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Supported silver clusters as nanoplasmonic transducers for protein sensing

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ABSTRACT

Transducers for optical sensing of proteins are prepared using cluster beam deposition on quartz substrates. Surface plasmon resonance phenomenon of the supported silver clusters is used for the detection. It is shown that surface immobilisation procedure providing adhesion of the silver clusters to quartz and functionalisation of cluster surfaces for antibody coupling are the key issues for cluster stability and protein detection. Focus was put on these tasks and the processes have been optimised. In particular, conditions for coupling of the antibodies to the clusters are developed providing an enhancement of the plasmon absorption band used for the detection. Atomic force microscopy study allows to suggest that immobilisation of antibodies on silver clusters has been achieved, thus giving a possibility to incubate and detect an antigen of interest. Hence, by applying the developed preparation stages and protein immobilisation scheme the sensing of protein of interest can be assured using a relatively simple optical spectroscopy method.

1. Introduction

Development of nanosensors is a rapidly growing field of research. The increasing interest arises from the unique physical characteristics and properties on the nanoscale that are not present in bulk materials. Therefore, nanodevices are able to deliver sensitivity, which is orders of magnitude higher compared to conventional sensor technologies, and supply additional performance advantages like short response time and portability [1]. Nanosensors also allow for building integrated systems, thus providing a platform for intelligent devices having significant data storing, processing and analyzing power. Intelligent nanosensors have a great potential to become very attractive as autonomous systems or to be spread out in a large number to form networks.

Among nanosensors, biorecognition systems are of significant importance for environmental, bioprocess and food quality controls as well as for medical and pharmaceutical applications [2,3]. A biosensor is an analytical device that interfaces a biological object to be recognised with a physical or chemical transducer to generate a signal which is then registered and analysed. There are a number of various approaches in realisation of detection [4]. Localised surface plasmon resonance (LSPR) biosensors were among the first demonstrated and since then they have gradually become a very powerful label-free tool. One of the great advantages of label-free detection is that the target molecules are not altered, i.e. they are detected in their natural forms. Nanoparticles (NPs) are typically used as transducers generating optical signals. At the same time they are similar in size to some organic molecules such as enzymes and proteins, thus, being ideal transducers used in detection. Many state-of-the-art biosensors utilising LSPR were demonstrated to provide a relatively high degree of sensitivity [5]. However, there are still a number of aspects to be considered in order to produce a reliable and selective sensor. Among them formation of a stable transducer, design of the detection scheme and surface immobilisation chemistry are challenging tasks.

NPs prepared through sol–gel processes starting with different salt containing solutions are the most widely used as transducers. A major disadvantage of this approach is the relatively low stability and short shelf life time of the particles, leading to a rapid decay of their sensing properties. There is also a poor size selection and high tendencies to agglomeration of NPs. Additionally, it is hard to control surface coverage by NPs. Alternatively, NPs deposited from cluster beams have been demonstrated to be an attractive approach [6,7]. One of the main advantages of this technique is that the clusters are first formed in a gas phase that provides both a high level of flexibility and precision in the control of cluster composition and size. Thereafter, the clusters can be deposited on the required substrate with control of surface coverage. Deposition which is carried

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out in vacuum allows avoiding contamination. Moreover, the cluster beam technique gives a possibility to control the kinetic energy of the particles thus providing conditions for pinning of clusters or nanostructuring of surfaces [8,9], in other words widening the spectrum of possible applications.

In the current paper, we present first results on the formation of transducers for protein sensing using deposition of silver clusters on modified quartz surfaces. The research is focused on the development of the surface immobilisation procedure to provide reasonable adhesion of the silver clusters to quartz, functionalisation of cluster surfaces for protein coupling and testing the applicability of the sensing scheme utilising LSPR.

2. Experimental

Silver clusters were produced using the experimental setup based on magnetron sputtering which is described in detail in [10,11]. A silver target of 99.99% purity was used for the cluster production. Cluster deposition was carried out on quartz substrates with dimensions 10 mm × 10 mm at room temperature in high vacuum at a background pressure of ca. 1×10^{-8} mbar. Thus, one produces pure supported silver NPs on the quartz surface. Deposited at low kinetic (so-called thermal) energies NPs preserve almost spherical shape with a slight tendency to oblate [10]. The setup allows for size selection of clusters with a relative standard deviation of ~9–13% for particles of various diameters in the range between 5 and 23 nm [11]. However, to test the principle of detection and develop methods for surface immobilisation, the precise size of clusters is considered to be not essential for the first experiments. Therefore, silver clusters were deposited without exact size selection in this work. Mean sizes and the size distribution will be described below.

