions

2.1. Ligand layer structure - general considerations

The structure of surface-grafted polymers or polymer brushes is of great interest because it dictates the interfacial properties of the coated material. However, detailed structural data are not easy to obtain. Such data necessarily have to be measured in situ because drying or other treatments for ex situ characterization perturb the structure of the grafted polymer layer. In solution, the structure is dictated by the grafting density of the polymer chains and their interactions with the solvent as described within the Alexander-de Gennes (AdG-) theory.^{[9]–^[14]} In good solvents, the interactions with the solvent are preferred over interactions with other polymer chains. At low grafting densities, i.e. without lateral constraints, the polymers adopt a swollen, random coil conformation, behaving like a free polymer in solution. The Flory-radius describes the size of the random coil that is formed by such a polymer in solution.^[15] For a surface grafted polymer half of the conformational space is occupied by the substrate (here: the AuNP). Accordingly, the thickness of the ligand layer is close to the Flory-radius of the polymer.^{[16][17]} The resulting structure of the polymer layer is then called mushroom conformation. In contrast, at high grafting densities, the polymer chains are forced into a stretched, so-called brush conformation to minimize polymer-polymer contacts and to maximize polymer-solvent contacts. In the case of PEGylated materials, it has been shown that these conformations critically affect the immune response and the protein adsorption in general.^{[18]–[22]} The grafting density and structure of the polymer layer, or more generally ligand layer, also dictate the chemical and colloidal stability of nanoparticles.^{[4][7][23]–[25]} Probing the structure of the ligand layer in solution is only possible with a few experimental methods. In case of AuNP, UV/vis-spectroscopy can be used to qualitatively detect the

formation of a ligand layer, when the change of refractive index at the AuNP surface causes a small shift of the localized surface plasmon resonance band (LSPR).^{[25]–[27]} This provides no structural information though and other processes, especially aggregation, can also cause shifts of the LSPR. Complementary characterization by dynamic light scattering (DLS) or related methods can be used to prove the absence of aggregates. For well-defined samples DLS can also be used to assess the thickness of the ligand layer but its resolution and accuracy are limited. For example, the increase of the hydrodynamic diameter from AuNP coated with citrate (shell thickness $s \approx 1$ nm) over AuNP coated with 2 kDa PEG (AuNP@PEG2k, $s \approx 6$ –10 nm) to AuNP@PEG5k ($s \approx 10$ –14 nm) can be easily detected,^[7] but small changes of $\Delta s \approx 1$ nm of the hydrodynamic diameter cannot be detected with confidence. Additionally, even slight disturbances, for instance caused by contaminations, aggregates, turbidity, microbubbles, viscosity or adhesion to vessel walls can compromise the results. Other approaches to determine the thickness of ligand layers include comparative gel electrophoresis experiments, differential centrifugal sedimentation and size exclusion chromatography.^{[28]–[31]} Compared to those SAXS is especially useful for in situ measurements in various solvents, buffers and complex sample environments. SAXS can provide an accurate measurement of AuNP size and dispersity.^[32] We have shown that for very concentrated solutions of PEG-coated AuNP a structure factor contribution allows determining the shell thickness of the AuNP@PEG conjugates.^[8] Besides the in situ structural information that can be obtained by a SAXS measurement, the structure factor contribution additionally allows studying interparticle interactions.

Scheme 1. Illustration of the ligands PEGMUA and MUA, ligand layer structures and their response to pressure. The coadsorption of MUA (grey) results in a lower grafting density of PEG-moieties (blue) in a less stretched conformation. The effect of high pressure is expected to be lower for the ligand layer in a less stretched conformation.

2.2. Model system

As a model system for SAXS-experiments we employed AuNP, functionalized with α -methoxypoly(ethylene glycol)- ω -(11-mercaptopundecanoate) (PEGMUA). This ligand provides superior chemical stabilization compared to simple PEG-thiols (without C10 alkylene spacer) commonly used for AuNP stabilization.^{[25][33]} It also allows for the controlled synthesis of mixed ligand layers, as shown for mixtures of PEGMUA and 11-mercaptopundecanoic acid (MUA).^[7] The structure of the ligands and the assumed ligand layers are illustrated in **Scheme 1**.

