

Guidelines To Predict Binding Poses of Antibody–Integrin Complexes

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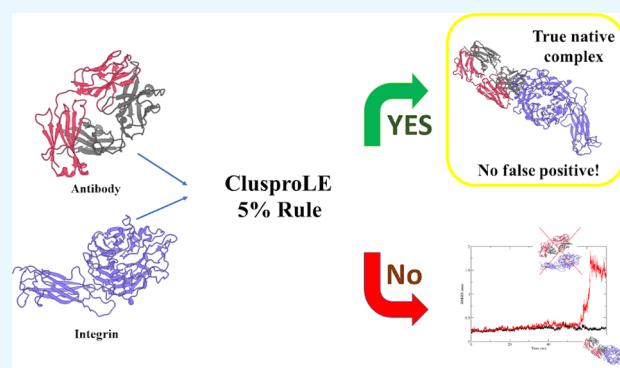


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ABSTRACT: Integrins are cell adhesion receptors that transmit bidirectional signals across the plasma membrane. They are noncovalently linked heterodimeric molecules consisting of two subunits and act as biomarkers in several pathologies. Thus, according to the increase of therapeutic antibody production, some efforts have been applied to produce anti-integrin antibodies. Here, we purposed to evaluate methods of generation and identification of the binding pose of integrin–antibody complexes, through protein–protein docking and molecular dynamics simulations, and propose a strategy to assure the confidence of the final model and avoid false-positive poses. The results show that ClusPro and GRAMM-X were the best programs to generate the native pose of integrin–antibody complexes. Furthermore, we were able to recover and to ensure that the selected pose is the native one by using a simple rule. All complexes from ClusPro in which the first model had the lowest energy, at least 5% more negative than the second one, were correctly predicted. Therefore, our methodology seems to be efficient to avoid misranking of wrong poses for integrin–antibody complexes. In cases where the rule is inconclusive, we proposed the use of heated molecular dynamics to identify the native pose characterized by RMSDi <0.5 nm. We believe that the set of methods presented here helps in the rational design of anti-integrin antibodies, giving some insights on the development of new biopharmaceuticals.



INTRODUCTION

Integrins are transmembrane heterodimeric glycoproteins consisting of two subunits, α and β .¹ Currently, 18 types of α -subunits and 8 of β -subunits are reported, forming 24 different integrins, described by now, from the combination of these subunits.² Such combinations between the α and β subunits allow each integrin to recognize one or more ligands present in the extracellular matrix (ECM) or cell surfaces and play a role in survival, proliferation, migration, transmigration, and apoptosis.³

Structurally, each integrin subunit is made of one cytoplasmic tail, one transmembrane helix, and an extracellular portion made of different ectodomains.⁴ The ectodomains responsible for ligand recognition is the β -propeller at subunit α and the β -I at subunit β .⁵ The interface between these ectodomains comprises the binding site, called metal ion-dependent adhesion site due to the presence of ions at the β -I, of some integrins such as $\alpha 4$, $\alpha 5$, and αv .⁴ Other integrins, such as $\alpha 1$, $\alpha 2$, and αL , have another ectodomain described, the α -I, which, in this case, is responsible for ligand recognition when it occurs.⁶

Integrins are considered biomechanical sensors of the microenvironment due to their capacity of recognizing changes

at the ECM, mediating specific cell responses to this, and, therefore, mitigating important physiological processes as embryo morphogenesis, wound healing, or regeneration.⁷ However, in addition to the importance of these receptors to biological processes, many integrins are biomarkers and mediators of different pathologies. Acute coronary syndrome, thrombosis, multiple sclerosis (MS), Crohn's disease, asthma, arthritis, platelet aggregation, psoriasis, glioblastoma, diabetic retinopathy, heart defects, atherosclerosis, melanoma, and prostate and pancreatic cancer are some diseases for which integrins are considered as markers to their progression and, therefore, for the development and prescription of anti-integrin drugs.⁸

The therapeutic antibodies market is in constant progress since 1986.⁹ In 2018, about USD 115.2 billion were allied to therapeutic monoclonal antibodies.¹⁰ Among the 94 therapeutic

