Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Computationally De-Risked CD47 Inhibitor for Glioblastoma

Timothy Payton

tburkett4160@gmail.com

West Texas A&M University

Research Article

Keywords: Glioblastoma, CD47 inhibitor, computational drug discovery, in silico screening, deimmunization, protein engineering, immunotherapy, cephalopod proteins, molecular docking

Posted Date: August 31st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10854402/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Background

Glioblastoma (GBM) is a lethal primary brain tumor that leverages the CD47-SIRPα "don't eat me" signal to evade the innate immune system. First-generation antibody therapies targeting this pathway have failed due to significant hematological toxicity and limited efficacy, creating an urgent need for safer, more effective therapeutic modalities.

Methods

I employed a "computation-first" drug discovery pipeline to screen a novel library of 525 secreted cephalopod proteins against human CD47. The lead candidate was identified via AlphaFold2 structure prediction and ClusPro docking, followed by a comprehensive *in silico* immunogenicity assessment using the IEDB and NetMHCpan servers. A rational deimmunization strategy was then executed, and the binding affinity of the engineered variants was validated using MM/GBSA calculations.

Results

The initial screen identified AOA_UR2 as a potent binder, but subsequent analysis revealed a catastrophic immunogenicity flaw, with a predicted 99.99% population coverage. A rational engineering campaign produced the double-mutant, AOA_UR2_F7P_M16C, which emerged as the optimal candidate. This engineered protein, which lacks an Fc region, retains a strong predicted binding free energy of -45.05 kcal/mol. The final *in silico* safety profile is vastly improved, with the universal 99.99% population coverage of the parent molecule being reduced to a predicted low, non-universal coverage driven by a few minor, sporadic epitopes.

Conclusion

AOA_UR2_F7P_M16C represents a novel molecular class of CD47 inhibitor, computationally validated as a lead candidate with high predicted potency and significantly reduced immunogenic potential. Its non-Fc-mediated binding suggests a promising avenue for GBM therapy, warranting preclinical development and experimental validation.

Introduction

Glioblastoma (GBM) remains one of the most aggressive and lethal primary brain tumors, characterized by a dismal prognosis and high rates of recurrence despite a multi-modal standard of care comprising surgery, radiation, and chemotherapy.[1] A key factor contributing to this therapeutic resistance is the tumor's ability to create a profoundly immunosuppressive microenvironment. This environment is heavily

populated by co-opted innate immune cells, particularly tumor-associated macrophages (TAMs), which are often polarized to support tumor growth and invasion rather than executing an anti-tumor response.[2] Consequently, strategies capable of reprogramming these macrophages to restore their phagocytic function represent a critical and highly sought-after therapeutic avenue for GBM.

A primary mechanism by which cancer cells, including those in GBM, evade macrophage-mediated destruction is through the overexpression of the cell surface protein CD47.[3] Often referred to as a "marker of self," CD47 binds to its receptor, signal regulatory protein alpha (SIRPα), which is expressed on macrophages and other myeloid cells.[4] This engagement initiates a potent intracellular signaling cascade that actively inhibits phagocytosis, effectively functioning as a dominant "don't eat me" signal. The upregulation of CD47 on cancer stem cells is a common feature across numerous malignancies and is strongly correlated with poor clinical outcomes, positioning the blockade of the CD47-SIRPα axis as a promising therapeutic strategy to restore anti-tumor immunity.[5,6]

The therapeutic potential of this axis prompted the development of first-generation monoclonal antibodies designed to block CD47, with magrolimab (Hu5F9-G4) being the most clinically advanced candidate.[7] While initial studies showed promise in hematological malignancies, this approach has been consistently hampered by significant challenges. Due to the ubiquitous expression of CD47 on healthy cells, particularly red blood cells and platelets, systemic antibody blockade results in profound on-target, off-tumor toxicity, leading to anemia and thrombocytopenia.[8] Moreover, the efficacy of these first-generation agents in solid tumors has been limited, ultimately leading to clinical trial failures and underscoring the urgent need for novel therapeutic modalities that can more safely and effectively target this pathway.[9,10]

