Design of aptamer conjugates as monoclonal antibody mimics

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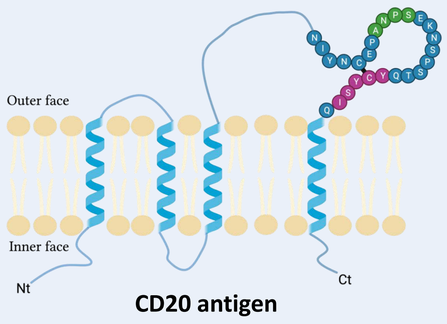
Introduction

- In the late 90's, monoclonal antibodies (mAbs) became a major tool for therapeutic purposes. However, some restrictions provided strong arguments for the development of alternative agents like aptamers that integrate the benefits of mAbs while circumventing these limits.
- We propose to design **small synthetic mimics** of Rituximab, which is used to treat some lymphomas by targeting the CD20 antigen.
- **DNA aptamers** were chosen as recognition elements because of their **low immunogenicity**, their **high affinity** for their target, their chemical **stability** and the fact that their production is carried out by organic synthesis. Among methods used to produce aptamers, CE-SELEX (capillary electrophoresis-systematic evolution of ligands by exponential enrichment) technique was preferred due to its multiple benefits such as less consumption of samples, natural binding environments, and a higher screening efficiency.

CD20 synthesis



CD20 antigen

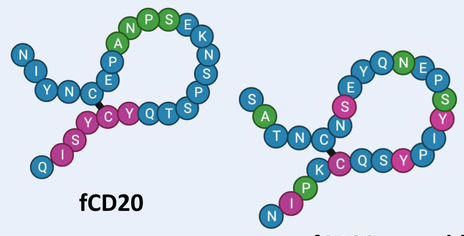
Solid Phase Peptide Synthesis

Nt–NIYNCEPANPSEKNSPSTQYCYSIQ–Ct

Nt–SATNCNSEYQNEPSYIPYSQCKPIN–Ct

- Fragment of the antigen (fCD20) (Mw=2848 g.mol^{-1})
- Scramble peptide as negative control

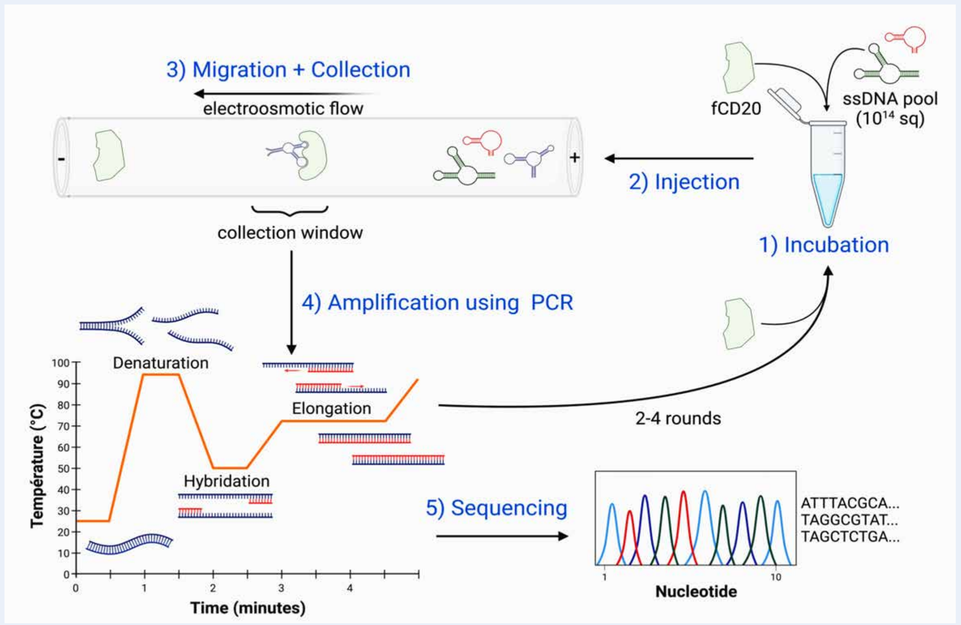
Disulfide bridge formation



fCD20 **fCD20 scramble**

CE-SELEX

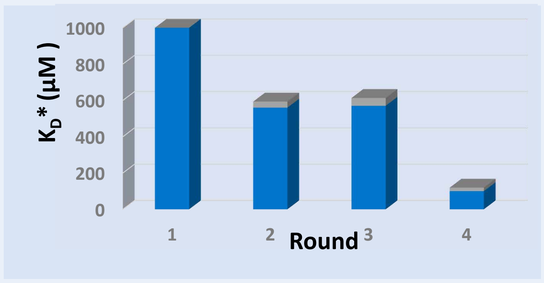
Combination of a **separative method (CE)** and a **selection process (SELEX)**



Schematic representation of the CE-SELEX process

Conditions for CE-SELEX process

Conditions	Round	[fCD20] (µM)	[ADN] (µM)	[fCD20]/[ADN]
TGK Buffer Tris 25 mM, Glycine 192 mM, KH_2PO_4 5 mM, **pH 8.3, 40 min**	**1**	1000	30	33.3
	2	0.5	0.17	3
	3	0.3	1	0.3
	4	0.03	1	0.03



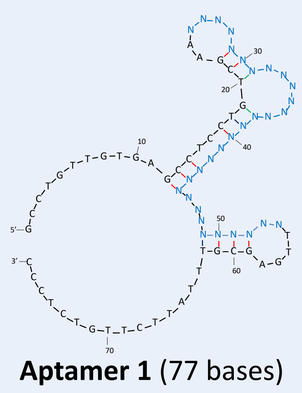
* Estimated dissociation constants (K_D) were calculated on one point from the decrease of the unbound DNA fractions

