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Nanomaterials: Chemistry  $\dot{\sigma}$  Physics

# Extending SERS to Dispersions for Applications in Catalysis

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#### Abstract

Dispersible SERS-active superstructures have been prepared as a tool in the study of liquidphase catalytic reactions by Raman spectroscopy. These superstructures are composed of functionalized  $\mathrm{SiO}_2$  nanospheres that are densely covered by Au nanoparticles (NPs) to ensure the presence of interparticle gaps of a few nanometers. Upon illumination with laser light electromagnetic hot spots are formed in these gaps, which results in an enhancement of Raman scattering. An ultrathin (1-2 nm) SiO<sub>2</sub> shell protects these plasmonic superstructures and enables the adsorption of catalytic NPs. Raman scattering of molecules involved in reactions at the catalytic surface is enhanced by the hot spots. Since these superstructures are dispersible, liquid-phase catalytic reactions could now be studied. In the second part of this thesis, another approach to obtain SERS-active structures is discussed, in which Au NP clusters are formed using an organic linker molecule. The synthesis of these superstructures and Au NP clusters is followed by characterization with TEM, UV-Vis spectroscopy and DLS. With these techniques the formation of functionalized  $SiO_2$  spheres with a dense layer of Au NPs on the surface was demonstrated. Successful cluster formation was indicated as well. With Raman spectroscopy the SERS activity of the superstructures and Au NP clusters is confirmed.

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# 1 Introduction

Conversion of biomass into transportation fuels and valuable chemicals has the potential to reduce the consumption of the shrinking supply of fossil fuels.<sup>1</sup> Currently, crude oil is favored as a feedstock for these products owing to the low costs and the high efficiency of the production process, which is mainly caused by the use of high-quality catalysts.<sup>2</sup> Additionally, the infrastructure supporting the use of fossil fuels as a feedstock is already present. Competition with this feedstock by biomass demands fast, efficient and safe conversion of biomass into transportation fuels and valuable chemicals.<sup>3</sup> The development of new catalytic materials is necessary to fulfill these demands. To enable rational catalyst design an in-depth knowledge of the underlying reaction mechanisms is essential.<sup>4</sup>

Many techniques have already been utilized to gain more knowledge on catalytic reactions. Especially *in situ* spectroscopy experiments are able to provide essential information on structural changes of the catalytic material and on the molecular structure of reaction intermediates.<sup>5</sup> The field of *in situ* catalysis research is dominated by four different spectroscopy techniques:

- 1. Nuclear Magnetic Resonance (NMR) spectroscopy. With this technique the atomic nuclei in molecules are exposed to a magnetic field. The effect of the magnetic field is probed with radio waves to determine the molecular structure.<sup>5,6</sup>
- 2. UV-Vis spectroscopy. Here, absorption of light in the UV to visible range is used to investigate the electronic energy levels of conjugated molecules and metal-ions.<sup>5</sup>
- 3. X-ray spectroscopy. Using this technique the electronic as well as the geometric structure of materials is probed by analysis of the absorption of X-ray radiation. Especially, samples without long-range order are frequently studied with X-ray spectroscopy, e.g. metallic nanoparticles.<sup>7</sup>
- 4. Vibrational spectroscopy (Raman and infrared (IR) spectroscopy). These techniques are used to investigate the vibrational energy levels in molecules and solids, which is useful for the determination of their structures.

The use of *in situ* NMR spectroscopy in catalysis research mainly consists of the analysis of the products formed during a reaction. For example, the conversion of <sup>13</sup>C enriched fructose to valuable chemicals (i.e. hydroxymethylfurfural (HMF), levulinic acid (LA) and formic acid (FA)) over different catalysts was monitored with *in situ* <sup>13</sup>C NMR spectroscopy, which provided a reaction mechanism.<sup>8</sup> With *in situ* UV-Vis spectroscopy catalytically active metal-ion centers are monitored during catalysis. The formation of coke or other aromatic species can be investigated as well.<sup>5,9</sup> Zakzeski *et al.* demonstrated this by utilization of *in situ* UV-Vis spectroscopy to follow the oxidation state of cobalt salts during the oxidation of lignin model compounds in ionic liquids.<sup>10</sup> In situ X-ray spectroscopy is used to study the geometric and electronic structure of catalytically active metal NPs.<sup>7</sup> Daniel *et al.* used this technique to follow the reduction of Pt with H<sub>2</sub> in Pt-Re nanoparticles (NPs), which resulted in an active catalyst for the hydrogenolysis of glycerol to propanediol.<sup>11</sup> With *in situ* vibrational spectroscopy measurements structural changes in

the catalyst during a reaction can be followed. The formation of products of the catalytic reaction can be monitored as well.<sup>5</sup> For instance, during the acid-catalyzed hydrolysis of cellulose the formation of HMF, FA, LA and glucose was studied with in situ ATR-IR spectroscopy.<sup>12</sup> However, all above-mentioned techniques have their limitations in the exact determination of a reaction mechanism. In general, NMR spectroscopy is less suitable for the analysis of product mixtures or large molecules, since spectrum interpretation is difficult for complex samples. UV-Vis spectroscopy only probes electronic transitions, which provides insufficient information to determine an exact molecular structure. With X-ray spectroscopy mainly data on the catalyst and its interaction with an adsorbate is obtained. However, the molecular structure of the adsorbate is difficult to derive. IR spectroscopy generally suffers from the presence of water. Another issue is the low infrared signal of vibrations that lack a change in dipole moment, e.g. metal-adorbate vibrations. In contrast, in spectra obtained with Raman spectroscopy no water peak is observed. Consequently, Raman spectroscopy can be applied to a wide range of reaction conditions.<sup>13</sup> Furthermore, in Raman spectroscopy a change in polarizability (i.e. ability to form a dipole moment) is necessay for a vibration to give a measurable signal. In general, Raman active molecules exhibit a low infrared activity and vice versa.<sup>14</sup> Thus, the two vibrational spectroscopy techniques are complementary. Therefore, Raman spectroscopy is an essential addition to the available tools for unraveling catalytic reaction mechanisms.



Figure 1: Energy diagram of different electronic processes: Rayleigh scattering (a), Stokes Raman scattering (b), anti-Stokes Raman scattering (c), and fluorescence (d). Reproduced from Moura *et al.*<sup>15</sup>

In a conventional Raman spectroscopy experiment, monochromatic coherent laser light is used to illuminate a molecule, which excites ground state electrons to a virtual state. A dominant fraction of the electrons falls back to the ground state, resulting in the emission of light with the same energy as the incident light, which is called elastic (Rayleigh) scattering (see Figure 1(a)). However, a small part of the incident light is inelastically (Raman) scattered and contains information on the molecular structure (see Figure 1(b) and 1(c)). To obtain a spectrum, the elastically

scattered light is separated by a filter and the Raman scattered light is detected. A wide variety of catalytic systems were successfully probed with such experiments.<sup>16-18</sup> In particular, the technique is suitable to extract structure-activity relationships of metal-oxide catalysts. Since the metal-oxide vibration generally is highly Raman active, the chemical properties of the catalytic active site can be determined.<sup>13</sup> However, Raman measurements can suffer from fluorescence (see Figure 1(d)), which causes a background emission that complicates observation of the signal of interest. Another issue is the degradation of the sample as a result of excitation with laser light. Furthermore, the sensitivity of Raman scattering is low: approximately only 1 out of 10<sup>8</sup> scattered photons is inelastically scattered.<sup>19</sup> Therefore, high concentrations of involved species are required to obtain a measurable Raman signal (0.3 M for pyridine, see Figure 24(a)). Several additional Raman spectroscopy techniques have been developed to tackle these drawbacks and enhance its versatility. One of these techniques is Resonance Raman Spectroscopy (RRS), which is used to improve the selectivity and sensitivity with respect to normal Raman spectroscopy.<sup>20</sup> In RRS electrons are excited close to an electronic state, which increases the Raman signal and allows analysis of the specific molecule.<sup>21</sup> In contrast to RRS, background fluorescence is absent in Coherent Anti-Stokes Raman Spectroscopy (CARS). With CARS two different pump lasers are used to induce anti-Stokes Raman scattering, i.e. scattering of light with a higher frequency than the incident light. This results in a 10<sup>5</sup>-10<sup>10</sup> enhancement of the Raman signal.<sup>22</sup> However, the sensitivity of RRS and CARS is still insufficient to study the molecular structure of catalytic reaction intermediates, which generally are present in extremely low concentrations.<sup>21–23</sup> A promising phenomenon to dramatically increase the sensitivity of normal Raman spectroscopy up to single molecule detection is Surface-Enhanced Raman Scattering (SERS).<sup>17,24</sup> Using this technique an extremely intense signal can be detected in the far-field caused by Raman scattering of a locally enhanced electromagnetic field, which is emitted by illuminated metallic nanostructures. Consequently, the molecular structure of adsorbates in catalytic reactions can be followed, leading to the discovery of new mechanisms.<sup>25,26</sup>

In a typical SERS experiment the investigated molecules are close to the surface of a metallic nanoparticle (NP), i.e. within a few nanometers. Upon irradiation of the particle with an electromagnetic field, the collective oscillations of electron density in the metal (plasmons) resonate with a fraction of the applied field, which is called Localized Surface Plasmon Resonance (LSPR). Consequently, the NP emits a highly localized enhanced field that increases Raman scattering by molecules near the NP surface. In practice, a sample, containing metallic NPs and molecules of interest, is illuminated with monochromatic laser light allowing the detection of a higher Raman signal in the far-field. The strongest Raman signal is observed for Ag NPs, but also Cu NPs show a strong SERS-effect<sup>27,28</sup> However, Ag and Cu are both easily oxidized under atmospheric conditions. Therefore, Au NPs are preferred owing to their inert nature, while a strong Raman signal is observed as well. Despite their inert nature, Au NPs are catalytically active in a number of chemical reactions, where the reaction rate increases with decreasing particle size.<sup>29,30</sup> In contrast, enhancement of the Raman signal increases with increasing NP size.<sup>31</sup> Therefore, it is impractical to simultaneously use a Au NP as both catalyst as well as a SERS substrate. However, combining small and large Au NPs in one hybrid structure enables the investigation of Au-catalyzed reactions

with SERS. Xie *et al.* demonstrated this by an *in situ* SERS measurement of the liquid-phase reduction of 4-nitrothiophenol (4-NTP) to 4-aminothiophenol (4-ATP) catalyzed by a Au/Au-SiO<sub>2</sub> plasmonic superstructure.<sup>32</sup> Here, SERS-activity was provided by a 80 nm Au NP, which was coated with an ultrathin SiO<sub>2</sub> shell of 1.5 nm. Subsequently, catalytically active 5 nm Au NPs are deposited on the SHell-Isolated Nanoparticle (SHIN) to form the Au/Au-SiO<sub>2</sub> structure. Since illumination with on-resonance light of a Au NP results in a strongly enhanced electromagnetic field, plasmonic side reactions are prevented by coating the NP surface with an inert material. Thus, to purely study the surface chemistry of the catalytic particle, coating of the SERS-active Au NP with SiO<sub>2</sub> is necessary.<sup>32, 33</sup> It should be noted that isolated Au NPs provide insufficient local field enhancement to yield a detectable Raman signal.<sup>34</sup> However, a further enhancement of the incoming electric field can be generated by interparticle gaps of a few nanometers (i.e. hot spots) as a result of LSPR coupling.<sup>17</sup> Consequently, in the Au/Au-SiO<sub>2</sub> structure a Raman signal is detected due to hot spots between the large and small particles.<sup>32</sup> This resulted in the elaboration of a reaction mechanism of the reduction of 4-NTP. Beside this specific reaction, more Au-catalyzed reactions can be studied with this structure as well.



Figure 2: Schematic representation of a dispersible  $SiO_2$  coated plasmonic structure with catalytically active NPs attached to the  $SiO_2$  layer.

To investigate Ag- and Cu-catalyzed reactions plasmonic superstructures similar to Au/Au-SiO<sub>2</sub> could be used, since these metals exhibit a SERS effect that is comparable to Au. However, the SERS effect in transition metals (e.g. Ni, Pt, Rh, Ru) is small or negligible, which limits the *in situ* study of reactions catalyzed by these metals.<sup>35</sup> A potential method to avoid this limit is by using nanocompositions consisting of a SERS-active substrate with catalytically active NPs bound to it. Zhang *et al.* produced such a composition by the charge-induced self-assembly of Pt NPs on Au SHINs forming Pt-SHINs.<sup>18</sup> Subsequently, the Pt-SHINs were dried on a Si substrate, which resulted in aggregated Pt-SHINs. Consequently, a small fraction of the Pt-catalyzed reactions

takes place in hot spots, which dramatically enhance the Raman signal. In this way, gas-phase reactions catalyzed by Pt were monitored. Similar compositions were synthesized by Hartman *et al.* using a wet impregnation of Au SHINs with Ru and Rh precursors.<sup>36</sup> A thermal reduction of the dried impregnated SHIN dispersions resulted in the formation of Ru and Rh NPs on the Au SHINs.<sup>36</sup> With these structures CO adsorption and hydrogenation were studied. A disadvantage of these methods is the poor compatibility with liquid-phase reactions. Therefore, dispersible SiO<sub>2</sub>-coated structures of aggregated Au NPs with catalytically active NPs adsorbed on the SiO<sub>2</sub> shell are required to study a wider range of catalytic reactions *in situ* (see Figure 2).

Figure 2 schematically represents the simplest form of such structures, in which a dimer of Au NPs with a small interparticle gap is coated with an ultrathin SiO<sub>2</sub> layer enabling attachment of catalytically active NPs. In theory, the hot spot in the junction of the dimer sufficiently enhances Raman scattering of catalytic species to give a measurable signal. Since the SiO<sub>2</sub> layer prevents plasmonic side reactions, purely the catalytic reaction can be studied. In this thesis, colloidal particles are used to obtain such structures in two different methods. The first method involves functionalization of SiO<sub>2</sub> NPs to enable dense adsorption of Au NPs on the surface. Due to the high density of Au NPs on the SiO<sub>2</sub> surface the interparticle distance is sufficiently small to form hot spots upon illumination. Subsequently, the Au NPs are coated with an ultrathin (1-2 nm) layer of SiO<sub>2</sub> forming SHell-Isolated Plasmonic Superstructures (SHIPSs). Ni NPs are then adsorbed on these SHIPS to enable the study of liquid-phase catalytic reactions. In the second approach, Au NPs are clustered with a linear molecule containing a sulfur group at both ends (see 1,4-MMTPEB in Figure 9), which is able to form a bond with the Au surface. Since the length of the conjugated molecule is  $\sim$ 2 nm, hot spots between linked Au NPs can be formed. After successful cluster formation isolation of these structures with SiO<sub>2</sub> would allow adsorption of catalytically active NPs. Characterization of both structures was performed with electron microscopy, UV-Vis spectroscopy, light scattering techniques and vibrational spectroscopy, which is discussed in Section 4. First, background information on SERS, the properties of colloidal particles and their synthesis is provided in Section 2. This is followed by the experimental procedures in Section 3. Finally, a conclusion and an outlook are given in Section 5.

# 2 Background

Enhancement of Raman scattering by SERS-active structures originates from the interaction of plasmonic structures with electromagnetic radiation. To fully understand the enhancement of Raman scattering by these structures, detailed descriptions of Raman spectroscopy and the SERS effect are provided in this section.

In this thesis, these SERS-active structures are prepared via the controlled cluster formation of colloidal particles. Typical colloidal properties are utilized to form these clusters. Therefore, the theory of colloidal synthesis and colloidal properties are extensively discussed in this section as well.

# 2.1 Raman & IR Spectroscopy

Interaction of an electromagnetic field with a molecule induces two different inelastic scattering events: Stokes Raman scattering and anti-Stokes Raman scattering. Both events are characterized by a frequency shift of the scattered field with respect to incident field. In Stokes Raman scattering an electron is exited to a virtual state, which falls back to a vibrational level above the ground state leading to the emission of a red-shifted photon (see Figure 1(b)). On the other hand anti-Stokes Raman scattering involves excitation of an electron from a vibrational level above the ground state to a virtual state. Subsequently, the electron falls back to the ground state, which results in the emission of a blue-shifted photon (see Figure 1(c)).<sup>37</sup> Since the population of these vibrational levels is determined by Boltzmann statistics, a dominant fraction of the electrons is in the ground state. Therefore, Stokes Raman scattering is the main inelastic scattering event.<sup>38</sup>

In Raman spectroscopy the (Stokes) Raman scattered field of an illuminated molecule is detected. Subsequently, the frequency difference of the incident and detected field is determined. Since this frequency difference is characteristic for a specific vibration, the molecular structure can be resolved. However, the number of vibrations that are effectively detected with Raman spectroscopy is restricted by selection rules. These rules dictate that an electron in a virtual state can only fall back to the ground state or a state that involves a change in polarizability. Therefore, to determine the Raman activity of a specific vibration, the distribution of electrons around the chemical bond has to be regarded. If electrons are evenly distributed around the chemical bond, a vibration is accompanied by a change in polarizability. One example is the C=C stretch vibration, which generally corresponds to an intense peak in the Raman spectrum.<sup>38</sup> Another intrinsic issue of Raman spectroscopy is the low cross section of Raman scattering, which leads to a poor signal of low-concentration species. A frequently used method to increase the signal is to adsorb the molecule of interest on a metallic nanostructures, which leads to surface-enhanced Raman scattering (see Section 2.2.3).

The vibrational levels of a molecule can also be occupied as a result of absorption of infrared (IR) radiation, which is utilized in IR spectroscopy. With this technique a molecule is exposed to IR radiation and the non-absorbed part of the incident field is detected, which is another method

to obtain information on the vibrational levels of the probed sample. Absorption of infrared radiation is subjected to selection rules as well, which dictate that a change in dipole moment is required for a vibration to be IR-active. For example, in a water molecule two hydrogen atoms with a low electronegativity vibrate with a highly electronegative oxygen atom. Consequently, the dipole moment in the O-H bond continuously changes upon vibration, which leads to a high IR-activity.

