

Substrate independent adsorption of gold nanoparticles by using polypeptides as adhesive

Master of Science Thesis in the Master Degree Programme, Materials Chemistry and Nanotechnology

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Master of Science Thesis

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Cover:

[Gold nanoparticles bound to a Mefp-1 coated gold surface, for explanation
see page 15-16.]

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Abstract

The increasing resistance of bacteria to antibiotics is a well-known problem. To get around bacterial adhesion to surfaces, especially in the medical devices field, antimicrobial surfaces is of growing interest. By attaching antimicrobial peptides (AMPs) to a surface this can be achieved. However attaching/ grafting AMPs with controlled surface coverage and intermolecular spacing is not trivial. In theory you coat the substrate with adhesive polypeptides, bind gold nanoparticles (AuNPs) to them, at controlled spacing, and then the AMPs can be attached to the AuNPs. The problem is how to make this the best way in practice. AuNPs size and surface coverage on polypeptide covered substrates were in focus in this project.

The adhesive polypeptides used in this study are poly-L-lysine and Mefp-1. Mefp-1 is an adhesive mussel protein and the hypothesis is that it can attach to many types of surfaces exactly like the mussel (*Mytilus Edulis*). Citrate stabilized gold nanoparticles were produced according to the Turkevich et al. method, with some modifications. The goal was to create AuNPs sized 40-60 nm with space between the particles. It is assumed that this will make the AMP surface efficient against the bacteria. It was also of interest to see if the size of the AuNPs, when produced, could be changed by just changing the temperature.

The polypeptides were coated on four different substrates; gold, glass, PMMA and PS. The AuNPs were bounded to the polypeptides. UV-VIS, DLS and SEM analysis were done to study the size of the AuNPs. The AuNP-polypeptide coated surfaces were analysed with SEM. In addition the binding time of the polypeptide and the AuNPs were studied with QCM-D.

The AuNPs size dependence of temperature turned out to be minor. Both the UV-VIS and the SEM showed that it was possible to create citrate stabilized particles with a size of 40-60 nm. The results from the DLS indicated that the solutions were polydisperse and therefore it was hard to find out the accurate average size of AuNPs.

SEM analysis of the AuNP coated substrates indicates that gold and glass substrates can be nicely covered both with lysine and Mefp-1. The surface coverage of AuNPs on lysine was higher than on Mefp-1. Two different kinds of PMMA substrates were also used; one more hydrophilic and one more hydrophobic. The hydrophilic PMMA surface was coated with lysine while Mefp-1 was not able to coat it. On lysine AuNPs were attached. Both the hydrophobic PMMA and the PS substrates coated with lysine gave a very uneven surface distribution of particles. The coating with Mefp-1 gave a low surface coverage on hydrophobic PMMA while on PS it gave the highest one. According to the QCM-D measurements the binding time of AuNPs on lysine was long enough but on Mefp-1, it was too short.

Keywords: gold nanoparticles, polypeptides, poly-L-lysine, Mefp-1, gold substrate, glass substrate, PMMA substrate, PS substrate, antimicrobial surface

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List of abbreviations

| | |
|----------|---|
| AuNPs | Gold nanoparticles |
| AMP | Antimicrobial peptide |
| Lysine | Poly-L-lysine |
| Mefp-1 | Mytilus edulis foot protein 1 |
| UV-VIS | Ultraviolet – Visible spectroscopy |
| SEM | Scanning Electron Microscope |
| DLS | Dynamic Light Scattering |
| QCM-D | Quartz Crystal Microbalance with Dissipation Monitoring |
| TOF-SIMS | Time-of-Flight Secondary Ion Mass Spectroscopy |
| PMMA | Polymethyl methacrylate |
| PS | Polystyrene |
| DOPA | 3,4-dihydroxyphenylalanin |

1. Introduction

A huge problem today in the medical devices field is the bacterial adhesion to biomaterials with corresponding infection. Another well-known problem is the increasing resistance of bacteria to antibiotics. It is desired to develop alternative ways to get around these problems. One way could be to create antimicrobial surfaces by covalently immobilize antimicrobial agents to surfaces. By using antimicrobial peptides (AMPs) it is possible to create a surface where bacteria can't live and will be destroyed. In addition the AMPs are not cytotoxic to eukaryotic cells. To immobilize the AMPs on the polypeptide surfaces gold nanoparticles (AuNPs) can be used. Thereby the degree of immobilization and the surface concentration of the AMPs can both be controlled through the surface coverage and size of the AuNPs. AuNPs size and surface coverage on polypeptide surfaces were investigated in this project.

Earlier studies have used PEG as a spacer between the surface and the AMPs which resulted in a small increase in efficiency compared to only using AMPs on the surface. [1] The AMPs loses efficiency when they are attached to the surface because they become too short, 5-20 nm, and thus can't penetrate the cell membrane of the bacteria. A high concentration of AMPs is needed on the surface to create the wanted effect. PEG is both too flexible and not rigid enough to increase the efficiency and there is a need for a more rigid spacer. Gold nanoparticles are rigid, non- flexible and has an advantage of attaching a self-assembly monolayer, which is easily modified.

To create clean AuNPs, the method described by Turkevich et al. was used, with some modifications. Previous studies have used 10 nm big AuNPs as a spacer between a surface and the AMPs. The result was that the cell membrane from the bacteria covered the particle with attached AMPs and the efficiency was low. [1] Therefore bigger particles were instead investigated in this work. The desired sizes of the particles are between 40-60 nm in diameter and with as small as possible size distribution. This will spread out the AMPs and make them more efficient against the bacteria. To confirm the size and distribution of the AuNPs different methods were used, such as, UV-VIS spectroscopy, SEM and DLS. The AuNPs with the right size were deposited on polypeptide films. Different ways can be used to attach AuNPs to surfaces such as direct binding, chemical modification and/or biological binding. To use as much biocompatible substances as possible, biological binding was the method used in this work. In biological binding a polypeptide with NH_3^+ groups are used and the negatively charged AuNPs are bound to it. The polypeptides used in this work were lysine and Mepf-1, also albumin was used as a reference. All polypeptide coated surfaces, with attached AuNP, were analysed in SEM. Also QCM-D analysis was done to see if the binding time was correctly chosen and to see how much AuNPs that was bound to the surface described in mass/cm^2 . The different surfaces materials evaluated were gold, glass, PMMA and PS.

1.1 Aim of the project

The aim of this diploma work is to be able to create citrate stabilized 40-60 nm big gold nanoparticles and to be able to create this specific size of particles only by changing the temperature. To make gold nanoparticles with a narrow size distribution (<20%) and to bind them to a polypeptide coated substrates is required. A nice surface coverage and an even distribution over the surface of AuNPs are wanted, this to be able to use them in future experiments as spacers between the polypeptide surface and an antimicrobial

peptide, figure 1. Two polypeptides are mainly used to coat the substrates; Mefp-1 and lysine. It is of interest to see if Mefp-1 can be used in a more general way, rather than lysine, since it should be able to bind to many more different surfaces.

It is also investigated if the gold nanoparticles distribution on the polypeptide surface can be controlled, only by changing the salt concentration in the AuNP solution upon binding.



Figure 1: A polypeptide coated substrate with attached AuNPs and AMPs.

2. Background

This section will give a short introduction to gold nanoparticles, the gold nanoparticle synthesis, the polypeptides used and to the characterization techniques that were applied.

2.1 Gold nanoparticles

The first metallic nanoparticles were prepared by Faraday in 1857. He discovered the optical properties of gold nanoparticles in solution and set the start for the growing interest in gold nanoparticles and their properties. [2] Today the method created by Turkevich et al. in 1951 [6] is one of the most used procedures to synthesise AuNPs in solution.

AuNPs in solution is not stable by themselves therefore different stabilizers are used. A stable solution of AuNPs can be created in two different ways either by steric stabilization or electrostatic. In steric stabilization bulky organic molecules are attached to the AuNPs which hinder them to aggregate [3]. The electrostatic stabilization can be created by using citrate as the stabilizer; this method was used in this project. The negatively charged citrate ions surround the AuNPs, generate repulsive Coulombic forces and thereby hinder the particles to aggregate. The electrostatic stabilization is based on the DLVO theory which is useful when describing the stability by the ionic double layer for spherical particles.

Gold nanoparticles have many different interesting applications due to their properties such as Surface Plasmon Resonance (SPR), biocompatibility, chemical stability and easy surface functionalization or bioconjugation [4]. These properties make them a great choice when it comes to creating antimicrobial surfaces. The size and the size distribution of the AuNPs are important to get the right amount of AMP to bind to it and to get the right interparticle distance between the AMPs. The AuNPs size can be detected by UV-VIS measurement due to the AuNPs SPR. AuNPs have their colour because of its SPR, which is caused by the atoms ability to absorb and reflect only certain frequencies of light. Spherical particles have a SPR at one single frequency while elongated particles show two different frequencies; this can easily be displayed by UV-VIS measurements. [5]

Gold has affinity for binding strongly to sulphur. The sulphur in a thiol group can easily bind to the gold surface and creates a self-assembly monolayer (SAM). [5] Also the nitrogen in the amino group has affinity for gold. By using this knowledge, molecules with interesting properties can be attached to gold, which creates new properties of the outer layer of the material. This is used for making the AuNPs antimicrobial; the AMP binds to the AuNPs which creates an antimicrobial surface. The AuNPs are used as a spacer between the coated surface and the AMPs.

2.2 Synthesis of gold nanoparticles

As already mentioned one of the most common methods today to create gold nanoparticles was established by Turkevich et al. already in 1951. This method was used because of its good ability to produce clean AuNPs and due to the fact that it is an environmentally friendly method, since citrate is used as the chemical reducing agent [6]. By chemically reducing $\text{HAuCl}_4(\text{aq})$ with trisodium citrate the AuNPs are formed. The size of the NPs can be varied by changing the temperature, stirring speed or the ratio of $\text{HAuCl}_4(\text{aq})$ to trisodium citrate.

2.3 Polypeptides

To be able to attach AuNPs to a surface it must have a certain chemistry that includes gold binding groups, like $-\text{SH}$ or $-\text{NH}_3^+$. Uncoated plastics that are used in the medical device field can't attract the AuNPs by themselves, therefore polypeptides are used to coat the plastics and enable the AuNPs to bind to them. Because of this the polypeptides must have good coating properties and be able to attract the AuNPs. Three different polypeptides; Poly-L-lysine, Mefp-1 and Albumin, were used in this project. Lysine is well known to coat glass surfaces and bind AuNPs to it. To get a more general approach a "new" polypeptide, Mefp-1, was tried out. Mefp-1 is more stable than lysine when it comes to attracting the AuNPs. The AuNPs bind to lysine only by the amine group while on Mefp-1 both the amine group and the DOPA group attract them. The DOPA group interacts with the AuNPs through the catecholic oxygen atoms [7]. Albumin is known to bind to gold and was used as a reference sample. The polypeptides are described in more detail below.

The polypeptides were coated on both hydrophobic and hydrophilic surfaces. The substrates used were gold, glass, PMMA and PS. Albumin is only coated on gold surfaces.

2.3.1 Poly-L-lysine

Lysine can coat many types of surfaces because of its NH_3^+ -group thus it also enhances the binding of other molecules to the surface [8]. The ability of lysine to cover the surface with a thin film in combination with its biocompatibility and its amino group makes it a good material to use when working with antimicrobial surfaces.

2.3.2 Mytilus Edulis Foot Protein

The polypeptide that makes it possible for the mussel to attach to almost any surface is mussel adhesive protein called Mefp-1. It is located in the byssus threads of the plaque. Mefp-1 consists of 80-85 repeating decapeptide units and has a molecular weight of 110 000 g/mol. Polar and basic amino acids are the main constituents of the decapeptide units. [9]

The crosslinking of the protein occurs if an oxidant is present or if pH is increased to over 8. The group that will take part in the crosslinking is 3,4-dihydroxyphenylalanine, DOPA. DOPA is also the group that will replace water on the surface and make the protein attach. [9]

The gold nanoparticles can chemically bind to Mefp-1 at two different locations, to the NH_2 -group or to the DOPA.

2.3.3 Albumin

Albumin from bovine serum was also used to coat the substrates. Albumin will bind strongly to gold through its thiol-groups. When albumin is coated on a gold substrate, most of the thiol-groups will be bound to it. The molecular weight is around 66 000 g/mol [10].

2.4 Characterization techniques

The size, shape and distribution of the AuNPs are important to know, since this affects the properties of the final antimicrobial surface. By using UV-VIS spectroscopy, SEM and DLS, the size, distribution and concentration can be investigated. In addition SEM measurements give information about the shape of the particles. QCM-D is used to measure the polypeptide and AuNP binding to the surface; it also gives information about the mass of AuNPs that sit on the surface.

2.4.1 UV-VIS

In UV-VIS spectroscopy a liquid sample is radiated with light of different wavelength. The wavelength that is absorbed by the sample is shown as an absorption peak in the absorbance – wavelength curve. Using Lambert-Beers law, the concentration of the particles in the liquid can be calculated. [11]

Gold nanoparticles in liquid are easy to measure with UV-VIS since they have their SPR. The SPR comes from the electrons in the particle, oscillating at neutral frequency. Depending on the structure of the particle the plasmon resonance occurs at one or two frequencies. If the particles are spherical only one plasmon resonance occurs and creates one peak in the absorption – wavelength spectra. With elongated particles two peaks will show, one for the transverse and one for the longitudinal surface resonance. [5] The location of the SPR absorption peak changes with the size of the particles and the absorbance maxima corresponds to the particle size.

2.4.2 SEM

The surface topography can be analysed in SEM. The samples are scanned by a fine beam of electrons, when the beam hits the sample various types of radiation are emitted. Backscattered electrons and secondary electrons are collected by a detector, which translate it to a picture of the surface. There are two requirements for successful analyse of a sample in SEM; the sample must be dry and conducting. Objects on the surface analysed can be seen all down to 2 nm. [12] SEM was used to look at the AuNPs size, shape, size distribution and surface coverage.

2.4.3 DLS

The technique is used to determine particle size in a solution. The size is related to the change in wavelength of the incoming light when a monochromatic light beam, such as a laser, hits the moving particles in the solution. The spherical particles move or

fluctuate due to Brownian motion. The Brownian motion is caused by particles randomly colliding with surrounding liquid molecules. By using the Stokes-Einstein equation together with the diffusion coefficient the mean hydrodynamic particle radius can be calculated. This radius is usually a little bigger than the actual particle radius due to the surface active components, such as diffuse solvent, ions or a surfactant layer. The data from the DLS measurements are presented as charts that describe the particle size distribution. [11]

DLS is a commonly used technique for studying the size of gold nanoparticles. The measurements also give information about if the solution is mono- or polydisperse.

2.4.4 QCM-D

In QCM-D gravimetric measurement is applied. By applying an oscillating electric field the quartz crystal starts to vibrate at certain frequencies. This frequency change is proportional to the mass of the material. The sample is placed on the quartz crystal and the vibrational frequency decrease when material is spread out over the sample. [13] This method gives information about how much of the AuNPs in the surrounding liquid that are reacted/bound to the sample and if the optimal reaction time is used.

3. Methods

In this section the experimental work is explained. First the synthesis of the AuNPs is described followed by the coating of the substrates. The last part includes the characterisation of the particles and substrates.

3.1 Synthesis of gold nanoparticles

As mention before, the Turkevich method for producing gold nanoparticles was used. By chemically reducing hydrogen aurate (HAuCl_4) with trisodium citrate as a reducing agent the AuNPs are formed. The HAuCl_4 and trisodium citrate was purchased from Sigma Aldrich and used as received without further purifications. To be able to create AuNPs in the size range of 40-60 nm, different gold salt to citrate ratios were tried out. The temperature and the gold to citrate concentration were changed and varied while the stirring speed was chosen to be the same in all experiments. The one that gave best results is described below. The other trials were done in the same way but with different gold to citrate ratios and they are discussed more in the result and discussion part.

A 50 ml 0,5mM HAuCl_4 solution and a 50 ml 0,58mM sodium citrate solution were separately brought to the desired temperature (50°C, 60°C, 70°C or 80°C). When both solutions had reached the selected temperature the sodium citrate solution was rapidly added under stirring to the gold salt solution and the colour changed from pale yellow to dark red under the first minutes. The solution was kept under constant temperature and stirring for one hour, to ensure that all $\text{HAuCl}_4(\text{aq})$ had been reduced. The nanoparticle solutions were stored at 5°C until use. All laboratory material was first washed in a Agua regia solution (3:1 $\text{HCl}:\text{HNO}_3$) followed by four times rinsing with Millipore water. Also a base piranha wash was done followed by four times rinsing with Millipore water to ensure as high as possible cleanliness. If the laboratory material or storing material is not clean the AuNPs can aggregate on the walls of the containers [14].

The AuNP solutions were washed by centrifugation to get rid of the extra citrate ions in the solution. 10 ml of stock solution was concentrated by centrifugation at 2500 rpm for 10 minutes directly followed by 2000 rpm for 30 minutes and the particles were re-

dispersed in Millipore water to remove the excess of citrate. The solution was put on a Vortex for 10 minutes to ensure perfect mixture. The centrifugation followed by mixing was repeated twice. The AuNP pellet was finally re-dispersed in up to 2 ml with Millipore water in an Eppendorf tube and put on a Vortex for 10 minutes.

3.2 Coated surfaces

All substrates were coated with different polypeptide films so that the AuNP could bind to them. All coated surfaces were dried with nitrogen gas and stored dry in Eppendorf tubes until the SEM measurements.

The AuNPs size, shape and surface coverage was analysed with SEM. Gold surfaces were used to enable SEM analyses without the need of any extra coverage with a leading material. Also glass, PMMA and PS surfaces were used; these on the other hand need to be coated with a leading material before SEM measurements. The surfaces were coated with a 5 nm thick film of gold.

Cysteamine was used as the coating material of the substrates only to look at the AuNPs size, shape and distribution. Three different kinds of polypeptides were then studied; lysine, Mefp-1 and albumin. Albumin was used as the reference sample since it binds stronger to solid gold than citrate stabilized AuNPs. Both lysine and Mefp-1 are proteins that can likely be used in the medical coating industry. The molecular weight of lysine is $>30\ 000\ \text{g/mol}$ [8]. Also cross-linking of Mefp-1 was tried as coating on the substrates. Cysteamine, albumin and lysine was purchased from Sigma Aldrich and used as received without future purifications. Mefp-1 was generously donated by the company Biopolymer Products of Sweden AB.

The stability of the AuNPs on the polypeptide film is of big importance. To be able to ensure that the particles will stay on the polypeptide film when used in for example the human body, the coated surfaces were separately exposed to a solution of $0,1\text{M}\ \text{Na}_2\text{CO}_3$ with pH 11,6 and one solution of $0,1\text{M}$ fibrinogen. The surfaces were put in the two solutions, one hour in each, and rinsed with Millipore water afterwards followed by drying with nitrogen gas. The surfaces used were gold and both lysine and Mefp-1 coated surfaces were tried out. SEM measurements were also done before the surfaces were exposed to these solutions, to get good references.

3.2.1 Cysteamine

To be able to look at the particles in SEM the AuNPs were attached to a gold surface with cysteamine as a coupler between the gold surface and the AuNPs. The gold surfaces were cleaned in an UV/ozone oven during one hour, rinsed with Millipore water and washed in base piranha solution for five minutes followed by rinsing with Millipore water again. The clean surfaces were put in a 1mM solution of cysteamine and ethanol for two hours. Surfaces were rinsed with ethanol and afterwards with Millipore water and were for two hours put in AuNPs solution to react. The AuNPs solutions were centrifuged in advance and the AuNPs were redispersed in Millipore water. AuNPs solutions produced at the different temperatures ($50, 60, 70$ and 80°C) were used.

3.2.2 Polypeptides

Three different polypeptides were used to evaluate the binding of AuNPs to them. The cleaning process for the gold, glass and PMMA surfaces was the same as for cysteamine. The cleaning process makes the surfaces hydrophilic. To test the possibility

of coating hydrophobic surfaces with polypeptides, substrates of PMMA and PS were used. To keep their original hydrophobicity only rinsing with ethanol and Millipore water were used to clean the surfaces. The cleaned surfaces were put in 2 ml of polypeptide solution for 15 minutes followed by careful rinsing with Millipore water. All polypeptide solutions had the same concentration, 1 μ M, and were prepared just before usage. The polypeptide coated surfaces were put in AuNP solution for one hour.

The polypeptide solutions were individually prepared and require different procedures. Both albumine and lysine were individually solved in Millipore water. The Mefp-1 solution was prepared by adding 1,56 ml of Millipore water to an Eppendorf tube followed by adding 0,22 ml Mefp-1 and carefully shaking the sample. 0,22 ml of Na_2CO_3 was added to the solution and mixed.

