# Design and characterization of monoclonal antibody peptide mimics

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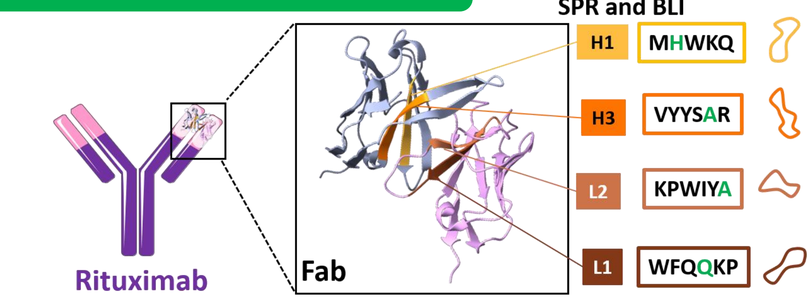
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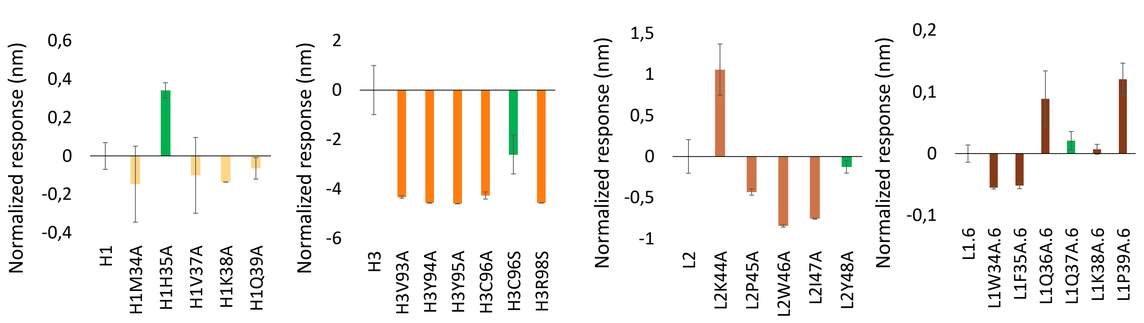
## Introduction

Since their introduction in the 1990s, **monoclonal antibodies** (mAbs) have emerged as **essential therapeutic agents**, although their clinical use can be limited by factors such as restricted tissue penetration and immunogenicity. To address these challenges, we are developing **peptide-based mimics of Rituximab** designed to target the CD20 antigen, a key marker in immunotherapy. These mimics consist of **selected peptide sequences** assembled onto a **multivalent scaffold** to enhance target recognition. Their binding to CD20 is characterized using sensitive biophysical techniques, including **BLI** and **SPR**, while their specificity is further assessed through **biological assays**.

## Study of RTX/CD20 interaction




- **Identification of key residues** involved in the RTX/CD20 interaction by ***ala-scan***




***Ala-scan* results obtained on a BLI biosensor.** Normalized responses were calculated by subtracting the $R_{max}$ of the *wt* peptide. $R_{max}$ values were determined using the Steady State model.

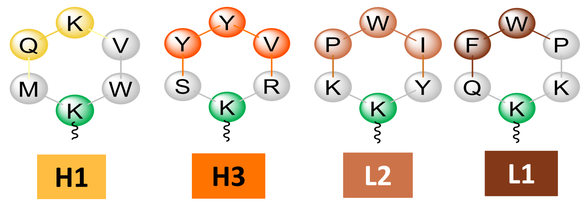
- **Aromatic** (W, F, Y) and **basic** residues (K, R) are critical for binding

| | Essential amino acids |
|---|---|
| Heavy chain | $K^{38}$, $V^{93}$, $Y^{94}$, $Y^{95}$, $C^{96}$, $R^{98}$ |
| Light chain | $W^{34}$, $F^{35}$, $P^{45}$, $W^{46}$, $I^{47}$, $Y^{48}$ |

