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Design and characterization of CD20 antigen surfaces for the selection of rituximab peptide mimics

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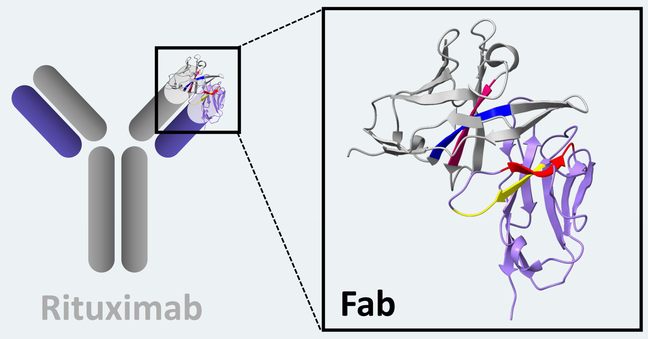
Introduction

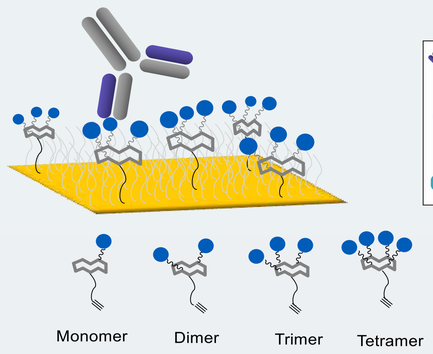
Since the 90s, **monoclonal antibodies** (mAb) have emerged as a promising class of pharmaceuticals and were successfully used for cancer therapy. However, several **limitations** related to the nature of mAbs such as their cost, their low tissue penetration and immunogenicity limits their extensive clinical use. So, **new technological solutions**, as small organic mAb mimics, have to be explored. In this context, we are interested in the **development of mAb mimics** that recognize the CD20 antigen, which is expressed on B cells and is a key target for several cancer and autoimmune diseases. In this context, the **mAb Rituximab (RTX) that target CD20**, is routinely used to treat some Lymphoma. Herein, we propose to **design macromolecular compounds comprising cyclopeptides selected from the RTX Fab as recognition elements for CD20** in combination with a detection element and/or a cytotoxic unit for therapeutic applications. For this pupose, we **developed and characterized biomimetic surfaces** to mimic the surface of B cells in order **to screen and select the RTX mimics** before moving on to cell-based assays.



Development of antigenic surfaces for SPR

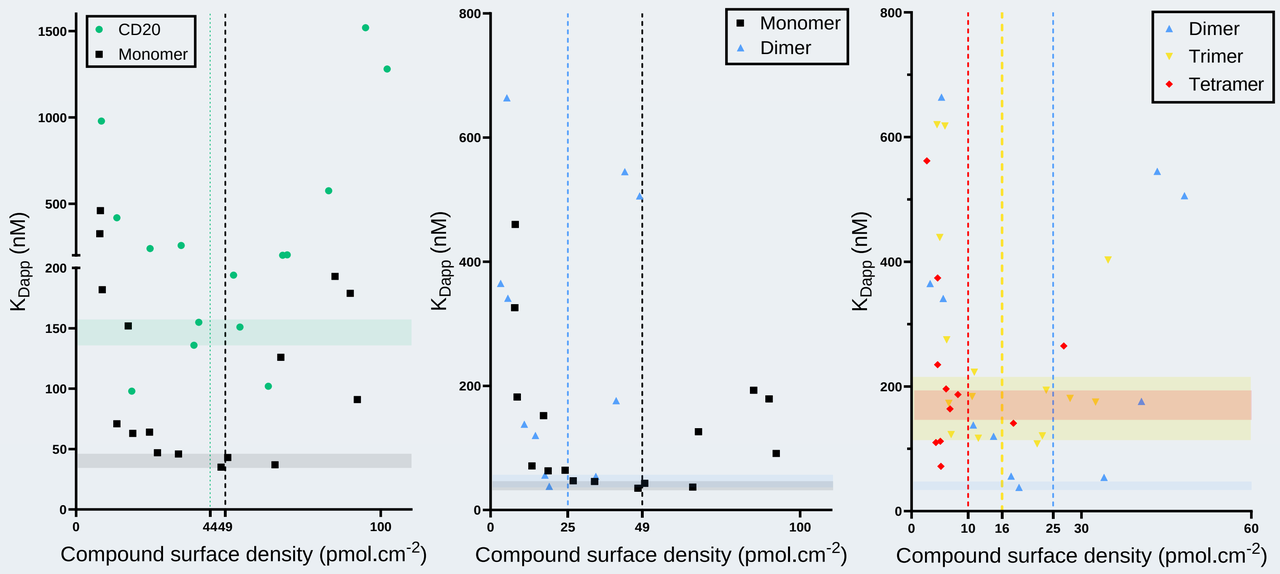
- The **biomimetic surfaces** were **characterize by Spectroscopic ellipsometry** (SE) coupled with **quartz microbalance** (QCM-D)

