

DiffBind: A SE(3) Equivariant Network for Accurate Full-Atom Semi-Flexible Protein-Ligand Docking[†]

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Molecular docking, a key technique in structure-based drug design (SBDD), plays a pivotal role in hit identification for specific targets. Accurate prediction of protein-ligand binding mode is important for precise scoring and rational molecular optimization. Notwithstanding its significance, modelling precise and physically plausible binding conformations is a largely unsolved problem in the real-world docking scenario. Flexible docking is a daunting task as modeling protein conformation changes upon ligand binding is extremely computationally expensive and inaccurate. Currently available deep learning docking methods ignore protein flexibility and fail to ensure the physical plausibility and detailed interactions. In this study, we present DiffBind, a comprehensive full-atom diffusion-based semi-flexible docking model that operates over the product space of ligand movements (translation, rotation, and torsion) and pocket side chain torsion changes. Evaluations reveal that DiffBind has considerably higher accuracy in producing native-like binding structures with physically plausible and detailed interactions than traditional docking methods and other deep learning-based approaches. Even in the AlphaFold2 modeled structures, DiffBind still demonstrates superior advantages in accurate pose prediction and structure refinement. DiffBind should be useful for modeling the pocket-ligand binding structure with significant side chain flexibility and virtual screening.

1 Introduction

The primary paradigm of drug discovery involves identifying and designing molecules that target key proteins within disease pathways. Historically, screening compound libraries using biochemical platforms has been the predominant approach for identifying novel drugs¹. Since the 1990s, high-throughput screening (HTS) has been employed on libraries ranging from 500,000 to 10^8 molecules^{2,3}, leading to the discovery of several drugs. While the HTS libraries represent a significant advancement over traditional lab-designed ones, they encompass only a fraction of potential drug-like molecules⁴. Given the challenges and expenses associated with synthesizing such a vast chemical space, computational methods for screening virtual libraries are frequently employed in drug discovery, allowing exploration of chemical spaces comprising tens of billions of molecules or even more^{5–7}.

Structure-based virtual screening (SBVS) enables rapid and cost-effective modeling of target-molecule binding structures from large-scale compound libraries, as well as the evaluation of their binding affinities, thereby identifying potential hits^{8–10}. Molecular docking is one of the most frequently employed techniques for SBVS. It is utilized to predict ligand binding poses, characterize protein-ligand binding strength, and identify key interactions^{11,12}. In general, conventional docking softwares, in-

cluding AutoDock4¹³, AutoDock Vina^{14,15}, Smina¹⁶, Glide¹⁷, and GOLD¹⁸, leverage heuristic search algorithms, to explore a variety of potential ligand conformations. Scoring functions with simplified terms are utilized for the fast estimation of the binding affinity and the priority of ligand poses.

Classical molecular docking methods describe protein-ligand interactions based on the lock-and-key model¹⁹, wherein a rigid receptor binding pocket serves as the "lock" and the molecular docking algorithm primarily optimizes the ligand's conformation to find a complementary "key". Such rigid docking methods, for the trade-off between accuracy and computational efficiency, always strive to determine the optimal and complementary binding conformation. When known complex structures are available, and ligand molecules are removed and then re-docked into native Holo pockets, rigid docking often achieves impressive success rate²⁰. However, in real-world cross-docking scenarios, binding pockets may undergo different structural alterations upon various ligands binding²¹. Even when known ligand-bound structures (Holo) are unavailable for investigated targets, drug screening and design on its unbound state (Apo) or computational modeled structures usually gives unsatisfied hit rate^{22–27}. If a docking method does not account for pocket flexibility, its performance can drastically decrease in such cases²⁸, which may cause potential drugs get ruled out during the early stage of drug discovery. Although AlphaFold2²⁹ is capable of accurately modeling target protein structures, traditional docking methods that overlook potential side-chain flexibility may still fail to perform effectively when applied to these structures³⁰.

Currently, there are two main strategies to address the flexibility of protein pockets. The first involves inducing local conformational rearrangements of the target using force fields or scor-

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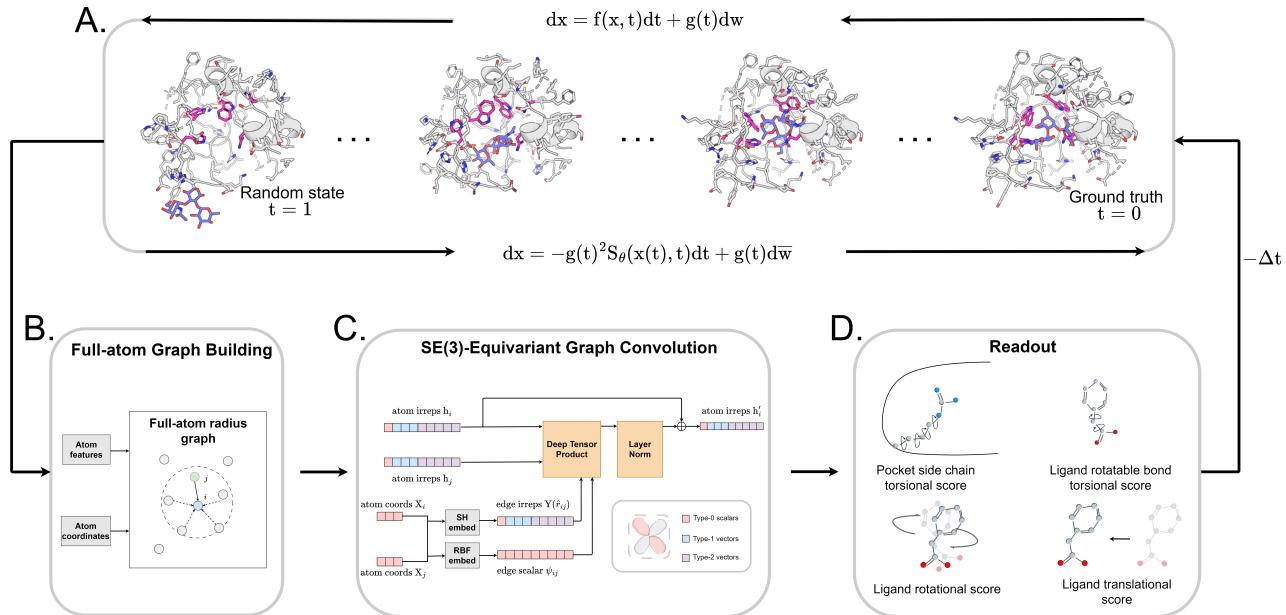


