

Structure-Based Molecular Docking Study of a Designed Cationic Peptide Sequence KRLVAGLIRKLA Against Targeting the Extracellular Domain of HER2 in HER2-Positive Breast Cancer

Sajida Gull*

Department of Chemistry, Faculty of Science, The Superior University, Lahore, Pakistan

Dr. Rashid Mahmood

Department of Chemistry, Faculty of Science, The Superior University, Lahore, Pakistan

ABSTRACT

Overexpression of the human epidermal growth factor receptor 2 (HER2) protein occurs in a significant number of breast cancers and serves as a validated therapeutic target but suffers from the problems of resistance, expense and toxicity when using an antibody for this purpose. Peptides present a more compact, economical alternative. In this study, a 12-residue cationic peptide, KRLVAGLIRKLA (charge +4), was studied as a ligand of the extracellular domain of HER2 (PDB ID 3MZW) through molecular docking with AutoDock 4.2.6 in 100 runs using the Lamarckian genetic algorithm. These 100 runs were organized into 60 clusters of conformation and had estimated binding energies between -9.82 and -3.15 kcal/mol (average -6.07 ± 1.42 kcal/mol). The most favorable pose (run 58; -9.82 kcal/mol; predicted K_i 63.7 nM) came from the biggest cluster (10 runs) and formed two salt bridges (Lys1–Asp395 and Lys10–Glu373) and three directional hydrogen bonds, interacting with 98 atom pairs within 4 Å of 13 HER2 residues. . While van der Waals, hydrogen bonds, and desolvation energy accounted for much more of the variance in the score compared to electrostatics (Spearman $\rho = 0.79$ versus 0.61), compared to the 26-amino-acid binding area of the affibody Z(HER2:342) crystallized in complex, the top-scoring docking poses were located towards the edge of this binding area and shared only four amino acids with it. In conclusion, the docking results support peptide binding in the vicinity of the affibody side of HER2 but do not provide any evidence of the specific and stable binding.

Keywords: HER2; Breast Cancer; Therapeutic Peptide; Molecular Docking; Autodock; Affibody; Structure-Based Drug Design

Introduction

Breast cancer continues to be one of the major causes of cancer diagnosis and death among women globally, and the problem is expected to persist [1]. Approximately 15-20% of cases of breast cancer have been found to overexpress human epidermal growth factor receptor 2 (HER2/ERBB2), which is a receptor tyrosine kinase with

amplification associated with rapid tumor growth and an unfavorable prognosis [2]. HER2 homodimerization and heterodimerization, which are not ligand-dependent, activate downstream signaling cascades including PI3K/AKT and MAPK pathways [2,3].

Drugs based on antibodies such as trastuzumab and pertuzumab that target different epitopes of the HER2 ECD [4] and antibody-drug conjugates have revolutionized HER2-positive cancers. However, drug resistance, cardiotoxicity, high price and poor penetrability of antibodies to tissues are some factors that inhibit their use. This has led to the exploration of smaller alternatives that do not consist of antibodies.

Peptide therapeutics offer an ideal balance between small molecule and biological agents. They provide high target specificity together with low molecular weight, efficient tissue penetrability and easy chemical synthesis of their structures, and an increasing number of approved peptide drugs is already available [6-8]. Designed peptide sequences based on scaffold engineering are particularly promising for HER2 targets. Affibody Z(HER2:342) shows high affinity for HER2 ECD, and the crystal structure of this interaction (PDB ID 3MZW) was solved [9]. Previous research has led to the identification of HER2-targeting peptides and peptidomimetics using antibody and affibody structures through structural analysis, docking, and molecular dynamics (MD) simulations [10-12].

Structure-based computational approaches make it possible to screen such candidate compounds prior to their synthesis. In the current study, a 12-amino acid peptide, KRLVAGLIRKLA, was designed to be the minimal HER2 binder and docked against the HER2 ECD from 3MZW. This investigation aimed to answer four questions: (i) whether this peptide docks favorably and consistently; (ii) what energy terms and interactions determine the best docking poses; (iii) whether there is any similarity between the docked poses and the affibody footprint of the protein from the crystal structure; and (iv) which docking poses can be investigated further by MD simulations.

Materials and Methods

Target preparation and peptide model

The X-ray structure of the HER2 ECD bound to the affibody Z(HER2:342) (PDB ID: 3MZW) [9] was acquired from the RCSB Protein Data Bank (Figure 1). Crystallization waters, non-protein heteroatoms and duplicated chains were stripped, missing heavy atoms were modeled, hydrogens were added at pH 7.4, and the system was energy-minimized to resolve steric clashing. The affibody binding surface was delineated by the 26 HER2 amino acids whose any atoms were less than 4.0 Å away from the affibody. A linear peptide KRLVAGLIRKLA consisting of 12 residues was constructed (Table 1).

Table 1. Properties of the designed peptide and the docking set-up.

Item	Value
Sequence	Lys-Arg-Leu-Val-Ala-Gly-Leu-Ile-Arg-Lys-Leu-Ala (12 residues)
Molecular weight	about 1,338 Da
Net charge (pH ~7)	+4 (two Lys, two Arg, no Asp/Glu)
Hydrophobic residues	7 of 12 (58%); Kyte–Doolittle GRAVY +0.54
Atoms / heavy atoms; torsions	124 / 94; 15 active torsions
Receptor	HER2 ECD from PDB 3MZW
Search algorithm	Lamarckian genetic algorithm, AutoDock 4.2.6
Runs; evaluations per run	100 runs; 2.5 million energy evaluations each
Clustering tolerance	2.0 Å ligand RMSD
Search box	Centred 2.8 Å from the centre of the affibody footprint

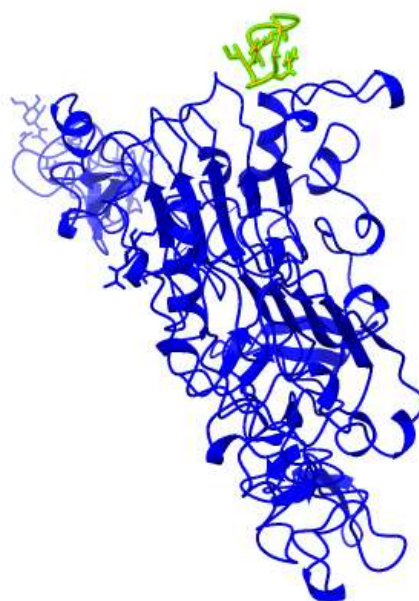


Figure 1. Three-dimensional structure of the HER2 extracellular domain (blue) in complex with the affibody Z(HER2:342) (green), PDB ID 3MZW, used as the docking target.