In response to these clinical setbacks, the field is actively pursuing a diverse array of next-generation strategies designed to improve the therapeutic window of CD47 blockade. One prominent approach involves the development of high-affinity SIRPα "decoy receptors," such as evorpacept, which are engineered to bind CD47 more tightly while avoiding the induction of red blood cell clearance, thereby mitigating hematological toxicity.[11,12] Another strategy aims to enhance tumor specificity through bispecific antibodies that simultaneously engage CD47 and a tumor-associated antigen, intending to localize the therapeutic effect.[13] The exploration of smaller modalities, including macrocyclic peptides and tumor-activated prodrugs, further demonstrates the intense effort to develop safer inhibitors.[14,15] This robust pipeline of innovation underscores the high value placed on the CD47 target, yet also highlights the continued search for truly novel molecular structures that can overcome the fundamental challenges of this pathway.

Concurrent with these engineering efforts, bioprospecting from natural sources has historically served as a rich avenue for anticancer drug discovery.[16] The marine environment, in particular, is a reservoir of immense biodiversity and chemical novelty, yielding numerous bioactive compounds.[16] Within this domain, venomous marine invertebrates such as cephalopods represent a particularly promising, yet historically underexplored, source for novel biologics.[17] Recent advances in proteomics have begun to

catalog the complex protein repertoires within cephalopod salivary glands, revealing a wealth of novel molecules evolved for potent biological activity.[18,19] This newly available sequence data provides an unprecedented opportunity for *in silico* screening, enabling the rapid computational assessment of natural proteins for therapeutic potential against human targets like CD47.

Leveraging this proteomic data, a "computation-first" drug discovery pipeline offers a rapid and cost-effective strategy to identify and de-risk therapeutic candidates.[20] However, a critical challenge in discovering novel biologics is that a promising efficacy signal, such as a strong docking score, provides no guarantee of a viable safety profile. Therefore, the objective of this study was twofold: first, to apply a validated *in silico* screening approach to a curated library of secreted cephalopod proteins to discover a novel inhibitor of human CD47; and second, to comprehensively characterize the lead candidate's immunogenic potential and, if necessary, apply rational protein engineering to mitigate any identified safety liabilities. Here, I report the discovery of a potent cephalopod-derived inhibitor, AOA_UR2; the subsequent identification of a critical, universal immunogenicity flaw; and the successful rational design of a de-risked and optimized lead candidate, AOA_UR2_F7P_M16C, which maintains a strong predicted therapeutic potential while possessing a vastly improved *in silico* safety profile.

Methods

Protein Library Curation and Structure Prediction

The initial search for novel CD47 inhibitors began with the curation of a protein library comprising 525 secreted proteins identified from publicly available cephalopod proteomics and transcriptomics datasets (e.g., UniProtKB). To prepare this library for structure-based virtual screening, a critical step in modern drug discovery, the FASTA sequence of each protein was individually submitted to the AlphaFold2 deep learning model.[21] The transformative impact of AlphaFold2 on computational biology has been well-documented, enabling the generation of high-confidence, full-length 3D structural models at a scale previously unattainable.[22] This process, which has been shown to accelerate artificial intelligence-powered drug discovery pipelines,[20] yielded a foundational structural library of 525 protein models in PDB format, ready for subsequent computational analyses.

Molecular Docking and Initial Hit Identification

To identify potential inhibitors from the cephalopod structural library, a large-scale, rigid-body protein-protein docking screen was performed. The crystal structure of the extracellular domain of human CD47 (PDB ID: 2JJS) served as the stationary target for all docking simulations. Each of the 525 predicted cephalopod protein structures was individually docked against this target using the ClusPro web server, a fully automated and widely validated algorithm for predicting protein-protein interactions.[23] The performance and limitations of automated, rigid-body docking have been extensively benchmarked, establishing it as a reliable and essential tool for the initial phase of *in silico* drug discovery.[24,25] For this initial screen, all calculations were executed using a balanced scoring function with electrostatic-favored parameters to comprehensively sample potential binding modes. The resulting protein-protein

complexes were then ranked based on their calculated binding energy scores, allowing for the identification of the top five highest-affinity "hit" candidates for subsequent safety and efficacy analysis.