Cross-binding of Mefp-1 requires one more step in the procedure. After the surface was laid in the Mefp-1 solution the surface was rinsed with Na_2CO_3 followed by Millipore water and was transferred to a 5 mM sodium periodat (NaIO_4) solution. After 10 minutes in this solution the surface was rinsed with Millipore water and placed in AuNP solution for one hour.

3.2.3 Gold nanoparticle binding

Gold nanoparticles solved in Millipore water gives a specific surface coverage over the coated surface. To be able to control the surface coverage of the AuNPs citrate buffer was used. Three different concentrations of citrate buffer were tested; 10mM, 1mM and 0,1mM. These AuNP solutions were centrifuged as mentioned before (section 3.1) but are after two times centrifugation dispersed in citrate buffer instead of Millipore water.

The polypeptide coated substrates were exposed to the AuNP solution for one hour. The substrates were placed vertical in an Eppendorf tube containing 2 ml of AuNP solution, figure 2. After one hour the coated substrates were rinsed with Millipore water and dried with nitrogen gas. The dry substrates were stored in Eppendorf tubes until SEM measurements.

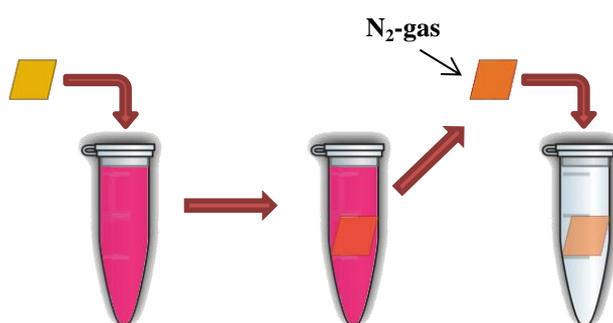


Figure 2: Eppendorf tube containing AuNP solution where the substrate was exposed to the solution for one hour. The coated substrate was dried with nitrogen gas and stored in an empty Eppendorf tube.

3.3 Characterization

To be able to evaluate the AuNPs size three different methods were used; UV-VIS, SEM and DLS. The protein coated surfaces were all examined by SEM to investigate the surface coverage of the AuNPs on the film. In addition QCM-D measurements of the binding of polypeptide and AuNPs to the surface were performed.

3.3.1 Gold nanoparticles

As mentioned before, the size and the size distribution of the produced AuNPs are important. The particle solutions were measured with UV-VIS, SEM and DLS to get information about the particle size, distribution and concentration. This also gave a better understanding for which method is to prefer when doing these kinds of measurements.

3.3.1.1 UV-VIS

The measurements were done using a Perkin-Elmer Lambda 9 spectrophotometer for each batch of AuNPs. All measurements were performed in disposable plastic cuvettes and radiated by light in the absorbance range of 400-800 nm. Millipore water was used as reference.

The wavelength from the absorbance maxima, the SPR, of the intensity-absorbance curve is related to the particle diameter. The particle diameter and concentration could be determined by using the supporting material described in an article from Haiss et al. [15].

3.3.1.2 SEM

The dry surfaces were analysed in SEM at a magnitude of 15 000 and 65 000 to get both an overview of the surface and a more detailed picture. The SEM instrument was a ZEISS SUPRA™ 40VP. All the SEM pictures were evaluated in the data program ImageJ where particle size, particle distribution and surface coverage were calculated.

3.3.1.3 DLS

The AuNP solutions, one batch of each temperature (50°C, 60°C, 70°C and 80°C), were measured with DLS. Measurements were performed on a Brookhaven ZetaPALS with a scattering angle of 90°. The wavelength and temperature were 676nm and 23°C respectively.

To get the particle size the Cumulant and the CONTIN analysis, done by the data program, were looked at. The solutions were both measured after centrifugation and without. All solutions were diluted with filtered Millipore water to a counting rate between 70 and 300 on the machine, in order to get good statistics in the correlation curve.

3.3.2 Polypeptide coated surfaces

The AuNP-polypeptide coated surfaces were examined in SEM to get information about the surface coverage. QCM-D measurement gives information about the mass of AuNPs reacted to the surface and indicates if the reacting time is correct. The surfaces were cleaned by using UV/ozone combined with base piranha wash or rinsing with ethanol.

3.3.2.1 SEM

The SEM measurement was done in the same way as for the cysteamine coated surfaces. When using non-leading surfaces under the coating of polypeptides and AuNPs the surfaces must be sputtered with a leading material to enable SEM analysis. Therefore a thin layer of gold was spread out over the AuNP – polypeptide coated surfaces. The gold layer was set to 5nm and makes the AuNPs appear bigger in the SEM pictures. The non-leading surfaces used were glass, PMMA and PS. The SEM pictures were evaluated in ImageJ where the surface coverage was of major interest.

3.3.2.2 QCM-D

The QCM-D used was a Q-Sense D300 (Q-Sense AB, Göteborg, Sweden) with a temperature controlled fluid cell. Surfaces used in the instrument were quartz crystals covered with gold. The measurements were done in the following way; a stable baseline was established with a buffer of Millipore water for 3-5 minutes, the polypeptide was added to the sample chamber and the adsorption was observed for 15 minutes, washing with Millipore water for 3 minutes, adding AuNP solution and the adsorption was studied for one hour. The time periods were chosen to be the same as when covering surfaces for analyses in SEM.

4. Results and discussion

The gold nanoparticle synthesis resulted in particles in the desired size range, 40 – 60nm. The results from three different analyse methods and a comparison between them are described below. The results for the polypeptide covered surfaces and the stability of them are also discussed.

4.1 Synthesis of gold nanoparticles

To be able to check how the temperature and gold to citrate ratio affects the size of the AuNPs, different trials were carried out. In the beginning the size of the particles was only decided by UV-VIS measurements. The purpose was to create AuNPs in the size range of 40-60 nm and to be able to control the size only by changing the temperature. The temperature interval from the start was 50-70°C and was later expanded to 50-80°C.

Four different trials, see figure 3, were made before it lead to one where the results could be repeated. The first trial resulted in AuNPs with a size of 56 nm. This was in the desired size range but since the particles were quite big and produced only at 60 °C the gold to citrate ratio was changed. An article written by J. Kimling et al. [16] suggests that the gold concentration should be 1,0 mM or below to give rise to stable particles. Trial two, three and four all had the same gold to citrate ratio, one, but with varying gold concentration. The second trial had a gold concentration of 1 mM, the third 0,8 mM and the fourth 0,5 mM. Unfortunately the second trial lacked in reproducibility, the third resulted in particles with very similar sizes when performed at different temperatures and the fourth gave too big particles (size range of 74-91 nm).

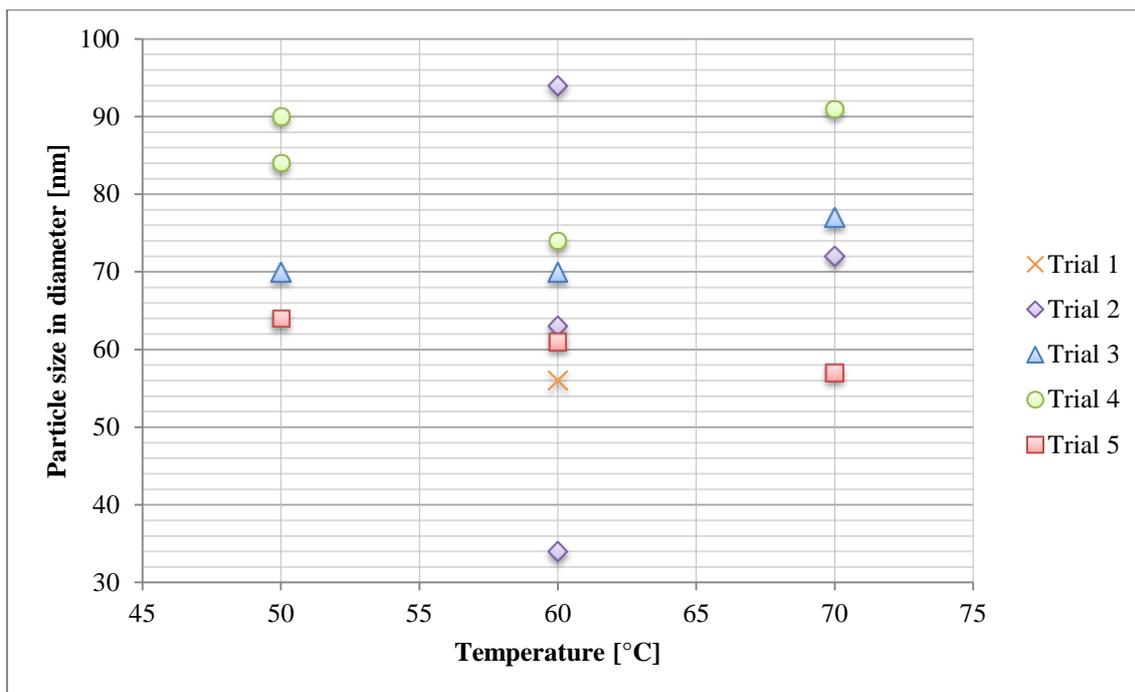


Figure 3: UV-VIS measurements displayed in particle size. Trial 1-5 with the different temperatures tested.

The fifth trial, Figure 3, had higher reproducibility and gave the temperature size dependence that was anticipated, namely lower temperature gives rise to bigger particles. In this trial the gold concentration was set to 0,5 mM and the gold to citrate ratio was 0,862. The particles produced at 70°C had a diameter of 55-57 nm and was the ones that were used in the following studies and analysis.

Since the purpose was to control the size of the AuNPs only by changing the temperature the gold to citrate ratio and the stirring speed was kept constant. The particles in trial five were in the same size range 50 to around 70 nm which indicates that the temperature makes a difference to the particle size but that it does not have a large effect. When trial five and four are compared it looks like the gold to citrate ratio affects the particle size more than the temperature. However, this is based only on the UV-VIS measurements.

4.2 Characterisation of gold nanoparticles

The AuNPs used in this section were all produced as in trial five and the temperature interval was expanded to 50-80°C. The particles were analysed with UV-VIS, SEM and DLS to get information about the size of the particles.

4.2.1 UV-VIS results

In the UV-VIS measurement only one absorption peak was visible which indicates that the AuNPs possesses a more spherical than elongated shape. The measurements also showed that the particle size decreases with increasing temperature. This result follows what was expected. The temperature dependence on the size of the AuNPs was more carefully studied at temperatures from 50°C to 80°C. Also one batch at 90°C and one at 100°C was made to investigate if also temperatures near boiling and boiling resulted in the same size-temperature behaviour. Both experiments resulted in particles of 51 nm diameter which is smaller than the particles at 80°C. If the assumption that higher temperature gives smaller particles was all true also the particles created at 100°C would be smaller than the one created at 90°C. From the table it is also noticed that both

particle solutions made at 70°C and 80°C create particles in the same size range. This can indicate that at this temperature range the particle sizes aren't affected so much by the temperature. The UV-VIS measurement displayed as size to temperature can be seen in figure 4.

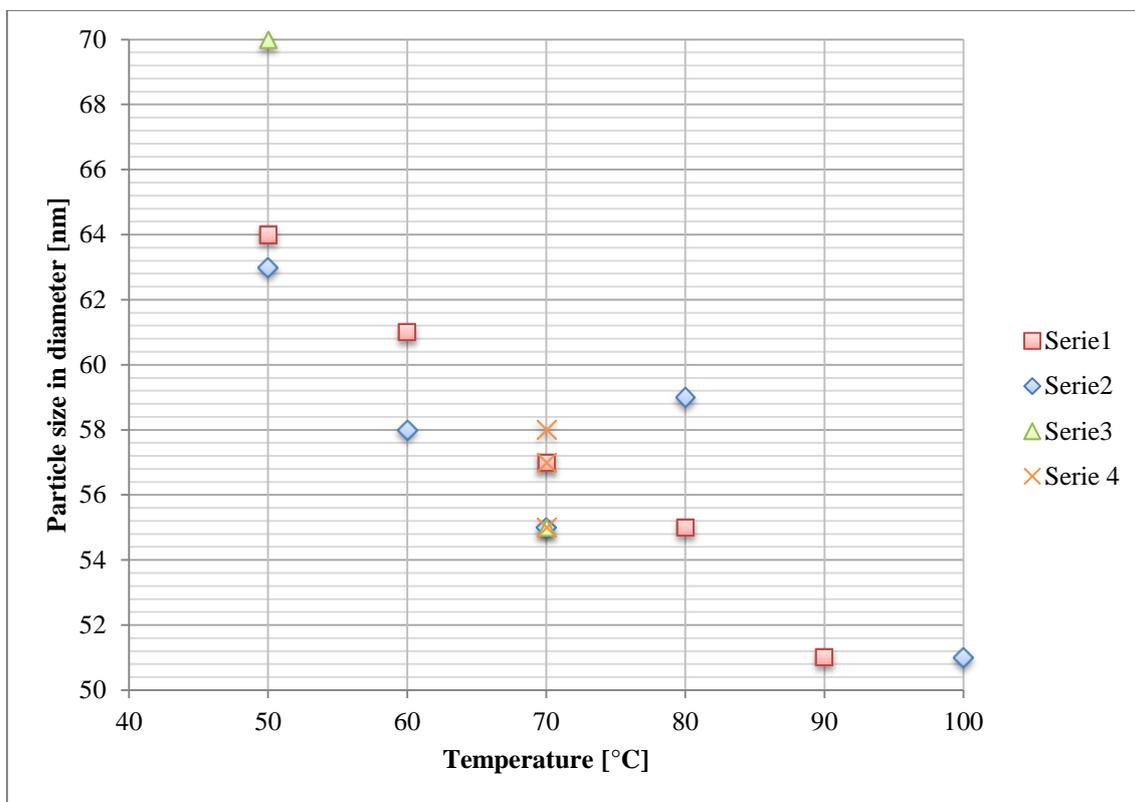


Figure 4: UV-VIS results where the AuNP size is related to the temperature they are created at. The different series includes the different AuNP stock solutions made.

4.2.2 SEM results

To be able to look at the particle size in SEM the AuNPs has to be attached to a surface and dried. The choice of surface and chemical film is important to be able to get easy and nice measurements. Gold surfaces were used to get around the inconvenient of sputtering (coverage of a thin layer of leading material) the sample before SEM analyse. Cysteamine was used as the chemical film due to its known good ability to bind to AuNPs [1].

The size, the size distribution and the agglomeration of AuNPs were evaluated from the SEM measurements. By using the data program ImageJ on the SEM pictures both the particle size and the surface coverage was calculated. The SEM picture is turned into a black and white picture in the program, by using the threshold tool which looks at the brightness intensity for the objects. The threshold is set at a level such that the particles are the only objects measured. Since the threshold can be changed according to the operators opinion, the outcome can vary, depending on who is creating the picture. Therefore the particles sizes have a margin of error, but since the same operator has analysed all the pictures this error shouldn't be big. Appendix I illustrates the change in appearance of a SEM picture when using ImageJ.

Figure 5 show AuNPs bound to a cysteamine coated gold surface, the particles are produced at 80°C. The figure illustrates well how the particles bind to cysteamine. SEM pictures with particles produced at other temperatures look similar. The SEM

measurements show mostly well distributed separated particles but there are also some agglomerations. The agglomerations can either come from the solution of AuNPs or they could be created when the particles are bound to the cysteamine film. If there were large amounts of agglomerations in the samples it would be a problem. The agglomerations would make it impossible to get good distribution of AuNPs over the surface. A good distribution of AuNPs is needed for future surface modifications. If the distribution isn't right it could for example make the surface only partially antimicrobial.

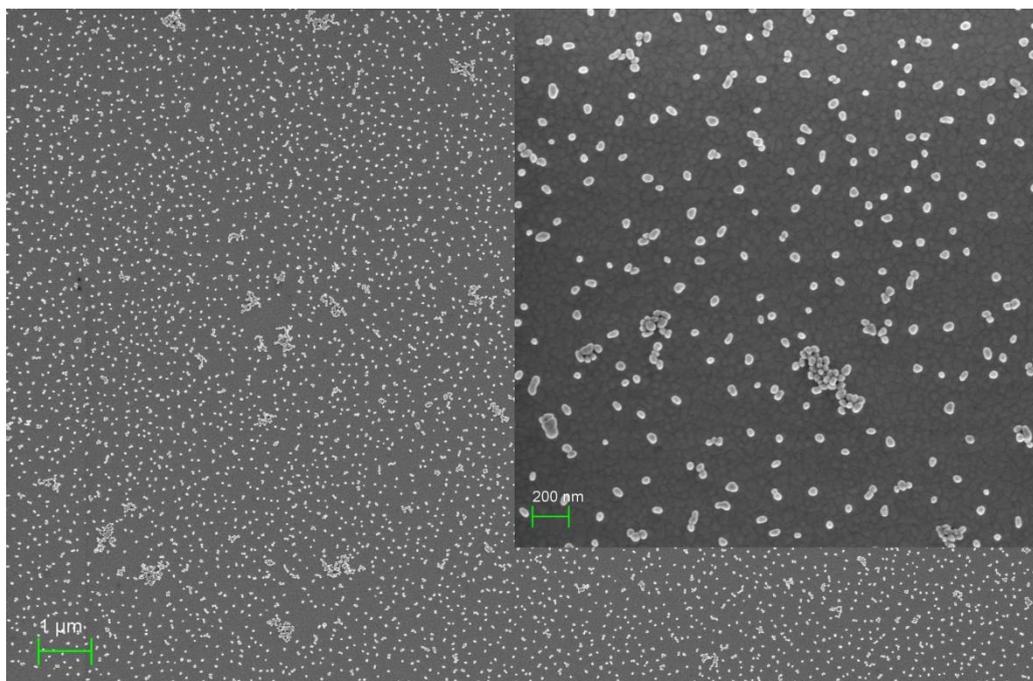


Figure 5: Cysteamine coated gold surface with attached AuNPs to it. The SEM picture is from particles made at 80°C.

The AuNPs produced at 50°C, 60°C, 70°C and 80°C were compared and the results are presented in table 1. These results do not follow the assumption that lower temperature results in bigger particles.

Table 1: Particle size calculated from the SEM measurement.

| Temperature [°C] | Particle diameter size [nm] |
|------------------|-----------------------------|
| 50 | 49,9 |
| 60 | 51,3 |
| 70 | 53,5 |
| 80 | 53,4 |

The size distribution was calculated on 1200 particles per AuNP solution and is displayed in figure 6 and also in Appendix II in a more detailed way. It should be noticed that the particle diameter size is an average of the major and the minor diameter since the particles are not completely spherical. The different solutions made at the different temperatures all showed a peak around 55nm. A more detailed measurement showed that there were most particles of size 49nm attached to the surface from solutions made at 50°C and 80°C and from solutions made at 60°C and 70°C there were most particles of size 53nm. Probably this is mainly a result from the fact that only 1200

particles were measured and if more SEM pictures and particles would have been evaluated it would likely have changed the results.

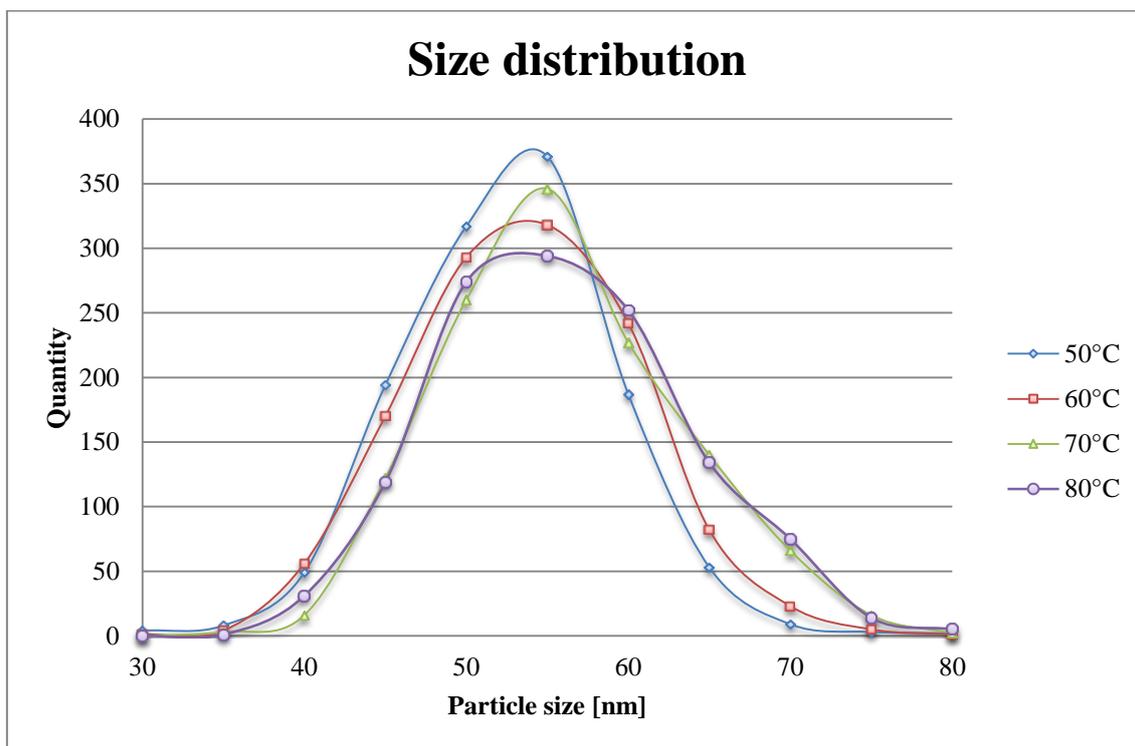


Figure 6: Size distribution for AuNPs created at 50, 60, 70 and 80°C. Amount of particles measured is 1200.

