

# Shape-controlled synthesis and functionalization of gold nanoparticles for hyperthermia applications

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## ABSTRACT

Control of nanoparticle shape may initially seem like a scientific curiosity, but its goal goes far beyond aesthetic appeal. The shape not only influences the chemical and optical properties of metal nanoparticles, but also their relevance for medical applications. By changing the shape from spherical to branched, one can change the absorption wavelength from visible to near-infrared. These NIR absorbing nanoparticles could be useful for hyperthermia treatment of cancer cells. First of all, it is important to synthesize nanoparticles with special shapes in a controlled way. Secondly, these nanoparticles need to be coated with functional groups for the covalent attachment of biomolecules. Finally, the applicability of these functionalized nanoparticles for hyperthermia must be studied. In this paper, we report the successful synthesis of functionalized non-spherical gold nanoparticles with a plasmon absorption band in the near-infrared of the electromagnetic spectrum.

**Keywords:** hyperthermia, branched nanoparticles, near-infrared, functionalization

## 1 INTRODUCTION

The past two decades have witnessed rapid advances in the ability to structure matter at the nanoscale with control over the size, shape, composition and morphology. At the nanoscale, materials exhibit unique optical, electronic and magnetic properties not seen at their bulk scale. This makes nanostructures attractive for a wide range of applications. The combination of these unique properties with the appropriate size scale has motivated the introduction of nanostructures into biological applications. Indeed, the cell dimensions are situated in the sub-micron to micron size scale, while proteins can be classified in the nanometer range. Thus nanoparticles from a few to a hundred nanometers become ideal for incorporation into biological systems. This opens the door to a spectrum of applications in biology and biomedicine such as cancer therapy [1].

Conventional surgical treatment of solid tumors can efficiently be used for the removal of well-defined and accessible tumors located in the non-vital tissue regions of the human body. However, for small, poorly defined tumors within vital regions, this traditional treatment is not

suitable. Hyperthermia therapy can provide a less invasive alternative to conventional surgical treatment of tumors. This type of treatment uses a lethal dose of heat to kill malignant tissue. Unfortunately, simple heating techniques (e.g. by supplying energy with radiofrequency, microwave, ultrasound or laser radiation) have trouble discriminating between the tumor and the surrounding healthy tissues [2]. Functionalized nanoparticles that can generate a local heat and specifically target the tumor tissue can provide the solution to this problem.

Noble metal nanoparticles have fascinated scientists since historic times because of their unique size and shape dependent optical properties. The bright colors of noble metal nanoparticles are the result of the presence of a plasmon absorption band. This band occurs when the frequency of incoming photons is in resonance with the collective excitation of the conducting electrons of the particles. The oscillating electric field of an incoming light wave causes the conducting electrons to oscillate. When the electron cloud is displaced relative to the nuclei, a restoring force arises from coulomb attraction. This effect is termed "Localized Surface Plasmon Resonance" (LSPR) [3,4].

The resonance frequency and the resultant position of the plasmon absorption band are mainly determined by the particle size, the shape and the nature of the surrounding medium [4]. Small spherical gold nanoparticles show a characteristic LSPR band around 530 nm. By increasing their size, the LSPR band will shift to higher wavelengths, but still in the visible region of the electromagnetic spectrum. However, these visible light absorbing nanoparticles can not be used for in vivo hyperthermia treatment. This application requires light in the near-infrared (NIR) region where the tissue (hemoglobin and water) has the highest transmissibility. The light penetration depth can be up to a few centimeters in the spectral region 650-900 nm, also known as the biological NIR window. By changing the nanoparticle shape from spherical to branched, one can tune the LSPR absorption wavelength from visible to near-infrared. These non-spherical nanoparticles absorb the NIR light followed by rapid conversion of this light into released heat [1]. The ability of these nanoparticles to convert absorbed light efficiently into localized heat can be exploited for hyperthermia treatment of cancer. Selective targeting of nanoparticles to biomarkers on cancer cells increases the specificity and decreases the laser dose necessary to kill tumor cells without injuring the surrounding healthy tissue.

