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# Development of an aptamer-based affinity purification method for vascular endothelial growth factor

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ABSTRACT

Since aptamers bind their targets with high affinity and specificity, they are promising alternative ligands in protein affinity purification. As aptamers are chemically synthesized oligonucleotides, they can be easily produced in large quantities regarding GMP conditions allowing their application in protein production for therapeutic purposes. Several advantages of aptamers compared to antibodies are described in general within this paper. Here, an aptamer directed against the human Vascular Endothelial Growth Factor (VEGF) was used as affinity ligand for establishing a purification platform for VEGF in small scale. The aptamer was covalently immobilized on magnetic beads in a controlled orientation resulting in a functional active affinity matrix. Target binding was optimized by introduction of spacer molecules and variation of aptamer density. Further, salt-induced target elution was demonstrated as well as VEGF purification from a complex protein mixture proving the specificity of protein-aptamer binding.


## 1. Introduction

Affinity separation is a frequently used method in downstream processing of recombinant proteins for biomedical or pharmaceutical application because it offers a high product quality and purity. Currently, natural or recombinant affinity ligands from animal or bacterial source including antibodies, Protein A or heparin are commonly used as capture molecules linked to the stationary chromatography phase [6]. However, especially the production of proteins for therapeutic purposes demands purification methods without any cell-based material to avoid potential contaminations with endotoxins, viruses or other pathogens associated with these components. In contrast to antibodies or heparin, aptamers are in vitro selected and chemically synthesized single-stranded DNA or RNA oligonucleotides and therefore fulfill this requirement.

In 1990, nucleic acid oligomers were first time selected in a process named SELEX (systematic evolution of ligands by exponential enrichment) by their ability to bind a certain molecule with high affinity [3,26]. Due to intramolecular interactions between their nucleobases, these oligonucleotides, named aptamers, fold into distinct three-dimensional structures which enable them to recognize and to bind their corresponding target molecules. Aptamers bind their targets with affinity and specificity comparable to those of antibodies making them an attractive alternative in affinity chromatography [29], sensing applications [8,14,16,25,27] and also for therapeutic purposes [2]. Furthermore, aptamers exhibit many advantages over antibodies: They are cheap in production, have a high thermal stability, can be produced regarding GMP conditions and offer the potential for regeneration after denaturation [9,13]. Aptamers can be chemically modified at either 3′ or 5′-terminus enabling a covalent immobilization in a controlled orientation on the solid phase. This may prevent loss of aptamer binding activity and aptamer leakage during the purification process [28]. If necessary, it is also possible to introduce molecular spacers at the aptamers' termini to avoid interactions with the solid surface that may interfere with the correct three-dimensional aptamer conformation and in consequence target binding [30]. Aptamer folding is highly influenced by surrounding buffer conditions such as pH, ionic strength or presence of specific ions. Thus, buffer exchange is an appropriate strategy not only for aptamer folding but also for unfolding and therefore target elution as well as aptamer regeneration. Moreover, during the SELEX process the selection conditions can be adapted with respect to the application to obtain aptamers with desirable properties for protein purification [9,29].

In 1999, the application of aptamer affinity purification was firstly reported for an L-selectin-immunoglobulin fusion protein [21]. Over the past few years, similar methods utilizing DNA aptamers have been developed for many other proteins, including *Taq*-polymerase [19], thyroid transcription factor [17], lysozyme [7] and also

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histidine affinity tag [11,31]). Although, since 1994 several aptamers binding to the human vascular endothelial growth factor (VEGF) have been selected [1,4,5,10,18,22], there are no reports about aptamer-based VEGF affinity purification, yet. Instead, heparin is used commonly as affinity ligand for VEGF purification.

In this work the VEGF-binding DNA aptamer V7t1 [18] was covalently coupled to magnetic beads, generating a functionalized affinity matrix phase for VEGF purification (Fig. 1). The aptamer is directed against the receptor-binding domain of the protein. Since different VEGF isoforms share a common receptor binding domain, the aptamer is able to bind the different isoforms which has been demonstrated for $VEGF_{121}$ [18]. While this cross-reactivity might be limiting for diagnostic application of the aptamer, it is not restrictive for the downstream processing of recombinant proteins. Oriented immobilization of the aptamer via 3′-terminal amino groups was chosen for maintenance of molecular flexibility and three-dimensional aptamer folding. Furthermore the aptamer immobilization was optimized with regard to aptamer density and the influence of spacers between aptamer and surface was investigated. The functionalized magnetic beads were utilized in protein binding experiments for characterization of VEGF binding.

## 2. Material and methods

All buffer salts were purchased from Fluka (Switzerland) and Sigma (Germany) and were of per analysis quality. All solutions were prepared with deionized water (ARIUM, Sartorius Stedim Biotech, Germany) and were filtered (0.2 μm) prior to use.