Fig. 1 The architecture of DiffBind. (A). Overview of score-based generative modeling through SDE for semi-flexible docking. The semi-flexible docking process is decomposed into ligand translation, rotation, bond torsion and pocket side chain torsion. (B). Construction of full-atom interaction graph. According to the real-time coordinates of each atom, we build the spatial graph as model input. (C). The architecture of $SE(3)$ equivariant graph convolution. It serves as the trunk block of DiffBind network. h_i and X_i are the irreducible representations (Irreps) and coordinates of atom i , respectively. The distance and vector between atom i and atom j are embedded through Gaussian radial basis (RBF) and spherical harmonics basis (SH) respectively, to get their edge scalar representations ψ_{ij} and edge vector Irreps $Y(\hat{r}_{ij})$. Then, Deep Tensor Product from e3nn is served as message-passing module to gather messages from neighborhood, followed by an equivariant Layer Normalization (Layer Norm) module to get the updated Irreps h'_i . (D). The output readouts of DiffBind network contains the predicted score of pocket side chain torsion, ligand rotatable bond torsion, ligand rotation and ligand translation. These scores are used to solve the reverse SDE.

ing function. For instance, rDock³¹ allows movements of functional groups that result in hydrogen bonds including -OH and -NH3. AutoDockFR (AutoDock for Flexible Receptors)³² allows users to specify up to 14 flexible side chains in advance and samples reasonable side chain dihedral angles from a rotamer library. Despite its better performance than AutoDock Vina in cross-docking experiments with Apo structures, it is considerably time-consuming and requires prior knowledge of potentially critical side chains in the pocket, which limits its application in SBVS. The second approach is the recently developed deep learning-based methods³³, which coarsen the representation of protein pockets, typically encoding only the protein's backbone atoms. This representation is not sensitive to minor pocket backbone flexibility and side chain adaptability. Earlier works, like DeepDock³⁴, TankBind³⁵, and EDM-Dock³⁶, predicted pocket residue-molecule distance map, which is used to reconstruct the binding structure. Leveraging powerful equivariant neural networks like EGNN³⁷, geometric deep learning³⁸ models such as EquiBind³⁹, LigPose⁴⁰, E3Bind⁴¹, Uni-Mol⁴², and KarmaDock⁴³ could iteratively predict the three-dimensional coordinates of ligands directly around the whole protein (blind docking) or predefined pocket. Recent SOTA blind docking method DiffDock⁴⁴ based on the diffusion generative modelling⁴⁵ employed the $SE(3)$ equivariant neural network⁴⁶ to denoise the rotation, translation, and bond torsion of ligand, and then rank poses by additional confidence model. However, existing deep learning-based docking approaches face limitations in effectively handling protein flexi-

bility. In addition, the generated ligand poses are often implausible for deep learning-based methods⁴⁷. Not only are there issues with bond lengths, angles, and torsion angles, leading to high intra energies of the ligand, but also optimizing the conformations generated by RDKit alignment^{43,48} also cannot alleviate clashes between the ligand and protein. Furthermore, ignoring the flexibility and invalidity of ligand poses makes it challenging for these deep learning-based methods to capture key interactions during cross-docking⁴⁷. Such short-comings impede subsequent steps, including experts post-optimizing ligands based on these detailed interactions or conducting further studies through molecular dynamics simulations.

Early Apo-Holo pair analysis has shown general consensus that upon ligand binding protein pocket undergoes significant side chain conformation heterogeneity while backbone is relatively rigid in most cases^{49–51}. Therefore, in most cases, side chain flexibility modelling is enough for flexible docking. In this study, we developed a full-atom semi-flexible docking model, DiffBind, based on the diffusion framework (Fig.1). We formulated semi-flexible docking as a problem of learning the joint denoising process of four variables in their tangent space: ligand rotation R , translation T , rotatable bond torsion τ , and pocket side-chain torsion χ . Following the VE-SDE (variance exploding stochastic differential equation) paradigm⁵², starting from the crystal complex $P(x(0)) = P(R(0), T(0), \tau(0), \chi(0))$, the forward process of the diffusion model, $P(x(t)|x(0))$, involves uniformly and continuously sampling time step $t \in [0, 1]$ and injecting noise to the four kinds

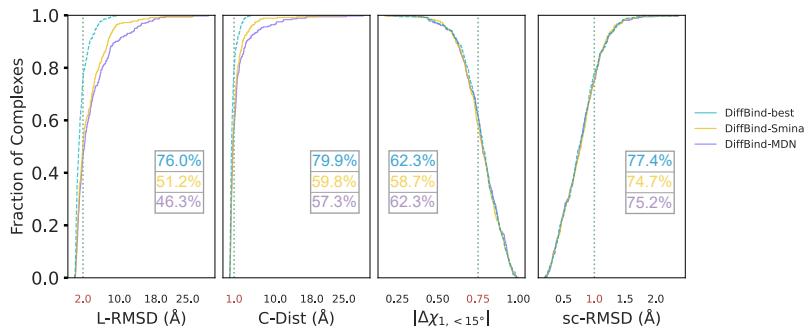


Fig. 2 Performance of DiffBind in PDBBind time-split test set. For each complex, 40 poses are generated. Distributions of L-RMSD, C-Dist, χ_1 and sc-RMSD are computed between DiffBind generated poses and ground-truth complex poses. Here, "DiffBind-best" means certain metrics are from the pose with the lowest L-RMSD generated by DiffBind model; "DiffBind-Smina" represents the DiffBind generated top-1 poses for each complex ranked by Smina scoring function; "DiffBind-MDN" represents the DiffBind generated top-1 poses for each complex ranked by MDN confidence model.

of movement operator to achieve binding structure perturbation. DiffBind is a SE(3)-equivariant generative model, following the message-passing paradigm⁵³ of graph neural network, and encodes the intricate interactions between the full-atom pocket and ligand, predicting the scores $\nabla_{x(t)} \log P_t(x(t))$ ⁵². At the docking procedure, starting from the randomly initialized binding conformation, the scores predicted by DiffBind are used to solve the reverse VE-SDE process⁵² to implement denoising sampling. With physics-based scoring function Smina¹⁶ or mixture density neural network (MDN)³⁴ serving as confidence model, binding structures sampled by DiffBind can be ranked, and then the top-1 complex pose can be selected as the final prediction. In the comprehensive evaluation, starting from pocket conformations with randomized side chain torsion angles, DiffBind outperforms state-of-the-art (SOTA) deep learning techniques and traditional docking methods. DiffBind can not only accurately recover protein pocket side-chain conformations, but also generate precise and highly physically plausible ligand binding poses. In cross-docking benchmark, DiffBind, due to its explicit consideration of pocket side-chain flexibility, significantly outperforms traditional semi-flexible docking methods like AutoDock VinaFlex³² and rDock³¹. It also demonstrated superior performance in generating both accurate and valid binding structures compared to other deep learning models that utilize coarse-grained protein structural representations.