Quartz substrates were modified (functionalised) prior to the deposition. A series of earlier experiments led us to the elaboration of the methodology to improve the cluster adhesion to the substrates in relation to stability against dipping in solutions used in the following steps of transducer formation and protein deposition. Quartz substrates have been cleaned with ethanol and subsequently treated for 30 min in an ozone cleaner to remove residual organic materials and to increase the surface density of hydroxyl groups. Directly after the ozone treatment the samples were placed in a desiccator and evacuated (subjected to low vacuum) in the presence of a mixture of toluene/3-aminopropyltrimethoxysilane (APTMS) at a ratio of 3:1. The gas phase deposition was carried out for 30 min to cover the surface with approximately one monolayer of APTMS. A schematic picture of the quartz surface modification is shown in Fig. 1. After this surface functionalisation with positively charged amine groups, the quartz substrates have been used for cluster deposition as described above. The presence of the amine groups was found to be significantly improving the silver NPs adhesion to the substrate.

Substrates with as-deposited clusters were incubated with a 1 mM 11-mercaptoundecanoic acid (11-MUA) solution in ethanol

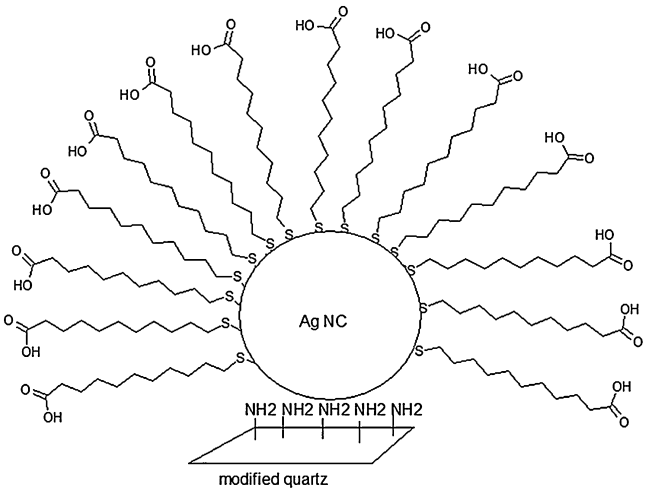

Fig. 2. Functionalisation of silver cluster surfaces using 11-MUA.

for 30 min and subsequently washed with pure ethanol to remove residual not reacted 11-MUA. The 11-MUA modified substrates have been dried under a stream of nitrogen. 11-MUA becomes selectively bound to silver NP by the sulphur-containing end and provides reaction groups for coupling of proteins (see Fig. 2). To activate these groups the samples were incubated with a freshly prepared 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) mix ratio 1:1 for 20 min. Subsequently the mix has been removed from the substrates and a protein solution has been added on top of the substrates. The incubation period for protein solutions was 30 min. After the incubations the samples have been thoroughly rinsed. All proteins used for the experiments are commercially available and they have been used without further purification.

Three series of samples were prepared: the first one follows classical antibody–antigen scheme (with anti-chicken egg albumin antibody and chicken egg albumin as antigen), the second one is of inversed sequence of protein deposition (first chicken egg albumin, then the corresponding antibody) and the third one is also inversed scheme but with lysozyme as the antigen, which should not be recognised by the anti-chicken egg albumin antibody. The proteins used in this work are chosen only to test the applicability of the developing detection approach and they are not of high practical importance.

The samples were characterised after each of the above-mentioned steps in preparation of the transducer system using atomic force microscopy (AFM) and optical transmission spectroscopy. For AFM studies, an *Ntegra-Aura* (NT-MDT) system was utilised. The measurements were performed in tapping mode using commercial Si cantilevers with curvature radius of tip better than 10 nm and a spring constant of approximately 26 N/m. Optical transmission spectra were obtained by a Perkin Elmer High Performance Lambda 1050 spectrometer in the interval of wavelengths

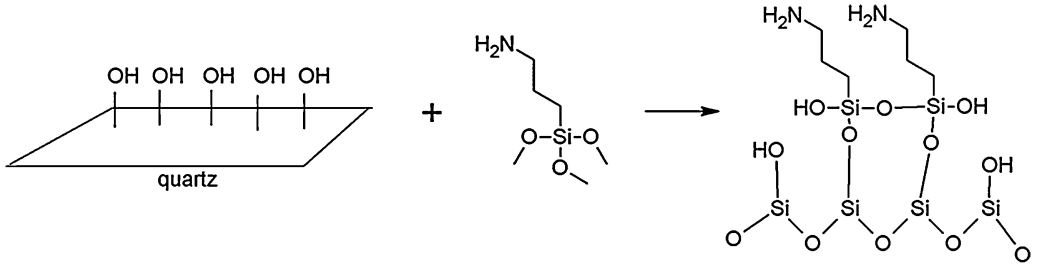

Fig. 1. Gas phase deposition of APTMS on quartz (surface functionalisation).