Clustered antigen architectures enhance monoclonal antibody adhesion: insights from biomimetic surfaces

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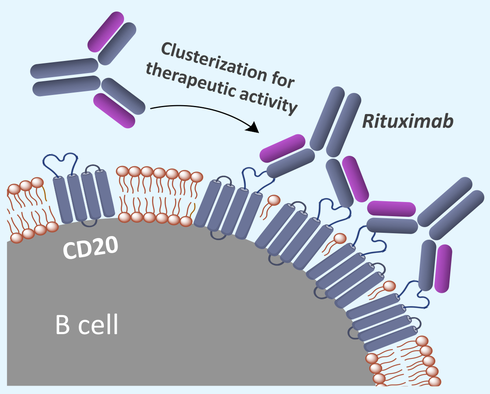
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Introduction

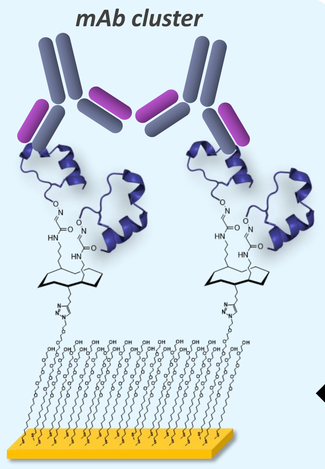
Since the 90s, **monoclonal antibodies** (mAb) have emerged as a promising class of pharmaceuticals. **Understanding antigen–antibody interactions is important to many applications** including diagnostics and treatment of diseases. The **CD20 antigen expressed by B cells has received significant attention** as it is a target for immunotherapy of B cell-related cancers but also autoimmune diseases. The CD20 antigen is the target of several therapeutic mAbs including rituximab. It is well-known that antigen-binding activity of mAbs, and associated biological efficacy, depends on several factors such as antigen density, association, and dissociation rates. It was shown that the **levels of CD20 expression at the cell surface may determine antibody-mediated cell death**.[1] We and others reported an optimal inter-CD20 spacing of nearly 2 nm on surfaces for efficient interaction with RTX.[2-4] To systematically study the effect of CD20 density on antibody recognition**, we designed self-assembled monolayer (SAM) functionalized gold surfaces that display tunable CD20 epitope densities**.[2-5] Quartz crystal microbalance with dissipation monitoring (QCM-D) and surface plasmon resonance (SPR) techniques were chosen to analyze the binding of the mAb rituximab.

Design of clustered CD20 surfaces

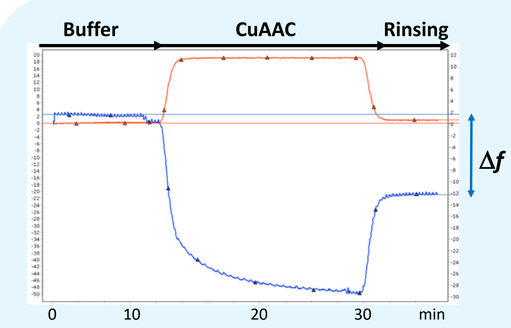


Biological context: CD20 antigen forms clusters at the cell surface upon interaction with mAbs which is essential for therapeutic activity

Highly biomimetic surface

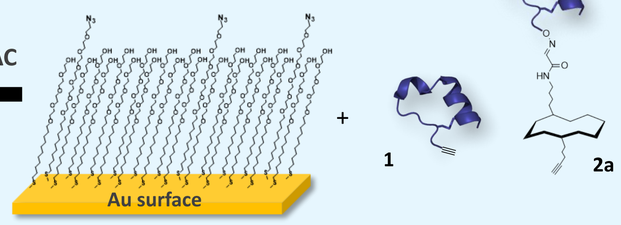


Characterizing Biomolecular Interactions *via* Label-Free QCM-D and SPR

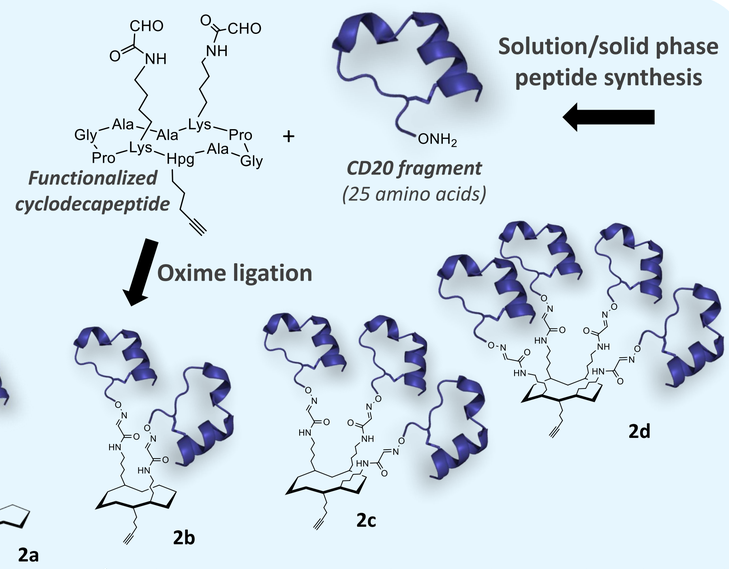


Real-time QCM-D monitoring of conjugate **2b** immobilization on alkane-thiol SAM

