

# Functionalized PEG gold nanoparticles (AuNPEGs) with excellent resistance towards agglomeration

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## Introduction

Gold nanoparticles have received a lot of interest in the research community [1] and also found applications in commercial operations. The instability of the bare gold particles can be improved by ligand coating. PEGylation is a common method to increase stability and robustness. In this work we present Polyethylene glycol (PEG) coated gold nanoparticles (AuNPEG) to improve on several handling issues. By using functionalized PEG derivatives, particles can be further modified or conjugated to biomolecules. We have used avidin as a tetrameric multivalent "glue" to demonstrate cluster formation between biotinylated AuNPEGs.

## Materials and Methods

### AuNPEG synthesis and purification

PEGylated gold nanoparticles were synthesized by a two-step method in order to control particle size. HAuCl<sub>4</sub> and monodisperse PEG disulfides were dissolved in 200ml MilliQ water, thoroughly mixed and continuously stirred in a water cooled reactor. NaBH<sub>4</sub> was dissolved in 100ml ice cold water and added drop wise into the reactor during 45minutes. The reduction proceeded for 1 hour, followed by a second addition of PEG (see below for details) and mixed for an additional 60 minutes. After maturation overnight, the AuNPEGs were repeatedly washed with PBS buffer (pH 7.4) and separated from the solution by centrifugation.

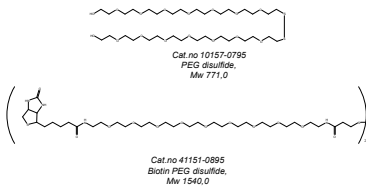
Several different PEG derivatives (-CH<sub>3</sub>, -OH, -COOH - biotin and mixtures thereof) have been linked to the AuNPEGs in the second step of the synthesis. The scope of this work has been to demonstrate the affinity of the biotin labeled AuNPEGs.

	Inert AuNPEG (G363B)	Biotin AuNPEG(G363C)
1st PEG addition	Ratio PEG disulfide:Au 1:1000	Ratio PEG disulfide:Au 1:1000
2nd PEG addition	Ratio PEG disulfide:Au 1:1	Ratio PEG disulfide:Au 1:1 Ratio PEG biotin disulfide:Au 1:1000

Table summarizing the PEG composition for the AuNPEGs described in this work.

### Monodisperse PEG derivatives

In this study we utilize standard catalogue products from Polypure. The compounds are single length and show high chemical integrity. In order to ensure monodispersity of the PEG molecules, all compounds were purified by preparative chromatography. Purity and structure were confirmed by HPLC and MS (data not shown).



### Monitoring the surface plasmon band

Absorbance measurements were performed using a Pharmacia Ultraspec LKB Spectrophotometer. Absorbance spectra were recorded in single use plastic cuvettes between 300 and 800nm. Samples were centrifuged at 13 000 RPM in a Heraeus Sepatech Biofuge A, for 5 to 20 minutes, followed by absorbance measurement of the supernatant.

Gold core size was calculated by applying the Stoke's law for a settling particle in rotating flow, assuming that 50% of the particles migrate 50% of the length of the centrifugation tube, when absorbance is reduced to half.

$$V_s = \frac{1}{18\mu} \Omega^2 r^2 (\rho_p - \rho_l) d^2$$

$$V_s = \frac{0.5L}{t_{1/2}}$$

$V_s$  = terminal velocity (m/s)  
 $\mu$  = dynamic viscosity (Pa·s)  
 $\Omega$  = rotation (rad/s)  
 $r$  = centrifuge radius (m)  
 $\rho_p$  = particle density (kg/m<sup>3</sup>)  
 $\rho_l$  = liquid density (kg/m<sup>3</sup>)  
 $d$  = particle diameter  
 $L$  = centrifugation tube length (m)  
 $t_{1/2}$  = time at half absorbance (s)

### Preparation of biotin-SAM

Quartz crystals were washed in a 1:1:5 solution of NH<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>O, heated to approximately 80°C for 5 minutes. After rinsing with 18.2 MΩ milliQ water and blown dry with nitrogen gas, the crystals were cleaned in an custom made UV-Ozone chamber for minimum 10 minutes (Grid lamp, model 8S-9102-02 BHK Inc). When the crystals were removed from the chamber, they were again rinsed with milliQ water, blown dry with nitrogen, before being immersed in a 100 μM aqueous solution containing PEG disulfide (90% w/w) and biotin PEG disulfide (10% w/w).