4.2.3 DLS results

The DLS measurements resulted in two different mean diameters depending on which evaluation method that were used. In all cases the Cumulant method gave lower values of the particle diameter than the CONTIN method. The volume curve indicated that in all solutions there was a large amount of small particles in the size range of about 1-5 nm. The result of this was that the mean particle diameter from the volume curve was much smaller than the one from the intensity curve. The different mean particle diameter from AuNP stock solutions that were produced at 50, 60, 70 and 80°C are presented in table 2. The measurement results, displayed in table 2 include one value where the bare stock solutions were measured and one where centrifugation of the stock solutions was done before the measurement. The centrifugation process was the same as the one described before. The measurements were done during two or four minutes depending on the results from the first two minutes. If the result from the first two minutes showed more than two different size groups of particles the measurement was done for two more minutes. The time of the measurements affected the results from the DLS measurements. Short measurements were usually enough to get the information of the particle size, increasing the time would have given more correct results but if dust or aggregates is in the solution this will show and give rise to worse results. Therefore the measurements were chosen to be run only for two to four minutes. Appendix III shows a more complete picture of all the results from the measurements.

Table 2: The mean diameter of AuNPs in nm. Results from the Cumulant method and CONTIN analysis. Stock solution from AuNP created at 50-80°C. Yes means with stock solution centrifuged, No means without centrifugation of the stock solution.

| | | 50°C | | 60°C | | 70°C | | 80°C | |
|--------------------|--------------------|-------------|------|-------------|------|-------------|------|-------------|------|
| | | Centrifuged | | Centrifuged | | Centrifuged | | Centrifuged | |
| | | Yes | No | Yes | No | Yes | No | Yes | No |
| Mean diameter [nm] | Intensity (CONTIN) | 46,9 | 45,5 | 52,3 | 32,8 | 47,7 | 45,8 | 77,0 | 60,5 |
| | Volume (CONTIN) | 3,2 | 2,2 | 7,5 | 2,0 | 4,3 | 4,6 | 2,2 | 2,1 |
| | Cumulant | 35,3 | 33,8 | 38,2 | 30,5 | 29,1 | 30,6 | 50,9 | 44,7 |

The large amount of small particles in combination with the groups of bigger particles makes the solutions polydisperse and not monodisperse as wanted. But it could be that the small particles are not gold nanoparticles; it is also possible that it is either unreacted gold salt or small citrate complexes. In almost all experiments the centrifugation of the solutions gave an increase in the particles mean diameter which is due to the loss of small particles. Only centrifuged AuNP solutions (the yes columns) were used in the SEM analyses and these results were therefore the ones of most interest.

Since there were a lot of small particles in the solutions it was hard to get results that were comparable with the results from the UV-VIS and SEM measurements. Therefore I would not recommend this method to decide the size of the particles; this method was on the other hand useful to get information about the cleanness of the solutions.

4.2.4 Evaluation of analysis techniques

To decide the size of the AuNPs three different analysis techniques were used and compared, in order to evaluate which one that serves this purpose best. All techniques gave different results on the size of the AuNPs, see table 3.

Table 3: Result of the AuNPs average sizes from the different analyse techniques.

| | 50°C | 60°C | 70°C | 80°C |
|-----------------|------|------|------|------|
| UV-VIS | 68 | 64 | 59 | 57 |
| SEM | 49,9 | 51,3 | 53,5 | 53,4 |
| DLS (Intensity) | 46,9 | 52,3 | 47,7 | 77 |

The hypothesis was that with lower temperature bigger particles would form. This assumption seemed true only looking at the results from the UV-VIS measurements. SEM indicates rather the opposite behaviour with a very small difference between the AuNPs created at 70 and 80°C. The DLS measurements did not show any trend. In this comparison the data from the CONTIN, intensity measurement, were the only one included. The reason for this is that the small particles interfere too much in the other technique. If only looking at the data from SEM and DLS the particles sizes are in the same size range in all cases except at 80°C. At 80°C the particles seems much bigger in the DLS measurement compared to the SEM result. This result is hard to explain but one reason could be that there are aggregates in the solution which could affect the result.

It is hard to say which one of these methods that gives the most correct size of the particles. However, in both SEM and DLS there are parameters that can be changed to get a more accurate final value. In SEM more pictures could be taken and a larger amount of particles could be calculated. Also the chemical film of the substrate could be changed; cysteamine may not be the best chemical to use. In DLS the running time could be optimized and a cleaning (further centrifugation or dialysis) of the solutions could be done, to get around the problems caused by the small “particles”. This is built on the assumption that the small “particles” are not small AuNPs but some other species.

If a quick particle size analyse is desired the UV-VIS would be the choice. The SEM measurements are good for getting additional information about the particles. If the only interest is the particle size SEM is too time consuming because of the sample preparations. DLS is used and recommended by many when it comes to deciding size of small particles in solution. If the solutions had been monodisperse, DLS would have been a good way to measure the particle size. DLS is on the other hand more time consuming than UV-VIS measurements but is faster than SEM.

4.3 Polypeptide coated surfaces

This section deals with the coating of lysine, Mefp-1 and albumin on different surfaces and attaching the AuNPs to them. The results from the binding of AuNP in water solution and in solutions with different amount of citrate are presented. The binding of AuNPs to cross-linked Mefp-1 was also examined and will be presented.

Four different substrate materials were used; gold, glass, PMMA and PS. The surfaces were primarily analysed with SEM but also some QCM-D measurements were done. In SEM mainly the surface coverage was analysed. With QCM-D the mass of AuNPs bound to the surface and the binding time of both the polypeptide and AuNP were analysed. QCM-D measurements were only done on gold surfaces.

4.3.1 SEM results

In all experiment with the different surfaces (gold, glass, PMMA and PS) a control surface, of the same material as tested, was used to be sure that the AuNPs couldn't bind to the surface without the polypeptide coating. The SEM pictures of these experiments are shown in Appendix IV. The reference pictures all show that there was no binding of AuNPs to the bare substrate.

The SEM pictures below, where the scale bares are 1 μm and 200 nm respectively, is taken at a magnification of 15000 respectively 65000.

4.3.1.1 Gold surface

The polypeptide – AuNPs covered gold surfaces, see figure 7-9, resulted in attachment of AuNPs on the lysine and Mefp-1 surface while with the albumin coating almost no AuNPs bound to it. This was an expected result. The surface coverage of lysine was 7,5 % while for Mefp-1 it was 5,6 %. This indicates that lysine binds a higher amount of AuNPs than Mefp-1. When the albumin coated gold surface is exposed to the AuNP solution, almost no binding of AuNPs to albumin happens, figure 9. This indicates that the thiol-groups on albumin are already occupied at the gold surface. Albumin can therefore be used as reference sample. Figure 7-9 show pictures where the AuNPs were dispersed in Millipore water and attached to the polypeptide film.

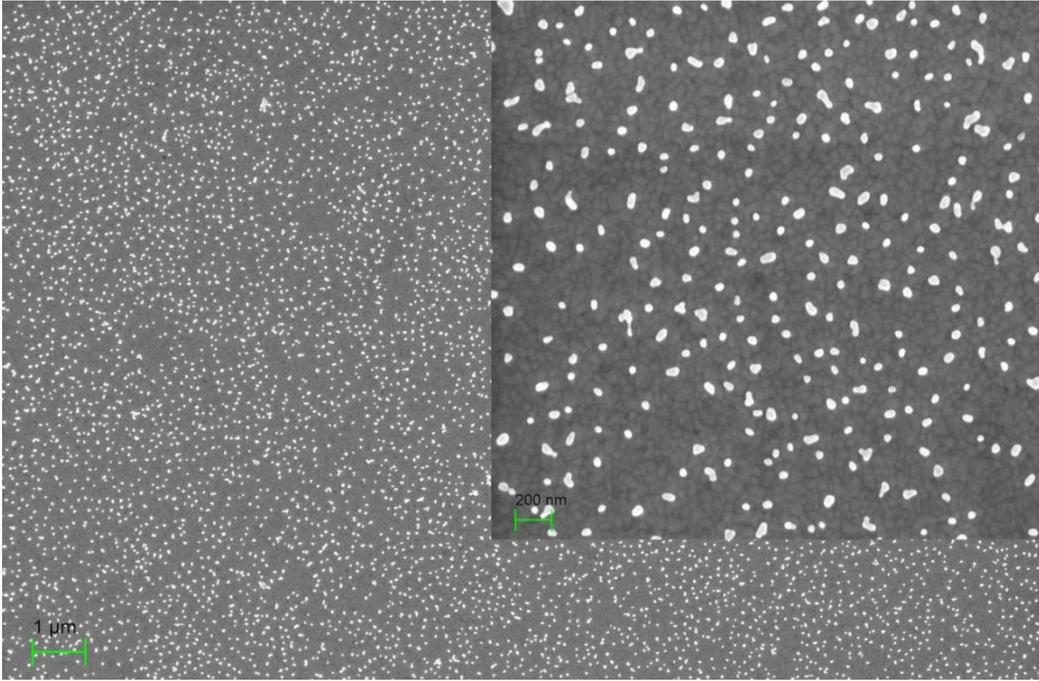


Figure 7: Lysine coated gold surface with AuNPs bound to it.

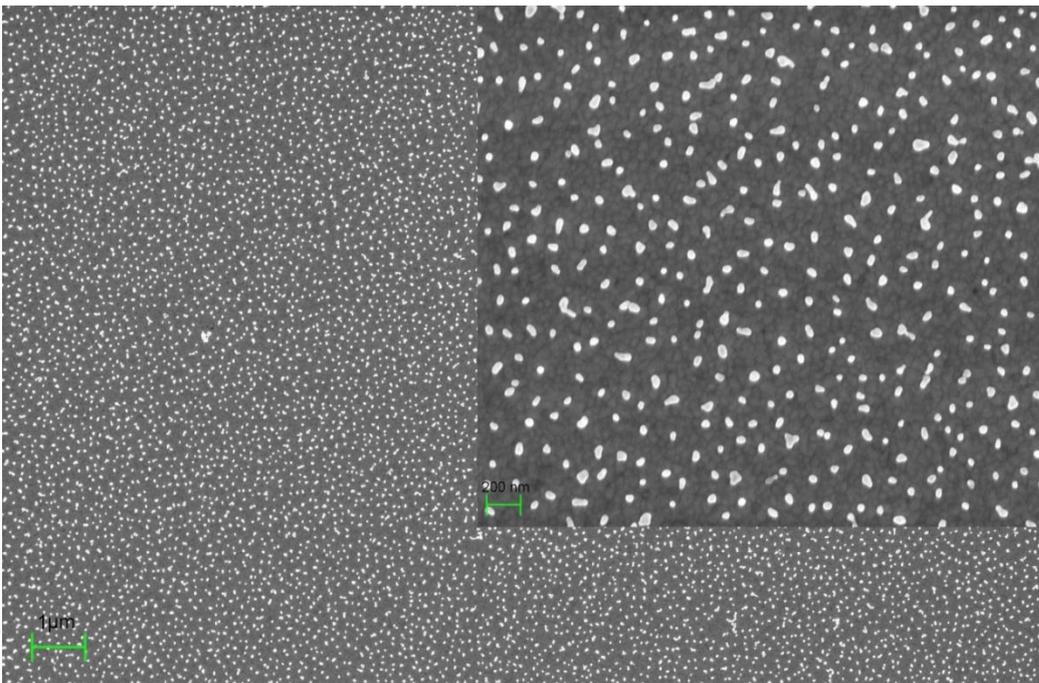


Figure 8: Mefp-1 coated gold surface with AuNPs bound to it.

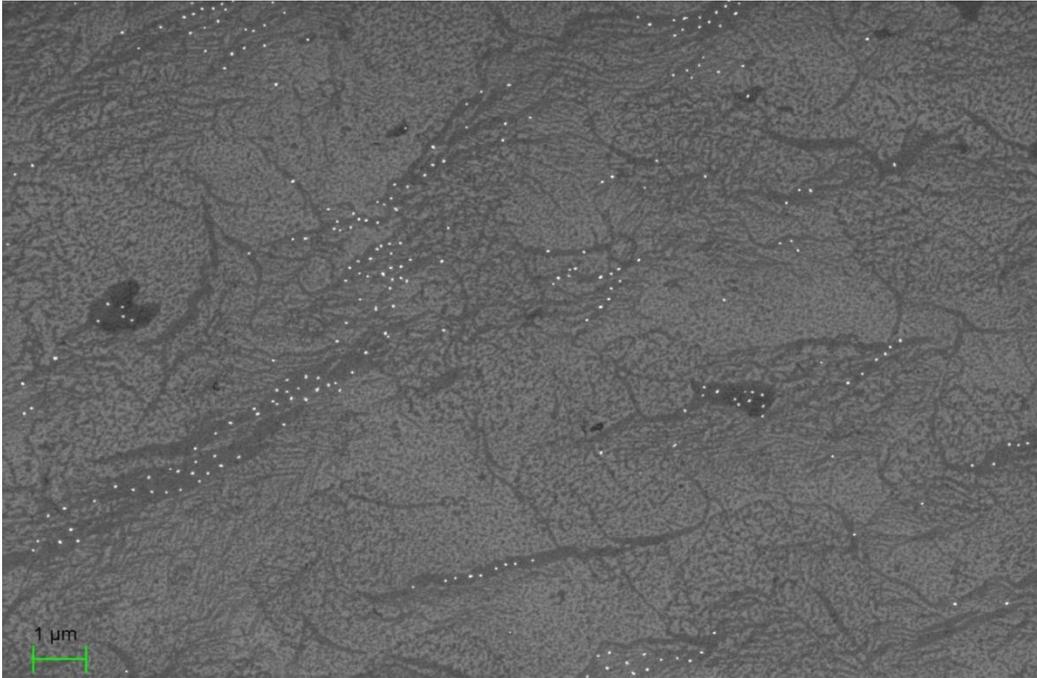


Figure 9: Albumin coated gold surface with AuNPs bound to it.

The following SEM images illustrate polypeptide – AuNPs covered surfaces where different salt concentrations were used in the AuNP solution. The result of salt concentration of 1 mM, figure 10-11, and 0,1 mM, figure 12, are presented below.

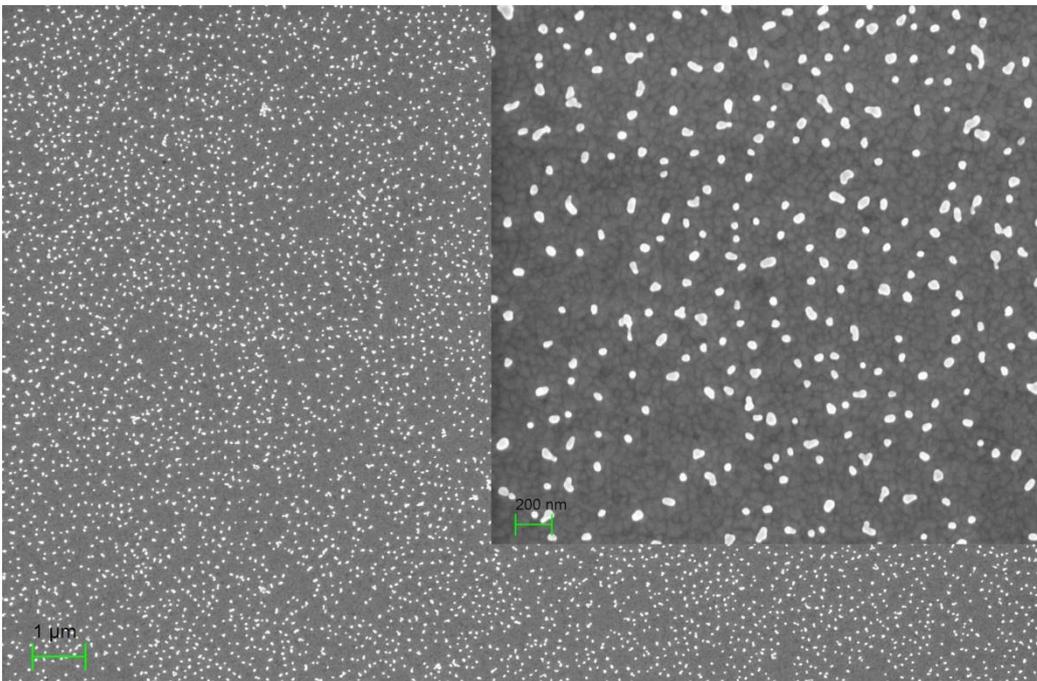


Figure 10: Lysine coated gold surface with AuNPs bound to it. The AuNPs were bound to the lysine film in 1mM citrate solution.

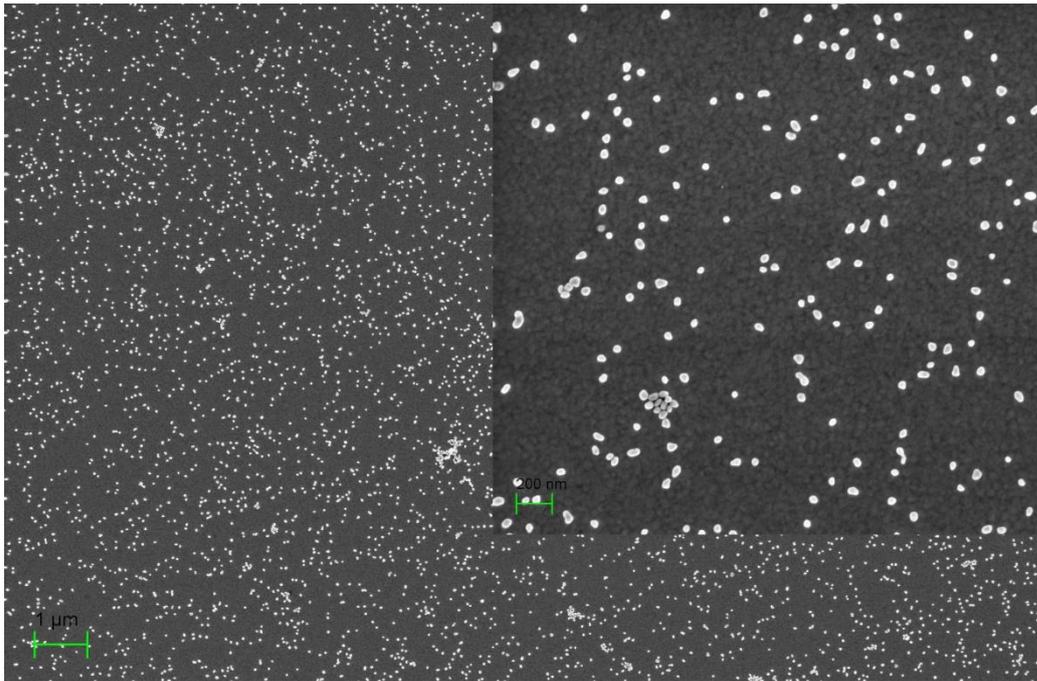


Figure 11: Mefp-1 coated gold surface with AuNPs bound to it. The AuNPs were bound to the Mefp-1 film in 1mM citrate solution.

