Build your own optical biosensor!

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Introduction

The purpose of the lab exercise is to show the working principle of a simple plasmonic biosensor that detects changes in refractive index on a metal surface. The physical biosensor principle is the same as in commercial surface plasmon resonance instruments, but based on gold nanoparticles instead of surface plasmons in a thin gold film. As a model system, the protein avidin will be detected when it binds to biotin on the sensor surface.

You can only get the grade "passed" on this lab exercise, but passing is mandatory for getting a grade on the full course. To pass the lab exercise you should not only perform the exercise itself, but also present solutions to the tasks described in this guide.

IMPORTANT: Tasks in RED can and SHOULD be solved already BEFORE conducting the experiments. This is in order to make you aware what the exercise is about and to understand why you do the different steps. You may be allowed to perform the lab exercise even if you have not found all the right answers, but you must show you have made a serious attempt. Tasks in BLUE should be solved DURING the lab exercise and are based on the results you get. Tasks in GREEN are NOT mandatory to solve and present answers to, but you should check them later.

There is no need to write a report and after the lab exercise the work is done. However, be aware that questions related to this lab exercise may appear in the exam.

You are encouraged to work in groups in order to solve the tasks!

In general, the supervisor will instruct you what do to during the exercise so you do not have to be afraid. (We will not tell you to "start working" and then leave the room.) You will be instructed on all practical details.

Nanoparticle Synthesis

Gold nanoparticles can be synthesized by the so called Turkevich method.[1] One lets a gold salt (ions in solution) react with a reducing agent like citrate in hot water. The reducing agent also forms negatively charged groups on the particle surface which stabilizes them, i.e. aggregation is prevented because the particles repel each other. The size of the particles is controlled by the molar ratios of Au and citrate according to the graph in Fig. 1.

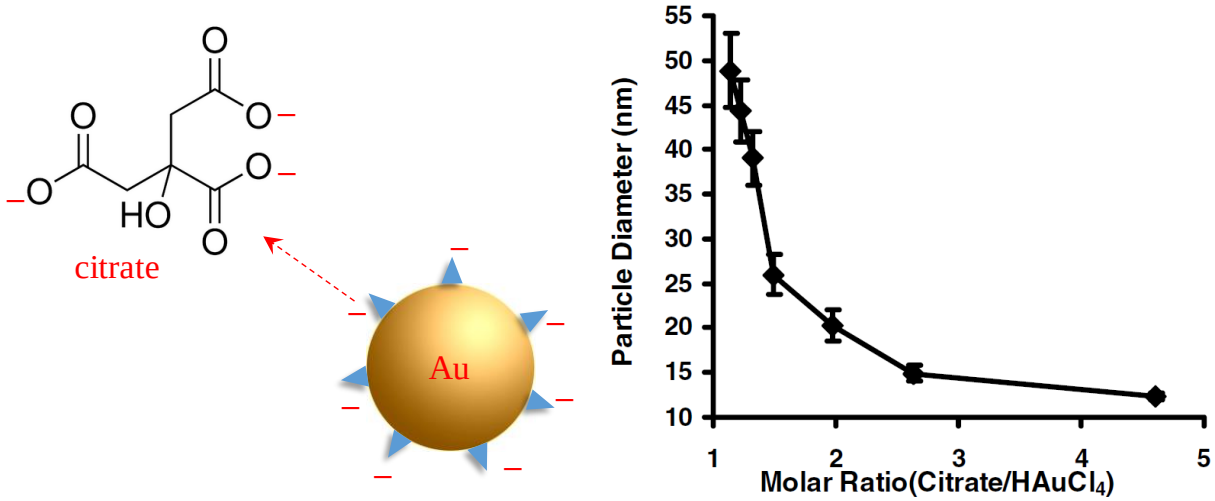

Figure 1. The citrate-capped gold nanoparticles and their diameter as a function of the molar ratio between gold chloride and sodium citrate. Graph adopted from literature.[2]

We want to prepare 20 nm particles at a concentration of 1.0×10^{18} m^{-3}. Our gold source is a (10 g total) 30% (weight) solution of $HAuCl_4$, which has a density of 1.637 g/cm^3. The nanoparticles can be assumed to have the same material properties as bulk Au, which has a density of 19.3 g/cm^3. Water will be heated to the boiling point under stirring. The gold salt is added first, followed by the citrate which is taken from a 100 mM (in water) stock solution.

