In Silico Designing and Optimization of the Anti-EGFR Scaffolds by CDRs-Grafting Technique

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Abstract

Monoclonal antibodies are attractive therapeutic agents in a wide range of human disorders that bind specifically to their target through their complementary-determining regions (CDRs). Small proteins with structurally preserved CDRs are promising alternatives to antibodies. In this study, we presented new antibody mimetics against colorectal cancer marker epidermal growth factor receptor (EGFR) created by CDRs grafting technique. Ten potential graft acceptor sites that efficiently immobilise the grafted CDR loops were selected from three small protein scaffolds by computational identification. The three most involved CDR loops in antibody-receptor interactions extracted from panitumumab monoclonal antibody against domain III of EGFR (EGFR DIII) crystal structure were then grafted to the selected scaffolds through the loop randomization technique. The combination of three CDR loops and 10 grafting sites revealed that three of the 36 combinations showed specific binding to EGFR DIII by binding energy calculation. Thus, the present strategy and selected small protein scaffolds are promising in designing new binders against EGFR with high binding energy.

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Running title: In Silico Designing of new EGFR binding scaffolds

Abstract

Monoclonal antibodies are attractive therapeutic agents in a wide range of human disorders that bind specifically to their target through their complementary-determining regions (CDRs). Small proteins with structurally preserved CDRs are promising alternatives to antibodies. In this study, we presented new antibody mimetics against colorectal cancer marker epidermal growth factor receptor (EGFR) created by CDRs grafting technique. Ten potential graft acceptor sites that efficiently immobilise the grafted CDR loops were selected from three small protein scaffolds by computational identification. The three most involved CDR loops in antibody-receptor interactions extracted from panitumumab monoclonal antibody against domain III of EGFR (EGFR DIII) crystal structure were then grafted to the selected scaffolds through the loop randomization technique. The combination of three CDR loops and 10 grafting sites revealed that three of the 36 combinations showed specific binding to EGFR DIII by binding energy calculation. Thus, the present strategy and selected small protein scaffolds are promising in designing new binders against EGFR with high binding energy.

Key Words : EGFR; panitumumab; molecular dynamic simulation; CDR grafting technique

Key points:

1. New anti-EGFR binders developed by CDR grafting technique.
2. scFv structure originated from panitumumab antibody binds to the EGFR with high affinity.
3. Panitumumab mimetics scaffolds computationally bind to the EGF binding site on EGFR.

Statements and Declarations

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1. Introduction

Antibodies, ~150 kDa glycoproteins, consist of two light and two heavy polypeptide chains with six CDRs for specific binding to their targets [1]. However, their therapeutic use is limited due to the low tissue penetration. Recently, advances in the structural engineering of proteins led to the construction of many small-size proteins (3-20 kDa) with the ability to bind to epitopes recognized by mono antibodies [2]. Among all the engineered and recombinant proteins, single chain variable fragments (scFvs) composed of VH and VL, with the same antigen-binding specificity can retain the affinity of a whole antibody molecule [3]. Low-molecular weight, high rate of excretion, good tissue penetration, and low costs for production of these antibody mimetics converted them into a promising alternative for targeted therapies [4]. Currently, amino acid conversion on the surface area of non-immunoglobulin scaffolds resulted in the generation of various small antibody mimetics [4]. However, length, conformation, and the conserved amino acid residues of the hypervariable CDRs loop play an essential role in native binding site definition [5]. On the other hand, 30 kDa fragments of antibody mimetics developed by the antibody fragmentation technique restricted their simple chemical synthesis [4]. Using the CDRs grafting strategy, protein scaffolds with variable loops that mimic the conformation of CDRs can be extracted to generate small scaffolds with improved affinity. Nicaise et al. developed some protein scaffolds against lysozyme which harbored CDRs from a specific antibody. However, designed scaffolds showed various affinities towards lysozyme, indicating that selected scaffolds directly affect the affinity of grafted CDRs [6]. Therefore, for designing antibody mimetics, selecting proper protein scaffolds and determining appropriate CDRs peptide sequences is essential [7]. Among many introduced non-immunoglobulin scaffolds, anticalins with a smaller size of 180 amino acids consisting one polypeptide chain, a beta-sheet structure, and four flexible loops without any glycosylated structure can be produced in prokaryotic hosts [8]. Another example of protein scaffolds is the tenth domain of human fibronectin type III (Fn3, also called Adnectin). Adnectin is a 94-residue monomeric protein with a β-sandwich fold structure and six internal loops without disulfide bonds. This protein mimics the interaction of CDRs with the target through three loops on the tip of molecules [8]. The third structure selected for this study is VHH, with small molecular weight, low aggregation, and high solubility, which contains three variable loops [5]. This existing assortment represents an ideal CDR acceptor repertoire for our approach. In addition to the structure of the CDRs acceptor, the framework plays a vital role in CDR orientation and conformation. Therefore, framework residues should be considered in designing a protein scaffold with high binding affinity to the target [9]. Here, we aimed to simulate the CDRs conformation in three selected scaffolds of humanized VHH, Fn3, and lipocalin with at least three flexible loops for grafting CDRs isolated from the target antibody. Three CDRs peptides of H2, H3, and L3 that play an important role in the recognition of the target were extracted from the monoclonal antibody panitumumab that binds specifically to the EGFR DIII. These CDRs were then grafted through loop randomization in all these scaffolds. Apart from scFv, which carries all 6 CDRs in their native structure, 36 anti-EGFR DIII protein candidates were created (24 candidates from lipocalin scaffold, 6 structures of Fn3, and 6 candidates of humanized VHH).

