In Silico Design of a Bispecific DuoBody Antibody Targeting S100A4 and T-Cell Receptors to Enhance Immune Infiltration in Glioblastoma

AISHWARYA VIKRAM[1] AND GAURAV SHARMA[2]

[1]*Wiregrass High School, Wesley Chapel, FL*
[2]*Eigen Sciences, Apex, NC*

Published Mar, 2026

Glioblastoma (GBM) is the most aggressive and lethal primary brain tumor in adults, characterized by rapid progression and resistance to current therapies. The S100A4 protein, secreted by GBM cells, plays a critical role in tumor invasion, metastasis, and immune evasion. To address these challenges, this study focuses on the in silico design of a bispecific DuoBody antibody capable of simultaneously binding to the S100A4 protein and T-cell receptors (TCRs). By bridging T cells directly to the tumor microenvironment, the designed antibody aims to enhance immune infiltration and antitumor activity. Using computational tools such as AlphaFold 3 for structure prediction, P2Rank and ScanNet for binding site identification, HDOCK for molecular docking, and PLIP/PRODIGY for interaction and binding energy analysis, we evaluated the structural feasibility and binding affinity of candidate antibody complexes. Preliminary results suggest that antibody 1IGT demonstrated the strongest interaction with S100A4, supporting its potential as a therapeutic candidate. This work highlights the promise of DuoBody antibodies as next-generation immunotherapeutics for GBM, though experimental validation remains necessary.

1. INTRODUCTION

Glioblastoma is the most common form of brain cancer in adults. It is highly aggressive and fast-growing.(Affinito et al., 2020) Some symptoms of glioblastoma are headaches, particularly ones that hurt the most in the morning, seizures, nausea, vomiting, changes in mental function, vision changes, weakness or balance problems, speech difficulties, fatigue, drowsiness, and reduced sense of touch.(Alifieris & Trafalis, 2015) Current treatment methods for glioblastoma are surgical resection, radiation therapy, chemotherapy, immunotherapy, and tumor-treating fields. These methods are not very good because the glioma stem cells have the ability to regenerate tumors after treatment and resist therapy. Some challenges with surgery are that glioblastoma tumors have an invasive growth pattern which makes surgical removal challenging.(Alifieris & Trafalis, 2015)

A tumor microenvironment is the ecosystem that surrounds a tumor inside of the body. It consists of cancer cells, blood vessels, immune cells, extracellular matrix, and various other molecules. It plays an active role in tumor development.(Alnefaie et al., 2022) Duo body antibody has two binding sites, which enables it to bind to two different targets.(Engelberts et al., 2020)

A DuoBody antibody is an antibody that has two binding sites, which allow it to bind to two different targets, Figure 1. (Engelberts et al., 2020; Koopman et al., 2021) It can be used to help TCR to enter the tumor microenvironment and reach the glioblastoma cells. S100a4 is secreted by the glioblastoma cell and helps the cancer progress. The S100A4 receptor is located on the S100A4. The TCR receptor is located on the surface of T cells. TCR binds to other antigens and plays a crucial role in the immune response. In this research, we have performed computational modeling of the duobody antibody targeting the S100A4 protein in GBM. These dual-body antibodies can help direct the T cell to the tumor microenvironment, resulting in cancer cell apoptosis.

2. METHOD

Uniprot is a free online protein database that provides protein sequences and functional data.(Consortium, 2014) It combines information from Swiss-Prot, TrEMBL, and PIR-PSD databases. It included cross-references, detailed annotations, and featured information such as protein names, sequences, taxonomies, and literature citations. We used Uniprot to retrieve the amino acid sequences for S100A4. Alphafold 3 is an advanced AI system that was developed by Google DeepMind and Isomorphic Labs that can accurately predict the 3D structure of proteins based on their amino acid sequences.(Abramson et al., 2024) It can also be