## Design of RTX mimics

**Recognition Elements:**

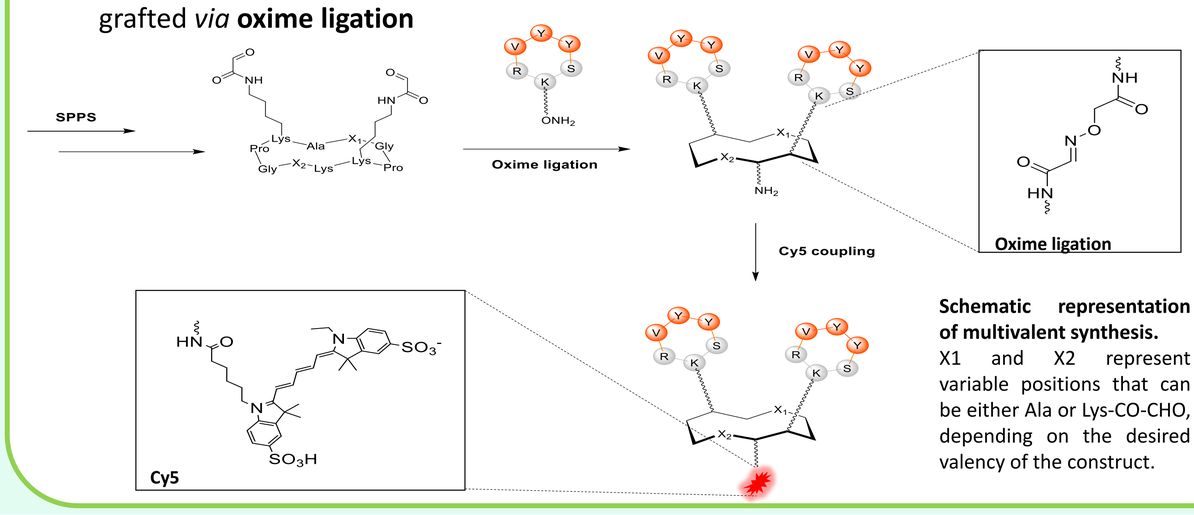
- **Selected cyclohexapeptides** synthesized *via* **SPPS**
- **Non-essential residue** were replaced by a **modified lysine for anchoring** to multivalent scaffolds




**Recognition peptide derived from the RTX paratope, engineered for grafting onto a peptidic scaffold**. Modified lysines are shown in green. Other colored amino acids highlight the most critical side chains for CD20 binding.

**Multivalent Constructs:**

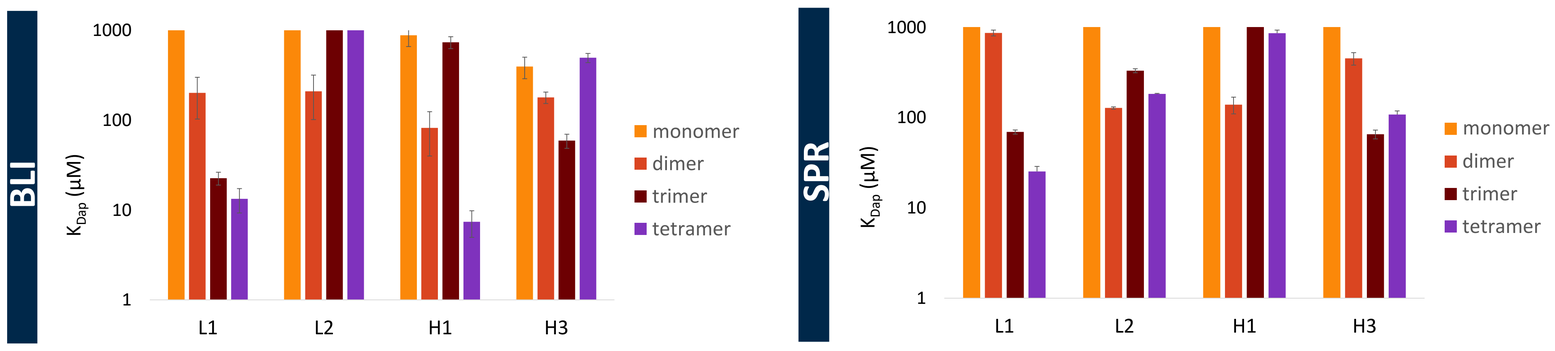
- **Decapeptide scaffolds** synthesized by **SPPS**
- Cyclopeptides mimicking RTX paratope were grafted *via* **oxime ligation**
- Multivalent mimics contain **2 to 4 copies of the same motif**




**Schematic representation of multivalent synthesis.** X1 and X2 represent variable positions that can be either Ala or Lys-CO-CHO, depending on the desired valency of the construct.