This way it is possible to modify the grafting density σ_g of PEG-moieties without reducing the chemical stability of the conjugates. Because of the ligands' structure, the grafting density for pure PEGMUA layers is very high, $\sigma_g > 1 \text{ nm}^{-2}$. Thus, the ligand layers can be described as being in a dense brush conformation.^[22] We note, that curvature effects are not expected to play a significant role for the particle sizes studied herein.^{[7][34]} The colloidal stability of the conjugates with pure PEGMUA- as well as with mixed PEGMUA/MUA-layers is sufficiently

high to prepare purified dispersions with very high concentrations by repeated centrifugation.^[7] Typically, 1000 ml of citrate-stabilized AuNP ($c = 3\text{-}6\text{ nm}$) with high uniformity and low dispersity are functionalized with the according ligand or ligand mixture and subsequently concentrated to volumes $< 1\text{ ml}$. This way, the concentration is increased from the nanomolar to the micromolar range, that corresponds to volume fractions of the conjugates (AuNP + ligand layer) in the percental range (1-12 %).

2.3. Grafting density

The AuNP used in this study had a core radius $R = 6\text{ nm}$ with a size polydispersity of 5-8 %, based on TEM and SAXS form factor measurements (Supporting Information, **Table S1**). PEGMUA with 2 or 5 kDa was used to prepare AuNP conjugates with pure ligand layers (AuNP@PEGMUA2k or AuNP@PEGMUA5k) or with mixed ligand layers (AuNP@PEGMUA2k_MUA or AuNP@PEGMUA5k_MUA). **Figure S1** shows an exemplarily TEM measurement of the AuNP, absorbance spectra of the conjugates before and after pressure experiments and results from a thermogravimetric analysis (TGA), used to determine the grafting density of PEGMUA2k and -5k on AuNP.

AuNP@PEGMUA2k_MUA and AuNP@PEGMUA5k_MUA were synthesized with molar ratios of 50 % MUA and 50 % PEGMUA (see Scheme 1). The coadsorption of MUA can be verified by FTIR-spectroscopy as described recently^[7] (Supporting Information, **Figure S2**) but not quantified. By TGA the grafting density can be assessed for the pure ligand layers (Figure S1c)) but not for the mixed ligand layers because no quantitative information of the mixed layer composition is available. Quantitation based on NMR-spectroscopy failed because of peak broadening due to limited diffusion that prevented a confident analysis of the spectral fine structure.^[35] Limited diffusion results from the grafting of the ligands to the AuNP and from the viscosity of samples with AuNP-conjugate concentrations that are sufficiently high for NMR analysis. An estimate of the relative MUA coverage can be

obtained by comparing mixed AuNP-conjugates with different PEGMUA/MUA-ratios by FTIR and gel electrophoresis.^[7]

For pure PEGMUA2k a grafting density of $\sigma_g = 3.0\text{-}3.3 \text{ nm}^{-2}$, for PEGMUA5k a grafting density of $\sigma_g = 1.7\text{-}2.0 \text{ nm}^{-2}$ was determined with TGA. For PEGMUA2k the grafting density agrees very well with the ones determined for the same ligand on gold nanorods.^[33] For pure MUA ligand layers, values in the range $\sigma_g = 4\text{-}6 \text{ nm}^{-2}$ were reported.^{[36][37]} Literature values for PEG-thiols on gold vary in a broad range. A very systematic study of PEGylated AuNP based on TGA was presented by Rahme et al.^[38] In this study, regular PEG-thiols (PEGSH) without a MUA-spacer as in PEGMUA were used and compared to those, the grafting densities of PEGMUA ligands are significantly higher for the same molecular weight. These higher values are in agreement with various experimental findings that PEGMUA ligands provide a much higher chemical stability for AuNP and gold nanorods than PEGSH ligands. A decreasing grafting density with increasing molecular weight found here and in Ref. [38] is reasonable, considering geometrical constraints.