### Quartz Crystal Microbalance (QCM) evaluation of binding

The functionalization of the AuNPEGs were evaluated with Quartz Crystal Microbalance, the Attina 100 system [2].

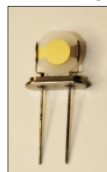


Figure 1 Quartz Crystal used in the QCM evaluation

Biotin modified quartz crystals, were rinsed with milliQ water and inserted into the instrument. After an equilibration period of up to one hour, samples were injected into the flow loop.

Throughout the entire experiment PBS buffer pH 7.4 (Sigma) was used as running buffer at 100 μl/min. Avidin (Sigma) was aliquotted in PBS buffer and stored at 20°C until use. For each experiment, one aliquot was thawed, diluted and used within 3 days. In all experiments the injection volume was set to 43 μl.

## References

- [1] Daniel and Astruc, *Chemical Reviews*, **104** (1), (2004)
- [2] www.attina.com
- [3] Demers, L. M. Et al *Anal. Chem.*, **72**, 5535- (2000)
- [4] Hook, F. et al. *JCIS* **208**, 63-67 (1998)
- [5] Kadri-Aslan, et al. *J. Phys. Chem. B* **108** (40), 15631-15636 (2004).
- [6] G. Mie, *Ann. Phys.* **330**, 377-445 (1908).

## Results & Discussion

### AuNPEG Characterization

In order to estimate the size of the gold core, a combined sedimentation-absorbance method was applied. The decrease in absorbance follows linearly with centrifugation time (figure 2) By using Stoke's law modified for rotating flow, it is possible to estimate a particle diameter when a density has been set. The density of gold is 19.3 g/cm<sup>3</sup> while PEG has a density close to water. It is assumed that the PEG layer will have no influence on the centrifugal force acting upon the AuNPEGs and therefore the density for gold is used. Given these assumptions, the gold core size was found to be 7.3 nm.

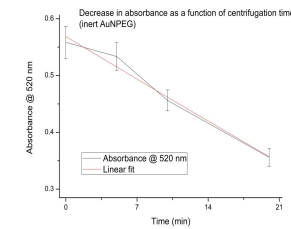


Figure 2: decrease in absorbance as a function of centrifugation time and linear fit.

### Size characterization by absorbance

Using the Beer-Lambert law for light absorption, an approximate extinction parameter of 4.2·10<sup>8</sup> M<sup>-1</sup> cm<sup>-1</sup> [3] and a characteristic length of 1cm, the concentration of gold spheres was found to be 5.07nM. By further assuming that the reaction went to completion and that all Au atoms present are bound in equally sized perfect spheres, a diameter of 15 nm could be calculated. Given the ratio of biotin-PEG used in the synthesis, there should be ~100 biotin groups per biotin-AuNPEG.

### Evaluation of biotin functionalization with QCM

After saturating the biotin-SAM crystal with avidin, the surface showed very low interaction with the inert AuNPEG while the biotin-AuNPEG bound strongly (figure 3). The response of the avidin binding was shown to be two to threefold that of the response of the biotinylated nanoparticle. This may reflect increased QCM response from a globular protein form compared to the more flexible structure of the PEGylated nanoparticle. [4] The same pattern is observed when repetitive injections of avidin, inert respectively biotinylated AuNPEGs are done (figure 4). The layer-by-layer formation indicates that surface bound structures are still accessible for further affinity binding.

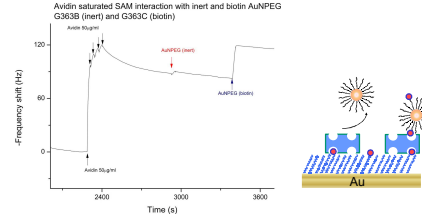


Figure 3: Left: Interaction between avidin, inert, biotinylated AuNPEGs and the QCM chip. The surface was saturated with avidin after 5 injections of 50 μg/ml. It is evident that the inert AuNPEG did not bind, whereas the biotin functionalized AuNPEG did bind to the avidin on the surface. Right: cartoon showing how the authors visualize the surface

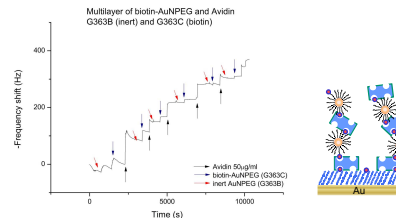


Figure 4: Left: Multilayer formation of avidin and biotin-AuNPEGs. There is no binding to the avidin of the inert AuNPEG, but when the biotinylated AuNPEG is injected there is an increase in signal. Right: cartoon of the multilayer.