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tic antibodies approved for the FDA.¹¹ (Food and Drug Administration), four are anti-integrins: Abciximab, Etaracizumab, Natalizumab, and Vedolizumab. Abciximab is a chimeric monoclonal Fab antibody that binds α IIb integrins, prevents myocardial ischemia, and controls unstable angina.¹² Etaracizumab is a monoclonal antibody against α v β 3 integrins for the treatment of stage IV metastatic melanoma.¹³ Natalizumab is a monoclonal antibody specific for α 4 integrins used for the treatment of MS and Crohn's disease.¹⁴ Vedolizumab is also a monoclonal antibody used for the treatment of Crohn's disease through the selective recognition of α 4 β 7 integrin.¹⁵

Given the relation of integrins with many diseases and the increase of therapeutic antibody production, some efforts have been applied to produce anti-integrin antibodies. As mentioned before, there are currently four approved anti-integrins¹¹ and at least four other antibodies are in advanced clinical trials. Therefore, the development of these antibodies is a promising strategy to treat or improve the treatment of integrin-related diseases.

The computational design of antibodies has been largely used in the past years despite the classical method of antibody production as animal immunization and large-scale library screening.¹⁶ One successful strategy for the design of antibodies is based on specific antigen–antibody interactions.¹⁷ The understanding of these interactions is dependent on the three-dimensional structure of the antibody–antigen complex, which can be achieved by experimental methods, such as crystallography or by computational methods, as protein–protein docking.

Molecular docking is a computational technique used to predict noncovalent interactions between macromolecules or, even more often, between a macromolecule (receptor) and a small molecule (ligand). Great progress has been made to improve protein–protein docking tools, allowing the obtaining of different protein complexes and the study of interactions involved in them.¹⁸ However, there are still some limitations at protein–protein docking functions and programs, which make obtaining of a truly binding-pose model of a complex difficult.¹⁹ The first problem is to perform the sampling of the possible protein–protein complexes, which is a hard task when the system is flexible or there are considerable conformational change upon binding. Once the possibilities are generated, the scoring function must be robust enough to identify the correct binding conformation of the complex.

Although, the identification of native antibody–antigen interaction for further studies of computational design of antibodies through docking tools is still limited. Once the right geometry is identified, further optimization on antibody structure can be done to optimize the specificity and affinity with its antigen.

Another computational method that assists the antibody design process is the molecular dynamics (MD) simulation. Thus, through such methodology, the dynamic behavior of molecules and complexes is simulated, with the monitoring of their temporal evolution from a classical force field, which describes bonded and nonbonded parameters of molecules, according to numerical resolution of Newtonian classical mechanics equations.²⁰

In addition, MD simulations have been used to facilitate the choice of the correct docking pose, in cases of protein–ligand,²¹ protein–carbohydrate,²² and protein–protein complexes.²³ It was noticed that the incorrect complex generally disrupts during the simulation.

Here, we aimed to evaluate methods of generation and identification of the binding pose of integrin–antibody complexes, through protein–protein docking and MD simulations and propose a strategy to assure the confidence of the final model as the correct one.

RESULTS AND DISCUSSIONS

Docking Using Single-Structure Native Conformation. Initially, we did a search for the three-dimensional structure of the approved therapeutic anti-integrin antibodies: Abciximab, Etaracizumab, Natalizumab, and Vedolizumab at Protein Data Bank (PDB) database.²⁴ The only antibody–integrin structure found was Natalizumab– α 4 integrin crystalized complex, under PDB ID 4IRZ. Because of the therapeutic importance of Natalizumab, we defined 4IRZ as our reference crystalized structure in this study.

Once we set our reference complex, we aimed to find which protein–protein docking programs were able to reproduce the native binding pose between Natalizumab and α 4-integrin based on 4IRZ. Then, we selected five protein–protein docking programs: ClusPro,^{25,26} PatchDock,²⁷ ZDOCK,²⁸ Hex,²⁹ and GRAMM-X,³⁰ and submit Natalizumab chains as receptors and α 4 chain as the ligand. For the docking runs in ClusPro and PatchDock, we used the antibody mode and, then, set the antibodies as receptors and integrins as ligands. Other parameters were set as default. For ZDOCK, GRAMM-X, or HEX, no residue to guide the docking were defined. For GRAMM-X and HEX, one hundred output models were set. Also, for HEX, shape + electrostatic OPLS minimization algorithms were defined to the docking runs.