CuAAC



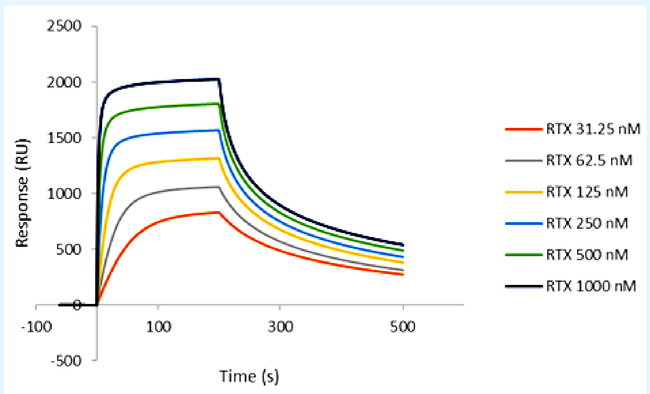
✓ **Determination of CD20 density** by using the Sauerbrey equation: $m_{QCM} = -C\Delta f$ and additional spectroscopic ellipsometry experiments (*data not shown*).



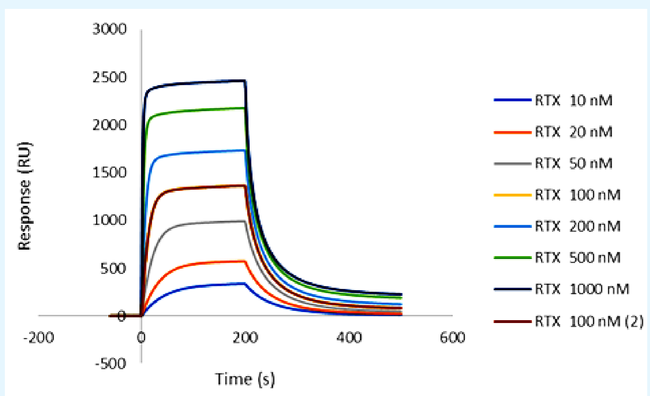
✓ **Cyclodecapepides were functionalized with orthogonal chemoselective functions**. Aldehyde functions were introduced for grafting the CD20 epitope, while alkyne functions allowed surface attachment through copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC).

Impact of clustered CD20 on RTX adhesion

➢ SPR sensorgrams recorded on surfaces functionalized with CD20 derivatives

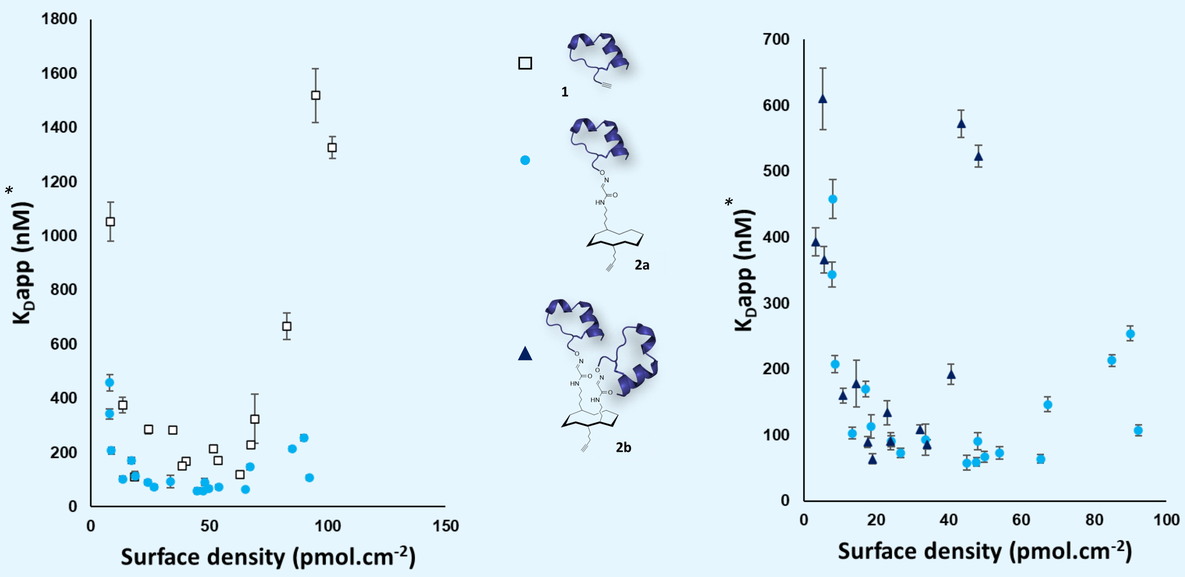


SPR binding profiles of 2a with RTX. Sensorgram was recorded on a surface functionalized with **CD20 monomer 2a** at 26.3 pmol.cm^{-2}. The RTX concentrations were 31.25, 62.5, 125, 250, 500, 1000 nM.



