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Optimization of Aptamer Immobilization for Protein Microarray Application

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INTRODUCTION

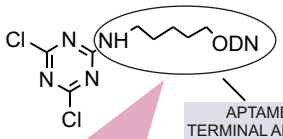
Aptamers represent an interesting alternative to antibodies concerning their application for protein microarrays as capture molecules. Aptamers are short single-stranded oligonucleotides, which are able to bind a wide range of molecules with high affinity and specificity. Compared to antibodies aptamers are easier to produce and more stable. The problem with aptamer microarrays is that the immobilized aptamers need to be able to fold into their correct 3D-structure to provide the binding of the target-protein. Therefore an immobilization strategy, which is based on chemical modification of the aptamer itself and the microarray, was developed[1].

Besides the difficulties in immobilization of the aptamers, it is also a problem to perform multiplex analyses with aptamer microarrays. For a detection of more than one protein in solution with one microarray-experiment various aptamers need to be immobilizied on one microarray. The challenge of this matter is to provide that each aptamer is able to adopt its correct 3D-structure, although almost all aptamers are synthesized under different conditions concerning buffer composition and pH-value. The aim of this study is to characterize different aptamers and find conditions in which these aptamers are able to work as capture molecules side by side on one protein microarray.

MODIFICATION OF MICRO-ARRAY WITH PEI

APTAMER MICROARRAY EXPERIMENT

ACTIVATION OF APTAMERS WITH CYANURIC CHLORIDE



APTAMER WITH TERMINAL AMINO-LINKER

PREVENTS STERIC HINDRANCE

PREVENTS UNSPECIFIC BINDING

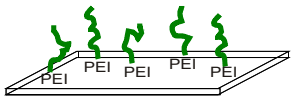
SPOTTING

1. REVERSION OF SURFACE-POLARITY WITH SUCCINIC ANHYDRIDE

2. DENATURATION OF THE APTAMERS

PREVENTS ELECTROSTATIC INTERACTIONS

UNFOLDED APTAMERS



FOLDING IN SELECTION-BUFFER

PROBLEM: MULTIPLEX ANALYSES

- every aptamer is synthesized under various conditions during selection
- universal folding-buffer needed

MICROARRAY READY FOR ANALYSIS

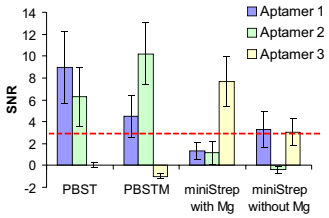
MULTIPLEX CHIP- EXPERIMENT

- three aptamers were immobilizied with the optimized strategy on one microarray (aldehyde-modified slide)
- folding was performed in four different buffers
- the microarrays were incubated with each of the target protein (Cy3-labelled)

Aptamer	Target	Selection-buffer
1	His -Tag	PBST
2	His -Tag	PBST
3	Streptavidin	miniStrep

Tab. 1: Aptamers used in this study.

RESULTS AND DISCUSSION



- this diagramm shows the obtained SNR (signal to noise ratio) against the four various folding-buffers
- each aptamer shows different results after incubation in the four diverse buffers

OUTLOOK

Further investigations concerning multiplex analyses with aptamer protein microarrays necessary:

- characterization of aptamer-folding in various buffer-systems
- multiplex chip-experiments with aptamers, which are selected under similar conditions
- further studies with different chip-surfaces

[1] J. Walter, Ö. Kökpinar, K. Fries, F. Stahl, T. Scheper. Systematic Investigations of Optimal Aptamer Immobilization for Protein Microarray Applications. *Analytical Chemistry.* August 27, 2008.