

Chapter VI. Conclusions

This thesis examined oligonucleotide immobilisation strategies, the stability of colloidal gold suspensions, the electrodeposition of the biorecognition nanomodules and the signal amplification for electrochemical hybridisation detection. The most important findings in each one are described.

Regarding oligonucleotide probe immobilisation:

- Several methods to immobilise oligonucleotides on gold electrodes as probes for DNA sensors were studied. The low reactivity of cystamine and thioctic acid SAMs on gold, demonstrated using redox mediators and cyclic voltammetry, led to the direct self-assembling of oligonucleotide-thiol probes. This self-assembling was demonstrated by piezoelectric and colourimetric techniques. The strategy provided a rapid, uniform and homogeneous immobilisation with only one step.
- A simple system to demonstrate oligonucleotide immobilisation and hybridisation detection before the use of biorecognition nanomodules as probes was used. Although several operating parameters, such as blocking agent type and concentration, mixed monolayer concentrations, and hybridisation time, were optimised, the high limits of detection suggested that signal amplification was necessary for the hybridisation detection.

Regarding colloidal gold suspension stability and conjugations:

- The best stabilising buffer conditions for bare gold and for resuspension of the centrifuged biorecognition nanomodules were found, which allowed to modify the colloidal gold maintaining the stability of the suspensions.
- Two model thiol-oligonucleotides, with FITC and digoxigenin as labels, were successfully conjugated on colloidal gold, resulting in stable and reproducible colloidal gold suspensions. Additionally, the conjugation was stable under hybridisation temperatures and times and the nanoconjugates were functional and able to differentiate 4-point mutations in 19-mer oligonucleotide sequences, allowing their use as probes for DNA sensors and arrays.

Regarding the site-directed electrodeposition of functional nanomodules:

- Colloidal gold was site-directed on several electrode surfaces by electrodeposition and characterised by several methods. Light and electron microscopy and electrochemistry demonstrated the selective electrodeposition on photolithographed gold IDEs of 5- μm width and gap electrodes, which proved the viability of the strategy for array manufacturing with photolithographic resolution. Spectrophotometric characterisation of the deposition of colloidal gold on ITO electrodes demonstrated the benefits of using short arraying times. Colloidal gold selective deposition was also demonstrated by piezoelectric techniques.

-
- After the "proof-of-concept" with bare colloidal gold, light microscopy, colourimetric and electrochemical techniques demonstrated the site-directed electrodeposition of oligonucleotide biorecognition nanomodules on photolithographed gold IDEs and 3-electrode arrays, and carbon screen-printed electrodes. Despite the clear electrodeposition over the non-specific adsorption, the values from the system were too close to the values from the blanks, indicating that signal amplification was required.
 - The electrodeposited oligonucleotide biorecognition nanomodules were functional and able to detect 4-point mutations in 19-mer oligonucleotide sequences, demonstrating their suitability as probes in DNA sensors. Again, the low current intensities showed the necessity for signal amplification.
 - Preliminary experiments suggested the possibility to decrease the non-specific adsorption yields by exposing the conjugate-modified electrodes to a flowing buffer solution at the appropriate E .

Regarding electron transfer kinetics optimisation:

- The effect of the global charge and the redox potential of the osmium complexes, as well as the effect of the pH and the ionic strength of the media on the electron transfer rate constant between mediators and redox enzymes were evaluated, in order to subsequently apply the acquired knowledge to the design of electrochemical signal amplification schemes for DNA sensors.
- The study of the global charge of the osmium mediator led to the conclusion that more positively charged mediators presented higher electron transfer rate constant as a consequence of the electrostatic interactions, and the existence of electron conducting "pathways". The pH dependence, which provided higher electron transfer rates at higher pH, was explained in terms of higher driving force and the effect on the pK_a of the enzyme active site and His residues. The effect of the redox potential correlated satisfactorily with the Marcus theory. The ionic strength showed an effect according to the screening of the charges of enzyme and mediator. The evaluation of the mentioned effects yielded several system parameters, such as the theoretical maximum electron transfer rate constant, the Marcus reorganisation energy, the electron transfer decay constants, the infinite ionic strength rate constants, and the radius and the charge of the enzyme. These parameters were used to understand the mechanisms of electron transfer for subsequent application in electrochemical signal amplification schemes for amperometric DNA sensors.

Summarising, the biorecognition nanomodules were successfully synthesised, and the stability of the suspensions, the functionality of the conjugates and the thermal stability of the modification were demonstrated. These biorecognition nanomodules were selectively electrodeposited on electrodes with photolithographic resolution. Consequently, the initial proposed strategy of selective electrodeposition of biorecognition nanomodules as arraying method for DNA sensors is viable.