

Electric Field Effect Detection of Biomolecular Interactions

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We here report on the optimization of electrical detection of molecular interactions. More specifically, interactions that occur when biomolecules attached to a metal (probe molecules) are exposed to their bioconjugates (target molecules). The ability to detect such reactions is essential in fields such as genomics and proteomics. One commonly employed method is the optical detection, which employs labeling of the targets with fluorescent tags and expensive instrumentation.

The development of a generic platform for label-free electrical detection of biomolecular interactions could prove useful for a wide range of applications. By reducing cost and improving portability, a large market in the field of genomics and proteomics can be targeted, from small-scale medical research laboratories to large-scale pharmaceutical companies.

We have investigated the application of field effect detection to the sensing of DNA hybridization. The technique is applicable in principle to all biomolecular interactions that affect the surface potential at a metal gate/electrolyte interface. The presence of immobilized chemical species results in a change of the interfacial dipole, which in turn affects the potential drop across the electrochemical double layer. This modulates the voltage applied to the gate of a field effect device, resulting e.g. in a change of the Capacitance-Voltage (C - V) characteristics for a Metal-Insulator-Semiconductor (MIS) capacitor. When the probe molecules interact with their bioconjugate, further changes in the interfacial dipole and the double layer potential drop are expected and therefore changes in the C - V curves are also expected.

We employed MIS capacitors made on single crystalline silicon. Single stranded DNA (ssDNA) with 18 or 20-base pairs are self-assembled on the gate together with spacer molecules. The C - V characteristics are measured before and after DNA immobilization. Upon immobilization of ssDNA the curves show a significant shift in the direction of negative voltages (with respect to an Ag/AgCl reference electrode immersed in the electrolyte) as expected from the immobilization of negative charges on the gate. Upon hybridization with a fully complementary DNA strand a further significant shift is observed in the same direction as before, which is consistent with the significant increase of negative charges on the gate brought about by the hybridization. When a non-complementary DNA strand is placed on substrates containing only the probe ssDNA and spacer using the same conditions as before, no significant shift is observed in accordance with the fact that no extra charged molecules are immobilized on the surface [1]. A non-complementary strand with only 3 bases mismatch can be detected in this way. Here we report on the sensitivity of the technique and the possibility of relating single-base mismatches to incomplete hybridization shifts.

The effect of the electrolyte used on such measurements has also been investigated. The concentration of the measuring buffer will change the Debye length and therefore part of the DNA charges will be screened upon increasing buffer concentration. On the other hand the pH of the solution can alter the net

biomolecular charge. A systematic study was carried out on the effect of buffer concentration and pH on the shifts observed in order to optimize the detection process.

The same detection mechanism can be applied to different biological systems where a change on the charge configuration occurs upon biomolecular interaction. Of major interest is the detection of protein interactions and we will also discuss the applicability of the MIS detection to such systems.

In order to promote the immobilization and hybridization of DNA, electric fields can be applied so as to offset the repulsion of the negative charge of probe and target. We carried out a study on the effect of electric fields using DNA labelled with fluorescence tags. It is observed that under a dc voltage, the time necessary for the formation of a dense ssDNA layer is dramatically reduced and hybridization is strongly promoted [1]. A systematic study of the effect of electric fields was also carried out using electrochemical quartz crystal microbalance (QCM) measurements in order to determine the density of DNA and efficiency of hybridization upon electric fields applied during variable time periods.

The same detection mechanism was extended to a MIS Field Effect Transistor (MISFET) fabricated using polycrystalline silicon thin film transistor (poly-Si TFT) technology. Poly-Si TFTs can be used to inexpensively fabricate the sensing devices as well as the integrated electronics for signal conditioning and detection. Active-matrix displays are currently produced using this technology and parallel development will enable the fabrication of microarrays where each channel is individually addressed. Another added advantage of poly-Si TFTs is that they can be fabricated onto glass or plastic substrates, which are ideal for biosensor applications due to insulation and disposability. We have previously demonstrated the applicability of FET structures without a metal gate as pH sensors and as penicillin sensors [2]. By using a metal gate we show that we can successfully detect DNA hybridization using polysilicon MISFETs, observing shifts on the transfer characteristics in excess of 300 mV. Issues related to passivation of FETs for the different types of sensors (DNA sensors and enzyme-based sensors) are discussed.

- [1] P. Estrela, P. Migliorato, H. Takiguchi, H. Fukushima and S. Nebashi, submitted to Biosens. Bioelectron.
- [2] F. Yan, P. Estrela, Y. Mo, P. Migliorato, H. Maeda, S. Inoue and T. Shimoda, submitted to Appl. Phys. Lett.