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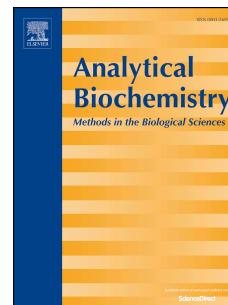
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# 1 Trypsin Enhances Aptamer Screening: A Novel Method for Targeting Proteins

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15 **Abstract:** A novel screening method for protein aptamer selection was developed in  
16 this study. Aptamers with high affinity and specificity to the surface recombinant  
17 antigen of *Helicobacter pylori* (HP-Ag) and to tumor markers carcinoembryonic  
18 antigen (CEA), cancer antigen 125 (CA125) and cancer antigen 19-9 (CA19-9 ) were  
19 screened using trypsin enhanced screening method. Briefly, the target proteins above  
20 were immobilized onto 96-well polystyrene plates and incubated with a  
21 single-stranded DNA (ssDNA) library for aptamer selection. Then, trypsin was  
22 introduced to digest the proteins and obtain ssDNA that bound to the target proteins  
23 with high specificity. The concentration of ssDNA that shed from protein-ssDNA  
24 complexes was detected. After sequencing, the enrichment of target-specific aptamers  
25 was monitored and the affinity of each aptamer was analyzed. Urea, which has been  
26 reported in other article, was used to compare with trypsin. The results revealed that  
27 trypsin was more effective than urea for protein aptamer selection. The protocol used  
28 in this study provided a novel method for generating aptamers.

29

30 **Keywords:** Trypsin, Method, Protein, Aptamer

## 31 1 Introduction

32 Nucleic acid aptamers are functionalized oligonucleotide sequences that can form  
33 three-dimensional structures [1], and they bind to many types of molecules with high  
34 affinity and specificity [2], such as proteins [3, 4], peptides [5], toxins [6], metal ions  
35 [7, 8], and cells [9, 10]. Since they are low molecular weight molecules with strong  
36 specificity and high stability [11], aptamers have been widely used in many fields,  
37 including biosensors [12, 13], biopharmaceuticals [14, 15], and molecular biology and  
38 technology [16, 17], since their discovery in 1900 [1, 18]. Generally, aptamers are

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