Computational Design of Bispecific Antibodies Targeting CD3 and VEGFA Receptors Enhances T-cell Mediated Cancer Therapy

SHRINJOY CHAUDHURI, GAURAV SHARMA

Monta Vista High School, Cupertino, CA, North Carolina, Apex, NC

Published September, 2025

DuoBody antibodies are a type of bispecific antibody designed to target two different antigens or epitopes. These antibodies can simultaneously bind to two targets and enhance CAR T-cell efficacy and specificity. We hypothesize that the bispecific DuoBody antibody can induce potent T-cell-mediated killing of malignant B cells by simultaneously targeting the CD3 receptor on T cells and VEGFA receptors on malignant B cells. The research aims to identify the most promising bispecific antibodies for inducing T-cell-mediated killing of malignant B cells. In this current work, we conducted simulations to design bispecific antibodies targeting CD3 and VEGFA receptors on the surface of CAR T-cell and cancer cells, respectively. AlphaFold 3 was used for the structural elucidation of CD3 and VEGFA receptors, while the antibody structures were sourced from the Protein Data Bank. Molecular docking simulations were performed to investigate receptor-antibody interactions. A total of 30 simulations were conducted, involving 15 antibodies for each receptor. The antibodies were screened based on binding site prediction using a graph neural network, binding energy calculations, and the number of hydrogen bonds formed between the receptor-antibody complexes. The results demonstrated that the antibodies bind to the specific region of the receptors, as predicted by the graph neural network. Further interaction analysis pinpointed the 1IL1 antibody as the most effective for the CD3 receptor and 2HRP antibodies for the VEGFA receptor. The designed DuoBody antibody targeting B-cell malignancies holds promise for treating complex diseases by improving specificity and efficacy while minimizing off-target effects.

1. INTRODUCTION

A duo-body antibody is a bispecific antibody that simultaneously binds to two antigens or epitopes. (1) This dual-binding nature allows duo-body antibodies to bring two separate targets close together, creating novel treatment approaches. (1) For instance, in cancer therapy, a duo-body antibody may bind to the antigen of a tumor cell with one arm and the antigen of an immune cell with the other. (1) This successfully connects the immune and tumor cells, facilitating immune-mediated killing.(2) Producing duo body antibodies often uses recombinant DNA technology, combining two different antigen-binding fragments (Fabs) into a single antibody molecule.(2) This revolutionary method can enhance the efficiency and precision of medical procedures for several diseases, such as autoimmune disorders and cancer.(2) Figure 1 shows the schematic of the bispecific antibodies. This figure outlines three steps: Firstly, the bispecific DuoBody antibody enters the site of action and has two antigen binding sites specially designed to latch on to the receptors on both the effector and cancer cells. In step two, after the antibody successfully binds to both cells, the effector cell can release granzymes and perforins to destroy the cancer cell. Finally, the cancer cell dies.

Molecular docking is a computational process used to determine which orientation a molecule (usually a small molecule or ligand) would prefer when it attaches to another molecule (usually a protein or enzyme) to create a stable complex.(3) This simulation of how they interact with target proteins is crucial in identifying possible drug candidates during drug discovery and testing.(3) During the docking procedure, algorithms that estimate the molecules' binding affinity test many poses, orientations, and configurations.(3) The concluding information helps to understand binding processes and optimize ligand chemical structure for better effectiveness and selectivity.(4)

The CD3 receptor is a group of proteins on the surface of T cells and is essential to immune response.(5) It is a part of the T-cell receptor (TCR) complex, which includes the CD3γ, CD3δ, CD3ε, and CD3ζ chains.(6) This receptor complex is vital since T cell activation and signal transmission depend on the receptor.(6) After the TCR identifies an antigen, the CD3 complex sends activation signals to the T Cell, causing it to multiply, differentiate, and launch immune functions.(5) because of its critical function in regulating T cell activity, the CD3 receptor is a crucial target

