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In silico engineering towards enhancement of Anti-CD20 nanobody binding affinity

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Motivation and Aim: CD20 is a membrane non-glycosylated protein (33-37 kDa) that expressed on the surface of normal and malignant B lymphocytes, and encoded by *MS4A1* Gene that is located on 11q12.2 (1). The efficacy of the anti-CD20 monoclonal Antibodies (like rituximab), in treating B-cell disorders such as cancer and autoimmunity diseases has been remarkable. Despite the clinical potential, became major detriments to their efficacy their immunogenicity and large size (∼150 kDa). The discovery of heavy-chain only antibodies (HcAbs) in camelids was amazing. HcAbs consist of just two heavy chains, with a single variable domain (VHH) that is as known the antigen-binding region. VHHs or nanobodies (Nbs) could retain full antigen binding potential upon isolation, establishing them as the smallest, naturally-derived antigen binding fragment (2). The unique properties of Nbs are small size (∼15kDa), affinity (in the nanomolar or picomolar range), high thermal and chemical stability, fast tissue penetration and rapid blood clearance, Recognition of hidden epitopes and sequences shared with human VH. Different approaches have been described for the enhancement binding affinity of Nbs. One of the ways to increase the efficiency of nanobodies is to use the Site-Directed Mutagenesis method.

Methods and Algorithms: in this study is used site-directed mutagenesis coupled with docking simulations for anti-CD20 nanobody to investigate how specific amino acid substitutions impact ligand-protein interaction. We created an anti-CD20 Nanobody with CDR grafting method and its modeling was performed by SWISS MODEL server. Then we introduced a point mutation on 101 residue of the nanobody that Tyrosine was converted to Arginine. In addition, we mutated the Tyrosine (non-polar hydrophilic aa) 101 and 107 to Arginine (polar hydrophilic aa) in the CDR3 region, the most involved region in binding to CD20, using PyMOL software and docked their to the CD20 using the HADDOCK program. PyMOL was also used to evaluate the amino acids involved in the interaction.

Results: By analyzing the structures, connectivity features were improved. The best structure designed in HADDOCK was selected based on the HADDOCK Score and energy level between normal and mutated nanobodies and the electrostatic and van der Waals energies between the CD20 and the nanobodies were calculated. The amount of van der Waals energy decreased (–47.1 (Normal) into –46.1(Y101R) and –38.9 (Y101.107R)) and the number of amino acids involved in the connection (Fig. 1–3) and the amount of electrostatic energy increased (–119.2 (Normal) into –180.3 (Y101R) and –187.3 (Y101.107R)).

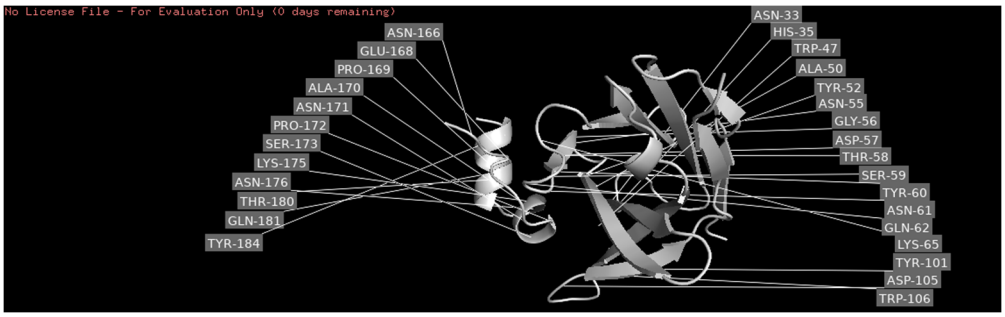

Fig. 1. Normal nanobody residues interaction

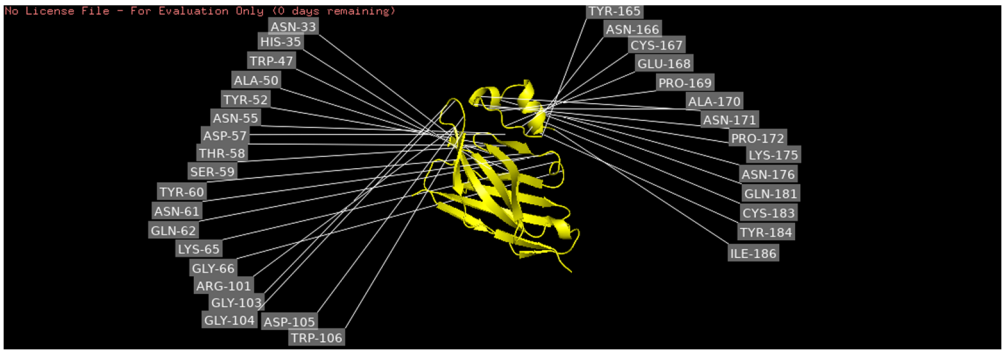

Fig. 2. Mutated nanobody (Y101R) residues interaction

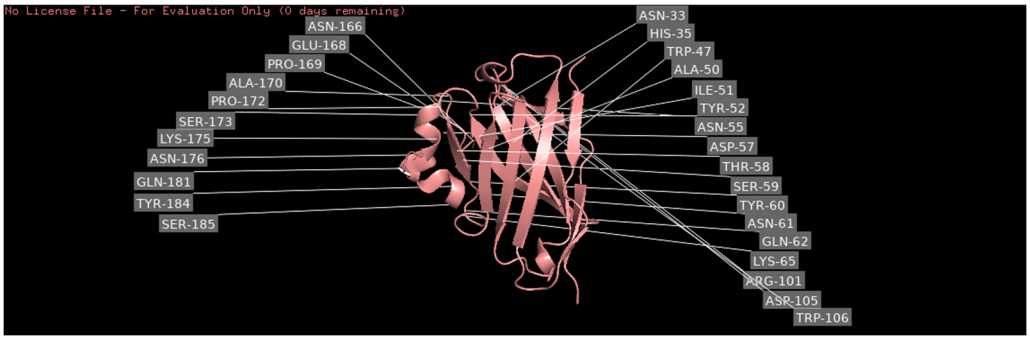

Fig. 3. Mutated nanobody (Y101.107R) residues interaction

Conclusion: Collectively, results obtained suggest that the binding of CD20 to the ligand (nanobody) was improved by converting non-polar hydrophilic amino acids into polar hydrophilic amino acids. Base of our results, this mutations increased the solubility and specificity, therefore, improved the binding affinity. However, further investigation is essential to clarify this binding.

References

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