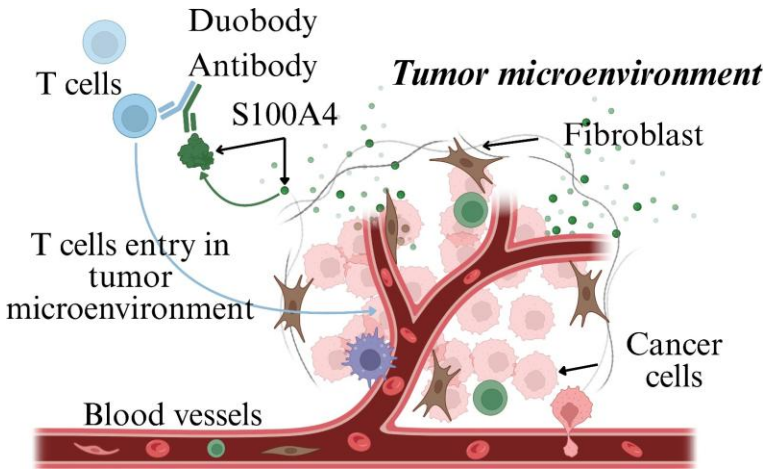

Fig. 1. This figure shows the duobody antibody binding to the T cell and S100A4, which allows the T cell to enter the tumor microenvironment with the S100A4. The image was generated by student using BioRender.

used to predict the structure of DNA, RNA, and other biological molecules, as well as their interactions. We used it to predict the full 3D structures of S100A4 and TCR. P2Rank and Scanet allows for the prediction and visualization of protein-ligand binding sites.(Tubiana, Schneidman-Duhovny, & Wolfson, 2022) We used them to predict potential binding pockets of S100A4 and TCR. These predictions guided docking site selection for the bispecific antibody. PLIP is used for automated detection and visualization of relevant non-covalent protein-ligand contacts in 3D structures. We used PLIP to analyze protein-ligand interactions.(Salentin, Schreiber, Haupt, Adasme, & Schroeder, 2015) Prodigy predicts the binding energy of protein-protein complexes based on intermolecular contacts.(Vangone & Bonvin, 2017) We used prodigy to calculate the binding energy of top docking complexes and to validate docking strength and stability.

3. RESULTS

Predicted Binding Sites:

Binding site analysis using P2Rank and ScanNet identified several potential interaction pockets on both S100A4 and the T-cell receptor (TCR) (Figure 2). These sites were used as docking targets for candidate antibodies, guiding the selection of optimal antibody–protein complexes. The location of the s100A4 is shown in Figure 3. Predicted binding sites are specific regions on a protein or molecule where another molecule, such as a drug, ligand, antibody, or aptamer, is likely to bind. These sites are identified using computational tools based on structural, chemical, and electrostatic properties of the molecule.

Interaction analysis

Interaction profiling with PLIP showed that 1IGT formed the highest number of stabilizing interactions: Hydrogen bonds and hydrophobic interactions were most frequent in the S100A4–1IGT complex (Figures 4-5). A hydrogen bond is a weak attractive interaction between a hydrogen atom and an electronegative atom such as oxygen, nitrogen, or fluorine. Hydrogen bonds play an important role in stabilizing protein structures and molecular binding interactions. Applications of hydrogen bonds include stabilizing protein and DNA structures, improving drug–target binding, and enhancing molecular recognition in biological systems.

Salt bridges and π–π stacking interactions further sup- ported the stability of these complexes. The TCR–antibody com- plexes also demonstrated multiple stabilizing contacts, confirm- ing effective binding at both ends of the bispecific DuoBody antibody. Salt bridges are electrostatic interactions between positively and negatively charged amino acids that help stabilize protein structures and molecular complexes. π–π stacking refers to attractive interactions between aromatic rings, contributing to the stability and binding affinity of molecular interactions.

Binding energy evaluation

Binding free energy calculations with PRODIGY revealed that the S100A4–1IGT complex had the most favorable binding energy compared to other antibody candidates (Figure 6). Negative binding energy values indicated strong and stable complex formation, supporting the hypothesis that 1IGT is the best antibody choice. Binding energy is the amount of energy required to separate two bonded particles or molecules. Higher binding energy indicates stronger and more stable interactions between them.

