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Nano-particles: their application in the development of electrochemical molecular beacon biosensors

Reporting

Project Information

NANOSENS

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Final Report Summary - NANOSENS (Nano-particles: their application in the development of electrochemical molecular beacon biosensors)

Project rational/introduction:

The aims of the NANOSENS project (Nano-particles: their application in the development of electrochemical molecular beacon biosensors) were to investigate the possibility of using nano-particles and or electrodeposited nano-domains as physical mask for the electrochemical patterning of molecular monolayer grafted onto conductive substrate. The proposed approach was applied in the preparation of transducers surface of DNA sensors (genosensors) and more specifically in the development of reagent-less devices. In order to achieve this goal the use of electrochemical molecular beacon (E-MB) was also envisaged. Molecular beacons are single stranded oligonucleotides probes characterised by a peculiar stem-loop structure; their design offers various advantages over the traditional probe design:
i) the possibility to carry an inherent signal transduction mechanism,
ii) the possibility of detecting a hybridisation event without the need of separating the unhybridised probes/targets and
iii) an enhanced specificity.

Several ways of transducing the inherent signal modulation of molecular beacon have been presented based on electrochemical or fluorescence. Between them electrochemistry is extremely attractive due to the fact that this is very easily implemented in low-cost miniaturised/portable devices; this transduction is achieved by the functionalisation of the molecular beacon at one end with a redox label and at the other with a grafting element to allow the immobilisation onto the transducer surface. In immobilised E-MB in the absence of target, the redox label will be forced to be in close proximity with the electrode surface, allowing efficient electron transfer and signal generation. In the presence of target the beacon opens up, physically moving the redox label away from the electrode surface, resulting in a quantitative loss in electron transfer efficiency and consequently of signal. As can be clearly deduced from the mechanism of signal generation the mobility of E-MB is fundamental for the recognition/transduction event; in this context the integration of E-MB with nano-patterned surfaces was seen, in the NANOSENS project, as a potentially advantageous approach.

Results summary:

At the beginning of the project several subtasks were identified in order to fully understand the different aspects involved in the preparation of the nano-patterned transducer surface with integrated the E-MB. Two different approaches to generate the metallic nano-domains on the conductive substrate were identified: electrodeposition of the metals and electrochemical grafting of in-bulk solution synthesised nano-particles.

In the case of the direct electrodeposition of the metal Cu was investigated. This was chosen due to its characteristic to electrodeposit onto Au surface following the mechanism known as underpotential deposition (deposition of a metal monolayer followed by bulk deposition) and its ability to promote spontaneous assembling of thiol modified molecules.

In the NANOSENS project a three steps electrodeposition protocol was proposed in order to allow deposition of nanometric metallic domain; this consisted of i) deposition of the metal onto the transducer surface, followed by ii) partial stripping of it and iii) growth of the remaining nucleation sites. The possibility of patterning the surface using electrochemically of particles was proved by the use of Ag nano-particles; this were drop-cast onto the electrode surface and then stably grafted on it using sequencial oxidation-reduction short pulses.

Formation of nano-domain was followed by electrochemical and imaging techniques.

Electrochemical patterning of the surface is based on the stripping of the metal from the transducer surface, after the functionalisation of it with protective layer (alkane thiol SAM). In this work the assembling and removal of different length alkanethiols (our protective layer model) onto and from Au, Ag, Cu and mix metal surfaces were evaluated. As expected the different alkane thiol self assembled onto the different metallic surfaces; furthermore the presence of the thiol resulted in a stabilisation of the metal deposits making their electrodesorption more difficult until this was not possible when 16 CH_2 alkanethiol were self assembled onto the mix metal surface.

In order to prove the formation of vacancy in the monolayer as a result of the electrochemical removal of the metal nano-deposit the assembling of thiol modified electroactive molecule (Ferrocene). This evaluation clearly showed that a significant assembling of the molecule was occurring only as a result of the electrochemical removal of the metallic nano domains.

Two different mutations of CF were chosen as model in the design/evaluation of the molecular beacons: DF508, the most abundant mutation and V520F a single point mutation.

Preliminary optimisation of the MB design (recognition element, spacer and stem structures) was performed using UV techniques. Optimised the design of the MBs the effect of their grafting on surfaces, on their mobility and as a result on the hybridisation efficiency, was studied by comparing the in-solution fluorescence MB (DF508 was used in this case) with analogous grafted onto Au nanoparticles. Clearly a loss in efficiency was recorded stressing the importance of creating suitable environment for the MB to change its spatial organisation.

The optimised fluorescence MBs designs were them translated in E-MB by replacing the fluorophore with an electroactive moiety; different moieties have been tested but only methylene blue provided the desired level of stability/reproducibility.

Preliminary test of the performances of the so designed MBs was performed by assembling them, following conventional methods (back filling and co-immobilisation) onto Au electrode surface. The E-MBs presented good specificities and robustness.

Following this characterisation the attempt of assembling the E-MBs onto surfaces structured with the developed approach was performed; no significant response was recorded in this case. In order to check if the no-response of the final device was due to problem in immobilisation of the E-MB the assay was repeated using probes with similar structure of the E-MB but without the electroactive moiety and using an enzymatic reaction (conventional sandwich assay format) to transducer the recognition event. This set of experiments showed that the genosensor prepared according to the proposed approach had performance analogous to those obtained with sensor prepared using conventional approaches; this result seems to indicate that in the proposed sensing platform immobilisation of MBs occurred but their density was very low with resulting low response level.

Conclusion:

In the NANOSENS project a full protocol for the structuring of conductive surface, using exclusively electrochemical techniques, has been proved. The proposed approach was successfully applied in the functionalisation of conductive surfaces in the development of electrochemical genosensor. The proposed approach is very interesting allowing the patterning, at nano level, of conductive surface in a very rapid, versatile and cost effective way. Possible application of the developed approach could be in the microelectronics industry or in the preparation of nano sensors. The E-MB developed in the project clearly confirmed the ability of these types of sensing element in the preparation of low cost diagnostic tools.

Related documents

Final Report - NANOSENS (Nano-particles: their application in the development of electrochemical molecular beacon biosensors)

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