2 Results and discussion

2.1 Performance on the PDBBind time-split test set

The performance of DiffBind is initially assessed using the PDBBind time-split test set^{39,54}. We employed four metrics, including ligand Root Mean Square Deviation (L-RMSD), geometric center deviation (C-Dist), proportion of pocket residues with $|\Delta\chi_1| < 15^\circ$ ($|\Delta\chi_1| < 15^\circ$) and side chain Root Mean Square Deviation (sc-RMSD) for the semi-flexible docking evaluation. As is depicted from Fig.2 (DiffBind-best), among the best poses (with lowest L-RMSD) from 40 DiffBind-generated poses for each complex, 76.0% of the ligand poses achieve successful docking (L-RMSD $< 2 \text{ \AA}$). Of these successful DiffBind-best poses, 79.9% correctly identify the binding sites (C-Dist $< 1 \text{ \AA}$); 62.3% demonstrate effective χ_1 recovery (more than 75% within $|\Delta\chi_1| < 15^\circ$); 77.4% ex-

hibit reliable side chain recovery (sc-RMSD $< 1 \text{ \AA}$). The results of ablation experiments conducted on the hyperparameters related to network sampling and denoising within the DiffBind framework are detailed in Supplemental Fig.S2. However, DiffBind-best represents the optimal scenario achievable by the DiffBind model, assuming a perfect confidence model could identify all the best poses. To enhance pose selection, we have developed two confidence models for ranking the generated poses (Fig.3.(A)). The first model (DiffBind-Smina) employs the all-atom physics-based scoring function Smina¹⁶, and the second one (DiffBind-MDN) utilizes a MDN (Mixture Density Network) network trained on the PDBbind time-split training set by this work. Using Smina to select the top-1 binding poses, DiffBind-Smina attains a success rate of 51.2% in the PDBbind time-split test set. Among the top-1 poses ranked by Smina, 59.8% can accurately locate binding positions; 58.7% show effective χ_1 recovery; 74.7% have reliable side chain recovery. The results demonstrate that DiffBind can accurately reconstruct the side chain conformations consistent with true pocket-ligand interactions, allowing the Smina to effectively select high-quality binding poses. When ranking sampled poses via the MDN model, DiffBind-MDN achieves a little bit lower success rate of 46.3% compared to DiffBind-Smina. Among the top-1 poses ranked by MDN confidence model, 57.3% correctly identify binding positions; 62.3% demonstrate effective χ_1 recovery; 75.2% have reliable side chain recovery.

Subsequently, the performance of traditional and recent deep learning-based methods is evaluated for comparison. The top-1 docking poses of each method, selected based on its confidence model or scoring function, are analyzed. DiffBind-Smina and DiffBind-MDN significantly outperform other deep learning-based pocket docking methods (Fig. 3.(B)), including KarmaDock with RDKit⁴⁸ ligand conformation alignment (KarmaDock Align) and TankBind with predefined pocket (TankBind-Pocket). This highlights the advantage of our full-atom based model. Even when compared to the traditional rigid docking method AutoDock Vina and Glide, with the experimentally determined true side chain conformations (redock), DiffBind-Smina achieves a marginally higher success rate without knowing the side chain conformations in the complex. We further used Rosetta⁵⁵ to repack side chain conformations in these Holo structures to simulate

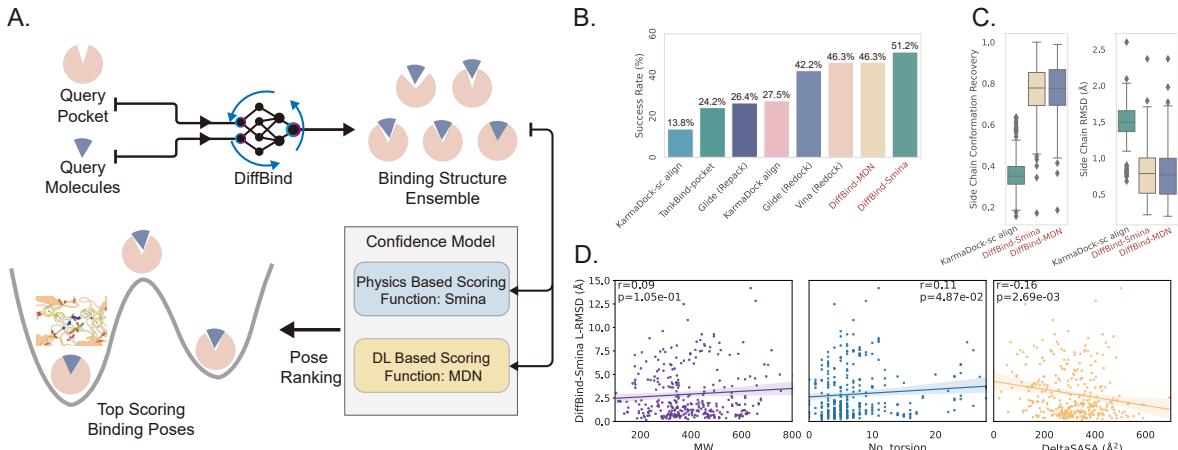


Fig. 3 (A). Overview of the DiffBind workflow. Various complex poses are generated by DiffBind network and confidence models are utilized to select the top-1 complex pose. **(B).** success rate of various pocket docking methods. **(C).** The distribution of $|\Delta\chi_1| < 15^\circ$ and sc-RMSD of KarmaDock-sc Align, DiffBind-MDN and DiffBind-Smina. **(D).** The correlation between L-RMSD from DiffBind-Smina and molecular weight (MW), number of rotatable ligand bonds (No. torsion) and variation of solvent accessible surface area caused by binding (DeltaSASA).