2.4. Refinement of SAXS data

The SAXS curves of concentrated solutions from all four samples are shown in **Figure 1a**). At the studied concentrations, the contribution of a structure factor to the scattering signal is present originating from interparticle interactions in solution. The data were analyzed by refinement with the SHS model, as described recently.^[8] Exemplarily structure factors at different pressures and the according fits are shown in **Figure S3**. The refinement yields the effective radii R_{SHS} of the conjugates as well as the volume fractions ϕ and the so-called stickiness parameter which characterizes the interparticle interactions. The effective radii represent the sum of the radii of the gold cores R_{core} and the thickness of the ligand layers. As the core radii are known and the dispersity of the cores is small (<8%), the thicknesses s of the ligand layers can be derived from the obtained data, i.e. $s = R_{\text{SHS}} - R_{\text{core}}$. The radii R_{core} and R_{SHS} as well as the ligand layer thicknesses s are shown in Figure 1b). The values agree

with the expectations based on the structural model depicted in Scheme 1. PEGMUA5k results in a thicker ligand layer ($s \approx 13.5$ nm) than PEGMUA2k ($s \approx 9.5$ nm) despite its slightly lower grafting density. Coadsorption of MUA reduces the thickness of the ligand layers by reducing the grafting density of PEG-moieties ($s \approx 6$ nm and $s \approx 8$ nm for the 2k and 5k species, respectively). The ligand layer thickness does not increase linearly with PEG-length because conformational freedom increases with distance from the anchoring points on the AuNP surface and due to the slightly lower grafting density of PEGMUA5k.

Figure 1. SAXS results for AuNPs with different PEGMUA coatings. a) SAXS curves for all four samples together with refinement using the SHS model (solid lines). The data were shifted vertically for clarity. b) The different sizes of the samples at 1 bar: Effective radius determined from the structure factor R_{SHS} , radius of the AuNP core R_{core} , ligand shell thickness s . c) Ligand shell thickness s as a function of pressure.

It is instructive to compare the obtained values with expectations based on the AdG-theory as well as a simple density model. **Figure 2** shows the expected ligand layer thicknesses as a function of grafting density.

Figure 2. Thickness of the ligand shell s as a function of grafting density σ_g calculated with the density of PEGMUA2k and -5k and water. The number of water molecules per PEG-ligand used for the calculations are indicated with the color code. The expected brush height, i.e. shell thickness based on the Alexander-de Gennes (AdG) theory is shown in blue for PEGMUA2k. The Flory radii R_F are indicated as dashed lines. The green areas indicate the range of experimental values obtained by SAXS (s) and by TGA (grafting density σ_g) for AuNP@PEGMUA2k (a) and AuNP@PEGMUA2k_MUA (b) or -5k respectively. The values obtained by the AdG-theory strongly overestimate the experimental values for PEGMUA5k and are therefore not shown. The grafting densities for b are not accessible by TGA and were therefore estimated as 50 % of the value for a. This estimate is supported by comparative gel electrophoresis experiments presented recently.^[7] The dashed arrows indicate the effect of pressure, and the green dashed circles the values for s at 4000 bar. The grafting density is expected not to change with pressure. Note the different scaling for s .

The values were calculated based on a simple model using the densities of PEG and water and the amount of adsorbed PEGMUA based on the grafting density (the detailed description can be found in the experimental section). The values for a “dry” PEG-shell without any water and for different numbers of water molecules per adsorbed PEGMUA ligand are shown. Additionally, the theoretical expectations for s based on the AdG- theory as well as the values of the Flory radii R_F are given.^[16] A thickness s in the range of R_F would suggest a mushroom conformation of the ligand layer. The experimental data (indicated by the green circles in Figure 2) suggest that all ligand layers are in the brush conformation even with MUA coadsorption. Accordingly, for the mixed ligand layers, the conformation remains brush-like, but with a reduced density of the brush that results in a significantly reduced thickness Δs of ~ 3 -4 nm for the 2k coatings and ~ 5 -6 nm for the 5k coatings. This is related to a significant difference in hydrodynamic radius considering that the nominal conformation is the same. It

