Scalable Ligand-Receptor Binding Affinity Landscape: A Case Study with Ziconotide and $Ca_V2.2$

Wei Li *

Posted Date: 11 September 2024

doi: 10.20944/preprints202409.0877.v1

Keywords: Ligand-Receptor Binding Affinity; ziconotide; CaV2.2; Structural Biophysics; Peptide Design

 doi:10.20944/preprints202409.0877.v1

Article

Scalable Ligand-Receptor Binding Affinity Landscape: A Case Study with ziconotide and $Ca_V2.2$

Wei Li

Contrebola Institute of Computational Interstructural Biophysics, No. 88, Fuxing East Road, Nantong City 226000, Jiangsu Province, People's Republic of China

Abstract: The optimization of ligand-receptor binding affinities is essential for enhancing therapeutic efficacy and specificity, particularly in the design of peptide-based drugs. ziconotide, a potent blocker of the N-type calcium channel $Ca_V2.2$, has demonstrated therapeutic potential in the treatment of moderate to severe chronic pain. However, there is limited structural and binding affinity data available for scaling the design of ziconotide analogues with improved efficacy and specificity. In this study, I present a structural biophysics-based approach to develop a scalable in silico framework for generating and analyzing ligand-receptor binding affinity landscapes, focusing on ziconotide and its receptor, $Ca_V2.2$. This framework integrates advanced computational structural tools and binding affinity calculations to produce high-accuracy structural and intermolecular binding data. The insights gained from this scalable approach will guide the design of ziconotide analogues with enhanced efficacy, offering a powerful computational workflow for high-throughput ligand optimization. Additionally, when combined with artificial intelligence (AI) algorithms, this computational workflow generates high-accuracy structural and biophysical data, towards an AI- and structural biophysics-driven paradigm shift in peptide discovery and design.

Keywords: Ligand-Receptor Binding Affinity, ziconotide, $Ca_V2.2$, Structural Biophysics; Peptide Design

1. Introduction

The design of peptide-based drugs, particularly in the context of ion channel blockers such as ziconotide, has emerged as a promising therapeutic avenue for the treatment of chronic pain [1–3]. ziconotide, a selective blocker of the N-type $Ca_V2.2$ calcium channel, has been clinically validated for its potent analgesic effects, offering an alternative to traditional opioid therapies [4]. Nonetheless, limitations such as narrow therapeutic windows and specificity [1,5] have necessitated the development of next-generation analogues that retain efficacy while minimizing side effects. Central to these efforts is a deeper understanding of the molecular structural interactions that govern the binding affinity between ziconotide and $Ca_V2.2$ [6–9].

To date, drug discovery and design remains a complex multiparameter optimization challenge [10,11]. For instance, the optimization of ligand-receptor binding affinity has relied heavily on experimental approaches such as mutagenesis, crystallography, and biophysical assays [12]. However, these experimental methods are time-consuming, labor-intensive, and often limited in their ability to explore a wide range of analogues and binding conformations [13]. To address these limitations, computational approaches have become increasingly important, providing a high-throughput alternative to experimental workflows. In particular, the advent of in silico methods for generating binding affinity landscapes has opened new possibilities for the rational design of drugs, enabling the exploration of structural modifications with unprecedented efficiency and reasonable accuracy [14].

Here, this article presents a scalable in silico framework for generating and analyzing ligand-receptor binding affinity landscapes [15], with a specific focus on ziconotide and its receptor, $Ca_V2.2$. By systematically exploring the sequence space at the binding interface between ziconotide and $Ca_V2.2$, this article aims to build a scalable ziconotide-$Ca_V2.2$ binding affinity landscape for the design of ziconotide analogues with overall improved therapeutic efficacy and specificity.

 doi:10.20944/preprints202409.0877.v1

2. Materials and Methods

Ziconotide is a synthetic version of a peptide found in the venom of a marine snail, *Conus magus* [4]. Specifically, it is a peptide consisting of 25 amino acids [5]. As of September 11, 2024, there is a total of three ziconotide-related structures in the Protein Data Bank (PDB) [16,17], as listed in Table 1.

Table 1. Experimentally determined ziconotide-related structures in PDB as of September 11, 2024, QUERY code: QUERY: Full Text = "ziconotide".