To evaluate the output models generated by each docking experiment, we used the DockQ program.³¹ The combination of interface root mean square deviation (RMSDi), Lrms (Ligand rms), and native contacts (F_{nat}) parameters to measure quality of the docking models in CAPRI (a Critical Assessment of PRedicted Interactions)³² comprises the DockQ score, which represents how similar a generated docking model is to the native complex structure. DockQ allows classifying docking models in incorrect, acceptable, medium, and high quality models.³² Figure 1 shows integrin–antibody docking models generated by ClusPro aligned with native structures and their respective DockQ classification.

We compared the docking models from the five programs with the native complex 4IRZ to evaluate which of them reproduce the proper binding pose. Four programs were able to generate at least one docking model with high quality. Only

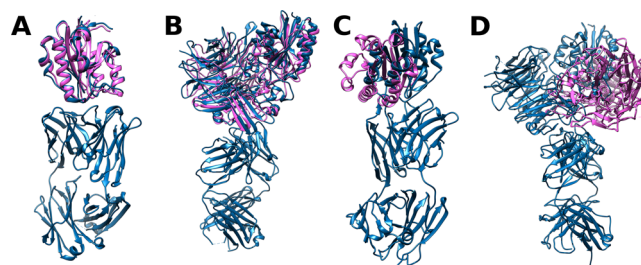


Figure 1. Illustration of (A) High (DockQ = 0.8), (B) Medium (DockQ = 0.5), (C) Acceptable (DockQ = 0.3), and (D) Incorrect (DockQ < 0.1) poses of integrin–antibody complexes obtained with ClusPro.

Hex did not generate any model with acceptable or better classification at DockQ.

Given the good performance of ClusPro, PatchDock, ZDOCK, and GRAMM-X, we increased the number of the evaluated antibody–integrin complexes to select which of them were able to reproduce the correct binding pose more frequently. Thus, we looked for “human integrins” at PDB, and we manually selected the entries with integrins complexed with antibodies. We found 35 complexes and picked 16 of them as our training group, based on crystal resolution, avoiding redundancy, and sampling diversification. The PDB IDs of the chosen structures are 2VDL, 4O02, 3ZE2, 2VDM, 2VDR, 1MHP, 6AVU, 6DJP, 3HI6, 3V4P, 3NIG, 3Q3G, 3EOA, 4Z7N, 3T3P, and 4IRZ itself. The respective antibodies and integrins of each complex were submitted to docking at the four programs. It is important to remark that only the β -propeller and the β -I or the α -I portions of each integrin were used in the docking.

As shown at Table 1, ClusPro reproduced the native binding pose in 81.25% of the cases, which represents 13 of the 15

Table 1. Percentages of Proper Binding Poses Predicted by Each Docking Program and Quality of the Obtained Models According to DockQ

program	correct pose (%)	DockQ quality model (%)		
		high	medium	acceptable
ClusPro	81.25	15.38	76.92	7.69
PatchDock	18.75	66.67	33.33	0.00
ZDOCK	56.25	44.44	44.44	11.11
GRAMM-X	75.00	91.66	0.00	8.33

complexes evaluated. Most of the reproduced docking models (76.92%, or 10 of the 13 correct complexes) from ClusPro were of medium quality. We observed that GRAMM-X reproduced the proper binding pose for 75% of the complexes and of which 91.67% were high-quality models. Therefore, we discarded from the further steps of evaluation the PatchDock and ZDOCK softwares, which generated correct models with a frequency lower than 60%. The percentages according to the DockQ Quality classification (Table 1) refer only to the complexes that reproduced, at least once, the native binding pose.