Antibody docking outcomes

Docking simulations with HDOCK revealed that multiple antibodies were capable of binding to the target proteins. Among these, antibody 1IGT demonstrated the strongest and most stable binding to S100A4, while also forming favorable interactions with the TCR (Figure 7). This suggests that 1IGT is a promising candidate for integration into the bispecific DuoBody design. Antibody docking outcomes describe the predicted binding interactions, binding affinity, and stability between an antibody and its target antigen obtained through computational docking simulations.

Molecular dynamics simulations

To further test stability, molecular dynamics (MD) simulations were performed using GROMACS. Results showed that the antibody–protein complexes maintained stable root-mean-square deviation (RMSD) values throughout the simulation, confirming structural stability under dynamic conditions.

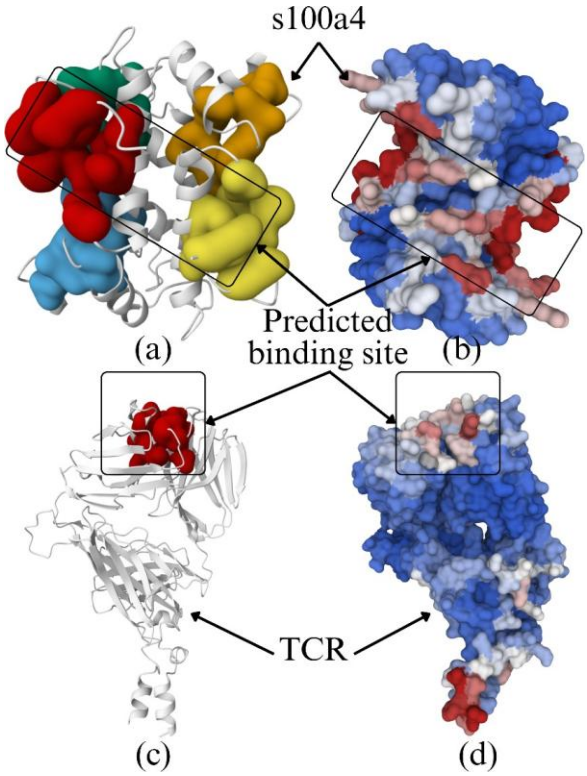

Fig. 2. (a) and (b) show the predicted binding sites of the S100A4 protein visualized using surface and electrostatic representations. (c) and (d) show the predicted binding regions on the T-cell receptor (TCR). The highlighted regions indicate potential interaction pockets identified using computational binding site prediction tools, including P2Rank and ScanNet.