# 2.2 Optical and Electronic Properties of Metallic NPs

Enhancement of the Raman signal can be accomplished with metallic NPs via the SERS effect. In this section, the effect of electromagnetic radiation on these NPs and the resulting enhancement of Raman scattering by the NPs are discussed.

### 2.2.1 The Optical Properties of Isolated Metallic Nanospheres

Extinction (absorption and scattering) of light by metallic materials strongly depends on the size of the illuminated object. Whereas bulk gold possesses a bright yellow color, caused by its decreased reflectivity of red light, Au nanospheres of ~ 10 nm have a wine-red color that changes to purple, when the particle size is increased.<sup>39</sup> The latter phenomenon was first calculated by Gustav Mie in 1908.<sup>40</sup> In his approach the interaction of light with metallic nanospheres was evaluated using the Maxwell equations, which describe the propagation of electric and magnetic fields through dielectric media. To properly do this a homogeneous sphere and surroundings were assumed. In addition, both media, NP and solvent, possess the same dielectric properties as the bulk material.<sup>41</sup> By solving the Maxwell equations with these assumptions it was found that the free electrons in metallic nanospheres are polarized by an electromagnetic field, which results in an overall polarization of the sphere. This polarization induces a dipole moment and the ability to form this dipole moment is defined as the polarizability ( $\alpha$ ):

$$\alpha = 4\pi\varepsilon_0 R^3 \frac{\varepsilon - \varepsilon_m}{\varepsilon - 2\varepsilon_m} \tag{1}$$

where R is the radius of the sphere,  $\varepsilon$  is the dielectric constant of the sphere, and  $\varepsilon_m$  is the dielectric constant of the medium, in which the sphere resides.<sup>41</sup> The formation of a dipole moment by an electromagnetic field thus depends on the size of the particles, the metal and the medium. Without an electromagnetic field the free electrons move through the particle generating differences in electron density. These oscillations in electron density are more commonly referred to as surface plasmons. Under influence of an electromagnetic field the induced dipole moment resonates with the incident field, which is called localized surface plasmon resonance (LSPR).<sup>28</sup> Consequently, the resonance frequency is absorbed and the rest of the electromagnetic field is scattered. In this way, absorption of green light and scattering of the remaining incoming light is the origin of the ruby red color of a dispersion of spherical Au NPs. Furthermore, non-spherical Au NPs like Au nanorods possess two different resonance frequencies, since a longitudinal and a transverse plasmon are present in these particles.<sup>42</sup> The longitudinal plasmon has the lower resonance frequency and corresponds to fluctuations in electron density in the length of the rod. In the same way, the width of the rod determines the transversal plasmon with the higher resonance frequency. By changing the particle size, the solvent, or the particle morphology the frequency of absorption (i.e. plasmon frequency) can be tuned.<sup>43,44</sup> This relation also follows from Equation 1, since the plasmon frequency is related to the polarizability via the induced dipole moment.<sup>17</sup>

### 2.2.2 Local Enhancement of the Electromagnetic Field



Figure 3: The optical and electronic effects of an electromagnetic wave on a Au NP. A dipole moment emitting an enhanced electromagnetic field (indicated by the negative and positive charges) is induced by SPR.

Upon plasmon resonance with an incoming electromagnetic field large amounts of energy are absorbed. A metallic colloidal NP can lose this energy via electron-phonon relaxations, which result in lattice vibrations, i.e. heat. Via diffusion this heat is dissipated into the solution in a few picoseconds.<sup>41</sup> However, the main energy loss mechanism of metallic NPs is radiation of a strongly enhanced electromagnetic field. Due to illumination with light that matches the plasmon frequency a dipole is induced within the NP. Consequently, a positively and negatively charged part are simultaneously present that are separated by a neutral segment (see Figure 3). Therefore, the induced dipole can be effectively described as a nanoantenna. Illumination with on-resonance light continuously drives the antenna leading to emission of an electromagnetic field with the same frequency as the resonance frequency. The strength of this field depends on the incident field ( $E_0$ ) via

$$E \approx E_0 \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m} \tag{2}$$

Thus, a much stronger field E is radiated by the antenna, when the resonance condition  $\varepsilon + 2\varepsilon_m \rightarrow 0$  is fulfilled.<sup>45</sup> Since the strength of this field scales with  $\sim 1/r^3$ , the enhancement solely is a local effect.<sup>17</sup> Furthermore, an additional enhancement is obtained upon particle aggregation as a

result of hot spot formation. In these hot spots surface plasmons overlap and couple to form a gap-mode plasmon. A very strong electric field is radiated as a result of the coupled plasmons.<sup>28</sup> Beside aggregation, hot spots can be formed by self-assembly or lithography techniques as well. Moreover, the strength of the additionally enhanced field depends on the size of the interpaticle gap. With increasing gap size the plasmon coupling becomes negligible and no additional enhancement is observed.<sup>46</sup> However, a very small interparticle distance (<1 nm) might lead to electron tunneling, which dramatically decreases the strength of the radiated field.<sup>17</sup> Hence, a 1-2 nm gap is favored for a strong enhancement.

#### 2.2.3 Surface-Enhanced Raman Scattering

Molecules residing near the surface of an illuminated metallic NP experience the same electromagnetic field as the NP as well as an enhanced field, which is emitted by the induced dipole. Consequently, a larger absolute number of photons is inelastically scattered, leading to an enhanced Raman signal. The intensity of this surface-enhanced Raman scattering (SERS) is given by:

$$I_{SERS} = I_0 I_{Raman} = |E_0|^2 |E_{Raman}|^2$$
(3)

where  $E_{Raman}$  is the field that is the result of Raman scattering of E and  $E_0$  by the molecule.  $I_{SERS}$ ,  $I_0$  and  $I_{Raman}$  correspond to the intensities of SERS, the incoming field and the Raman scattered field, respectively. Especially, for the high-frequency fraction of visible light the approximation  $E_0 \approx E_{Raman}$  holds, which leads to the  $I_{SERS} \approx |E_0|^4$  relation.<sup>17</sup> Furthermore, not only this electromagnetic enhancement but also a chemical enhancement contributes to the far-field radiation emitted by the molecule. The chemical enhancement includes all contributing factors that result from metal-molecule interactions. Here, three subclasses can be distinguished: a molecular resonance mechanism, a charge-transfer mechanism and a nonresonant chemical mechanism.<sup>47</sup> Molecular resonance takes place when the frequency of the incoming light matches a molecular electronic transition. Adsorption of a molecule on a metallic NP can shift the energy levels of the molecule leading to the emergence of a molecular resonance. Since resonant Raman scattering has a higher efficiency than normal Raman scattering, this can result in an enhanced Raman signal.<sup>17,48</sup> Second, charge-transfer of a metal to an adsorbed molecule leads to more inelastically scattered photons as well. In this mechanism a high-energy electron is transferred from the metal to the LUMO of the molecule. Subsequently, the electron loses part of its energy by radiation of a inelastically scattered photon, which is accompanied by an increased Raman intensity.<sup>47</sup> Finally, a small contribution to the overall enhancement is an effect of a nonresonant chemical mechanism. In contrast with the other mechanisms, no illumination of the NP and the adsorbed molecule is required for this mechanism to take place. Thus, nonresonant chemical enhancement merely consists of the ground-state interactions of the molecule with the metallic surface. It is presumed that the local environment of the molecule is the determinative for the impact of this mechanism.<sup>49</sup> Overall, these enhancements are quantified by the enhancement factor (EF):

$$EF = \frac{I_{SERS}/N_{surface}}{I_{bulk}/N_{bulk}} \tag{4}$$

with  $N_{surface}$  and  $N_{bulk}$  being the number of molecules near the surface of the metallic NP and the number of molecules in a bulk sample, respectively.  $I_{bulk}$  is the Raman scattering intensity of the bulk sample.<sup>50</sup> In general, a dominant fraction of the EF (~10<sup>5</sup> or higher) is attributed to electromagnetic enhancement, while the chemical enhancement mechanisms contribute a relatively small part to the EF (~10<sup>1</sup>-10<sup>2</sup>).<sup>45</sup>

### 2.3 **Properties of Colloidal Particles**

In this thesis, SERS-active structures are prepared using colloidal particles, which are macromolecular entities with typically one dimension in the 1-1000 nm size range. In a colloidal dispersion, these particles are uniformly distributed in a dispersion medium, e.g. a solvent. Two examples of a colloidal dispersions are the aqueous dispersions of Au and SiO<sub>2</sub> particles (see Section 2.4.1 and 2.4.2). To understand the relevant physical and chemical properties of these systems the concept of colloidal stability needs to be treated. In addition, the basic principles of colloidal synthesis are discussed to understand Au and SiO<sub>2</sub> particle formation. The size and surface charge of these particles can be characterized using light scattering measurements, which are explained afterwards.



Figure 4: A Au NP stabilized by negatively charged (mainly citrate) and positively charged (mainly Na<sup>+</sup>) species, which form the EDL. The locations of the Stern layer and slipping plane boundary are indicated with arrows.

### 2.3.1 Stability

In a stable colloidal dispersion a metastable dispersed phase of single colloidal particles is indefinitely maintained. Stability is favored by repulsive interparticle forces, which prevent flocculation or coagulation. However, attractive forces are inevitably present in every colloid as well. Predominantly, interaction between colloidal particles consists of interatomic forces of the atoms in different particles. First, the presence of attractive forces is a result of van der Waals attraction between the nuclei of atoms. Attraction increases with decreasing internucleus separation or increasing polarizability of the atom.<sup>51</sup> Second, a repulsive force is induced at a small interparticle separation owing to the interaction of electron clouds (i.e. Born repulsion).<sup>52</sup> Furthermore, the surface properties of a colloidal particle influence the stability of the system.<sup>53</sup> Here, an important quantity for colloidal stability is surface charge, which is mainly determined by the ions distributed around the surface. Depending on the surface potential of the colloidal particle, positively or negatively charged ions strongly adsorb on the surface, forming the so-called Stern layer (see Figure 4). To compensate the charge of the adsorbed ions a second layer of ions is diffusely bound to the colloidal particle. The strongly and diffusely bounded ions together form the Electrical Double Layer (EDL).<sup>54</sup> According to Coulumb's Law, a repulsive force is present between two equally charged species.<sup>51</sup> Therefore, EDLs with a charge of equal sign repel each other, which is the origin of electrostatic stabilization.

The van der Waals interaction  $(G_{vdW})$  and the EDL interactions  $(G_{electrostatic})$  were combined in the Derjaguin–Landau–Verwey–Overbeek theory (DLVO) to theoretically predict the stability of a colloid.<sup>53,55</sup> Following this theory, the total free energy of interaction in a colloid (*G*) is described by:

$$G = G_{vdW} + G_{electrostatic} \tag{5}$$

Here, the size and the content of the colloidal particle determine the strength of the van der Waals interaction. In addition,  $G_{electrostatic}$  depends on the thickness of the EDL, which decreases with increasing electrolyte concentration.<sup>51</sup> Therefore, the contribution of  $G_{vdW}$  to G is relatively constant, while  $G_{electrostatic}$  strongly depends on the dispersion medium.

Another effect that influences colloidal stability is steric repulsion. Instead of the formation of an EDL, the surface of a colloidal particle can also be coated with neutrally charged molecules that are adsorbed. In dispersion these particles collide as a result of Brownian motion leading to interpenetration of the adsorbed surface layers. Consequently, the concentration of adsorbed molecules increases at the collision site. Then, an in-flow of the dispersion medium, caused by osmosis, leads to expansion of the adsorbed surface layers, which drives the colloidal particles apart.<sup>51,53</sup> Thus, this steric repulsion can be the origin of colloidal stability as well. In the DLVO theory the contribution of steric repulsion is not included in the free energy of interaction (G). other forces are known to influence colloidal stability as well, e.g. hydrophobic and solvation forces.<sup>55</sup> Therefore, a third term should be included in G to obtain a more complete description of colloidal stability:

$$G = G_{vdW} + G_{electrostatic} + G_{non-DLVO}$$
<sup>(6)</sup>

Here,  $G_{non-DLVO}$  accounts for the non-DLVO forces, including steric, hydrophobic and solvation forces.

#### 2.3.2 Colloidal Synthesis

A dispersion of particles in the colloidal size range can be obtained by breaking down a bulk material or by building up particles with atomic or molecular monomers (i.e. the nucleation method).<sup>51</sup> The latter is preferred for the production of colloidal particles of Au and SiO<sub>2</sub> owing to the convenient synthesis procedure and the equal size of the obtained particles (i.e. monodispersity). In this method colloidal particles are formed in a solution with a monomer concentration (C) above the saturation concentration ( $C_{sat}$ ). In such supersaturated solutions the Gibbs free energy change ( $\Delta G$ ) of particle formation is determined by the degree of supersaturation ( $C/C_{sat}$ ) and the surface tension of a particle ( $\sigma$ ) leading to

$$\Delta G = -nk_B T ln[C/C_{sat}] + \sigma b n^{2/3} \tag{7}$$

Here, n is the number of monomers,  $k_B$  is the Boltzmann constant, T is the temperature and b is a geometric factor.<sup>51,56</sup> It follows from Equation 7 that at a sufficiently high degree of supersaturation particle formation (i.e. nucleation) is favored over preservation of monomers in solution. These particles are the first thermodynamically stable clusters of monomers and are defined as nuclei.<sup>57</sup> After nucleation the nuclei grow by monomer addition to reach the final particle size. In addition, at a higher  $C/C_{sat}$  the effect of the surface tension becomes less significant and smaller nuclei are formed, which results in the production of a larger amount of smaller final particles.<sup>51,56</sup>



Figure 5: LaMer's model for the growth of colloidal NPs. Reproduced from LaMer et al.58

A frequently used model to describe nucleation and growth was first proposed by LaMer (see Figure 5).<sup>56,58</sup> In this model nucleation is preceded by an induction period, in which the degree of supersaturation increases by the formation of monomers. At a specific concentration of monomers ( $C_{min}$ ) nuclei are rapidly formed from the monomers during a burst of nucleation.<sup>56</sup> Due to this fast consumption of monomers a maximum concentration ( $C_{max}$ ) is reached and decreases to below  $C_{min}$ . Now, the concentration of monomers is too low for nucleation to occur and already formed nuclei grow by monomer addition to reach the final particle size. During the growth stage particles of different sizes are present, which causes a process called Ostwald ripening. In this process the overall particle surface is minimized by the growth of large particles at the expense of small particles.<sup>51,56</sup>

### 2.3.3 Light Scattering

In section 2.2.1, the ability of an electromagnetic field to induce a dipole moment in a metallic NP was already described. The induced dipole is driven by resonance with surface plasmons in the particle, which results in radiation of a locally enhanced field.<sup>17</sup> However, formation of a dipole moment upon illumination also occurs in NPs without resonance.<sup>51</sup> This non-resonant dipole emits radiation of the same frequency as the incoming field, which is known as elastic scattering (Rayleigh scattering). Using the classical approach of Rayleigh-Debye-Gans (RDG) theory elastic scattering by a colloid can be approximated, assuming

$$m = n_p/n_m, \qquad |m-1| \ll 1, \qquad \frac{2\pi R}{\lambda} |m-1| \ll 1,$$
 (8)

Here,  $n_p$  and  $n_m$  are the complex refractive indices of the particle and the dispersion medium, respectively, and  $\lambda$  is the wavelength of the incident light.<sup>59</sup> Therefore, similar refractive media are required for the RDG theory. A suitable particle size with respect to  $\lambda$  is necessary as well to ensure that the whole particle is subjected to the same field strength at a given moment. When above conditions are fulfilled, the scattering intensity of a single colloidal particle follows from  $\lambda$ , the scattering angle and characteristics of the colloid  $(n_p, n_m, R \text{ and particle morphology})$ .<sup>59</sup> If the RDG conditions are not fulfilled, reflection at the solvent-particle interface ( $|m - 1| \ll 1$ ) or formation of multiple dipoles within the particle  $(R/\lambda \approx 1)$  takes place.<sup>51</sup> In the latter, these dipoles interact resulting in intraparticle multiple scattering, which dramatically complicates computations.<sup>60</sup> In the non-RGD regime, elastic scattering by a colloidal particle is more accurately described by an electrodynamic approach using Mie theory.<sup>51,59</sup>

Regarding a dispersion of colloidal particles, scattering of the individual particles can be simply summed to obtain its scattering pattern. However, for this summation a dilute dispersion is required, because interparticle multiple scattering is negligible in this regime. Notably, the obtained scattering pattern fluctuates dynamically over time, since the position of the individual particles changes as a result of Brownian motion.<sup>51</sup> Detection of this fluctuating pattern is the basis of Dynamic Light Scattering (DLS) (i.e. photon correlation spectroscopy). In this technique, monochromatic polarized laser light is radiated onto a dilute dispersion.<sup>61</sup> Consequently, a scattering pattern can be detected in the far-field that changes during irradiation. The change in detected intensity correlates with the motion of the particles. Via auto-correlational computations this motion can be analyzed to yield the diffusion coefficient (*D*). Subsequently, the hydrodynamic radius ( $r_h$ ) of the particles in dispersion can be determined with the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi \eta r_h} \tag{9}$$

Here,  $\eta$  is viscosity of the dispersion medium.<sup>59</sup> It should be noted that  $r_h$  is the radius of a hard sphere with an equal diffusion coefficient as the measured colloidal particle. Therefore, accurate size determination of non-spherical particles by DLS demands additional theoretical experiments.<sup>62</sup> Finally, a change in EDL thickness, resulting from variations in electrolyte concentration, can influence the hydrodynamic radius as well.<sup>63</sup>

### 2.3.4 Zeta potential

An important property of electrostatically stabilized colloidal particles is the surface charge, since this determines the stability of the dispersion (see Section 2.3.1). Under influence of an electric field these particles move carrying the diffuse layer of ions around the surface with them (see Figure 4).<sup>51</sup> The outer boundary of this diffuse layer is called the shear plane or slipping plane. The electrical potential at this plane is defined as the zeta potential( $\zeta$ ), which is used as a measure of the net surface charge.<sup>64</sup> To calculate the zeta potential the Henry equation is used,

$$u_e = \frac{2\zeta\varepsilon_m Ef(\kappa R)}{3\eta} \tag{10}$$

Where the  $f(\kappa R)$  factor is called the Henry function, which takes the inverse Debye length ( $\kappa$ ) and the particle radius (R) into account. In general, the value of the Henry function is 1.5 for aqueous dispersion media (Smoluchowski approximation) and 1 for non-aqueous dispersion media (Huckel approximation).<sup>65</sup> In addition,  $u_e$  is defined as the electrophoretic mobility of a colloidal particle under influence of an electric field (E).<sup>51</sup>

Experimental determination of the electrophoretic mobility is generally used to calculate the zeta potential of colloidal particles. Here, one frequently utilized technique is Laser-Doppler Electrophoresis (LDE).<sup>65</sup> A typical experiment consists of the application of an electric field to a colloid. Depending on the surface charge, the colloidal particles move along or against the field direction. Subsequently, a monochromatic laser is focused on the moving particles, which leads to elastic scattering of the incident light. Since the particles possess velocity, the frequency of the scattered light is slightly shifted as a result of the Doppler effect. After detection of the scattered light the electrophoretic mobility can be determined, because the Doppler frequency shift and the velocity (i.e. mobility) are related. Using Equation 10, the zeta potential is then obtained. Typically, the values of the zeta potential range from -100 mV to +100 mv. However, it is generally considered that for a stable colloid the absolute value of the zeta potential is equal to or larger than 30 mV.<sup>51</sup>

### 2.4 Synthesis of NPs

The production of the colloidal Au and  $SiO_2$  NPs involves solution-processed bottom-up particle growth. In this thesis, the Turkevich method<sup>66</sup> is used for the synthesis of Au NPs and spherical  $SiO_2$  NPs are synthesized with the Stöber method.<sup>67</sup> Although particle growth is comparable to LaMer's model described in Section 2.3.2, the exact mechanism is fundamentally different. Therefore, a detailed discussion of both mechanisms is provided in the following sections.