Grafting by **CuAAC** on a Self-Assembled Monolayer (SAM) of alcane thiols (N_3 or OH)
Antigen density is controlled by the N_3/OH ratio



Compound	Surface density SE (pmol.cm^{-2})	Surface density QCM-D (pmol.cm^{-2})	Hydration (%)
Monomer *	37 ± 1	32 ± 6	66 ± 5
Dimer **	41 ± 4	32 ± 11	47 ± 13

Characterization of CD20 surfaces by SE coupled to QCM-D. SE areal densities were determined by *De Feijter* equation and QCM-D areal densities were extracted from *Sauerbrey* equation. * n = 2, ** n = 3



Impact of epitope surface density and clustering on RTX/CD20 affinity in SPR. K_{Dapp} were determined via the Heterogeneous Ligand (HL) model by the k_{off}/k_{on} ratio and areal densities by *Jung*'s formula.

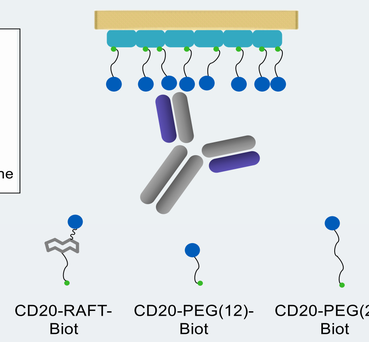
Affinity of rituximab against CD20 at optimal surface density in SPR. K_{Dapp} were determined *via* the Heterogeneous Ligand (HL) model by the k_{off}/k_{on} ratio and areal densities by *Jung*'s formula. For the determination of inter-ligand distances, the projected surface of the compounds is considered circular. [a] n = 3, [b] n = 4

Compound	Surface density (pmol.cm^{-2})	K_{Dapp} * (nM)	Inter-ligand spacing (nm)
CD20 [a]	44 ± 8	147 ± 10	2.2 ± 0.2
Monomer [b]	49 ± 13	40 ± 5	2.1 ± 0.3
Dimer [a]	25 ± 11	49 ± 10	3.1 ± 0.6
Trimer [b]	16 ± 6	165 ± 50	3.8 ± 0.7
Tetramer [b]	10 ± 6	172 ± 25	5.0 ± 1.1

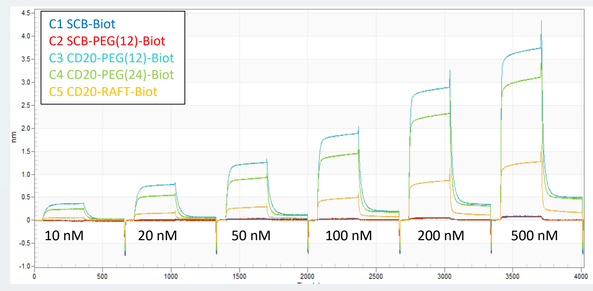
* K_D values ***in vitro*** **: K_{Dapp} ≈ 5 - 19 nM**

Development of biosensors for BLI

Grafting by **Streptavidin/Biotin** interaction
Antigen density is controlled by the time of grafting



- **BioLayer Interferometry** (BLI) **biosensors** to **screen** RTX sequences to design the RTX mimics.



Compound	Loading response max (nm)	K_{Dapp} (nM)
SCB-Biot	0.8 ± 0.1	> mM
SCB-PEG(12)-Biot	1.3 ± 0.1	> mM
CD20-Biot	1.1 ± 0.1	> mM
CD20-PEG(12)-Biot	1.5 ± 0,1	250 ± 20
CD20-PEG(24)-Biot	1.5 ± 0.1	320 ± 10
CD20-RAFT-Biot	0.9 ± 0.1	560 ± 20

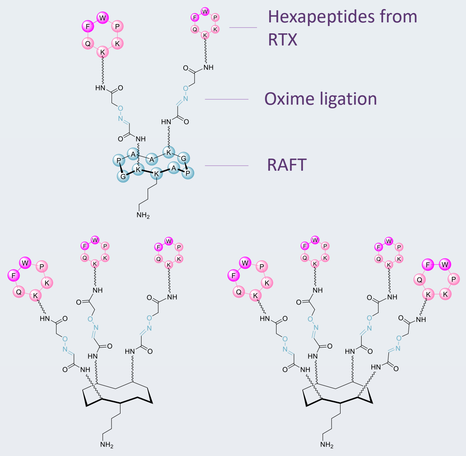
Impact of linker length on RTX/CD20 affinity in BLI. K_{Dapp} were determined *via* the Heterogeneous Ligand (HL) model by the k_{off}/k_{on} ratio.