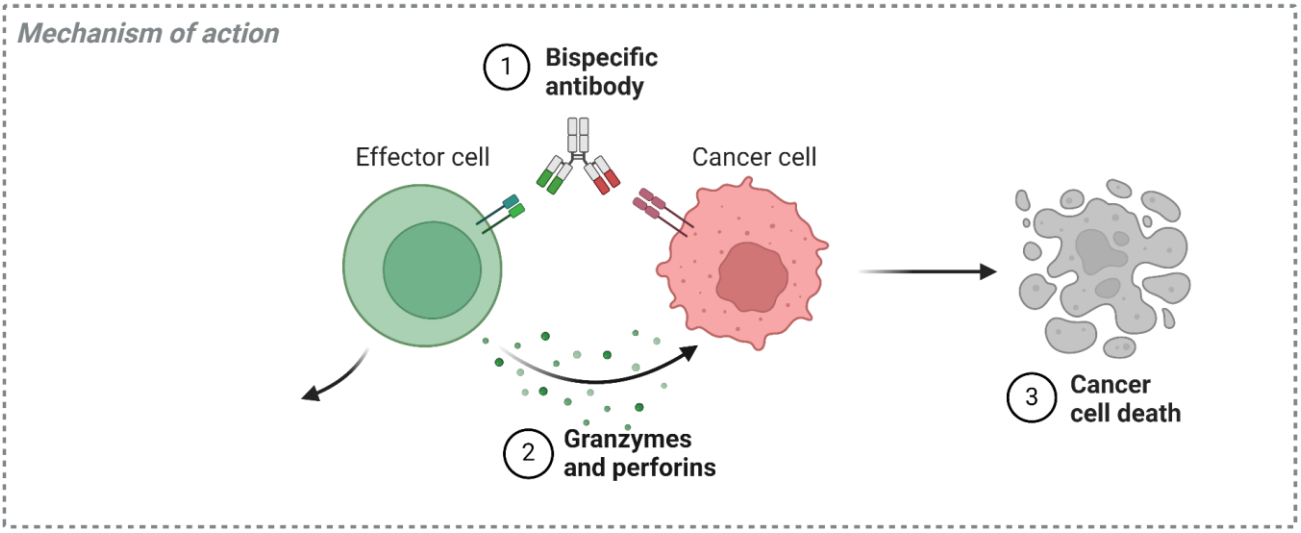

Fig. 1. Function of a bispecific antibody in connecting the effector and the cancer cell to expedite cancer cell death. The figure was created on biorender.com.

for numerous immunotherapies, including monoclonal antibodies used to treat cancer and autoimmune illnesses.(5) CD20 is a transmembrane protein found on the surface of B cells from the early pre-B cell stage to maturity, but it is not found on plasma cells (pro-B cells). This protein works as a calcium channel and is essential for controlling B cell activation, multiplication, and differentiation. Since CD20 is only expressed in B cells, it is a perfect candidate for monoclonal antibody treatments for treating autoimmune diseases like rheumatoid arthritis and B cell malignancies like non-Hodgkin lymphoma and chronic lymphocytic leukemia. Therapeutic antibodies that target CD20 function, like rituximab, work by attaching to the CD20 antigen on B cells, leading to their death through processes such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and triggering apoptosis.

We hypothesize that the variable region of the antibody binds to the specific region of CD3 and VEGFA receptors that trigger the stronger bispecific binding. We have performed computational simulations in the current research to find duo body antibodies binding to CD3 and VEGFA receptors. Variable regions of multiple antibodies were docked on these receptors to find the best pair of variable regions. Later, these variable regions were combined using AlphaFold 3 to get the duo body structures. Docking simulations predicted combining 1LK3 and 2HRP variable regions is the best binder for CD3 and VEGFA receptors, respectively. The present study will help in designing novel duo-body antibodies against various cancers.

2. METHOD

For this investigation, the receptors CD3 and VEGFA were used for simulation. The receptor amino acid sequences were obtained from the UniProt website and uploaded on the AlphaFold 3.(7) Using AlphaFold3, their three-dimensional (3D) structures were predicted.(8) GrASP was used to indicate the binding sites for the receptors to find possible interaction locations.(9) The HDOCK software was used to conduct molecular docking simulations to investigate the interactions between the receptors and putative binding partners. (10) The PRODIGY web server was used to calculate the binding energy of receptor-antibody interactions. (11) Fifteen antibody variable sections were docked onto the VEGFA and CD3 receptors to determine which combination would best build the DuoBody. The best antibody variable regions found from the docking studies were incorporated into the design of the duo body antibody utilizing AlphaFold3. The 3D structure of the receptor-antibody complexes was visualized using the ChimeraX software. (12)

3. RESULTS

In this research, we have used computational modeling techniques to identify how DuoBody Antibodies can be used to show the binding process to VEGFA receptors and CD3 receptors on cancer cells and T-cells, respectively. AlphaFold 3 was used to obtain the 3D structure of CD3 and VEGFA receptors. AlphaFold 3 is a machine learning system developed by Google's Deepmind to predict the structure of proteins and nucleic acids from their amino acids and bases, respectively. The binding site of both receptors was obtained using the GrASP web server shown in Figure 2. GrASP is a web server that uses the Graph-based residue neighborhood strategy to predict binding sites on the surface of a protein. Using the ChimeraX software, a molecular visualization program, the electrostatic surface potential (ESP) was computed, Figure 2. The electrostatic surface potential of the CD3 molecule was primarily negative, and the electrostatic surface potential of the VEGFA molecule was mainly positive.