Among all these structures, three of them were selected for further analysis in molecular dynamic simulation.

2. Materials and Methods

2.1 Identification of antigen-binding CDRs

The structure of the EGFR DIII/Panitumumab complex (PDB entry 5SX4) was used as the initial complex for extracting the affective CDRs in interaction with the receptor. Two CDRs, H2 and H3, from the heavy chain and L3 from the light chain were selected for grafting into three scaffolds of Lipocalin (PDB entry 3dwt), Fn3 (1TTF), and VHH (3BX7). The sequence of these CDRs defined by Parapred software [10], each with two residues per site (residues on the upper and lower core of variable domains affecting CDRs conformation) [9].

2.2 Identification of grafting loops

The initial coordinates of three selected graft acceptors were taken from PDB accession codes 3BX7 (Scaf1), 1TTF (Scaf2), and 3dwt (Scaf3). Three-dimensional models of 36 designed structures after immobilizing CDR peptides into variable loops were generated by Modeller software (Mod10.2). The best models with the lowest DOPE score from each 36 designed scaffolds were selected for further analysis. The scFv model of the panitumumab antibody, also generated as the second reference molecule beside the whole structure of the antibody, extracted from 5SX4 PDB entry, to be used for binding energy comparison. Then, the quality of models was assessed by online web servers, including PDBSum (*http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html*) and ProSA-web [11]. The model structures were evaluated by Ramachandran Plot Assessment using the PDBSum server.

2.3 Docking

To find potential candidates against the EGFR DIII, molecular docking was carried out to screen conformations of designed ligands with high stability at the binding site of EGFR DIII by the HADDOCK 2.2 web server [12]. Initially, molecular docking analysis was performed with the reference molecules in the active site of EGFR to re-produce the same conformation similar to the co-crystallized ligand (5SX4). The result of scFv docking with EGFR DIII was also used as the second reference molecule. Among all generated receptor-ligand complexes, models with lower binding energy were selected for MD simulation.

2.4 Molecular Dynamic Simulation

The MD simulation is widely used to determine the structural stability of the protein and protein-ligand complexes under physiological conditions [13]. To evaluate the stability of 2 obtained structures from docking analysis, molecular dynamic simulation was applied using GROMACS 4.6.5 suits [14]. GROMOS96 force field and simple point charge (SPC) as water model was used to generate topology files. All simulations were analyzed at 343 K. To neutralize each system, some water molecules were randomly replaced by ions. After being neutralized, the system's energy was minimized using the steepest descent algorithm. Then all systems were simulated for 100 ps under NVT at 300 K and NPT ensembled at the pressure of 1 bar within periodic boundary conditions and restraint force of 100 KJ/mol using modified Berendsen thermostat and Parinello-Rahman barostat algorithms, respectively. The electrostatic interactions were evaluated using the Particle Mesh Ewald (PME) algorithm. Ultimately, the MD run was done with no restraint for 50 ns to evaluate the stability of structures.

2.5 Binding free energy

Eventually, a thorough analysis of the binding free energy ([?]G_{bind}) of all selected scaffolds was carried out using Molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) protocol by the g-mmpbsa package [15]. MM-PBSA analysis gives a detailed estimation of the binding affinity of protein-ligand interaction. To calculate the total [?]G_{bind}, the free polar solvation energy, SASA energy, and potential energy (Van der Waals and electrostatic interactions) of each protein-ligand complex were analyzed.

3. Results

3.1 Strategy for creating anti-EGFR scaffolds

Selecting an appropriate scaffold for grafting effective CDRs in binding is crucial to mimic the antibody-receptor interaction. Accordingly, we computationally designed small antibody mimetics with high affinity against EGFR DIII based on the panitumumab antibody. Ideal traits of scaffold candidates as CDRs acceptors include a suitable size (less than 180 amino acids) with a minimum of three variable loops, low disulfide bonds, and non-immunogenicity. Then active CDRs in ligand-receptor interaction were determined and immobilized randomly into variable loops of each scaffold. All designed structures were analyzed (Table 1) and after docking against EGFR DIII, the best ones from each scaffold were selected for MD analysis. The Ramachandran plots of scFv and three selected scaffolds are depicted in Fig.1, and the selected scaffolds' residue information is shown in table 2.