Molecular docking

Docking was done using AutoDock 4.2.6 [13]. Hundred docking runs employing the Lamarckian genetic algorithm were performed using 2.5 million energy evaluations. The free energy of binding (ΔG) was determined as the sum of intermolecular energy and ligand internal energy plus torsional penalty minus unbound ligand internal energy. Since the unbound ligand is assumed to have the same conformation as the bound ligand, the internal energy terms cancel out and ΔG is equal to the

intermolecular energy plus a constant torsional penalty of 4.47 kcal/mol. Poses were clustered at 2.0 Å RMSD while the clusters were sorted based on lowest energy. Inhibition constants (Ki) were predicted using ΔG at 298 K.

Pose and interaction analysis

The best conformation (run 58) and the lowest energy conformer from each of the ten best clusters were evaluated with geometric analysis. Contacts were made up of atom pairs within 4 Å apart; hydrogen bonds were identified based on the distance and angle between donor–acceptor atoms; salt bridges were identified based on oppositely charged N $\cdots$ O pairs; and hydrophobic contacts were represented by side-chain carbon–carbon pairs within 4.0 Å. Interactions of the crystal affibody were derived from a PLIP [14] report of 3MZW. Ligand efficiency was evaluated as the ratio $-\Delta G/\text{number of heavy atoms}$. Relationship between variables was determined with Spearman rank correlation; significance between groups was performed with Mann–Whitney U test; and confidence intervals for the mean were estimated through bootstrap resampling method. The value of $p < 0.05$ was regarded as significant. Docking results are theoretical predictions and not experimental affinities.

Position of this analysis in the design pipeline

The current docking analysis is carried out using a nine-stage structure-based approach: protein preparation, binding site identification, peptide design based on a template, prediction of peptide structure, molecular docking, MD simulation, trajectory analysis, calculation of the binding free energy with residue-wise decomposition, and in silico peptide property prediction. The design stages are iterative, whereby any problem encountered in one generation of peptides influences the design of the subsequent generation. This work addresses only the docking step of the first designed peptide and serves as a basis for MD simulation; the peptides were neither synthesized nor tested.

Results

Docking energy landscape

For the 100 runs, there were binding free energies that varied from -9.82 to -3.15 kcal/mol (runs 58 and 10, respectively), with a mean of -6.07 ± 1.42 kcal/mol and a median of -5.92 kcal/mol (Table 2, Figure 2). The best run was 2.6 standard deviations below the mean, which was an outlier on the favourable side but not a too strange outlier amongst 100 runs. There were 22 runs scoring at or better than -7 kcal/mol, 10 at or better than -8 kcal/mol, and 6 at or better than -9 kcal/mol; all six of the last group were in one cluster. Energy did not correlate with run number (Spearman $\rho = -0.04$, $p = 0.67$), which is consistent with independent random starting points. Ki values spanned from 63.7 nM (run 58) to 4.9 mM, with a median value of around 45 μM , and 9 runs were below 1 μM . Ki being based on ΔG gives no independent information.

Online ISSN

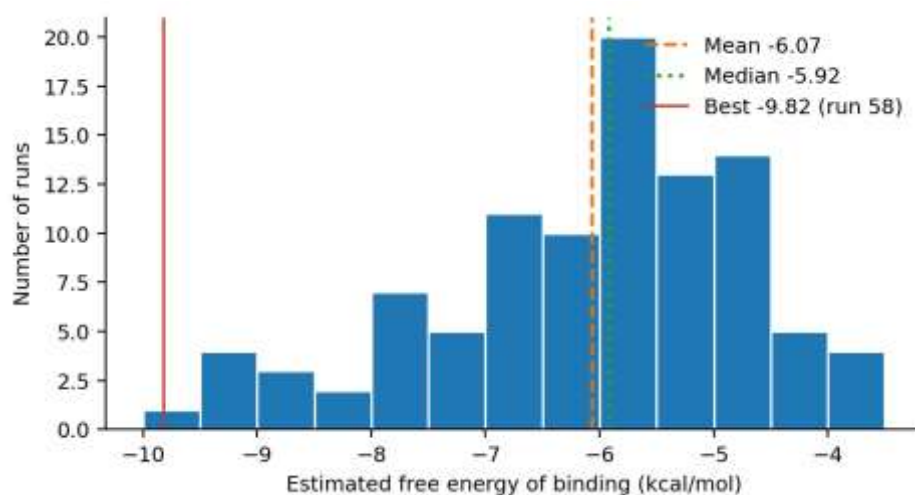
3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>**Table 2.** Summary of the estimated binding free energy (kcal/mol) over 100 docking runs.

Statistic / threshold	Value	Comment
Best (run 58) / worst (run 10)	−9.82 / −3.15	Range of 6.67 kcal/mol
Mean ± SD (95% bootstrap CI)	−6.07 ± 1.42 (−6.34 to −5.79)	Close to the median
Median (quartiles)	−5.92 (−6.66 / −5.08)	Slight tail towards strong binding
Runs ≤ −7 kcal/mol	22 (22%)	Clear lower tail
Runs ≤ −8 kcal/mol	10 (10%)	Six from cluster 1
Runs ≤ −9 kcal/mol	6 (6%)	All from cluster 1
Predicted $K_i < 1 \mu\text{M}$	9 runs	Median K_i about 45 μM

**Figure 2.** Distribution of estimated binding free energies for the 100 docking runs (0.5 kcal/mol bins). Dashed line: mean; dotted line: median; red line: the best run.

