Nanostructured Surface Preparation for Enhancement of Sensitivity of Electrochemical DNA Sensors

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Biosensor signals can be enhanced by specifically designed transducer surfaces for anchoring the recognition molecules. For electrochemical DNA biosensors, the spacing and orientation of immobilized DNA probes is critical for maximal hybridization with target and to achieve low detection limit [1-3]. We have developed a nanostructured electrode surface preparation method that can create a favorable environment for DNA probe molecule attachment to electrode surface and creates suitable orientation for maximal hybridization with the complementary target. The approach is based on sequential gold nanoparticles electrochemical nucleation on glassy carbon surface [4]. Briefly, the first stage of gold nanoparticles are nucleated on clean glassy carbon electrode by applying a potential pulse. The nucleated nanoparticles are then insulated by forming self assembled monolayer (SAM) of thiolated probe DNA. For effective insulation of the nanoparticles, 2-mercaptoethanol is used as a back filler SAM. Then, the second stage of gold nanoparticles nucleation is made on glassy carbon area that was not covered with gold nanoparticles in the first deposition stage. The method enables an increase of the number density of nanoparticles deposited by preventing them from aggregation as well as to create isolated domains for probe molecule for effective hybridization with target molecules there by eliminating problems associated with steric hindrance. For 10 nM target nucleotide consisting of 63 bases detected by sandwich assay using HRP labled marker probe, hydrogen peroxide as a substrate and hydroquinone redox mediator. A 15 fold higher signal was observed with the nanostructured electrode than planar polycrystalline gold electrode. Currently the work is in progress to further optimize the system in order to determine the lowest detection limit.

References:

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Figures:

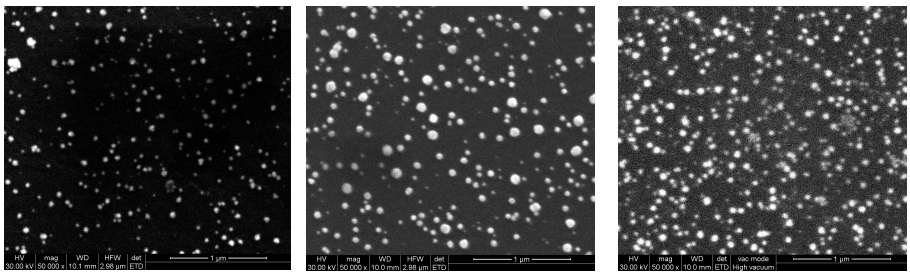

Figure 1. Scanning electron microscopy images of gold nanoparticles electrochemically deposited on glassy carbon electrode at 0V versus Ag/AgCl from 0.1 mM $KAuCl_4$ in 0.5 M H_2SO_4
(A) first deposition stage (5 seconds),
(B) Three deposition rounds of 5 s each with out SAM protection
(C) Three deposition rounds of 5 s each with SAM of 2-mercaptoethanol protection. The increase in particle number density due to protection of the particles with a SAM in

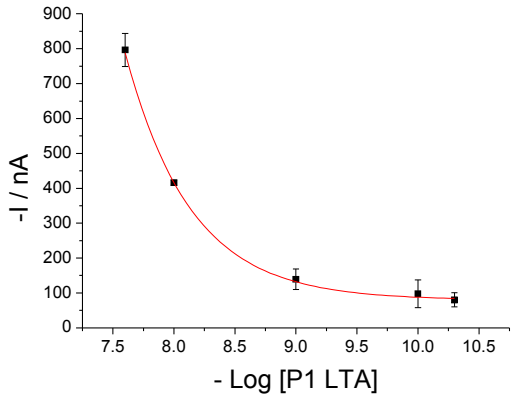

Figure 2. Preliminary result
Calibration curve for determination of P1-LTA (63 bases long) single strand target DNA by sandwich assay using horseradish peroxidase conjugated marker probe. Optimization of the procedure is in progress in order to achieve detection of even lower concentration of target. (error bar standard deviation for triplicate determinations)