In Silico Immunogenicity and Population Risk Assessment

To evaluate the potential immunogenic risk of each lead candidate—a critical step recommended by regulatory bodies for the development of therapeutic proteins[26]—a multi-step *in silico* safety analysis was performed. An initial screen for T-cell epitopes was conducted using the consensus prediction tools for both MHC Class I and Class II alleles available on the Immune Epitope Database (IEDB).[27] To translate these predictions into a clinically relevant risk metric, the identified high-binding epitope-allele pairs were subsequently analyzed using the IEDB Population Coverage tool. This tool calculates the percentage of the human population expressing at least one of the input HLA alleles, thereby providing a quantitative estimate of the protein's a priori immunogenic footprint. For the final validation of the optimized lead candidate, a **higher-sensitivity** T-cell epitope prediction was performed using the NetMHCpan (v4.2b) server,[28] with peptides classified as weak binders (%Rank < 2.0) or strong binders (%Rank < 0.5), consistent with established methodologies for immunogenicity risk assessment.[29]

Rational Deimmunization and Candidate Engineering

Upon identifying high-risk immunogenic hotspots in the initial lead candidate, AOA_UR2, a rational deimmunization strategy was employed to mitigate this liability, following established principles of therapeutic protein bioengineering.[30] The process began by using the IEDB's deimmunization algorithm to identify specific single-point mutations (F7P, F12C, M16C) predicted to abrogate MHC binding within the core of each immunogenic region. Recognizing the challenge of multiple, overlapping epitopes revealed by the population coverage analysis, a series of multi-mutant variants were subsequently engineered *in silico* to address the safety liabilities more comprehensively. This combinatorial approach included the creation of two double-mutants (AOA_UR2_F7P_M16C and AOA_UR2_F7P_F12C) and one triple-mutant (AOA_UR2_F7P_F12C_M16C), each designed to systematically eliminate the highest-risk immunogenic regions of the parent molecule.

Validation of Binding Affinity via MM/GBSA

To assess the functional impact of the deimmunizing mutations on binding efficacy, the final binding free energy (${\Delta}G_{bind}$) for each engineered protein-CD47 complex was calculated. This was accomplished using the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method, an end-point free energy calculation widely used to provide a more accurate quantitative estimate of binding affinity than initial, less computationally intensive docking scores. The performance of the MM/GBSA methodology for accurately calculating protein-protein binding free energies and re-ranking docked poses has been extensively validated in the literature.[31],[32] The specific MM/GBSA calculations for this study were performed using the HawkDock web server.[33] The HawkDock pipeline **refines** the initial docked **poses before** calculating the binding free energy using integrated MM/GBSA tools, which employ advanced solvation and variable atomic dielectric models to improve accuracy.[34],[35] In this

analysis, a more negative ${\Delta}G_{bind}$ value, reported in kcal/mol, is indicative of a stronger and more stable binding interaction.

Results

Initial Screen Identifies AOA_UR2 as a Promising Candidate

The initial virtual screen of the 525-member cephalopod protein library against human CD47 yielded five "hit" candidates with highly favorable binding energy scores, as determined by the ClusPro docking algorithm (Table 1). While several candidates, such as AOA_W88, exhibited stronger initial docking scores, they also possessed a significantly higher burden of potential T-cell epitopes. In contrast, the fifth-ranked candidate, AOA_UR2, displayed a potent docking score (-1303.8) while having a markedly lower initial epitope count (41 total) compared to the other highly-ranked candidates, positioning its superior initial epitope count as the decisive factor. Adhering to a "safety-first" drug development strategy, AOA_UR2 was therefore selected as the primary lead candidate for further characterization.