Task 1 (red): If we synthesize the particles in a total volume of 50 mL, how much $HAuCl_4$ solution should we take? Also, calculate the volume of sodium citrate solution which should be added to the reaction mixture to get the desired particle size. (You may assume that the volumes you will add are much smaller than the 50 mL you start with.)

Task 2 (green): What will happen to the particles at sufficiently low pH?

Extinction Spectroscopy

Extinction (absorbance, optical density etc.) measurements are commonly used, for instance in biochemistry, to determine the concentration of a molecule in a solution. The amount of light that passes through a sample solution can be derived by considering an infinitesimal slab of the sample where the entities that interact with light cannot shadow each other (see lecture on optical techniques). If I_0 is the intensity going in and I is the transmitted intensity we can define the extinction Ω in logarithmic units by the Lambert-Beer law:

$$\Omega(\lambda) = \log\left(\frac{I_0(\lambda)}{I_1(\lambda)}\right) = \sigma(\lambda)Cl \qquad (1)$$

Here we have emphasized the dependence on wavelength. The point of the equation is to relate the microscopic property of extinction cross section to the macroscopic property of concentration.

You will measure the extinction spectrum of your gold colloid suspension using a cuvette with 1 cm pathlength (l).

Task 3 (blue): Calculate the extinction cross section of a 20 nm spherical Au particle in water, at the resonance wavelength, using your extinction spectrum and Equation 1. Compare the cross section value and the measured resonance wavelength with theory. Compare also with the geometrical cross section area.

Task 4 (green): How does the spectrometer measure the reference intensity $I_0(\lambda)$ and account for the false photon counts in the detector?

Particle Stability

You will gradually add more and more NaCl to a part of the colloid suspension.

Task 5 (blue): What happens eventually to the colloidal suspension and why?

Task 6 (green): Given the approximate salt concentration you introduced, consider if one can use DLVO theory to make a rough estimate of the number of citrate groups on the particle surface (at high pH)! What parameters are needed and what assumptions must be done?

Biosensor Test

First, the particles will be attached to a glass surface in a liquid flow-cell. Since glass is negatively charged in water due to deprotonation of -OH groups on the surface (unless pH is very low), these gold colloids will not attach. Therefore, the glass will first be made positively charged through adsorption of poly(L-lysine), a peptide containing only the amino acid lysine which is positive at neutral pH.

Task 7 (red): Derive an expression for the extinction of a monolayer of nanoparticles. Each particle still has a cross section of σ, but instead of a concentration C and a path length l the relevant parameter is now the surface coverage Γ of particles.

You will analyse the spectrum of the nanoparticle surface and measure changes in real time using a standard spectrophotometer. First, you will measure the extinction spectrum of just the nanoparticles on glass.

Task 8 (blue): What has happened to the extinction spectrum now that the particles are on a glass surface instead of being suspended in water and why?

Task 9 (blue): Estimate the surface coverage Γ using your previously derived expression. Make sure to set a proper baseline for the extinction so that you only get the nanoparticle contribution.

