Abstract

The goal of this thesis has been to design, characterise and optimise an electrochemical DNA sensor array. In order to investigate the oligonucleotide probe immobilisation and the hybridisation detection, preliminary experiments with an easy system were performed. This system demonstrated the suitability of oligonucleotide self-assembled monolayers (SAMs) on gold as immobilisation method. Due to the rapid DNA sensor development towards DNA arrays, a modified strategy was proposed. This strategy was based on the site-directed and selective electrodeposition of biorecognition nanomodules on electrodes of photolithographic resolution. These biorecognition nanomodules, oligonucleotide-modified colloidal gold particles, were rationally synthesised previously studying the conditions under which colloidal gold suspensions were stable. Fluorescence and colourimetric techniques proved the effectiveness of the conjugation, the functionality of the conjugated probes, and the thermal stability of the modification, which made the biorecognition nanomodules suitable for hybridisation detection. After their characterisation, the biorecognition nanomodules were electrodeposited on different electrode surfaces and the site-directed immobilisation was clearly demonstrated by several techniques, such as light and electron microscopy, and colourimetric, piezoelectric and electrochemical techniques. Additionally, the site-directed deposited biorecognition nanomodules were functional and able to differentiate 4-point mutations in 19-mer oligonucleotides. Despite the promising results, which demonstrated the viability of the directed electrodeposition as arraying technique, the necessity for signal amplification was observed, as system values were very close to blank values. Following two parallel objectives for the electrochemical signal amplification (to intrinsically increase kinetic rates between enzymes and electrodes, and to optimise electrochemical recycling systems), osmium complexes were rationally designed and the kinetics of electron transfer with redox enzymes was evaluated. These kinetic studies showed that more positively charged mediators and with higher redox potentials yielded higher rates, also favoured at high pH and low ionic strength, demonstrating the possibility to amplify electrochemical signals.

This thesis is structured in seven chapters. Chapter I establishes the basis of DNA sensors and arrays, colloidal gold stability, conjugations and deposition, and signal amplification. It also presents the thesis. Chapter II describes the preliminary system for the immobilisation characterisation and hybridisation detection. Chapters III, IV and V correspond to the synthesis and characterisation of the functional biorecognition nanomodules, the site-directed electrodeposition of these nanomodules, and the electron transfer kinetics optimisation for signal amplification. Finally, Chapters VI and VII summarise the conclusions and the future work.