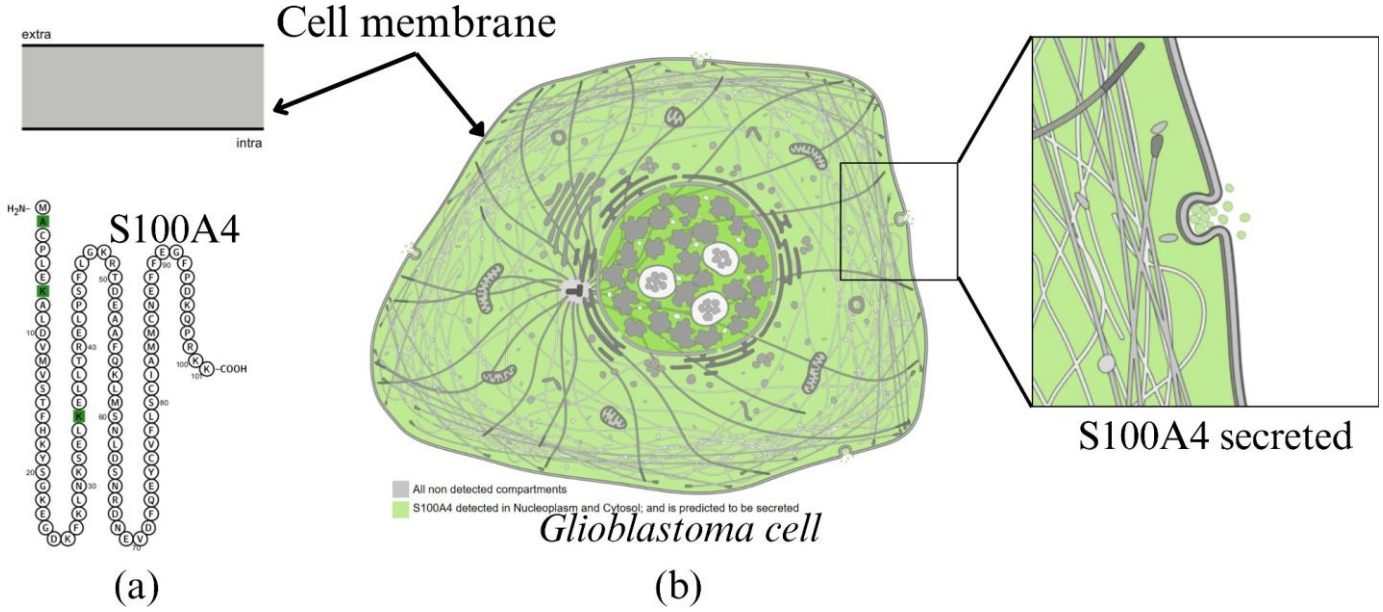

Fig. 3. (a) Shows the membrane topology and structural orientation of the S100A4 protein. (b) Illustrates the localization and secretion of S100A4 from the glioblastoma cell into the tumor microenvironment. The figure highlights the extracellular release of S100A4, which may contribute to tumor progression and immune modulation. Generated using the PROTTER web server.

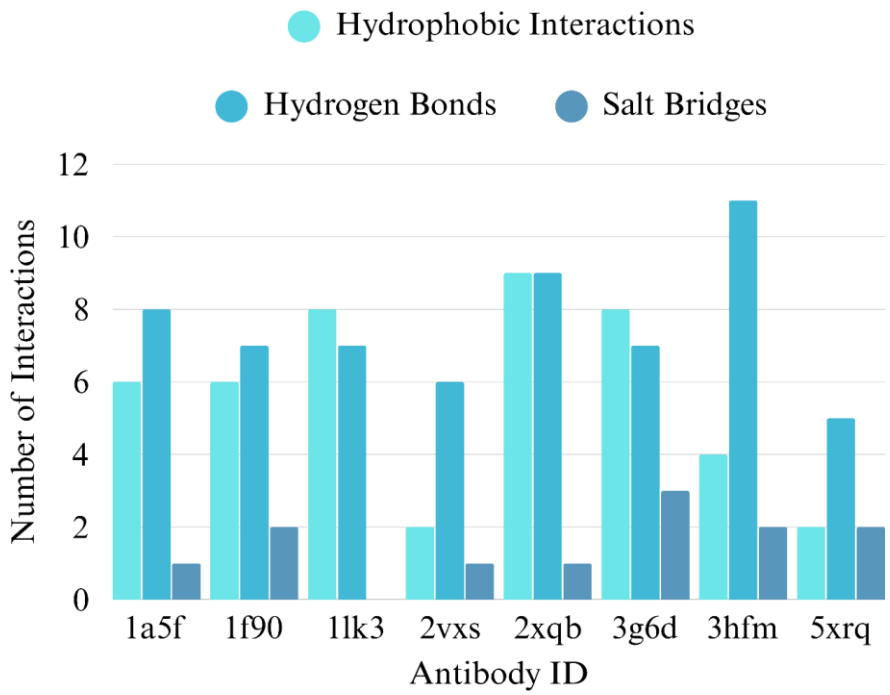

Fig. 4. Shows the number and types of intermolecular interactions formed between different antibodies and the target protein, including hydrogen bonds, hydrophobic interactions, and salt bridges. Antibody 3hfm demonstrated the highest number of hydrogen bond interactions, indicating strong binding stability. The figure was generated using the PLIP web server.