Cross-Docking Using Multiple Structures. In real cases of determining the antibody–antigen mode of binding by computational methods, proteins in the available experimental/modeled structures are probably in different conformation than those expected for the formation of desired complex. To assess capability of the GRAMM-X and ClusPro to identify the correct binding mode of integrin–antibody complex from non-optimal conformation, we applied cross-docking validation method. In this study, we performed nine antibody–integrin dockings, where the antibody and the integrin structures were obtained from distinct crystallographic structures, therefore, different PDBs. The cross-docking antibody–integrin combinations were: 2VDL-3FCU, 2VDL-3ZE2, SOPY-3IJE, SOPY-4O02, 4O02-6AVU, 3HI5-3EOA, 3EO9-3HI6, 4IRZ-3V4P, and 3QA3-3Q3G.

As cross-docking results, we observed that GRAMM-X did not generate correct models for any of the cross-docking runs (Table 2), whereas ClusPro reproduced the correct binding pose at 44.4% of the cases (Table 2). Despite the high quality of models generated by GRAMM-X in ideal situation where

Table 2. Crossdocking Performance: Percentages of Proper Binding Poses Predicted by ClusPro and GRAMM-X and Percentages of Distinguishing the Correct Model by 5% ClusPro Rule

program	correct binding poses predicted	models with high quality	models with medium quality	models with acceptable quality	5% ClusPro rule
ClusPro	44.4	25	25	50	50%
GRAMM-X	0	–	0	0	–

integrin and antigen conformation of binding is known, this program showed to be highly dependent on the initial conformation of the proteins, which is a considerable issue for prediction of the protein–protein binding mode. Otherwise, ClusPro showed to be less dependent on initial conformation, once it could recover almost 50% of the complexes tested in condition of cross-docking. Given the difficulties of performing cross-docking validation for protein–protein systems, these results represent one important advantage for using ClusPro to generate binding poses for integrin–antibody complexes.

Rescoring of Docking Poses. Once we set the programs to generate the correct docking models (Figure 1), our next challenge was to define a method to distinguish the proper docking model among all output files produced by the programs. ClusPro standard creates 30 output models for each docking run, on average, whereas GRAMM-X generates as many models as defined by the user. In our case, we defined a value of 100 output models to each docking. At first, we used two software to rank the models from both programs and point the correct binding pose: FireDock³³ and DockScore.³⁴

The FireDock and DockScore servers create a list with ranked models based on interaction scores. For each program, we verified if the best ranked model corresponds to the correct binding pose reproduced by ClusPro or GRAMM-X generated using the native complexes as input. As Table 3 shows,

Table 3. Percentages of Distinguishing the Correct Model by Each Ranking Methodology for ClusPro and GRAMM-X Poses

ranking method	software to generate pose	
	ClusPro	GRAMM-X
FireDock	58.33	75.00
DockScore	8.33	0.00
GRAMM-X rank	–	75.00
lowest energy (ClusPro)	83.33	–
cluster size (ClusPro)	50.00	–
5% ClusPro rule	83.33	–

FireDock could distinguish and rank the proper model in first place in 58.33 and 75% of the complexes in which the correct pose was proposed by ClusPro and GRAMM-X, respectively. On the other hand, DockScore recovered less than 10% of the correct pose for both structure-generating programs. Therefore, for our study, FireDock had a better performance than DockScore to rank and distinguish a correct binding pose from a poor one among the output docking models.

However, the use of FireDock or DockScore to rank models has a critical limitation factor. Both programs rank the generated complexes even when there are no correct docking models. This can be a huge problem due to the false positive

results generation. Therefore, we looked for a method to distinguish the native binding pose and signaling when there is no correct model.

5% Rule to Identify True Binding Conformations. We then evaluate the internal score values provided by ClusPro. No further analysis was carried out using GRAMM-X because of its bad performance on cross-docking evaluation. We used the ClusPro values of cluster size (CS, highest values are in the top of the rank) and lowest energy (LE, lowest values are in the top of the rank) to classify docking models and distinguish the proper models. As Table 3 shows, the LE score ranked the correct model in 83.33% of the cases, despite 50% of CS. However, LE per se is also incapable to distinguish incorrect models. To solve this limitation, we created a simple analysis strategy, based on LE, called “5% ClusPro rule” (Table 3).