PDB ID	Structure Title (release date from newest to oldest)
7MIX [6,7]	Human N-type voltage-gated calcium channel $Ca_V2.2$ in the presence of ziconotide at 3.0 Angstrom resolution
7MIY [6,7]	Human N-type voltage-gated calcium channel $Ca_V2.2$ at 3.1 Angstrom resolution
7VFU [8,9]	Human N-type voltage gated calcium channel $Ca_V2.2$-α2/δ1-β1 complex, bound to ziconotide

Among the three, there are two ziconotide-$Ca_V2.2$ complex structures with PDB IDs 7MIX [6,7] and 7VFU [8,9]. Despite the differences in their resolutions, both ziconotide-$Ca_V2.2$ complex structures are determined experimentally with Cryo-EM [6,7,18] and deposited in Protein Data Bank with a standardized data format for biomolecular structures, making them both suitale to be used as starting structural templates for the scalable structural biophysical workflow [15,19–21]. Thus, this study here chooses PDB entry 7MIX (Figure 1) [6,7]) as a structural template to define and build a scalable ligand-receptor binding affinity (K_d) landscape [15] with ziconotide-$Ca_V2.2$ complex structure as an example.

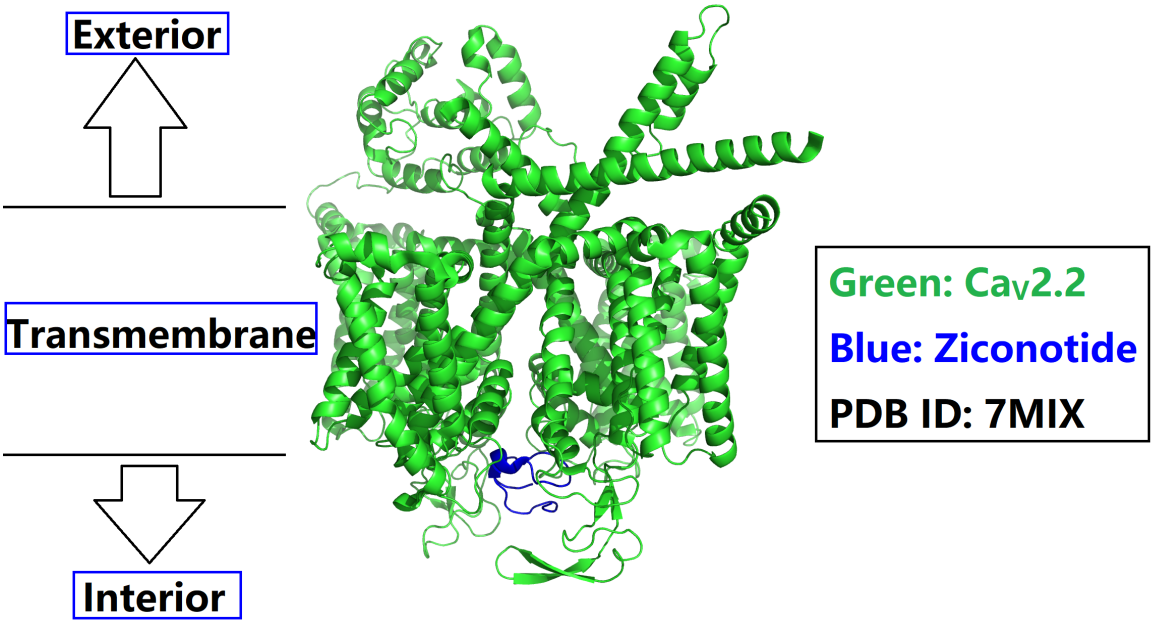

Figure 1. An overall structure of the ziconotide-$Ca_V2.2$ complex structure with PDB ID 7MIX. In this figure, Exterior and Interior represent the extracellular and intracellular region with respect to the ziconotide-$Ca_V2.2$ complex structure, while Transmembrane represents the transmembrane region of the ziconotide-$Ca_V2.2$ complex structure.

With PDB entry 7MIX [6,7] (Table 1) as an initial input, subsequent structural modeling (Modeller) [19] and physics-based K_d calculations (Prodigy) [20,21] consists of an automated in silico generation of synthetic homology structural and K_d data, as illustrated in Figure 2 and described previously in

 doi:10.20944/preprints202409.0877.v1

detail [14]. Briefly, Modeller [19] was employed to build a total of 500 (25 × 20) homology structural models one site-specific missense mutation introduced to the amino acid sequence of ziconotide (PDB entry 7MIX [6,7]).