3.2 Molecular docking analysis

To screen newly designed scaffolds targeting the EGFR on the surface of cancer cells, a molecular docking study was performed using the HADDOCK webserver. The results showed the binding of the ligands in the same position and orientation with acceptable binding scores as compared to the reference complexes. It verified that the selected docking parameters were optimal. Docked scaffolds are ranked based on their docking scores. Docking results revealed that three selected scaffolds out of 36 had docking scores between the range of -99 to -145.8, in which Scaf1 showed the best docking score in comparison to scFv and 5SX4 complex. The complex of all three selected scaffolds and the reference of scFv with EGFR DIII were analyzed in molecular dynamic analysis.

3.3 Molecular Dynamics Simulation

The molecular dynamic method was used to analyze the physical movements of atoms and molecules and to study conformational change at the atomic level. To assess the binding stability and determine binding-free energy against EGFR active site, three scaffolds and scFv were subjected to 50 ns molecular dynamic simulation. Further, all four scaffolds were analyzed by RMSD, RMSF, H bond, and MMPBSA calculation to examine the protein stability and dynamic behavior throughout the simulation period. The variation of the all scaffolds-receptor complex was determined by the root mean square deviation (RMSD) during the 50 ns MD simulation. RMSD of alpha carbon was calculated (Fig.2), and results indicated that all four complexes remained stable throughout the simulation with an average RMSD 0.75, 2.65, 0.2, 2.7 for scFv (Orange), Scaf1 (Gray), Scaf2 (Blue), and Scaf3 (Red), respectively. A slight fluctuation in the case of the scFv complex was noted during the first 6 ns, which achieved the equilibrium and remained stable throughout the simulation. Measuring the average movement of the atom position at the specific temperature and pressure was performed by root mean square fluctuation (RMSF) analysis. The fluctuations in the constituents' residues were observed for all four structures and plotted to compare the flexibility of each residue in complexes (Fig.3). RMSF was calculated for EGFR DIII, SCFV, and three selected scaffolds. Low RMSF values obtained for all four complexes indicate good stability of the system. Although there are little fluctuations in some residues of the scFv and Scaf2, all grafted residues in designed scaffolds had low RMSF values. Otherwise, the fluctuation during all four CDR loops-receptor interactions was below 0.2 nm, which is perfectly acceptable and shows good stability of designed structures.

3.4 Hydrogen bonding analysis

Hydrogen bonding between a ligand and receptor stabilizes their interaction [16]. It also shows the specificity, metabolization, and adsorption of the drug. To further explain the conformational stability, the total number of hydrogen bonds between each scaffold-receptor was analyzed (Fig.4). It indicated that the scFv and EGFR DIII complex shows strong bonding interaction throughout the 50 ns of MD simulations. Analyzing the reference complex of scFv with the receptor, around 13 hydrogen bonds (Orange) were observed in the complex. At the same time, 13, 5, and 4 residues in Scaf1 (Gray), Scaf2 (Blue), and Scaf3 (Red) were involved in hydrogen interaction with the receptor. The above detailed H-bond analysis showed that Scaf1 was bound to the EGFR DIII as effectively and tightly as the reference molecule scFv.

3.5 Molecular interaction of the top scaffolds with EGFR

The 3D interaction of the top 3 scaffolds and the reference molecules of the panitumumab and scFv were visualized by PyMOL software [17]. The docked poses demonstrated that all 6 CDR loops in the panitumumab structure interacted in 12 hydrogen bonds with EGFR D3. Asp50, Phe91, Asp92, Lue94 in light chain and Asp33, Tyr35, Tyr55, Asn58, Thr59, Asp100, and Thr103 in heavy chain are involved in H bonds. Analyzing the SCFV complex showed that CDR regions H1, H2, L1, L2, and L3 interacted with EGFR by several residues, among which Tyr35, Asn58, and Asp327 are in common with the panitumumab complex. Selected scaffolds interacted with receptor through hydrogen bonds of residues in L3, H3, and H2 CDR loops in Scaf1, H3, L3 in Scaf2, and L3, H2 in Scaf3 (Fig.5). Analyzing these three scaffolds indicated that Tyr131, Asn134 in Scaf1, Thr28, Phe60, Asp61 in Scaf2, and Asn107 are in common with reference molecules. These scaffolds also form hydrophobics contacts with EGFR DIII, which are presented in table 4. From the visualization study of all three scaffolds, we observed that the L3 CDR loop involved H-bonding interactions similar to the reference molecules. It suggests that this loop plays an important role in scaffold-receptor hydrogen interaction. From the findings, it can be seen that Scaf1 mimics the conformation of the protein-receptor complex with all three CDR loops involved.