is also evident that all PEG-layers are “heavily hydrated”, a well-established property of PEG.^[39] A confident quantification of the hydration is impossible with the available data. The estimated number of ~230 water molecules per PEGMUA2k-chain agrees with literature values for free PEG2k,^[40] however, the conformations of surface-grafted and free PEG are very different. Thus, a direct comparison is not meaningful, but the magnitude of hydration obtained by the simple geometric model seems realistic. For PEGMUA2k the AdG model describes the data reasonably well, the predicted values differ approximately 1-2 nm from the experimental values. In contrast, for PEGMUA5k the AdG-model strongly overestimates the shell thicknesses by about 10 nm (data not shown). Shell thicknesses based on DLS tend to be lower than those obtained by the SHS-model (**Figure S4**). However, as discussed earlier, the accuracy of DLS is limited and the ranges for s are broad. Considering the radial polymer segment density distribution, i.e. that there is no sharp “edge” of the ligand layer,^{[11][13][16]} it seems reasonable that the thickness of the ligand layer can be determined experimentally just within certain boundaries, regardless of the method. The observed trends, however, agree well with the structural model hypothesized for the different AuNP-conjugates.

2.5. Stability trends

In general, the observed stability of the pure PEGMUA conjugates was higher than for the mixed ligand layer conjugates. The conjugates with mixed ligand layers exhibited a stronger tendency to agglomerate during centrifugation and longer sonication was necessary to completely redisperse the pellets. The presence of aggregates was probed by DLS measurements after the SAXS measurements (**Figure S4**). Indication of slight aggregation was observed only for the mixed ligand layer conjugates. In the case of AuNP@PEGMUA2k_MUA the upturn of the SAXS signal at small angles also indicates the presence of stronger attractive interparticle interactions promoting the formation of aggregates (**Figure 1a**). With UV/Vis-spectroscopy no indications of aggregation were observed (**Figure S1b**). Significant aggregation of AuNP typically results in a redshift of absorption (blueshift

of transmission) due to plasmonic coupling.^{[26][27]} Plasmonic coupling is strongly distance-dependent, though, and if the interparticle distances within the aggregates are sufficiently large, it is possible that no optical response can be observed. Large interparticle distances within aggregates are possible when the aggregation is caused by ligand-ligand interactions or mediated cross-linking.^[41] In contrast, aggregation caused by disintegration of the ligand layer or insufficient steric stabilization is driven by strong but short-ranged attractive Van-der-Waals (VdW) interactions of the AuNP-cores and the minimization of surface energy and will result in small interparticle distances or even coalescence.^{[6][23][25][42]} Therefore such -usually irreversible- aggregation results in a strong optical response of the sample. E.g. AuNP ($R \sim 5$ nm or larger) coated with just MUA are electrostatically stabilized in solution but the steric stabilization provided by MUA is not sufficient to prevent irreversible aggregation during repeated centrifugation that is accompanied by a strong redshift of absorption. For AuNP with $R \sim 6$ nm we identified the minimum molecular weight for sufficient steric stabilization with PEG ligands to be ~ 2000 g/mol.^[7] The data obtained by the SHS-refinement and the optical properties of the samples indicate that the (partial) aggregation, if present, of the AuNP@PEG-conjugates (pure and mixed) is ligand-mediated. Possibly it can be interpreted as a gelling effect that becomes more prominent and likely at higher concentrations. For the mixed ligand layers, interpenetration and interparticle ligand interactions are more likely because of the lower grafting densities. Since the thicknesses of the mixed ligand layers are still quite large (> 4 nm), it is unlikely that core-core VdW-interactions dominate the aggregation. The thickness of the pure PEGMUA2k layer is in the same range as the thickness of the PEGMUA5k_MUA mixed layer (9.5 and 8 nm, respectively) but the pure layer provides higher stability. Thus, the thickness of the ligand layer cannot explain the difference in stability for these samples. It has to be stressed, that the extent of aggregation is very low for all samples and the main populations are not affected. For the pure ligand layers no aggregation was detectable.

