A dynamic biochip platform for real-time detection and quantification of proteins

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Introduction

Motivation

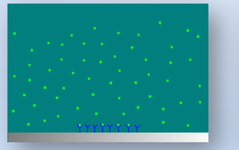
Protein analytics based on immunoassays such as ELISA play an important role in today's clinical diagnostics. However, large sample consumption, long incubation time and tedious handling impede applications in point of care units in vicinity of the patient. With ongoing miniaturization microarray based immuno tests were established permitting multiparametric diagnostics of a single sample [1]. Nevertheless, a severe drawback of conventional microarrays is its limited mass transport. Analyte is transported to capture molecules solely by means of diffusion, resulting in long incubation times [2].

Goals

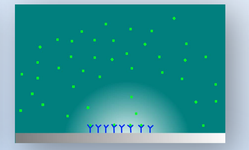
- significantly reduced assay time
- quantitative measurements
- compatibility with clinically relevant proteins
- dynamic real-time detection

Strategy

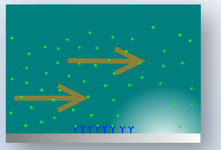
- use pressure driven flow of analyte solution to shift the depletion zone
- proof of principle based on a simple flow cell based setup in a direct assay with human IgG
- analyze binding interaction in real-time



Initial state: equal distribution of analyte in solution



Formation of a depletion zone in close vicinity of the capture antibodies

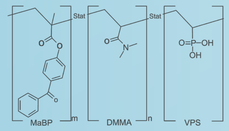


Lateral shifting of the depletion zone via pressure driven flow of analyte solution

Technology

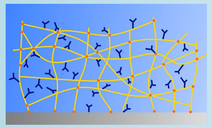
Protein Immobilization

Capture antibodies (anti-human IgG) are immobilized on PMMA substrates via a surface attached water swellable polymer network. The methacrylamide based copolymer is spotted on the substrate with capture antibodies in PBS print buffer following crosslinking in a photo radicalic reaction under UV exposure (254 nm)



Chemical structure of the copolymer:

DMMA: monomer, basis for the polymer chains
MABP: photo radicalic cross linker
VPA: enhances solubility in water



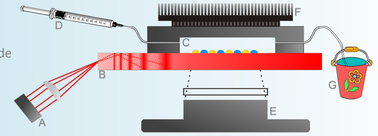
Schematic illustration of a spot containing antibodies immobilized in polymer network

Fluorescence measurements & incubation

Read-out of the microarrays was performed in a home-built biochip reader. Method of detection is based on TIRF (total internal reflection fluorescence) [3]. Laser light is coupled into the microarray substrate, which acts as a waveguide. A homogeneous evanescent field is built at the surface area of the microarray. The intensity of this field characteristically decays exponentially in direction perpendicular to the substrate. It permits real-time monitoring of the binding interaction of the entire array during incubation. Incubation was performed in a flow cell (65 µl) integrated in the biochip reader

Schematic illustration of the biochip reader:

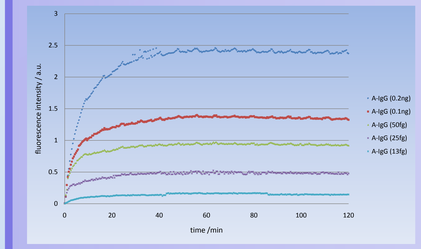
A: light source (laser)
B: substrate=waveguide
C: flow cell
D: syringe pump
E: CCD camera
F: peltier element
G: reservoir



Results and Discussion

Scaling parameters

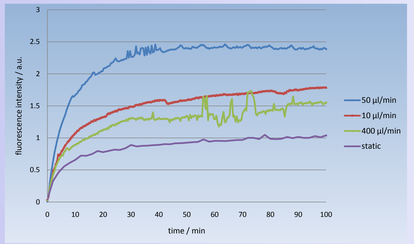
The concentration range of clinically relevant proteins ranges over more than 8 orders of magnitude. In contrast, the dynamic range of the detection system is usually limited considerably. Adjustment of signal intensity can be achieved by printing an applicable concentration of antibodies per spot or dispensing larger drop volumes (not displayed). In both cases a linear relation with regard to measured fluorescence intensity was found for the range of concentrations used. Scaling laws are fundamental for design of multiparametric microarray platforms.



Dependence of amount of capture antibodies per spot on the fluorescence signal. Concentration of Cy5-labeled human IgG in solution: 8 nM

Continuous actuation

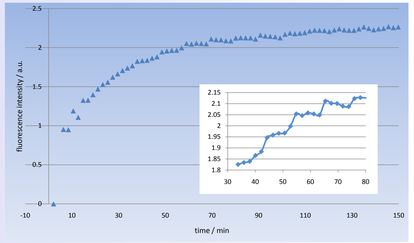
The influence of pressure driven flow on binding kinetics was investigated for several flow rates. As illustrated below, an adequate fluidic actuation improves the transport of analyte molecules to their corresponding binding partners. An equilibrium of the binding reaction could be obtained in a time frame as short as one hour, compared to more than 8 hours in a static assay under same conditions. An optimum value for the flow rate can be derived: For low flow rates mass transport is insufficient, for high flow rates stress on the polymer network becomes significant.



Dependence of the flow rate on the fluorescence signal. Concentration of Cy5-labeled human IgG in solution: 8 nM

Stop-flow actuation

For further determination of the influence of convective flow during incubation on the binding reaction, a series of alternating static (duration: 5 min) and dynamic incubation periods (1 min) was performed („stop-flow"). The concentration of analyte is periodically renewed in close vicinity of capture spots, which is depleted during the static phases without convective flow [4]. A clear improvement of mass transport was determined represented by a steeper slope of the measured signal as a result of actuation by pressure driven flow.



Dependence of stop-flow actuation on the fluorescence signal. Concentration of Cy5-labeled human IgG in solution: 8 nM. Duration static phase: 5 min, dynamic phase: 1 min

Conclusion

- a proof of concept of a new dynamic biochip platform for protein microarray applications was demonstrated.
- the dynamic immunoassay provides significantly reduced assay times compared to conventional static methods
- immobilization of antibodies in polymer network based on a surface-attached polymer proved to provide a sufficient anchoring under convective flow conditions
- a broad range of clinically relevant proteins can be detected: assays on HbA1c, IgG, cytokines were tested successfully.

References

[1] Hoheisel JD (2006): Microarray technology: beyond transcript profiling and genotype analysis. Nat Rev Genet 7:200-210.

[2] Kusnezow W, Syagailo YV, Ruffer S, Klenin K, Sebald W, Hoheisel JD, Gauer C, Goychuk I (2006): Kinetics of antigen binding to antibody microspots: strong limitation by mass transport to the surface. Proteomics 6:794-803.

[3] Lehr HP, Reimann M, Brandenburg A, Sulz G, Klapproth H (2003): Real-time detection of nucleic acid interactions by total internal reflection fluorescence. Anal Chem 75:2414-2420.

[4] Lionello A, Josserand J, Jensen H, Girault HH (2005): Dynamic protein adsorption in microchannels by "stop-flow" and continuous flow. Lab Chip 5:1096-1103.