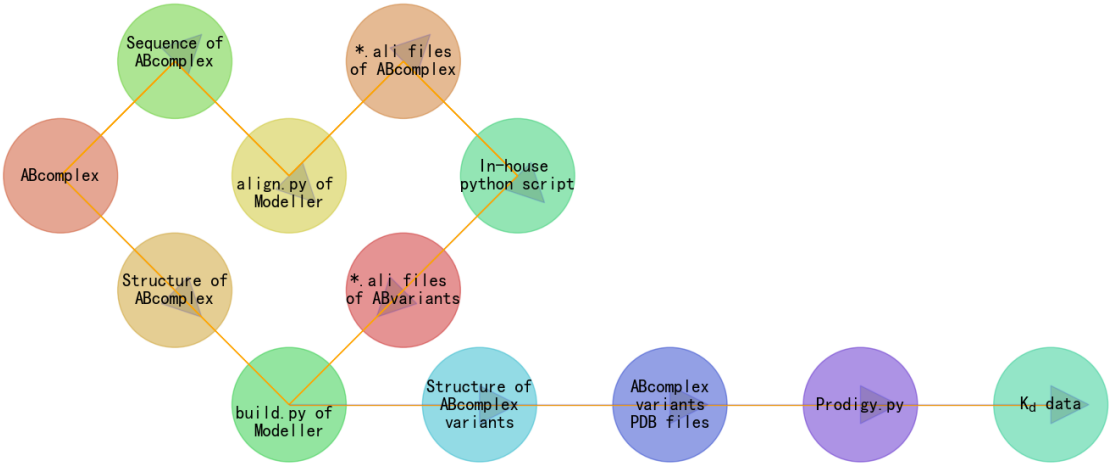

Figure 2. A scalable Modigy (Modeller + Prodigy) workflow for automated in silico generation [14] of synthetic structural (Modeller [19]) and K_d (Prodigy [20,21]) data with reasonable accuracy [15].

Afterwards, the binding affinities were calculated using Prodigy [20,21] for all structural models of ziconotide-$Ca_V2.2$ complex analogues. With PDB entry 7MIX [6,7] as template, all structural modeling [19] and physics-based K_d calculations [20,21] were repeated thirty times (500 × 30) on Wuxi Taihu Lake High Performance Computing platform.

3. Results

3.1. A Scalable Ziconotide-$Ca_V2.2$ Binding Affinity Landscape Based on Computational Structural Biophysics

With Modeller [19] and Prodigy [20,21], a set of structural physics-based calculations were performed for the native experimental ziconotide-$Ca_V2.2$ complex structure PDB entry 7MIX. As shown in Figure 3, the K_d between native ziconotide and $Ca_V2.2$ is 4.8×10^{-8} M (vertical red line in Figure 3), while the K_d values ziconotide analogues and $Ca_V2.2$ possess a much wider distribution, ranging from 4.6×10^{-6} M to 5.0×10^{-9} M, according to the structural biophysics-based Prodigy [20,21] calculations of the 30 × 500 homology structural models of $Ca_V2.2$ bound to ziconotide analogues.