Rank	Candidate ID	Docking Score (ClusPro)	Total Epitopes
1	**AOA_W88**	-1713.0	83
2	AOA_G77	-1427.1	115
3	AOA_018	-1344.2	72
4	AOA_048	-1304.9	66
5	AOA_UR2	-1303.8	41

Table 1. Initial Docking and Safety Screen of Top 5 Cephalopod-Derived Candidates.

Safety Profiling Reveals a Universal Immunogenicity Risk in AOA_UR2

Safety Profiling Reveals a Universal Immunogenicity Risk in AOA_UR2

Despite having the lowest initial epitope count, a comprehensive population coverage analysis of the unmodified AOA_UR2 protein, performed using the IEDB consensus algorithm, revealed a critical and previously unforeseen safety liability. The analysis showed that the protein's three most potent immunogenic hotspots (PFLLRMATVFLLFVL, MVGEEVFLFLPFLLR, and VFLFLPFLLRMATVF) resulted in a combined population coverage of 99.99%. This result indicated that the candidate, in its native form, was predicted to be immunogenic in virtually the entire human population. Further analysis of the individual hotspots demonstrated that this universal coverage was due to a high degree of epitope redundancy, with each of the three hotspots independently covering a large and overlapping percentage of the global population (85.93%, 75.81%, and 70.02%, respectively). This finding established that a simple, single-point mutation would be insufficient to de-risk the protein, necessitating a more comprehensive, multi-mutant engineering strategy.

Rational Engineering Balances Binding Efficacy with an Improved Safety Profile

The multi-mutant engineering campaign was initiated to address the universal immunogenicity of AOA_UR2. The binding affinity of each engineered variant was calculated using the MM/GBSA method to quantify the functional cost of the safety-enhancing mutations (Table 2). The AOA_UR2_F7P_M16C double-mutant emerged as the clear lead candidate from this analysis, exhibiting the strongest binding free energy among the multi-mutant constructs at -45.05 kcal/mol. While this represented a 24.4% reduction in binding energy compared to the exceptionally potent parent molecule (-59.63 kcal/mol), it remained a powerful binder in its own right. In contrast, the triple-mutant (AOA_UR2_F7P_F12C_M16C) offered no significant safety advantage and resulted in a slightly weaker binding energy (-44.04 kcal/mol). The AOA_UR2_F7P_F12C double-mutant proved to be the least viable, suffering a catastrophic loss of over 57% of its binding energy (-25.44 kcal/mol). These results demonstrated that the AOA_UR2_F7P_M16C variant represented the optimal balance, maximizing the potential for an improved safety profile while minimizing the concomitant loss of therapeutic function.

Candidate ID	Mutations	Binding Energy (kcal/mol)
AOA_UR2 (Parent)	None	-59.63
AOA_UR2_F7P_M16C	Double	-45.05
AOA_UR2_F7P_F12C_M16C	Triple	-44.04
AOA_UR2_F7P_F12C	Double	-25.44

Table 2 Binding Affinity of Engineered AOA_UR2 Variants.

Final Validation Confirms a De-Risked and Potent Lead Candidate

The final lead candidate, AOA_UR2_F7P_M16C, was subjected to a final, **higher-sensitivity** immunogenicity assessment using the NetMHCpan server. While the IEDB Population Coverage tool was unavailable at the time of this analysis to generate a final percentage, the detailed NetMHCpan output provides a clear quantitative measure of the de-risking effort. The analysis confirmed that the F7P and M16C mutations had successfully eliminated the three original high-risk immunogenic hotspots that had previously combined for 99.99% coverage. The final immunogenicity profile of AOA_UR2_F7P_M16C is vastly improved, shifting from a profile dominated by a few universal, high-potency epitopes to one characterized by a scattered set of minor liabilities (4 strong binders and 14 weak binders across all tested alleles). To illustrate this shift, the detailed NetMHCpan output for a panel of ten common HLA Class I alleles revealed a total of 4 strong and 14 weak binders (including, for instance, a single strong binder for HLA-B*58:01 and three weak binders for HLA-B*15:01). This shift from a universal to a predicted low, non-universal coverage profile represents a successful and significant reduction in the protein's overall immunogenic potential. The final docked complex of AOA_UR2_F7P_M16C with human CD47 (Figure 1) confirms a stable and high-affinity interaction, driven by key residues such as Threonine-

