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Aptamers: Universal capture units for lateral flow applications

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ABSTRACT

The present work demonstrates the implementation of aptamers as capture molecules for a wide range of target classes in lateral flow assay applications. The targets were chosen in order to cover a wide range of target classes (small sized - metabolite, medium sized - protein, and large sized - whole cell/spore). For each target class one target molecule was selected as representative and appropriate aptamers were used for lateral flow assay development. The work points out that the implementation of aptamers as capture molecules in a universal lateral flow test platform was successful independent form target size. Furthermore, the limit of detection for *p*-aminohippuric acid in urine (200 ppm), lysozyme in white wine (20 ppm), and *Alicyclobacillus* spores in buffered orange juice (>8 CFU/mL) were determined using aptamers as capture molecules. The whole approach is considered as a proof of concept, regarding the ability of aptamers as an alternative to antibodies (in conjunction with directive 2010/63/EU on the protection of animals used for scientific purposes) in lateral flow applications.

1. Introduction

Lateral flow devices (LFDs) provide a prompt detection, which is featured by an easy usage and the suitability for simple yes or no decisions. Furthermore, they can be used *in-field*, without the requirement of complex analytical knowledge or equipment. The application range of LFDs includes both the prompt detection of e.g. toxins, pathogen microorganisms or poisonous residues/contaminates in comestible goods or raw materials and the clinical diagnosis like determination of drug residues or the well-known pregnancy test [1,7,13,29]. Most established lateral flow test systems share the common attribute that *in vivo* generated antibodies were used as capture and detection molecules, due to the fact that antibodies are enabled to interact highly specific with their target molecules. Considering the directive 2010/63/EU on the protection of animals used for scientific purposes, which entered into force 2013, alternatives for immunological based applications are desired. Herein aptamers possess a promising *in vitro* alternative to antibodies.

Aptamers are short single stranded DNA or RNA oligonucleotides, which are usually selected from a high diverse oligonucleotide library via the SELEX (systematic evolution of ligands by exponential enrichment) process. Their single stranded conformation enables the aptamers to form distinct three dimensional structures, which are the basis for high affine interactions - comparable to those of antibodies [9,10,32,34]. Aptamers, once generated, can be used in a similar manner for a wide range of applications, like trapping, detection, labeling or sensing [6,20,21,24,25,31]. Moreover aptamers provide some advantages, like selection towards non-immunogenic or toxic targets or base precise modifiability during aptamer synthesis.

The present work demonstrates the usage of aptamers as universal capture molecules for several target classes in lateral flow applications. Thereby three different target classes were chosen to demonstrate the all-round suitability. As target classes small molecules (here: *p*-aminohippuric acid), proteins (here: lysozyme), and spores (here: *Alicyclobacillus* spores) were chosen to prove the practicability for different sized targets. Thereby each target class own its contribution to the field as their representatives can be (i) biomarkers e.g. for proving authenticity or medical issues, (ii) toxins, (iii) pesticides, (iv) pathogen or spoilage inducing microorganisms.

The research approach could be seen as a novel proof of concept, to extent the application range of aptamers. So far various aptamers for several target classes (e.g. toxins, ions, whole cells, metabolites, antibiotics, and proteins) are published. To extend the field of applications the present research proves the general usage of aptamers for lateral flow applications.

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Among the general suitability proof, the applications were adapted and the detection limits were successfully determined in real samples, to demonstrate the usage under realistic conditions. Thus the present work justifies that aptamers are in general appropriate capture units for lateral flow purposes. Compared to other published lateral flow applications, the shown method is adaptable to further approaches within a short period of time, due to the fact that, in combination with a suitable FITC (fluorescein isothiocyanate) probe, every previously selected and published aptamer could be utilized as capture molecule.

2. Materials and methods

To include a wide target range, earlier published aptamers with an affinity towards lysozyme (selected in white wine simulating buffer) and *Alicyclobacillus* spores (selected in orange juice simulating buffer) were used [18,19]. In addition aptamers towards the small molecule *p*-aminohippuric acid were selected via recently published *just in time*-Selection as it was shown, that the *just in time*-Selection method is suitable for selecting aptamers towards small molecules [12,19]. All aptamers used for lateral flow development are listed with their sequence and regarding dissociation constant in Table 1.

2.1. Selection of aptamers towards p-aminohippuric acid

Selection of aptamers towards *p*-aminohippuric acid (Merk Millipore, Purity $\geq$ 98%) was performed using *just in time*-Selection [19]. Following buffer composition was used for defined interaction conditions during SELEX process: 0.5 mM EDTA, 1 M NaCl, 5 mM Tris-HCl, pH 7.5. The aptamer pool for the first selection round consisted of the following sequences: 5′-CATCCGTCACACCTGCTC-$(N)_{40}$-GGTGTTGGCTCCCGTATC-3′ (Thermo Fisher Scientific Inc., Darmstadt, Germany). For the FISHing process, which is part of the *just in time*-Selection process, the target was immobilized on the surface of carboxylated magnetic particles (SiMAG-Carboxyl, chemicell GmbH, Berlin, Germany) regarding to the manufactures protocol (1-Step Method, see Supplementary Material). FISHing includes several washing steps and a final elution step for each selection round. The resulting eluate contains the enriched aptamers. This semi-automated FISHing was performed using the KingFisher Duo (Thermo Fisher Scientific Oy, Vantaa, Finland).