2.6. Response to pressure

The application of pressure in the kbar-range allows probing the volumetric response of soft matter and, in particular, to get insight into the degree of hydration.^{[43]–[46]} The response of the different conjugates to pressures up to 4000 bar was monitored with SAXS which showed a decrease of ligand shell thickness with increasing pressure (Figure 1c and Figure S3). In recent works, this observed decrease of the polymer shell thickness was attributed to a deswelling.^[8] In their analysis of pressure-induced deswelling of poly(*N*-isopropylacrylamide) (pNIPAM) microgel particles, Lietor-Santos et al. concluded that the entropy of mixing, and in consequence the Flory polymer-solvent interaction parameter, depend on the hydrostatic pressure.^[44] With increasing pressure, solvent-solvent contacts are increasingly favored over polymer-solvent contacts, resulting in a deswelling. The deswelling effect (Δs) is stronger for AuNP@PEGMUA (2k and 5k) ($\Delta s_{2k} = 5.5$ nm; $\Delta s_{5k} = 3.5$ nm at $p = 4000$ bar), and the compression of conjugates with PEGMUA5k seems to be stronger in the pressure range 1–1000 bar. The weaker response of the mixed-layer conjugates ($\Delta s_{2k} = 2.0$ nm; $\Delta s_{5k} = 3.0$ nm) might be attributable to their lower hydration because less PEG is present in the ligand shell. From a structural point of view, the PEG moieties in the mixed layer are in a less stretched conformation and therefore more conformations of similar thickness are available to evade the pressure as illustrated in Scheme 1. As a result, the change of layer thickness Δs is smaller for mixed-layer conjugates. The differences of PEGMUA2k and -5k conjugates indicate that the pressure response depends on the PEG-length. Considering that the total water content of PEGMUA5k ligand shells is higher and the PEG-segment density is lower at larger distances from the AuNP-surface, it can be reasoned that a fraction of the PEG-associated water is already removed at comparably low pressures up to 1000 bar. At the maximum pressure probed, 4000 bar, the shell thickness of AuNP@PEGMUA5k is still approximately 10 nm, significantly larger than it would be expected for a mushroom conformation ($s \approx$ Flory radius) or a dry PEG-shell (compare Figure 2), suggesting that not all water molecules are removed

from the PEG-shell at this pressure. In contrast, for all other samples the shell thicknesses at 4000 bar are in the range predicted for dry PEG shells. The pressure effects were reversible and no significant destabilization of the samples was observed (Figure S1b) and Figure S4).

3. Conclusion

In conclusion, we have shown that a model system based on PEG-coated AuNP probed by SAXS allows studying the structure of ligand layers at high concentrations and their response to pressure. Our results indicate that the grafting density is more important than the PEG length ($> 2k$) for the stability of the colloidal dispersion at high concentrations. The pure PEG layers with high grafting densities are in a dense brush conformation and provide high stability whereas coadsorption of MUA decreases the PEG-grafting density, leading to a less dense conformation. This is accompanied by a slight but significant decrease in colloidal stability at high concentrations. The lower grafting density is also related to a significant decrease in ligand layer thickness. However, the reduced thickness cannot explain the differences in stability. In particular, the pure PEGMUA2k layer is characterized by a higher stability than the PEGMUA5k_MUA mixed layer although their thicknesses are in the same range. The structural data obtained by SAXS, complemented with TGA data, correspond well with a simple geometrical model based on PEG and water densities. The PEG shells are strongly hydrated; deswelling can be induced by hydrostatic pressure. The decrease of ligand layer thickness due to pressure-induced deswelling is more pronounced for pure PEG ligand layers. PEG-ligand layers are usually regarded as a “passive” coating. Our results underline, however, that the details of the PEG-layer structure can have significant impact on basic properties as colloidal stability. For applications in complex environments, as in nanomedicine, it is crucial to understand the structure of the PEG-layer and how it affects interactions. Complex mixed ligand layers including PEG-ligands are often studied in the context of nanomedicine, challenging proper characterization and understanding. The controllable synthesis and surface chemistry of AuNP conjugates in combination with SAXS

as a powerful method for in situ characterization of colloids allow studying the structure of such ligand layers and their response to external stimuli. In future studies the properties of PEG-layers in more complex environments like biologically relevant fluids and media should be addressed. Contributing to the understanding of these systems will help to improve the stability of polymer coated nanoparticles, the control of their surface chemistry and the reproducibility of their syntheses and applications.

