Design of a Soluble *de novo* CD20 Antigen for Therapeutic Antibody Discovery

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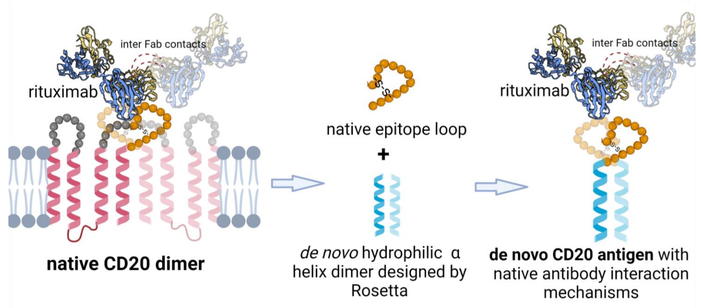
Introduction

CD20 is a target for leukemia and non-Hodgkin lymphoma. It is a transmembrane cellular protein with hydrophobic helix structure. However, due to the insolubility of this protein, the native CD20 binding structure with therapeutic antibodies is challenging to study. We used Rosetta, a computational protein design tool, to engineer a *de novo* antigen which maintains the native antigen binding features while altering features to increase the stability and solubility to make it compatible for use in antibody development.

In this study, we validated the expression and binding of the *de novo* antigen with anti-CD20 Fragment antigen-binding antibodies (Fabs) and the ability to express the *de novo* antigen in bacterial cells. We expressed the *de novo* antigen in Expi293F mammalian cells and E. Coli. The *de novo* CD20 antigen has good expression and a high yield (around 33 mg/L). We also demonstrated that it had a reasonable binding affinity with three different Fabs. The bacterial expression had a lower yield although there was in fact an expression.

De novo CD20 Antigen Design:

We used Rosetta, a computational software, to design a water-soluble *de novo* CD20 antigen.



Hypothesis

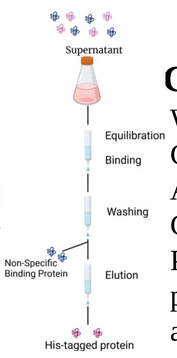
We are looking to validate the binding and stability of the *de novo* antigen, using anti-CD20 fragment antigen-binding (Fab) antibodies. We are also looking to validate a bacterial expression of the *de novo* CD20. We are hypothesizing that our *de novo* protein is able to bind to commonly used CD20 antibodies and has an affinity for them. We also hypothesize that the *de novo* CD20 antigen can be expressed in E. Coli.

Methods


Antibody


Fab

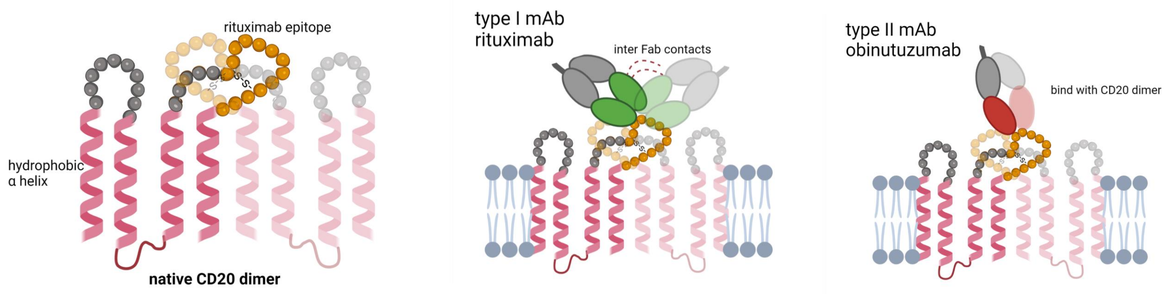
CD20 Fab antibodies were used to validate *de novo* antigen binding affinity. Fabs are composed of one constant and one variable domain of each of the heavy and the light chain. Fabs are beneficial because they penetrate tissues more efficiently due to their smaller size.

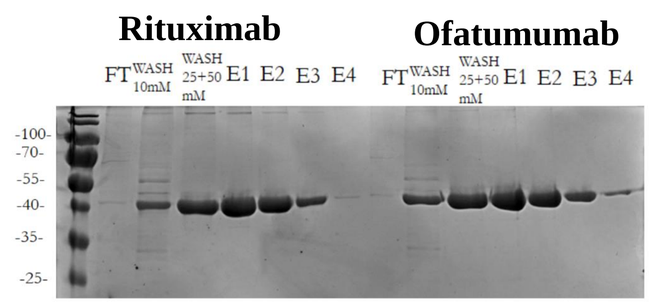


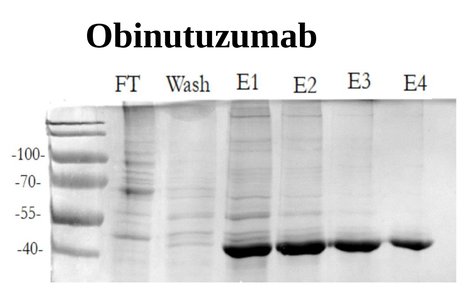
Cell Culture:
We used Nickel-Column Affinity Chromatography Purification to purify the Fabs and the Bacteria.