Molecular docking simulations using the HDOCK software were performed to understand the binding interactions between the receptor and antibody. The docked structures are displayed in Figure 3. The antibodies were selected based on the following criteria: the first step was using the ChimeraX software to visually inspect the receptor-antibody complexes. The criteria were that the binding of the antibody should match the predicted binding site, which we found using the GrASP web server. The VEGFA molecules that fit this criterion were 2XQB, 1A5F, 1IL1, 1IQW, and 2HRP. The CD3 molecules that fit this criterion were 1B4J, 1LK3, 2VXS, 2XQB, 3D85, and 3G6D. The next step was to take these molecules, which satisfy the first criteria, and calculate the number of hydrogen bonds between the antibody and receptor using an in-house Python script. The antibodies are arranged in decreasing order of hydrogen bonds. The hydrogen bonds are shown in Figure 4. For VEGFA, the satisfactory molecules are 2HRP, 1IL1, 2XQB, 1A5F, AND 1IQW. For CD3, the satisfactory molecules are 1B4J, 1LK3, 2VXS, 2XQB, 3D85, and 3G6D. The last criterion was to take all these molecules and calculate the binding energy. The molecules with the strongest binding energy were the best. For this criterion, the satisfactory molecules for VEGFA were 2HRP and 2XQB. The CD3 molecules that were satisfactory were 1B4J and 1LK3. In molecular systems, the binding energy is often reported in kilojoules per mole (kJ/mol). Negative binding energy values indicate a stable system, as energy would be required to break the bonds within the molecule.

4. DISCUSSION

DuoBody antibodies are used in various biomedical research and therapy areas, such as treating cancer, autoimmune diseases, infectious diseases, and central nervous system diseases.(1) They are widely applied in cancer treatment to simultaneously target two different antigens, improving the specificity and efficiency of immunotherapies.(2) In autoimmune diseases, the antibodies can modulate two pathways simultaneously to reduce inflammation.(2) Similarly, in infectious diseases, antibodies target multiple viral or bacterial epitopes, enhancing pathogen neutralization.(2) They are also explored in central nervous system diseases like Alzheimer's and Parkinson's, where they can simultaneously target neurotoxic proteins like beta-amyloid and tau.(2,13,14)

Limitations: The current research will help target the CD3 receptors on T-cells and VEGFA receptors on cancer cells, directing the T cell to kill the cancer cell. One limitation of the current research is the need for more experimental validation. In future research, we will perform lab experiments to compute the binding energy, including surface plasmon resonance and isothermal calorimetry.(15,16) In addition, we will increase the number of antibodies used in this research to improve the statistical reliability of our findings. Finally, we will explore the application of these antibodies targeting VEGFA against other malignancies or cancers.

5. CONCLUSION

In this research, we have performed computational modeling of bispecific antibodies binding to CD3 and VEGDA receptors. The 3D structures of the CD3 and VEGFA receptors were obtained, and 15 antibodies were downloaded from the PDB website and docked on them. Based on the docking results, we have found that 1IL1 was the most effective candidate for CD3 receptor and 2HRP for VEGFA receptor. We used multistep selection criteria, starting with a simple visual inspection and then calculating the molecules' hydrogen bonds and binding energy. Understanding the antibody binding mechanism to the two receptors will help us effectively use it in future treatments.