## 2 MATERIALS & METHODS

### 2.1 Materials

All materials and reagents were used as commercially received. Hydrogen tetrachloroaurate ( $\text{HAuCl}_4$ ) and sodium citrate were purchased from Acros Organics. Bis-(*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium (BSPP) was supplied by Strem Chemicals. Sodiumhydroxide ( $\text{NaOH}$ ) was obtained from Merck, while hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) 30 % was purchased from Air Products. 11-mercapto-1-undecanoic acid (11-MUA), 16-mercapto-1-hexadecanoic acid (16-MHA) and vitamin C were supplied by Sigma-Aldrich. 2-(2-[2-{2-(2-[2-{11-mercapto-undecyloxy}-ethoxy]-ethoxy)-ethoxy}-ethoxy]-ethoxy) carboxylic acid ( $\text{SH}-(\text{CH}_2)_{11}-\text{PEO}_6-\text{COOH}$ ) was obtained from Sensopath Technologies.

### 2.2 Instrumentation

UV-Vis spectroscopic studies were carried out using a Shimadzu UV-1601PC instrument with a slit width of 2 nm and a data interval of 0.1 nm. Fourier transform infrared (FTIR) spectra were measured with a Bruker IFS 66v/S instrument containing a  $\text{N}_2$ -cooled MIR-MCT detector. For every sample, 2048 scans were recorded at a resolution of  $1 \text{ cm}^{-1}$ . Transmission electron microscopy (TEM) images of the nanoparticles were obtained using a Tecnai F30 instrument operating at 300 kV. The samples were prepared by depositing a few droplets of the nanoparticles suspension on a carbon copper grid.

### 2.3 Synthesis of the branched gold nanoparticles

The synthesis of branched gold nanoparticles is based on a previously described procedure [5]. Briefly, before synthesis of the nanoparticles all glassware was cleaned with  $\text{HNO}_3/\text{HCl}$  (1:3) and rinsed with  $\text{H}_2\text{O}$  prior to use. 50 mL of aqueous sodium citrate ( $6.8 \cdot 10^{-3} \text{ M}$ ) and 2 mg BSPP were mixed and 100  $\mu\text{L}$  of 30% (v/v)  $\text{H}_2\text{O}_2$  was added. Next, under constant stirring  $\text{HAuCl}_4$  (0.05 M) was added slowly at room temperature. After several minutes, the color of the suspension changed from colorless to blue, which indicates the formation of branched gold nanoparticles. At first, this procedure was carried out manually and afterwards with an automated titration setup to get more reproducible results. Finally, the influence of different parameters such as the addition rate of the gold salt, the age of the mixture of sodium citrate and BSPP and the concentration of  $\text{HAuCl}_4$ , BSPP and  $\text{H}_2\text{O}_2$  were studied.

### 2.4 Electroless plating of the branched gold nanoparticles

In this procedure 2.5 mL of the branched gold nanoparticles were mixed with 1.5 mL of ascorbic acid

(0.01 M). Then 20 mL of  $\text{H}_2\text{O}$  was added under vigorous stirring. Finally, different amounts (0  $\mu\text{L}$  to 2.5 mL) of  $\text{HAuCl}_4$  (0.01 M) were added.

### 2.5 Chemical functionalization of the branched gold nanoparticles

Functionalization of the branched nanoparticles is possible by deposition of thiol molecules onto the particle surface by place exchange. In this procedure 2 mL of branched gold nanoparticle suspension was mixed with 20  $\mu\text{L}$  of  $\text{NaOH}$  (0.5 M) to adjust the pH to a value of 11. Next, 200  $\mu\text{L}$  of thiol solution (12 mM in ethanol) was added and the mixture was stirred for at least 3 h. Three different thiol molecules were used: 11-MUA, 16-MHA and  $\text{SH}-(\text{CH}_2)_{11}-\text{PEO}_6-\text{COOH}$ . Finally, the suspension was centrifuged for 10 minutes at 4500 rpm, the supernatant was removed and the nanoparticles were resuspended in water. Centrifugation and washing was repeated 3 times.

