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Single-molecule detection on a protein-array assay platform for an absolute instead of relative quantification of protein-protein interactions

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Abstract

Based on a total internal reflection configuration, a fluorescence-linked, single-molecule sensitive analytical platform was established that permits the detection of individual binding events on standard protein arrays. Apart from the unsurpassed sensitivity, the process permits truly quantitative measurements, since the number of binding events can be counted, thereby improving on relative measurements, which do not yield absolute values. Also, a universally applicable labelling and purification process was established to prepare biologically active proteins with a stoichiometric 1:1 ratio of attached dye-label.

The process was initially used for the detection of tuberculosis markers in human plasma and urine samples. No sample preparation had to be done; no signal amplification step was required; and no washing steps were needed during analysis. Biological samples containing about 600 antigen molecules per microliter produced a distinct signal.

In extension, we now use the process also for quantitative measurements of both expression and structural variations of proteins by means of complex antibody microarrays of more than a thousand cancer-specific binder molecules. Body liquids as well as tissue and cell extracts are being studied for the identification of personal differences. For the development of new therapeutic approaches, we pursue particularly the identification of structural variations of protein isoforms that are specific for a disease status and do not occur in healthy patients.

Biography

Jorg D. Hoheisel is Head of the *Division of Functional Genome Analysis* and Chairman of the Scientific Council at the Deutsches Krebsforschungszentrum (DKFZ; German Cancer Research Center) in Heidelberg, Germany. Apart from publishing more than 300 scientific papers, the division filed 47 patents. Jorg Hoheisel is co-founder of four companies; another three companies were set-up by former group members. Prior to joining DKFZ, Jorg Hoheisel worked for five years at the Imperial Cancer Research Fund in London, UK, the initial two years funded by an EMBO fellowship. Before, he had obtained his PhD at the University of Constance, Germany.