AC electrokinetic immobilization of single biomolecules on nano-electrode arrays

Xenia Knigge[1,2], Christian Wenger[3], Frank F. Bier[2], Ralph Hölzel[1]

Ralph.Hoelzel@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalysis and Bioprocesses (IZI-BB), Am Mühlenberg 13, 14476 Potsdam, Germany
[2]University of Potsdam, Karl-Liebknecht-Straße 24 – 25, 14476 Potsdam, Germany
[3]IHP GmbH, Leibniz Institute for High Performance Microelectronics, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany

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Introduction

A key element in the construction of a biosensor is the immobilization of the bioreceptor. In common biosensors the bioreceptor molecules are immobilized as randomly oriented objects on one transducer, leading to a comparatively low ensemble signal due to the combined measurement of all analytes. Immobilizing individual, addressable biomolecules would allow to exactly locate the signal's origin achieving a new level of biosensing. This goal can be achieved by a label-free method of immobilization: Dielectrophoresis (DEP). DEP is a phenomenon in which a dipole is induced in a polarizable particle in an inhomogeneous (AC) electric field. By the right choice of voltage and frequency, this particle can be moved without damage and can be immobilized. Enzyme activity and antibody binding function are preserved after DEP application [1, 2] and biomolecules can be immobilized oriented parallel to the electric field lines [3].

Results and Discussion

Using regular arrays of many thousands of vertical silicon- or tungsten-based nano-electrodes with tip diameters ranging from 500 nm down to about 1nm it is possible to gain statistical information since thousands of experiments are carried out in parallel. Using nanospheres of different sizes as a model system, immobilization of just a single object on each electrode is ensured by choosing appropriate electrode dimensions and shapes in relation to the objects' size. Immobilization of exactly one particle at each electrode tip has been demonstrated for electrode tip diameters with half the particle size [4].

These results have been transfered to the successful DEP-immobilization of autofluorescent R-PE proteins as individual biomolecules on nano-electrode arrays. The proof of immobilization and singling is done via fluorescence microscopy in combination with a histogram method, revealing the blinking of molecules, immobilized as few or singles on the electrodes of the array. In this way the ensemble averaging of the analyte signal is avoided, still a statistically large sample size is investigated.

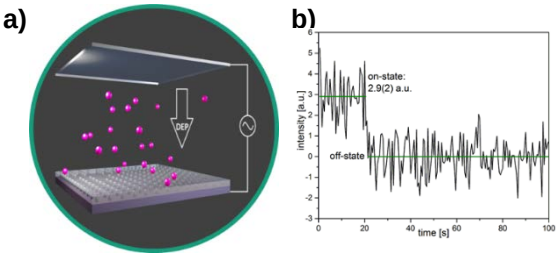

Figure 1: *a) Experimental setup; b)Time trajectory showing a single molecule event*

Conclusions

Electrically controlled immobilization and singling of nanospheres and proteins on nano-electrodes has been shown. The method is efficient at low voltages, fast, as the immobilization takes place after minutes, and highly parallel, so thousands of experiments can be carried out simultaneously. This leads to the possibility of single molecule investigations with still good statistics and points to new approaches in the field of biosensing.

References

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