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Evaluation of the effect of site-directed mutagenesis on the affinity of anti-PD1 nanobody by *in silico* modeling

Mirzaei M.[1], Mirhoseini S.[1], Heidari M.M.[1], Khatami M.[1], Orlov Y.L.[2, 3]

[1] *Department of Biology, Faculty of Sciences, Yazd University, Yazd, Iran*

[2] *Institute of Digital Medicine I.M. Sechenov First Moscow State Medical University, Moscow, Russia*

[3] *Novosibirsk State University, Novosibirsk, Russia*

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Motivation and Aim: Variable domain of heavy chain (VHH) or single domain antibody, also called nanobody (Nb), is the smallest fragment of an antibody (15 kDa) that can bind to antigen [1]. Nbs consist of three complementary determining regions (CDRs) and four framework regions that separate CDRs. CDRs are regions that bind to the antigen [2]. Low immunogenicity, high stability and solubility are some advantages of Nbs. Also, they are well expressed in microorganisms. Nbs have many applications, especially in medicine, which is utilized to diagnose and treat diseases [3,4]. A method for the production of Nb is grafting CDRs from non-camelid antibodies to Nbs frameworks [5]. Programmable cell death 1 (PD-1) is an immune checkpoint protein on T cells that plays a key role in modulating immune responses. The interaction of PD-1 with its ligands (PD-L1 and PD-L2) induces common inhibitory signals which leads to the reduction of the activity of T cells. PD-L1 and PD-L2 have increased expression in tumor cells, enabling tumor cells to escape T cell-induced immune responses [6]. Blocking the interaction of PD-1 with its ligands by anti-PD1 Nbs can increase the activity of T cells, which is effective in treating cancer. The type of amino acids at the site of antigen and CDR interaction is important. CDR3 is a significant site of antigen binding. Substitution of the neutral or negative amino acid with a positive amino acid in CDR3 is one way to improve the affinity of CDR3 to the target antigen [7, 8].

Methods and Algorithms: In this study, CDR grafting method was utilized to produce anti-PD1 Nb. SWISS-MODEL server (http://swissmodel.expasy.org) was used for modeling the three-dimensional structure of anti-PD1 Nb, PD1 antigen and mutated anti-PD1 Nbs. Pymol software was applied to determine the Nb-antigen binding site. Then, HADDOCK server was used to investigate the interaction between Nbs and antigen. HADDOCK requires the atomic structures of each of the subunits of the complex in the PDB format which were obtained from the SWISS-MODEL. The HADDOCK score is a weighted sum of buried surface area and various energies, including van der Waals, electrostatic, desolvation, and restraint violation energy. Electrostatic and van der Waals forces play an important role in the interaction between two proteins. The electrostatic force represents the bond created between two charged amino acids of proteins. The van der Waals force is less powerful, but it is especially important because of the large number of links. The more negative score of these two energies and the more positive score of the buried surface area indicates the better affinity of the Nb.

Results: HADDOCK results showed that some of the mutated Nbs improved the affinity (Table 1). Evaluation of optimization strategies for the affinity of Nb indicated that conversion of three tyrosines of CDR3 to arginine improved affinity (Fig. 1–4).

Table 1. Comparison of affinity of Nbs according to HADDOCK

	Van der Waals energy	Electrostatic energy	Buried Surface Area
Anti-PD1 Nb	-44.7 +/- 2.8	-89.7 +/- 32.1	1249.6 +/- 98.4
Mutated Nb (1)	-45.1 +/- 0.6	-102.5 +/- 15.7	1298.9 +/- 126.2
Mutated Nb (2)	-45.8 +/- 3.6	-112.5 +/- 13.8	1388.6 +/- 86.0
Mutated Nb (3)	-52.6 +/- 4.5	-123.9 +/- 6.9	1434.9 +/- 65.6

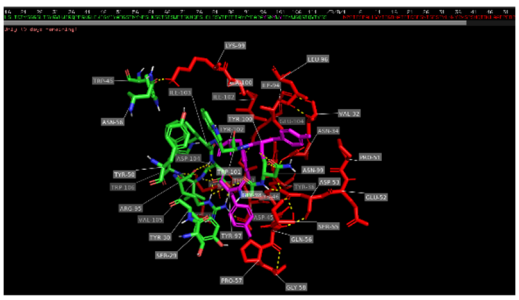

Fig. 1. Overview of the interaction of anti-PD1 Nb and it's antigen in Pymol software

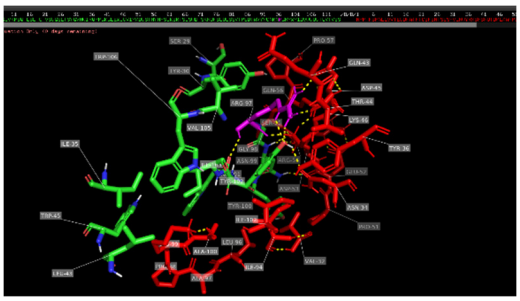

Fig. 2. Overview of the interaction of mutated anti-PD1 Nb (1) and it's antigen in Pymol software

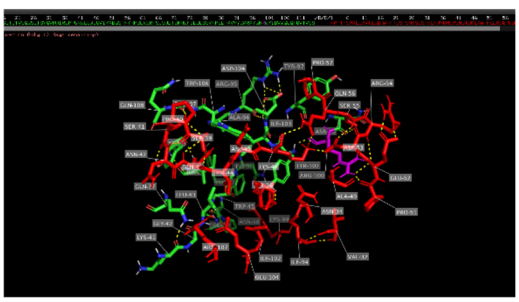

Fig. 3. Overview of the interaction of mutated anti-PD1 Nb (2) and it's antigen in Pymol software

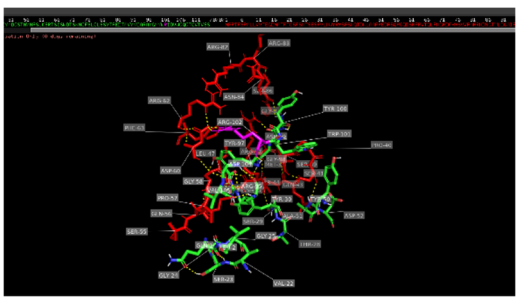

Fig. 4. Overview of the interaction of mutated anti-PD1 Nb (3) and it's antigen in Pymol software

Conclusion: Nbs have many advantages and applications, especially in medicine. Optimization strategies provide the possibility of improvement of the Nbs. This study indicates that site-directed mutagenesis can improve the affinity and function of Nbs.

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