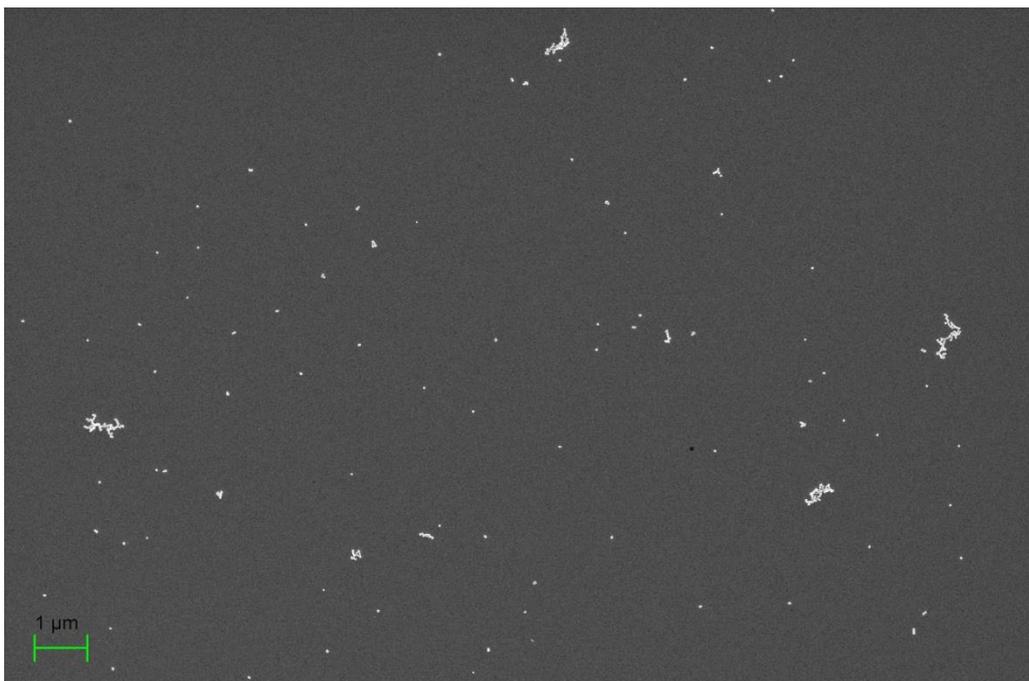


Figure 12: Mefp-1 coated gold surface with AuNPs bound to it. The AuNPs were bound to the Mefp-1 film in 0,1mM citrate solution.

By comparing the Mefp-1 pictures it could be seen that decreasing salt concentration makes the particles separate more. At 0,1mM salt concentration the distance between the particles increased while at 1mM the particles are more unevenly spread. Many of the particles lie close together but it looks like the particles have attached in lines where bigger spaces are located between the lines. The surface where Millipore water was used has particles with a more even space between the particles and a more even surface coverage.

The surface coverage on lysine with 1mM salt concentration was 5,1 % and for Mefp-1 5,0 %. With lower salt concentration, 0,1mM, the surface coverage decreased to 0,3 %

for Mefp-1 and also more agglomerations was seen on the surface. Salt concentration of 0,1mM was not tried on lysine. Higher salt concentration, 10mM, was tried, but when adding the citrate buffer to the AuNP solution the AuNPs aggregated. The reason is that the distance between the citrate stabilized AuNPs decreased and the repulsive forces was no longer enough to separate them.

Using Millipore water as the solvent for the AuNPs resulted in higher surface coverage than when using citrate buffer as solvent. The surface coverage of AuNPs on lysine decreased from 7,5 to 5,1 % and for Mefp-1 from 5,6 to 5,0 % when using citrate buffer instead of Millipore water. By using different salt concentrations it is possible to change the surface coverage of AuNPs. The citrate buffer affects the distance between the particles and the surface coverage of AuNPs has to be investigated further to be able to say if this is a good method to use to change the coverage on the surface. In addition the time of the substrates in AuNP solution might not be the optimal when using salt solution instead of pure water.

The crosslinking of Mefp-1, before reacting AuNPs to it, resulted in a lower surface coverage (4,7 %), than without crosslinking (5,6 %). Compare figure 13 and figure 8. The cross linking of Mefp-1 makes the DOPA groups crosslink, the AuNPs are both attached to the DOPA group and the NH_3^+ group. The lower surface coverage of AuNPs can be explained by less DOPA groups that are available for binding the AuNPs. Another explanation can be that since the DOPA group is cross-linked the time for binding AuNPs to it needs to be prolonged. This could be tested by extending the time that the surface is in the AuNP solution. From the SEM pictures the cross-linking of Mefp-1 seems to get a more even distribution of AuNPs, which is good. The even distribution is important to be able to get an even antimicrobial surface when attaching the AMPs to the AuNPs.

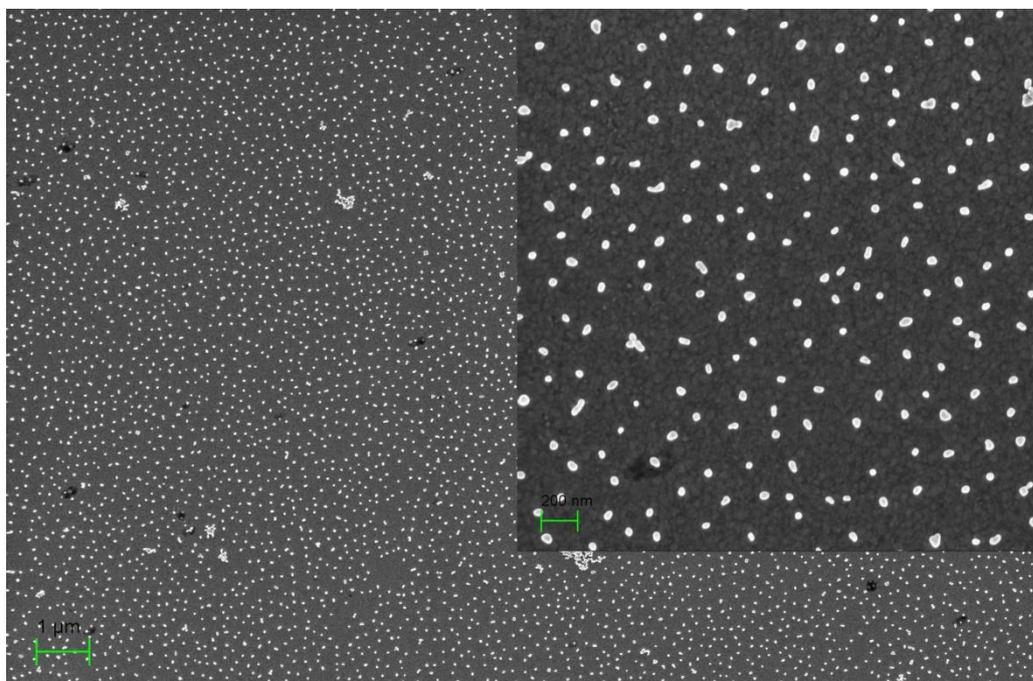


Figure 132: Cross-linked Mefp-1 with AuNPs bound to it.

4.3.1.2 Glass surface

The surface coverage of AuNPs on glass substrates is displayed in figure 14-15. The glass surfaces are coated with lysine respectively Mefp-1. The binding of AuNPs to the different polypeptide films on glass could easily be seen, the glass slides turned red after the time in the AuNP solution. The surface coverage of the lysine coated surface was 7,7 % and for Mefp-1 5,8 %. The values are very similar to the ones seen when coating on gold surfaces. The surface coverage of AuNPs on both lysine and Mefp-1 looks more even when coating on glass than on gold.

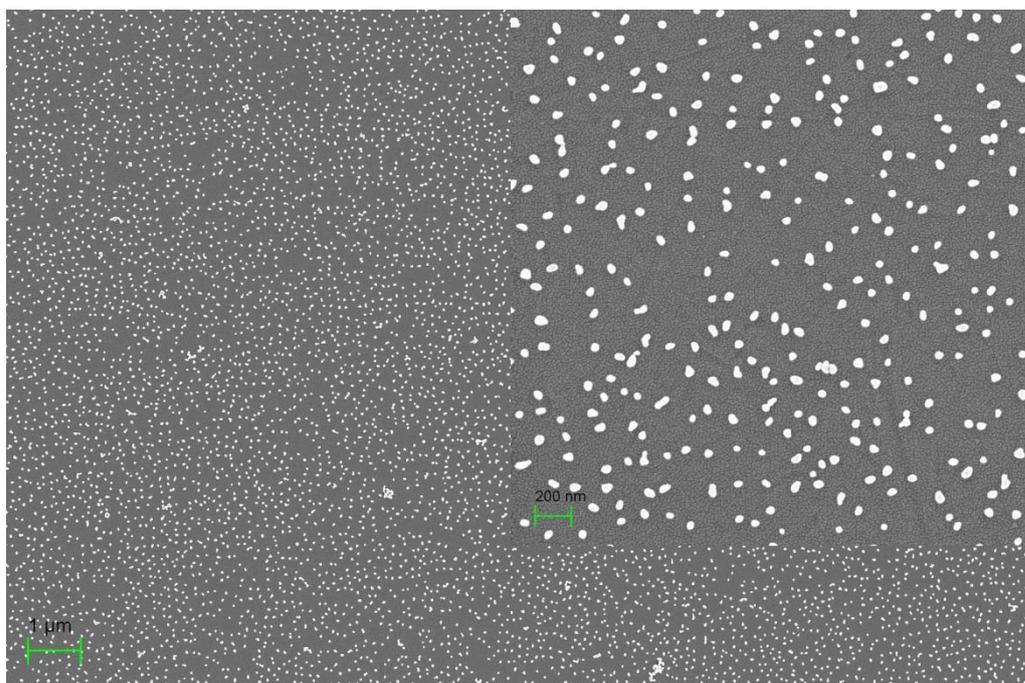


Figure 143: Lysine coated glass substrate with AuNPs bound to it.

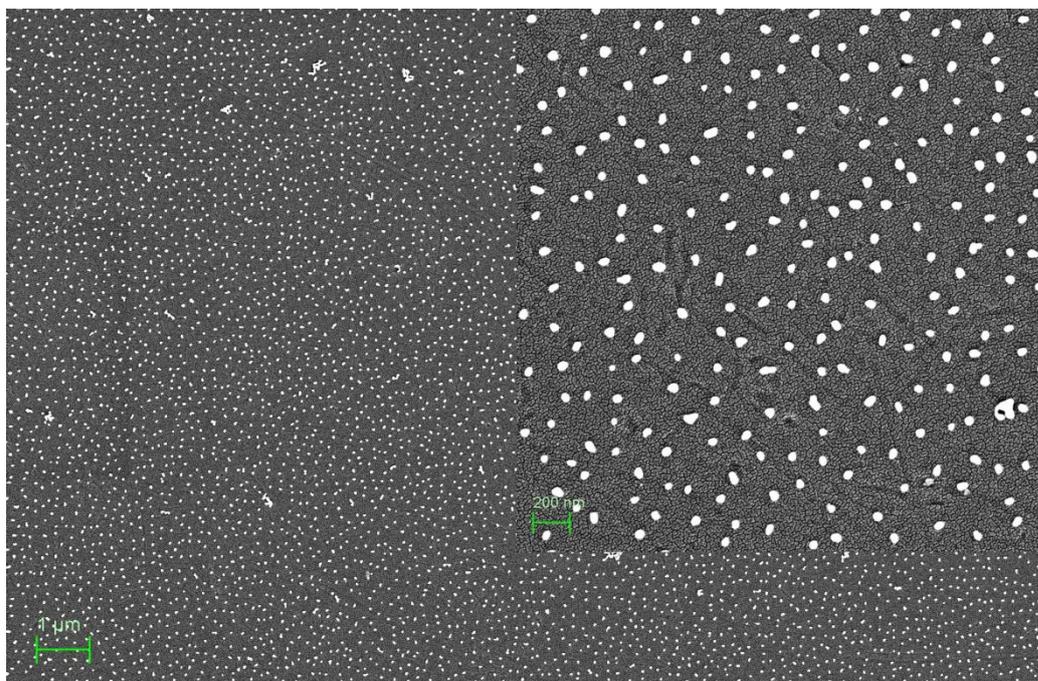


Figure 15: Mefp-1 coated glass substrate with AuNPs bound to it.

4.3.1.3 PMMA surface

Figure 16-17 shows PMMA surfaces with UV/ozon and base piranha wash, this cleaning procedure makes the surface hydrophilic. PMMA surfaces only cleaned by rinsing with ethanol was created and illustrated in figure 18-19. The PMMA surfaces washed with only ethanol are hydrophobic and were individually coated with lysine and Mefp-1. The hydrophobicity of a material can be crucial to if the polypeptide film attaches to it, or not.

The lysine coated hydrophilic PMMA surfaces showed that AuNPs bound to it, but on the Mefp-1 coated surface no AuNPs could be seen, figure 16-17. The highly porous structure of the hydrophilic PMMA comes from the partial brake down of the material in the base piranha wash. Because of the height differences in the surface, the surface coverage of AuNPs on lysine could not be calculated. It is likely that Mefp-1 has not coated the surface and therefore no AuNPs are attached. Both the gold and glass substrate have been hydrophilized by the base piranha wash and therefore it is odd that Mefp-1 should not be able to coat the hydrophilic PMMA surface. The only visible difference between the surfaces is that the gold and glass surfaces are flat and the PMMA surface is highly porous. A tentative speculation is that the Mefp-1 is too big and bulky to bind effectively to such a micro porous surface.

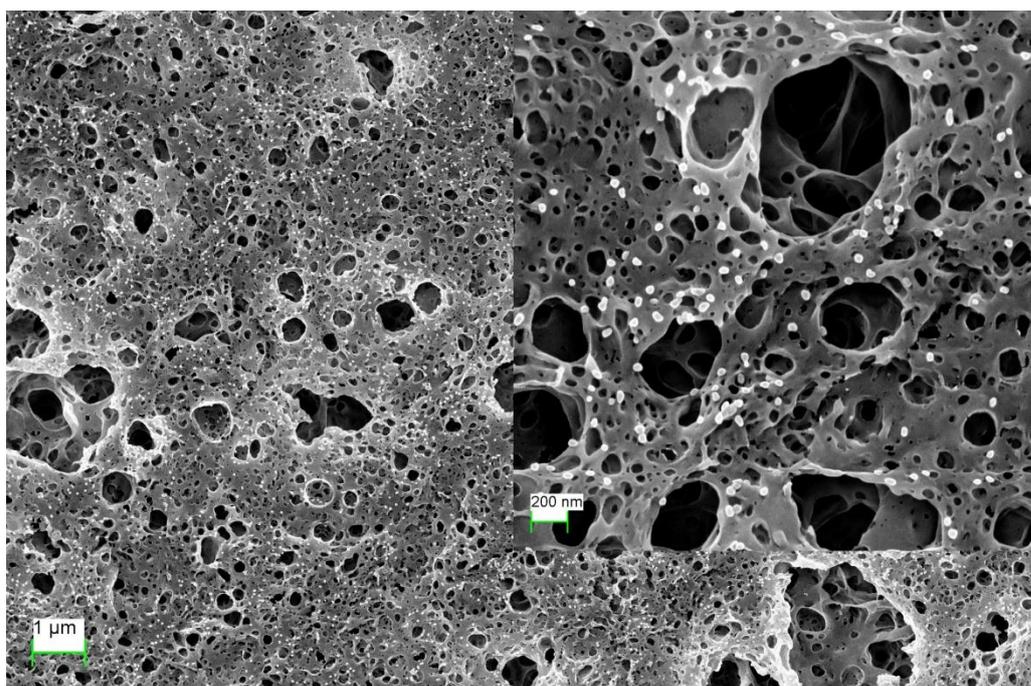


Figure 164: Lysine coated hydrophilic PMMA substrate with AuNPs bound to it.

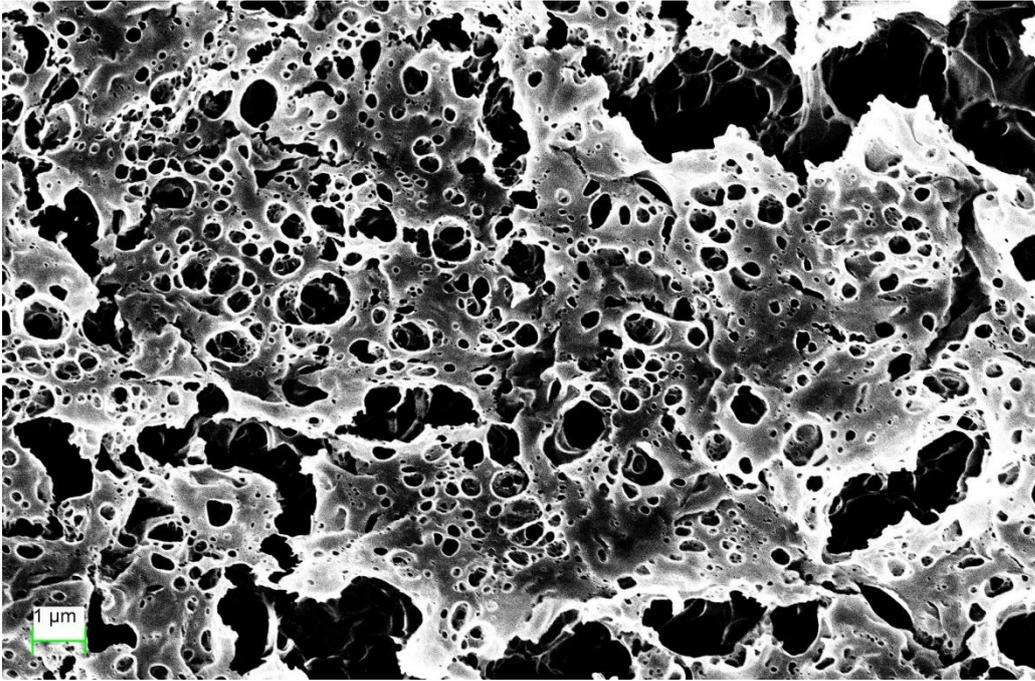


Figure 17: Mefp-1 coated hydrophilic PMMA substrate. No AuNPs are bound to it.

The hydrophobic PMMA surfaces showed a different behaviour, figure 18-19. AuNPs could bind both to the lysine coated surface and to the Mefp-1 coated. For the lysine coated one the spreading of AuNPs is very uneven and is changing from parts with no surface coverage to parts with high surface coverage. The AuNPs bound to Mefp-1 are more evenly spread out and the surface coverage is 0,8 %. It is much lower than the surface coverage on Mefp-1 when coated on gold or glass substrates.

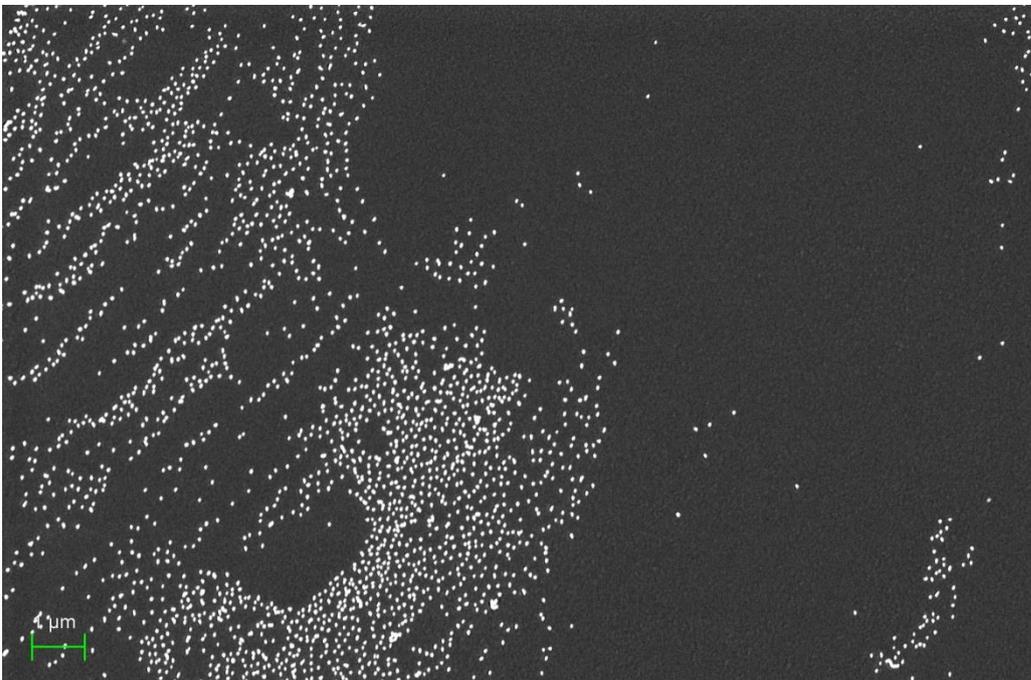


Figure 185: Lysine coated hydrophobic PMMA substrate with AuNPs bound to it.