During the following BEAMing step, the amplification of enriched aptamers takes part. A strand separation is included during the solid-phase-based BEAMing protocol, to receive new single stranded aptamers. After 15 rounds of selection the enriched aptamer pool was ligated (TOPO TA cloning kit, Thermo Fisher Scientific Inc., Darmstadt, Germany) and transformed into chemically-competent cells (*E. coli* XL1). The plasmids were isolated (QIAprep Spin miniprep kit, Qiagen GmbH, Hilden, Germany), and sequenced by GATC Biotech AG (Constance, Germany) using M13 forward primers. To obtain details about the binding parameters of selected aptamers, dissociation constants were determined via fluorescence binding assay, analogous as described in detail elsewhere [12]. Thereby a good interaction behavior is indicated by a low dissociation constant.

2.2. Determination of aptamer selectivity via fluorescence assay and surface plasmon resonance

2.2.1. p-aminohippuric acid

The selectivity of the aptamer towards *p*-aminohippuric acid (*p*-aha Apt 1, used for the following lateral assay development) was determined towards further more or less similar small molecules/metabolites (hippuric acid, adipic acid, phenylalanine, taurine, creatine, and phytosphingosine) via fluorescence binding assay. Therefore, the metabolites (each 25 μg) were linked to the surface of magnetic particles (SiMAG-Carboxyl, chemicell GmbH, Berlin, Germany) using the manufacturers protocol applied already during aptamer selection. Further as reference for normalization a blank measurement (particle containing no covalent-coupled target) was performed similar and considered during data evaluation.

Afterwards the aptamer was fluorescently labeled in accordance to the BEAMing protocol as published before by Ref. [12] using a modified forward primer (100 μM, 5′-Alexa488-CATCCGTCACACCTGCTC-3′, Life Technologies GmbH, Darmstadt, Germany). Afterwards the concentration of labeled aptamer was determined using the NanoDrop®ND-1000 (Thermo Fisher Scientific, Waltham, USA).

The fluorescently labeled aptamer (50 nM) was incubated with the seven different target particles. Therefore 10 μL of target particles ($1.8 \cdot 10^8$ particles/mL) were incubated with 90 μL aptamer solution for 30 min under agitation and exclusion of light. Subsequently the solution was centrifuged twice for 10 min at 14800 rpm. The supernatant was removed and the second centrifugation was carried out after a washing step using 100 μL selection buffer to remove unspecific/non-bound aptamers. The resulting particle pellet was resolved in 100 μL ddH_2O and used for fluorescence measurement (SpectraMaxx®2, extinction 485 nm, emission: 525 nm, Molecular Devices Analytical Technolgies GmbH, Ismaning, Germany).

As the aptamer was selected towards *p*-aminohippuric acid, obtained fluorescence signals were considered relative to the fluorescence signal from aptamers incubated with the *p*-aminohippuric acid containing particles.

2.2.2. Lysozyme

The selectivity of the used aptamer towards lysozyme (LysApt4)

Table 1
Sequences of used *p*-aminohippuric acid, lysozyme and *Alicyclobacillus* spores aptamers and FITC-probes (primer regions are marked in italics). Estimated dissociation constants are given in nM [18,19].

Aptamer	Sequence (5′-3′)	Dissociation constant [nM]
***p*-aminohippuric acid**		
p-aha Apt 1	*CATCCGTCACACCTGCTC*ACGTCATCCGTCACACCTGCTCACGTCATCCGTCACACCTGCTC*GGTGTTCGGTCCCGTATC*	**99.8**
p-aha Apt 2	*CATCCGTCACACCTGCTC*CACTCGTCACACCCATCCGTCACACCTGCTCCCCCCACTG*GGTGTTCGGTCCCGTATC*	**290.5**
***Alicyclobacillus* spores**		
AliApt5	*CATCCGTCACACCTGCTC*CCCAGCGTGGCGTCGACCCGGACCCTGTCAGCCCCCTCGCG*GGTGTTCGGTCCCGTATC*	**2.3**
Lysozyme		
LysApt4	*CATCCGTCACACCTGCTC*TTGTTATTTTTTGTTGATGTAGGTTTGATGATGTATTTCC*GGTGTTCGGTCCCGTATC*	**21.7**
Used probes		
Probe 1	FITC-GATAC	-
Probe 2	FITC-GATACGGGAG	-
Probe 3	FITC-GATACGGGAGCCAACACC	-