#### 2.4.1 The Turkevich Method

Semi-spherical Au NPs are produced with the Turkevich method, which consists of the reduction of chloroauric acid (HAuCl<sub>4</sub>) with trisodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) in an aqueous solution. A nucleation and growth model, first proposed by LaMer, is frequently used to elucidate the mechanism behind particle formation (see Figure 5 and Section 2.4.2).<sup>58,66</sup> However, x-ray spectroscopy and scattering studies provide a more decisive insight, in which the seed-mediated growth model is proposed.<sup>68</sup>

(a) 
$$[\operatorname{AuCl}_4]^{-}$$
 +  $\begin{bmatrix} \operatorname{HOOC-H}_2C, \operatorname{CH}_2\operatorname{-COOH} \\ \operatorname{HO} & \operatorname{COO} \end{bmatrix}^{-} \xrightarrow{-\operatorname{Cl}} \begin{bmatrix} O \\ O \\ H \\ \operatorname{Cl} & \operatorname{CH}_2\operatorname{-COOH} \\ \operatorname{Cl} & O \\ \operatorname{Cl} & \operatorname{Cl} \end{bmatrix}^{-} \\ \xrightarrow{-\operatorname{Cl}} [\operatorname{AuCl}_4]^{-} + \operatorname{HOOC} \xrightarrow{O} \\ \xrightarrow{-\operatorname{COOH}} \\ \xrightarrow{-\operatorname{COOH}} + \\ \xrightarrow{-\operatorname{Cl}} \begin{bmatrix} \operatorname{AuCl}_4 \end{bmatrix}^{-} + \operatorname{HOOC} \xrightarrow{O} \\ \xrightarrow{-\operatorname{COOH}} \\$ 

(b)  $C_6H_8O_7 \xrightarrow{pH=3.1} [C_6H_7O_7]^7 \xrightarrow{pH=4.8} [C_6H_6O_7]^{2^-} \xrightarrow{pH=6.4} [C_6H_5O_7]^{3^-}$ 

(c) 
$$[AuCl_4]$$
  $\xrightarrow{\text{pH}=5.4}$   $[AuCl_3OH]$   $\xrightarrow{\text{pH}=6.4}$   $[AuCl_2(OH)_2]$   $\xrightarrow{\text{pH}=7.5}$   $[AuCl(OH)_3]$   $\xrightarrow{\text{pH}=8.3}$   $[Au(OH)_4]$ 

Figure 6: Reaction mechanism of the most reactive  $[Au]^{3+}$  reduction pathway (a). The dominant citrate species at the indicated pH values (b). The dominant Au complexes at the indicated pH values (c).

In the first step of the seed-mediated growth model the citrate reduction of  $HAuCl_4$  produces  $Au^0$  monomers, which form small Au clusters. An essential parameter in this synthesis is the

pH. In Figure 6(a) the most reactive pathway of [Au]<sup>3+</sup> reduction is shown, which predominantly takes place at a pH<4.8.<sup>69</sup> At this pH  $[AuCl_4]^-$  is mostly present, which is the most reactive  $[AuCl_{4-x}(OH)_{x}]^{-}$  complex (see Figure 6(c)). However, during the standard Turkevich procedure the pH changes upon citrate addition resulting in different pHs at each stage of the synthesis.<sup>57</sup> In general, the synthesis starts by preparation of a HAuCl<sub>4</sub> solution with a pH<5.4, since predominantly  $[AuCl_4]^-$  ions are present.<sup>69</sup> To this solution  $[C_6H_5O_7]^{3-}$  ions are added enabling the reduction of  $[AuCl_4]^-$ . As a result of  $[C_6H_5O_7]^{3-}$  addition the pH of the reaction mixture was increased (see Figure 6(b)). This leads to the hydroxylation of  $[AuCl_4]^-$  forming the less reactive Au complexes. However, this hydroxylation is slow compared to the reduction rate of Au<sup>+</sup>. Therefore, a large amount of Au<sup>0</sup> monomers is produced right after citrate addition followed by a significant decrease of the reduction rate caused by hydroxylation of the Au complexes.<sup>57</sup> Simultaneously, Au<sup>0</sup> monomers form small clusters, which mature into seeds of  $\sim$ 1.5 nm by coalescence during the second step of the seed-mediated growth model.<sup>57,68</sup> Here, colloidal stability of the seeds is provided by the EDL, which is mainly composed of Na<sup>+</sup>, citrate and  $[AuCl_{4-x}(OH)_x]^-$  (see Figure 4 and Section 2.3.1). In this way, coalescence with other seeds is prevented. Another possible growth mechanism in this step is Ostwald ripening.<sup>68</sup> In the third step the seeds slowly grow by the reduction of  $[AuCl_{4-x}(OH)_x]^-$  in the EDL. Here, the growth rate is limited by the low reaction rate of  $[AuCl_{4-v}(OH)_{v}]^{-1}$  reduction.<sup>68</sup> During the fourth step the final size of the Au NPs is reached by fast monomer consumption. The increased reaction rate is attributed to an autocatalytic effect of the gold surface.<sup>57</sup>

After the formation of stable seeds in steps 1 and 2 the Au NPs grow during steps 3 and 4 to their final size, which mainly depends on the HAuCl<sub>4</sub> : Na<sub>3</sub>Cit ratio. Variation of this ratio yields a final particle diameter in the range of 9 nm to 120 nm.<sup>44</sup> However, nonspherical particles with an increasing polydispersity are observed for larger Au NP sizes. To maintain a spherical shape and a narrow size distribution, a seeded-growth procedure is used, in which control over the growth stage is gained. In this approach small Au NPs (~ 15 nm) are produced via the Turkevich method. Subsequently, a mild reducing agent, e.g. hydroxylamine, and an additional amount of Na<sub>3</sub>Cit and HAuCl<sub>4</sub> are added to the previously synthesized Au NPs (~ 15 nm) to reach the final particle size.<sup>70,71</sup> Separated and slow addition of the precursor solutions further increases the control over the growth stage, which results in monodisperse nearly spherical Au NPs with a large diameter (30-300 nm).<sup>72</sup>

The fundamental difference between the described seed-mediated growth model and the nucleation and growth model lies in the early stages of both models. In the nucleation and growth model a burst of nucleation forms a large amount of nuclei with a size that corresponds to the first thermodynamically stable cluster size (typically a few atoms).<sup>57</sup> Subsequently, the nuclei grow to reach their final particle size, while maintaining a constant number of particles (see Section 2.3.2). In the seed-mediated growth model coalescence results in a significant reduction of the amount of particles. Another difference is the large size of the seeds ( $\sim$  1.5 nm) compared to the typical nucleus size. Therefore, gold NP formation via the Turkevich method deviates from the nucleation and growth model and the seed-mediated growth model should be employed for a more precise description.

#### 2.4.2 Synthesis of SiO<sub>2</sub> Nanospheres

In the Stöber method, monodisperse spherical  $SiO_2$  NPs are formed by hydrolysis of alkyl silicates (e.g. tetraethyl orthosilicate (TEOS)) followed by condensation of the products.<sup>73</sup> The reaction is catalyzed by aqueous ammonia and takes place in an alcoholic solution via the following reaction mechanism:

Hydrolysis



Condensation

Figure 7: Reaction mechanism of hydrolysis and condensation of alkyl silicates to form a Si-O-Si bond, R = H,  $C_n H_{2n+1}$ .

Figure 7 summarizes all the possible pathways to form Si-O-Si bonds. Notably, both water condensation as well as alcohol condensation can occur. Since every silicon atom is tetra-substituted, all these condensation pathways lead to the generation of a network of silicon and oxygen atoms, eventually resulting in SiO<sub>2</sub> particle formation. The particle size and size distribution of the particles can be tuned by varying the reaction parameters. From theory it follows that an increase in temperature results in a decrease in particle size. An increase in the feed rate of TEOS to an ammonia and water mixture also leads to smaller particles.<sup>74,75</sup> Furthermore, it is possible to tune the properties of the SiO<sub>2</sub> particles by changing the solvent, alkyl silicate or catalyst.<sup>73,76,77</sup> However, for this work variations in the ammonia and TEOS concentration are the most interesting changes of reaction parameters. Therefore, these are discussed in more detail.

The ammonia concentration influences the final particle size owing to its catalytic activity in both hydrolysis as well as condensation.<sup>78</sup> During hydrolysis a hydroxide ion formed by ammonia is able to react with the silicon center, which forces one of the ethoxy groups to leave. Subsequently, in the condensation reaction ammonia deprotonates the formed silanol group, which is able to form a Si-O-Si bond with another silanol or silicate (see Figure 7). Increasing the ammonia concentration increases the rate of hydrolysis and condensation forming more monomers. According to LaMer's model of nucleation and growth (i.e. monomer-addition model) an increase in monomer concentration results in a higher nucleation rate (see Section 2.3.2). This leads to

smaller particles, since more nuclei are formed and thus the nuclei have less monomers to grow from. However, when the ammonia concentration is increased, the opposite is empirically determined.<sup>79</sup> This result is explained by the stimulating effect an increase of ammonia has on the condensation rate. The hydrolysis rate benefits less from an increase in ammonia than the condensation rate, which leads to a decrease in nucleation rate.<sup>80</sup> A lower number of nuclei during the growth stage leads to larger particles. So an increase in ammonia concentration leads to an increase in SiO<sub>2</sub> particle size.<sup>81</sup>



Figure 8: Schematic representation of the mechanism of  $SiO_2$  particle growth following the aggregation-only model.

By the same reasoning, an increase in TEOS concentration leads to larger SiO<sub>2</sub> particles, since this also promotes the hydrolysis and condensation rate.<sup>79,82</sup> However, when the [TEOS]/[NH<sub>3</sub>] ratio becomes too large, a shortage of ammonia limits the hydrolysis and condensation reactions, which leads to a multimodal size distribution.<sup>81</sup> This phenomenon is hard to understand using the monomer-addition model. However, another method of describing the formation of SiO<sub>2</sub> particles by hydrolysis and condensation of TEOS is the aggregation-only model. In this model it is assumed that growth by monomer addition does not happen and that newly formed subparticles are likely to aggregate. Particle growth thus only occurs via aggregation of multiple subparticles or aggregation of a large particle with a subparticle, which is shown in Figure 8.<sup>83–85</sup> When [TEOS]/[NH<sub>3</sub>] is large, subparticles are constantly being produced. These subparticles are not fully consumed by large particles, because the rate of aggregation of subparticles with large particles is lower than the subparticle-subparticles aggregation rate. Therefore, the excess of subparticles enables to formation of smaller large particles, resulting in a bimodal distribution.<sup>81,84</sup>

The monomer-addition and the aggregation-only model both fail to fully explain all empirically determined trends. The effect of changes in ammonia concentration are explained very well by the monomer-addition model, which is inadequate for understanding the effect of variations in TEOS concentration. For this phenomenon the aggregation-only model is more suitable. The basic assumptions of the models are not unambiguous. The colloidal instability of subparticles and the growth of particles via surface reactions of monomers are two examples of topics that cause dissension.<sup>86,87</sup> However, a combination of both models is more widely accepted. In the

combined model aggregation of subparticles leads to the formation of large particles. Subsequently, monomers and small siloxane clusters react with the surface of the large particles to form a smooth surface and reach the final particle size.<sup>77,87</sup>

In summary, the Turkevich method and the Stöber method, yielding Au and  $SiO_2$  NPs respectively, involve two fundamentally different mechanisms of colloidal particle growth. In the next sections, the preparation and characterization of these particles is discussed in detail.

# 3 Materials & methods

# 3.1 Materials

Hydroxylamine hydrochloride (98.0 %) (NH<sub>2</sub>OH·HCl), triethylamine (99 %), magnesium sulfate (99.5 %) (MgSO<sub>4</sub>), (3-aminopropyl)trimethoxysilane (97 %) (APTMS), silver nitrate (99.0 %) (AgNO<sub>3</sub>), copper iodide (98.0 %) (CuI), (3-mercaptopropyl)trimethoxysilane (95 %) (MPTMS), sodium silicate solution (~27 % SiO<sub>2</sub> in 14 % NaOH), N,N-diisopropylethylamine (99 %), 1,4-diethylbenzene (96%), acetone (ChromaSolv, 99.9 %), bis(triphenylphosphine)palladium(II) dichloride (99.99 %)  $(Pd(PPh_3)_4Cl_2)$ , poly(ethyleneimine) solution (~50 % in H<sub>2</sub>O) (PEI), sodium hydroxide (EMSURE,99 %) (NaOH), ammonia solution (EMSURE, 28-30 %) (NH<sub>3</sub>(aq)) and hydrogen chloride (EMSURE, 37 %) (HCl) were purchased from Sigma-Aldrich. Trisodium citrate dihydrate (99 %), ethanol (99.5 %) (EtOH) and sodium sulfate (99.0 %) were obtained from Acros Organics. Hexane (mixture of isomers), dichloromethane (practical grade) (CH<sub>2</sub>Cl<sub>2</sub>) and ethyl acetate were bought from Interchema. A dispersion of Au NPs (Au40) was provided by BBI Solutions. Acetonitrile (99.95 %) was bought from BioSolve. N-Methyl-2-pyrrolidone (99 %) (NMP) was bought from Fisher Scientific. Chloromethyl methyl ether (95 %) (MOMCl) was obtained from abcr GmbH. Chloroform-D (D, 99.8 %) (CDCl<sub>3</sub>) was provided by Cambridge Isotope Laboratories, Inc. MilliQ water (MQ) with a resistivity of 18.2 M $\Omega$ ·cm at 25 °C, filtered by a Direct-Q 3 UV (Millipore), was used. 4bromothiophenol (98 %) and HAuCl<sub>4</sub>·3 H<sub>2</sub>O were purchased from Alfa Aesar. Pure HAuCl<sub>4</sub>·3 H<sub>2</sub>O was stored under inert atmosphere in a glovebox. A stock solution of HAuCl<sub>4</sub>·3 H<sub>2</sub>O in MQ (26.4 mM) was kept outside the glovebox for direct use in the following synthesis procedures.

# 3.2 Superstructures of Au and SiO<sub>2</sub> NPs

The first step in the production of the superstructures is the synthesis of the building blocks, being the Au and  $\text{SiO}_2$  NPs. Next, the  $\text{SiO}_2$  NPs are functionalized to increase the affinity between  $\text{SiO}_2$  and Au NPs. This is followed by the adsorption of the Au NPs on the functionalized  $\text{SiO}_2$  NP, forming the superstructures. The last step is coating the superstructures with an ultrathin layer of  $\text{SiO}_2$ .

# 3.2.1 Synthesis of Au NPs

For the production of Au NPs an adjustment of the Turkevich method was used.<sup>66</sup> Prior to the synthesis all glassware was cleaned with aqua regia (1:3  $\text{HNO}_3$ :HCl), MQ and ethanol followed by drying in the oven. Subsequently, a yellow 2.64 mM  $\text{HAuCl}_4 \cdot 3 \text{ H}_2\text{O}$  solution was prepared by dilution of the stock solution with MQ. 3.48 mL of  $\text{HAuCl}_4 \cdot 3 \text{ H}_2\text{O}$  (2.64 mM) was added to 30 mL of MQ in a 250 mL round-bottom flask (RBF). The mixture was heated to 100 °C under magnetic stirring (800 rpm) and 0.18 mL of 5 wt% trisodium citrate dihydrate was added to the boiling solution. The reaction was allowed to continue for 15 minutes. During the reaction a ruby red color arose, which indicated the formation of Au NPs (Au17). The Au17 dsipersion was cooled down to room temperature. Next, 0.94 mL of this Au17 solution and 0.11 mL of 5 wt% trisodium citrate dihydrate were added to 90 mL of MQ in a 250 mL RBF. Under vigorous stirring (2000)

rpm) 0.1 mL of NH<sub>2</sub>OH·HCl (2.64 mM) and 0.1 mL of HAuCl<sub>4</sub>·3 H<sub>2</sub>O (2.64 mM) were alternatingly added in a dropwise manner up to a total of 5 mL of each precursor in 30 minutes. The mixture was stirred for an additional 5 minutes to complete the seeded-growth of Au17 yielding large Au NPs (Au56 and Au63).