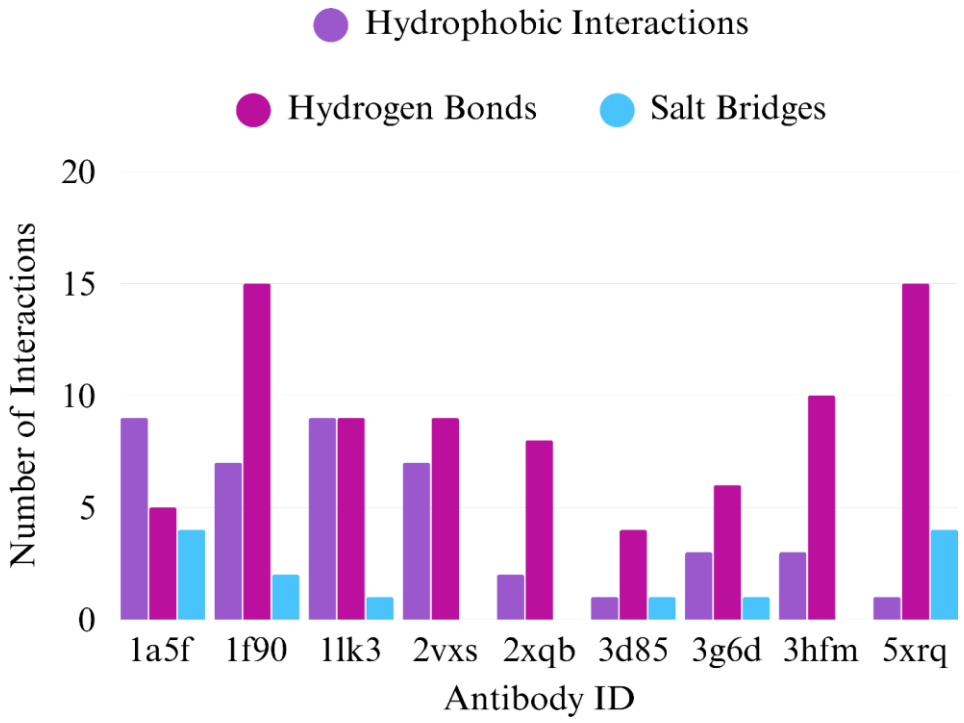

Fig. 5. Shows the number and types of intermolecular interactions formed between S100A4 and different antibody candidates, including hydrogen bonds, hydrophobic interactions, and salt bridges. Antibodies 1f90 and 5xrq exhibited the highest number of hydrogen bond interactions, suggesting strong binding affinity and stable complex formation. The figure was generated using the PLIP web server.