4. Experimental

AuNP-conjugate synthesis. Citrate stabilized AuNP with 12 nm diameter, the ligands PEGMUA2k and -5k, conjugates AuNP@PEGMUA and AuNP@PEGMUA/MUA were synthesized as described previously.^{[7][25][47]} Concentrated solutions were prepared by repeated centrifugation at 20,000 g starting with 2 h centrifugation of 12 x 45 ml aliquots in 50 ml tubes (Rotilabo® centrifuge-tubes, Carl Roth, Germany). The supernatants were carefully removed and the remaining AuNP-solution transferred to 1.5 ml reaction tubes (SafeSeal tubes, Sarstedt, Germany) followed by 30 min of centrifugation. The pellets were combined in one tube. Two additional rounds of centrifugation (30 min, 20 000 g) were applied for purification and further concentration. After each centrifugation step the redispersed pellets were treated for 5-10 min with ultrasound. The final pellets were redispersed in 200-800 µl water and treated with ultrasound for 15-30 minutes. The stability of the conjugates was not affected by long ultrasound treatment as tested at temperatures up to 55° C.

Small-angle X-ray scattering (SAXS). High pressure small angle X-ray scattering measurements in a pressure range of 1 – 4000 bar have been performed at beamline I22, Diamond Light Source, Didcot, U.K., employing a high pressure sample cell.^[48] To penetrate two sealing diamond windows, an X-ray beam of 160 x 300 ($v \times h$) µm² size and energy of $E = 18$ keV (wavelength $\lambda = 0.68$ Å) was used. Two dimensional SAXS patterns were recorded by a Pilatus 3-2M detector at a sample-detector-distance of 6.704 m and an exposure time of 1

s per frame. No radiation damage was detectable as checked by repeating the 1 bar measurements.

The so-obtained SAXS curves can be described as $I(Q) \propto P(Q)S(q)$, wherein $Q = 4\pi/\lambda \sin\theta$ denotes the modulus of the wave vector transfer and 2θ the scattering angle. Details on the refinement can be found in Ref. [8]. In short, the form factor $P(Q)$ of log-normal distributed spheres was used yielding average particle radii of $R_{core} = 6$ nm and size polydispersities of 6-8 %. The values for the different samples are listed in Table S1. For modeling the structure factor $S(Q)$ the sticky hard sphere model was used.^{[8][49][50]} The experimental curves were refined in terms of the stickiness parameter which characterizes the interparticle interactions, the volume fraction, and the effective particle size R_{SHS} . As shown previously, R_{SHS} allows us to determine the ligand shell thickness s , although the scattering contrast of the ligand shell is very weak.^[8]

Thermogravimetric analysis (TGA). For TGA a Netzsch TG 209 F1 was used with 85 μ l Al_2O_3 -crucibles. The temperature program was as follows: Under nitrogen flow (40 ml/min) 293 K - 373 K at 20 K/min, 10 min at 373 K, 373 K - 523 K at 10 K/min, 10 min at 523 K, 523 K - 873 K at 20 K/min. Under air flow (80 % N_2 , 20 % O_2 , 20 ml/min) 10 min at 873 K, 873 K - 1023 K at 20 K/min. The temperature program was tested with the pure ligands PEGMUA2k and 5k. After the program the residual mass was 0-2 % indicating their complete combustion. Baselines were recorded with empty crucibles before preparing samples in them. AuNP were coated with PEGMUA2k or PEGMUA5k, concentrated and purified as described above. The concentrations of the final solutions were determined by the methods described by Haiss et al. (based on the absorbance of the diluted samples at 450 nm) or Scarabelli et al. (based on the absorbance of the diluted samples at 400 nm).^{[51][52]} The concentrations determined with these methods differed by ~15 %, with lower concentration values obtained with the latter method. Defined volumes of the AuNP-conjugate solutions were pipetted into the crucible and dried in a drying oven at 65 °C, if necessary in several steps. For