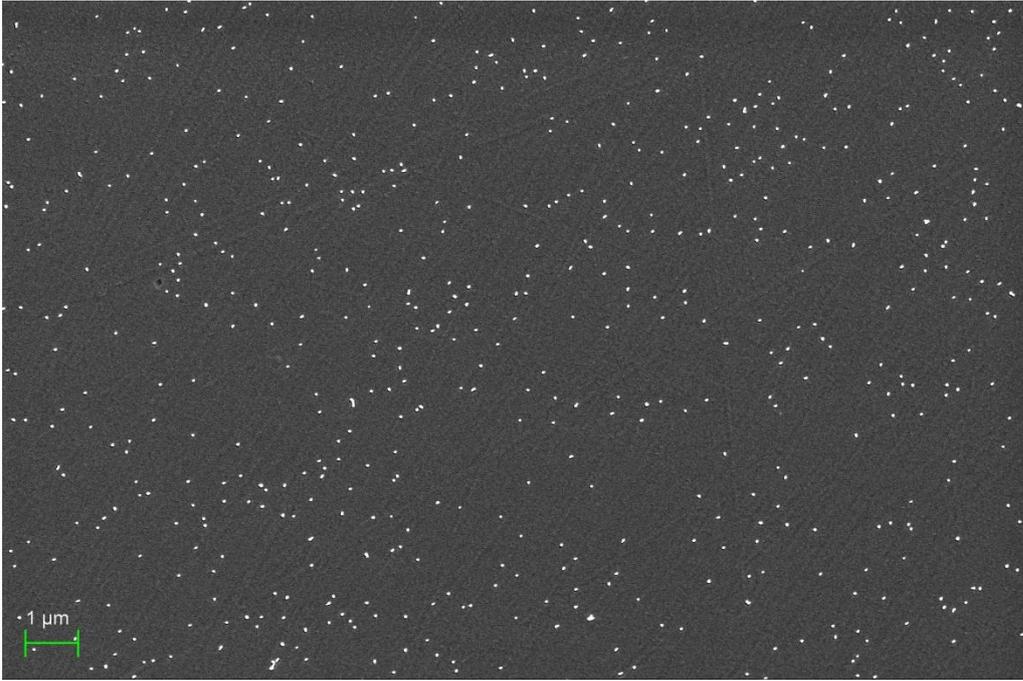


Figure 196: Mefp-1 coated hydrophobic PMMA substrate with AuNPs bound to it.

4.3.1.4 PS surface

The AuNP binding to lysine and Mefp-1 coated PS substrates can be seen in figure 20-21. The PS surfaces were only rinsed with ethanol as cleaning procedure. The binding of AuNPs to lysine resulted in uneven binding; the surface was partially covered in elongated narrow slices while other parts of the surface were empty from particles, figure 18. When Mefp-1 was used it instead gave a surface coverage of 7 %, with a relatively even distribution of particles, figure 21. The degree of AuNPs surface coverage on PS is higher than on both gold and glass.

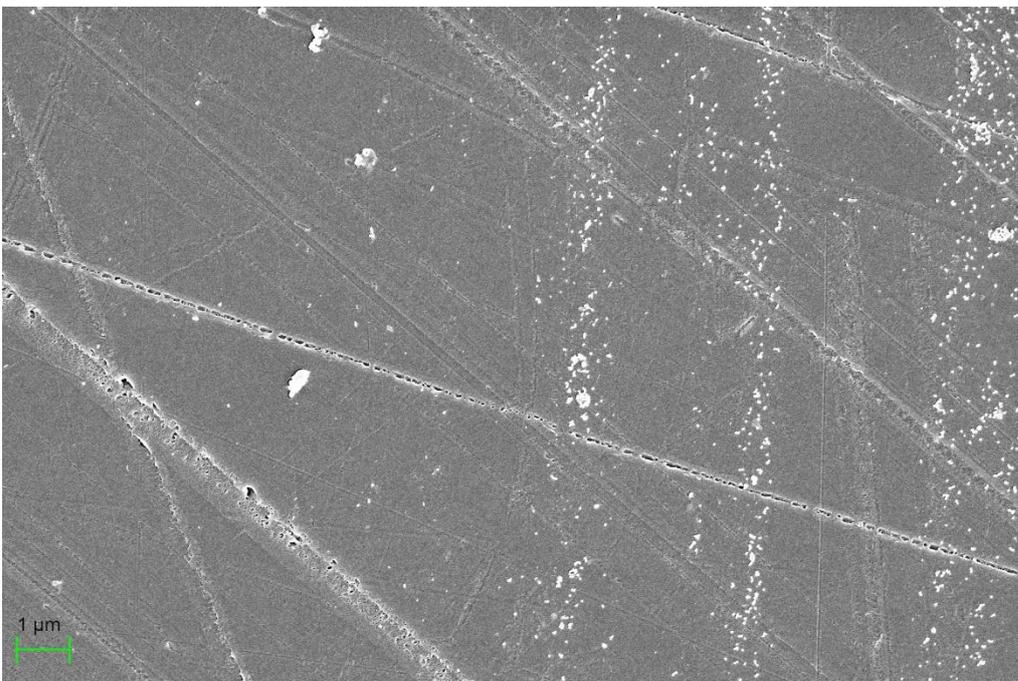


Figure 207: Lysine coated hydrophobic PS substrate with AuNPs bound to it.

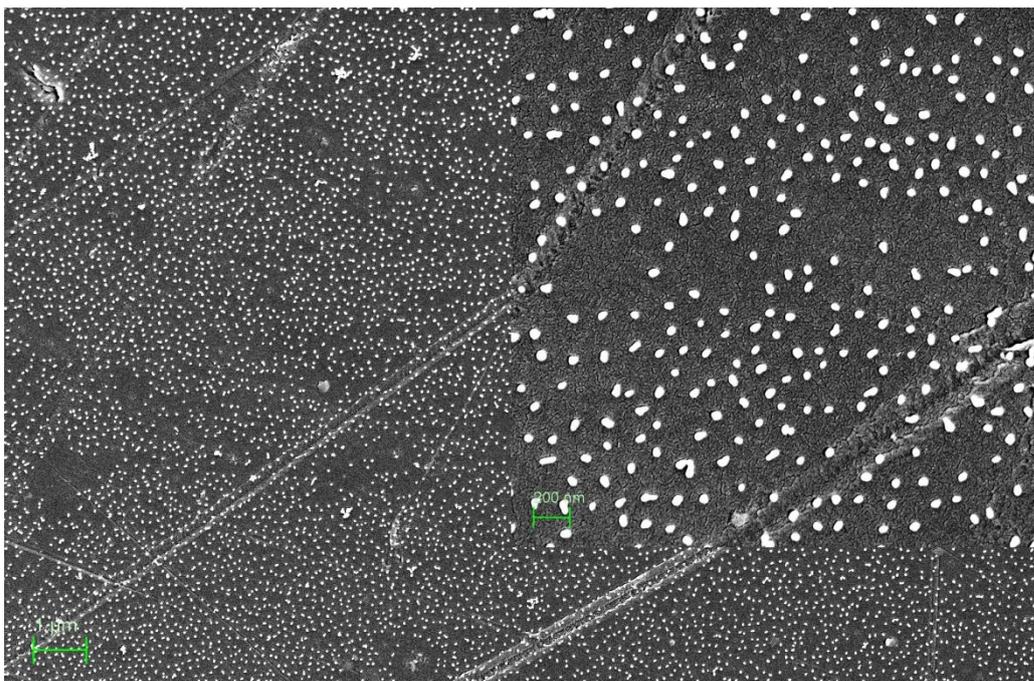


Figure 21: Mefp-1 coated hydrophobic PS substrate with AuNPs bound to it.

4.3.2 QCM-D results

The QCM-D results gave information about if the reaction time for the binding of the polypeptides and the AuNPs was optimal. The measurements also made it possible to calculate how much AuNPs, defined in mass, were attached to the surface. Both Mefp-1, lysine and the binding of AuNPs to them was studied. The QCM-D measurements showed that for both the polypeptides the binding time was long enough. The coating of the surface happened almost immediately after contact with the polypeptide solution. In the case of lysine the binding time of AuNPs, 1 hour, was enough but on Mefp-1 this binding time was too short. The graphs with the QCM-D results can be found in Appendix V. To be able to compare the surface coverage of AuNPs on the polypeptide films it is crucial that the right reaction time is used. The QCM-D results show that the AuNPs bind much faster to the lysine film than to the Mefp-1 film. It is assumed that if the binding time of AuNPs was extended (longer time for the polypeptide coated surface in the AuNP solution) a higher surface coverage would appear.

The first QCM-D measurement with AuNPs bound to lysine gave a very unstable curve, Appendix V – Figure 1, due to too short stabilization time of the instrument. This measurement resulted in 18,99 ng/cm² AuNPs bound to the surfaces. Since the measurement was so unstable one more try was done; this resulted in no binding of AuNPs to the lysine film, Appendix V – Figure 2. A possible explanation was that the AuNP solution used had too low concentration of AuNPs to generate the binding to the surface. Therefore an AuNP solution, provided by Mats Hulander CMB Gothenburg University (using the same protocol), with higher concentration and lower particle size, 38 nm, was tried. This resulted in an attached AuNP mass of 20,96 ng/cm², Appendix V – Figure 3. The mass difference from the first try, with particles produced in the project, was not so big. This result also points towards that the AuNPs on lysine has reached saturation on the surface. The concentration of the AuNPs in the solution seems to be of big importance. If a too low particle concentration is used when binding AuNPs to the substrate it can cause the particles not to bind to the coated substrate. On the other hand the result from the SEM analysis always gives a surface coverage of AuNPs to lysine when gold substrates are used. This can be an indication that QCM-D is not the optimal

technique to use when looking at the binding of AuNPs to a polypeptide coated substrate. Since the QCM-D machine is not uniquely used for this analysis it can contain contaminations from earlier analysis, which can cause failure to the measurements.

The measurements on AuNPs bound to the Mefp-1 film were done with both the particles produced in the project with lower concentration, and the provided smaller particles with higher concentration. The amount AuNPs bound to the Mefp-1 film was 52,5 ng/cm² and 139,7 ng/cm² respectively, Appendix V – Figure 4 and 5. The different concentrations in the AuNP solutions resulted in a much larger difference between the mass of AuNPs bound to the Mefp-1 film than to the lysine film. The reason for this can be that the AuNPs at lysine has reached saturation (even if the reaction time is prolonged no more AuNPs can bind to the surface) while for Mefp-1 the higher concentration of AuNPs reduces the needed binding time to reach saturation on the surface.

The frequency response of lysine is much lower than of Mefp-1; this does not necessary mean that Mefp-1 has much higher amount of AuNPs bound to it. The stronger frequency response could arise because of the polypeptide configuration on the substrate. Mefp-1 is much bulkier and gives the surface more structure than lysine which probably lays flat on the substrate. Another explanation can be that when rinsing the AuNP-Mefp-1 surface with Millipore water, which was done in the experiment, particles that are not bound strongly to Mefp-1 is washed away. This can't be seen in the QCM-D measurements. According to the SEM results with the gold substrates, lysine binds a higher amount of AuNPs to the surface than Mefp-1. This is the opposite of what the QCM-D analyse shows. This indicates that QCM-D may not be the best method to measure the mass of AuNPs attached to the surface. On the other hand it is good for measuring the binding time.

Albumin was not investigated since almost no binding of AuNPs was registered in the SEM measurement. The QCM-D measurements were only done on gold surfaces.

4.3.3 Surface coverage – a summary

The surface coverage of AuNPs on polypeptide coated substrates has been presented in an earlier section. The result from the QCM-D measurements on a gold substrate has also been described. This section will summarize the results and discuss the correlation between them.

The surface coverage of AuNPs on the polypeptide films on the different substrates used is presented in table 4. Only the values where AuNPs were solved in Millipore water are displayed.

Table 4: The surface coverage of AuNPs (in %) on the different substrates coated with polypeptides.

| | | Lysine | Mefp-1 |
|--------------|--------------------|---------------|---------------|
| Gold | | 7,5 | 5,6 |
| Glass | | 7,7 | 5,8 |
| PMMA | Hydrophilic | --- | 0 |
| | Hydrophobic | --- | 0,8 |
| PS | | --- | 7 |

It is of interest to investigate if Mefp-1 could be used to coat surfaces in a more general way than lysine. This is not possible to say at this moment. More different substrates must be covered and tested to be able to draw such a conclusion. What can be said is that lysine has a higher surface coverage on gold and glass substrates, but since the QCM-D results showed that the binding time of AuNPs on Mefp-1 was too short it is possible that the results from the surface coverage can change if the binding time was prolonged. Lysine worked better on hydrophilic PMMA than Mefp-1. On hydrophobic PMMA and PS the coating of lysine resulted in very uneven surface coverage, which is not good if the surface shall be antimicrobial. The hydrophobic PMMA had low surface coverage on Mefp-1 while the Mefp-1 coated PS substrate gave the highest measured surface coverage in this project.

Gold was used to check if it was possible to bind AuNPs on the polypeptide films. When making bacterial studies with light microscope it is essential to have a transparent surface, this is why the tests were also done on a glass substrate. The coating with a polypeptide film works great on both the hydrophilic substrates. The AuNP surface coverage for gold and glass is very similar on lysine and the same applies to Mefp-1. The values are a little higher on the glass substrate than on the gold.

PMMA is used as bone cement when a hip or knee replacement is needed. If the PMMA implant could be made antimicrobial it would decrease the bacterial infections post-surgery. PMMA is both hydrophilic due to the carbonyl group and hydrophobic because of the methylene group, it is more hydrophilic than PS but more hydrophobic than gold and glass. When making the PMMA surface hydrophilized by the base piranha wash it partially destroys the surface structure and makes it more porous. When the surface is covered with the different polypeptides only lysine coats the surface and enables the AuNPs to bind to it. The surface coverage of the AuNPs is difficult to calculate because of the porous structure. The hydrophobic PMMA surface coated with lysine showed only partial binding of AuNPs to it while the Mefp-1 coated one showed AuNPs attached to it. The surface coverage was low and it should be tested if a prolonged time in the AuNP solution could give a higher surface coverage. With this low surface coverage it is probably not possible to create an antimicrobial surface.

PS is not yet a plastic used for spare parts in the human body; it is anyhow interesting to investigate the coating of polypeptides on it. The coating of lysine and AuNPs looks similar to the hydrophobic PMMA surfaces, with very uneven coating. PS has electrically neutral and nonpolar groups and is hydrophobic. The coating of Mefp-1 and AuNPs was good on PS and the surface coverage of AuNPs was higher than all the others.

When calculating the surface coverage of AuNPs in ImageJ it also provides the average particle size. The SEM measurements on lysine and Mefp-1, result in nearly the same particle size as the measurements done with UV-VIS. The difference was only approximately 0,5nm. This confirms that UV-VIS can be used as a fast technique to measure the particle size. The particle size from the SEM measurements on AuNPs attached to cysteamine and the measurements on lysine and Mefp-1 do not give the same results. This is an indication that cysteamine is not the best chemical to use when looking at particle size.

4.4 Stability of gold nanoparticles coated on polymer films

The stability of the samples was only tested on lysine coated and Mefp-1 coated gold surfaces with AuNPs bound to them. The AuNPs used were dissolved in a Millipore water solution. Two different kinds of stability were tried; one affinity test and one chemical test. The final goal is to be able to create antimicrobial surfaces that will be used on medical devices in the body. Therefore the surfaces were exposed to fibrinogen which is a compound in our blood system. If the AuNPs has higher affinity for the solution of fibrinogen than for the polypeptide surface they sit on, the stability of the AuNPs on the surfaces are not good enough. The chemical exposure was done to investigate if the binding between the NH_3^+ groups on the polypeptide and the AuNPs were strong enough. Exposing the surface to a strong base, in this case Na_2CO_3 with a pH of around 11, will change the NH_3^+ groups to NH_2 groups. If the polypeptide surface loses the AuNPs when exposed to a strong base it is not as stable as hoped for.

The surface coverage and average particle size of the fibrinogen and Na_2CO_3 exposed surfaces are displayed in table 5.

Table 5: The surface coverage and average particle size before and after exposure to fibrinogen and Na_2CO_3

| | | Surface coverage [%] | | Average particle size [nm] | |
|--------------------------|--------|----------------------|-------|----------------------------|-------|
| | | before | after | before | after |
| Fibrinogen | Lysine | 7,4 | 8,2 | 59,7 | 59,5 |
| | Mefp-1 | 9,6 | 7,5 | 58,2 | 56,5 |
| Na_2CO_3 | Lysine | 7,1 | 0 | 57,1 | --- |
| | Mefp-1 | 5,1 | 5,9 | 52,7 | 56,7 |

The surface coverage of both lysine and Mefp-1 is about the same before and after exposure to fibrinogen. The difference in surface coverage on the surface can be due to uneven surface distribution, the SEM pictures are not taken on the same spot before and after exposure. When the surfaces were subjected to Na_2CO_3 in the result was that the lysine coated surface lost almost all AuNPs while Mefp-1 was still intact. The AuNPs are bound only by electrostatic bonds to the lysine surface while for Mefp-1 the AuNPs binds both electrostatically and through the catecholic oxygen atoms on the DOPA group.

The average particle size was measured to exclude the possibility that particles with certain sizes lost their binding to the surface more than others. The particle sizes before and after exposure were so similar that this effect could be ruled out.

5. Conclusion

It is possible to create particles in the size range of 40-60nm. The temperature dependence is not as big as anticipated. It has an influence but the size effect is probably more affected by varying the gold salt to citrate ratio instead. The stirring speed can probably also affect the particle size.

To investigate the size of the AuNPs it is not the best option to use cysteamine as a film. It binds AuNPs well through the thiol-group but more aggregates are found in the SEM pictures. The UV-VIS and the SEM measurement gave different results of the particle size. Using lysine or Mefp-1 as the coating material instead resulted in very similar outcome from the measurements.

Deciding the particle size with UV-VIS works well and this method is recommended if a fast answer is needed. It correlates nicely with SEM measurements. The SEM measurements show nice round particles with no odd structures, such as triangles or rods. SEM is a great method to look at distribution of particles on the surface. The DLS measurements gave the information that the sample was; clean, polydisperse and that it consists of a lot of small species. Since a large amount of small species were in the sample the method is not so good for determination of the mean particle size. Although the CONTIN analyse works better than the Cumulative one, it is still not good enough.

The QCM-D showed that longer binding time of AuNPs to Mefp-1 is needed. It is hard to compare the surface coverage of AuNPs on the lysine coated surface with the Mefp-1 coated one since the binding time was too short for Mefp-1. QCM-D measurements with prolonged binding time of AuNPs to Mefp-1 are of interest to get comparable results. The surface coverage of the AuNPs on the polypeptide films should be investigated further. In this study it is shown that dissolving the AuNPs in different concentrations of salt reduce the surface coverage of AuNPs on the polypeptides

From the surface analysis made in this project it is still hard to say if Mefp-1 is better than lysine and can work in a more general way to coat different substrates. More substrates should be tested and the binding time of AuNPs should be prolonged.

The stability tests on lysine and Mefp-1 indicates better stability of the AuNPs bound to Mefp-1 than lysine. This means stronger binding of particles to Mefp-1 and thus a better resistance to different environments.

6. Further studies

This project has opened up many new ideas on how it is possible to use polypeptides to attach AuNPs on different kinds of substrates. Improvements of the methods can be done and suggestions will follow. The most important improvements concern the synthesis of the AuNPs.

It was shown that the temperature impact on the particle size wasn't so large. It is instead interesting to see how changes of the gold salt to citrate ratio and the stirring speed would affect the particle size. The particles optimal size and the distance between them are not yet known, and should be investigated further. This must be done through bacterial studies after attaching the AMPs to the AuNPs. To see if the AMPs have attached to the AuNPs TOF-SIMS analysis could be used. The outcome from the bacterial studies will give the answer if the particle size or the distance between them has to be changed. The distance between the particles can be changed by the salt concentration in the surrounding particle solution. A wider range of salt concentrations and their effect on the distance between the AuNPs should be analysed in SEM.

The coating of substrates with polypeptides was only studied on gold, glass, PMMA and PS. To get a better understanding for the coating with Mefp-1 and if it can attach to a

wider range of substrates than lysine, more substrates need to be tested. To investigate if antimicrobial surfaces could be used in the human body, more substrates, such that are used in the medical device field should be tested, for example silicone substrates.

The QCM-D measurements in this study indicated that the binding time of the AuNPs were too short on Mefp-1. Therefore the binding time should be prolonged and studied with QCM-D to discover the optimal reaction time. In addition measurements on the other substrates, glass, PMMA and PS, should be performed to see if the binding time was correct for them. The optimal concentration of AuNPs in the solution, in the reaction with the polypeptides, especially lysine, should also be tested.

The stability of the AuNPs to the polypeptide coated gold surface was only studied by exposing the samples to fibrinogen and strong base separately. Further stability tests should be made by exposing the samples to strong acids as well.