an Apo-like state for each target protein. The docking success rate of Vina and Glide significantly decreases in these Apo-like proteins, underscoring the limitations of rigid docking methods in handling side chain movements and the importance of semi-flexible docking in virtual screening. To illustrate the challenges of semi-flexible docking, we retrained the KarmaDock by integrating a ResNet module⁵⁶ (Supplemental Fig. S3) for predicting side chain torsion angles, resulting in a new model named KarmaDock-sc Align. DiffBind significantly surpasses KarmaDock-sc Align in terms of side chain recovery (Fig. 3.(C)). Compared to KarmaDock, the performance of KarmaDock-sc Align significantly declines (Fig. 3.(B)) due to the difficulty in balancing ligand coordinate recovery with side chain torsion recovery, highlighting the complexity inherent in semi-flexible docking. As is widely recognized, factors like the number of heavy atoms and rotatable bonds in a ligand profoundly impact the success rate of conventional docking programs⁵⁷. Hence, we examine the relationship between L-RMSD from the DiffBind model and various ligand characteristics, such as molecular weight, rotatable torsion bonds, and changes in solvent accessible surface area (DeltaSASA) upon binding. Contrary to traditional methods^{14,17,31,58}, as the ligand complexity and size increase, the performance of DiffBind-Smina does not deteriorate significantly (Fig.3.(D)).

2.2 Performance on the Posebusters test set

To evaluate the physical plausibility of poses generated by DiffBind, we compared its performance to other baseline methods using the Posebusters test set and the Posebusters suite⁴⁷, a tool designed to assess the validity of ligand-protein complexes based on criteria including bond length, planarity of aromatic rings in ligands, and clashes between ligands and proteins. Success in docking is redefined as a pose having an L-RMSD less than 2 Å and simultaneously passing the physical validity check by Posebusters, with this success rate termed as the PB-success rate. The Posebusters test set comprises 428 distinct complexes released since 2021, with no overlap with the PDBBind v2020 dataset. To

demonstrate that the success rate of DiffBind is not solely due to local ligand energy relaxation and its superior in side chain packing, the performance of other methods is evaluated with stricter energy minimization for the ligand, given the true side chain conformation. For poses generated by DiffBind, ligand energy minimization is conducted using the side chains as predicted by the model. The energy minimization is performed using the AMBER ff14sb force field⁵⁹ for protein and the Sage force field⁶⁰ for ligand in OpenMM⁶¹, as used in the Posebusters paper⁴⁷. Fig.4.(A) shows that traditional rigid docking methods like Glide perform best on re-docking when provided with the correct Holo pocket environment, followed by Vina and Gold, with most of their generated docking poses being physically valid. However, their performance significantly deteriorates when docking with Rosetta-repacked proteins, highlighting their heavy dependence on side chain conformations. Traditional semi-flexible docking methods rDock and VinaFlex are also involved in comparison. VinaFlex, heavily reliant on predefined flexible side chains, performs the worst in our scenario where information about flexible side chains is assumed unavailable. rDock, capable of optimizing functional groups prone to forming hydrogen bonds in side chains, achieves higher success rate in repacked proteins compared to Vina and Glide, but lower success rate in proteins with ground-truth side chains. For these traditional methods, their PB-success rate is only slightly lower than their overall success rate, indicating that most generated poses are validated by Posebusters suite due to the physical components in their scoring functions. Therefore, the post ligand optimization using force field does not cause obvious impact to their PB-success rate. Among blind docking methods, DiffDock shows better performance (success rate of 38%) than TankBind (16%) and EquiBind (2%), but most of their generated poses are invalid due to ignoring protein side chains (Fig.4.(A)). Ligand energy minimization significantly improves the PB-success rate of DiffDock (35%). TankBind and EquiBind also see improvements in PB-success rate with energy minimization, but still lag behind DiffDock (Fig.4.(B)). Although blind docking is a tough

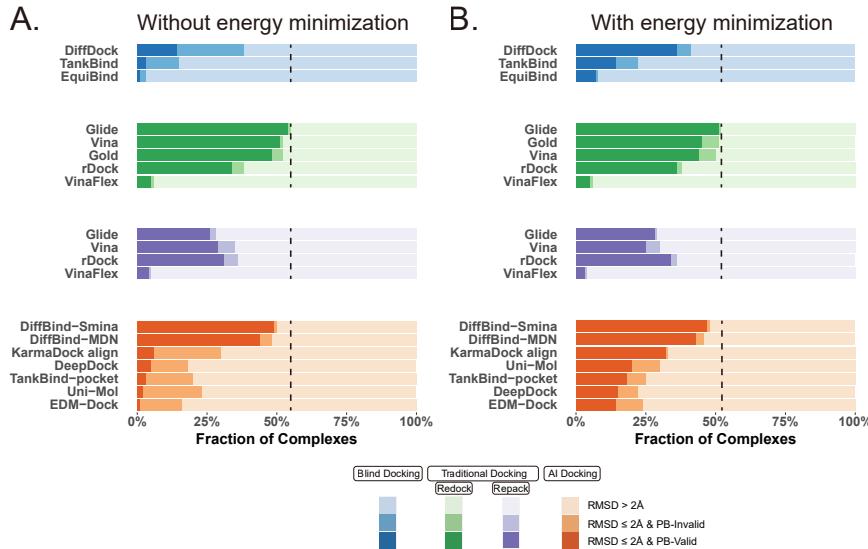


Fig. 4 Comparison of DiffBind with other method boosted by force field optimization in Posebusters test set. The left column (A) shows the performance of methods without energy minimization, and the right one (B) shows the performance of methods with energy minimization. The lightest color represents the failure rate for docking, the moderate color represents the success rate, and the darkest color represent the PB-success rate. The blue color, green color, purple color and orange color represents performance of blind docking methods, traditional docking methods in redocking, traditional docking methods using repacked target proteins as input and deep learning-based docking methods, respectively.