 doi:10.20944/preprints202409.0877.v1

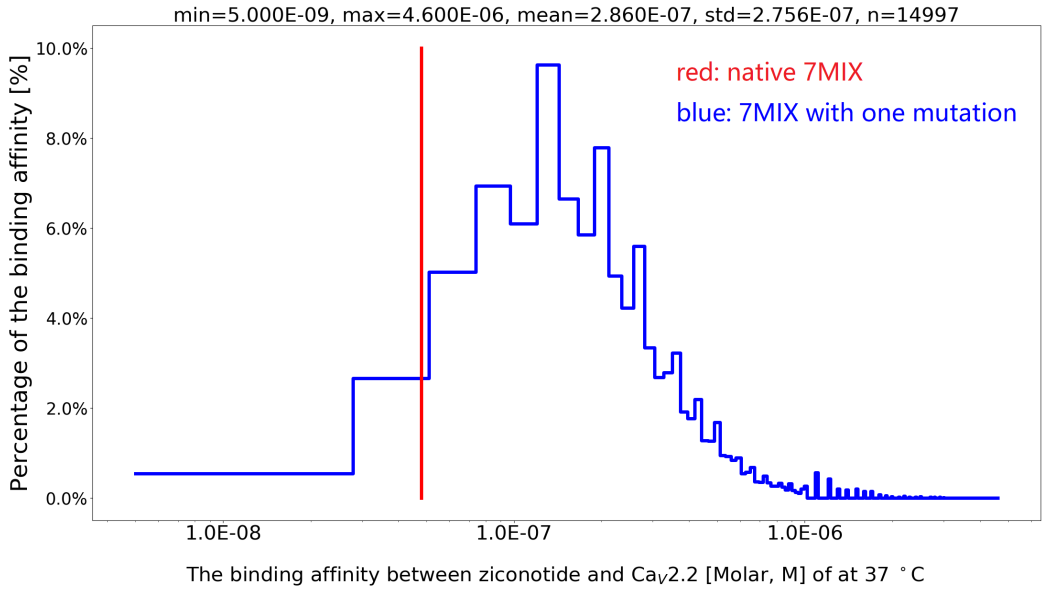

Figure 3. A histogram of the distribution pattern of the ziconotide-$Ca_V2.2$ binding affinities of PDB entry 7MIX with one site-specific missense mutation introduced to the sequence of ziconotide. The vertical red line marks the K_d of the native ziconotide-$Ca_V2.2$ complex structure of PDB entry 7MIX.

In short, this study starts from an experimental ziconotide-$Ca_V2.2$ complex structure (PDB entry 7MIX) to build a scalable ziconotide-$Ca_V2.2$ binding affinity landscape based on computational structural biophysics (Figure 3). This ziconotide-$Ca_V2.2$ binding affinity landscape is scalable because:

1. the Modigy (Figure 1) workflow [14] is applicable to biomolecular structure databases such as PDB [16] and AFDB [22–25].
2. the Modigy (Figure 1) workflow [14] introduced only one site-specific missense mutation to the sequence of ziconotide (PDB entry 7MIX), where the number could be larger, provided that the overall accuracy is reasonable for the synthetic structural and biophysical data [14].
3. the ziconotide-$Ca_V2.2$ binding affinity landscape (Figure 3) includes only site-specific mutants of ziconotide, but not site-specific mutants of the receptor, i.e., $Ca_V2.2$, highlighting the use of this in silico workflow [14] in high-throughput generation of synthetic structural and biophysical data for other drug targets (GPCRs [26], ion channels [27], etc.) to train AI models for the discovery and design [28] of not just peptides, but also of small molecule compounds [29,30].
4. method-wise, in addition to the structural modeling [19] and physics-based K_d calculations [20,21] employed here, this Modigy (Figure 1) workflow [14] is also able to integrate molecular dynamics simulations [19,31–34] to further enhance the accuracy of the structural biophysics-based K_d calculations [13,28,35] in drug discovery and design [10–12,36–38].

3.2. Designing Ziconotide Analogues with Over Two Orders of Magnitude Enhanced $Ca_V2.2$ Affinity

As described in the Materials and Methods section as above, the key feature of this Modigy workflow [14] lies in its scalability, allowing the generation of homology models for a wide array of site-specific point mutations. This capability is critical in scenarios where the computational design of therapeutic peptides demands extensive exploration of the mutation landscape to optimize therapeutic properties such as improved efficacy and specificity [39–41].

Here, in this article, with an automated in silico generation of synthetic structural and K_d data [14], a huge set of ziconotide analogues were designed with the scalable Modigy (Figure 1) workflow [14]. After a ranking of the ligand-receptor K_d values, five ziconotide analogues (Table 2) stood out, with binding affinity to $Ca_V2.2$ ranging from 1.4×10^{-10} M to 1.9×10^{-10} M, according to the structural biophysics-based Prodigy [20,21] calculations of homology structural models of $Ca_V2.2$ bound to ziconotide analogues with five site-specific mutations introduced to its amino acid sequence. Compared

 doi:10.20944/preprints202409.0877.v1

to the K_d between native ziconotide and $Ca_V2.2$ is 4.8 $\times$ 10^{-8} M (vertical red line in Figure 3), the binding affinities between the five ziconotide analogues (Table 2) and $Ca_V2.2$ are at least increased over two orders of magnitude of ligand-receptor binding affinity.