## 3 RESULTS AND DISCUSSION

### 3.1 Optimization and characterization of the synthesis of branched gold nanoparticles

As described in the experimental section, the synthesis procedure was first carried out manually. However, this led to irreproducible results. To avoid this problem, the procedure was automated by constructing a titration setup for controlled addition of the gold salt, which is a crucial step in the synthesis procedure. With this setup the procedure could be optimized and tuning of the plasmon absorption band of the nanoparticles into the near infrared was possible. In this optimization process, the influence of different parameters on the position of the plasmon absorption band was studied.

First, the addition rate of  $\text{HAuCl}_4$  was investigated. This gold salt is the precursor salt that must be reduced in order to form gold nanoparticles. Slow addition of the gold salt is necessary in order to obtain nicely branched nanostructures. The addition rate was varied from 5 to 40  $\mu\text{L}$  per minute. The slower the gold salt was added, the more the plasmon absorption band red-shifted (Figure 1). The suspension color changes from red to purple to blue when going from faster to slower addition rates respectively (Figure 2). Optimizing this parameter, the plasmon absorption band could be shifted up to 56 nm to higher wavelength.

Secondly, the age of the mixture of sodium citrate and BSPP played an important role. Sodium citrate was added together with  $\text{H}_2\text{O}_2$  as a reducing agent for gold salt at room temperature. BSPP is a detergent that plays a significant role in the formation of the branched structures. The mixture of citrate and BSPP should be made freshly. In this way the branched nanoparticles show the best characteristics and the plasmon absorption band is shifted 15 nm to higher wavelength. When using a mixture that is

one week old, the absorption band is shifted to lower wavelengths.

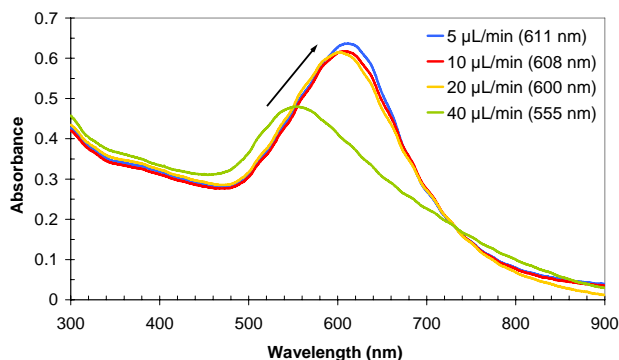


Figure 1: UV-Vis absorption spectra of branched gold nanoparticles synthesized with different addition rates of HAuCl<sub>4</sub>.



Figure 2: Suspensions of branched gold nanoparticles with a color going from red to blue.

Finally, the influence of the concentration of three different agents was studied. The concentration of HAuCl<sub>4</sub> was varied from 0.05 M to 0.0125 M for an addition rate of 10 µL/min. The best results were obtained with 20 µL of HAuCl<sub>4</sub> (0.05 M), where the absorption band was shifted 32 nm to higher wavelength. The amount of BSPP was varied from 0.2 to 1.6 mg in 10 mL of sodium citrate solution. The best result concerning the position of the absorption maximum was obtained with 0.2 mg of BSPP, which led to an increase of 15 nm in wavelength position. The amount of H<sub>2</sub>O<sub>2</sub> was varied from 5 to 80 µL in 10 mL of citrate/BSPP solution. The best results were obtained with 20 µL of H<sub>2</sub>O<sub>2</sub>, which resulted in a shift of 57 nm of the plasmon absorption band. After this optimization procedure, the branched gold nanoparticles show a plasmon absorption band around 610 nm, the beginning of the near infrared region.