Conformational clustering

The 100 structures formed 60 clusters with RMSD of 2.0 Å, with 17 clusters containing more than one run (Figure 3), where 43 clusters were from a single run, which is typical for a flexible molecule with 15 rotatable bonds. The first cluster was the most energetically favorable and the largest (10 runs; mean energy = −8.53 kcal/mol). Inside cluster 1, there were six structures (runs 58, 52, 98, 18, 33, and 20), being 0.8 Å apart from run 58 and having the energy range from −9.82 to −9.00 kcal/mol, as well as four structures being 1.3–1.9 Å apart with energy range of −7.79 to −6.65 kcal/mol. Thus, the difference in geometry costs 1.5–3 kcal/mol, which means that energy of the structure depends on a few particular contacts. The ten members of the cluster 1 are presented in Table 4 and Figure 4, whereas Table 3 gives the most energetically favorable structure from each of the first ten clusters. Energies of clusters 2–7 differed by less than the typical precision of the AutoDock scoring

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

function.

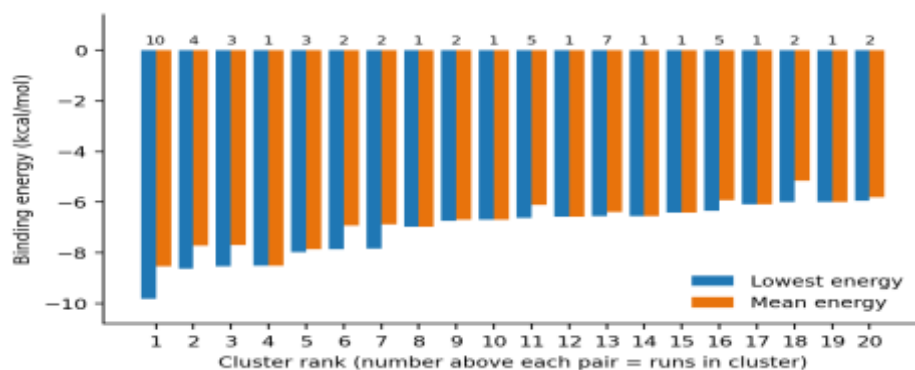


Figure 3. Lowest and mean energy of the 20 best-ranked clusters. The label above each pair gives the number of runs in the cluster.

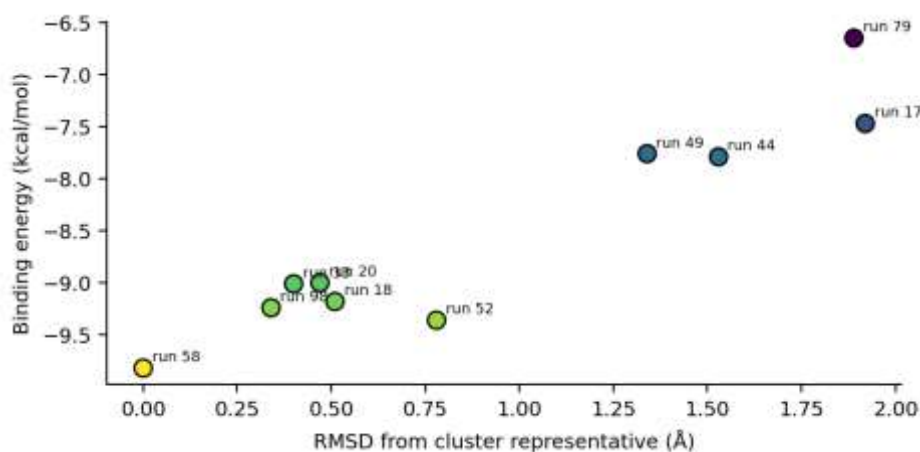


Figure 4. Members of cluster 1: binding energy against RMSD from the cluster lowest-energy member (run 58). Colour also encodes energy.

Table 3. Members of cluster 1 (ten runs).

Run	Rank in cluster	ΔG	RMSD from run 58 (Å)	Predicted Ki	Contacts	Dist. to footprint centre (Å)
58	1	-9.82	0.00	63.7 nM	98	19.1
52	2	-9.36	0.78	137 nM	98	19.0
98	3	-9.24	0.34	169 nM	99	19.0
18	4	-9.18	0.51	188 nM	99	18.9
33	5	-9.01	0.40	250 nM	103	19.0
20	6	-9.00	0.47	253 nM	94	19.1
44	7	-7.79	1.53	1.94 μ M	88	18.9
49	8	-7.76	1.34	2.06 μ M	84	19.4
17	9	-7.47	1.92	3.33 μ M	85	19.0
79	10	-6.65	1.89	13.4 μ M	101	18.9

Table 4. Lowest-energy representative of each of the ten best clusters.

Cluster	Run	Runs	ΔG	Pred. Ki	Contacts	HER2 res.	H-bonds	Salt br.	Dist. (Å)
1	58	10	−9.82	63.7 nM	98	13	3	2	19.1
2	62	4	−8.63	474 nM	108	16	1	2	19.0
3	82	3	−8.54	553 nM	78	9	3	1	17.7
4	96	1	−8.50	592 nM	101	10	2	2	18.0
5	45	3	−7.98	1.41 μ M	87	11	3	2	17.6
6	7	2	−7.85	1.75 μ M	72	10	7	2	18.9
7	91	2	−7.83	1.83 μ M	79	12	1	2	18.8
8	4	1	−6.98	7.65 μ M	84	13	6	2	11.7
9	68	2	−6.73	11.6 μ M	85	13	1	1	7.6
10	86	1	−6.69	12.6 μ M	70	9	3	2	14.5

ΔG in kcal/mol; contacts = atom pairs within 4 Å; Dist. = distance of the pose from the centre of the affibody footprint.

Energy decomposition

The intermolecular energy comprised two terms, namely the van der Waals + hydrogen bond + desolvation and electrostatic terms (Table 5). Both terms were negative in all runs, meaning that the cationic peptide was attracted by HER2 independent of the poses. However, the former term exhibited greater variance (standard deviation 1.13 compared with 0.81 kcal/mol) and correlated more strongly with ΔG (Pearson $r = 0.82$ compared with 0.60; Spearman $\rho = 0.79$ compared with 0.61; Figure 5). The lowest van der Waals term value (−9.84 kcal/mol) was obtained in run 58 among the 100 runs, whereas run 58 ranked 12th regarding the electrostatic term (−4.45 kcal/mol). Thus, the superior performance of run 58 can be attributed primarily to packing effects rather than attractive charge interactions.