3.6 Binding free energy

The binding free energy (MM-PBSA) calculated from MD trajectories showed that the total binding energies of all complexes were observed in an acceptable range. The results of MM-PBSA are given in table 5. In particular, complex Scaf1-EGFR DIII presented the lowest binding free energy and higher binding affinity with the receptor, suggesting a more stable protein conformation. These free energy calculations validated the molecular docking results, showing that this scaffold was favorably binding to EGFR DIII and can be determined as a suitable CDR acceptor to mimic the panitumumab-EGFR interaction.

4. Discussion

Antibody mimetics is a new technique based on highly-structured scaffolds originating from natural proteins. Mutation in their key residues, especially in variable loops, can alter their affinity towards new targets [18]. In the present study, we conducted a CDR grafting technique to find new binders with a high affinity toward EGFR. In our report, we retrieved the extracellular domain 3 of EGFR from EGFR/panitumumab complex (5SX4) to be used as the target in our structure-based virtual screening method for 36 CDR-acceptors designed by loop alteration technique. Further, the docking hits were applied for molecular dynamic (MD) simulation, and the result was analyzed by different parameters such as RMSF, H-bond, and MMPBSA. Therefore, the current study was undertaken to analyze the behavior of the designed complexes using protein-receptor complex against domain III EGFR. In addition to the scFv structure originating from panitumumab, three grafting scaffolds among all designed ones were selected to be docked against EGFR DIII. Then, the complex of protein-EGFR DIII was simulated through molecular dynamic analysis to be compared in their binding site. Based on the previous research on the crystal structure of the panitumumab-EGFR complex[19], three CDR loops H2, H3 from the heavy chain, and L3 from the light chain of panitumumab with the most effective interaction against receptor selected to be grafted in new scaffolds. The docking scores and analyzing the CDR loop positions against receptor were used as the main criteria for the binder selection. Therefore, we focused on the three top hits with the best docking scores that share a similar binding site to the reference molecules. The epidermal growth factor protein binding by EGFR occurs within a cleft between two extracellular domains, DI and DIII [20]. The binding site of panitumumab partially overlaps the EGF binding site and prevents the dimerization of the EGFR ectodomain and its activation [19]. The overall binding of panitumumab to domain III is similar to cetuximab. Based on a mutational analysis, Voigt et al. described critical amino acids overlap in panitumumab and cetuximab epitopes with EGF binding site: K443 and D355 in panitumumab and D355, K443, Q408, H409, S468 in cetuximab binding site [21]. The panitumumab complex with EGFR DIII shows that upon binding, all 6 CDRs in the heavy and light chains interact without any conformational alteration. According to previous studies, CDRs L3, H2, and H3 commonly make the largest contribution to antigen binding [9]. The L3 CDR lines up adjacent to the final B-strand of domain III with two interactions of D92 and L94. The heavy chain

showed most of the specific hydrogen bond interactions made by H2 and H3. Most notably, three tyrosine residues interacted with the B-strand of domain III at the same position as EGF. Y54, Y55, N58, and T59 in H2 interacted with N384, D420, K443, respectively, while in H3, D100 has a water-mediated hydrogen bond with S468. H3 makes an additional hydrogen bond between T103 and K465 of domain III [19]. It can be visualized that interaction with S468 had the highest interaction rate (Fig.5). This residue interacts with EGF, panitumumab, cetuximab, scFv, and all three designed scaffolds. The scFv structure interacted with critical residues of S468, Q408, and H409 through 5 H-bonds in CDR regions which overlap with the cetuximab binding site. Scaf1 interacted with the same epitope as cetuximab through 3 H-bonds in inserted CDR loops against S468 and Q408. Scaf2, in addition to S468, interacted with D355, which is involved in both panitumumab and cetuximab binding sites. It indicates that this scaffold mimics the function of these two antibodies. In contrast to the other two scaffolds, Scaf3 could not make any hydrogen interaction in CDR regions with critical residues in the panitumumab binding site. However, Q1 in Scaf3 interacted with S468 and made hydrophobic contact with F30 against F412 in the panitumumab binding site. It shows that Scaf3 can be used as an alternative to panitumumab but with lower binding affinity. Therefore, all three scaffolds that exhibit the interaction with the same amino acid residues (S468, D355, Q408, and H409) are subjected to a 50 ns simulation process. The MD results confirmed the stability of scFv and three selected scaffolds throughout the simulation. The RMSF was calculated for domain III of EGFR, scFv, and three designed binders. It refers to the stability of the complex as high fluctuations related to more flexible and unstable bounds. Although there are some fluctuations in Scaf2 amino acid residues, they are not at the inserted CDR loops nor involved in protein interaction. The fluctuation during all interactions was below 0.2 nm, which is totally acceptable. To validate the docking energy of the protein-receptor complex, an MMPBSA calculation was performed. The designed structures presented comparatively acceptable MM-PBSA scores compared to the scFv structure. The calculated binding free energy of these binders were -26.4, -39.89, -9.47, -13.51 KJ mol^{-1}, for scFv, Scaf1, Scaf2, and Scaf3, respectively. Therefore, they represent excellent candidates for further investigation in vitro analysis. The only exception is the Scaf3 complex which showed a slightly lower MMPBSA value which is in accordance with its visual analysis results. By analyzing the binding energy and stability through dynamic simulation, we have shown that scFv, Scaf1, and Scaf2 may be potential binders that mimic the function of panitumumab. We tested *in vitro* and *in vivo* function of scFv structure against EGFR (unpublished data), which confirmed the results of *in silico* report. However, we also believe that the function of the other three designed scaffolds in vitro also should be investigated to approve their binding potential to EGFR protein.