### 2.1. Aptamers

All DNA oligonucleotides were purchased from IDT Integrated DNA Technologies BVBA, Belgium. The following DNA sequences were used:

V7t1: 5′-TGT GGG GGT GGA CGG GCC GGG TAG A-3′

V7t1_14B: 5′-TGT GGG GGT GGA CGG GCC GGG TAG ATA GTA TGT GCA ATC-3′

V7t1_14T: 5′-TGT GGG GGT GGA CGG GCC GGG TAG ATT TTT TTT TTT TTT-3′

V7t1_12EG: 5′-TGT GGG GGT GGA CGG GCC GGG TAG A/dodecaethylene glycol/-3′

Random DNA: 5′-AAA CCG CGT CTC TAC GAC CGG TGC TCG ATT TAA TTT CGC TGA CGT GA-3′

The sequences of VEGF binding aptamers V7t1 and V7t1_14B were taken from literature [18]. V7t1_14B corresponds to the minimal aptamer V7t1 which is 3′-terminal elongated with 14 bases of the originally selected aptamer Vap7. V7t1_14T accords to the V7t1 sequence elongated with 14 thymine nucleotides at 3′-terminus and V7t1_12EG accords to the V7t1 sequence expanded with a dodecaethylene glycol spacer at 3′-terminus. The spacer length of 14B, 14T and 12EG are estimated to be 5.29 nm, 7.28 nm and 4.58 nm, respectively (calculation available in the supporting information). The random DNA oligonucleotide used as negative control is a randomized oligonucleotide sequence with statistical base distribution. Oligonucleotides used for immobilization carry a 3′ terminal amino group, those for electrophoresis mobility shift assay and microscale electrophoresis experiments are labeled with cyanine 3 (Cy3) at the 3′-terminus.

### 2.2. Proteins

Bovine serum albumin (BSA), α-chymotrypsin and myoglobin were obtained from Sigma–Aldrich GmbH, Germany. Human $VEGF_{165}$ was produced as fusion protein with an N-terminal His-tag. Gene sequence coding for the mature VEGF (amino acids 27-191 according to Acc. No.: **AAA35789**) was cloned into pET16b plasmid (Merck KG, Germany) and afterwards brought into *E. coli* BL21(DE3). Cultivation was performed in 2 L baffled shake flasks at 37 °C and 150 rpm using terrific broth medium (12 g/L tryptone, 24 g/L yeast extract, 4 mL/L glycerol, 100 mL/L potassium phosphate buffer) supplemented with 100 μg/mL carbenicillin. For main-cultivation, 500 mL medium were inoculated with *E. coli* cell suspension to a starting $OD_{600}$ of 0.05. At $OD_{600} \approx 0.5$ protein expression was induced with 1 mM IPTG before the culture was grown for at least further four hours at 37 °C. Protocols for isolation and resolubilization of inclusion bodies (IB) were developed on the basis of procedures described in the literature [20,24]). Cells were harvested by centrifugation (6000 g, 4 °C, 15 min), resuspended in lysis buffer (50 mM Tris/HCl, 1 mM EDTA, 1% Triton X-100, 10 mM $MgCl_2$, 10 μg/mL DNase II, pH 8), incubated at 20 °C for 30 min and disrupted by ultra-sonication (Labsonic M, Sartorius AG, Germany; parameters: 3 × 30 s at 70% amplitude and 0.6 s cycle). Lysates

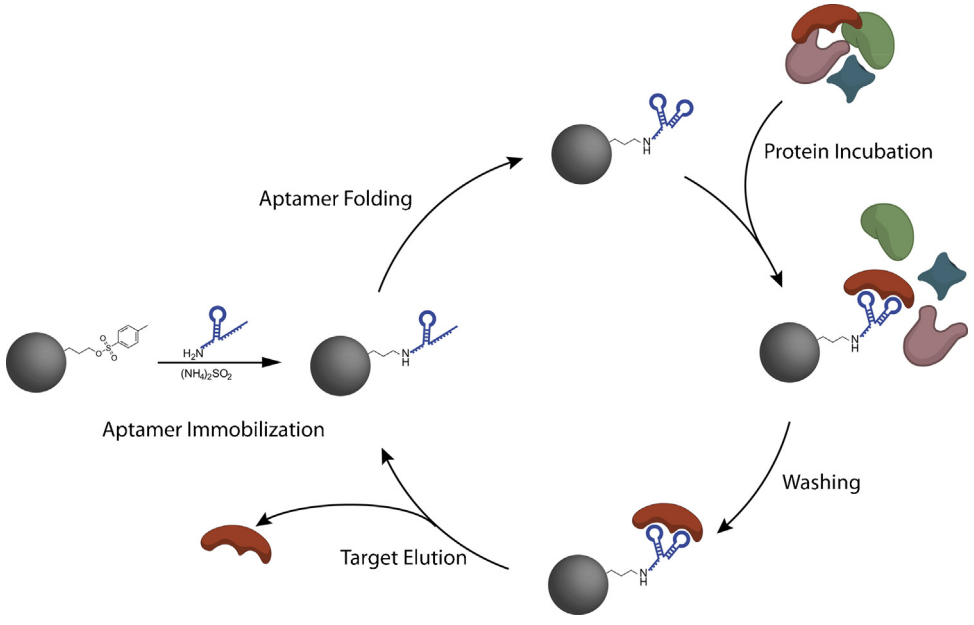


**Fig. 1.** Principle of protein purification process using aptamer-modified magnetic beads.