task for its broad searching space in the whole protein, but semi-flexible docking like DiffBind, denoising a chaotic side chain conformation into a well-packed conformation having valid interaction with the ligand, has much more objectives for prediction. DiffBind, utilizing Smina scoring function or MDN network as the confidence model to select the top-1 pose from 40 generated ones, outperforms all other deep learning-based methods in both blind and pocket docking. DiffBind-Smina and DiffBind-MDN demonstrate both high success rate (50.2% for DiffBind-Smina and 48.1% for DiffBind-MDN) and PB-success rate (48.1% for DiffBind-Smina and 44.4% for DiffBind-MDN), with lower penalties by Posebusters compared to other deep learning-based methods, showcasing the capability of DiffBind in generating accurate and physically plausible complex poses. The performance of DiffBind is comparable to traditional rigid docking methods using known ground-truth side chain conformations for redocking. As is depicted from Supplemental Fig.S5, DiffBind shows its effectiveness in binding site identification and pocket side chain recovery on Posebusters test set, as well. Force field optimization has minimal impact on DiffBind generated structures, which also demonstrates the high physical plausibility of DiffBind generated poses. Among other deep learning-based pocket docking methods, KarmaDock Align achieves the highest success rate (30.4%) but a very low PB-success rate (6.1%). Force field optimization of ligands rescues most poses with L-RMSD < 2 Å into good physical validity. KarmaDock Align, EDM-Dock, TankBind-pocket, Uni-Mol, and EDM-Dock, which focus on fitting the RMSD of the ligand and during training, tend to ignore the intra energy of the generated poses and protein side chains, as is shown from supplemental Fig.S7. Indeed, force field optimization is not allowed in realistic docking to meet the demands of high-throughput screening.

Four specific cases from the Posebusters test set (supplemen-

tary Fig. S8), never trained or seen by DiffBind, are presented to highlight its superiority over other methods focusing solely on ligand coordinates while neglecting ligand conformation validity. In complexes with PDB ID 6TW5, 7PRM, 7T1D, and 7CD9, DiffBind successfully docks ligands into precise positions with valid conformations and recovers pocket side chains into good interaction with ligands. In contrast, KarmaDock Align, EDM-Dock, and TankBind fails to predict correct binding ligand poses, and their generated poses cannot pass the physical plausibility check of the Posebusters suite. As is shown from Supplementary Table. S2, ligand poses generated by EDM-Dock and TankBind exhibit both internal invalidity (including internal steric clash, bump aromatic ring, etc) and steric clash with proteins, while KarmaDock Align, due to using RDKit for ligand pose alignment, frequently fails in ligand-protein clash.

2.3 Performance on the CD cross-dock test set

To showcase the exceptional capabilities of DiffBind in semi-flexible docking, we evaluate its performance in the more challenging task of cross-docking. We use a self-curated benchmark called the CD test set, which includes various cross-docking scenarios such as Apo-Holo and cross-docking between different Holo states with various protein families. CD test set contains six subsets, ApoRef²⁴, CASF2016⁵⁴ with target proteins in the Apo state, GPCR-AF2 set with Apo-like proteins predicted by AlphaFold2²⁹, and Ensemble sets featuring prominent docking targets including CDK2, EGFR and FXA. RMSD of binding site backbone (within 5 Å cutoff away from crystal ligand) conformational changes in these subsets predominantly range between 0-2 Å, as shown in Supplemental Fig.S2.

In these subsets, when considering L-RMSD alone, traditional rigid docking methods such as Vina, Smina, LinF9⁶², and Glide

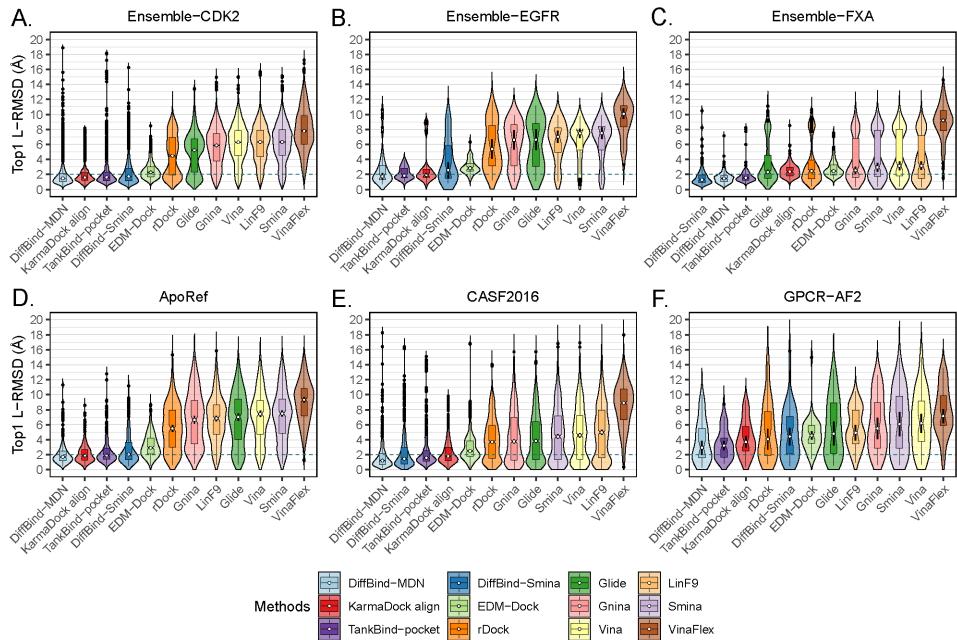


Fig. 5 The L-RMSD distribution of various methods in CD cross-dock test set.

Table 1 Success rate of various methods on CD cross-dock test set.