SPR binding profiles of 2b with RTX. Sensorgram was recorded on a surface functionalized with **CD20 dimer 2b** at 37.5 pmol.cm^{-2}. The RTX concentrations were 10, 20, 50, 100, 200, 500, 1000 nM.

➢ Impact of epitope surface density and clustering on RTX/CD20 affinity



** K_{Dapp} values were determined using a steady-state model based on the equilibrium response of the sensorgrams*

✓ The presence of **the cyclodecapeptide scaffold in monomer 2a enhances RTX recognition** at comparable surface densities: 60 nM compared to ~150 nM for surfaces modified respectively with **2a** or **1** at 50 pmol·cm^{-2}.

✓ Introducing the cyclodecapeptide spacer improves epitope accessibility and promotes antibody binding.

✓ Surfaces functionalized with **CD20 monomer 2a and dimer 2b exhibited similar minimum K_{Dapp} values**, with slightly enhanced affinity for the dimer at their respective optimal surface densities (50 and 20 pmol·cm^{-2}).

✓ The minimum K_{Dapp} values for trimer **2c** and tetramer **2d** (~120 nM) were higher than that of the dimer. This reduced affinity is the result of increased steric hindrance and conformational rigidity imposed by packing multiple CD20 units onto a single cyclodecapeptide scaffold.

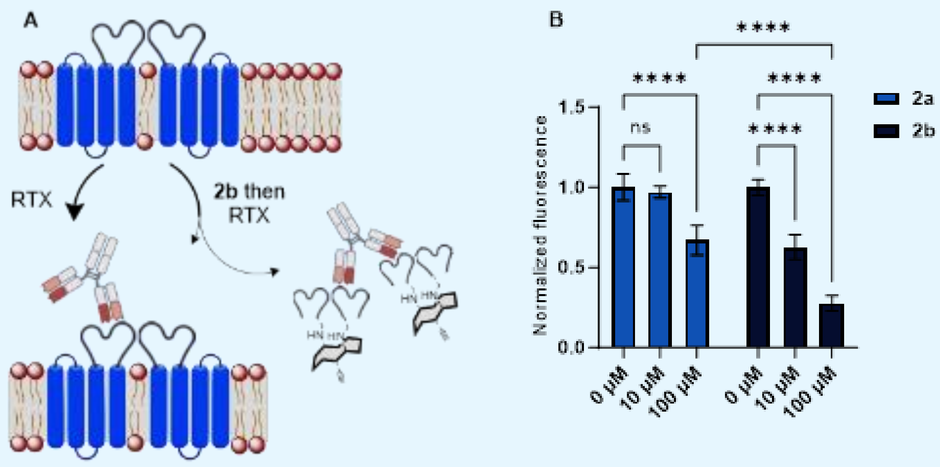
➢ Apparent dissociation constants of anti-CD20 mAbs on mono- and dimeric CD20-functionalized surfaces

mAb	Compound 2a (K_{Dapp} nM)	Compound 2b (K_{Dapp} nM)
RTX	86 ± 16	45 ± 3
MEM-97 **	41 ± 6	311 ± 19
L26 ***	ND	ND

*** MEM-97 binds a different extracellular epitope from RTX*
**** L26 recognizes an intracellular epitope and was used as a negative control*

✓ **Formation of a more favorable interaction between RTX and CD20 dimer 2b**, which faithfully mimics the prerequisite 2 nm distance between two CD20 proteins at the cell surface.

➢ Inhibition of cell–antibody interaction with multivalent CD20 constructs



(A) Schematic of the competition assay between Raji cells and synthetic CD20 dimer **2b**. (B) Flow cytometry analysis of competition between Raji cells and synthetic CD20 dimer **2b**. Raji cells were co-incubated for 30 min at 4 °C with increasing concentrations of **2b** and RTX, followed by incubation with fluorescent secondary antibody for 30 min at 4 °C.

*Statistical significance was assessed using a two-way ANOVA test (comparison across concentrations). ****: $p < 0.0001$ and ns: $p > 0.123$*

✓ **The results indicate enhanced competition for dimeric constructs 2a**, supporting their relevance to CD20 organization and binding stoichiometry at the cell surface.

✓ The synthesized CD20 constructs appear to be **promising candidates for the affinity-based selection of RTX mimics**.

Conclusion & Outlooks

❖ Conclusion

- We designed and characterized **biomimetic surfaces** displaying clustered multivalent CD20 architectures based on cyclodecapeptide scaffolds.
- We demonstrated that both the **spatial organization and valency of CD20** epitopes strongly **influence RTX binding affinity and specificity**.
- Dimeric CD20 constructs offers the best compromise between epitope accessibility and multivalent interaction.

❖ And now ?

- Design of **clustered CD20 on lipid bilayer surface**.
- Impact of the lateral mobility on rituximab adhesion.
- **Screening of mAb mimics** on CD20 biomimetic surfaces.

References

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