5. Conclusion

This study aims to identify novel antibody mimetics scaffolds against the EGFR DIII whose binding sites overlap with EGF. Herein, molecular docking and MD simulation were successfully performed to design novel binders to EGFR DIII based on the CDR grafting technique. A set of 36 binders designed by loop randomization was screened by the molecular docking method. Eventually, the relative stability of the three selected scaffolds was validated by MD analysis. The scFv structure of panitumumab was designed to be compared with other designed binders. Trajectories analysis showed that all four complexes were structurally stable during the 50 ns MD run. From this study, two Scaf1 and Scaf2 showed promising high affinity against EGFR in comparison to 5SX4 and scFv complexes. Thus, the results of this study indicate that the anti-EGFR activity of these compounds could pose a great deal of significance against highly overexpressed EGFR cancers. These detailed analyses indicate that the computational antibody mimetics approach through the CDR grafting technique is very efficient. This in silico study may offer the opportunity to explore these scaffolds in vitro against EGFR.

References

1. Woof JM, Burton DR. Human antibody–Fc receptor interactions illuminated by crystal structures. Nat. Rev. Immunol. 2004; https://doi.org/10.1038/nri1266

2. Ito T, Nishi H, Kameda T, Yoshida M, Fukazawa R, Kawada S, et al. Combination Informatic and Experimental Approach for Selecting Scaffold Proteins for Development as Antibody Mimetics. Chem.

Lett. 2021; https://doi.org/10.1246/cl.210443

3. Kang TH, Seong BL. Solubility, stability, and avidity of recombinant antibody fragments expressed in microorganisms. Front. Microbiol. 2020; https://doi.org/10.3389/fmicb.2020.01927

4. Kadonosono T, Yimchuen W, Ota Y, See K, Furuta T, Shiozawa T, et al. Design strategy to create antibody mimetics harbouring immobilised complementarity determining region peptides for practical use. Sci. Rep. 2020; https://doi.org/10.1038/s41598-020-57713-4

5. Chiu ML, Goulet DR, Teplyakov A, Gilliland GL. Antibody structure and function: the basis for engineering therapeutics. Antibodies. 2019; https://doi.org/10.3390/antib8040055

6. Nicaise M, Valerio-Lepiniec M, Minard P, Desmadril M. Affinity transfer by CDR grafting on a non-immunoglobulin scaffold. Protein Sci. 2004; https://doi.org/10.1110/ps.03540504

7. Löfblom J, Frejd FY. Alternative scaffolds as bispecific antibody mimetics. In: Bispecific antibodies. Springer. 2011; https://doi.org/10.1007/978-3-642-20910-9_7

8. Garg P. Selective Preference of Antibody Mimetics over Antibody, as Binding Molecules, for Diagnostic and Therapeutic Applications in Cancer Therapy. Biointerface Res Appl Chem. 2021; https://doi.org/10.33263/briac113.1067910689

9. Wagner HJ, Wehrle S, Weiss E, Cavallari M, Weber W. A two-step approach for the design and generation of nanobodies. Int J Mol Sci. 2018; https://doi.org/10.3390/ijms19113444

10. Liberis E, Veličković P, Sormanni P, Vendruscolo M, Liò P. Parapred: antibody paratope prediction using convolutional and recurrent neural networks. Bioinformatics. 2018; https://doi.org/10.1093/bioinformatics/bty305

11. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007; https://doi.org/10.1093/nar/gkm290

12. Van Zundert G, Rodrigues J, Trellet M, Schmitz C, Kastritis P, Karaca E, et al. The HADDOCK2. 2 web server: user-friendly integrative modeling of biomolecular complexes. J. Mol. Biol. 2016; https://doi.org/10.1016/j.jmb.2015.09.014

13. Mathpal S, Joshi T, Sharma P, Joshi T, Pundir H, Pande V, et al. A dynamic simulation study of FDA drug from zinc database against COVID-19 main protease receptor. J. Biomol. Strut. Dyn. 2022; https://doi.org/10.1080/07391102.2020.1821785