Online ISSN

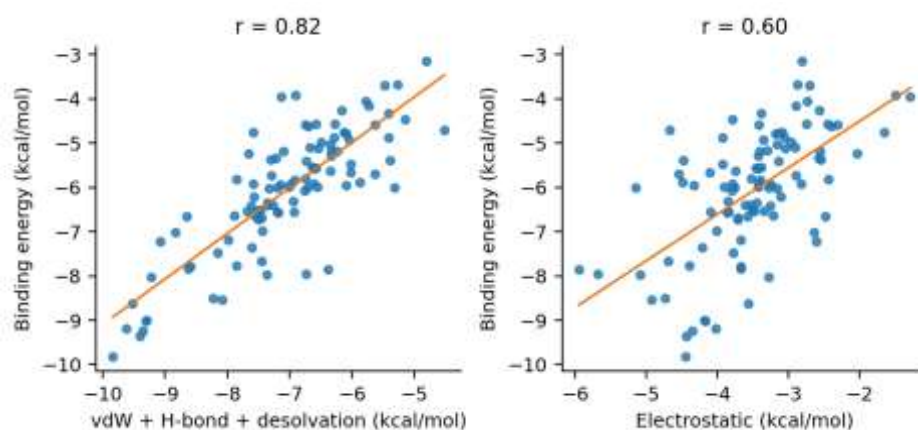
3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>**Table 5.** Energy terms across the 100 runs (kcal/mol).

Term	Mean	SD	Median	Lowest	Highest	Run 58
Intermolecular	-10.54	1.42	-10.40	-14.29	-7.62	-14.29
vdW + H-bond + desolvation	-7.05	1.13	-6.96	-9.84	-4.52	-9.84
Electrostatic	-3.49	0.81	-3.43	-5.95	-1.29	-4.45
Ligand internal	-2.56	0.46	-2.50	-3.46	-1.23	-2.03
Torsional penalty (constant)	4.47	0.00	4.47	4.47	4.47	4.47
Estimated ΔG	-6.07	1.42	-5.92	-9.82	-3.15	-9.82

**Figure 5.** Estimated ΔG against the van der Waals + H-bond + desolvation term (left) and the electrostatic term (right). Orange line: least-squares fit; r : Pearson coefficient.

3.4 Interactions of the best pose

In run 58 the peptide formed 98 atom pairs within 4 Å distance of 13 HER2 amino acid residues organized in three clusters: Phe359-Glu373, Trp393-Ser396, and Gly425-Leu426 (Figure 6). The most atom pairs (17) were provided by Asp395, followed by Pro394 (13) and Asp360, Gln371, and Trp393 (11 each). Only 6 out of 12 residues of the peptide interacted with HER2 (Lys1, Arg2, Leu3, Val4, Lys10, and Ala12), implying that both N- and C-terminal parts of the peptide lay on the surface while residues 5-9 and Leu11 were bent into the solvent. The binding was stabilized by two salt bridges (Lys1 NZ-Asp395 OD2, 2.54 Å and Lys10 NZ-Glu373 OE1, 2.50 Å) and three directional hydrogen bonds (Table 6). Six hydrophobic pairs were found; however, several of them involved the aliphatic side chains of Lys and Arg residues. No contacts were detected for the central hydrophobic cluster Leu7-Ile8. The salt bridge distances are shorter than the 2.8-3.0 Å characteristic of crystalline proteins, a typical feature of docking predictions to be relaxed during MD simulation.

Table 6. Polar interactions of the best pose (run 58).

Type	Peptide atom	HER2 atom	Distance (Å)	Angle (°)
Salt bridge	Lys1 NZ	Asp395 OD2	2.54	—
Salt bridge	Lys10 NZ	Glu373 OE1	2.50	—
H-bond (peptide→HER2)	Arg2 NE	Phe359 O	2.69	124
H-bond (peptide→HER2)	Lys10 NZ	Gln371 OE1	2.99	139
H-bond (HER2→peptide)	Lys1 O	Ser396 N	3.40	153

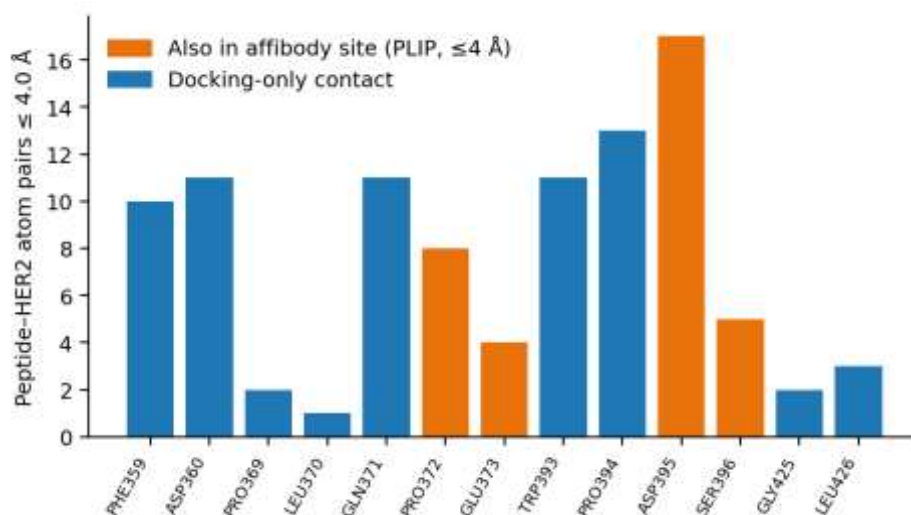


Figure 6. Peptide-HER2 atom pairs within 4 Å for each HER2 residue in run 58. Orange: residue also within 4 Å of the affibody in the crystal structure; blue: contact seen only in the docked pose.