6. REFERENCES

1. Kontermann, R.E. et al. "Bispecific antibodies." Drug Discovery Today, vol. 20, no. 7, 2015, pp. 838-847.
2. Sheridan, C. "Bispecific antibodies poised to deliver wave of cancer therapies." Nature Biotechnology, vol. 39, no. 3, 2021, pp. 251-255.
3. Fan, J. et al. "Progress in molecular docking." vol. 7, 2019, pp. 83-89.
4. Ghasemi, J.B. et al. "Molecular docking challenges and limitations." Pharmaceutical sciences: Breakthroughs in research and practice, IGI Global, 2017, pp. 770-794.
5. Clevers, J. et al. "The t cell receptor/cd3 complex: A dynamic protein ensemble." vol. 6, 1988, pp. 629-662.
6. Marrack, P. et al. "The t cell receptor." vol. 238, no. 4830, 1987, pp. 1073-1079.
7. Consortium, T.U. "Uniprot: A hub for protein information." Nucleic Acids Research, vol. 43, no. D1, 2014, pp. D204-D212.
8. Abramson, J. et al. "Accurate structure prediction of biomolecular interactions with alphafold 3." Nature, vol. 630, no. 8016, 2024, pp. 493-500.
9. Smith, Z. et al. "Graph attention site prediction (grasp): Identifying druggable binding sites using graph neural networks with attention." Journal of Chemical Information and Modeling, vol. 64, no. 7, 2024, pp. 2637-2644.
10. Yan, Y. et al. "Hdock: A web server for protein–protein and protein–DNA/rna docking based on a hybrid strategy." Nucleic Acids Research, vol. 45, no. W1, 2017, pp. W365-W373.
11. Vangone, A. et al. "Prodigy: A contact-based predictor of binding affinity in protein-protein complexes." Bio Protoc, vol. 7, no. 3, 2017, p. e2124.
12. Pettersen, E.F. et al. "Ucsf chimerax: Structure visualization for researchers, educators, and developers." Protein science, vol. 30, no. 1, 2021, pp. 70-82.
13. Roshanbin, S. et al. "Reduction of αsyn pathology in a mouse model of pd using a brain-penetrating bispecific antibody." Pharmaceutics, vol. 14, no. 7, 2022, p. 1412.
14. Rofo, F. et al. "A brain-targeting bispecific-multivalent antibody clears soluble amyloid-beta aggregates in alzheimer's disease mice." Neurotherapeutics, vol. 19, no. 5, 2022, pp. 1588-1602.
15. Hou, W. et al. "A review of surface plasmon resonance-enhanced photocatalysis." Advanced Functional Materials, vol. 23, no. 13, 2013, pp. 1612-1619,

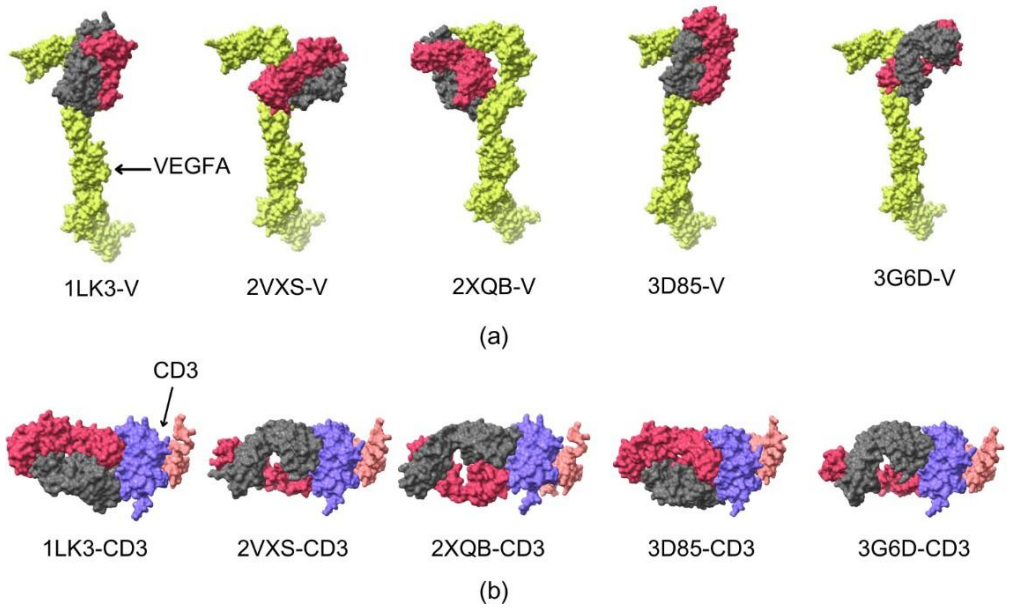

Fig. 2. Molecular docked structures of receptors and antibodies. The CD3 molecules that fit this criterion were 1B4J, 1LK3, 2VXS, 2XQB, 3D85, and 3G6D.