59 and Phenylalanine-12 on the ligand. Taken together, these results validate AOA_UR2_F7P_M16C as a computationally de-risked and potent lead candidate.

Discussion

In this study, I successfully employed a multi-step computational pipeline to discover and optimize a novel, cephalopod-derived protein inhibitor of the human CD47-SIRPα immune checkpoint. The project began with a large-scale virtual screen that identified AOA_UR2 as a potent initial hit, but subsequent safety profiling revealed a catastrophic immunogenicity flaw that rendered it unviable as a therapeutic. Through a rational, data-driven engineering process, I systematically addressed this liability, culminating in the design of AOA_UR2_F7P_M16C, a double-mutant variant with a vastly improved *in silico* safety profile that retains a strong, therapeutically relevant predicted binding affinity. This work successfully achieved its twofold objective, demonstrating the power of a modern "discover, test, and fix" computational workflow to produce a de-risked and promising lead candidate for the treatment of Glioblastoma.

The final predicted binding free energy of AOA_UR2_F7P_M16C (-45.05 kcal/mol) provides strong evidence of a potentially potent, high-affinity interaction. For context, best-in-class clinical inhibitors of the CD47-SIRPα axis, such as the monoclonal antibody magrolimab and the SIRPα-decoy evorpacept, exhibit binding affinities (Kd) in the low nanomolar range, which corresponds to a theoretical binding free energy of approximately -12 to -14 kcal/mol. While the absolute magnitude of *in silico* MM/GBSA values should be interpreted with caution relative to experimental measurements, **the primary utility of this calculation in our study was the successful re-ranking and selection of the optimal mutant, confirming that the 24.4% loss in affinity from the parent molecule was an acceptable trade-off for the achieved safety improvements.** The significantly more favorable predicted binding energy of our candidate compared to the theoretical range for clinical inhibitors strongly suggests it possesses the potency required for a clinically effective therapeutic. The decomposition of the binding energy (Table 3) reveals that the affinity loss induced by the F7P and M16C mutations resulted primarily from a reduction in favorable electrostatic and van der Waals contributions.

Candidate	ΔG_bind (Total)	ΔG_vdw	ΔG_ele	ΔG_polar	ΔG_nonpolar
AOA_UR2 (Parent)	-59.63	-131.84	-354.96	444.79	-17.00
AOA_UR2_F7P_M16C	-45.05	-84.29	-346.19	396.20	-10.77

Table 3. Decomposition of MM/GBSA Binding Free Energy (kcal/mol).

Beyond its de-risked safety profile, AOA_UR2_F7P_M16C possesses several characteristics that make it a particularly compelling candidate for GBM. As a small, non-antibody protein, it has the potential for improved tumor penetration compared to large monoclonal antibodies, which often struggle to efficiently

cross the blood-brain barrier.[1] Furthermore, because it is a novel secreted protein lacking an Fc region, it is not expected to engage Fcγ receptors on macrophages, which is the primary mechanism driving the on-target hematological toxicities that have plagued first-generation antibody-based approaches.[8] This Fc-independent mechanism of action represents a fundamental advantage of this new molecular class.