14. Hess B, Kutzner C, Van Der Spoel D, Lindahl E. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J. Chem. Theory Comput. 2008; https://doi.org/10.1021/ct700301q

15. Kuzmanic A, Zagrovic B. Determination of ensemble-average pairwise root-mean-square deviation from experimental B-factors. Biophys J. 2010; https://doi.org/10.1016/j.bpj.2009.11.011

16. Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci Adv. 2016; https://doi.org/10.1126/sciadv.1501240

17. DeLano WL. The PyMOL molecular graphics system. http://www pymol org. 20.

18. Richards DA. Exploring alternative antibody scaffolds: Antibody fragments and antibody mimics for targeted drug delivery. Drug Discov Today Technol. 2018; https://doi.org/10.1016/j.ddtec.2018.10.005

19. Sickmier EA, Kurzeja RJ, Michelsen K, Vazir M, Yang E, Tasker AS. The panitumumab EGFR complex reveals a binding mechanism that overcomes cetuximab-induced resistance. PloS one. 2016; https://doi.org/10.1371/journal.pone.0163366

20. Martin-Fernandez ML, Clarke DT, Roberts SK, Zanetti-Domingues LC, Gervasio FL. Structure and dynamics of the EGF receptor as revealed by experiments and simulations and its relevance to non-small cell lung cancer. Cells. 2019; https://doi.org/10.3390/cells8040316

21. Voigt M, Braig F, Göthel M, Schulte A, Lamszus K, Bokemeyer C, et al. Functional dissection of the epidermal growth factor receptor epitopes targeted by panitumumab and cetuximab. Neoplasia. 2012; https://doi.org/10.1593/neo.121242

Figure legends

Fig.1 Ramachandran plots of generated models by Modeller software. (A) scFv structure; (B) Scaf1; (C) Scaf2; (D) Scaf3.

Fig.2 RMSD profile of all protein-receptor complexes for 50 ns MD simulation period. scFv-EGFR DIII (Orange), Scaf1-EGFR DIII (Gray), Scaf2-EGFR DIII (Blue), and Scaf3-EGFR DIII (Red).

Fig.3 The graph representing the RMSF values of Cα atoms for 50 ns trajectories. (A) scFv structure, (B) Scaf1, (C) Scaf2, (D) Scaf3, (E) EGFR DIII.

Fig.4 Diagram representing the dynamics observed in the hydrogen bonding patterns for scFv-EGFR as the reference molecule (Orange), Scaf1-EGFR DIII (Gray), Scaf2-EGFR DIII (Blue), Scaf3-EGFR DIII (Red).

Fig.5 3D structures of scFv and three top ligands in complex with EGFR. (A) scFv-EGFR DIII complex and hydrogen bonds in CDR regions, (B) Scaf1-EGFR DIII complex and hydrogen interaction in three inserted CDR loops, (C) Scaf2-EGFR DIII complex and its hydrogen interactions in grafted CDRs, (D) Scaf3-EGFR DIII complex and residues involved in hydrogen bonding.