Method	Ensemble-CDK2			Ensemble-EGFR			Ensemble-FXA			ApoRef			CASF2016			GPCR-AF2			
	RMSD Mean / Å	RMSD Median / Å	PB-SR	RMSD Mean	RMSD Median	PB-SR	RMSD Mean	RMSD Median	PB-SR	RMSD Mean	RMSD Median	PB-SR	RMSD Mean	RMSD Median	PB-SR	RMSD Mean	RMSD Median	PB-SR	
Traditional rigid docking methods	Vina	6.12±2.63	6.26	0.079	6.37±2.34	6.90	0.060	4.35±3.17	3.02	0.344	6.98±3.35	7.22	0.082	4.93±3.62	4.46	0.294	7.42±3.99	7.77	0.136
	LinF9	6.12±2.50	6.31	0.061	6.31±2.43	6.63	0.119	3.88±2.90	2.96	0.365	6.59±3.08	6.67	0.089	4.99±3.56	4.52	0.272	5.66±2.54	5.18	0.076
	Smina	6.15±2.65	6.28	0.079	6.33±2.53	6.90	0.090	4.17±3.16	2.70	0.362	7.08±3.47	7.43	0.077	4.88±3.69	4.38	0.304	7.25±4.15	7.41	0.152
	Gnina	5.67±2.63	5.78	0.099	6.02±2.69	6.29	0.090	3.83±2.99	2.44	0.394	6.67±3.65	6.51	0.082	4.57±3.56	3.74	0.320	6.72±3.95	6.06	0.106
	Glide	4.81±2.60	5.21	0.154	6.87±3.63	7.67	0.090	3.49±2.72	2.31	0.271	6.62±3.51	6.85	0.091	4.28±3.20	3.74	0.219	5.37±3.63	4.57	0.182
Traditional semi-flexible docking methods	VinaFlex	8.04±2.92	7.88	0.013	9.03±2.47	9.446	0.000	9.19±2.22	9.42	0.005	8.81±2.94	9.06	0.015	8.80±2.98	9.07	0.023	7.36±3.33	7.07	0.045
	rDock	4.61±2.76	4.62	0.257	5.45±2.98	4.70	0.134	3.29±2.66	2.28	0.440	5.46±3.02	5.31	0.157	4.11±2.82	3.58	0.296	5.37±3.60	5.12	0.212
Deep learning-based docking methods	TankBind-pocket	2.17±1.85	1.62	0.100	2.21±0.96	1.82	0.015	1.73±0.96	1.50	0.110	2.63±1.88	1.97	0.040	2.37±2.38	1.63	0.123	3.74±1.98	3.42	0.015
	EDM-Dock	2.62±1.27	2.32	0.051	2.95±1.05	2.66	0.015	2.68±0.98	0.45	0.009	3.33±1.55	3.09	0.011	3.06±1.92	2.55	0.064	4.72±1.95	4.61	0.00
	KarmaDock Align	1.89±1.20	1.58	0.135	2.65±2.06	1.93	0.045	2.43±0.92	2.30	0.009	2.47±1.56	2.04	0.047	2.36±1.57	1.89	0.136	4.30±2.20	3.91	0.015
	DiffBind-Smina	2.31±1.80	1.73	0.564	3.44±2.53	2.57	0.403	1.54±1.14	1.30	0.789	2.96±2.32	2.10	0.434	2.30±2.28	1.58	0.566	4.92±4.12	4.02	0.227
	DiffBind-MDN	1.85±1.34	1.48	0.674	2.58±2.15	1.80	0.478	1.42±0.70	1.35	0.794	2.32±1.73	1.76	0.476	1.87±1.93	1.25	0.636	3.64±2.59	2.74	0.212

Best performance in bold. RMSD Mean and RMSD Medium, lowest; PB-SR, highest. RMSD Mean and RMSD Median denote the average \pm standard deviation and median of L-RMSD for top-1 generated ligand poses from each complex, respectively. PB-SR denotes PB-success rate.

underperform, compared to deep learning-based methods that use main-chain coarse-grained representations of proteins. As indicated in Fig.6 and Table.1, the L-RMSD median for methods like TankBind-pocket, EDM-Dock, and KarmaDock Align hover around 2 Å across the subsets, whereas for traditional rigid docking methods, it even surpasses 5 Å in subsets like CDK2, EGFR, and ApoRef. However, when physical plausibility is taken into account, the PB-success rate for TankBind-pocket, EDM-Dock, and KarmaDock Align drops to levels similar to traditional rigid docking methods (below 10%). Notably, in the Ensemble-FXA subset, methods such as Vina, LinF9, Smina, Gnina, and Glide performs significantly better, achieving PB-success rates of 34.4%, 36.5%, 36.2%, 39.4%, and 27.1%, respectively. Traditional semi-flexible methods such as VinaFlex and rDock, developed for cross-dock scenarios, were also evaluated. VinaFlex shows poorer performance than rigid docking methods in both

L-RMSD distribution and PB-success rate, due to its reliance on predefined side chains and limitations on the number of flexible side chains. The semi-flexible method rDock, capable of optimizing side chains conducive to hydrogen bonding, outperforms all traditional rigid docking methods in L-RMSD distribution and has higher PB-success rate than TankBind-pocket, EDM-Dock, and KarmaDock Align. We observed that both traditional rigid and semi-flexible docking methods perform better in subsets like Ensemble-FXA and CASF2016, where pocket backbone conformational changes are minimal (mostly between 0-0.5 Å). Our method DiffBind, leveraging a full-atom based neural network to learn additional side chain movements, marginally outperforms all other methods in accurately recalling ligand coordinates and ensuring the validity of complex poses. On the CD test set, the MDN network surpasses the Smina scoring function for pose ranking. DiffBind-MDN achieves state-of-the-art PB-success rate of

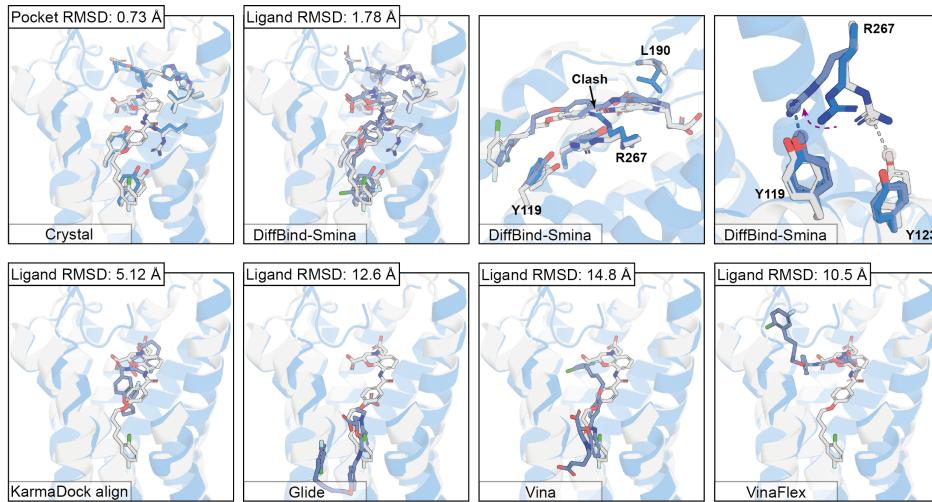
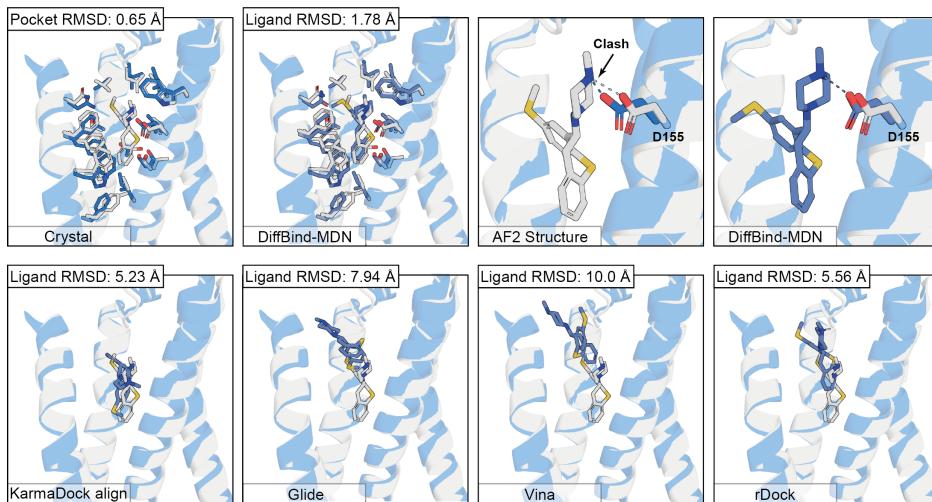
A.**B.**