Besides characterization with UV-Vis absorption spectroscopy, the branched nanoparticles were also characterized with TEM. Figure 3 shows the TEM images of the gold nanoparticles synthesized with the optimized procedure with an average size of 50 nm.

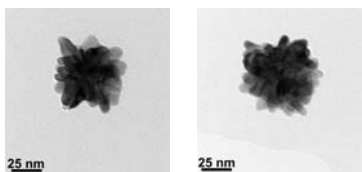


Figure 3: TEM images of branched gold nanoparticles.

### 3.2 Characterization of electroless plating of branched gold nanoparticles

The plasmon absorption band of these branched gold nanoparticles must be shifted as far as possible into the near infrared region of the electromagnetic spectrum for hyperthermia applications. In order to achieve this goal, an alternative method was applied to modify these branched gold nanoparticles i.e. electroless plating with gold.

In this procedure, gold salt added in different amounts, was reduced with vitamin C in the presence of the branched gold nanoparticles. Upon addition, the plasmon absorption band first shifted to higher wavelength (with addition of 50 µL HAuCl<sub>4</sub>) and then moved back to lower wavelength (Figure 4). This indicates that the branched structure remains until addition of 50 µL of gold salt and then disappears. The suspension color changes from blue to red (Figure 4-inset).

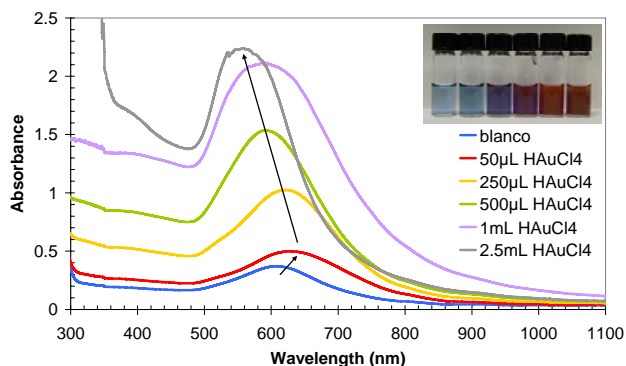


Figure 4: UV-Vis absorption spectra of branched gold nanoparticles plated with gold. Inset: corresponding gold nanoparticles suspensions.

To get a better understanding of this electroless plating method on the branched gold nanoparticles, the initial blue shift to higher wavelength, when adding only 50 µL HAuCl<sub>4</sub>, was further investigated in detail. A total amount of 250 µL of gold salt was added in little steps of 25 µL. Hereby, the plasmon absorption band shifts to higher wavelength going from 610 to 670 nm until approximately 100 µL of gold salt was added. After further addition of gold salt the absorption band goes back to lower wavelengths (Figure 5).

Indicated by the UV-Vis absorption spectra and confirmed by TEM the branched morphology will first remain and then disappear and go back to a spherical one (Figure 6).

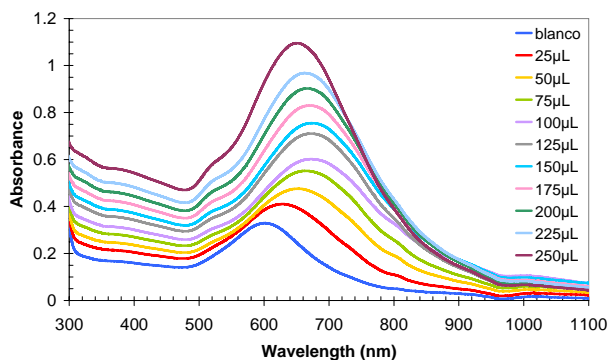


Figure 5: UV-Vis absorption spectra of branched gold nanoparticles synthesized with addition of gold salt in little steps.