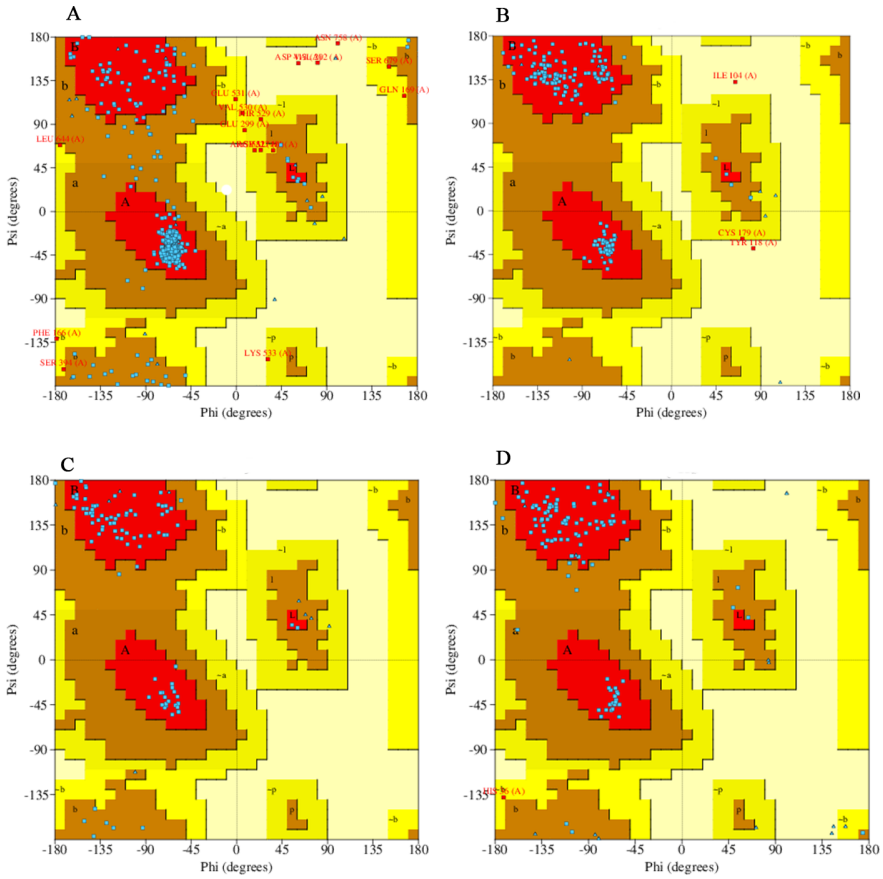

Fig.1

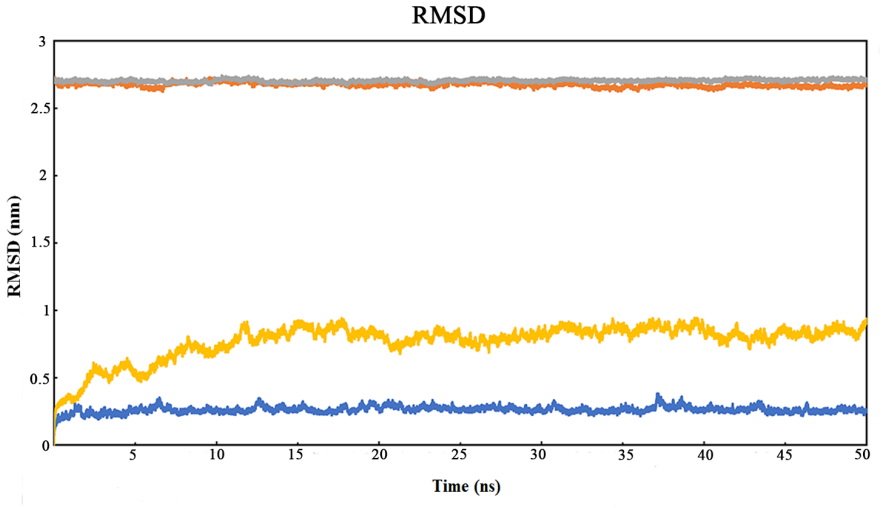

Fig. 2

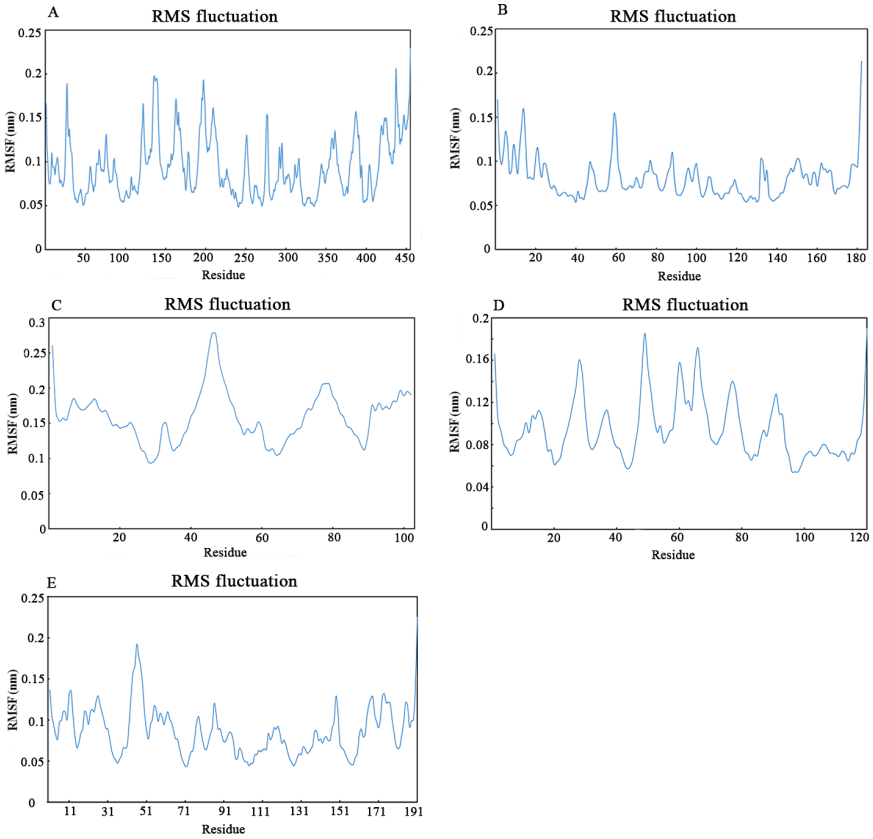

Fig. 3

