# Generation of chemically modified aptamers to capture human C5 complement component in myasthenia gravis treatment

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## OBJECTIVE

**The aim of this study is to develop chemically modified aptamers designed to selectively recognize, bind and remove the C5 complement protein from myasthenia gravis patients' plasma. Pure Biologics' proprietary selection platform PureApta™ was used for the identification of DNA aptamers.**

## INTRODUCTION

Myasthenia gravis is an autoimmune disease caused by disrupted neurotransmission at neuromuscular junctions, characterized by weakness and fatigue of muscles, possibly leading to respiratory failure [1]. Currently available therapies are associated with adverse events, therefore effective treatment remains a clinical challenge. A new class of drugs diminishes the autoimmune response by targeting the complement cascade. Our goal is to develop a novel aptamer-based therapeutic medical device, which will selectively remove the C5 complement component from patients' plasma while leaving other blood components intact and therefore support conventional treatment, especially during myasthenic crisis.

## SELEX PROCESS USING PureApta™ PLATFORM

*In vitro* aptamer selection was performed using Pure Biologics' proprietary modular platform PureApta™. In the SELEX campaign, a chemically modified ssDNA library was used, in which deoxythymidines were replaced with an unnatural hydrophobic uridine derivative (Figure 1). The selection consisted of six rounds with progress monitored via qRT-PCR and ELONA with polyclonal sequence pools. Results after the 4$^{th}$ round indicated sequence enrichment, therefore NGS analysis was applied and five aptamer candidates (MG.S5.1-5) were identified. However, the selection was continued, resulting in the identification of an additional five sequences (MG.S5.6-10).

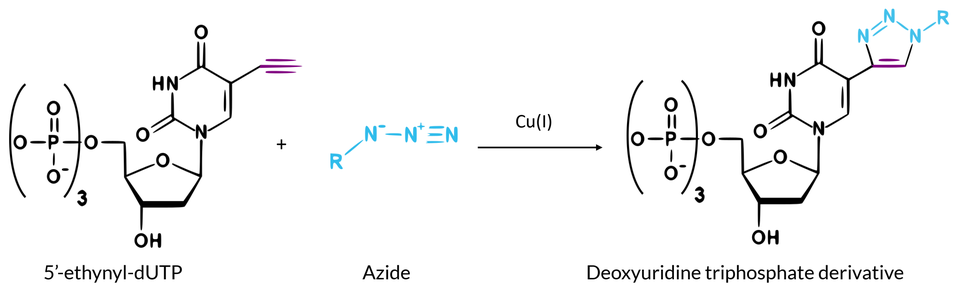


**Figure 1**. Click chemistry reaction method.

## ELONA

Ten sequences demonstrated strong and specific binding capacity towards the molecular target – purified human C5 complement component – and no interactions with another complement protein. Moreover, concentration-dependent signals were observable for all tested clones. Eight aptamers also effectively bind to native C5 protein captured directly from human plasma with a C5-specific monoclonal antibody, immobilized on a plate (Figure 2).