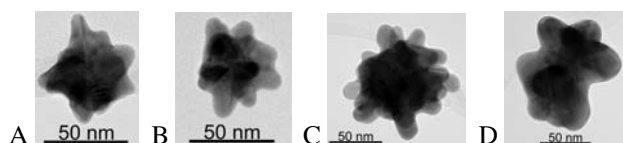


Figure 6: TEM images of branched gold nanoparticles with A) blanco B) 25  $\mu\text{L}$   $\text{HAuCl}_4$  C) 100  $\mu\text{L}$   $\text{HAuCl}_4$  D) 250  $\mu\text{L}$   $\text{HAuCl}_4$

### 3.3 Functionalization with thiols via place exchange

The branched gold nanoparticles must be functionalized with a reactive end group to achieve further coupling of biomolecules. Thiol molecules with different end groups can be used for this purpose. We investigated the place exchange reactions using 11-MUA, 16-MHA and  $\text{SH}-(\text{CH}_2)_{11}-\text{PEO}_6-\text{COOH}$ . These thiols incorporate  $\text{COOH}$ -groups that can be used for covalent attachment of biomolecules using  $\text{NH}_2$ -groups. In the UV-Vis spectra a successful exchange can be observed for the three different thiol molecules. This is indicated by a shift of the plasmon absorption band to higher wavelength after addition of the thiol molecule (Figure 7). This shift is caused by a local change of the environment around the nanoparticle surface.

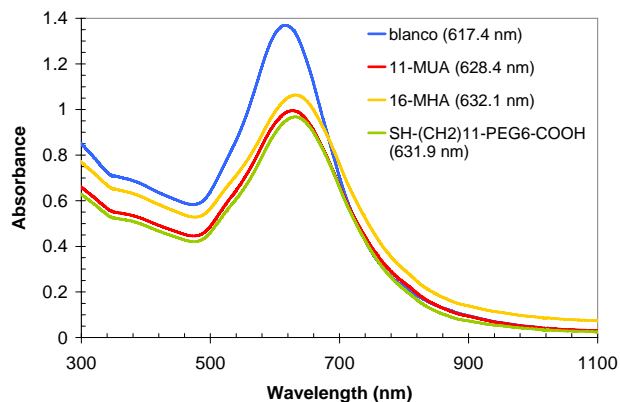


Figure 7: UV-Vis absorption spectra of branched gold nanoparticles after exchange with thiol molecules.

Besides UV-Vis absorption spectroscopy, FTIR was used to prove the presence of thiol molecules onto the surface of the different branched gold nanoparticles. All three spectra showed the characteristic bands for the corresponding thiols. Important bands of  $\text{CH}_2$  and  $\text{COOH}$  stretching vibrations were present around  $2900\text{ cm}^{-1}$  and  $1730\text{ cm}^{-1}$  respectively for all three nanoparticles. Additional bands for the PEO chains around  $1132\text{ cm}^{-1}$  were present in the spectrum of the nanoparticles modified with  $\text{SH}-(\text{CH}_2)_{11}-\text{PEO}_6-\text{COOH}$ . (Data not shown)

Finally, TEM was used to verify the successful exchange of thiols onto the branched nanoparticles. A thin white layer is visible around the nanoparticle surface, indicating the presence of a less dense layer such as thiol molecules. (Data not shown)

## 4 CONCLUSIONS

In this study, the synthesis of branched gold nanoparticles was optimized with as main goal to tune the plasmon absorption band into the near infrared. Hereto, different parameters of the synthesis procedure were optimized and further tuning of the plasmon band was performed by electroless plating of the nanoparticles. After this optimization we could obtain a shift of the plasmon absorption band from 555 nm to 670 nm, which is in the near infrared region of the electromagnetic spectrum. Subsequently, thiol molecules were successfully attached onto the nanoparticle surface using place exchange. This chemical functionalization of the branched nanoparticles will allow to covalently immobilize biomolecules for hyperthermia applications. In the future, the applicability of these functionalized nanoparticles for hyperthermia treatment will be studied.

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