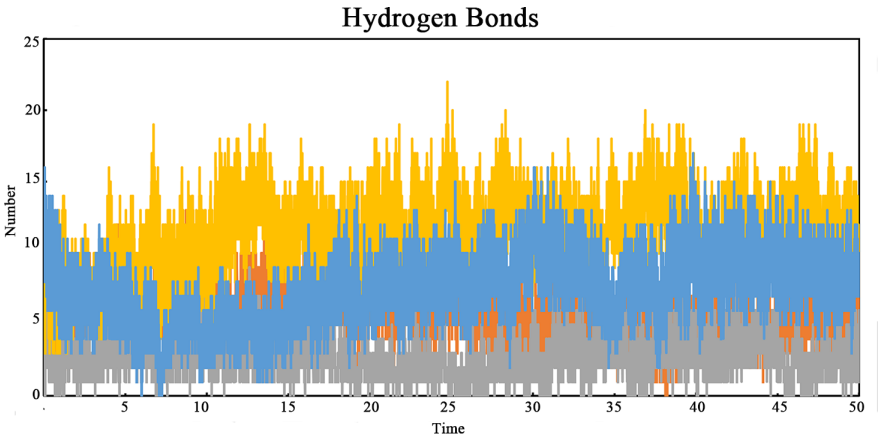

Fig. 4

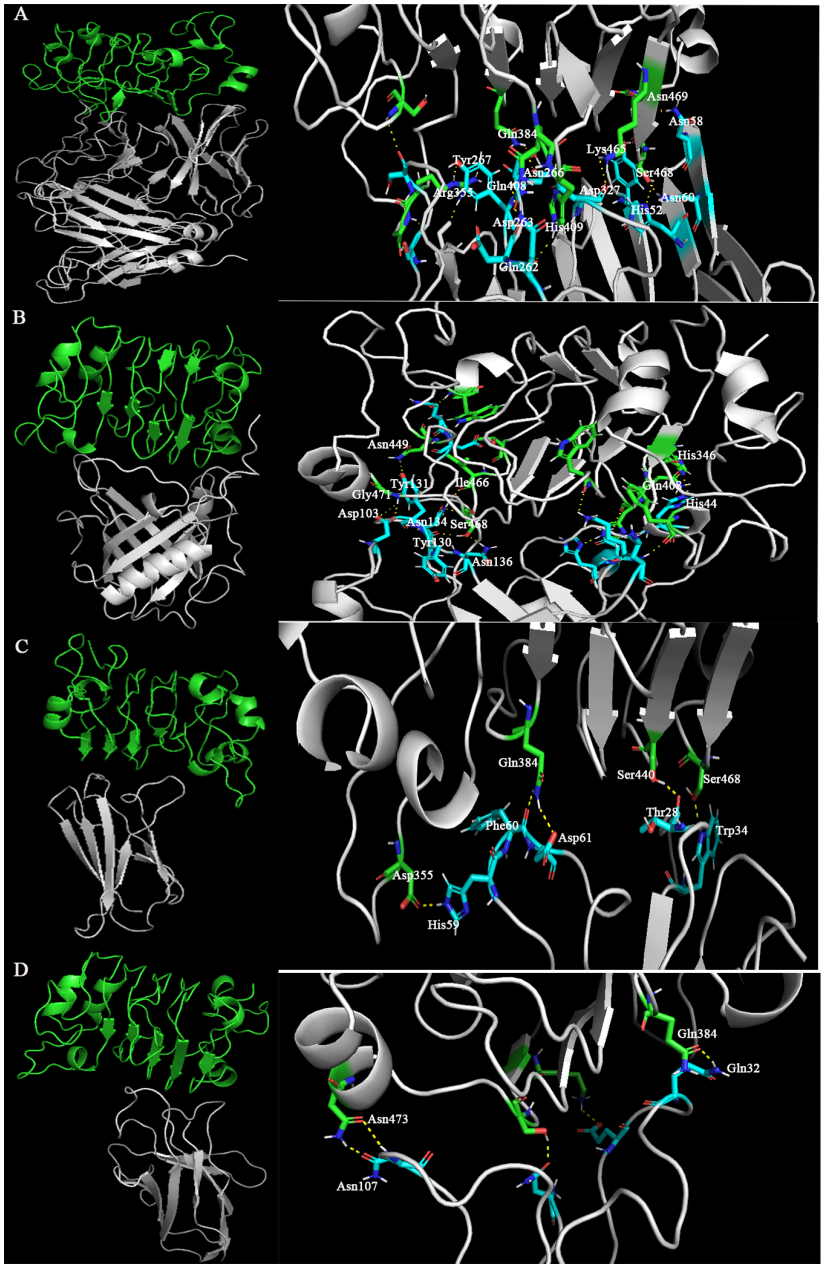

Fig. 5

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