The rule says that if the difference of LE between the first and the second models in the energy rank is equal or higher than 5%, the first model in the LE rank is correct. When the difference is below 5%, the first model is not the proper one and two situations are possible: the correct pose was not generated or the program is not being able to recover it using LE score terms. The rule ranked the correct binding pose in nine of the 16 integrins (62.5%). In three integrins (18.75%), for which no correct models exist, the difference was below 5% and the rule indicated that the first model was not correct, leading to one of the possible mentioned situations. For another three integrins (18.75%), the rule correctly pointed that the first model was not correct, but it was not able to rank the proper model.

When the analysis is restricted just to complexes with correct model prediction within the docking outputs, the rule was able to rank the proper model in 83.33% of the cases (Table 3). Therefore, it is very important to point out that the rule did not generate false positives in any of the tested complexes; it recovered the correct model in 62.5% of the cases and signaled wrong models in 37.5% of the cases, half of which belonged to docking outputs without correct models. In other words, this simple rule showed to be a good indicator of how reliable the first pose recovered by the LE score is.

To ensure the capacity of the rule to distinguish correct models without producing false positives, we set other 17 antibody–integrin complexes from the PDB search as our testing group to evaluate ClusPro and the 5% ClusPro rule as a pipeline. The PDB IDs of the complexes in the testing group are: 2VDK, 2VDN, 2VDO, 2VDP, 2VDQ, 2VC2, 3NID, 3NIF, 3T3M, 3V4V, 3ZDZ, 3ZE0, 3ZE1, 4Z7O, 4Z7Q, 5HDB, and 6AVR. The integrins and antibodies were submitted to ClusPro in the same way as the training group, the rule was applied, and the result was checked with DockQ.

We observed that ClusPro generates correct models in 15 (88.23%) of the 17 cases. The rule points the proper model in 13 (86.66%) from the 15 docking runs with correct models found, and in two cases (13.33%), it was not able to distinguish the proper model. For the two docking runs with no correct models generated (11.76%), all LE difference values were lower than 5%, which means that in any of the complexes tested till now, the rule pointed a wrong model as a correct one.

Therefore, the ClusPro and 5% ClusPro rule were approved after evaluation with the testing group of antibody–integrin complexes. Furthermore, the “5% ClusPro rule” distinguished the correct model among 50% of the outputs with the correct binding pose predicted during cross-docking validation steps.

Despite the “5% ClusPro rule” performance, we noticed one false-positive case at the cross-docking study, with the 4O02–6AVU combination. In this case, the rule pointed one incorrect model as the proper one, in the absence of correct models. However, this was the only case of false positive among 42 dockings performed at this study. In addition, we observed that, in this case, there is a huge difference between LE and center energy scores, which is not observed in the other cases. Also, 6AVU is a PDB obtained by electronic microscopy with a poor resolution, which can interfere in the docking process. Thus, given ClusPro results obtained by now, we set it as the most appropriate docking program for antibodies–integrin studies.

Heated MD to Distinguish True from False-Positive Complexes. We observed that in some cases, the ClusPro program generated a proper model, but the rule could not rank it. To complement the ranking methods proposed till now and to overcome the remaining limitation to distinguish the correct binding pose, we use MD as a tool to validate and demonstrate the different behavior between wrong and correct models. We applied the methodology described by Radom and co-workers²³ which consists of increasing the temperature during a short simulation to disturb the interactions in the wrong models, resulting in an unstable and high (root-mean-square deviation) rmsd along the simulation. In our study, we call this method “heated dynamics”.

As we defined 4IRZ as our reference complex and used it to evaluate the docking methods, we also performed the heated dynamics for this complex in order to validate that methodology on our system. Thus, we took all models generated by ClusPro referring to Natalizumab– α 4 complex and did the simulations.