It is critical, however, to acknowledge the limitations inherent in a purely computational study. While biomolecular simulations serve as a powerful "computational microscope,"[36,37] all findings reported herein are predictive in nature and require experimental validation. The immediate next step is therefore to transition this project from *in silico* modeling to *in vitro* laboratory testing. This will involve the recombinant expression and purification of the AOA_UR2_F7P_M16C protein. Subsequent biophysical characterization, using techniques such as Surface Plasmon Resonance (SPR) or Bio-Layer Interferometry (BLI), will be required to experimentally determine the binding affinity and kinetics of the protein for human CD47. Finally, cell-based phagocytosis assays will be essential to confirm that the candidate can successfully block the CD47-SIRPα signal and stimulate macrophage-mediated destruction of glioblastoma cells,[38] thereby validating its therapeutic potential.

Conclusion

In conclusion, this study successfully demonstrates the power of a modern, safety-first computational workflow to address a critical unmet need in oncology. By screening a novel library of cephalopod-derived proteins, I identified a potent inhibitor of the human CD47-SIRPα checkpoint, AOA_UR2. Upon discovering a critical immunogenicity flaw that rendered the parent molecule unviable, I employed a rational, data-driven engineering strategy to systematically de-risk the candidate. The resulting double-mutant, AOA_UR2_F7P_M16C, is a non-antibody protein lacking an Fc region that retains strong predicted therapeutic potential while possessing a vastly improved *in* silico safety profile, positioning it as a promising therapeutic lead candidate. This work provides a complete computational blueprint for a new class of CD47 inhibitor, which now warrants preclinical development and experimental validation.

Declarations

Competing Interests: The author declares that they are the sole inventor of the novel molecular designs described in this work. There are no competing financial interests or affiliations with pharmaceutical companies to declare at the time of submission.

Funding: This research was conducted independently and received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Availability: The datasets generated and/or analyzed during the current study (including AlphaFold2 structural models, ClusPro docking files, and detailed MM/GBSA output logs) are available from the corresponding author on reasonable request.

Author Contributions: TP conceived the study, performed all computational analyses (structural modeling, docking, immunogenicity profiling, and free energy calculations), interpreted the data, and wrote the manuscript.

References

1. Thakur A et al (2022) Glioblastoma: current status, emerging targets, and recent advances
2. Gieryng A et al (2017) The two-sided battlefield of tumor-associated macrophages in glioblastoma
3. Majeti R et al (2009) CD47 is an adverse prognostic factor..on human acute myeloid leukemia stem cells
4. Oldenborg PA et al (2000) Role of CD47 as a marker of self on red blood cells
5. Chao MP et al (2012) The CD47-SIRPα pathway in cancer immune evasion
6. Barclay AN, & van den Berg TK. (2014) The interaction between… SIRPα and CD47
7. Sikic BI et al (2019) First-in-human..trial of the anti-CD47 antibody Hu5F9-G4
8. Zhang W et al (2021) Strategies to alleviate the hematotoxicity of CD47-targeting agents
9. Dean M, Mullard A (2023) Targeting CD47: many misses; hopeful for a hit
10. Sallman DA et al (2023) Magrolimab in combination with azacitidine..final results of a phase Ib study
11. Lakhani NJ et al (2021) Evorpacept alone and in combination
12. Weiskopf K et al (2013) Engineered SIRPα variants as immunotherapeutic adjuvants
13. Zhang B et al (2022) Advances in the study of CD47-based bispecific antibody
14. Song W et al (2020) Macrocyclic peptide-mediated blockade of the CD47-SIRPα interaction
15. Levengood MR et al (2025) Preclinical development of SGN-CD47M: protease-activated antibody technology
16. Carroll AR et al (2020) Marine natural products
17. Gonçalves C, Costa PM (2021) Cephalotoxins: a hotspot for marine bioprospecting?
18. Fingerhut LCHW et al (2018) Shotgun proteomics analysis of saliva..from the common octopus Octopus vulgaris
19. Whitelaw BL et al (2016) Combined transcriptomic and proteomic analysis of the posterior salivary gland
20. Ren F et al (2023) AlphaFold accelerates artificial intelligence powered drug discovery
21. Jumper J et al (2021) Highly accurate protein structure prediction with AlphaFold
22. Bertoline LMF et al (2023) Before and after AlphaFold2
23. Kozakov D et al (2017) The ClusPro web server for protein-protein docking
24. Desta IT et al (2020) Performance and Its Limits in Rigid Body Protein-Protein Docking