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Appendix I

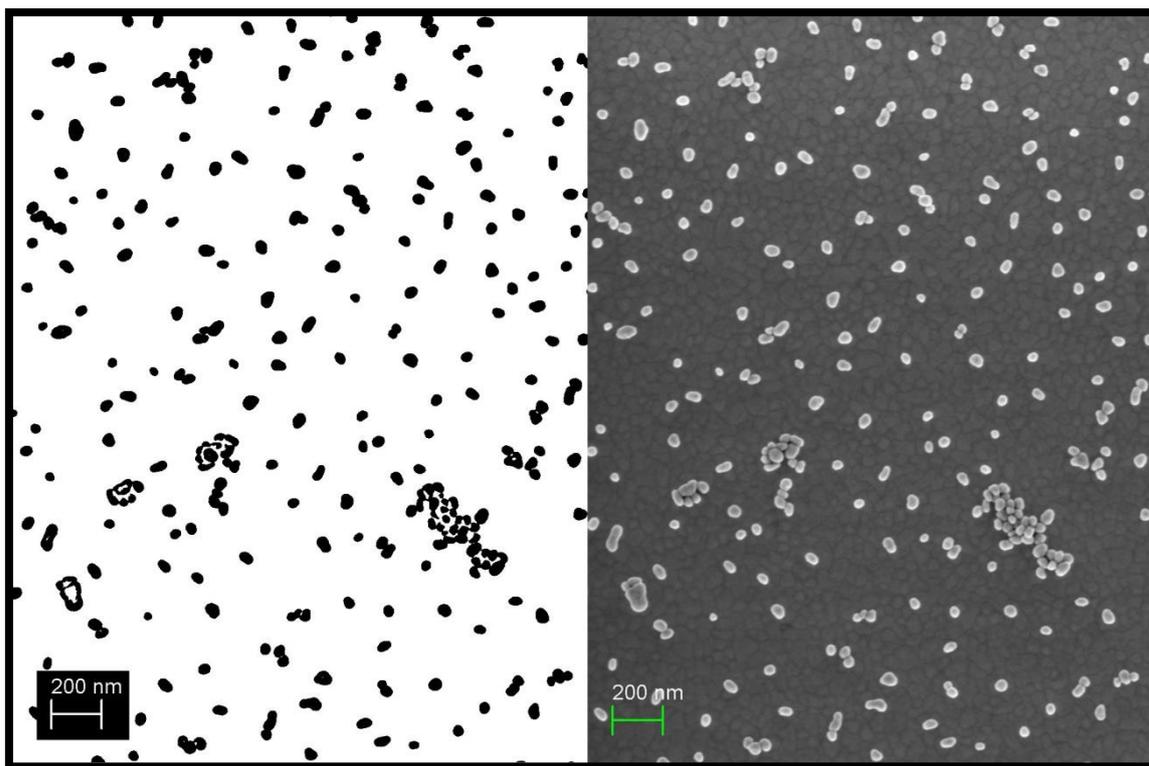


Figure 1: AuNP solution made at 80°C. The particles are bound to a cysteamine coated gold surface. The figure illustrates how the particles are analysed with ImageJ. The picture to the right is the original SEM picture and the one to the left is after using the threshold tool.

Appendix II

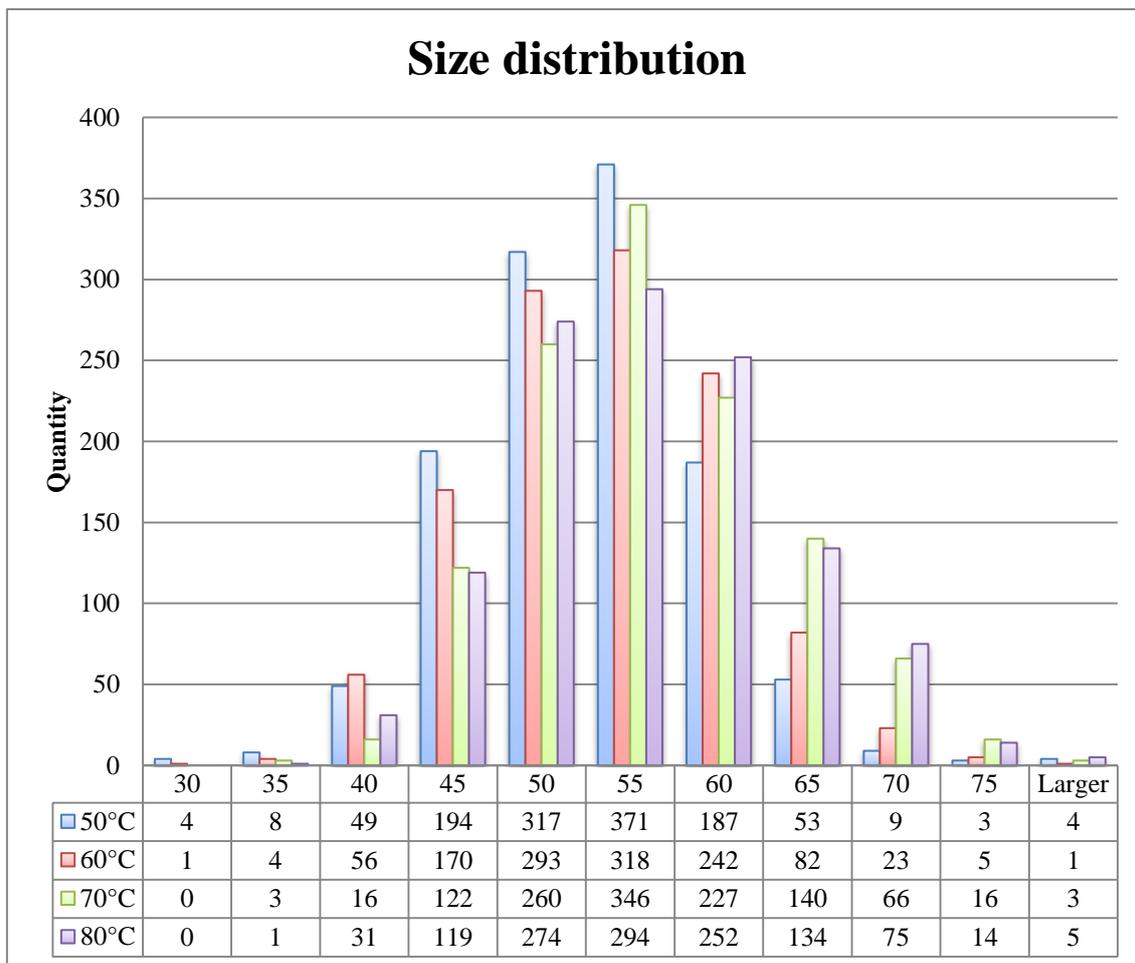


Figure 1: Size distribution of 1200 measured AuNPs. The particles were produced at different temperatures and bound to a cysteamine coated gold substrate.

Appendix III

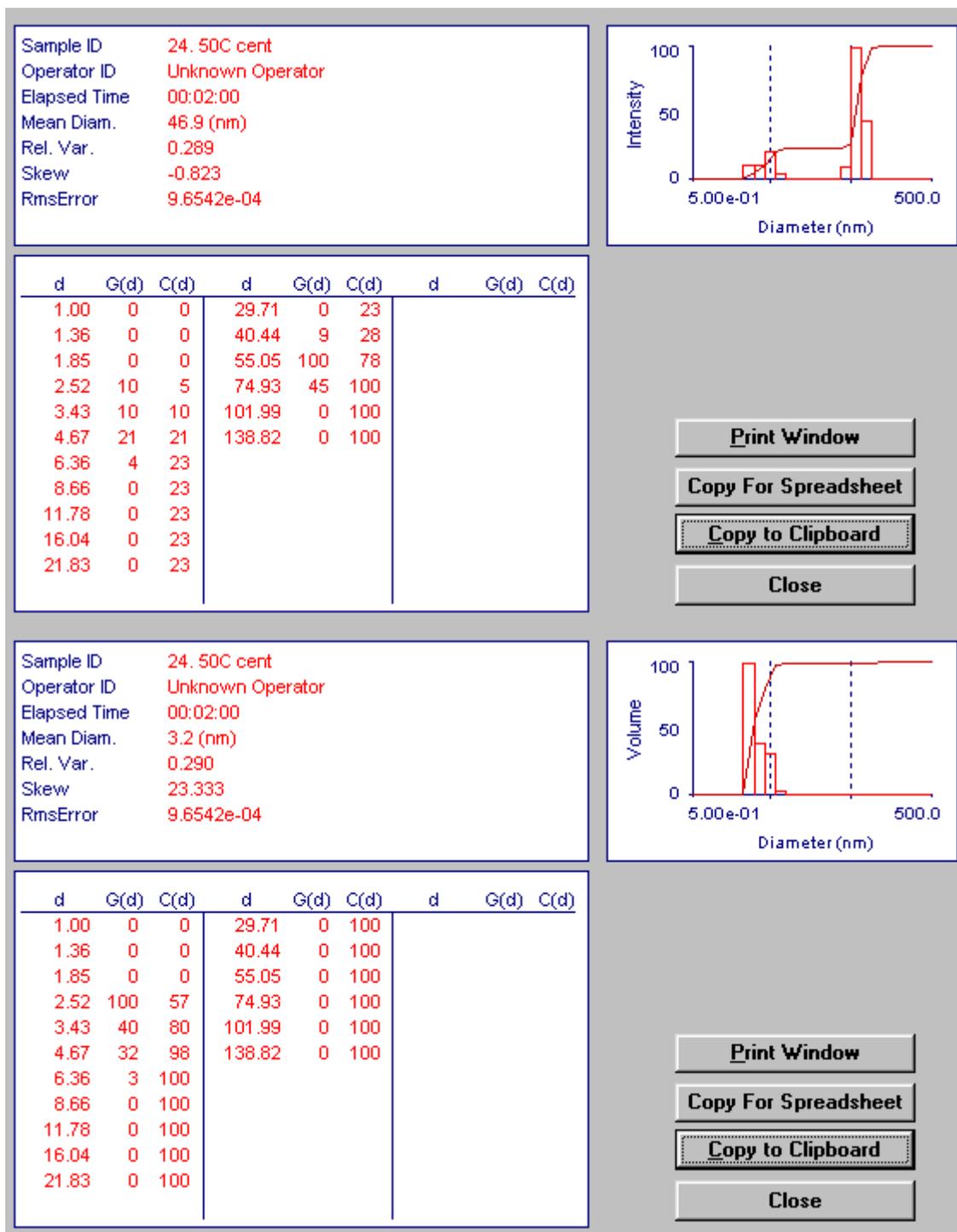


Figure 1: Results from DLS measurements. The figures show both the intensity and the volume results. The particles were produced at 50°C and centrifuged and re-dispersed in Millipore water.

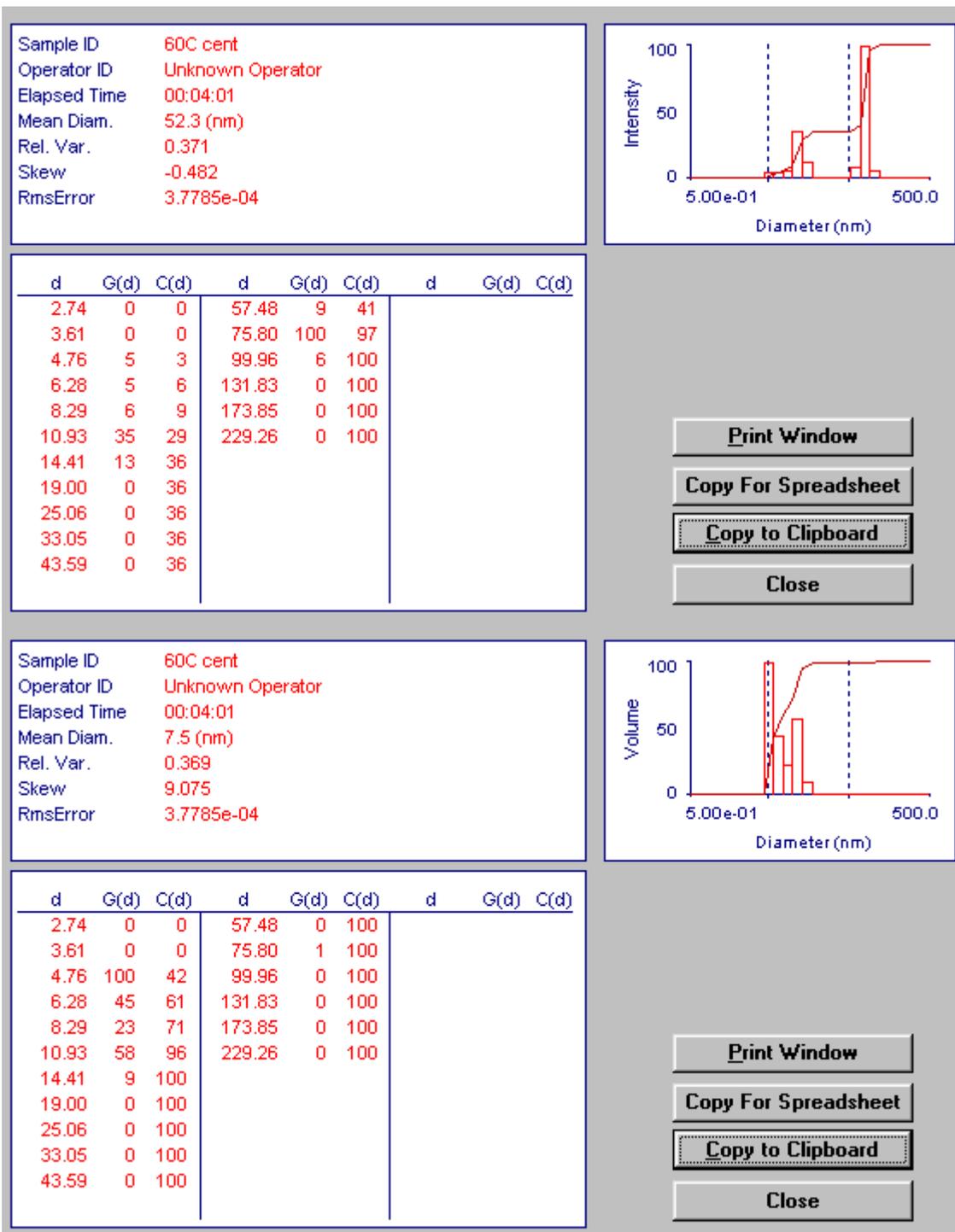


Figure 2: Results from DLS measurements. The figures show both the intensity and the volume results. The particles were produced at 60°C and centrifuged and re-dispersed in Millipore water.

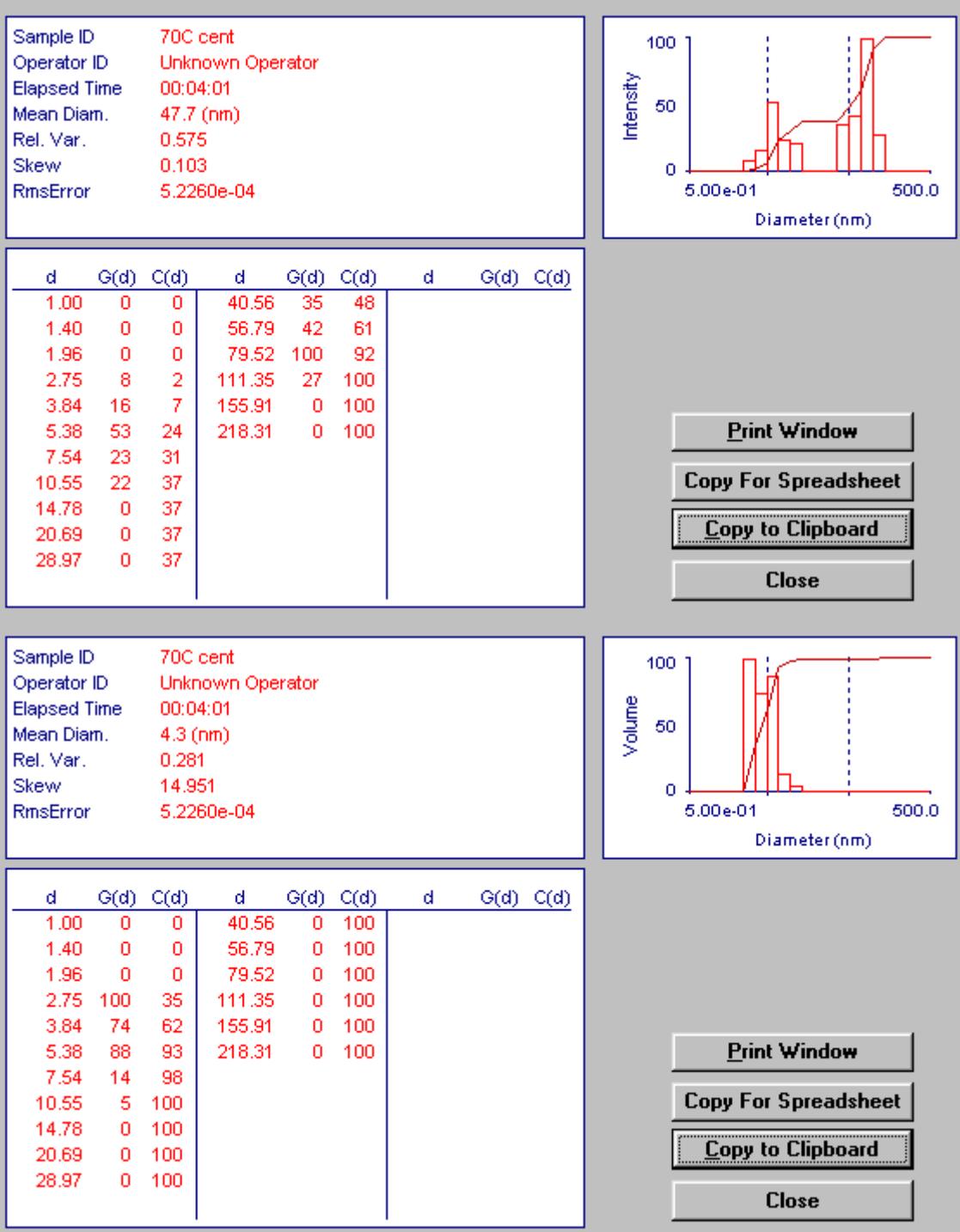


Figure 3: Results from DLS measurements. The figures show both the intensity and the volume results. The particles were produced at 70°C and centrifuged and re-dispersed in Millipore water.

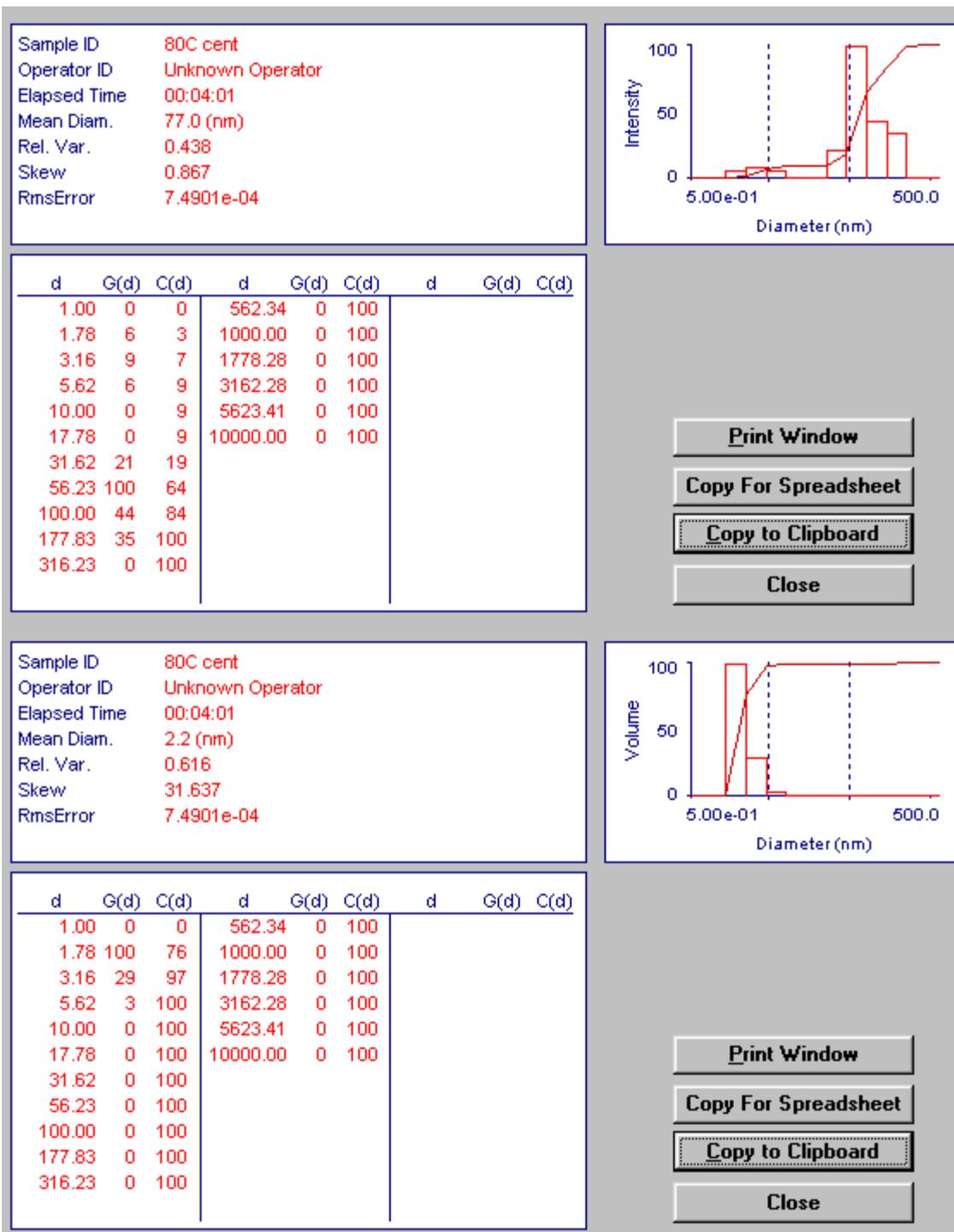


Figure 4: Results from DLS measurements. The figures show both the intensity and the volume results. The particles were produced at 80°C and centrifuged and re-dispersed in Millipore water.