We then carried out four consecutive heated dynamics simulations at 310, 330, 360, and 390 K for all poses obtained by ClusPro for 4IRZ complex. A better description of MD parameters is found in the Supporting Information section. In order to evaluate the stability of the binding pose during the simulation, we defined the RMSDi, the rms deviance of residues within 7 Å of protein–protein interface in the beginning of the MD procedure. Once the RMSDi value exceeded the cutoff of 5 Å, the binding pose was discarded.

Only in two of 28 simulations, the interface residues remained stable and below 5 Å after the MD simulation procedure, among them the native pose, as shown in Figure 2. Despite the complexity and size of integrin and Fab constructions of the antibody, our result is in good agreement with that of Radom and co-workers²³ and can be helpful to reduce the multitude of possible correct poses in cases where the previous pipeline is not able to rank the model with the correct binding pose.

Our study presents a combination of methods to ensure the reliability of the protein–protein complexes generated and then allow investigating the interaction between antibodies and integrins, giving guidelines (Figure 3) for further studies of computational evaluation and development of anti-integrin antibodies. As a pipeline to create antibody–integrin complexes, we propose the use of ClusPro protein–protein docking to generate docking models, the 5% ClusPro rule to rank the correct model and the heated dynamics to confirm the model pointed by the rule or to distinguish correct model from the wrong ones.

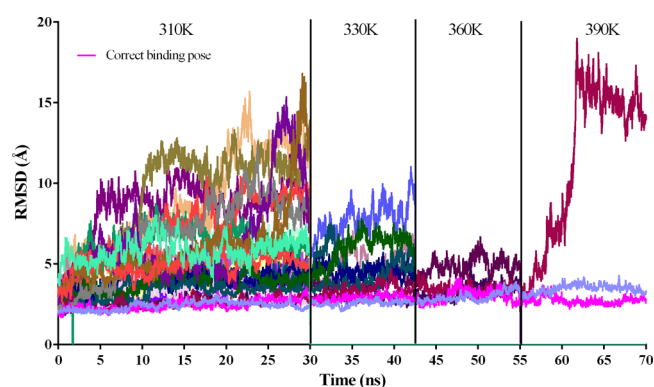


Figure 2. rms deviance of residues (rmsd) of the protein–protein interface for all models generated by ClusPro from the 4IRZ complex during the heated dynamics simulation. The y-axis shows the rmsd value in nm of the interaction interface for each model along the heated dynamics. The x-axis shows the time of simulation in ns. The vertical bars mark the temperature range during the simulation. Each color represents a model from ClusPro, and the black colored line represents the docking model with the correct binding pose.

DISCUSSION

The ClusPro software performs three basic steps in a docking run: rigid body docking, rmsd-based clustering of the 1000 LE score structures, and the removal of steric clashes by energy minimization.²⁵ The program received several improvements since the CAPRI meeting of 2017. This web-server automated program has been proved to be a useful tool to predict protein–protein interactions with accuracy and a user-friendly interface.³⁵ One considerable feature of ClusPro is an antibody–antigen mode for docking, which considers the proper symmetry for this kind of interaction.²⁶ This mode improves the accuracy of the docking results, and it is an advantage for the computational design of antibodies.

The 5% ClusPro rule presented here is helpful to distinguish correct models from wrong models without doing new computational experiments. For antibody–integrin systems, the rule was trustable, without any false-positive cases. It is noteworthy that the rule must be different for different kinds of protein complexes, and this should be tested for each case.

Even with the good performance of ClusPro, this program has a limitation that must be considered for the design of anti-integrin antibodies. ClusPro does not include heteroatoms, such as ions, in its docking run.²⁵ This can be a problem, once integrins have essential ions in their structures.³ All 35 complexes evaluated in this study do not have ions at the

binding-sites of the antibodies, allowing the use of ClusPro results to further steps. However, we strongly recommend the use of complementary docking programs, such as HADDOCK,³⁶ based on ClusPro outputs to generate binding poses which include ions at the docking run.

The heated dynamics, as expected, works to our system in a similar way as described by Radom and co-workers.²³ We applied the RMSDi method to evaluate the contact surface between the structures. RMSD values of full complexes are not useful in this case due to the presence of two domains that are very flexible with each other and there is possibility of antibody interaction with both domains. Also, direct calculation of the contact surface area should not work, as a restructuring of the complex may increase the measured value. The main conclusions about applying heated dynamics to discard wrong binding poses are that the contact surface of a truly protein–protein complex must be stable at increased temperatures and, apparently, this is also true for antibodies–integrin systems, as shown here.