## Evaluation of RTX mimics *in vitro*

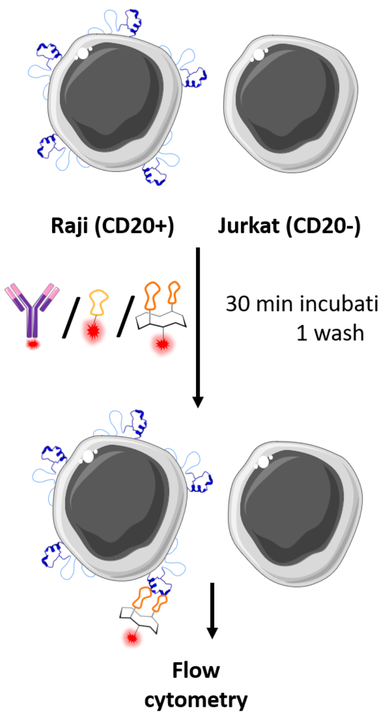
- **BLI** and **SPR** assays on biomimetic surfaces developed in the laboratory [1,2]
- **Dimerization** significantly **increased** binding affinity (statistical rebinding)
- **L1** displayed a **synergic effect** upon multimerization
- Tri- and tetrameric forms of L2, H1, and H3 exhibited limited improvement, likely due to intramolecular constraints



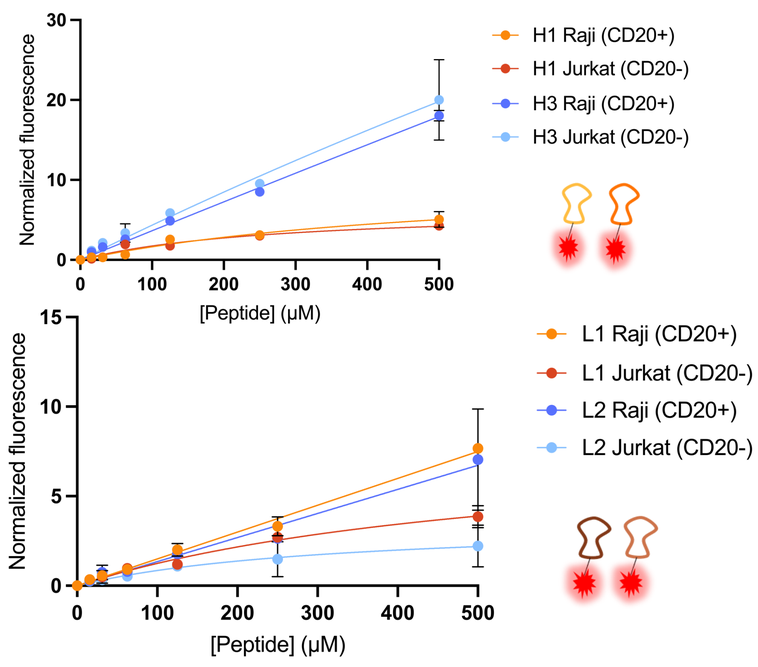

**Evaluation of mimic affinity by Biolayer Interferometry and Surface Plasmon Resonance**. The $K_{Dapp}$ were determined *via* the Steady State (SS) model. n = 3