Design and Evaluation of RTX mimics

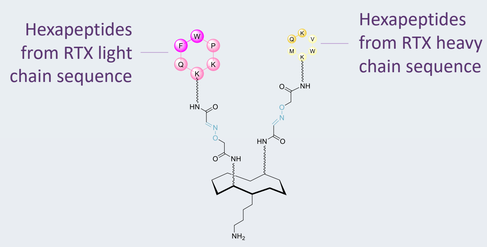
❖ RTX peptide mimic design

Two types of RTX mimics :
- **multivalent mimics** displaying 2 to 4 times the same cyclopeptide from RTX consensus sequence
- **Bispecific mimics** displaying 2 different cyclopeptides, one from the heavy chain and one from the light chain sequences.

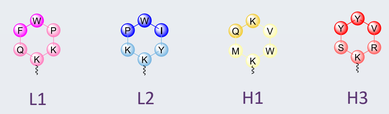
Multivalent RTX mimics



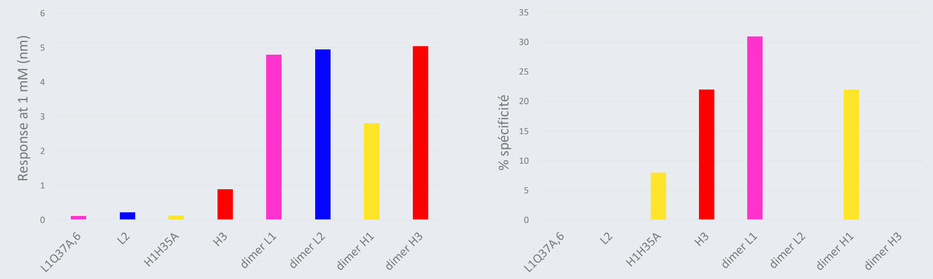
Bispecific RTX mimics



Hexapeptides from RTX containing a modified ketoaldehyde-lysine to carry out oxime ligation:



❖ Affinity of RTX peptide mimics for CD20

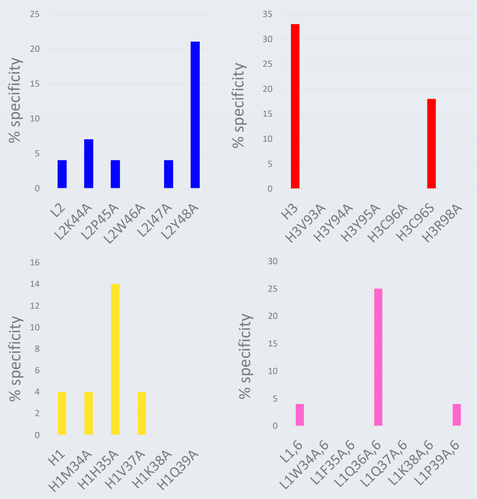


Impact of dimerization of RTX cyclopeptides on CD20 recognition by BLI model. The specificity percentage was determined by comparison with the "CD20-scramble" response at a concentration of 1 mM.

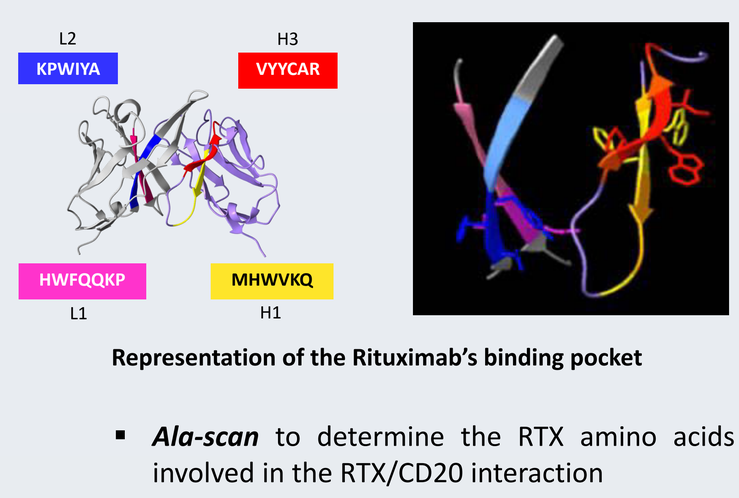
- **Dimerization** induces an **increase in response**
- **Dimerization** can **improve specificity** for the target

Study of RTX/CD20 interaction

❖ Amino acid significance of Rituximab-derived peptide sequences *via Ala-scan*



Ala-scan **peptide specificity results obtained on the BLI model.** The specificity percentage was determined by comparison with the "CD20-scramble" response at a concentration of 1 µM.



Representation of the Rituximab's binding pocket

- ***Ala-scan*** to determine the RTX amino acids involved in the RTX/CD20 interaction
- Presence of a **binding pocket** in RTX with two interaction areas

Conclusion & Outlooks

❖ Conclusion

- Design of antigenic surfaces with high specificity and affinity for its antibody
- Design of BLI biosensors for peptide screening
- Determination of the amino acids from RTX binding pocket involved in the interaction
- Design and synthesis of RTX mimics

❖ And now ?

- Evaluation of the affinity and the specificity of RTX mimics by BLI and SPR
- Biological assays with the hits

References

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