MD has been an important strategy to develop the computational design of antibodies.³⁷ The heated dynamics was enough to observe the behavior differences between complexes, to point one or two possible correct models.

One interesting pattern we found in this study was that ClusPro and GRAMM-X properly found the correct binding pose of all complexes made of α IIB integrins, whereas none of the evaluated programs predicted the binding pose of all complexes made of α v. The other integrins, α 1, α L, and α 4, had their complexes predicted in different frequencies among the programs. This observation gives some alerts and guidelines for the development of anti-integrin antibodies.

The computational prediction of a complex with α IIB integrins is well established, giving certain insurance to work with these integrins and applying the methods discussed here. However, computational studies with α v must take some care to validate a binding pose, as we saw that none of the tested program was able to generate correct docking models. We recommend, then, the use of other methods or programs for these integrins. To the other integrins, the methods presented here may be applied, but while taking some care to avoid working with unreal binding poses.

CONCLUSIONS

In this work, we displayed some guidelines to generate and distinguish binding poses of antibody–integrin complexes, which are potential targets for development of new biopharmaceuticals. We suggest that using ClusPro as the

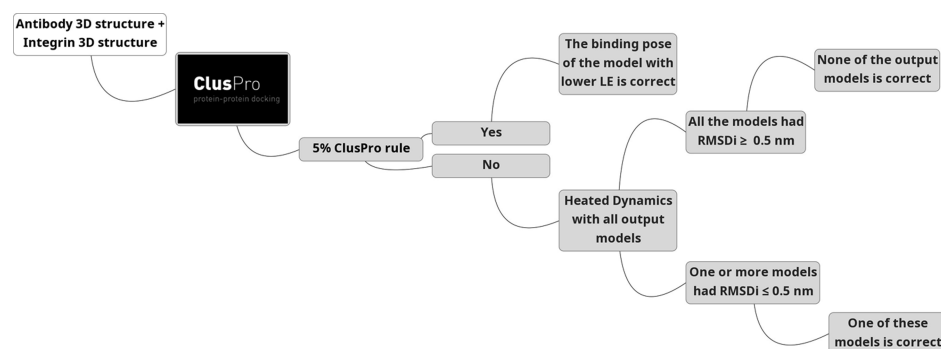


Figure 3. Pipeline proposed to generate an antibody–integrin complex with the correct binding pose.

docking program, the 5% rule to point the proper model and the heated dynamics to validate the model is a promisor strategy to work with this kind of protein complex. This methodology was built based on low conformational change of the integrin “head” upon binding of antibodies and the conserved folding through the family. These features allowed us to recover the correct pose even when non-native structures were used as input complexes for protein–protein interaction prediction. For different protein complexes, we strongly recommend the revalidation of these methods, in which our methodology may be used as a starting point to develop and fine-tuning of system-specific pipelines. Furthermore, we endorse the use of additional docking tools, such as HADDOCK, for some integrin complexes to consider ions at the docking run.

The set of methods presented here helps in the rational design of anti-integrin antibodies by proposing the best docking and ranking method to generate antibody–integrin complexes. A knowledge of the three-dimensional structure of these complexes is fundamental to investigate the main interactions involved, to predict hotspot residues and purposing mutations to regulate some antibody properties as affinity and specificity. In addition, our guidelines have shown to be reliable and reproducible.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c00226>.

MD simulation: force field, ensembles, and other parameters; RMSDi calculation: all criteria applied to obtain RMSDi; capability of docking programs to generate correct pose for 4IRZ complex and all complexes from the training set; ranking of Cluspro models using FireDock and DockScore score; ranking of GramXX models using FireDock and DockScore score; cross-docking result for GramXX and Cluspro; and validation of Cluspro Lowest Energy 5% Rule using 17 complexes from the test set (PDF)

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Notes

The authors declare no competing financial interest.

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