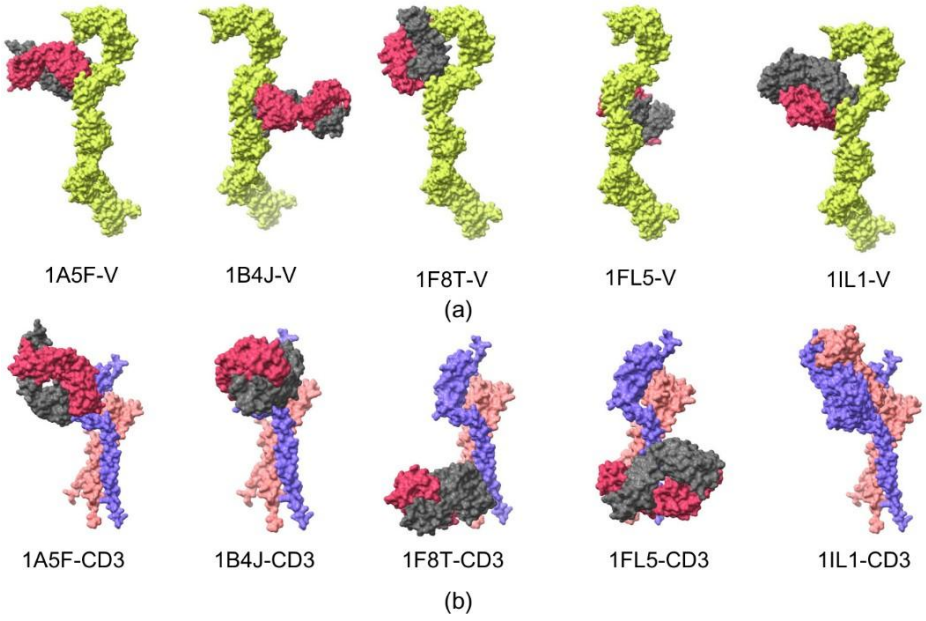

Fig. 3. Molecular docked structures of receptors and antibodies. The VEGFA molecules that fit this criterion were 2XQB, 1A5F, 1IL1, 1IQW, and 2HRP.

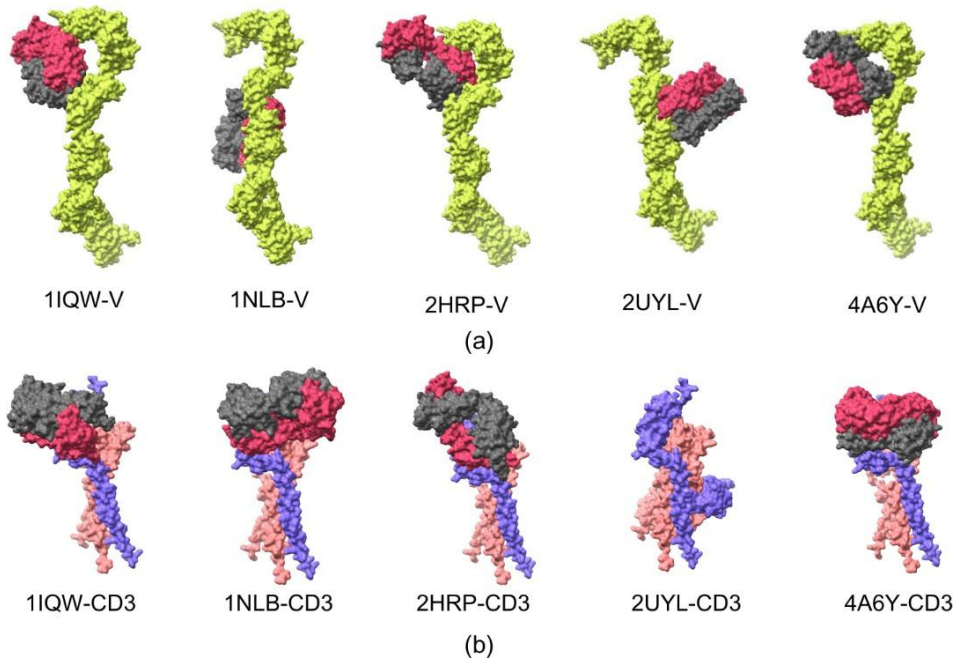

Fig. 4. Molecular docked structures of receptors and antibodies. The VEGFA molecules that fit this criterion were 2XQB, 1A5F, 1IL1, 1IQW, and 2HRP. The CD3 molecules that fit this criterion were 1B4J, 1LK3, 2VXS, 2XQB, 3D85, and 3G6D.

