QUALITATIVE AND QUANTITATIVE ANALYSIS OF BIORECOGNITION IN PIEZOELECTRIC BIOSENSORS

Rogério M. M. Rodrigues, Luis F.M. Rosa, Guilherme N.M. Ferreira
IBB-Institute for Biotechnology and Bioengineering
Centre for Molecular and Structural Biomedicine
University of Algarve, Campus Gambelas, 8005-139 Faro, Portugal
rogerodrigues@gmail.com

The formation of biomolecular complexes through specific recognition and consequent affinity interaction is an important biochemical process in nature and, among others, includes hybridization of complementary strands of DNA and RNA, antigen–antibody binding, and protein–nucleic acid binding. These processes represent the main source of molecular constructs for the development and design of bioanalytical micro-devices and methodologies. Thus, the detailed study of such devices and methodologies involving such processes, leading ultimately to the quantitative characterization of the involved kinetics, constitutes a major priority in the development of miniaturized devices to detect specific molecules, complex structures or processes.

We use piezoelectric devices to study biorecognition processes in aqueous systems. We demonstrate that these devices operating in liquid environments also sensitively respond to the properties of the solution and films which can easily lead to the misinterpretation of the measured data. We use impedance spectroscopy methods to construct equivalent electric circuits to model the system in order to enable the differentiation of all the contributions to the sensor final signal. We were able to distinguish mass load from acoustic energy viscoelastic losses and detect charge induced parasite/stray capacitive interferences. The quantification of such contributions enables the correction of frequency data measurements and its further use in models to study the biorecognition reaction leading to the determination of more accurate parameters such as kinetic data.

This communications focus the different aspects involved in the development of piezoelectric based biosensors to correctly measure bimolecular binding kinetics as well as to detect biomolecules in biological samples. The quantification of the effect of charged species, density, and viscosity is used to enhance and optimize the sensor signal and performance for binding monitoring and quantification.

We demonstrate the applicability of impedance analysis in quantitatively monitoring the immobilization of alkanethiols onto gold surfaces forming Self Assembling Monolayers, in the detection and quantification of streptavidin binding to biotin and in the development of recombinant antibody-based biosensors to detect HIV-1 virion infectivity factor (Vif). In the last case, the affinities of single chain (4BL) and single domain (VH and VHD) recombinant antibodies generated against HIV-1 Vif were compared. Recombinant antibodies were immobilized onto activated sensors surface and used as biorecognition material. We further demonstrate the potential of these sensors as tools for HIV-1 infection monitoring and follow-up through the successful selective detection of HIV-1 Vif from HEK293 cell culture extracts.

Acknowledgments:
The authors thank to Portuguese Foundation for Science and Technology (FCT) the financial support through the research project POCI/BIO/61912/2004 and the grants SFRH/BD/38136/2007 and SFRH/BD/36294/2007.