Results

Antibody Binding to Native CD20:



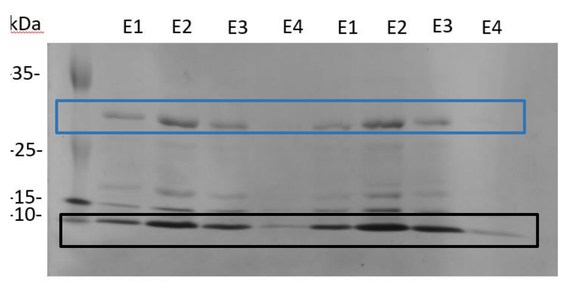




SDS-PAGE gels of the three Fabs that were used after they had been purified. Expression of all three of the Fabs was achieved by a process of optimization by varying elements such as time and the amount of heavy chain and light chain that was added.

Bacterial Expression:

Non-Reducing Gel. Black box indicates CD20 protein, Blue box indicates unknown band.

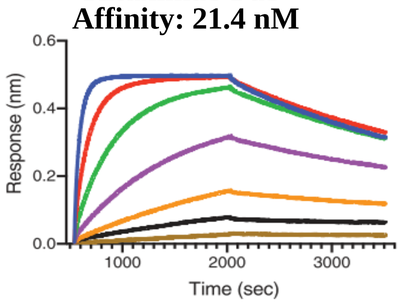


Reducing Gel. Black box indicates CD20 protein, Blue box indicates unknown band.

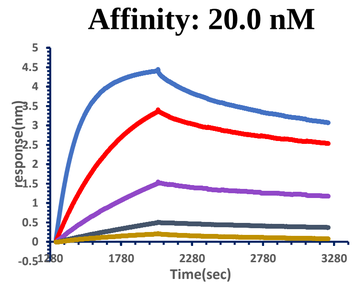
SDS-PAGE gels of the Bacterial Expression after the protein has been purified. The purification of the protein was achieved by using Nickel-Column Affinity Chromatography. This expression produced a lower protein yield than the mammalian cell expression and displayed unknown bands.

De novo CD20 Binding Affinity:

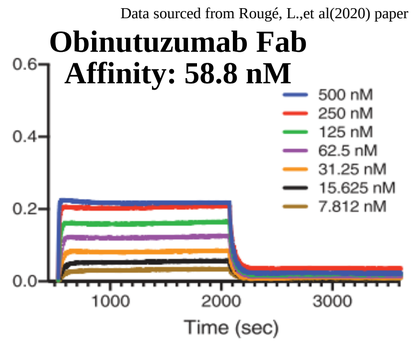
Native CD20:

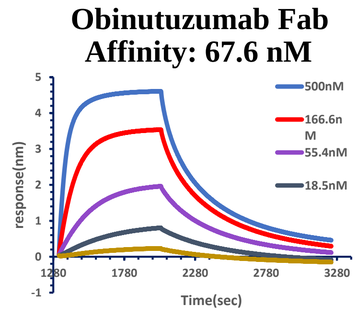


De Novo CD20:



Data sourced from Rougé, L.,et al(2020) paper





Biolayer Interferometry (BLI) was used to measure the binding affinity of the *de novo* CD20 and CD20 antibody Fabs. The *de novo* CD20 antigen had a similar or better affinity for the fab than the native CD20.

Expression and Yield:

Construct	Native CD20	De novo CD20	De novo CD20
Expression host	Insect cell	Expi293	E.Coli shuffleT7
Yield	0.5 mg/L	33 mg/L	2.9 mg/L

Conclusions & Future Directions

Our designed de novo CD20 antigen was successfully expressed in mammalian cells and E. Coli. The mammalian cells have a good binding affinity with CD20 antibodies. We were also able to validate the experimental viability of the engineered *de novo* antigen.

We are currently trying to produce a structure of the *de novo* antigen and the Fab antibody bound complex and to gain a further understanding of the CD20 binding mechanism to further validate the experimental viability of the engineered *de novo* antigen. The use of the soluble CD20 antigen can lead to the creation of more advanced and efficacious cancer therapeutic antibodies.

References

Rougé, L.,et al(2020). Science, 367(6483), 1224–1230
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Susa, K. J., et al(2021). Science, 371, 300−305
Images created with BioRender.com

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