Table 2. Inter-chain binding affinities calculated by the Prodigy server [20,21] for the ziconotide-$Ca_V2.2$ complex structural models, including native ziconotide and ziconotide analogues with five site-specific mutations introduced to its amino acid sequence.

Design of ziconotide analogues	Inter-chain K_d (M) at 37 °C	Supplementary file
Native (PDB entry 7MIX)	4,8 $\times$ 10^{-8}	PDB entry 7MIX
G18B_Y, G3B_Y, C1B_R, S9B_R, S19B_K	1.4 $\times$ 10^{-10}	zic1.pdb
G3B_W, S22B_W, G18B_W, C1B_H, S19B_K	1.4 $\times$ 10^{-10}	zic2.pdb
G3B_W, G18B_W, S19B_R, C1B_W, S9B_H	1.5 $\times$ 10^{-10}	zic3.pdb
G18B_Y, G3B_W, C1B_H, S9B_K, S22B_E	1.9 $\times$ 10^{-10}	zic4.pdb
G18B_Y, S22B_W, S19B_R, G3B_Y, C1B_H	1.9 $\times$ 10^{-10}	zic5.pdb

4. Conclusion and Discussion

To sum up, this study reports a scalable ziconotide-$Ca_V2.2$ binding affinity landscape based on computational structural biophysics, with a structural biophysical workflow [14] integrating structural modeling [19] and physics-based K_d calculations [20,21], tailored for computational drug design and discovery [15]. In addition, the scalable workflow [14] described here presents a technically feasible method for generating synthetic structural and biophysical data, which is of use for enhancing the specificity and efficacy of therapeutic peptides [28,42].

Overall, this scalable synthetic structural and biophysics data serve two purposes: (1), this scalable Modigy (Figure 1) workflow [14] creates a scalable antigen-antibody binding affinity landscape, which acts like a map to guide the design of peptides with improved efficacy and specificity [43,44]; (2), this scalable Modigy (Figure 1) workflow [14] generates useful training data [45,46] for AI-driven drug design (AIDD, Figure 1) models [10,11,28] towards the design of both biomolecule (e.g., peptide) [43,44] and small molecule compounds (Figure 1) with improved efficacy and specificity.

4.1. Implications for Peptide Design and Future Directions

The findings of this study underscore the importance of a precise understanding of ligand-receptor binding dynamics in the structural context of therapeutic peptide design [47]. As ziconotide and its analogues continue to be explored for pain management, the ability to fine-tune their binding affinity to $Ca_V2.2$ offers significant promise for improving their safety and efficacy profiles, based on the scalable ziconotide-$Ca_V2.2$ binding affinity landscape based on computational structural biophysics [14]. This scalable framework not only addresses current limitations in experimental throughput but also paves the way for more sophisticated computational peptide design and discovery pipelines.

Moreover, this work contributes also to the broader application of AI-driven drug discovery platforms. While the current framework relies on structural biophysics and binding affinity calculations [20,21], the integration of machine learning models trained on synthetic binding affinity data could accelerate the identification and discovery of optimal ligand-receptor pairs. Furthermore, expanding this approach to other receptor targets, particularly those in complex neural and pain-signaling pathways, could lead to breakthroughs in peptide and protein drug design and discovery in future.

 doi:10.20944/preprints202409.0877.v1

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, W.L.; methodology, W.L.; software, W.L.; validation, W.L.; formal analysis, W.L.; investigation, W.L.; resources, W.L.; data duration, W.L.; writing–original draft preparation, W.L.; writing–review and editing, W.L.; visualization, W.L.; supervision, W.L.; project administration, W.L.; funding acquisition, not applicable.

Funding: This research received no external funding.

Institutional Review Board Statement: No ethical approval is required.