Dissociation constant (K_D) evaluated after each round of CE-SELEX

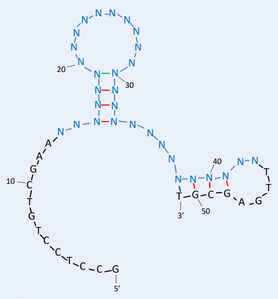
After sequencing, **two aptamers were selected** through CE-LIF analyses

Fragmentation from 2D structures

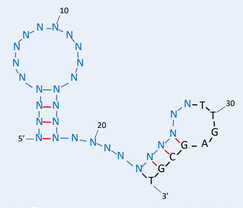
- **Secondary structures for Aptamer 1 and 2** simulated using mFold.
- **Three fragments** were designed.



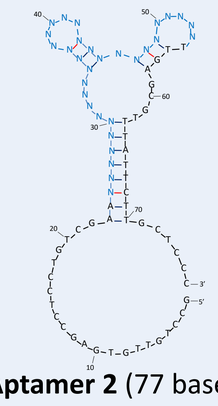
Aptamer 1 (77 bases)



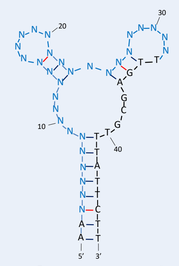
A1-fragment 1 (51 bases)



A1-fragment 2 (36 bases)



Aptamer 2 (77 bases)



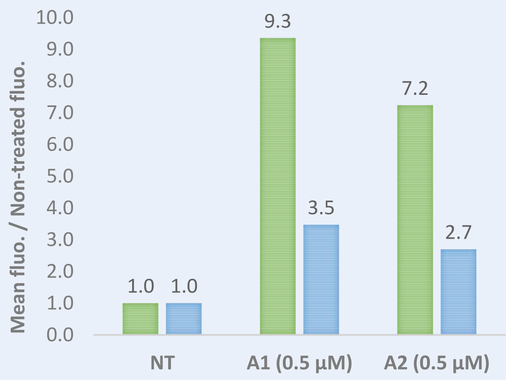
A2-fragment (48 bases)

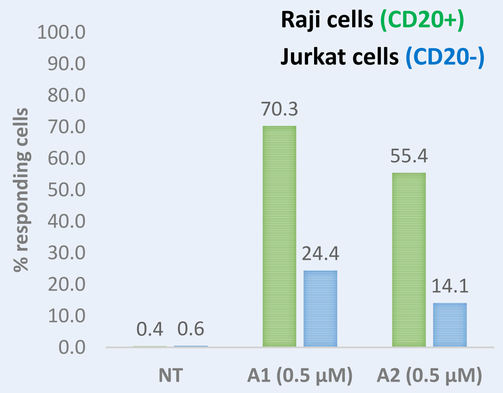
Isothermal Titration Calorimetry

	fCD20			fCD20 scramble		
Analyte	K_D (µM)	N (sites)	c	K_D (µM)	N (sites)	c
Apta 1	6.4 ± 0.9	0.86 ± 0.01	20.1	N/D	N/D	N/D
Apta 2	1.2 ± 0.2	1.49 ± 0.01	194	N/D	N/D	N/D
Apta 1-F1	9.0 ± 2.4	0.88 ± 0.02	14.7	> mM	N/D	N/D
Apta 1-F2	18.9 ± 3.3	1.07 ± 0.03	8.5	> mM	N/D	N/D
Apta 2-F	5.5 ± 1.3	1.46 ± 0.03	39.8	> mM	N/D	N/D

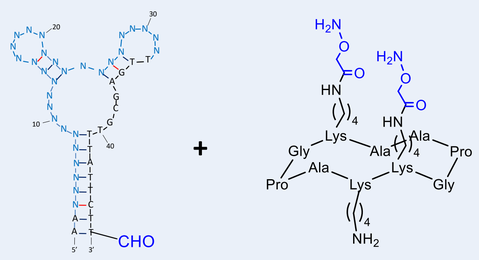
- The shrinking of the length led to a slight decrease of the affinity.
- The specificity remains high for the fCD20.

Flow cytometry

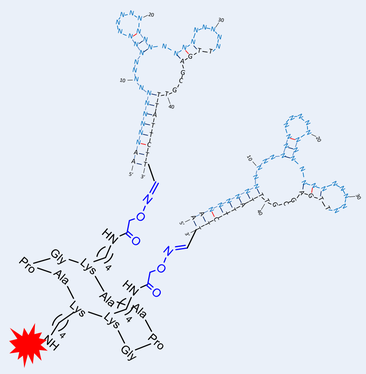




Mimic Design



To improve the affinity of the selected compounds, multivalent systems will be designed using cyclopeptides as scaffolds. Up to 4 aptamers and a fluorophore or cytotoxic agent will be then grafted through chemical ligations.



***In vitro, in vivo* tests**

Summary and Outlook

- The first part of this project enabled us to select two aptamers with a suitable affinity (1-5 µM) and a high selectivity for the CD20 antigen.
- From their 2D structures, three fragments were designed and characterized. Similar affinities were found with high selectivity.
- *In cellulo* tests using flow cytometry were performed with the full aptamers showing promising results.
- In parallel, we started the synthesis of dimers using peptide scaffolds to assess multivalent effects.

Reference

J. Cossu, C. Ravelet, V. Martel-Frachet, E. Peyrin, D. Boturyn, Bioorganic & Medicinal Chemistry (**2024**)
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