#### 3.2.2 Synthesis of SiO<sub>2</sub> NPs

For the synthesis of SiO<sub>2</sub> NPs of different sizes the Stöber method was used, in which an alkyl silicate is hydrolized and condensated in an ethanolic solution catalyzed by ammonia.<sup>73</sup> The exact reaction parameters yielding the different SiO<sub>2</sub> dispersion are listed in Table 1 and the dispersions are named according to the corresponding particle size (see Section 4.2). The synthesis procedures of SiO<sub>2</sub>461, SiO<sub>2</sub>564 and SiO<sub>2</sub>742 started by mixing a fraction of the EtOH with the NH<sub>3</sub>(aq) in a 100 mL RBF. All EtOH and NH<sub>3</sub>(aq) were mixed in the synthesis of SiO<sub>2</sub>125. H<sub>2</sub>O (MQ) was added to the EtOH-NH<sub>3</sub>(aq) mixture in the procedure of SiO<sub>2</sub>461. Separately, a mixture of TEOS and the remaining EtOH or pure TEOS (in the synthesis of SiO<sub>2</sub>125) was rapidly added to the (H<sub>2</sub>O-)EtOH-NH<sub>3</sub>(aq) mixture. Addition took place under vigorous stirring (500 rpm). During the reaction the color of the mixture changed from colorless to turbid white (the reaction times are listed in Table 1). Washing of SiO<sub>2</sub>461, SiO<sub>2</sub>564 and SiO<sub>2</sub>742 was performed with centrifuge-assisted sedimentation. The supernatant was discarded and a 1:1 ethanol:MQ mixture was added to the precipitate, which was repeated three times. The washed particles were redispersed in ethanol. In the procedure of SiO<sub>2</sub>125 washing was performed after functionalization.

Table 1: Reaction conditions for the synthesis of SiO<sub>2</sub> NPs of different sizes.

Name	TEOS	NH <sub>3</sub> (aq)	H <sub>2</sub> O	EtOH	Time	Comments
	(mL)	(mL)	(mL)	(mL)	(hours)	
SiO <sub>2</sub> 125	3.8	6.6	-	88.5	2	Stirred at 1200 rpm
$SiO_2461$	10.0	2.5	26	104	48	Solve TEOS in 50 mL EtOH
$SiO_2742$	1.0	10.0	-	50	2	Solve TEOS in 4 mL EtOH
SiO <sub>2</sub> 564	1.0	10.0	-	50	48	Solve TEOS in 4 mL EtOH

### 3.2.3 Functionalization of SiO<sub>2</sub> NPs

A slightly adjusted method of Claesson *et al.* was used for the functionalization of  $SiO_2$  NPs with propylamine and propylthiol.<sup>88</sup> For the functionalization of  $SiO_2125$  with propylamine an excess of APTMS (0.7 mL) was added directly after the synthesis of  $SiO_2125$  to the complete volume of the dispersion; an excess MPTMS (0.7 mL) was used for functionalization with propylthiol. The reaction mixture was stirred for 40 minutes. Next, the volume was reduced to one-third by distillation at ambient pressure at 110 °C using a Vigreux distilling column. Subsequently, the functionalized  $SiO_2125$  were washed with EtOH in three steps and the precipitate was redispersed in EtOH. For the functionalization of  $SiO_2461$  and  $SiO_2742$  with propylamine and propylthiol the volume of the  $SiO_2$  dispersions was diluted with 3 eq. ethanol in a 100 mL RBF. Furthermore, an

excess of ammonia (0.2 eq) was added to enable hydrolysis and condensation of the alkyl silicates with the surface of the NP. After addition of an excess of MPTMS or APTMS (0.1 eq.) the reaction is allowed to continue for 2 hours, followed by three washing steps with MQ and EtOH. Propylamine functionalized particles were redispersed in MQ; EtOH was used to redisperse propylthiol functionalized particles.

For the functionalization of SiO<sub>2</sub> NPs with PEI the procedure of Padmanabhan *et al.* was followed.<sup>89</sup> First, 300  $\mu$ L of SiO<sub>2</sub>564 or SiO<sub>2</sub>742 dispersion was diluted with 2.7 mL of MQ. A PEI solution was made by adding 1 mL of MQ to an excess of PEI (0.15 g). Prior to the addition of the PEI solution the pH of the diluted SiO<sub>2</sub> dispersion was adjusted to 11-12 with NaOH (0.1 M). Thereafter, the PEI solution was added to the SiO<sub>2</sub> dispersion and the mixture was stirred for 4 hours. NaOH and the excess PEI are removed by three washing steps and the PEI-functionalized particles were redispersed in MQ. A similar procedure with 0.5 g of PEI was used to coat SiO<sub>2</sub>461 with PEI.

### 3.2.4 Production of Superstructures

Table 2: Overview of the functionalized  $SiO_2$  particles and the superstructures reported in this thesis. In the last column the volume of the  $SiO_2$  dispersion is listed, which was used to prepare the corresponding superstructure. The added volume of Au NP dispersion was kept constant.

Name	SiO <sub>2</sub> dispersion	Coating	Au dispersion	Added amount of $SiO_2$ (µL)
SiO <sub>2</sub> -SH125	SiO <sub>2</sub> 125	propylthiol	-	-
SiO <sub>2</sub> -SH461	SiO <sub>2</sub> 461	propylthiol	-	-
SiO <sub>2</sub> -NH <sub>2</sub> 125	SiO <sub>2</sub> 125	propylamine	-	-
$SiO_2$ -NH <sub>2</sub> 461	SiO <sub>2</sub> 461	propylamine	-	-
SiO <sub>2</sub> -PEI461	SiO <sub>2</sub> 461	PEI	-	-
SiO <sub>2</sub> -PEI564	$SiO_2564$	PEI	-	-
SiO <sub>2</sub> -PEI742	$SiO_2742$	PEI	-	-
Au17SiO <sub>2</sub> -SH125	SiO <sub>2</sub> 125	propylthiol	Au17	45
Au17SiO <sub>2</sub> -SH461	SiO <sub>2</sub> 461	propylthiol	Au17	2.5
Au40SiO <sub>2</sub> -SH461	SiO <sub>2</sub> 461	propylthiol	Au40	2.5
Au63SiO <sub>2</sub> -SH461	SiO <sub>2</sub> 461	propylthiol	Au63	2.5
Au40SiO <sub>2</sub> -NH <sub>2</sub> 125	SiO <sub>2</sub> 125	propylamine	Au40	125
Au17SiO <sub>2</sub> -NH <sub>2</sub> 461	SiO <sub>2</sub> 461	propylamine	Au17	2.5
$Au40SiO_2$ - $NH_2461$	SiO <sub>2</sub> 461	propylamine	Au40	2.5
Au17SiO <sub>2</sub> -PEI461	SiO <sub>2</sub> 461	PEI	Au17	12.5
Au40SiO <sub>2</sub> -PEI461	SiO <sub>2</sub> 461	PEI	Au40	12.5
Au63SiO <sub>2</sub> -PEI461	SiO <sub>2</sub> 461	PEI	Au63	12.5
Au63SiO <sub>2</sub> -PEI564	$SiO_2564$	PEI	Au63	2.5
Au56SiO <sub>2</sub> -PEI742	SiO <sub>2</sub> 742	PEI	Au56	50

Superstructures were synthesized by mixing a Au NP dispersion with a functionalized SiO<sub>2</sub> dispersion. The number concentrations of the functionalized SiO<sub>2</sub> dispersion was unknown. Therefore, it was not possible to synthesize superstructures with a constant number of SiO<sub>2</sub> NPs. Instead, rough estimates on the concentration can be made, based on the obtained particle size with respect to the added reactant. However, the number concentration of Au NPs can be accurately determined with UV-Vis spectroscopy.<sup>90</sup> By addition of a large excess of Au NPs in the superstructure synthesis the number concentration of the functionalized SiO<sub>2</sub> dispersion becomes irrelevant, since adsorption of a maximum amount of Au NPs is ensured in this way.

A large set of different combinations of Au NP and SiO<sub>2</sub> NP dispersions are used in the formation of superstructures. The different combinations are listed in Table 2. The synthesis of the superstructures started by diluting the functionalized SiO<sub>2</sub> dispersion with MQ. Propylthiol-coated SiO<sub>2</sub> NPs were first dispersed in MQ. Dilution was performed by adding MQ to the functionalized SiO<sub>2</sub> dispersion (see Table 2 for the exact added volume). An equal volume of Au NP dispersion as the diluted SiO<sub>2</sub> dispersion was added and this was stirred (400-500 rpm) for 2 hours in a sample vial.

### 3.2.5 SHIPS Synthesis and Adsorption of Ni NPs

The synthesis procedure of Li *et al.* was used to coat the superstructures with  $SiO_2$ .<sup>33</sup> First, 2 mL Au63SiO<sub>2</sub>-PEI564 dispersion was diluted with 8 mL MQ. To the diluted Au63SiO<sub>2</sub>-PEI564 dispersion 13 µL freshly prepared 1 mM APTMS was added, which was prepared with MQ. To allow adsorption of APTMS to the Au surface the mixture was stirred (800 rpm) for 15 minutes. Separately, 1 mL of sodium silicate solution, 45 mL of MQ and 0.3 mL of HCl (1 M) were added to a 50 mL volumetric flask, which was filled to the mark with MQ. The pH of the diluted sodium silicate solution was kept below 11 (around 10.7). To start SiO<sub>2</sub> growth 108 µL of the diluted sodium silicate solution was added dropwise to the diluted superstructure dispersion and the reaction was continued for 48 hours forming (Au63SiO<sub>2</sub>-PEI564)-SHIPS. Washing was performed in three steps with MQ using centrifuge-assisted sedimentation (8 minutes at 4500 rpm). (Au63SiO<sub>2</sub>-PEI564)-SHIPS were resdispersed in MQ.

Colloidal Ni particles were synthesized by Wilbert Vrijburg following a modified procedure of Metin *et al.*<sup>91,92</sup> The surfactants of these particles are oleylamine and oleic acid, which provide colloidal stability.<sup>91</sup> Prior to adsorption on (Au63SiO<sub>2</sub>-PEI564)-SHIPS 0.0045 g of dried Ni particles were redispersed in 20 mL of NMP. 20  $\mu$ L of this dispersion is added to 0.5 mL of SHIPS dispersion. The mixture of particles was sonicated for 30 minutes to adsorp the Ni particles on the SiO<sub>2</sub> shell and form Ni-SHIPS. To remove non-adsorbed Ni particles this dispersion is washed using centrifuge-assisted sedimentation. The Ni-SHIPS were redispersed in 0.5 mL of MQ.

# 3.3 Au NP Clustering with a Linker Molecule

The synthesis of the linker molecule consists of the Sonogashira coupling of 4-bromothiophenol with a protected thiol group (see Figure 9). For this synthesis, the procedure of Sakamoto *et al.* 

was used.<sup>93</sup> Subsequently, the protected product of the Sonogashira coupling was added to Au63 to form Au NP clusters.



Figure 9: Reaction schemes of the synthesis of (4-bromophenyl)(methoxymethyl)sulfane (4-BPMMS) (a), 1,4-bis((4-((methoxymethyl)thio)phenyl)ethynyl)benzene (1,4-MMTPEB) (b) and 4,4'-(1,4-phenylenebis(ethyne-2,1-diyl))dibenzenethiol (1,4-PBEDDB) (c).

#### 3.3.1 Protection of 4-bromothiophenol

First, an ice-bath cooled reflux setup was built, since the reaction is exothermic and chloromethyl methyl ether (MOMCl) is a volatile toxic compound. Subsequently, 3.05 g of 4-bromothiophenol was dissolved in 15 mL of acetonitrile and 3.7 mL of N,N-diisopropylethylamine in the flask of the setup. 2.0 mL of MOMCl was added dropwise, while the temperature was kept below 10 °C. The mixture was stirred (500 rpm) for 3 hours at 0 - 2 °C. Then, 40 mL of ethyl acetate was added to extract the product, which was triply washed with 20 mL of brine (NaCl saturated water). The product layer was dried with MgSO<sub>4</sub> and the solvent was removed by rotational evaporation to yield 3.46 g of yellow liquid as product (92.5 % yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.41 (singlet, 3H), 4.92 (singlet, 2H), 7.35 (doublet, 2H), 7.40 (doublet, 2H) (see Appendix D.1), which corresponds with (4-bromophenyl)(methoxymethyl)sulfane (4-BPMMS) (see Figure 9(a)).

### 3.3.2 The Sonogashira Coupling Reaction

Prior to the synthesis the protected 3.46 g of 4-BPMMS was mixed with 180 mL of triethylamine. Subsequently, the mixture was degassed by bubbling  $N_2$  for 30 minutes. Separately, 1.02 g of Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> and 0.20 g of CuI were added to a reflux setup and degassed by three cycles of evacu-

ation and refilling with N<sub>2</sub>. The degassed solids were dissolved in the triethylamine solution, while N<sub>2</sub> was bubbled to ensure a degassed reaction mixture. Finally, 1.62 g of 1,4-diethynylbenzene was added and the mixture is degassed for an additional 5 minutes. Then, the reaction temperature was raised to 90 °C using an oil bath and the mixture was allowed to reflux for 20 hours. The reaction was terminated by removal of the heat source and the product was extracted with ethyl acetate. The product layer was triply washed with brine, dried with MgSO<sub>4</sub> and the solvent was removed by rotational evaporation to yield a red-brown solid. Further purification of the crude product was performed by SiO<sub>2</sub> column chromatography using hexane/CH<sub>2</sub>Cl<sub>2</sub> 2/1 as eluent. During the chromatography procedure the polarity of the eluent was increased to elute the desired product ( $R_f = 0.27$ ) from the column. The eluent was removed by rotational evaporation to obtain 0.63 g of a yellow-orange solid (19.5 % yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.45 (singlet, 6H), 4.96 (singlet, 4H), 7.40 (singlet, 8H), 7.48 (singlet, 4H) (see Appendix D.3), which corresponds to 1,4-bis((4-((methoxymethyl)thio)phenyl)ethynyl)benzene (1,4-MMTPEB) (see Figure 9(b)).

### 3.3.3 Deprotection of 1,4-MMTPEB

Recovering the thiol groups was started by placing 0.13 g of 1,4-MMTPEB under  $N_2$  atmosphere. Separately, a solution of 0.14 g of AgNO<sub>3</sub> in 40 mL of ethanol was made, to which 40 mL of  $CH_2Cl_2$  is added. The mixture was degassed by bubbling  $N_2$  for 10 minutes. The mixture was stirred overnight in the dark under inert atmosphere. Via Schlenk filtration the yellow precipitate was separated, which was washed with 80 mL of  $CH_2Cl_2$  in three steps. The washed precipitate was resuspended in 40 mL of degassed  $CH_2Cl_2$  and 40 mL of degassed HCl (6 M) was added. The mixture was stirred in the dark for 3 hours under inert atmosphere. The organic layer was triply washed with water and brine and dried over  $Na_2SO_4$ . Acquisition of pure 4,4'-(1,4-phenylenebis(ethyne-2,1-diyl))dibenzenethiol (1,4-PBEDDB) (see Figure 9(c)) was not successful (see Appendix D.4), despite variation of several reaction parameters (reaction time, light exposure and the use of inert or normal atmosphere).

### 3.3.4 Au NP Clustering Using a Linker Molecule

Particle linking by direct addition of 1,4-MMTPEB to a dispersion of Au NPs is obstructed by the insolubility of this molecule in water. To overcome its insolubility  $2 \cdot 10^{-3}$  g of 1,4-MMTPEB was dissolved in 10 mL of acetone. From this solution 10 µL was added to 1 mL of Au63 dispersion and 3 mL of MQ. The mixture was stirred for 2 hours yielding 1,4-MMTPEB-Au63. To obtain (0.2)1,4-MMTPEB-Au63 a similar procedure was followed with 0.2 eq of the 1,4-MMTPEB solution.

# 3.4 Characterization

UV-Vis absorption spectra were recorded on a Varian Cary-50 Conc UV-Visible spectrophotometer. Quartz cuvets with an optical path length of 10.00 mm were used for the measurements, which were filled with 2 mL sample. Prior to data collection a reference spectrum of MQ was taken, which was subtracted from the spectrum of the sample by the Cary WinUV software. Infrared spectroscopy measurements were performed on a Thermo Scientific Nicolet iS5 FT-IR spectrometer combined with the Thermo Scientific iD7 ATR accessory. Spectra were recorded from one droplet of sample, which was dropped with a Pasteur pipette on the ATR crystal. The OMNIC software was used for spectrum acquisition.

A Renishaw inVia Raman confocal microscope coupled to a 532 nm and 785 nm laser was used to measure Raman spectra. A 1200 lines/mm grating and a  $50 \times$  objective were used with varying laser power and exposure time. Samples were vacuum dried on a Si wafer. If it is mentioned, a pyridine solution (0.3 M) was added to the dried sample, which was vacuum dried as well. The microscope was focused on several spots on the wafer that contained dried particles. Prior to data collection, a reference spectrum of the Si wafer was measured. Spectra were obtained with the Renishaw WiRE 3.4 software.

Spin-coating of superstructure dispersions was performed on hydroxylated  $Si_3N_4$  wafers. Hydroxylation was performed by placing the  $Si_3N_4$  wafers in a 5:1:1 H<sub>2</sub>O:H<sub>2</sub>O<sub>2</sub>:NH<sub>4</sub>OH mixture with a temperature of 60 °C for 30 minutes. Next, the wafers are placed in boiling H<sub>2</sub>O for 30 minutes. The wafers are dried by a stream of dry N<sub>2</sub> followed by placement of the wafers in the oven for 30 minutes at 120 °C. Spin-coating was carried out by addition of 10 µL sample on the wafer, which was spinning at 2500 rpm.