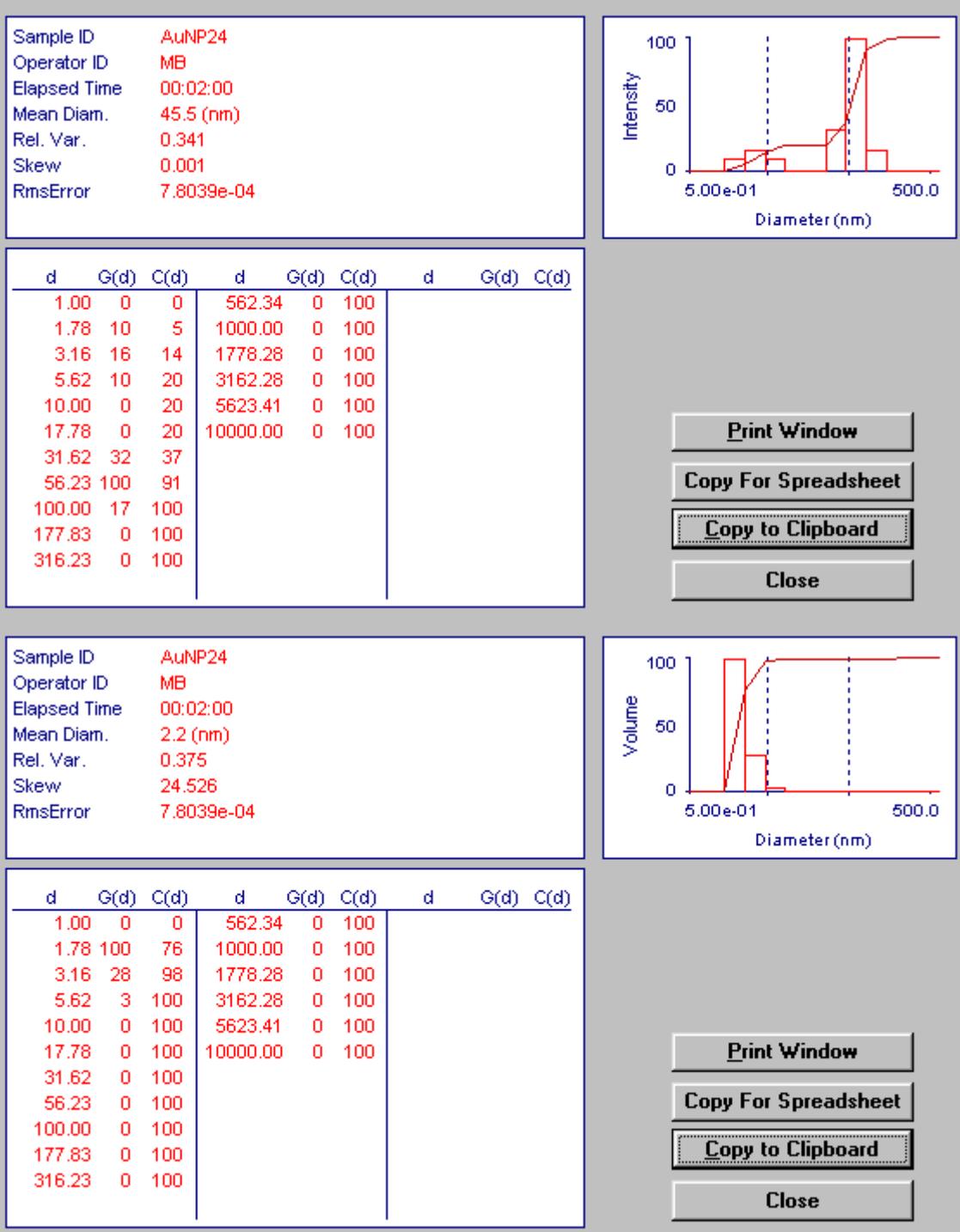


Figure 5: Results from DLS measurements. The figures show the intensity respectively the volume results. The particles were produced at 50°C.

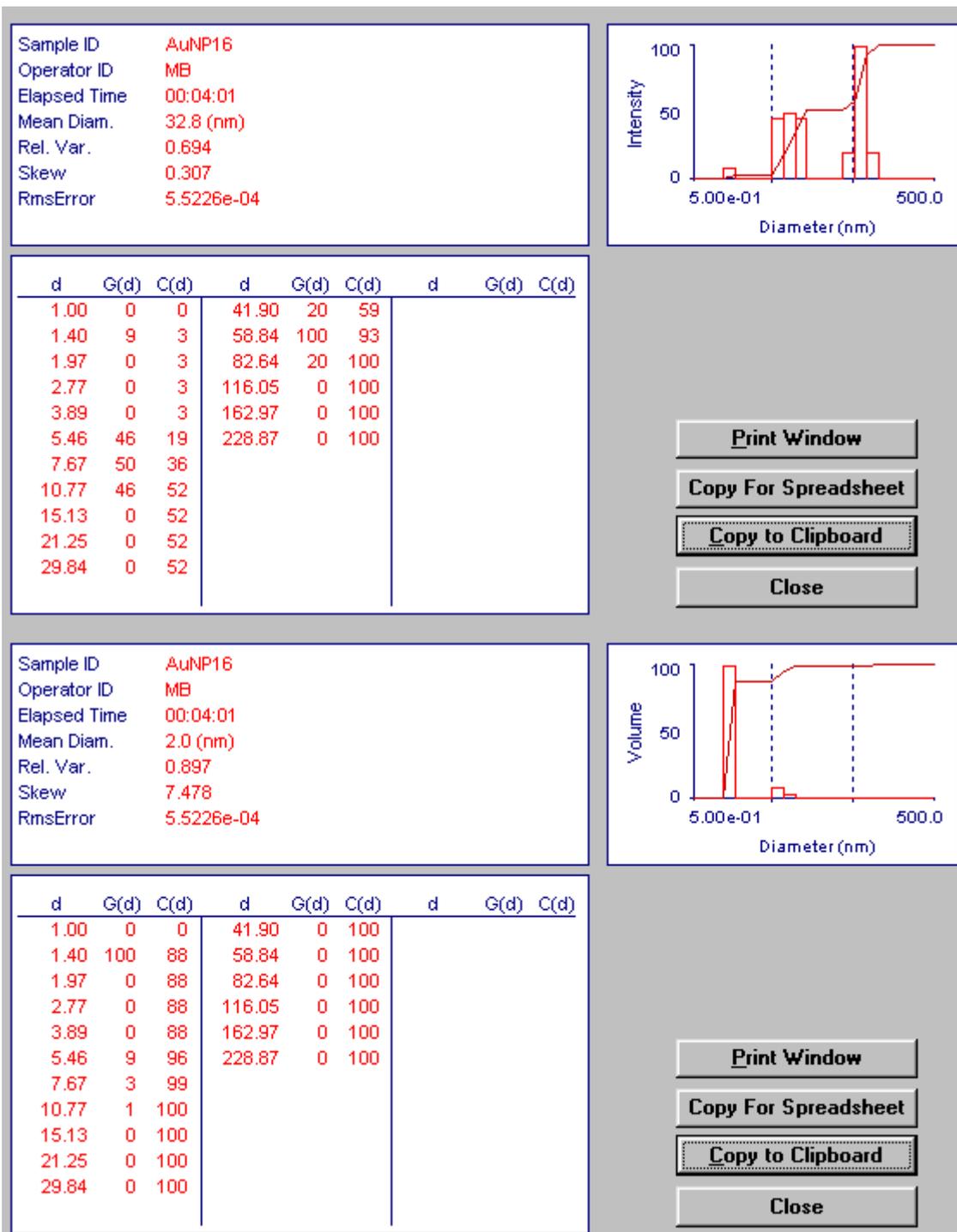
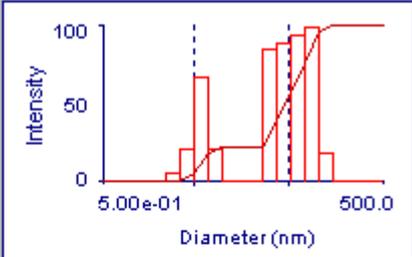


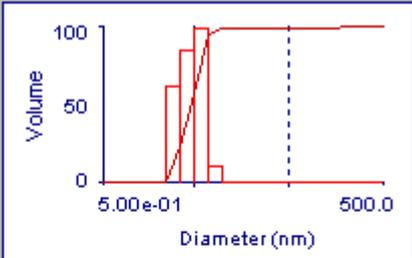
Figure 6: Results from DLS measurements. The figures show the intensity respectively the volume results. The particles were produced at 60°C.

Sample ID AuNP20
 Operator ID MB
 Elapsed Time 00:04:01
 Mean Diam. 45.8 (nm)
 Rel. Var. 0.436
 Skew 0.300
 RmsError 5.1567e-04



| d | G(d) | C(d) | d | G(d) | C(d) | d | G(d) | C(d) |
|-------|------|------|--------|------|------|---|------|------|
| 1.02 | 0 | 0 | 42.23 | 90 | 58 | | | |
| 1.43 | 0 | 0 | 59.25 | 95 | 77 | | | |
| 2.01 | 0 | 0 | 83.11 | 100 | 96 | | | |
| 2.82 | 6 | 1 | 116.59 | 19 | 100 | | | |
| 3.95 | 21 | 5 | 163.56 | 0 | 100 | | | |
| 5.54 | 68 | 19 | 229.44 | 0 | 100 | | | |
| 7.77 | 22 | 23 | | | | | | |
| 10.91 | 0 | 23 | | | | | | |
| 15.30 | 0 | 23 | | | | | | |
| 21.46 | 0 | 23 | | | | | | |
| 30.11 | 86 | 40 | | | | | | |

Sample ID AuNP20
 Operator ID MB
 Elapsed Time 00:04:01
 Mean Diam. 4.6 (nm)
 Rel. Var. 0.375
 Skew 13.110
 RmsError 5.1567e-04



| d | G(d) | C(d) | d | G(d) | C(d) | d | G(d) | C(d) |
|-------|------|------|--------|------|------|---|------|------|
| 1.02 | 0 | 0 | 42.23 | 0 | 100 | | | |
| 1.43 | 0 | 0 | 59.25 | 0 | 100 | | | |
| 2.01 | 0 | 0 | 83.11 | 0 | 100 | | | |
| 2.82 | 63 | 24 | 116.59 | 0 | 100 | | | |
| 3.95 | 86 | 57 | 163.56 | 0 | 100 | | | |
| 5.54 | 100 | 95 | 229.44 | 0 | 100 | | | |
| 7.77 | 11 | 100 | | | | | | |
| 10.91 | 0 | 100 | | | | | | |
| 15.30 | 0 | 100 | | | | | | |
| 21.46 | 0 | 100 | | | | | | |
| 30.11 | 1 | 100 | | | | | | |

Figure 7: Results from DLS measurements. The figures show the intensity respectively the volume results. The particles were produced at 70°C.

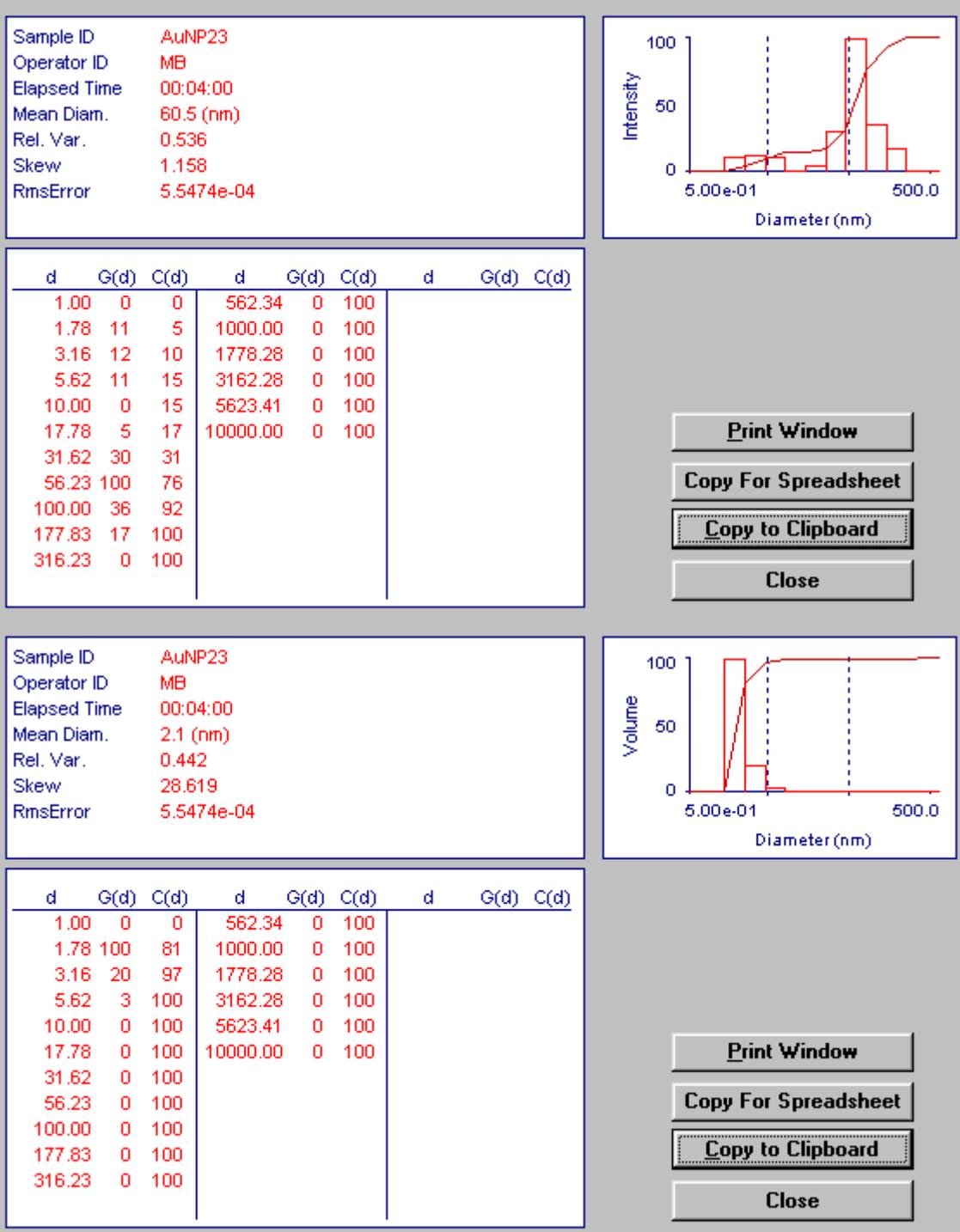


Figure 8: Results from DLS measurements. The figures show the intensity respectively the volume results. The particles were produced at 80°C.

Appendix IV

The figures show the results from the SEM measurement where no AuNPs was attached to the bare surface.

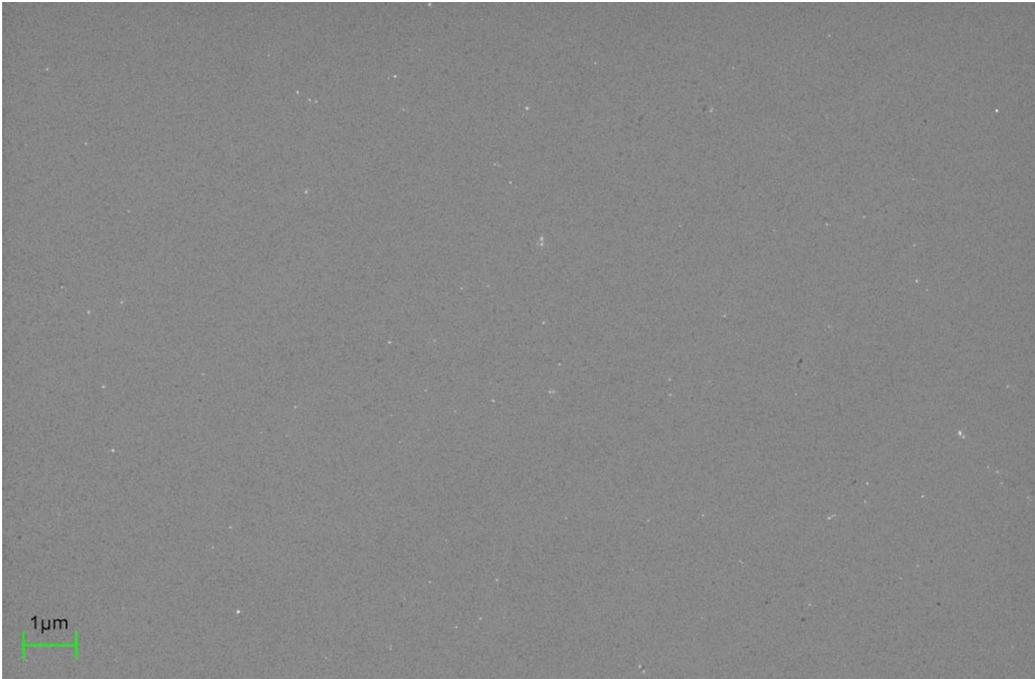


Figure 1: AuNPs on gold surface, reference sample

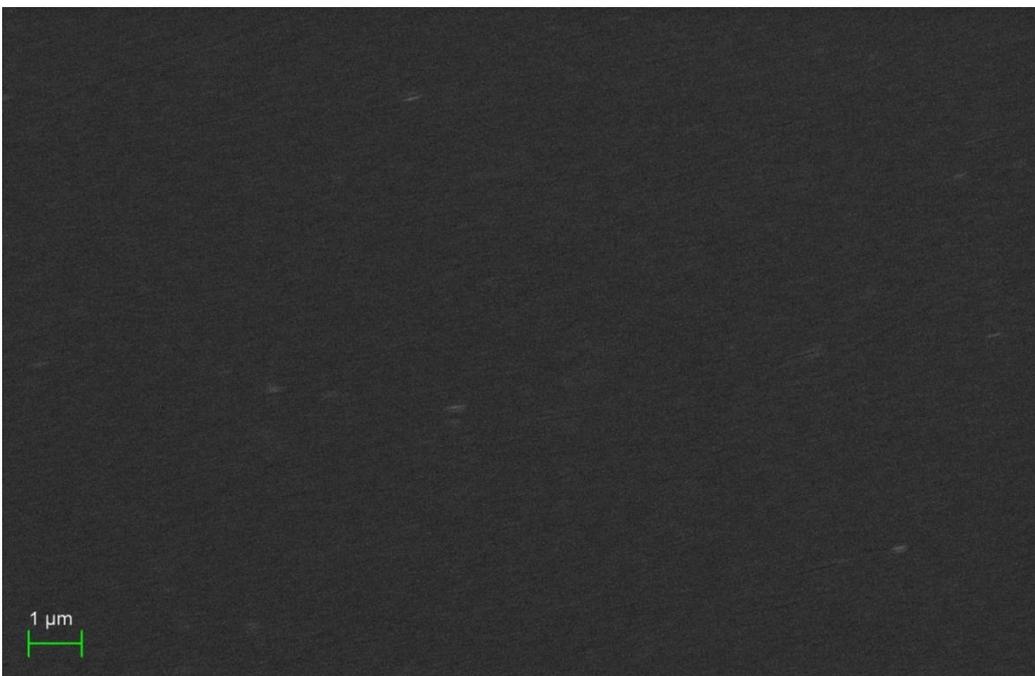


Figure 2: AuNPs on glass surface, reference surface

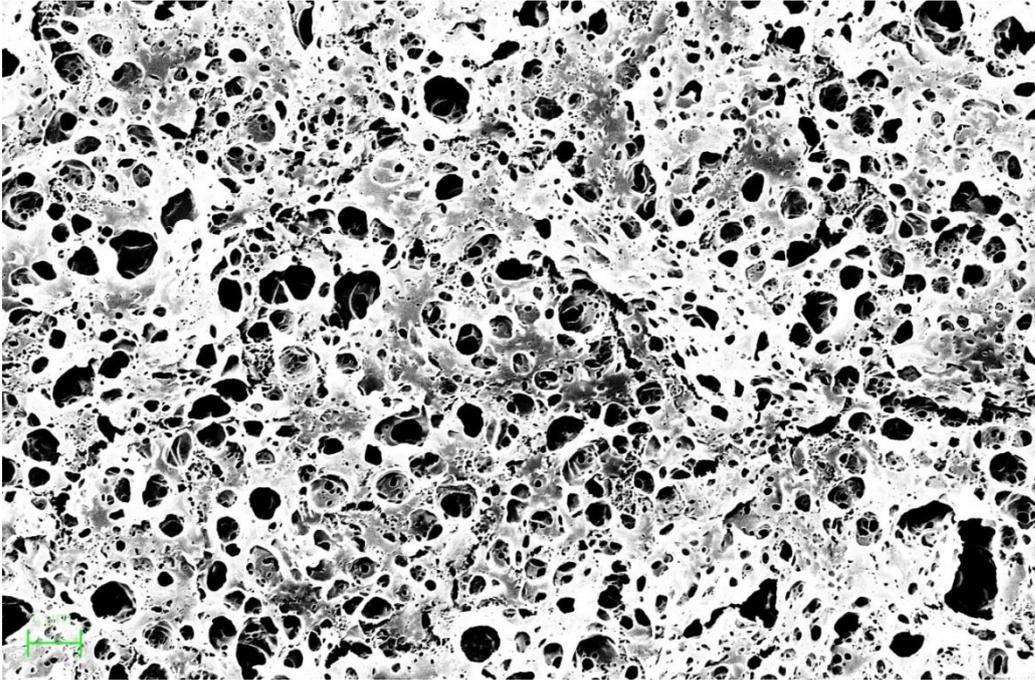


Figure 3: AuNPs on PMMA substrate, reference surface. The PMMA substrate was cleaned with base piranha wash.