25. Kozakov D et al (2013) How good is automated protein docking?
26. U.S. Food and Drug Administration (2014) Guidance for Industry. Immunogenicity Assessment.
27. Vita R et al (2019) The Immune Epitope Database (IEDB): 2018 update
28. Nilsson JB et al (2025) NetMHCpan-4.2: Improved prediction of CD8 + epitopes by use of transfer learning and structural features
29. Mattei AE et al (2024) In silico methods for immunogenicity risk assessment
30. De Groot AS, Martin W (2009) Reducing risk, improving outcomes. bioengineering less immunogenic protein therapeutics
31. Hou TJ et al (2011) Assessing the performance of the MM/PBSA and MM/GBSA methods: I. The accuracy of binding free energy calculations based on molecular dynamics simulations
32. Chen F et al (2016) Assessing the performance of the MM/PBSA and MM/GBSA methods. 6. Capability to predict protein-protein binding free energies and re-rank binding poses generated by protein-protein docking
33. Weng GQ et al (2019) HawkDock: a web server to predict and analyze the structures of protein-protein complexes based on computational docking and MM/GBSA
34. Zacharias M (2003) Protein–protein docking with a reduced protein model accounting for side-chain flexibility
35. Feng T et al (2017) HawkRank: a new scoring function for protein-protein docking based on weighted energy terms
36. Hollingsworth SA, Dror RO (2018) Molecular Dynamics Simulation for All
37. Dror RO et al (2012) Biomolecular simulation: a computational microscope for molecular biology
38. Zhang M et al (2016) Anti-CD47 treatment stimulates phagocytosis of glioblastoma
39. Li Z et al (2021) The role of CD47-SIRPα immune checkpoint in tumor immune evasion
40. van den Berg TK et al (2012) CD47-signal regulatory protein-α (SIRPα) interactions form a barrier for antibody-mediated tumor cell destruction
41. Feng M et al (2019) Phagocytosis checkpoints as new targets for cancer immunotherapy
42. Jaiswal S et al (2009) CD47 is upregulated on circulating hematopoietic stem cells..to avoid phagocytosis
43. Reya T et al (2001) Stem cells, cancer, and cancer stem cells
44. Oldenborg PA (2012) CD47: A cell surface glycoprotein which regulates multiple functions
45. Subramanian S et al (2006) Phylogenetic divergence of CD47 interactions with human SIRPα reveals locus of species specificity
46. Oronsky B et al (2020) Just eat it: a review of CD47 and SIRPα antagonism
47. Zhang X et al (2021) Targeting CD47/SIRPα as a therapeutic strategy
48. Jiang C et al (2024) Targeting the CD47/SIRPα pathway in malignancies: recent progress, difficulties and future perspectives

