QUARTZ CRYSTAL MICROBALANCE AS IMMUNOSENSORS FOR THE DETECTION OF LIGHT MOLECULES

R. Funari[1], B. Della Ventura[2], C. Altucci[1], R. Velotta[1]
[1]Dipartimento di Fisica, Università di Napoli "Federico II", Via Cinthia, 26 – 80126 Napoli
[2]Dipartimento di Fisica, Università di Roma "La Sapienza", Piazzale Aldo Moro, 5 – 00185 Roma

Since antibodies (Ab) are the main element of the immune system and their role is to bind and neutralize particular pathogens and toxic molecules, they are often exploited as a recognition element in sensing. Nevertheless, the recognition region of Ab is limited to the so-called variable part (Fab region) and one of the main issues in the development of low-cost, rapid and effective biosensors is the immobilization of Ab onto the sensor surface in a suitable way so that it is able to recognize the antigen, i.e. with its Fab oriented side-up. To this end, the so-called photonic immobilization technique (PIT), based on the UV activation of proteins, has been recently proposed [1], with the aim of tethering antibodies preferentially oriented onto the gold electrode of a QCM (quartz crystal microbalance) [2].
The antibodies are activated by breaking the disulfide bridge in the triad Trp/Cys-Cys, highly conserved in immunoglobulin superfamily [3], through absorption of ultrashort UV laser pulses. The free thiol groups so produced interact with the gold electrode of a QCM making the adsorbed antibodies preferentially oriented with its sensitive parts exposed to the environment, thereby greatly increasing the binding efficiency.
To exploit such advantage, PIT has been applied to develop a QCM based immunosensor for the detection of parathion, a forbidden pesticide, in aqueous solution. The detection of light and low soluble molecules is of paramount importance in biosensors development. Since parathion weighs only 291.26 Da, QCM is effective only if the antigen is made "heavier". We have faced such an issue by adopting two strategies: The first is based on the complex formation between parathion and bovine serum albumin (BSA). This big protein results to be able to absorb small molecule through nonspecific interactions [4]. The second mimics the sandwich configuration widely used in the ELISA assay and the antibody itself is used to ballast the antigen. With the latter method we have been able to reach a limit of detection of 1.5 ppb with a measurement requiring less than 15 minutes. Moreover, we also estimated the kinetic parameters of the immobilized antibodies. The sensor so realized is highly specific since it does not show any appreciable response against compounds similar to parathion. Since PIT only requires the presence of the structural triad Trp/Cys-Cys, this technique can be very useful in the functionalization of any thiol reactive surface.

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