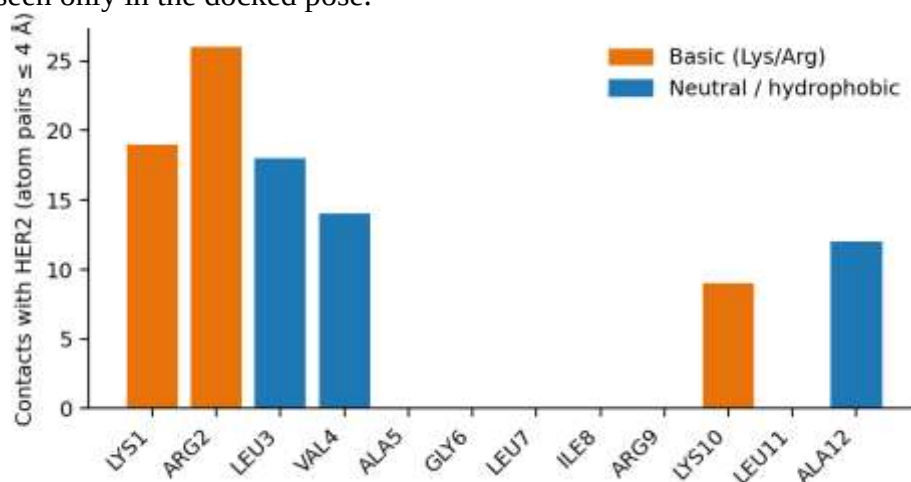


Figure 7. Contacts of each peptide residue with HER2 in run 58 (atom pairs within 4

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

Å). Orange: Lys and Arg; blue: other residues.

Only the termini participated in the interaction: Lys1 (19 pairs of atoms), Arg2 (26), Leu3 (18), Val4 (14), Lys10 (9) and Ala12 (12) interacted with HER2 in run 58 (Figure 7), while residues 5 to 9 and Leu11 did not have any atom in the vicinity of 4 Å from HER2.

Comparison with the affibody footprint

The crystal structure affibody has established 18 bonds with HER2 (8 hydrogen bonds, 7 hydrophobic interactions, 1 salt bridge, 1 water bridge and 1 π -stacking interaction), including arginine-acid pairings such as Arg28-Asp395 and Arg10-Glu379. In this way, the Lys1-Asp395 and Lys10-Egl373 pairs in the docked complex also seem to be similar to this arginine-mediated bonding (Figure 8). Affibody is a protein of 58 residues with about 430 heavy atoms compared to 94 in the case of peptide, so that higher number of hydrogen bonds (8 vs 3) and hydrophobic interactions (7 vs 6) were anticipated.

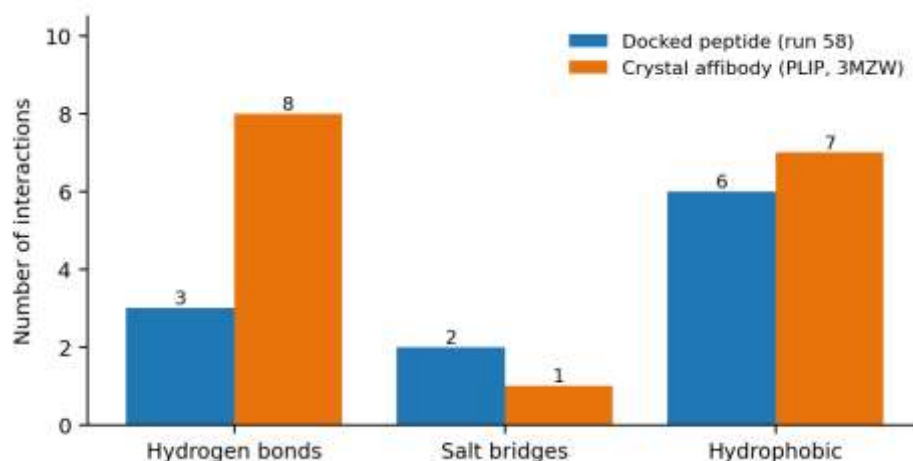


Figure 8. Hydrogen bonds, salt bridges and hydrophobic contacts of the docked peptide (run 58, geometric analysis) and the crystal affibody (PLIP). Counts come from different methods and are not a like-for-like test.

All 100 runs interacted with footprint residues because the search box was located 2.8 Å away from the footprint centre. The significant finding was how the poses were positioned in relation to the footprint. The poses were located 6.7–21.2 Å away from the footprint centre (mean 11.6 Å), and energy had an inverse relationship with distance ($\rho = -0.38$, $p < 0.001$) meaning that the poses farther from the centre were ranked better (Figure 9). Cluster 1 had the average distance of 19.0 Å from the centre, compared to 12.6 Å from all other runs (Mann–Whitney $p < 0.001$), whereas the 36 runs having less than 10 Å of distance from the centre varied in energy between -6.73 and -3.68 kcal/mol. In agreement with this, the number of footprint residues contacted had positive correlation with energy ($\rho = 0.33$, $p < 0.001$): the 50 runs contacting 8 or more footprint residues were characterised by the mean energy of -5.47 kcal/mol and minimum energy of -6.98 , whereas the other 50 runs averaged -6.67 kcal/mol.

Online ISSN

3007-3197

Print ISSN

3007-3189

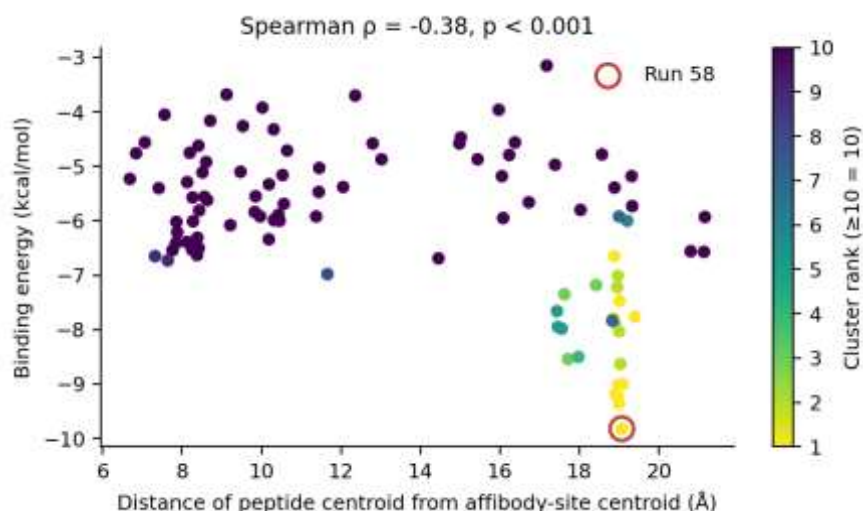
<http://amresearchreview.com/index.php/Journal/about>

Figure 9. Binding energy against the distance between the peptide centroid and the centroid of the affibody footprint. Colour: cluster rank (clusters above 10 shown as 10); red circle: run 58.

Table 7. Affibody-footprint residues most often contacted by the docked peptide (100 runs).