PEGMUA2k the AuNP diameter d_{AuNP} was 12.0 nm, the particle concentration was in the range 11.3-13.0 μM (corresponding to $c(\text{Au}) \sim 120 \text{ mg/ml}$) and 50 μl were dried yielding 7.99 mg. A residual mass of 6.15 mg was obtained after the temperature program, corresponding to a concentration of 11.7 μM AuNP in the 50 μl . Assuming an average molecular weight in the range 2000-2200 g/mol for PEGMUA2k, the mass fraction of ligands (23 %) corresponds to a footprint of 0.30-0.32 nm^2 and a grafting density $\sigma_g = 3.0\text{-}3.3 \text{ nm}^{-2}$. For PEGMUA5k, 200 μl of 3.9-4.6 μM AuNP@PEGMUA5k with $d_{\text{AuNP}} = 10.7 \text{ nm}$ were dried to yield 8.86 mg. A residual mass of 6.20 mg was obtained corresponding to 4.2 μM AuNP ($c(\text{Au}) \sim 30 \text{ mg/ml}$). Assuming an average molecular weight in the range 4500-5500 g/mol for PEGMUA5k, the mass fraction of ligands (30 %) corresponds to a footprint of 0.50-0.60 nm^2 and a grafting density $\sigma_g = 1.7\text{-}2.0 \text{ nm}^{-2}$.

UV/Vis-spectroscopy. Absorbance measurements were carried out using a Varian Cary 50 spectrometer. UV microcuvettes sealed with lids (Plastibrand, Carl Roth GmbH, Karlsruhe, Germany) were used for all experiments. The highly concentrated samples from the SAXS measurements ($c = 4\text{-}9 \mu\text{M}$) were diluted by factors 1000-16000 to yield suitable concentrations in the range 3-6 nM.

Calculation of layer thickness.

For the geometrical calculation we used:

$$s = \left(\frac{3V_{\text{shell}}}{4\pi} + R_{\text{core}}^3 \right)^{\frac{1}{3}} - R_{\text{core}}$$

with

$$V_{\text{shell}} = \sigma_g A_{\text{AuNP}} (V_{\text{PEG}} + N_{\text{H}_2\text{O}/\text{PEG}} V_{\text{H}_2\text{O}})$$

and

$$V_i = \frac{M_i}{N_A \rho_i}$$

V_{shell} is the volume and s the thickness of the ligand shell, R_{core} the radius of the AuNP core (based on TEM and SAXS), A_{AuNP} the surface of the AuNP assuming sphericity, σ_g the grafting density of the PEG-ligand, V_i , M_i and ρ_i are the volume, molecular weight and bulk density of the according molecular species (PEGMUA2k, PEGMUA5k or H₂O). $N_{H_2O/PEG}$ is the number of water molecules per PEG-ligand and N_A is the Avogadro constant. Molecular weights 2000 g/mol and 5000 g/mol were used for PEGMUA2k and -5k, respectively.

The Flory-radius R_F and AdG-brush height L were calculated as described in Ref [16] with a monomer length $a = 0.33$ nm and a degree of polymerization $N = 45$ for PEGMUA2k and $N = 116$ for PEGMUA5k, yielding $R_F = aN^{3/5}$ and $L = Na(a/D)^{2/3}$, where D is the distance between the grafting points of the polymers and thus $D = (\sigma_g^{-1})^{1/2}$. In the AdG-theory, the Flory-radius relates to the mushroom conformation and L describes the height of the brush. To account for the MUA part of the molecules, 0.7 nm were added to the geometrical calculations of R_F and L that account for the PEG moieties of the ligands. This value corresponds to literature values for the thickness of self-assembled MUA monolayers on gold.^{[53]–[55]}

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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