Fig. 6 The binding poses of two cases from the GPCR-AF2 subset in the CD test set. In all panels, Holo protein and ligand are shown in grey. AF2 modeled protein structure is shown in blue. DiffBind sampled ligand and pocket side chains are shown in silver lake blue. Note that DiffBind calculations were done using the AF2 modeled backbone structures. For each frame, the docking method used is given at the bottom left. The pocket C α RMSD between Holo and AF2 structure within 5 Å cutoff away from crystal ligand, and ligand RMSD are reported on left top, except the two right top frames in each panel for the comparison between AF2 modeled and DiffBind sampled side chain conformations. (A). Human cysteinyl leukotriene receptor 2 bound to its antagonist ONO-2570366 (PDB ID: 6RZ6). The side chains of Y119, Y123, L190 and R267 are shown; (B). 5-Hydroxytryptamine receptor 2A bound to its inverse agonist methiothepin (PDB ID: 6WH4). The side chain of D155 is shown.

67.4%, 47.8%, 79.4%, 47.6% and 63.6% in CDK2, EGFR, FXA, ApoRef and CASF2016, respectively. In the GPCR-AF2 subset, DiffBind-MDN exhibits slightly lower PB-success rate of 21.7% compared to DiffBind-Smina (22.7%). This reduced performance is attributable to higher penalties incurred from ligand-protein clashes.

Here, we present the docking poses of various methods on two examples from the GPCR-AF2 subset. The first example involves a crystal structure with the PDB ID 6RZ6, where we investigate the target protein from 6RZ6 identified as the human Cysteinyl leukotriene receptor 2⁶³. This receptor plays a role in regulating pro-inflammatory responses associated with allergic disorders. The ligand in this case is an antagonist, ONO-257036. We

predict the AF2 structure (Apo-like) of the receptor protein and then dock the ligand molecule into this AF2 structure. Following binding sites alignment, the predicted AF2 structure exhibited a binding site (within 5 Å cutoff away from crystal ligand) RMSD of 0.73 Å when compared with the crystal structure. However, in the Apo-like AF2 predicted structure, residues R267 and L190 is found to block ONO-25703 binding to pocket, preventing traditional rigid docking methods like Glide and Vina from locating the correct binding site. VinaFlex, despite its ability to leverage side chain flexibility, also fails to dock the ligand correctly. KarmaDock Align, similarly did not achieve correct docking, although it shows better L-RMSD than the methods mentioned above. In contrast, our semi-flexible docking method, DiffBind, coupled with Smina

and MDN confidence model can select the top-1 complex pose from the 40 poses. DiffBind adeptly repacks the side chain of the R267 residue, enabling the ligand to successfully dock into the correct binding position. However, DiffBind fails to accurately predict the position of the R267 side chain. In the crystal structure, the R267 side chain forms a hydrogen bond interaction with the Y123 side chain, whereas in the DiffBind-predicted structure, R267 forms a hydrogen bond interaction with the Y119 side chain, which can be attributed to the similarity in the spatial positions of the Y123 residue and the Y119 residue relative to R267.

The second example involves a crystal structure with the PDB ID 6WH4, where the target protein is the human 5-HT2A serotonin receptor, which is associated with the actions mediated by psychedelics⁶⁴, and the ligand is methiothepin. Following alignment of the binding sites, the AF2-predicted structure displays a pocket RMSD of 0.65 Å when compared to the crystal structure. In the AF2 structure, the side chain of the D155 residue has vdW clash (pair distance is 1.5 Å) with the ligand, leading to the failure of docking attempts by Glide and Vina. Although KarmaDock Align and the traditional semi-flexible docking method rDock predicts the ligand position with a smaller L-RMSD, they still did not achieve successful docking. In contrast, the top-1 complex pose selected by the MDN confidence model in DiffBind not only accurately reproduces the ligand position but also successfully repacks the side chain of D155 residue, enabling it to form electrostatic interactions with the ligand. This example further demonstrates the capability of DiffBind to effectively manage protein-ligand interactions, particularly in challenging docking scenarios.

We also present four cases from ApoRef subset where DiffBind successfully docks ligands into the Apo pockets of crystal structures (Supplementary Fig. S9), with these complexes not having appeared in the training set. The PDB IDs for these four cross-dock examples are as follows: Holo: 1ZGY, Apo: 1PRG; Holo: 2XIR, Apo: 1VR2; Holo: 3UVR, Apo: 1WFC; and Holo: 3RM6, Apo: 4EK3. In each of these instances, top-1 complex poses generated by DiffBind accurately recover the ligand poses, while also optimizing side chains in the Apo state pockets that would otherwise clash with the ligand. These results highlight the potential of DiffBind in aiding researchers to study detailed interactions in real scenarios when no complex structures are available and provide insights for further lead optimization.

3 Conclusions

In this research, we have developed a full-atom diffusion model, DiffBind, for semi-flexible pocket docking. DiffBind is capable of explicitly simulating the interactions of full atoms between the pocket side chains and the ligand molecules, which is extremely hard for previous docking methodologies. Our method not only surpasses traditional approaches in terms of the docking success rate but also achieves state-of-the-art (SOTA) levels in generating plausible docking conformations as compared to recent deep learning methods, as evidenced by evaluations conducted on the PDBBind and Posebusters test sets. Furthermore, starting from a random side-chain conformation, DiffBind can accurately dock molecules while concurrently recovering the side-chain conformations.