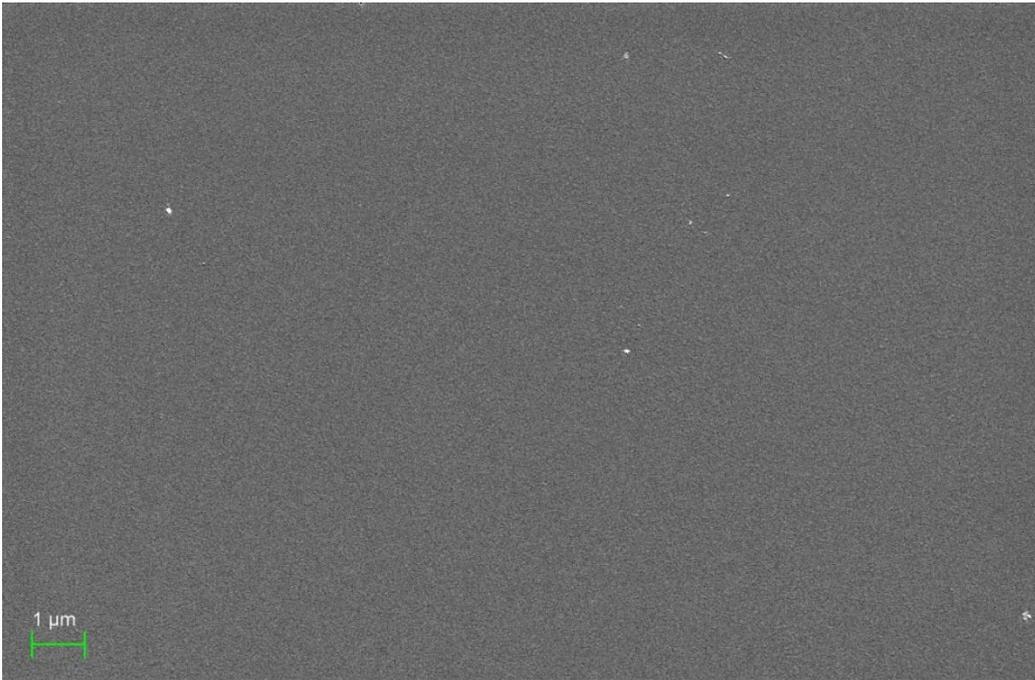


Figure 4: AuNPs on PMMA substrate, reference surface. The PMMA substrate was cleaned with ethanol.

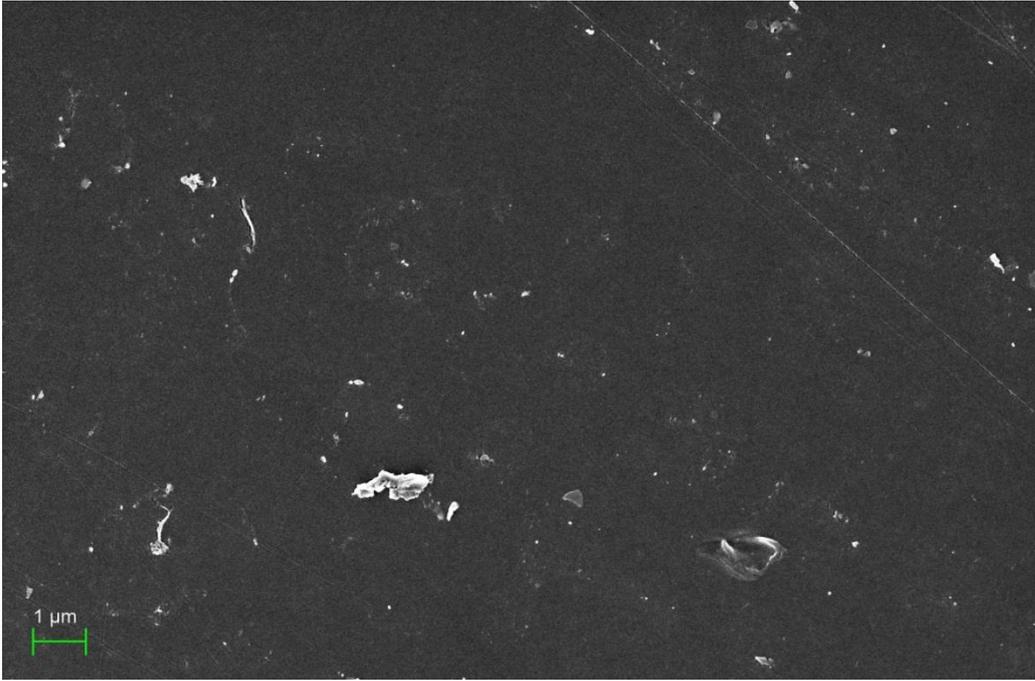


Figure 5: AuNP on PS substrate, reference surface. The PS substrate was cleaned with ethanol.

Appendix V

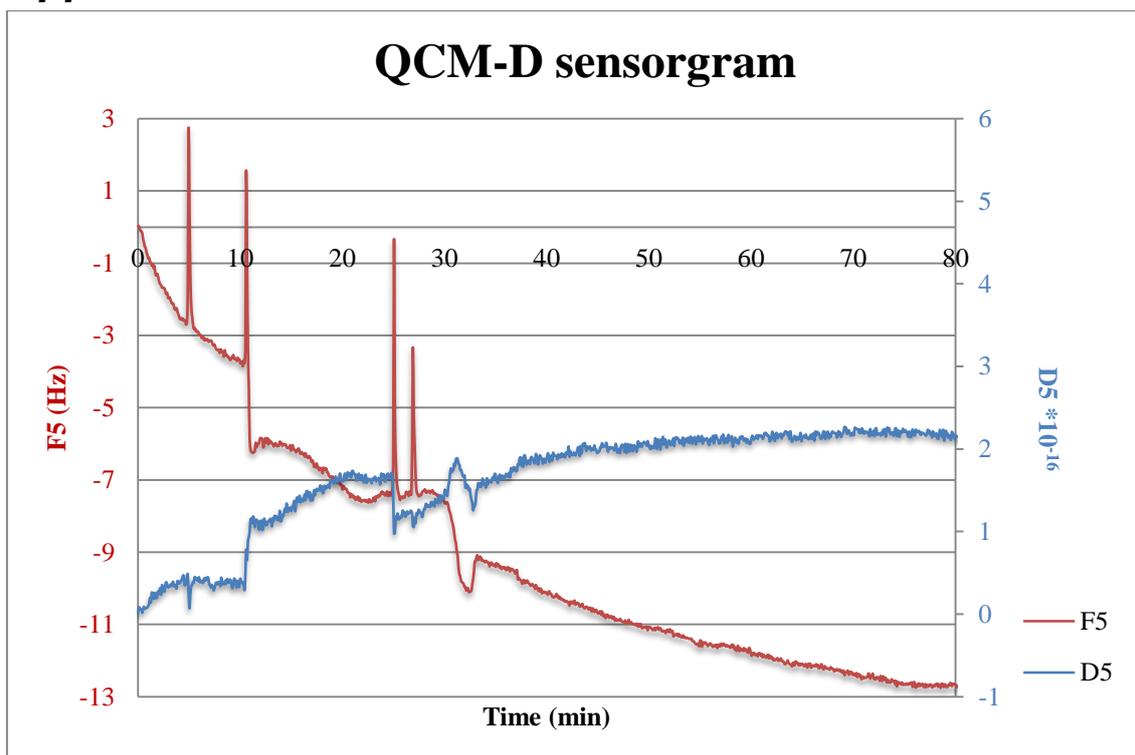


Figure 1: QCM-D sensorgram describing the binding of lysine to a gold surface and the binding of AuNPs to it. The particles were produced in this project, the particles had a lower concentration in the solution and was 58 nm in diameter big.

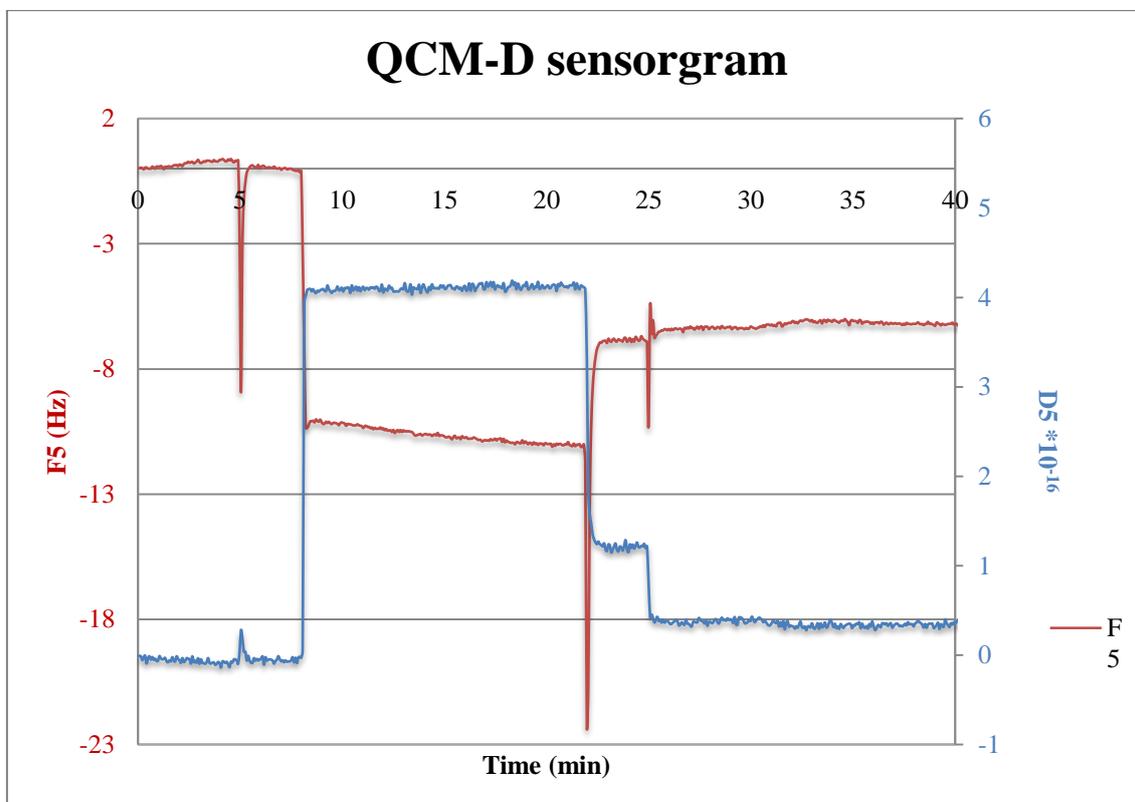


Figure 2: QCM-D sensorgram describing the binding of lysine to a gold surface. No binding of AuNPs could be seen in the sensorgram. The particles were produced in this project, the particles had a lower concentration in the solution and was 58 nm in diameter big.

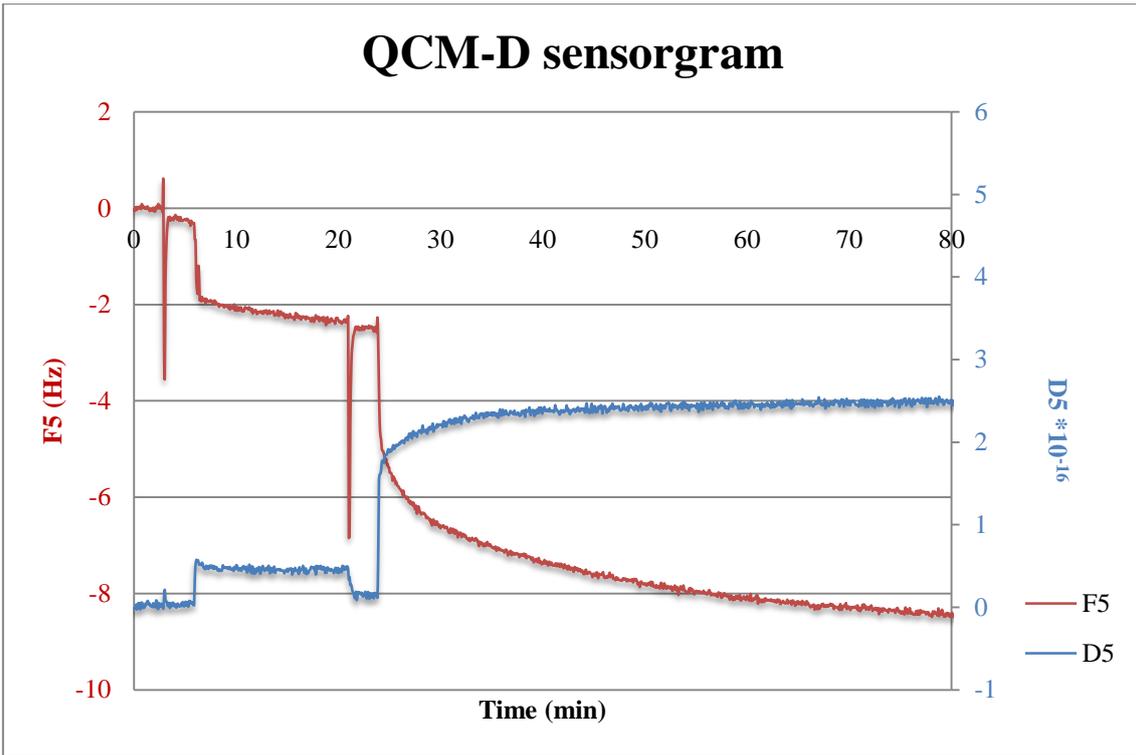


Figure 3: QCM-D sensorgram describing the binding of lysine to a gold surface and the binding of AuNPs to it. The particles were produced by Mats Hulander, the particles had a higher concentration in the solution and was 38 nm in diameter big.

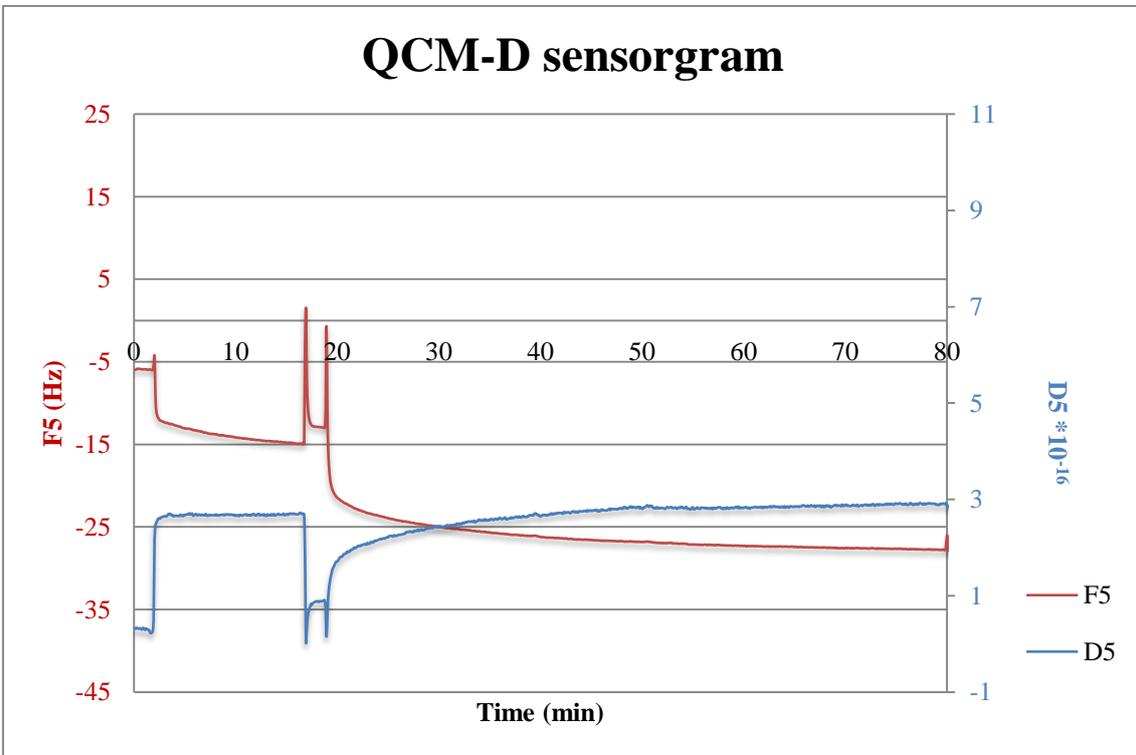


Figure 4: QCM-D sensorgram describing the binding of Mefp-1 to a gold surface and the binding of AuNPs to it. The particles were produced in this project; the particles had a lower concentration in the solution and were 58 nm in diameter big.

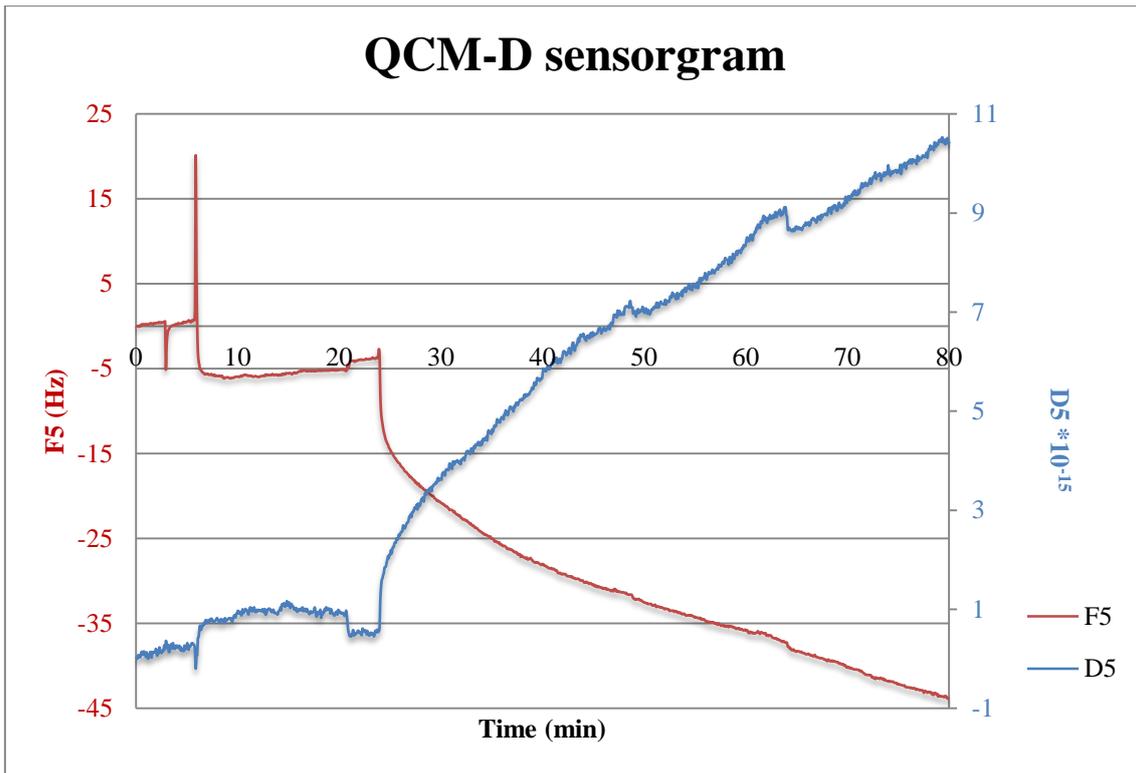


Figure 5: QCM-D sensorgram describing the binding of lysine to a gold surface and the binding of AuNPs to it. The particles were produced by Mats Hulander, the particles had a higher concentration in the solution and was 38 nm in diameter big.