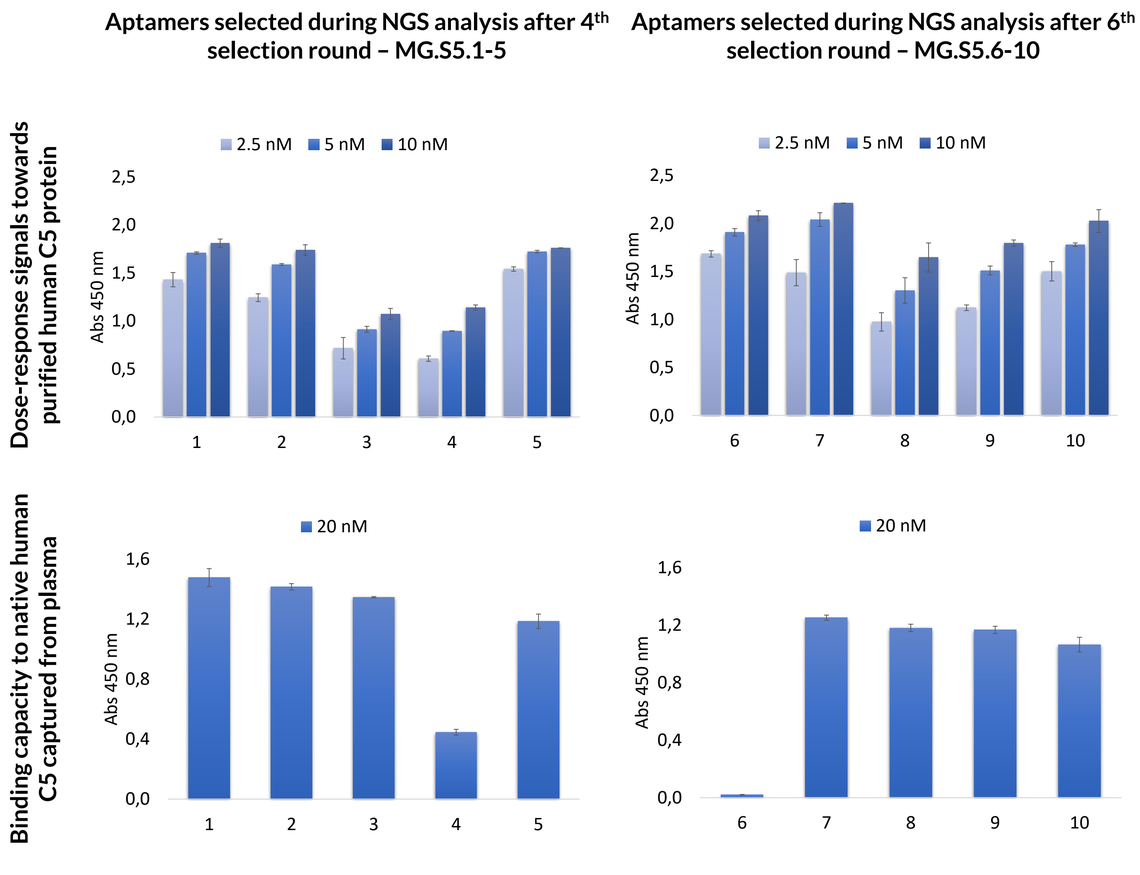


**Figure 2.** ELONA results for MG.S5.1-10 clones.

## BINDING AFFINITY OF MG.S5 APTAMERS FOR THE TARGET

Surface Plasmon Resonance technology (SPR) was used to assess binding between selected clones and their molecular target. It was determined that eight of ten tested sequences specifically bind to human C5 complement component in buffer solution, with $K_D$ values in the picomolar range (Table 1; Figure 3A). Affinity of the best binder, aptamer MG.S5.5, towards human plasma spiked with C5 protein was also confirmed, with a $K_D$ value of 158 pM (Figure 3B).

**Table 1.** Characteristics of aptamers obtained in the SELEX procedure towards the C5 complement protein with a chemically modified ssDNA library.

| Aptamer name | Length [nt] | $K_D$ |
|---|---|---|
| **MG.S5.1** | 76 | 143 pM |
| **MG.S5.2** | | 112 pM |
| **MG.S5.3** | | 612 pM |
| **MG.S5.5** | | 58.5 pM |
| **MG.S5.7** | | 196 pM |
| **MG.S5.8** | | 464 pM |
| **MG.S5.9** | | 154 pM |
| **MG.S5.10** | | 275 pM |

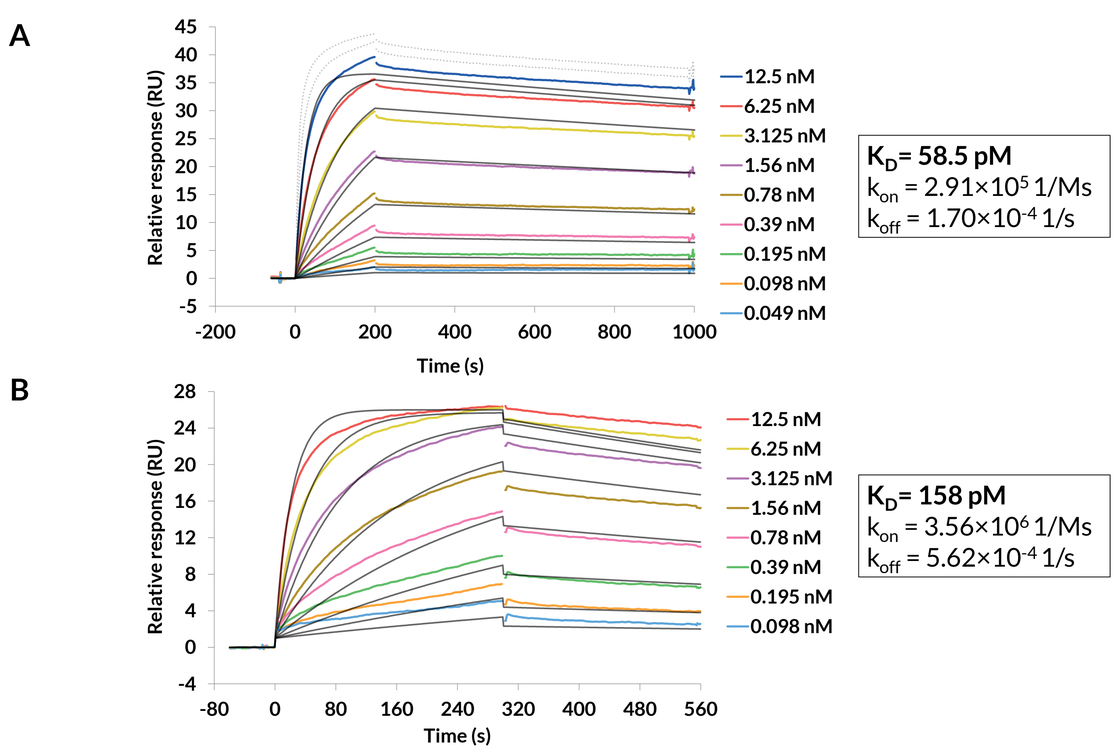


**Figure 3**. Affinity measurements and comparison of SPR results for aptamer MG.S5.5 in **(A)** buffer solution, **(B)** C5-depleted human plasma spiked with external C5 complement component. A 1:1 kinetic binding model was applied in a series of dose-response experiments.

## CONCLUSIONS

Pure Biologics' proprietary platform PureApta™ was used to identify aptamers with high affinity and specificity towards molecular target – the C5 complement component – intended to be removed from plasma to diminish damaging autoimmune response in myasthenia gravis patients. Our results suggest great potential of these molecules in the implementation of a novel aptamer-based therapeutic medical device for the treatment of myasthenia gravis. Leading sequences will undergo an optimization process to obtain aptamers which retain strong and specific binding to the molecular target, as well as have improved stability in human plasma.

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**References**

[1] Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015 Oct;14(10):1023-36.