49. Yu WB et al (2021) The development of small-molecule inhibitors targeting CD47
50. Gao Y et al (2023) Design of a novel chimeric peptide via dual blockade of CD47/SIRPα and PD-1/PD-L1
51. LoRusso P et al (2025) A phase 2..study of evorpacept (Evo) with trastuzumab
52. Advani R et al (2018) CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma
53. Whitelaw BL et al (2020) Adaptive venom evolution and toxicity in octopods
54. Khalifa SAM et al (2019) Marine natural products: a source of novel anticancer drugs
55. Cooke IR et al (2015) Toxicity in cephalopods
56. von Reumont BM et al (2014) Quo Vadis Venomics? A roadmap to neglected venomous invertebrates
57. El-Seedi HR et al (2025) Marine natural products as a source of novel anticancer drugs: an updated review
58. Imran MAS et al (2023) Insights into Common Octopus (Octopus vulgaris) Ink Proteome
59. Cornet V et al (2014) Dual role of the cuttlefish salivary proteome
60. Ford L (1992) Host defense mechanisms of cephalopods
61. Pérez-Polo S et al (2023) Identifying natural bioactive peptides from the common octopus..skin mucus
62. Chaisakul J et al (2016) Effects of animal venoms and toxins on hallmarks of cancer
63. Karthigayan S et al (2006) Anticancer principles from salivary gland extract of Octopus ageina
64. Librizzi M et al (2023) Natural anticancer peptides from marine animal species
65. Agüero-Chapin G et al (2024) Unveiling encrypted antimicrobial peptides from cephalopods' salivary glands
66. Borkakoti N, Thornton JM (2023) AlphaFold2..Implications for drug discovery
67. Lyu J et al (2024) Prospective assessment of AlphaFold2 predictions for structure-based drug discovery
68. Comeau SR et al (2004) ClusPro: a fully automated algorithm for protein–protein docking
69. Zhang XJ et al (2025) HawkDock version 2: an updated web server to predict and analyze the structures of protein–protein complexes
70. Chu L-S et al (2024) Flexible protein–protein docking with a multitrack iterative transformer
71. Hou TJ et al (2002) Empirical Aqueous Solvation Models Based on Accessible Surface Areas with Implicit Electrostatics
72. Sun HY et al (2014) Assessing the performance of MM/PBSA and MM/GBSA methods. 4. Accuracies of MM/PBSA and MM/GBSA methodologies evaluated by various simulation protocols using PDBbind data set
73. Wang E et al (2020) VAD-MM/GBSA: A Variable Atomic Dielectric MM/GBSA Model for Improved Accuracy in Protein–Ligand. Binding Free Energy Calculations

74. Liu X et al (2019) Accurate and Efficient Calculation of Protein-Protein Binding Free Energy-Interaction Entropy. with Residue Type-Specific Dielectric Constants

Figures

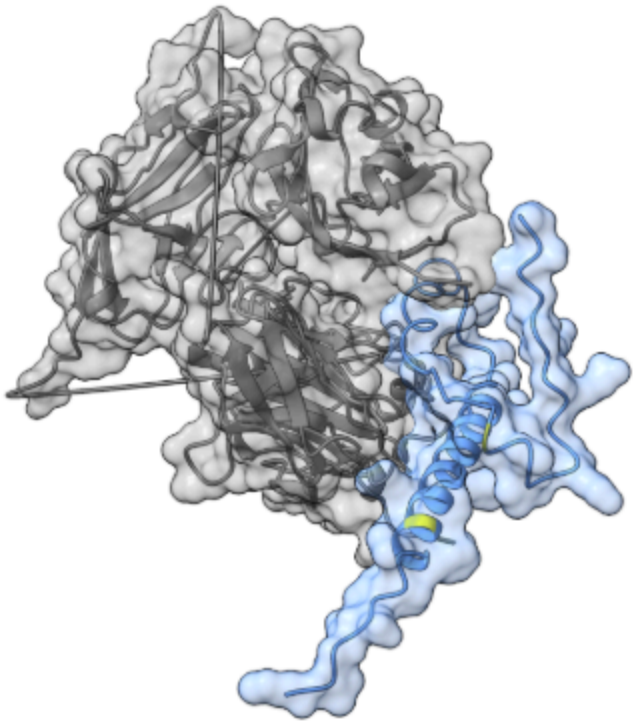


Figure 1

The predicted binding mode of the engineered protein AOA_UR2_F7P_M16C with human CD47. The image shows the lowest energy docked conformation as predicted by the HawkDock server. The engineered ligand, AOA_UR2_F7P_M16C (shown in blue), forms a stable, high-affinity complex with its target, the extracellular domain of human CD47 (shown in gray). The key interacting residues at the binding interface, including Threonine-59 and Phenylalanine-12 on the ligand, are highlighted in yellow, illustrating the specific molecular interactions that drive the protein's potent inhibitory function.