Raman measurements of NP dispersions were performed with an Avantes AvaSpec-Hero spectrometer couple to an integrated 785 nm Raman probe manufactured by Innovative Photonic Solutions. A 1200 lines/mm grating and 4.6 mW laser power were used. Spectra were obtained using the AvaSoft 8 software.

Particle size and aggregation were studied with Transmission Electron Microscopy (TEM) on a FEI Tecnai-10 (100 kV), Tecnai-12 (120 kV) or Tecnai-20FEG (200 kV) microscope. Images were attained from 10  $\mu$ L of the sample that was vacuum dried on Formvar Cu-grids (300 mesh) obtained from Van Loenen Instruments. The particle size was quantitatively determined using the iTEM software by averaging the size of 100-300 particles. Grid preparation for cryo-TEM was performed with a FEI Vitrobot, in which 3  $\mu$ L of the sample was dropped on glow-discharged Quantifoil R2/2 grid in a H<sub>2</sub>O saturated atmosphere. The grid with wet sample was then blotted for 2 seconds and rapidly injected in liquid ethane to freeze the liquid sample film. A Gatan 626 cryo-transfer holder was used for grid transport and loading of the grid in the Tecnai-20FEG microscope.

Scannning Electron Microscopy (SEM) images were obtained with a FEI Helios nanolab 600 DualBeam microscope using standard microscope control software. An operation voltage of 2.0 kV at a current of 0.2 nA were used. Before the SEM measurements were performed, all samples were coated with Pt in a Cressington 208 HR sputter coater using a Pt target.

DLS and LDE data were acquired with a bifunctional Malvern Instruments Zetasizer Nano-ZS using the Malvern Instruments software. For a DLS measurement samples were prepared by dilution with MQ. With a laser pointer light scattering by the diluted sample was tested and adjusted, if necessary. The samples were loaded into polystyrol/polystyrene cuvets with an optical path length of 10 mm. Forward scattering measurements at a detection angle of 13° and backward scattering measurements at a detection angle of 173° were performed. Data from backward scattering measurements was used in further analysis unless stated differently. Particle size distributions are obtained by averaging 5-10 intensity (%) distributions. The peak positions of the

distributions corresponds to the hydrodynamic diameter of the particles in the measured dispersion. In the presentation of the DLS results the particle size distributions are smoothened with a B-spline interpolation.

DTS1070 cells were used to obtain the zeta-potential in LDE measurements. Zeta-potentials were measured over a pH-range with a Malvern Instruments MPT-2 Autotitrator using 0.001-1 M HCl and NaOH solutions. In the presentation of the LDE results the particle size distributions are smoothened with a B-spline interpolation.

# 4 Results and Discussion

# 4.1 Au NPs

In this section the synthesis and characterization of small (17 nm), intermediate (40 nm) and large (63 nm) Au NPs are discussed. The intermediate citrate stabilized Au NPs are provided by BBI solutions and are used without pretreatment. Small and large Au NPs are obtained from the colloidal synthesis procedure, described in Section 3.2.1.

In the first step of the synthesis procedure, small Au NPs are formed by reduction of  $HAuCl_4$  with citrate, which is accompanied by several color changes of the reaction mixture. First, the yellow color changes to blue-purple shortly after the addition of citrate followed by the emergence of a ruby red color at the final stage of the procedure. Here, light absorption by  $[AuCl_{4-x}(OH_x)]^-$  complexes causes the yellow color of the reaction mixture.<sup>94</sup> The change of color to blue-purple is likely the result of adsorption of  $HAuCl_4$  on the surface of the Au NPs, which affects the dielectric properties of the medium.<sup>95</sup> Finally, the ruby red color is caused by scattering of non-resonant light and this indicates successful formation of the small Au NPs.

In the second step of the synthesis procedure small Au NPs are grown by a seeded growth approach. First, a diluted dispersion of small Au NPs is prepared, which possesses a light ruby red color. Upon dropwise addition of  $HAuCl_4$  and  $NH_2OH \cdot HCl$  solutions an intense pink color emerges. The color change is the result of a red-shift of the LSPR caused by the increasing particle size. The color intensification is a result of the increasing particle size as well, since the total scattered light energy scales quadratically with the volume of the scattering particle.<sup>96</sup>



Figure 10: TEM images of semi-spherical Au NPs; (a) Au17, (b) Au40, and (c) Au63.

Experimental data on the size and morphology of the Au NPs is obtained with TEM. In Figure 10 TEM images of the vacuum-dried dispersions are presented. Regarding all three dispersions, the particle morphology is semi-spherical. However, more anisotropic shapes are observed for larger particles, e.g. the truncated triangles in Figure 10(b) and 10(c). Furthermore, in Figure 10(a) particle sintering has occurred owing to the unstable surface of these relatively small Au NPs and

the high energy of the electron beam used in TEM.<sup>97</sup> Finally, particle sizes of 16 nm, 39 nm, and 62 nm were found for Au17, Au40, and Au63, respectively. For all Au NP dispersions the standard deviation of this size determination is below 10 %, which is an indication for a low polydispersity. Moreover, the standard deviation increases with increasing particle size (see Table 4).<sup>71</sup>



Figure 11: (a) UV-Vis absorption spectra of Au17, Au40, and Au63. The position of the peak corresponds to a particle size of 15 nm, 36 nm, and 60 nm for Au17, Au40, and Au63, respectively.<sup>90</sup> (b) Particle size distributions of Au17, Au40, and Au63 obtained by DLS measurements. The peaks of the distributions are located at 21 nm, 46 nm, and 67 nm with a peak width of 6 nm, 10 nm and 13 nm for Au17, Au40, and Au63, respectively.

With UV-Vis spectroscopy light absorption by the dispersions of Au NPs is measured. The obtained absorption spectra are shown in Figure 11(a), which all contain a peak in the 450 nm to 650 nm region. As explained in Section 2.2.1, this peak originates from resonance of the incident electromagnetic field with the surface plasmons in the Au NPs. In addition, in the wavelength region below 450 nm an increased absorption is observed compared to wavelengths above 650 nm. This increased absorption is the result of interband transitions of d-electrons.<sup>98</sup> Light with a wavelength above 650 nm hardly resonates with surface plasmons of these Au NPs nor can it excite d-band electrons. Therefore, nearly no light absorption is observed in this region. Concerning the individual spectra of the different dispersions, the peak wavelength increases with increasing particle size, which is in agreement with the theory treated in Section 2.2.1. Utilizing the work of Haiss *et al.* the average size of the Au NPs in dispersion is determined based on the absorption peak position.<sup>90</sup> The particles sizes obtained with this approach are 15 nm, 36 nm, and 60 nm for Au17, Au40, and Au63, respectively, and are listed in Table 4.

Particle size distributions of the Au NPs in dispersion are provided by DLS measurements (see Figure 11(b)). The peak positions of the distributions are 21 nm, 46 nm, and 63 nm for Au17, Au40, and Au63, respectively. Here, the size distribution broadens with increasing particle size as well (see Table 4). It should be noted that the standard deviation in particle size calculated from TEM

measurements and the peak width of a DLS measurements are two different statistically determined measures for the physical width of the particle size distribution. Moreover, all distributions are slightly asymmetrical due to a broadening in the large particle size regime. As discussed in Section 2.3.3, DLS is a bulk technique that converts a dynamically changing scattering pattern into a particle size distribution using auto-correlational calculations and the Stokes-Einstein equation (Equation 9). The intensity in the obtained distribution is based on the detected amount of scattered light. Since the effectiveness of light scattering increases with increasing particle size, an overvaluation of large particles can be observed in size distributions obtained from DLS. This is one possible explanation for the asymmetrical distributions in Figure 11(b). Additionally, the formation of small aggregates can result in the measurement of larger particles, which leads to an asymmetrically shaped distribution as well. Furthermore, it was found that dilution of the Au17 dispersion with MQ resulted in the measurement of larger particles (see Appendix A.1). It should be noted that dilution with MQ lowers the ionic concentration of the Au dispersion. Since the colloidal Au NPs are stabilized by ions, dilution with MQ could induce aggregation.<sup>51</sup> This possibly is an explanation for the measurement of larger particles as well.



Figure 12: Zeta potential distributions of Au17, Au40, and Au63 obtained by LDE measurements.

Name	pН	Zeta potential (mV)	Zeta potential st. dev. (mV)
Au17	5.8	-39	36
Au40	7.3	-45	20
Au63	3.8	-34	12

Table 3: Zeta potentials of the Au17, Au40, and Au63 at the as-obtained pH of the dispersions.

To determine the zeta potential of the Au NP dispersions LDE measurements were performed. All zeta potential distributions were obtained without dilution of the Au NP dispersion at the as-obtained pH. In Figure 12 the zeta potential distributions of Au17, Au40, and Au63 are shown.

The measured zeta potential with the standard deviation is given in Table 3. Importantly, all Au NPs have a zeta potential below -30 mV, which is generally considered as a limit for colloidal stability (see Section 2.3.4). Furthermore, an inverse correlation between pH and zeta potential is observed in Table 3. This can be explained by considering the effect of pH on the stabilizing agent of the Au NPs, i.e. citrate anions. Since a higher pH leads to deprotonation of the carboxylic acid groups, a more negative zeta potential is the expected result.<sup>99</sup>

Name	TEM size	TEM st. dev.	UV-Vis size	DLS peak size	DLS peak width	Average
	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)
Au17	16	1	15	21	6	17
Au40	39	3	36	46	10	40
Au63	62	4	60	67	13	63

Table 4: Particle size of Au NPs determined by different techniques.

In summary, the particle sizes measured with TEM, DLS, and UV-Vis spectroscopy are listed in Table 4 and the average size is used in naming the Au NP dispersions. For all Au NPs DLS measurements give the largest particle size. As discussed in Section 2.3.3, the hydrodynamic particle size is determined with DLS. In this quantity solvent and EDL effects are included, which are not incorporated in the size determination with TEM and UV-Vis spectroscopy. Consequently, a lower diffusion coefficient is calculated leading to a hydrodynamic radius that is slightly higher than the actual particle radius.<sup>51</sup> Furthermore, in UV-Vis spectroscopy the particle size is calculated using the position of the absorption peak that is related to resonance with the plasmon of the shortest dimension. In contrast, size determination with TEM is performed by measuring the longest dimension of 100-150 particles. Therefore, the Au NP size determined with TEM (D<sub>TEM</sub>), UV-Vis spectroscopy (D<sub>UV-Vis</sub>), and DLS (D<sub>DLS</sub>) follows the order D<sub>DLS</sub>>D<sub>TEM</sub>>D<sub>UV-Vis</sub>. Moreover, a low polydispersity of all the Au dispersions is indicated by the standard deviation of the size determination with TEM. This standard deviation as well as the peak width of the particle size distribution determined with DLS both increase with increasing particle size (see Table 4). In general, this phenomenon is described as Ostwald ripening, which is defined as growth of large particles by the consumption of small particles.<sup>71</sup> Finally, the Au dispersions can be considered colloidally stable, since the zeta potential is lower than -30 mV.

### 4.2 Functionalized SiO<sub>2</sub> Spheres

Dispersions of  $SiO_2125$ ,  $SiO_2461$ , and  $SiO_2742$  were synthesized with the Stöber method by varying reactant concentrations and reaction time. Subsequently, these dispersion reacted with MPTMS, APTMS, or PEI to produce  $SiO_2$ -SH,  $SiO_2$ -NH<sub>2</sub>, or  $SiO_2$ -PEI, respectively. Here, the functionalization with MPTMS and APTMS takes place via hydrolysis and condensation of the trimethoxysilane (TMS) groups with silanol groups of the  $SiO_2$  NP. This results in propylthiol and propylamine chains sticking out of the  $SiO_2$  surface. The functionalization of  $SiO_2$  NPs with PEI is based on adsorption of the PEI polymer on the surface. In this section, the size, morphology, and effect of functionalization of the  $SiO_2$  NPs are discussed.



Figure 13: TEM images of  $SiO_2$  NPs; (a)  $SiO_2125$ , (b)  $SiO_2461$ , and (c)  $SiO_2742$ .

The size and morphology of bare SiO<sub>2</sub> NPs were investigated with TEM. For SiO<sub>2</sub>125, SiO<sub>2</sub>461, and SiO<sub>2</sub>742 a particle size of 87  $\pm$  17 nm, 415  $\pm$  27 nm, and 642  $\pm$  22 nm was found, respectively. The standard deviations are relatively low, which indicates the formation of SiO<sub>2</sub> with a low polydispersity. Since damage of the SiO<sub>2</sub> structure by the electron beam leads to particle shrinkage in TEM measurements, the particle size provided by DLS is used in naming the dispersions of SiO<sub>2</sub> NPs (see Table 5).<sup>100</sup> Furthermore, the shape of the SiO<sub>2</sub> particles varies from spherical to semi-spherical. In Figure 13(a) the shape of SiO<sub>2</sub>125 particles can be observed. A dominant fraction of these particles have a semi-spherical shape, which is the result of a rough surface. In the monomer-addition model a rough surface is formed, when large amounts of partially hydrolyzed species (e.g.  $Si(OC_2H_5)_2(OH)_2$ ) are involved in surface reactions.<sup>101</sup> Consequently, branched particle growth hinders the formation of a smooth surface. On the other hand, the aggregation-only model assumes that a rough surface is generated by aggregation with subparticles (see Section 2.4.2).<sup>78</sup> In Figure 13(b) a perfect spherical shape of SiO<sub>2</sub>461 is shown, which is frequently reported for the Stöber method.<sup>67</sup> Furthermore, in Figure 13(a) and 13(c) slightly "peanut-shaped" particles are observed, which is the result of aggregation during particle growth.<sup>102</sup> Similar results are obtained for SiO<sub>2</sub>564 (see Appendix B.1). Finally, functionalization of the bare SiO<sub>2</sub> NPs barely changed the particle size determined with TEM. As an example a TEM image of SiO<sub>2</sub>-NH<sub>2</sub>461 with the measured particle size is shown in Appendix B.3.
Name	TEM size	TEM st. dev.	DLS peak size	DLS peak width
	(nm)	(nm)	(nm)	(nm)
SiO <sub>2</sub> 125	87	17	125	23
$SiO_2461$	415	27	461	54
$SiO_2742$	642	22	742	78

Table 5: Particle size of  $SiO_2$  NPs determined by TEM and DLS.



Figure 14: Particle size distributions of  $SiO_2125$ ,  $SiO_2461$ , and  $SiO_2742$  obtained by DLS measurements. The peaks of the distributions are located at 125 nm, 461 nm, and 742 nm for  $SiO_2125$ ,  $SiO_2461$ , and  $SiO_2742$ , respectively.

In Figure 14 the particle size distributions obtained with DLS are shown. The peaks of the distributions are located at 125 nm, 461 nm, and 742 nm for  $SiO_2125$ ,  $SiO_2461$ , and  $SiO_2742$ , respectively (see Table 5). A slightly asymmetrical shape of the distribution can be seen, which was also observed in the particle size distribution of the Au NPs (see Figure 11(b)). Here, aggregation by sample preparation or overvaluation of large particles are two possible explanations of the asymmetric shape as well. However, in TEM slightly "peanut-shaped" particles were observed for  $SiO_2125$  and  $SiO_2742$ , which could account for the presence of larger particles in the size distribution. Consequently, an asymmetrical shape of this distribution might be the result. Finally, functionalization of the  $SiO_2$  NPs caused a change in the particle size determined with DLS (see Appendix B.4). An increase in size was observed for  $SiO_2$ -SH461 and  $SiO_2$ -NH<sub>2</sub>461 compared to  $SiO_2461$ . Since an excess of MPTMS and APTMS was added, several layers of propylthiol or propylamine functionalized  $SiO_2$  could have been formed on  $SiO_2461$  causing a size increase. Functionalization with PEI of  $SiO_2461$  resulted in a decrease in size. Here, adjustment of the pH to 11 in the coating procedure could have induced partial dissolution of  $SiO_2461$  causing a smaller particle size. However, this is not observed in the results of TEM measurements of  $SiO_2461$ -PEI (see Appendix B.5).



Figure 15: (a) Raman spectra of bare and functionalized  $SiO_2461$  measured with 532 nm laser with a laser power of 50 mW. The positions of the S-H, -NH<sub>2</sub>, and -NH- vibration are indicated. (b) Rescaled Raman spectrum of bare and functionalized  $SiO_2461$  measured with 532 nm laser.

To investigate the success of functionalization of the  $SiO_2$  surface several characterization techniques were used. First, Raman spectroscopy measurements with a 532 nm laser on bare and functionalized  $SiO_2461$  were performed, of which the obtained spectra are shown in Figure 15. Upon functionalization of  $SiO_2461$  with propylthiol two important differences arise in the Raman spectrum. First, a small peak is observed at 2580 cm<sup>-1</sup>, which is assigned to the S-H stretch vibrations.<sup>103, 104</sup> This indicates successful functionalization with propylthiol. However, an intense peak is expected for the S-H vibration. An explanation for the low intensity might be that the surface of  $SiO_2$ -SH is only partially functionalized. In addition, in the spectra of bare  $SiO_2461$  and  $SiO_2$ -SH461 a broad band is observed in the 3200-3600 cm<sup>-1</sup> region caused by -OH vibrations of surface silanol groups.<sup>105</sup> An increase in intensity is seen for  $SiO_2$ -NH<sub>2</sub>461 and  $SiO_2$ -PEI461 in this region, which also corresponds to -NH<sub>2</sub> and -NH- vibrations. Since these groups are present in propylamine and PEI, this points towards successful functionalization as well.