### Biotin-AuNPEG Avidin cluster

The extent of cluster formation can be controlled by mixing biotin-AuNPEGs with avidin in various concentrations. If the number of biotin groups is equivalent to the available binding sites, clustering proceeds until every available binding site is occupied. Smaller clusters are formed when the proportions are other than unity [5]. By monitoring the UV-vis spectra for samples with different concentration of avidin in combination with sedimentation-absorbance method, the effect of protein concentration on the cluster size can be measured. The effects we observe are only to a limited extent due to coupled plasmons between adjacent gold cores, since the UV spectra are virtually identical for different ratios (figure 5). Possibly the distance between the gold cores are too large for us to observe such an effect [6]. But when the avidin concentration vs. sedimentation speed is plotted, the primary influence of avidin on cluster formation is revealed (figure 6). As expected when going from low to high concentration of avidin, the maximum sedimentation speed is passed. The measured sedimentation speed can be normalized to the inert AuNPEG or at avidin concentration zero to give an estimate of number of particles in the various clusters (figure 7). Maximum cluster size is found when the concentration of avidin and AuNPEG are 43nM and 1.64nM respectively.

Surface Plasmon Band shift with increasing concentrations of Avidin

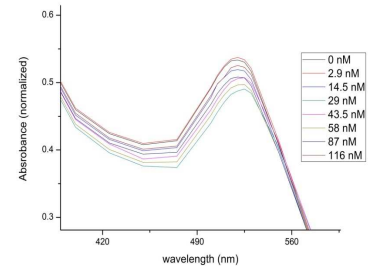


Figure 5: UV-Vis absorbance spectra for the biotin-AuNPEGs at different concentrations.

Sedimentation speed as a function of avidin concentration

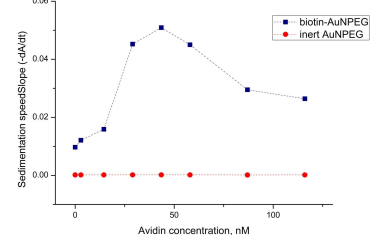


Figure 6: Sedimentation speed as a function of avidin concentration. Maximum speed is found at 43.5 nM avidin.

Number of gold cores per cluster Normalized to average inert core size

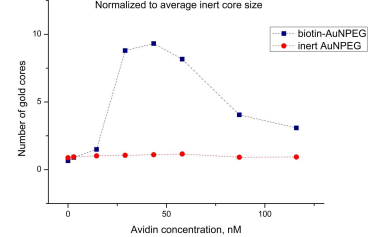


Figure 7: Number of gold cores per cluster. The largest cluster contained 9 gold cores.

### Agglomeration resistance

The PEG protective coating gave the particles good stability when subjected to harsh treatment. AuNPEGs were stable in strong acidic and basic solutions (pH 1-13), as well as in high salt concentration (3M NaCl). No agglomeration could be noticed by visual inspection or UV absorbance. They also showed good resistance to temperature variations. Neither freeze/thaw cycle nor boiling for extended periods of time appear to affect the properties of the particles. The amphiphilic nature of the PEG coated permitted the controlled partitioning in extraction procedures, and particles could be reconstructed in either aqueous or organic solvents from the dried state.



Figure 8: Left and middle: AuNPEG extracted from water to methylene chloride. Right two AuNPEG samples after overnight sterilization cycle in autoclave

## Conclusions

In this work we are presenting a convenient method for preparing highly stable PEGylated gold nanoparticles using monodisperse PEG derivatives. The successful functionalization of the AuNPEGs has been shown by the biotin-modified nanoparticle binding to surface immobilized avidin. The accessibility of multiple biotin moieties on the AuNPEGs have been demonstrated as well by the cluster formation with avidin. Thus the ability of the particles to withstand common chemistry procedure opens up for opportunities in further functionalization and refinement.

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