HER2 residue	Runs in contact	In PLIP set	Run 58 contact	Cluster 1 runs (of 10)
Ser396	56	No	Yes	10
Glu379	48	Yes	No	0
Ser401	48	Yes	No	0
Trp430	47	Yes	No	0
Pro372	41	No	Yes	10
Asp399	40	No	No	0
Gln376	39	Yes	No	0
Asp395	39	Yes	Yes	10
Glu373	31	Yes	Yes	9
Ile428	0	No	No	0

Residues listed are those touched by the largest numbers of runs, plus the never-contacted Ile428. Glu373 is shown because run 58 and nine members of cluster 1 contact it.

Of the footprint residues, the ones that came in contact the most were Ser396 (56 runs), Glu379 and Ser401 (48 each) and Trp430 (47), while the one that never came in contact was Ile428 (Table 7). Ten cluster representatives formed three classes based on the acidic patch of HER2 they interacted with (Table 8, Figure 11). Clusters 1-7 (energy -9.82 to -7.83 kcal/mol) interacted with the Asp395 patch and overlapped the footprint at 2-4 residues, predominantly Pro372, Asp395 and Ser396. Clusters 8 and 10 interacted with Glu379 patch, and cluster 9 with Asp399-Val402 patch, while

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

clusters 8 and 9 overlapped with 9 and 11 footprint residues, but had energy -6.98 and -6.73 kcal/mol respectively. Out of 25 residues that were contacted the most times by the ensemble, 17 were from the footprint, and 65 HER2 residues were contacted at least once.

Table 8. Footprint overlap of the ten best cluster representatives.

Cluster	Run	ΔG	HER2 residues	Footprint residues	Footprint residues contacted
1	58	-9.82	13	4	372, 373, 395, 396
2	62	-8.63	16	4	372, 395, 396, 427
3	82	-8.54	9	2	395, 396
4	96	-8.50	10	4	372, 395, 396, 427
5	45	-7.98	11	3	395, 396, 427
6	7	-7.85	10	2	395, 427
7	91	-7.83	12	4	372, 373, 395, 396
8	4	-6.98	13	9	376, 379, 401, 402, 434, 504, 505, 516, 517
9	68	-6.73	13	11	372, 373, 376, 379, 396, 397, 398, 399, 401, 402, 430
10	86	-6.69	9	4	376, 379, 434, 516

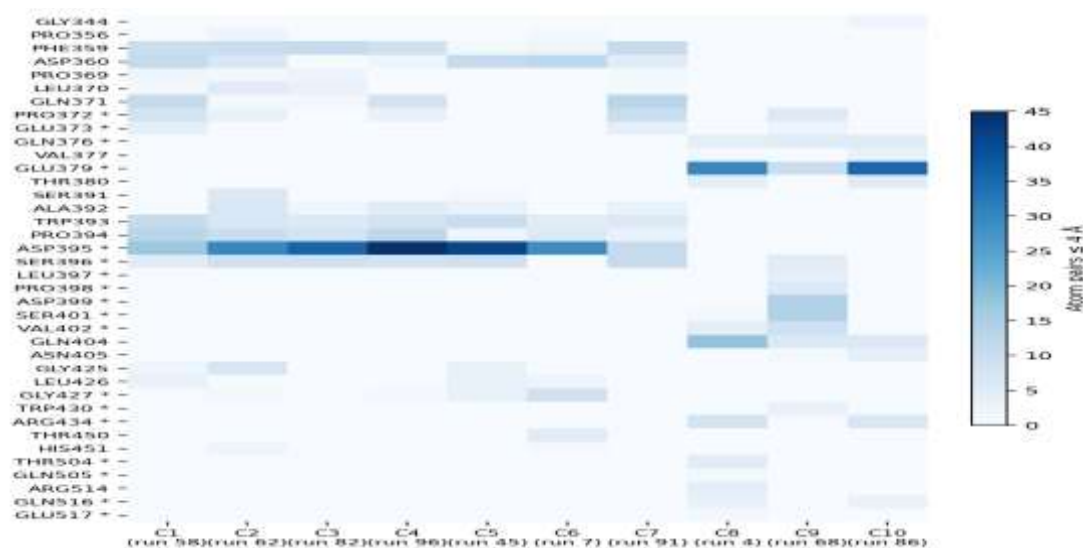


Figure 10. Atom pairs within 4 Å between the peptide and each HER2 residue for the lowest-energy member of each of the ten best clusters. An asterisk marks residues in the affinity footprint.

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

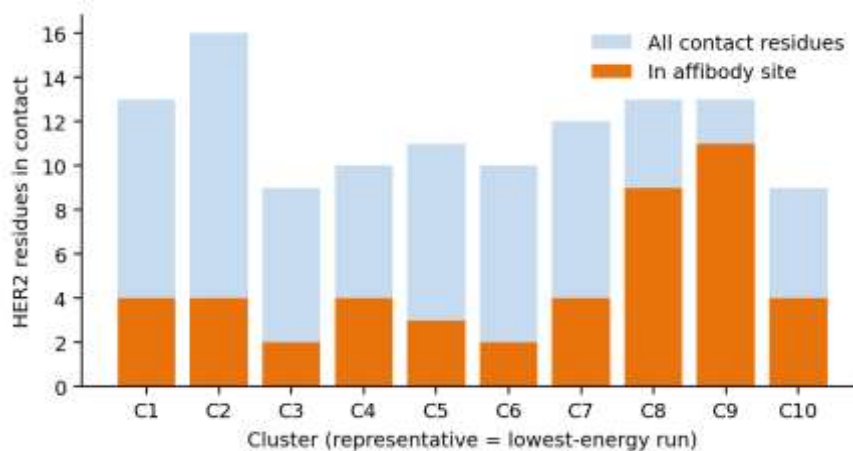


Figure 11. For the lowest-energy member of each of the top ten clusters: number of HER2 residues in contact (light blue) and how many belong to the affibody footprint (orange).