Second, several peaks can be seen in the 2700-3100 cm<sup>-1</sup> region, which is more clearly shown in Figure 15(b). The weak band in the spectrum of bare SiO<sub>2</sub> at 2940 cm<sup>-1</sup> is assigned to the -CH<sub>3</sub> vibrations, indicating the presence of unhydrolyzed ethoxy groups.<sup>87</sup> At the same frequency a sharp peak is observed in the spectra of SiO<sub>2</sub>-SH461 and SiO<sub>2</sub>-NH<sub>2</sub>461, which corresponds to the -CH<sub>2</sub>- vibration in the middle of the propyl chain.<sup>104,105</sup> Since a propyl chain is present in propylthiol and propylamine, this indicates successful functionalization with these groups. In addition, both spectra contain a peak at 2985 cm<sup>-1</sup>, which is assigned to the -CH<sub>2</sub>-S- and -CH<sub>2</sub>-N-vibration.<sup>105</sup> In these spectra, vibrations in a -Si-CH<sub>2</sub>- group could account for the band around 2900 cm<sup>-1</sup>.<sup>105</sup> Furthermore, the only distinct peak in this range of the spectrum of SiO<sub>2</sub>-PEI461 is observed at 2985 cm<sup>-1</sup>, which corresponds to vibrations in the -CH<sub>2</sub>-N- groups present in PEI.

The presence of the broad band in this spectrum might be caused by the inhomogeneity of the polymer.

From the spectra in Figure 15(a) and 15(b) it can thus be concluded that functionalization of  $SiO_2461$  with propylthiol, propylamine and PEI is successful. Raman measurements with a 785 nm laser and IR measurements were performed on the bare and functionalized  $SiO_2$  spheres as well (see Appendix B.6 and B.7). However, the spectral differences between bare and functionalized  $SiO_2$  are significantly smaller using these measurements, which complicated interpretation of the spectra.



Figure 16: Zeta potential of bare and functionalized  $SiO_2461$  over a pH-range of 1-12. The vertical error bars indicate the standard deviation in the zeta potential. The standard deviation in pH was below 0.1 in all measurements and is omitted.

Another method to probe surface modifications of colloidal particles is by determination of the zeta potential. In Figure 16 the results of LDE measurements of bare and functionalized SiO<sub>2</sub>461 over a pH range of 1-12 are presented. Here, bare SiO<sub>2</sub>461 and SiO<sub>2</sub>-SH461 both show a strongly fluctuating zeta potential at a pH > 10.5. In this regime also larger standard deviation of the zeta potential are found. Since at this pH SiO<sub>2</sub> starts to dissolve, the surface continuously changes causing a fluctuating zeta potential with large standard deviations. When the pH is below 10.5 for bare SiO<sub>2</sub>461, SiO<sub>2</sub>-SH461, and SiO<sub>2</sub>-NH<sub>2</sub>461, the zeta potential increases with a decreasing pH. In the case of bare SiO<sub>2</sub> the surface is covered with silanol groups, which are slightly acidic.<sup>78</sup> Thus, the silanol groups are predominantly deprotonated at pH > 7 causing the zeta potential of ~-70 mV (see Figure 16). With decreasing pH protonation of Si-O<sup>-</sup> groups leads to a less negative

surface charge. SiO<sub>2</sub>-SH461 follows the same pH-dependence of the zeta potential, which could be explained by the only partial functionalization of SiO<sub>2</sub>461 with propylthiol indicated by Raman spectroscopy measurements (see Figure 15). Consequently, the surface of SiO<sub>2</sub>-SH461 would still be partially covered with silanol groups causing a similar pH-dependence of the zeta potential as bare SiO<sub>2</sub>461. Furthermore, from the p $K_a$  values of silanol groups on a SiO<sub>2</sub> surface (p $K_a$  = 8.5)<sup>106</sup> and of the thiol group of propylthiol (p $K_a = 10.6$ )<sup>107</sup> it follows that the silanol groups are more acidic than the thiol groups. Therefore, the effect of pH on the zeta potential is dominated by the silanol groups, which might cause the overlap of the zeta potential between bare and propylthiol functionalized SiO<sub>2</sub>. Furthermore, the zeta potential of bare and propylamine functionalized  $SiO_2461$  are equal at pH > 8.5. At a lower pH the zeta potential of  $SiO_2$ -NH<sub>2</sub>461 gradually increases with decreasing pH, which is caused by the protonation of the amine groups. At pH  ${\sim}7$ SiO<sub>2</sub>-NH<sub>2</sub>461 passes its isoelectric point resulting in a positive zeta potential. In addition, the isoelectric point of SiO<sub>2</sub>-PEI461 is reached at pH  $\sim$ 10. Here, a positive zeta potential is observed at a lower pH as well, which is the result of protonation of the amine groups present in PEI. Moreover, significant fluctuations of the zeta potential are observed for SiO<sub>2</sub>PEI-461 over the measured pH range, which are likely caused by deformations of the polymer exposing or screening amine groups.

Altogether,  $SiO_2$  NPs with a low polydispersity were synthesized using the Stöber method. The particle morphology is predominantly spherical, although particles with rough surfaces and slightly "peanut-shaped" particles are observed as well. In addition, the  $SiO_2$  surface was functionalized with propylthiol, propylamine, and PEI. Successful functionalization with propylamine and PEI was shown by analysis with Raman spectroscopy and LDE. Moreover, the LDE measurements show that the zeta potential of  $SiO_2$ -NH<sub>2</sub>461 and  $SiO_2$ -PEI461 is positive below a pH of 7 and 10, respectively. A less pronounced effect of functionalization was observed for  $SiO_2$ -SH461, which is probably the result of only partial reaction of MPTMS with the  $SiO_2$  surface.

#### 4.3 Plasmonic Superstructures

A large set of plasmonic superstructures was obtained using combinations of the different Au and functionalized  $SiO_2$  NPs. Since no control over Au NP adsorption was obtained using bare  $SiO_2$  NPs (see Appendix C.1), only superstructures containing functionalized  $SiO_2$  NPs are discussed. In this section, TEM, UV-Vis spectroscopy, and DLS measurements are used to study the effect of the  $SiO_2$  functionalization on Au NP adsorption. In addition, the ratio of the Au and  $SiO_2$  NP size is investigated. Finally, the plasmonic superstructures with the most dense adsorption of Au56 (see Appendix A.3) and Au63, potentially yielding a structure with a high SERS-activity, are treated in more detail.

#### 4.3.1 The Effect of SiO<sub>2</sub> Functionalization



Figure 17: TEM images of Au40/SiO<sub>2</sub>-SH461 (a), Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 (b), and Au40/SiO<sub>2</sub>-PEI461 (c).

With TEM measurements the adsorption of Au NPs on the functionalized  $SiO_2$  surface is visualized. It should be noted that upon sample drying on the TEM grid particles tend to aggregate. Therefore, Au NP adsorption observed in TEM images might partially be the result of drying effects. In Figure 17 the TEM images of superstructures composed of Au40 and functionalized  $SiO_2461$  are shown. Adsorption of Au40 is observed on all three samples. However, the images of Au40/SiO<sub>2</sub>-SH461 (Figure 17(a)) and Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 (Figure 17(b)) also contain a large fraction of free Au40 particles. In Figure 17(c) an image of Au40/SiO<sub>2</sub>-PEI461 is shown, in which densely adsorbed Au40 on the SiO<sub>2</sub> surface is observed. Notably, no free Au40 is present in this sample. Since TEM is a qualitative technique, information on only a few superstructures is provided. Therefore, bulk techniques as UV-Vis spectroscopy and DLS are required.

Adsorption of Au NPs can be studied with UV-Vis spectroscopy, since at a high adsorption density of Au NPs on the  $SiO_2$  surface a second absorption band arises in the 600-900 nm region. Here, the small interparticle distances of Au NPs in densely covered superstructures causes coupling of the LSPR of individual particles. Consequently, the longitudinal plasmon is extended,

which results in the emergence of a second resonance frequency with a lower energy than the transversal plasmon.<sup>42</sup> Therefore, the presence of a second absorption band is an indication for dense adsorption of Au NPs on the SiO<sub>2</sub> surface.



Figure 18: (a) UV-Vis absorption spectra of Au40/SiO<sub>2</sub>-SH461, Au40/SiO<sub>2</sub>-NH<sub>2</sub>461, and Au40/SiO<sub>2</sub>-PEI461. (b) Particle size distribution of Au40/SiO<sub>2</sub>-PEI461 determined with DLS. The peak of the distribution is located at 446 nm with a width of 39 nm. The measurement was performed by detection of scattered light at the forward angle.

In Figure 18(a) the UV-Vis absorption spectra of superstructures of Au40 and functionalized  $SiO_2461$  are shown. The absorption spectra of Au40 and  $Au40/SiO_2$ -SH461 are equally shaped indicating no dense packing of Au NPs on the  $SiO_2$  surface. In addition, slightly increased absorption in the 600-900 nm region is present in the spectrum of  $Au40/SiO_2$ -NH<sub>2</sub>461. Furthermore, an intense second absorption band can be seen in the spectrum of  $Au40/SiO_2$ -PEI461, which indicates dense Au NP packing. A similar result is obtained for superstructures of Au17 and functionalized SiO<sub>2</sub>461 (see Appendix C.2).

Another suitable technique to study Au NP adsorption is DLS, in which the shape of the measured particle size distribution can give information on the presence of free Au NPs. In Figure 18(b) the size distribution of Au40/SiO<sub>2</sub>-PEI461 is presented, which shows a peak at 446 with a width of 39 nm. Notably, no peak is observed around 40 nm, which is the result of a negligible number of free Au40 NPs. This points towards adsorption of Au40 particles on the surface of SiO<sub>2</sub>-PEI461. It should be noted that the distribution in Figure 18(b) is obtained using the forward scattering detection angle, since backward scattering measurements failed to result in a reliable particle size distribution. At the forward detection angle, light scattering is more effective for larger particles. Therefore, in a dispersion of small and large particles the intensity of light scattering by the small particles can be insufficient for a DLS measurement if the concentration of the smaller particles is too low or the concentration of the large particles is too high. In Au40/SiO<sub>2</sub>-PEI461 a large excess of Au40 is used to ensure maximum adsorption on SiO<sub>2</sub>-PEI461, so the concentration of the large SiO<sub>2</sub>-PEI461 particles is unlikely to be too high. Since forward scattering measurements

of similarly concentrated Au40 samples yield a size distribution with an intense peak at 45 nm (see Appendix A.2), the concentration of small particles is not too low either. Therefore, the particle size distribution in Figure 18(b) implies that Au40 NPs are adsorbed on the SiO<sub>2</sub> surface in Au40/SiO<sub>2</sub>-PEI461. Finally, the size distributions of Au40/SiO<sub>2</sub>-SH461 and Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 are shown in Appendix C.3, which are both multimodal with a peak around 40 nm. One explanation is that the SiO<sub>2</sub> surface is completely covered and free Au40 NPs are not able to adsorb. Another possibility is that the affinity of SiO<sub>2</sub>-SH461 and SiO<sub>2</sub>-NH<sub>2</sub>461 for Au40 is not high enough for adsorption of a major fraction of Au40. The TEM and UV-VIS spectroscopy results of Au40/SiO<sub>2</sub>-SH461 and Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 indicate that the latter is true.

In summary, a low adsorption density of Au NPs on the SiO<sub>2</sub>-SH461 surface is demonstrated by TEM, UV-Vis spectroscopy and DLS results. A slightly stronger affinity between  $SiO_2$ -NH<sub>2</sub>461 and Au40 is observed, which is indicated by the increased absorption in the 600-900 region of the UV-Vis absorption spectrum. However, this difference with SiO<sub>2</sub>-SH461 is not directly observed in the results of TEM and DLS measurements. Furthermore, all three used techniques show that Au40 densely adsorbs on the surface of SiO<sub>2</sub>-PEI461. Thus, the following trend in the affinity between functionalized SiO<sub>2</sub> and Au40 can be determined: PEI>propylamine>propylthiol. This trend is expected from the zeta potential of functionalized SiO<sub>2</sub> NPs (see Figure 16). First, the zeta potential of Au40 is considered, which is -45 mV at a pH of 7.3 (see Table 3). At this pH the zeta potential of SiO<sub>2</sub>461-PEI, SiO<sub>2</sub>-NH<sub>2</sub>461, and SiO<sub>2</sub>-SH461 is +50 mV, -3 mV and -70 mV respectively. Since an attractive force is present between opposite charges, Au40 particles strongly adsorb on the positively charged SiO<sub>2</sub>-PEI461 surface.<sup>108</sup> On the other hand, the zeta potential of SiO<sub>2</sub>-NH<sub>2</sub>461 and SiO<sub>2</sub>-SH461 is negative, which results in repulsion of Au40 particles. However, the negative charge on  $SiO_2$ -NH<sub>2</sub>461 is relatively small, which is a possible explanation for the slightly stronger adsorption of Au40 on SiO<sub>2</sub>-NH<sub>2</sub>461 than on the highly negatively charged SiO<sub>2</sub>-SH461. Furthermore, the effect of the pH on the adsorption of Au NPs on functionalized  $SiO_2$  was investigated. Since at a lower pH the zeta potential of SiO<sub>2</sub>-NH<sub>2</sub>461 becomes more positive, a denser adsorption of Au NPs is expected. However, no significant change in the adsorption density of Au40 NPs is observed, when the pH of the Au40 dispersion is lowered prior to the synthesis of the Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 using HCl (10 mM) (see Appendix C.4). Besides the zeta potential of functionalized SiO<sub>2</sub> NPs, another effect of the functional groups thus influences the adsorption of Au NPs, explaining the PEI>propylamine>propylthiol trend. The affinity between amine, imine, and thiol groups and Au surfaces probably accounts for this additional effect.<sup>89,109</sup> Here, a similar affinity is expected between amine and imine groups and Au surfaces. Since PEI contains a large number of imine groups, the largest affinity between Au NPs and SiO<sub>2</sub>-PEI is expected. A lower affinity between Au NPs and SiO<sub>2</sub>-NH<sub>2</sub> due to the smaller number of amine groups. However, a higher affinity between SiO<sub>2</sub>-SH and Au NPs was anticipated, since the highest affinity between thiol groups and Au surfaces is present.<sup>109</sup> The lower adsorption density can be explained by the only partial functionalization of  $SiO_2461$  with propylthiol.

#### 4.3.2 The Effect of Au : SiO<sub>2</sub> NP Size Ratio



Figure 19: TEM images of Au40/SiO<sub>2</sub>-NH<sub>2</sub>125 (a), Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 (b), and Au17/SiO<sub>2</sub>-NH<sub>2</sub>461 (c). The inset shows the definition of  $\theta$  which is given for each sample in the bottom left corner of the image.

The size ratio of the Au and SiO<sub>2</sub> NPs is the second parameter that was varied in the synthesis of plasmonic superstructures. In Figure 19 TEM images of superstructures with different Au :  $SiO_2$ -NH<sub>2</sub> NP size ratios are shown. Here,  $\theta$  is used as a measure of this ratio, which is defined in the inset of Figure 19(a). In Figure 19(a) the image of a superstructure with  $\theta = 23^{\circ}$  is presented, in which a large amount of free Au NPs can be seen. In addition, a small amount of Au NPs is adsorbed on the SiO<sub>2</sub> surface. Furthermore, at  $\theta = 9^{\circ}$  (see Figure 19(b)) adsorption is observed for a larger amount of Au NPs, but a large amount of free Au NPs is present as well. Morevover, a superstructure with a densely covered SiO<sub>2</sub> surface is obtained if  $\theta = 4^{\circ}$  (see Figure 19(c)). From TEM measurements it can thus be semi-qualitatively determined that a small  $\theta$  is favored for Au NP adsorption. Additionally, similar results were obtained with superstructures composed of SiO<sub>2</sub>-SH (see Appendix C.5). Furthermore, changing  $\theta$  by exclusive variation of the Au NP size resulted in comparable results for SiO<sub>2</sub>-PEI461 as well (see Appendix C.6).

In Figure 20 UV-Vis absorption spectra of the superstructures visualized in Appendix C.6 are shown. All spectra contain a peak at the SPR wavelength of both the free Au NPs as well as an absorption band in the 600-900 nm region, which indicates dense coverage of the SiO<sub>2</sub>-PEI461 surface with Au NPs. The intensity of this band increases with decreasing Au NP size demonstrating again that Au NP adsorption is favored by a small  $\theta$ . Additionally, the same correlation between adsorption density and  $\theta$  is observed with TEM. For example, in Appendix C.6 a dense adsorption of Au17 in Au17/SiO<sub>2</sub>-PEI461 is shown. A less dense adsorption of Au NPs is observed in TEM images of Au63/SiO<sub>2</sub>-PEI461 (see Appendix C.6), which also contains free Au63 particles. The presence of free Au63 particles is another indication of the lower affinity between functionalized SiO<sub>2</sub> and large Au NPs.



Figure 20: UV-Vis absorption spectra of Au17/SiO<sub>2</sub>-PEI461, Au40/SiO<sub>2</sub>-PEI461, and Au63/SiO<sub>2</sub>-PEI461.

Summarizing, from TEM measurements a relation between the Au :  $SiO_2$  NP size ratio is semiqualitatively determined, which is supported by analysis with UV-Vis spectroscopy. Using these techniques a high adsorption density of Au NPs on the surface of functionalized SiO<sub>2</sub> was observed, when  $\theta$  is small.

#### 4.3.3 The Optimal Superstructure

In Section 4.3.1 and Section 4.3.2 several important parameters in the synthesis of the superstructures were found. First, superstructures with the most dense adsorption of Au NPs were synthesized by functionalized  $SiO_2$  with the most positive zeta potential and vice versa. The zeta potential of the functionalized  $SiO_2$  thus correlates with the adsorption density of Au NPs. However, by decreasing the pH of the reaction mixture, resulting in a more positive zeta potential of functionalized  $SiO_2$ , no significant increase in adsorption density was observed. Therefore, the affinity between the functional group and the Au surface is important as well. Finally, a correlation between a small Au :  $SiO_2$  NP size ratio and dense Au NP adsorption was found.

To obtain superstructures with the strongest SERS effect, Au NPs in the 50-70 nm size range are required, since these particles possess the highest SERS activity.<sup>110</sup> Taking into account the SERS activity and the above parameters, large SiO<sub>2</sub>-PEI as well large Au NPs are used in the synthesis of the optimal superstructure. In this way, preparation of superstructures with a dense adsorption of Au NPs is ensured and a high SERS-activity is provided. In this section, the characterization of superstructures composed of SiO<sub>2</sub>-PEI742 and Au56 is discussed (see Appendix A.3 for a detailed analysis of Au56).