Acknowledgments: I am grateful to the communities of structural biology, biophysics, medicinal and computational chemistry and algorithm design, for the continued accumulation of knowledge and data for drug discovery & design, and for the continued development of tools (hardware, software and algorithm) for drug discovery & design.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: During the preparation of this work, the author used OpenAI's ChatGPT in order to improve the readability of the manuscript, and to make it as concise and short as possible. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Deer, T.R.; Pope, J.E.; Hanes, M.C.; McDowell, G.C. Intrathecal Therapy for Chronic Pain: A Review of Morphine and Ziconotide as Firstline Options. *Pain Medicine* **2018**, *20*, 784–798. doi:10.1093/pm/pny132.
2. Rubiu, E.; Restelli, F.; Nazzi, V.; Mazzapicchi, E.; Bonomo, G.; Veiceschi, P.; Alfiero, T.; Agresta, G.; Locatelli, D.; Dario, A. A Benefit/Risk Assessment of Intrathecal Ziconotide in Chronic Pain: A Narrative Review. *Journal of Clinical Medicine* **2024**, *13*, 1644. doi:10.3390/jcm13061644.
3. Gomez, K.; Santiago, U.; Nelson, T.S.; Allen, H.N.; Calderon-Rivera, A.; Hestehave, S.; Rodríguez Palma, E.J.; Zhou, Y.; Duran, P.; Loya-Lopez, S.; Zhu, E.; Kumar, U.; Shields, R.; Koseli, E.; McKiver, B.; Giuvelis, D.; Zuo, W.; Inyang, K.E.; Dorame, A.; Chefdeville, A.; Ran, D.; Perez-Miller, S.; Lu, Y.; Liu, X.; Handoko.; Arora, P.S.; Patek, M.; Moutal, A.; Khanna, M.; Hu, H.; Laumet, G.; King, T.; Wang, J.; Damaj, M.I.; Korczeniewska, O.A.; Camacho, C.J.; Khanna, R. A peptidomimetic modulator of the $Ca_V2.2$ N-type calcium channel for chronic pain. *Proceedings of the National Academy of Sciences* **2023**, *120*. doi:10.1073/pnas.2305215120.
4. Lin, J.; Chen, S.; Butt, U.D.; Yan, M.; Wu, B. A comprehensive review on ziconotide. *Heliyon* **2024**, *10*, e31105. doi:10.1016/j.heliyon.2024.e31105.
5. Wermeling, D.P. Ziconotide, an Intrathecally Administered N-Type Calcium Channel Antagonist for the Treatment of Chronic Pain. *Pharmacotherapy* **2005**, *25*, 1084–1094. doi:10.1592/phco.2005.25.8.1084.
6. Gao, S.; Yao, X.; Yan, N. Structure of human Cav2.2 channel blocked by the painkiller ziconotide. *Nature* **2021**, *596*, 143–147. doi:10.1038/s41586-021-03699-6.
7. Yan, N.; Gao, S.; Yao, X. Human N-type voltage-gated calcium channel Cav2.2 in the presence of ziconotide at 3.0 Angstrom resolution, 2021. doi:10.2210/pdb7mix/pdb.
8. Dong, Y.; Gao, Y.; Wang, Y.; Zhao, Y. Human N-type voltage gated calcium channel CaV2.2-alpha2/delta1-beta1 complex, bound to ziconotide, 2021. doi:10.2210/pdb7vfu/pdb.
9. Dong, Y.; Gao, Y.; Xu, S.; Wang, Y.; Yu, Z.; Li, Y.; Li, B.; Yuan, T.; Yang, B.; Zhang, X.C.; Jiang, D.; Huang, Z.; Zhao, Y. Closed-state inactivation and pore-blocker modulation mechanisms of human CaV2.2. *Cell Reports* **2021**, *37*, 109931. doi:10.1016/j.celrep.2021.109931.
10. van de Waterbeemd, H.; Smith, D.A.; Beaumont, K.; Walker, D.K. Property-Based Design: Optimization of Drug Absorption and Pharmacokinetics. *Journal of Medicinal Chemistry* **2001**, *44*, 1313–1333.
11. Pennington, L.D.; Hesse, M.J.; Koester, D.C.; McAtee, R.C.; Qunies, A.M.; Hu, D.X. Property-Based Drug Design Merits a Nobel Prize. *Journal of Medicinal Chemistry* **2024**.
12. Li, W. Inter-molecular Binding Affinity Synthetic Data Augmentation Transforms the Landscape of Computational Biomolecule Design and Discovery **2024**. doi:10.20944/preprints202408.1713.v1.
13. Towards a purely physics-based computational binding affinity estimation. *Nature Computational Science* **2023**, *3*, 10–11. doi:10.1038/s43588-023-00396-4.
14. Li, W. In Silico Generation of Structural and Intermolecular Binding Affinity Data with Reasonable Accuracy: Expanding Horizons in Drug Discovery and Design **2024**. doi:10.20944/preprints202405.1739.v1.