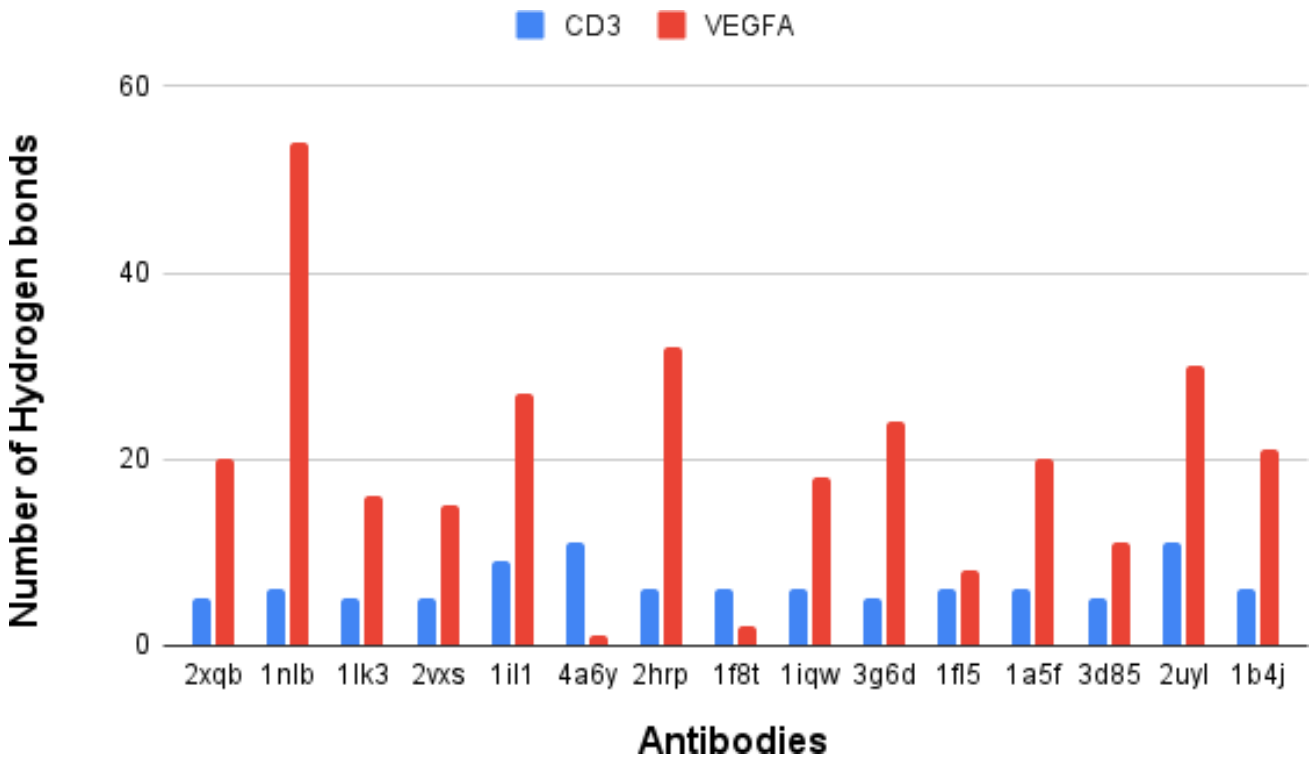

Fig. 5. Number of hydrogen bonds formed by antibodies with CD3 and VEGFA receptor.

Table 1. The binding energy for each CD3 and VEGFA was computed using the PRODIGY software.

CD3 Complex	CD3 Value	VEGFA Complex	VEGFA Value
1LK3	-11.9	1LK3	-11.5
2VXS	-10.3	2VXS	-12.1
2XQB	-9.9	2XQB	-13.2
3D85	-8.6	3D85	-22.4
3G6D	-11.3	3G6D	-11.0
1A5F	-14.0	1A5F	-13.2
1B4J	-10.9	1B4J	-12.6
1F8T	-12.2	1F8T	-14.1
1FL5	-14.1	1FL5	-14.4
1IL1	-15.2	1IL1	-11.8
1IQW	-9.0	1IQW	-12.4
1NLB	-13.8	1NLB	-13.9
2HRP	-11.6	2HRP	-14.5
2UYL	-19.3	2UYL	-23.5
4A6Y	-13.8	4A6Y	-13.9