Next, the sensor surface will be functionalized in a simple manner. The recognition element in our biosensor is the small molecule biotin (vitamin H). We will use a short thiolated oligo(ethylene glycol) molecule (thiol-OEG), where a fraction of the molecules has biotin attached to the other end. These molecules bind directly to the gold surface by forming Au-S bonds. The OEG molecule (without biotin) is commonly used to make gold surfaces inert.[3]

The target molecule which we will detect when it binds to the surface is avidin, a ~60 kD protein found in egg white. It can bind with very high affinity to biotin and this interaction is, unlike most biomolecular interactions, practically irreversible.[4] The dissociation constant depends on the exact version of avidin but can be as low as $K_D = 10^{-15}$ M. It is the dissociation rate constant which is particularly low, i.e. once a biotin-avidin complex has formed it will not dissociate during the timescale of any ordinary experiment. This makes the interaction quite useful in biotechnology. (Interestingly, if you eat a lot of egg-white, raw so that the protein is not denatured, you can suffer from vitamin H deficiency because the avidin binds all of it and makes it unavailable for your body.)

A simple specificity test can be done by adding another protein such as bovine serum albumin (BSA). BSA is a 66 kD protein abundant in blood serum (here from cows). It is commonly used as a "blocking agent" on surfaces and to test for interference with other interactions.

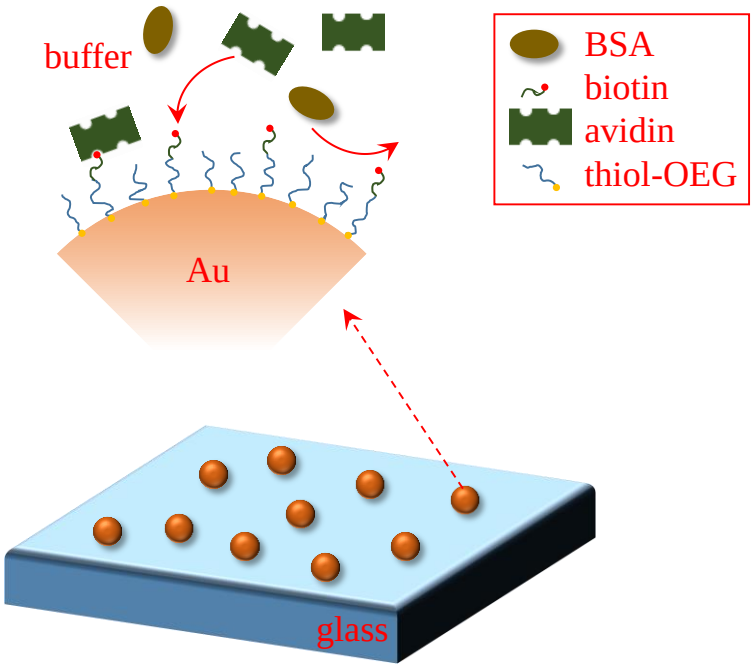

Figure 2. The biosensor design. Gold nanoparticles on a glass surface, functionalized with biotin, are used to capture avidin. The binding is detected through changes in the optical spectrum of the nanoparticles.

Task 10 (red): After introducing avidin you will see a binding curve where the sensor signal increases with time and it can be assumed that the response is proportional to surface coverage. How can you test if the binding kinetics are influenced by diffusion?

Task 11 (blue): Assume that the avidin proteins are spheres with a diameter of 5 nm. Estimate the detection limit of the biosensor in terms of protein surface coverage! Hint: Try to estimate a value for the areal coverage when the avidin binding has reached saturation.

Task 12 (blue): If you would measure on a single gold nanoparticle, would you be able to detect single avidin molecules? What is the required noise level?

Task 13 (green): Discuss advantages and disadvantages with this type of nanoparticle biosensor in comparison with SPR.

References

[1] J. Kimling et al. *Journal of Physical Chemistry B* **2006**, 110 (32), 15700–15707.

[2] N. Nath and A. Chilkoti *Analytical Chemistry* **2004**, 76 (18), 5370-5378.

[3] K.L. Prime and G.M. Whitesides *Science* **1991**, 252 (5009), 1164-1167.

[4] N.M. Green *Biochemical Journal* **1963**, 89 (3), 585-591.