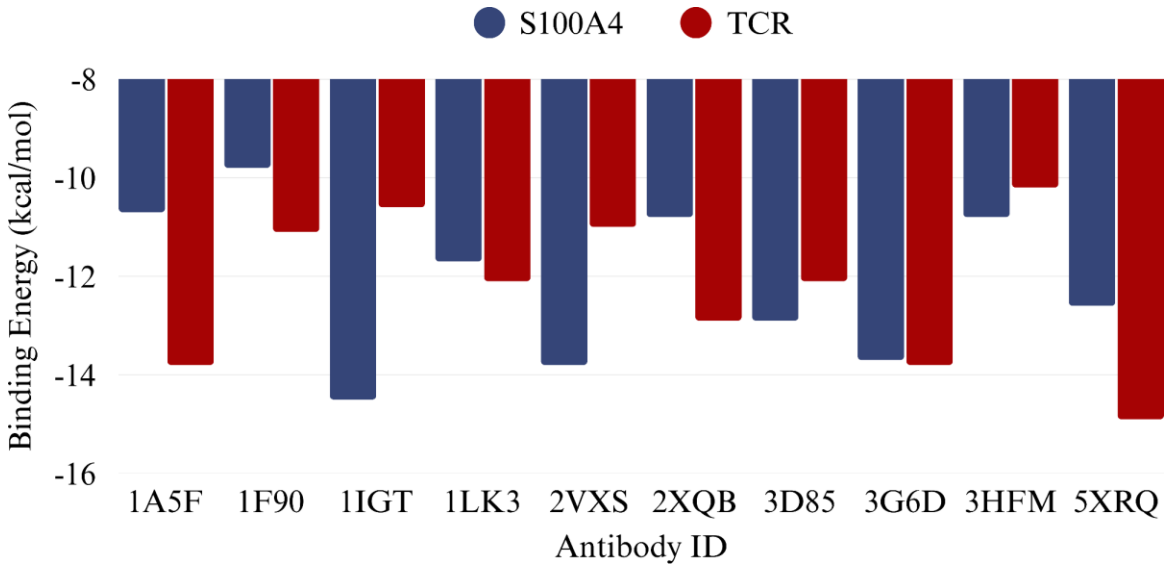

Fig. 6. Shows the binding energy values between different antibody candidates and the target proteins S100A4 and TCR. More negative binding energy values indicate stronger and more stable molecular interactions. Among the tested antibodies, 1IGT and 5XRQ demonstrated highly favorable binding affinities, supporting their potential as promising candidates for bispecific DuoBody antibody design. The figure was generated using PRODIGY software.

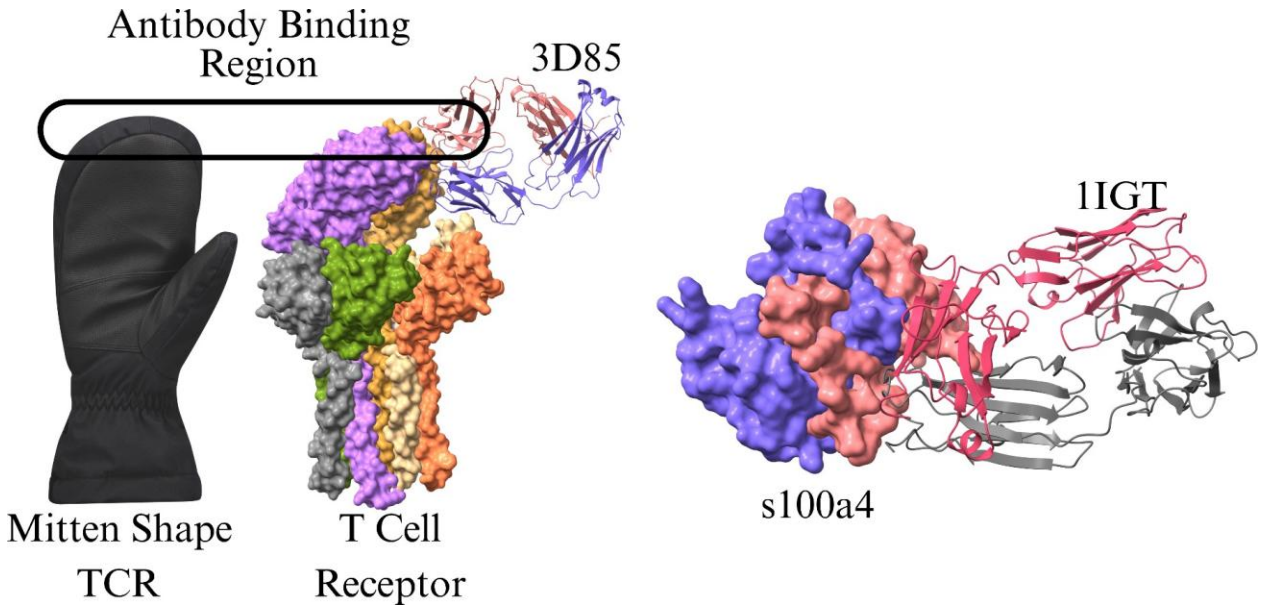

Fig. 7. The figure illustrates the docked structures of antibody candidates bound to the T-cell receptor (TCR) and S100A4 protein. The left panel shows antibody 3D85 interacting with the TCR binding region, while the right panel shows antibody 1IGT docked to the S100A4 protein. These docking results suggest favorable antibody–target interactions that may support the development of bispecific DuoBody antibodies for enhancing T-cell infiltration in glioblastoma.