 doi:10.20944/preprints202409.0877.v1

15. Li, W. Scalable Antigen-Antibody Binding Affinity Landscape: A Case Study with ENHERTU **2024**. doi:10.1101/2024.07.12.603351.
16. Berman, H.; Henrick, K.; Nakamura, H. Announcing the worldwide Protein Data Bank. *Nature Structural & Molecular Biology* **2003**, *10*, 980–980.
17. Subramaniam, S.; Kleywegt, G.J. A paradigm shift in structural biology. *Nature Methods* **2022**, *19*, 20–23.
18. Ruedas, R.; Vuillemot, R.; Tubiana, T.; Winter, J.M.; Pieri, L.; Arteni, A.A.; Samson, C.; Jonic, S.; Mathieu, M.; Bressanelli, S. Structure and conformational variability of the HER2-trastuzumab-pertuzumab complex. *Journal of Structural Biology* **2024**, *216*, 108095.
19. Webb, B.; Sali, A. Protein Structure Modeling with MODELLER. In *Methods in Molecular Biology*; Springer US, 2020; pp. 239–255.
20. Vangone, A.; Bonvin, A.M. Contacts-based prediction of binding affinity in protein-protein complexes. *eLife* **2015**, *4*.
21. Xue, L.C.; Rodrigues, J.P.; Kastritis, P.L.; Bonvin, A.M.; Vangone, A. PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. *Bioinformatics* **2016**, p. btw514.
22. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; Bridgland, A.; Meyer, C.; Kohl, S.A.A.; Ballard, A.J.; Cowie, A.; Romera-Paredes, B.; Nikolov, S.; Jain, R.; Adler, J.; Back, T.; Petersen, S.; Reiman, D.; Clancy, E.; Zielinski, M.; Steinegger, M.; Pacholska, M.; Berghammer, T.; Bodenstein, S.; Silver, D.; Vinyals, O.; Senior, A.W.; Kavukcuoglu, K.; Kohli, P.; Hassabis, D. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021**, *596*, 583–589.
23. Tong, A.B.; Burch, J.D.; McKay, D.; Bustamante, C.; Crackower, M.A.; Wu, H. Could AlphaFold revolutionize chemical therapeutics? *Nature Structural & Molecular Biology* **2021**, *28*, 771–772.
24. Ruff, K.M.; Pappu, R.V. AlphaFold and Implications for Intrinsically Disordered Proteins. *Journal of Molecular Biology* **2021**, p. 167208.
25. Higgins, M.K. Can We AlphaFold Our Way Out of the Next Pandemic? *Journal of Molecular Biology* **2021**, p. 167093.
26. Zhang, M.; Chen, T.; Lu, X.; Lan, X.; Chen, Z.; Lu, S. G protein-coupled receptors (GPCRs): advances in structures, mechanisms, and drug discovery. *Signal Transduction and Targeted Therapy* **2024**, *9*.
27. Tsien, R.W.; Hess, P.; McCleskey, E.W.; Rosenberg, R.L. Calcium channels: Mechanisms of Selectivity, Permeation, and Block. *Annual Review of Biophysics and Biophysical Chemistry* **1987**, *16*, 265–290.
28. Li, W.; Vottevor, G. Towards a Truly General Intermolecular Binding Affinity Calculator for Drug Discovery & Design **2023**.
29. Li, W.; Shi, G. How $Ca_V1.2$-bound verapamil blocks Ca^{2+} influx into cardiomyocyte: Atomic level views. *Pharmacological Research* **2019**, *139*, 153–157.
30. Li, W. Calcium Channel Trafficking Blocker Gabapentin Bound to the α-2-δ-1 Subunit of Voltage-Gated Calcium Channel: A Computational Structural Investigation **2020**.
31. Li, W. Characterising the interaction between caenopore-5 and model membranes by NMR spectroscopy and molecular dynamics simulations. PhD thesis, University of Auckland, 2016.
32. Du, X.; Li, Y.; Xia, Y.; Ai, J.; Wu, Y. Molecular dynamics simulations and free energy calculations of protein–ligand interactions: recent advances and future perspectives. *Current pharmaceutical design* **2017**, *23*, 4436–4450.
33. Jalily Hasani, H.; Barakat, K. Homology Modeling: an Overview of Fundamentals and Tools. *International Review on Modelling and Simulations (IREMOS)* **2017**, *10*, 129. doi:10.15866/iremos.v10i2.11412.
34. Muhammed, M.T.; Aki-Yalcin, E. Homology modeling in drug discovery: Overview, current applications, and future perspectives. *Chemical Biology & Drug Design* **2018**, *93*, 12–20. doi:10.1111/cbdd.13388.
35. Wan, S.; Bhati, A.P.; Zasada, S.J.; Coveney, P.V. Rapid, accurate, precise and reproducible ligand–protein binding free energy prediction. *Interface Focus* **2020**, *10*, 20200007. doi:10.1098/rsfs.2020.0007.
36. Gupta, R.; Srivastava, D.; Sahu, M.; Tiwari, S.; Ambasta, R.K.; Kumar, P. Artificial intelligence to deep learning: machine intelligence approach for drug discovery. *Molecular Diversity* **2021**, *25*, 1315–1360.
37. Trosset, J.Y.; Cavé, C. In Silico Drug-Target Profiling. In *Target Identification and Validation in Drug Discovery*; Springer New York, 2019; pp. 89–103.
38. Tinberg, C.E.; Khare, S.D.; Dou, J.; Doyle, L.; Nelson, J.W.; Schena, A.; Jankowski, W.; Kalodimos, C.G.; Johnsson, K.; Stoddard, B.L.; Baker, D. Computational design of ligand-binding proteins with high affinity and selectivity. *Nature* **2013**, *501*, 212–216.