Figure 21: TEM image of Au56/SiO<sub>2</sub>-PEI742 with the corresponding  $\theta$  in the bottom left corner.

In Figure 21 a TEM image of  $Au56/SiO_2$ -PEI742 is shown, in which dense packing of Au56 on the SiO<sub>2</sub> surface is observed. Furthermore, free Au NPs were barely present on the TEM grid, which indicates a high affinity between SiO<sub>2</sub>-PEI742 and Au56. In Section 2.2.2, it was described that upon illumination of aggregated Au NPs hot spots are formed, in which the electromagnetic field is dramatically increased. This is the result of an interparticle distance of a few nanometers. To obtain SERS-active superstructures the presence of hot spots during illumination between the Au NPs on the SiO<sub>2</sub> surface is required. Due to the dense adsorption of Au56 on SiO<sub>2</sub>-PEI742 visualized by TEM measurements the interparticle distance is likely to be a few nanometers in Au56/SiO<sub>2</sub>-PEI742 superstructures. Therefore, a high SERS-activity is expected for these structures.



Figure 22: (a) UV-Vis absorption spectra of Au56 and Au56/SiO<sub>2</sub>-PEI742. (b) Particle size distribution of Au56/SiO<sub>2</sub>-PEI742 determined with DLS. The measurement was performed with the forward detection angle. The peak of the distribution is located at 613 nm and the peak width is 44 nm.

A stronger indication for the presence of hot spots during illumination is provided by analysis with UV-Vis spectroscopy, since plasmon coupling between Au NPs in the superstructures can be probed with this technique. In Figure 22(a) the UV-Vis absorption spectrum of Au56 and Au56/SiO<sub>2</sub>-PEI742 are shown. A strong absorption band is observed in the 600-900 nm region for Au56/SiO<sub>2</sub>-PEI742, which indicates plasmon coupling between Au NPs in Au56/SiO<sub>2</sub>-PEI742. Since plasmon coupling only takes place between Au NPs with an interparticle distance of a few nanometers, the second band in the absorption spectrum implies that Au56/SiO<sub>2</sub>-PEI742 is SERS-active.<sup>41</sup>

With DLS measurements the size of the superstructures and the presence of free Au NPs in the Au56/SiO<sub>2</sub>-PEI742 dispersion is investigated. In Figure 22(b) the size distribution of Au56/SiO<sub>2</sub>-PEI742 is presented. The distribution contains a peak at 613 nm, which is low compared to the size of bare  $SiO_2742$ . Since a layer of Au NPs is adsorbed on the surface of  $SiO_2742$  particles, light scattering is significantly increased in the superstructure. Therefore, multiple scattering potentially took place during the measurements, which resulted in a lower measured particle size.<sup>111</sup> Another explanation for the small superstructure size is a measurement error in the forward scattering experiments. An example is provided in Appendix B.2, in which a difference in particle size of 122 nm is observed between forward and backward scattering measurements. More importantly, the size distribution of Au56/SiO<sub>2</sub>-PEI742 contains no peak that corresponds with free Au NPs. This demonstrates a high adsorption density of Au56 particles on  $SiO_2$ -PEI742, which is in agreement with the observations in Section 4.3.1, Section 4.3.2, and the TEM images of Au56/SiO<sub>2</sub>-PEI742 (see Figure 21).

Overall, dense adsorption of Au56 on SiO<sub>2</sub>-PEI742 was demonstrated by TEM and UV-Vis spectroscopy measurements. The second band in the absorption spectrum of Au56/SiO<sub>2</sub>-PEI742 implies that a high SERS-activity can be expected. Furthermore, the negligible amount of free Au NPs determined by analysis with TEM and DLS is the result of the high affinity between SiO<sub>2</sub>-PEI742 and Au56. Similar results were obtained for Au63/SiO<sub>2</sub>-PEI564 (see Appendix C.7).

#### 4.3.4 SERS Activity of the Superstructures

Following the results discussed in the last section, a high SERS-activity of  $Au56/SiO_2$ -PEI742 and  $Au63/SiO_2$ -PEI564 superstructures is expected. To prove this Raman spectroscopy measurements are required. In these measurements a 785 nm laser was used, since light of this wavelength is in resonance with the coupled gap-mode plasmons of the hot spots. Appendix C.8 shows the spectrum of  $Au63/SiO_2$ -PEI564 obtained with this procedure, which consists of multiple intense peaks. Since the  $Au63/SiO_2$ -PEI564 dispersion is dried on the substrate, aggregation is inevitable. Consequently, numerous intersuperstructure hot spots are randomly present all over the substrate during the measurement. Raman scattering of a vibration that takes place inside a hot spot is dramatically enhanced, which causes the presence of multiple peaks with high intensities. However, no information on the presence of intrinsic hot spots in the superstructures is obtained in this way, since intersuperstructure hot spots are present on the wafer as well. Therefore, methods that are able to exclusively study intrinsic hot spots in the superstructures are discussed in

this section.



Figure 23: Raman spectrum of one Au63/SiO<sub>2</sub>-PEI564 superstructure on a spin-coated wafer. 10  $\mu$ L Pyridine (0.3 M) was added as probe molecule and a laser power of 4.1 mW was used. The inset shows a Raman microscope image of the Si<sub>3</sub>N<sub>4</sub> wafer, which was spin-coated with Au63/SiO<sub>2</sub>-PEI564. The isolated superstructure corresponds with the dark spot.

One method to prevent the formation of intersuperstructure hot spots is to spin-coat the wafer with a superstructure dispersion. In this way, the superstructures are homogeneously distributed on the wafer. In Appendix C.9 a SEM image of a hydrophilic Si<sub>3</sub>N<sub>4</sub> wafer spin-coated with Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 is shown as an example, in which isolated superstructures are observed. This isolation of particles prevents hot spot formation due to drying. A similar image of a wafer spincoated with Au63/SiO<sub>2</sub>-PEI564 was obtained with the Raman microscope (see the inset of Figure 23). Since the size of this superstructure is above the resolution limit, focusing the microscope on isolated superstructures is possible.<sup>112</sup> Prior to acquisition of a Raman spectrum a diluted pyridine solution (0.3 M) was dropped on the spin-coated wafer. Since pyridine is highly Raman active, it can be used as a reference in the study of SERS activity of superstructures. Additionally, the access to hot spots for external molecules is probed with pyridine. In Figure 23 the Raman spectrum of one isolated Au63/SiO<sub>2</sub>-PEI564 superstructure is shown. The spectrum was measured after the addition of 0.3 M pyridine. The peaks at 1012 cm<sup>-1</sup> and 1032 cm<sup>-1</sup> are characteristic for pyridine (see Figure 24(a) for a reference spectrum of pyridine).<sup>113</sup> A Raman spectrum of dried Au63 with pyridine was also acquired (see Appendix C.10). In this spectrum intense peaks corresponding to vibrations in pyridine are observed, which is the result of the SERS effect caused by aggregated Au63 particles.<sup>41,114</sup> The intensity of the pyridine peaks in the spectrum of dried Au63

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and in the spectrum of spin-coated Au63/SiO<sub>2</sub>-PEI564 are similar, which indicates a strong SERS effect for Au63/SiO<sub>2</sub>-PEI564. It should be noted that the laser power used in the measurement of Au63/SiO<sub>2</sub>-PEI564 is ~40 times stronger. However, the EF of a substrate composed of a dried Au NP dispersion generally is ~10<sup>6</sup>, which is among the highest EFs known for Au NPs.<sup>114</sup> Therefore, the EF of an isolated Au63/SiO<sub>2</sub>-PEI564 superstructure is still expected to be significant, despite the fact that a higher laser power was used. An exact determination of the EF was not possible, since the acquisition of a spectrum of the pyridine solution was unsuccessful using the Renishaw Raman setup.



Figure 24: (a) Raman spectrum of a 0.3 M pyridine solution. (b) Raman spectrum of dispersed Au63/SiO<sub>2</sub>-PEI564 in a solution of 1.2 mM pyridine. The measurements were conducted with the Avantes Raman probe (785 nm).

Another way to prevent intersuperstructure hot spot formation is by measuring a Raman spectrum of the superstructures in dispersion. In Figure 24(b) the spectrum of the Au63/SiO<sub>2</sub>-PEI564 superstructure dispersion with a pyridine concentration of  $1.2 \cdot 10^{-3}$  M is shown. In this spectrum the characteristic pyridine peak is observed as well. Since the intensity of this vibration in an aqueous pyridine solution is known (see Figure 24(a)), an EF of  $\sim 5 \cdot 10^2$  was calculated for Au63/SiO<sub>2</sub>-PEI564 superstructures using Equation 4 (see Appendix C.11). The SERS-activity of free Au NP is negligible, which evidences the presence of intrinsic hot spots.<sup>34</sup>

Concluding, two Raman techniques were used to study the presence of intrinsic hot spots in Au63/SiO<sub>2</sub>-PEI564. First, spin-coating a Si<sub>3</sub>N<sub>4</sub> wafer with Au63/SiO<sub>2</sub>-PEI564 dispersion provided the ability to focus on an isolated superstructure, which was followed by the acquisition of a Raman spectrum using the Renishaw Raman setup. The formation of intersuperstructure hot spots was prevented by spin-coating. Therefore, the strong Raman signal was caused by the SERS effect demonstrating the presence of intrinsic hot spots in Au63/SiO<sub>2</sub>-PEI564. Second, in measurements of dispersed Au63/SiO<sub>2</sub>-PEI564 using the Avantes Raman probe an enhancement of the Raman signal was observed as well. Therefore, it can be concluded that during illumination intrinsic hot spots are present in Au63/SiO<sub>2</sub>-PEI564 superstructures.

# 4.4 SHIPS Synthesis and the Adsorption of Ni NPs

To study catalytic mechanisms using these SERS-active plasmonic superstructures isolation by a  $SiO_2$  shell is demanded to allow adsorption of catalytic particles and to prevent plasmonic side reactions.<sup>18,103</sup> In this section,  $SiO_2$  coating of Au63/SiO<sub>2</sub>-PEI564 is discussed. Subsequently, adsorption of colloidal Ni particles is treated.



Figure 25: (a) A magnified TEM image of (Au63/SiO<sub>2</sub>-PEI564)-SHIPS. (b) Schematic representation of the TEM image of (Au63/SiO<sub>2</sub>-PEI564)-SHIPS. Orange = Au NPs, grey = SiO<sub>2</sub> spheres and SiO<sub>2</sub> shell.

Figure 25(a) shows a TEM image of (Au63/SiO<sub>2</sub>-PEI564)-SHIPS. A schematic representation of this image is provided in Figure 25(b). In the TEM image of (Au63/SiO<sub>2</sub>-PEI564)-SHIPS an ultrathin layer ( $\sim 2 \text{ nm}$ ) of a material of lower contrast is observed on the Au NPS. Since the same procedure is used as the SHIN synthesis, this thin layer probably is the SiO<sub>2</sub> shell.<sup>33</sup> The grey spherical surface on the left side of the image is the PEI-functionalized SiO<sub>2</sub> surface indicating successful SiO<sub>2</sub>-coating of the superstructures.



Figure 26: (a) TEM image of Ni-(Au63/SiO<sub>2</sub>-PEI564)-SHIPS. (b) Magnification of the indicated part of the image.

The results of adsorption of 4 nm Ni NPs are visualized in Figure 26(a), in which the Ni-(Au63/SiO<sub>2</sub>-PEI564)-SHIPS are shown. Due to the low contrast of the Ni particles magnification of the image is necessary to observe the Ni NPs (see Figure 26(b)). In the magnified image three Ni particles are distinguished in the ultrathin  $SiO_2$  shell of the superstructure. This demonstrates the successful adsorption of Ni NPs on (Au63/SiO<sub>2</sub>-PEI564)-SHIPS. It should be noted that the Ni NPs did not selectively adsorb on the thin  $SiO_2$  shell, but were observed on the surface of  $SiO_2564$  as well (see Appendix C.12). Free Ni NPs were present on this TEM image as well. However, only Raman scattering by molecules involved in catalysis on the surface of the SERS-active superstructures can be detected. Therefore, free Ni NPs are not expected to disturb the investigation of catalytic reactions on the superstructures.

To obtain catalytically active metallic Ni NPs the oleylamine and oleic acid should be removed, which can be done by UV-ozone cleaning or reduction at elevated temperatures. Since the surface was passivated by a thin layer of NiO during a gradual and controlled exposure to oxygen, further reduction is required to obtain catalytically active metallic Ni NPs.

Summarizing, Au63/SiO<sub>2</sub>-PEI564 were isolated with an ultrathin SiO<sub>2</sub> shell with a thickness of  $\sim$ 2 nm. Ni NPs were adsorbed on these coated superstructures to form Ni-(Au63/SiO<sub>2</sub>-PEI564)-SHIPS. Ni-catalyzed reactions could be studied with the Ni-(Au63/SiO<sub>2</sub>-PEI564)-SHIPS after a pre-treatment consisting of reduction at elevated temperatures or of UV-ozone cleaning followed by a shorter reduction step.

# 4.5 Au NP Clustering Using a Linker Molecule

The Sonogashira coupling reaction was performed to obtain a linker molecule with two sulfurcontaining groups at both ends. Since sulfur atoms have a high affinity for Au, Au NPs can be clustered with this molecule.<sup>93,109</sup> In this section, the synthesis of the linker molecule and characterization of 1,4-MMTPEB-Au63 are discussed.

The first step in the synthesis of the linker molecule is protection of the thiol group present in 4bromothiophenol, which prevents poisoning of the Pd-catalyst used in the Sonogashira coupling reaction.<sup>115</sup> This step is performed by reaction of 4-bromothiophenol with MOMCl to obtain pure 4-BPMMS with a high yield (96.4 %) (see Appendix D.1). Subsequently, two 4-BPMMS molecules are coupled to the terminal alkyne ends of 1,4-diethynylbenzene in the Sonogashira coupling reaction (see Appendix D.2 for the mechanism), which results in the formation of 1,4-MMTPEB. Column chromatography was used to isolate 1,4-MMTPEB with a yield of 19.5 % (see Appendix D.3). The low yield is likely the result of homocoupling of 1,4-diethynylbenzene.<sup>116</sup> This is supported by the isolation of unreacted 4-BPMMS as a result of the absence of 1,4-diethynylbenzene. Furthermore, deprotection of the S-MOM group to recover the thiol was attempted by addition of an ethanolic AgNO<sub>3</sub> solution to a solution of 1,4-MMTPEB in chloroform followed by reaction with HCl. In this reaction, Ag<sup>+</sup> attacks sulfur, which caused precipitation of the Ag-1,4-MMTPEB complex.<sup>117</sup> Reaction with HCl was reported to decompose this complex and recover the thiol group.93 In this work, isolation of deprotected 1,4-MMTPEB (i.e. 1,4-PBEDDB) unfortunately was unsuccessful (see Appendix D.4). A possible explanation is the oxidation of the newly produced thiol groups by air exposure forming disulfide compounds.<sup>118</sup> Since isolation of 1,4-PBEDDB was unsuccessful, Au NP linking is performed with 1,4-MMTPEB. In this procedure an excess of 1,4-MMTPEB is used to investigate the feasibility of Au NP linking by this molecule.



Figure 27: Cryo-TEM images of Au63 (a) and 1,4-MMTPEB-Au63 (b).

TEM measurements were performed to study the effectiveness of 1,4-MMTPEB addition in Au63 linking. In Appendix D.5 a conventional TEM image of 1,4-MMTPEB-Au63 is shown, in which clustered Au63 particles are observed. In addition, organic material seems to be present between clustered Au NPs, which could be 1,4-MMTPEB. However, clustered particles are frequently observed with TEM as a result of aggregation during sample preparation. Since no specific distinction between linked and aggregated particles can be made, cryo-TEM measurements were performed. By instantaneously freezing the sample instead of drying, aggregation upon sample preparation is prevented in cryo-TEM. Therefore, only linked particles or particles that are aggregated before sample preparation appear clustered in a cryo-TEM image. In Figure 27(a) a cryo-TEM image of Au63 is shown, in which free particles are observed on the edge of a grid hole. In addition, grey blurs are present corresponding to small ice crystals. After reaction with 1,4-MMTPEB, both clustered as well as free Au63 NPs are observed on the cryo-TEM image (see Figure 27(b)), which is an indication of the successful Au63 cluster formation. The shape of these clusters varies from nearly spherical to highly anisotropic. Furthermore, the number of Au63 NPs in one cluster is in the range of 2-15 in this image. Finally, the number concentration of particles, i.e. free Au63 or clusters, is significantly lower in 1,4-MMTPEB-Au63 with respect to Au63 resulting from cluster formation. Hence, the concentration of 1,4-MMTPEB-Au63 was increased before the sample for a cryo-TEM measurement was prepared.



Figure 28: (a) UV-Vis absorption spectra of Au63 and 1,4-MMTPEB-Au63. (b) Particle size distribution of Au63 and 1,4-MMTPEB-Au63 determined with DLS. The peak of the distribution is located at 109 nm with a width of 47 nm.

Au NP linking can be studied with UV-Vis spectroscopy as well, since plasmon coupling between linked particles is expected to result in light absorption in the 600-900 nm region.<sup>119</sup> In Figure 28(a) the UV-Vis absorption spectra of Au63 and 1,4-MMTPEB-Au63 are presented, which predominantly overlap. The absence of absorption in the 600-900 nm region can be caused by unsuccessful Au63 clustering with 1,4-MMTPEB. Another explanation is that only a few Au63 particles are clustered. Consequently, the absorption by clustered Au63 is inadequately strong with respect to free Au63 to observe an increased absorption in the 600-900 nm region.