3. DISCUSSION

Some applications of this include developing it as a next-generation immunotherapy strategy for glioblastoma. It may also enhance T-cell infiltration into tumors by targeting S100A4-induced immune tumors. It also provides a generalizable model for designing DuoBody antibodies for other solid tumors. How- ever, there are several limitations. Computational simulations do not account for the variability in in vivo immune responses. In addition, the DuoBody construct is theoretical and requires experimental synthesis and validation. Finally, conformational flexibility of antibodies and proteins may not be fully captured in docking studies. Future work on this could include molecular dynamics simulations to validate the stability of the DuoBody-antigen complexes over time. It could also include conducting in vitro and in vivo experiments to assess the efficiency and safety of the designed DuoBody antibody. The findings of this study demonstrate the potential of bispecific DuoBody antibodies as a promising immunotherapeutic strategy for glioblastoma by simultaneously targeting S100A4 and T-cell receptors.

4. CONCLUSION

CAR-T cell therapy represents a powerful cancer treatment strategy, yet its success can be limited by the suppressive tumor microenvironment. This study highlights how targeting lactate metabolism through lipocalin proteins may help reduce tumor- driven immunosuppression and improve CAR-T cell activity. By focusing on metabolic vulnerabilities, such as the buildup of lactic acid, researchers can design therapies that not only attack cancer cells directly but also create a more favorable environment for immune function. Overall, this approach opens new possibilities for enhancing CAR-T therapy effectiveness and supports further research into combining metabolic regulation with immunotherapy.

5. REFERENCES

1. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., . . . Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature, 630(8016), 493-500.
2. Affinito, A., Quintavalle, C., Esposito, C. L., Roscigno, G., Giordano, C., Nuzzo, S., . . . Condorelli, G. (2020). Targeting Ephrin Receptor Tyrosine Kinase A2 with a Selective Aptamer for Glioblastoma Stem Cells. Molecular Therapy Nucleic Acids, 20, 176-185.
3. Alifieris, C., & Trafalis, D. T. (2015). Glioblastoma multiforme: Pathogenesis and treatment. Pharmacology & Therapeutics, 152, 63-82.
4. Alnefaie, A., Albogami, S., Asiri, Y., Ahmad, T., Alotaibi, S. S., Al-Sanea, M. M., & Althobaiti, H. (2022). Chimeric Antigen Receptor T-Cells: An Overview of Concepts, Applications, Limitations, and Proposed Solutions. Frontiers in Bioengineering and Biotechnology, 10.
5. Consortium, T. U. (2014). UniProt: a hub for protein information. Nucleic acids research, 43(D1), D204-D212.
6. Engelberts, P. J., Hiemstra, I. H., de Jong, B., Schuurhuis, D. H., Meesters, J., Beltran Hernandez, I., . . . Breij, E. C. W. (2020). DuoBody-CD3xCD20 induces potent T-cell-mediated killing of malignant B cells in preclinical models and provides opportunities for subcutaneous dosing. eBioMedicine, 52.
7. Koopman, L., Vries, L. S.-d., Egerod, F. L., Wubben, S., Houtkamp, M., Poot, S. D., . . . Breij, E. (2021). 863 In vitro and in vivo studies establish DuoBody®-CD3xB7H4 as a novel drug candidate for the treatment of solid cancers. Journal for ImmunoTherapy of Cancer, 9(Suppl 2), A904-A904.
8. Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F., & Schroeder, M. (2015). PLIP: fully automated protein–ligand interaction profiler. Nucleic acids research, 43(W1), W443-W447.
9. Tubiana, J., Schneidman-Duhovny, D., & Wolfson, H. J. (2022). ScanNet: an interpretable geometric deep learning model for structure-based protein binding site prediction. Nature Methods, 19(6), 730-739.
10. Vangone, A., & Bonvin, A. (2017). PRODIGY: A Contact-based Predictor of Binding Affinity in Protein-protein Complexes. Bio Protoc, 7(3), e2124.