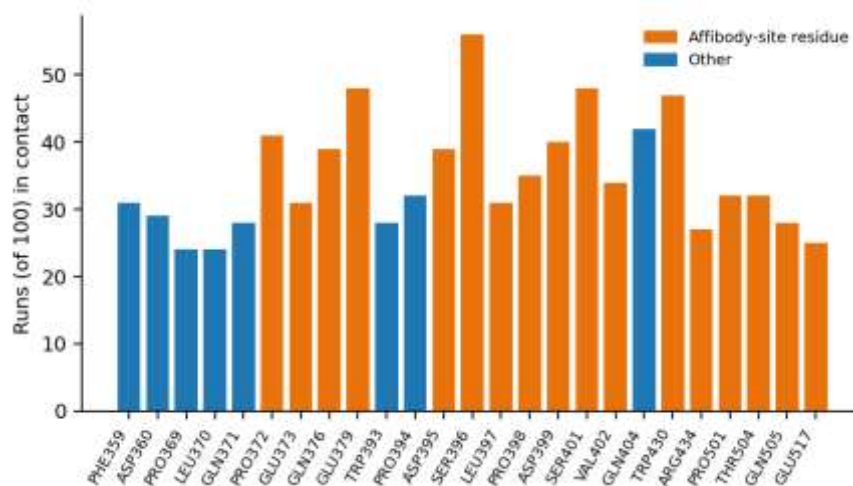


Figure 12. The 25 HER2 residues most often in contact with the peptide across all 100 runs. Orange: residues in the affibody footprint.

Peptide properties and ligand efficiency

The motif KRLVAGLIRKLA has four basic amino acids in positions 1, 2, 9, and 10, whereas the other hydrophobic amino acids are found in positions 3 through 8 and 11 through 12. This gives rise to the pattern of two charged pairs of amino acids in the N- and C-terminal ends, respectively, with a hydrophobic motif between them (Leu-Val-Ala-Gly-Leu-Ile) (Figure 13).

Online ISSN

3007-3197

Print ISSN

3007-3189

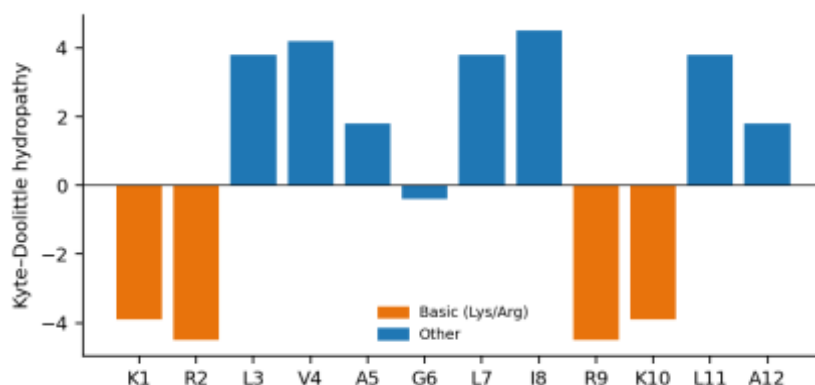
<http://amresearchreview.com/index.php/Journal/about>

Figure 13. Kyte–Doolittle hydropathy of each residue of KRLVAGLIRKLA (positive = hydrophobic). Orange: Lys and Arg.

The efficiency of the ligand for run 58 was -0.104 kcal/mol per heavy atom, which is 1.6 times greater than the average efficiency of all runs (-0.065). For peptides of this size, there is relatively poor efficiency in comparison to smaller molecules, making this efficiency ratio applicable only in comparing variants of similar size. Out of all 100 poses, the most reliable contacts were Lys1 (15.8 atom pairs), Leu11 (12.3), Leu3 (8.6), and Lys10 (8.2). Leu11 was the important contact for many high-ranking clusters, but not for run 58.

Discussion

Docking is commonly employed as an initial filter that suggests reasonable models and helps to select candidates for further simulation, which are more costly. The selected peptide sequence KRLVAGLIRKLA fulfilled this condition because it was docked with good score and had a reproducible binding mode instead of isolated one. Out of 100 independent docking simulations, ten produced cluster 1 binding mode, and six of them were less than 0.8 Å away from the best binding mode. This is the strongest argument that the minimum of the scoring function was found by the search.

Energy decomposition analysis revealed that the lead of the best conformation was derived mostly from packing, as can be seen from van der Waals + hydrogen bonds + desolvation component and not from strong electrostatic interaction. It is relevant in terms of design because HER2 ECD possesses more acidic residues (56 Asp/Glu) compared to basic ones (43 Lys/Arg), and consequently any cationic peptide tends to be attracted to it in some way. In terms of distinguishing specificity from non-specificity of the interaction, electrostatic interactions alone cannot do this while positive correlation of atom contacts number with the score ($\rho = -0.70$) indicates the feasibility of this approach.

That the highest scoring poses were on the edges of the affibody footprint, whereas poses that covered more of the footprint had lower scores should be taken into consideration. The docking box was placed around the centre of the footprint, thus some overlap would be pre-defined. In the docking box, the peptide liked to bind in the Asp395 acidic patch, which is where Arg28 of the affibody binds, and lysine and arginine side chains mimicked the Arg-acid pairing of the affibody. It is unrealistic for a 12-residue linear peptide to match the 58-residue affibody footprint interface because Trp14 and Tyr35 are buried in the interface and are absent in the peptide.

Moreover, these poses might be penalized due to the use of rigid-receptor model. All of these observations coincide with previous structure-based attempts when short interface-derived peptides possessed only partial affinity of their parent ligand and needed to be optimized [10–12,17].

Three design implications can be drawn. Firstly, Lys1 was found to be the most stable anchor and will be tested as the first residue in the redesign process. Secondly, due to the absence of interaction of the hydrophobic core (Leu7-Ile8) with the HER2 protein in the optimal pose, substitutions involving the introduction of hydrophobic or aromatic residues into contact with the target will be tested in accordance with the mechanism of binding of the affibody molecule. Thirdly, due to the similarity of clusters 2-7 which is less than the probable error of scoring, several representatives from each group need to be subjected to MD analysis.

The peptide sequence itself provides the explanation for the obtained outcomes. Indeed, the two charged residues at the N-terminal and C-terminal ends would allow these groups to interact with acidic residues on HER2, while the hydrophobic region in between would provide an additional means of energy of binding, which was not used until now as it was exposed to solvent. As for whether or not the peptide assumes a helical structure on binding as implied by its alternating pattern of the sequence, it is not evaluated in the current work but it could be done through helicity measurements using MD simulations.