DLS measurements were performed to probe the Au NP size after addition of 1,4-MMTPEB. In Figure 28(b) the measured particle size distributions of Au63 and 1,4-MMTPEB-Au63 are shown. The peak of the distribution shifts from 67 nm to 109 nm upon addition of 1,4-MMTPEB, which indicates successful Au NP clustering. In addition, broadening of the distribution to a particle size of 300 nm is observed demonstrating the formation of clusters of multiple Au NPs. In a spherical cluster of 300 nm, a maximum of 79 Au63 particles can be accommodated.<sup>120</sup> However, in the cryo-TEM images mainly anisotropic clusters were observed, for which determination of the exact cluster size is complicated.<sup>121</sup> Therefore, the results in Figure 28(b) only represent a rough approximation of the physical particle size distribution. In this work, dispersibility of the clusters and access of molecules to hot spots between clustered Au NPs are required to allow the study of catalytic reactions in solution. Considering these properties, a maximum cluster size of roughly 79 particles is acceptable. It should be noted that large particles are overvalued in DLS measurements. Thus, 1,4-MMTPEB-Au63 consists of only a relatively low amount of these large clusters. Additionally, in Figure 28(b) the presence of free Au63 NPs is indicated.

The effect of the amount of added linker was investigated with DLS measurements as well. Appendix D.6 shows the particle size distribution of (0.2)1,4-MMTPEB-Au63, in which 0.2 eq. of 1,4-MMTPEB was used. A nearly exact agreement with the particle size distribution of Au63 can be observed, which is an indication for unsuccessful cluster formation.

Peak (cm <sup>-1</sup> )	Assignment
1085	Vibration in the phenylthio moiety <sup>122</sup>
1128	Symmetric C–H bending in the benzene ring <sup>123</sup>
1179	Benzene ring stretch <sup>124</sup>
1584	Symmetric stretch of C=C in the benzene ring <sup><math>124</math></sup>
2208	$C \equiv C \text{ vibrations}^{123, 124}$

Table 6: Assignment of the five intense Raman vibrations of 1,4-MMTPEB.



Figure 29: Molecular structure of 1,4-MMTPEB with the position of the vibrations indicated in the figure.

Raman spectroscopy was used to analyze the SERS activity of 1,4-MMTPEB-Au63. First, a reference spectrum of 1,4-MMTPEB was obtained, which is shown in Figure 30(a). Five intense

peaks are observed in this Raman spectrum, of which the assignment is provided in Table 6. The position of the assigned vibrations in 1,4-MMTPEB is further clarified in Figure 29.



Figure 30: (a) Reference spectrum of 1,4-MMTPEB. (b) Raman spectrum of 1,4-MMTPEB-Au63 measured with a 785 nm laser with a power of 0.92 mW.

The Raman spectrum of 1,4-MMTPEB-Au63 shown in Figure 30(b) was obtained by drying the sample on a Si wafer. Compared to the reference spectrum of 1,4-MMTPEB a new peak at 1010 cm<sup>-1</sup> is observed, which is also present in the spectrum of bare Au63 (see Appendix A.4). Furthermore, the peak at 1085 cm<sup>-1</sup> is shifted to 1072 cm<sup>-1</sup>. Joo *et al.* observed a similar shift of this peak upon adsorption of benzyl phenyl sulfide on a substrate of dried 50 nm Au NPs.<sup>122</sup> In their work, a peak at 1090 cm<sup>-1</sup> assigned to the thiophenyl moiety shifted to 1076 cm<sup>-1</sup> as a result of Au–S bond formation. Since the same moiety is present in 1,4-MMTPEB, the peak shift in the Raman spectrum of 1,4-MMTPEB-Au63 (see Figure 30(b)) is attributed to a similar Au–S bond. Additionally, the intensity of the peaks corresponding to 1,4-MMTPEB is dramatically enhanced compared to intensity in the reference spectrum. To measure the reference spectrum ~10 mg 1,4-MMTPEB was deposited on a Si wafer, while only ~5 ng linker was present in the measured amount of 1,4-MMTPEB-Au63. This increase in intensity is caused by the SERS effect, which implies that a dried sample of 1,4-MMTPEB-Au63 is SERS-active.

In summary, the linker molecule 1,4-MMTPEB was synthesized with a Sonogashira coupling reaction, which was used to form Au NP clusters. Analysis with DLS indicates successful formation of clusters composed of 2-79 Au63 particles. The presence of clusters in 1,4-MMTPEB-Au63 is supported by cryo-TEM measurements. In these clusters the thiophenyl moieties of 1,4-MMTPEB are bonded to the Au surface, which was demonstrated by a peak shift in the Raman spectrum. However, the bonding of both thiophenyl moieties to two different Au NPs could not be confirmed. Therefore, only Au63 clustering is indicated and a more elaborated study is required to prove particle linking. Furthermore, the amount of added 1,4-MMTPEB is sufficient to saturate the Au surface in 1,4-MMTPEB-Au63. In the procedure to coat Au NPs with SiO<sub>2</sub>, bonding of APTMS to the Au surface is required (see Section 3.2.5). Saturation of Au surface possibly hinders the bonding of APTMS. Therefore, a significantly lower amount of 1,4-MMTPEB should be used to form Au NP clusters. When 0.2 eq. of 1,4-MMTPEB was used, cluster formation was not observed. Thus, no further experiments to obtain SERS-active structures by Au NP clustering with 1,4-MMTPEB were performed.

# 5 Conclusion and Outlook

In conclusion, two different dispersible structures have been prepared that extend the versatility of Raman spectroscopy in solution. Using hot spots between Au NPs the Raman signal of molecules involved in catalysis can be enhanced by these structures via the SERS effect, which could be used for applications in liquid-phase catalysis.

The first structure is composed of a functionalized  $SiO_2$  nanosphere with Au NPs densely adsorbed on the surface, which are coated with an ultrathin layer of  $SiO_2$  to allow the adsorption of Ni NPs. First, dispersions of semi-spherical Au NPs were synthesized with the Turkevich method. Separately,  $SiO_2$  nanospheres were produced, which was followed by functionalization with propylthiol, propylamine or PEI. The largest affinity between Au and functionalized  $SiO_2$  particles was found in superstructures containing PEI-SiO<sub>2</sub>. In addition, a correlation between a small Au :  $SiO_2$  NP size ratio and dense Au NP adsorption is found, since higher Au NP adsorption densities were observed for superstructures with a small  $\theta$ . By utilization of these findings superstructures with a high SERS activity were designed, which consist of large PEI-SiO<sub>2</sub> NPs (SiO<sub>2</sub>-PEI564 or SiO<sub>2</sub>-PEI742) and large Au NPs (Au56 or Au63). The SERS activity was investigated using Raman spectroscopy on wafers that were spin-coated with superstructure dispersions. Additionally, Raman measurements on the dispersions were performed as well using a Raman probe. With both techniques it was found that the superstructures were coated with an ultrathin layer of SiO<sub>2</sub>, on which colloidal Ni NPs were adsorbed.

One of the key challenges of future research on SERS-active superstructures composed of Au and SiO<sub>2</sub> NPs is the optimization of the synthesis procedure. Although Au NPs and functionalized SiO<sub>2</sub> particles are successfully synthesized, improvements in the formation of superstructures can be made. First, the adsorption density of Au NPs on the surface of SiO<sub>2</sub>-PEI742 and SiO<sub>2</sub>-PEI564 can be increased, since uncovered spots are present on the SiO<sub>2</sub> surface (see Figure 21). This can be achieved by increasing the added amount of Au NP dispersion or by decreasing the number concentration of SiO<sub>2</sub>-PEI742 or SiO<sub>2</sub>-PEI564 particles. Another large challenge of future research is to produce superstructures with a high level of robustness. In this way, these structures could be used in a wide range of potentially harsh catalytic reaction conditions. Therefore, more extensive TEM measurements are required to ensure the homogeneous coating of the superstructures with SiO<sub>2</sub>. Additionally, the SERS activity of the SHIPS and Ni-SHIPS should be investigated. Furthermore, pre-treatment of the Ni-SHIPS by UV-Ozone cleaning to remove the ligands or by high-temperature reduction to obtain metallic Ni might result in a loss of SERS-activity, since this is observed with SHINs as well.<sup>36</sup> Further research on the activated Ni-SHIPS should thus be performed. Another option is to adsorb different catalytically active NPs on the SHIPS, for which activation by pre-treatment is not required (i.e. Pt NPs).<sup>18</sup> Finally, catalysis could be studied with these improved SHIPS.

The second structure consists of Au NPs that are clustered by the addition of a linker molecule (1,4-MMTPEB). The formation of Au NP clusters was demonstrated by cryo-TEM and DLS mea-

surements. In addition, analysis with Raman spectroscopy indicated the formation of an Au-S bond between the Au surface and the thiophenyl moiety of 1,4-MMTPEB. Moreover, a dried sample of 1,4-MMTPEB-Au63 was found to be SERS-active. However, a surface saturating amount of 1,4-MMTPEB was used in the synthesis of MMTPEB-Au63. Lowering of this amount resulted in a lack of cluster formation. Since growth of a SiO<sub>2</sub> shell is possibly hindered by a saturated surface, these structures are currently inapplicable as a tool in catalysis research.

Future work on these Au NP clusters should be started by development of methods to lower the amount of added 1,4-MMTPEB while maintaining cluster formation. In addition, more research is required to determine the SERS activity of the Au NP clusters in solution. Finally, it might be interesting to study the effect of the length and the shape of the linker molecule.<sup>125</sup>

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# Appendices

# A Au NPs

# A.1 DLS Au17



Figure 31: Particle size distributions of Au17 with different concentrations determined with DLS.

# A.2 Forward scattering DLS measurements of Au NP dispersions



Figure 32: Particle size distributions of Au17, Au40, and Au63 determined with DLS. The measurements were performed by detection of scattered light at the forward angle.

### A.3 Au56



Figure 33: (a) TEM image of Au56 with a mean particle size of  $52 \pm 3$  nm. (b) UV-Vis absorption spectrum of Au56 with a peak position that corresponds with a Au particle size of 56 nm.



Figure 34: (a) Particle size distribution of Au56 determined with DLS. The peak of the distribution is located at 60 nm and the peak width is 11 nm. (b) Zeta potential distribution of Au56. The peak of the distribution is located at -40 mV and the peak width is 15 mV.

# A.4 Raman spectrum of Au63



Figure 35: Raman spectrum of a dried dispersion of Au63 NPs measured with a 785 nm laser with a power 0.11 mW.

# **B** Functionalized SiO<sub>2</sub> spheres

# B.1 TEM image of SiO<sub>2</sub>564



Figure 36: TEM image of SiO\_2564. The mean particle size is 500  $\pm$  20 nm.

# **B.2 DLS** measurements of SiO<sub>2</sub>564



Figure 37: Particle size distributions of  $SiO_2564$  determined with DLS. The measurement was performed with a forward detection angle (red) and backward detection angle (black). The peak of the distribution obtained with forward detection is located at 442 nm and the peak width is 27 nm. The peak of the distribution obtained with forward detection is located at 564 nm and the peak width is 63 nm.

# **B.3** TEM image of SiO<sub>2</sub>-NH<sub>2</sub>461



Figure 38: TEM image of SiO<sub>2</sub>-NH<sub>2</sub>461. The mean particle size is 420 nm  $\pm$  23 nm.



# **B.4 DLS data of bare and functionalized SiO**<sub>2</sub>461

Figure 39: Particle size distributions of bare and functionalized  $SiO_2$ . The distribution peak of bare  $SiO_2461$ ,  $SiO_2$ -SH461,  $SiO_2$ -NH<sub>2</sub>461, and  $SiO_2$ -PEI461 is located at 461 nm, 471 nm, 507 nm, and 440 nm with a peak width of 54 nm, 40 nm, 66 nm, and 50 nm, respectively.

# **B.5** TEM image of SiO<sub>2</sub>461-PEI



Figure 40: TEM image of  ${\rm SiO_2461}\text{-}{\rm PEI}.$  The mean particle size is 414 nm  $\pm$  24 nm

# B.6 Raman spectra of bare and functionalized SiO<sub>2</sub> spheres measured with a 785 nm laser



Figure 41: Raman spectra of bare and functionalized SiO $_2461$  measured with a 785 nm laser with a power of 34 mW.

# **B.7** ATR-IR spectra of bare and functionalized SiO<sub>2</sub>461



Figure 42: IR spectra of bare and functionalized SiO<sub>2</sub>461 measured with an ATR set-up.

# **C** Plasmonic superstructures

# C.1 TEM image of Au17 on bare SiO<sub>2</sub>461



Figure 43: TEM image of Au17 on bare  $SiO_2$ . In this image  $SiO_2$  deformation and uncontrolled Au NP adsorption are observed.

# C.2 UV-Vis absorption spectra of Au17 on functionalized SiO<sub>2</sub>461



Figure 44: UV-VIS absorption spectra of Au17, Au17/SiO<sub>2</sub>-SH461, Au17/SiO<sub>2</sub>-NH<sub>2</sub>461, and Au17/SiO<sub>2</sub>-PEI461.
# C.3 DLS measurements of Au40/SiO<sub>2</sub>-SH461 and Au40/SiO<sub>2</sub>-NH<sub>2</sub>461



Figure 45: Particle size distributions of Au40/SiO<sub>2</sub>-SH461 (a) and Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 (b) determined with DLS.

# C.4 TEM image of Au40/SiO<sub>2</sub>-NH<sub>2</sub>461 at a reduced pH



Figure 46: TEM image of Au40/SiO\_2-NH\_2461 synthesized at a pH of 3.9.

# C.5 TEM images of Au17/SiO<sub>2</sub>-SH125 and Au40/SiO<sub>2</sub>-SH125



Figure 47: TEM images of Au17/SiO<sub>2</sub>-SH125 and Au40/SiO<sub>2</sub>-SH125. The corresponding  $\theta$  is given in the left bottom corner of each image. Similar adsorption of Au NPs is observed for superstructures with equal  $\theta$ .

### C.6 TEM images of Au/SiO<sub>2</sub>-PEI461



Figure 48: TEM images of Au17SiO<sub>2</sub>-PEI461 (a), Au40SiO<sub>2</sub>-PEI461 (b), and Au63SiO<sub>2</sub>-PEI461 (c). The corresponding  $\theta$  is given in the left bottom corner of each image. Coverage of the SiO<sub>2</sub> surface with Au NPs decreases with increasing  $\theta$ .

# C.7 Au63/SiO<sub>2</sub>-PEI564



Figure 49: TEM image of Au63/SiO<sub>2</sub>-PEI564.



Figure 50: (a) UV-Vis absorption spectra of Au63 and Au63/SiO<sub>2</sub>-PEI564. (a) Particle size distribution of Au63/SiO<sub>2</sub>-PEI564 determined with DLS. The peak of the distribution is located at 451 nm with a width of 38 nm.



# C.8 SERS activity of the superstructures

Figure 51: Raman spectrum of Au63/SiO<sub>2</sub>-PEI564 dried on a Si wafer. The measurement was performed with a 785 nm laser with a power 0.11 mW.

### C.9 SEM image



Figure 52: SEM image of a  $\rm Si_3N_4$  wafer spin-coated with Au40/SiO\_2-NH\_2461.



### C.10 Raman spectrum of Au63 with pyridine

Figure 53: Raman spectrum of dried Au63, to which a 0.3 M pyridine solution was added. The spectrum was measured using a 785 nm laser with a power 0.11 mW.

#### C.11 Calculation of the EF

To quantify the SERS effect Equation 4 is often used:

$$EF = \frac{I_{SERS}/N_{surface}}{I_{bulk}/N_{bulk}}$$
(11)

$$\begin{split} I_{SERS} &= 400 \text{ counts} \\ I_{bulk} &= 200 \text{ counts} \\ N_{surface} &= (1.2 \text{ mM} \cdot 2 \text{ mL}) \cdot N_A = 1.4 \cdot 10^{18} \\ N_{surface} &= (0.3 \text{ M} \cdot 2 \text{ mL}) \cdot N_A = 3.6 \cdot 10^{21} \\ EF &= 500 \end{split}$$

The calculated EF is slightly lower than the actual EF, since at a concentration of 1.2 mM the Au surface is saturated. Hence, the real  $N_{surface}$  is lower than used in the calculation, which leads to a higher actual EF.

# C.12 TEM image of Ni-(Au56/SiO<sub>2</sub>-PEI564)SHIPS



Figure 54: TEM image of Ni-(Au56/SiO<sub>2</sub>-PEI564)SHIPS. In the image free Ni NPs and Ni NPs adsorbed on the SiO<sub>2</sub> sphere can be observed.

# D Au NP linking with an organic linker

# D.1 H<sup>1</sup>-NMR spectrum of 4-BPMMS



Figure 55: NMR spectrum of 4-BPMMS with a purity of 98.8 %.



# D.2 Catalytic cycle and mechanism

Figure 56: Reaction mechanism of the Sonogashira coupling reaction of 4-BPMMS and 1,4-diethynylbenzene catalyzed by  $Pd(PPh_3)_2Cl_2$ .



### D.3 H<sup>1</sup>-NMR spectrum of 1,4-MMTPEB

Figure 57: NMR spectrum of the 1,4-MMTPEB fraction of the Sonogashira coupling. Traces of water and grease are observed. The small peaks between 0.3 and 1.5 ppm correspond to hexane (mixture of isomers), which was used as eluent in column chromatography purification. The purity of 1,4-MMTPEB is 82.5 %



# D.4 H<sup>1</sup>-NMR spectrum of 1,4-PBEDDB

Figure 58: NMR spectrum of the product of 1,4-MMTPEB deprotection. No product peaks are observed, which indicates the unsuccessful isolation of 1,4-PBEDDB.

### **D.5** 1,4-MMTPEB-Au63



Figure 59: TEM image of 1,4-MMTPEB-Au63. The right part of the figure shows a zoom of the indicated part of the image.

#### D.6 Particle size distribution of (0.2)1,4-MMTPEB-Au63



Figure 60: Particle size distribution of (0.2)1,4-MMTPEB-Au63. A nearly perfect agreement with the particle size distribution of Au63 is observed (see Figure 11(a)).