 doi:10.20944/preprints202409.0877.v1

39. Pierce, B.G.; Hellman, L.M.; Hossain, M.; Singh, N.K.; Vander Kooi, C.W.; Weng, Z.; Baker, B.M. Computational Design of the Affinity and Specificity of a Therapeutic T Cell Receptor. *PLoS Computational Biology* **2014**, *10*, e1003478. doi:10.1371/journal.pcbi.1003478.
40. Kilar, C.R.; Sekharan, S.; Sautina, L.; Diao, Y.; Keinan, S.; Shen, Y.; Bungert, J.; Mohandas, R.; Segal, M.S. Computational design and experimental characterization of a novel β-common receptor inhibitory peptide. *Peptides* **2018**, *104*, 1–6. doi:10.1016/j.peptides.2018.04.001.
41. Bolon, D.N.; Grant, R.A.; Baker, T.A.; Sauer, R.T. Specificity versus stability in computational protein design. *Proceedings of the National Academy of Sciences* **2005**, *102*, 12724–12729. doi:10.1073/pnas.0506124102.
42. Xu, D.; Zhang, Y. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. *Biophys. J.* **2011**, *101*, 2525–2534.
43. Jin, Y.; Schladetsch, M.A.; Huang, X.; Balunas, M.J.; Wiemer, A.J. Stepping forward in antibody-drug conjugate development. *Pharmacology & Therapeutics* **2022**, *229*, 107917.
44. Kang, J.C.; Sun, W.; Khare, P.; Karimi, M.; Wang, X.; Shen, Y.; Ober, R.J.; Ward, E.S. Engineering a HER2-specific antibody–drug conjugate to increase lysosomal delivery and therapeutic efficacy. *Nature Biotechnology* **2019**, *37*, 523–526.
45. Liu, T.; Lin, Y.; Wen, X.; Jorissen, R.N.; Gilson, M.K. BindingDB: a web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Research* **2007**, *35*, D198–D201.
46. Wang, R.; Fang, X.; Lu, Y.; Yang, C.Y.; Wang, S. The PDBbind Database: Methodologies and Updates. *Journal of Medicinal Chemistry* **2005**, *48*, 4111–4119.
47. Vanhee, P.; van der Sloot, A.M.; Verschueren, E.; Serrano, L.; Rousseau, F.; Schymkowitz, J. Computational design of peptide ligands. *Trends in Biotechnology* **2011**, *29*, 231–239. doi:10.1016/j.tibtech.2011.01.004.