The degree of confidence to be placed in each of these statements varies and is indicated in Table 9. There is strong support for the convergence of the docking search, and the contacts for run 58 are precise for this particular pose, but the statements that run 58 represents the actual optimal pose, that the peptide favors binding to the affibody site more than other HER2 surface residues, and most importantly that it does so with nanomolar affinity, are poorly supported.

Table 9. How much weight each docking result can carry.

Statement	Confidence	Reason
The search converged on one best cluster	High	Ten independent runs agree within 2 Å and six within 0.8 Å
The best pose contacts Asp395, Glu373 and Ser396	High (this pose)	Measured directly from the coordinates
Run 58 is the true best binding mode	Low to medium	Energy lead of 1.2 kcal/mol over the next cluster is within scoring error
The peptide prefers the affibody site over other HER2 surfaces	Low	The docking box was centred on the footprint
The best poses sit at the footprint edge, not its centre	Medium	Consistent across clusters 1–7 and in correlation tests, but from one docking experiment
The peptide binds HER2 with nanomolar affinity	Not supported	Predicted K_i is a scoring-function output

Limitations

There are some limitations of these results. The work was performed entirely through computation. Docking scores and predicted K_i values are computed parameters, and the scoring function is precise up to $\pm 2-3$ kcal/mol only. The receptor was considered rigid, while HER2 demonstrates conformational flexibility upon interaction [18]. Only one peptide and one target conformation were studied; the search box used was responsible for docking to the affibody side of the protein and did not allow for the interaction to be analyzed using geometric approach. It was not tested whether the peptide assumes helical conformation upon binding. Lastly, the short distances between salt bridges show that charges in docking results represent idealized contacts. MD simulations, MM/GBSA calculations and finally binding studies, such as SPR and cell-based experiments are necessary before any claims on therapeutic properties can be done.

Conclusion

Binding of the designed peptide KRLVAGLIRKLA to the extracellular domain of HER2 was evaluated using molecular docking, which revealed the main, highly consistent binding pose (cluster 1, best $\Delta G = -9.82$ kcal/mol), stabilized by the salt bridge between Lys1-Asp395 and the salt bridge between Lys10-Glu373 and other packing interactions with 13 HER2 amino acids. Poses as a whole tended to concentrate around the surface of HER2 where the affibody Z(HER2:342) binds, while the best-scoring poses were located on the edge of the footprint, and poses covering more of the footprint received lower scores. Molecular docking confirmed the hypothesis about the ability of the designed peptide to bind HER2 in the vicinity of the affibody site but does not indicate any specific and stable binding. Further steps will be modeling of several poses from each pose group via MD simulation and peptide redesign to increase hydrophobic interactions.

Acknowledgements

We are grateful to the Department of Chemistry, The Superior University, Lahore for the financial support of this research.

References

- [1] Gu H, Wang R, Beeraka NM, et al. Global burden and trends of breast cancer: GLOBOCAN 2022 estimates of incidence and mortality in 185 countries. *Chin Med J*. 2026;139(3):404–414.
- [2] Cheng X. A comprehensive review of HER2 in cancer biology and therapeutics. *Genes*. 2024;15(7):903.
- [3] Pan L, Li J, Xu Q, et al. HER2/PI3K/AKT pathway in HER2-positive breast cancer: a review. *Medicine*. 2024;103(24):e38508.
- [4] Cruz VL, Souza-Egipsy V, Gion M, et al. Binding affinity of trastuzumab and pertuzumab monoclonal antibodies to extracellular HER2 domain. *Int J Mol Sci*. 2023;24(15):12031.
- [5] Guidi L, Pellizzari G, Tarantino P, Valenza C, Curigliano G. Resistance to antibody-drug conjugates targeting HER2 in breast cancer: molecular landscape and future challenges. *Cancers*. 2023;15(4):1130.

- [6] Ghaly G, Tallima H, Dabbish E, et al. Anti-cancer peptides: status and future prospects. *Molecules*. 2023;28(3):1148.
- [7] Zheng B, Wang X, Guo M, Tzeng CM. Therapeutic peptides: recent advances in discovery, synthesis, and clinical translation. *Int J Mol Sci*. 2025;26(11):5131.
- [8] Al Musaimi O, AlShaer D, de la Torre BG, Albericio F. 2024 FDA TIDES (peptides and oligonucleotides) harvest. *Pharmaceuticals*. 2025;18(3):291.
- [9] Eigenbrot C, Ultsch M, Dubnovitsky A, Abrahmsén L, Härd T. Structural basis for high-affinity HER2 receptor binding by an engineered protein. *Proc Natl Acad Sci USA*. 2010;107(34):15039–15044.
- [10] Geng L, Wang Z, Yang X, et al. Structure-based design of peptides with high affinity and specificity to HER2 positive tumors. *Theranostics*. 2015;5(10):1154.
- [11] Yang X, Wang Z, Xiang Z, et al. Peptide probes derived from pertuzumab by molecular dynamics modeling for HER2 positive tumor imaging. *PLoS Comput Biol*. 2017;13(4):e1005441.
- [12] Satyanarayanajois S, Villalba S, Jianchao L, Lin GM. Design, synthesis, and docking studies of peptidomimetics based on HER2–Herceptin binding site with potential antiproliferative activity against breast cancer cell lines. *Chem Biol Drug Des*. 2009;74(3):246–257.
- [13] Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30(16):2785–2791.
- [14] Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. PLIP: fully automated protein–ligand interaction profiler. *Nucleic Acids Res*. 2015;43(W1):W443–W447.
- [15] Torres PH, Sodero AC, Jofily P, Silva-Jr FP. Key topics in molecular docking for drug design. *Int J Mol Sci*. 2019;20(18):4574.
- [16] Sahu D, Rathor LS, Dwivedi SD, et al. A review on molecular docking as an interpretative tool for molecular targets in disease management. *Assay Drug Dev Technol*. 2024;22(1):40–50.
- [17] Banappagari S, Ronald S, Satyanarayanajois DS. A conformationally constrained peptidomimetic bound to the extracellular region of HER2 protein. *J Biomol Struct Dyn*. 2010;28(3):289–308.
- [18] Vacca S, Gragera M, Buschiazzo A, et al. Structural analysis of HER2–trastuzumab complex revealed receptor conformational adaptation. *Sci Adv*. 2025;11(30):eadu9945.