## Evaluation of RTX mimics *in cellulo*

- **Flow cytometry performed** on Raji (CD20+) and Jurkat (CD20-) cell lines



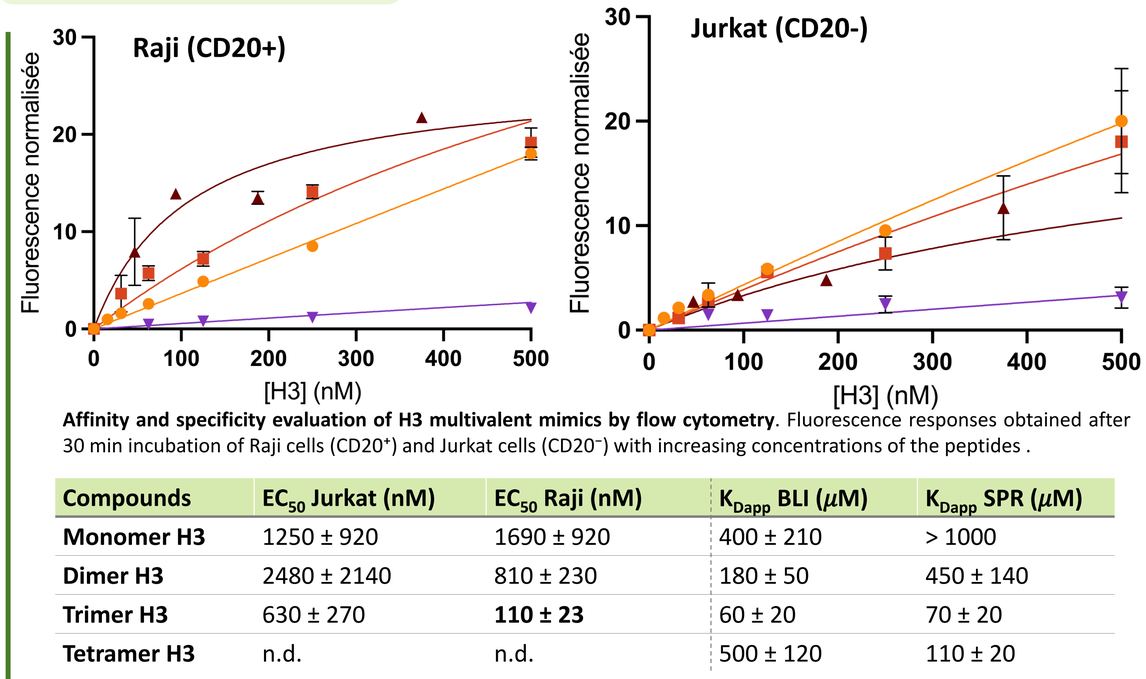

### Monomers




**Evaluation of monomer affinity and specificity by flow cytometry**. Fluorescence responses obtained after 30 min incubation of Raji cells (CD20+) and Jurkat cells (CD20-) with increasing concentrations of the peptides .

- The **H3** cyclopeptide exhibits the **strongest response**
- H1 and H3 display non-specific binding
- **L1** and **L2** show **specificity toward CD20**

### H3 multivalent mimics




**Affinity and specificity evaluation of H3 multivalent mimics by flow cytometry**. Fluorescence responses obtained after 30 min incubation of Raji cells ($CD20^+$) and Jurkat cells ($CD20^-$) with increasing concentrations of the peptides .

| Compounds | $EC_{50}$ Jurkat (nM) | $EC_{50}$ Raji (nM) | $K_{Dapp}$ BLI (µM) | $K_{Dapp}$ SPR (µM) |
|---|---|---|---|---|
| **Monomer H3** | 1250 ± 920 | 1690 ± 920 | 400 ± 210 | > 1000 |
| **Dimer H3** | 2480 ± 2140 | 810 ± 230 | 180 ± 50 | 450 ± 140 |
| **Trimer H3** | 630 ± 270 | **110 ± 23** | 60 ± 20 | 70 ± 20 |
| **Tetramer H3** | n.d. | n.d. | 500 ± 120 | 110 ± 20 |

**Comparison of cellular interaction parameters ($EC_{50}$) derived from flow cytometry experiments and apparent dissociation constants ($K_{Dapp}$) obtained by BLI and SPR**. $EC_{50}$ values were determined using a specific binding model (n = 2), whereas $K_{Dapp}$ values were determined using the Steady-State (SS) model (n = 3).

- **Similar responses** observed by **BLI** and **SPR** experiments
- Monomer and tetramer show non-specific binding
- **Specific binding** observed with the **dimer**, and particularly with the **trimer**
- **Multimerization improves binding affinity and specificity**

## Conclusion

1. **Rational identification of Rituximab paratope mimics :** four **key peptide sequences** were identified through an **Ala-scan**, highlighting the critical contribution of **some residues** to **CD20 binding**.
2. **Multimerization** resulted in a **significant increase in binding affinity**. It also **improved CD20 specificity especially for H3**, as demonstrated by **flow cytometry**.
3. **Promising cell specificity :** the **L1** and **L2** mimics showed **selective binding to CD20+ cells (Raji)**, with minimal interaction with CD20- cells (Jurkat), validated by **flow cytometry.**

## Nexts steps

- **Perform biological assays on the L1 series to confirm the synergistic effect.**
- **Develop a Dynamic Combinatorial Library (DCL)** to identify the best-performing **heteromultimers**.

## References

1. L. Bar et al, *Anal. Chem.* **2020**, *92*, 5396 – 5403.
2. O. Ricloux et al, *Anal. Chem.* **2026**, *98*, 19418 – 19426.