On the cross-docking benchmark, CD test set, DiffBind has also demonstrated superior performance. Notably, previous methods that employed deep learning to characterize protein pockets through coarse-grained main-chain representations also showed promise results, but they led to a lack of detailed information regarding the interactions between side-chain atoms and ligands. DiffBind that simulates side chains addresses this gap in deep learning methodologies. The physical validity of DiffBind generated complex poses, coupled with the simulated detailed three-dimensional interactions, provides users with correct interactions to facilitate further optimization.

4 Methods

4.1 Datasets

4.1.1 PDBBind time-split dataset

We use the PDBBind v2020 dataset⁵⁴ for training and evaluation. For each target protein-ligand pair within the PDBBind v2020 dataset, we define the protein pocket as any residues within 12 Å buffer of any heavy atoms in the ligand molecule. Following the time split strategy proposed by the work of EquiBind³⁹, where 363 complex structures uploaded later than 2019 serve as test set. After removing ligands that exist in the test set, the remaining 16739 structures are used for training and 968 structures are used for validation. The dataset is named as "PDBBind time-split dataset" in the article.

4.1.2 Posebusters test set

The PoseBusters test set⁴⁷ is a meticulously curated collection of crystal complexes sourced from the PDB. This set encompasses a diverse array of high-caliber, recent protein-ligand complexes characterized by drug-like molecules. With 428 distinct complexes, inclusive of unique proteins and ligands released since 2021, it ensures no overlap with the complexes found in the PDBBind v2020 dataset.

4.1.3 CD test set

Given the current absence of a large-scale benchmark dataset for cross-docking, especially to address various cross-docking scenarios (including Apo-Holo and cross-docking between different Holo states), we have established a benchmark dataset tailored for cross-docking evaluation, termed CD test set. We integrated ApoRef²⁴, a test set constructed by constrained MD for inducing Apo-like pockets into Holo-like pockets; several prominent ensemble docking targets including CDK2, EGFR, FXA; CASF2016⁵⁴ and GPCR-AF2³⁰ that contains 18 human GPCR complexes published after April 30, 2018. ApoBind database⁶⁵ and AHoJ⁶⁶ are utilized to search for corresponding Apo states based on queried protein-ligand pairs, thereby creating pairs for the Apo-Holo and Holo-Holo mixed cross-dock dataset. The detailed protocol of how to construct the CD test set can be found in Supplementary Section 1. The finalized CD test set comprises of 14,194 structural pairs for cross-docking benchmark tests.

4.2 Diffusion generative model

The diffusion model utilize the framework of stochastic differential equations⁶⁷ to diffuse the data distribution described as follows:

$$dx = f(x, t)dt + g(t)d\omega \quad (1)$$

For $x \in \mathbb{R}^D$, $f(x, t) \in \mathbb{R}^{D \times D}$ denotes a vector-valued function called the drift coefficient of $x(t)$, and $g(t) \in \mathbb{R}^{R \times R}$ denotes a scalar function called the diffusion coefficient of $x(t)$. The lack of canonical local coordinate system defined for ligand molecules, makes the drift coefficient hard to design for the ligand rotation. Consequently, the drift coefficient $f(x, t)$ is set to be 0, and the model becomes the score-based generative model⁵². The reverse diffusion running backwards in time, which is also known as the denoising process, is given by the following reverse-time SDE:

$$dx = -g(t)g(t)^T \nabla_{x(t)} \log P_t(x) dt + g(t)d\omega \quad (2)$$

To estimate $\nabla_{x(t)} \log P_t(x)$, we can train a score-based neural network $S_\theta(x(t), t)$ to fit it. The standard score-match loss function is as follows:

$$\begin{aligned} J(x) = & \mathbb{E}_t [\lambda(t) \mathbb{E}_{x(t) \sim P_{t|0}(x(t)|x(0))} [\|S_\theta(x(t), t) \\ & - \nabla_{x(t)} \log P_{t|0}(x(t)|x(0))\|^2]] \end{aligned} \quad (3)$$

$\lambda(t) = 1/\mathbb{E}_{x(t) \sim P_{t|0}(x(t)|x(0))} [\|\nabla_{x(t)} \log P_{t|0}(x(t)|x(0))\|^2]$ is the pre-computed weight factors.

4.3 Pose transformations and diffusion on the product space

We choose the specific SDE for forward diffusion process as follows:

$$dx = \sqrt{\frac{d\sigma^2(t)}{dt}} dw, \text{ where } \sigma(t) = \sigma_{\min}^{1-t} \sigma_{\max}^t, t \in [0, 1] \quad (4)$$

$\sigma(t) = \{\sigma_R(t), \sigma_T(t), \sigma_\tau(t), \sigma_\chi(t)\}$ denotes the noises that injected into ligand Rotation R , translation T , rotatable bond torsion τ and pocket side chain torsion χ . According to the specific group that each variable lies in, we would design the form of corresponding σ carefully for diffusion kernel and the score computation. For a ligand pose with n atoms, $X_l \in \mathbb{R}^{3 \times n}$, translation of a ligand pose $T \in \mathbb{R}^3$ lies in the 3D translation group $\mathbb{T}(3)$. The diffusion kernel for ligand is a Gaussian function with variance σ_T as follows, which is also utilized for computing the score of ligand translation $\nabla P_{t|0}(X_l(t)|X_l(0))$:

$$P_{t|0}(X_l(t)|X_l(0)) = \mathcal{N}(X_l(0), \sigma_T(t)) \quad (5)$$

As rotation of a ligand pose lies in the 3D rotation group $\mathbb{SO}(3)$, $IGSO(3)$ distribution^{68,69} was chosen as the diffusion kernel. In specific, rotation matrix $R \in \mathbb{R}^{3 \times 3}$ can be split into a unit vector $\hat{\omega} \in \mathfrak{so}(3)$ uniformly as the rotation axis and a axis-angle $\omega \in [0, \pi]$. Consequently, the functionality of σ_R can be replaced by $\hat{\omega}$ and ω . The diffusion kernel for ligand rotation is as follows:

$$P_{t|0}(R(t)|R(0)) = \mathbf{R}(\hat{\omega}, \omega)R(0) \quad (6)$$

The score of rotation diffusion can be computed according to

$$\nabla \ln P_t(R(t)|R(0)) = \left(\frac{d}{d\omega} \log(f(\omega)) \right) \hat{\omega} \quad (7)$$

$$f(\omega) = \sum_{l=0}^{\infty} (2l+1) \exp(-l(l+1)\epsilon^2/2) \frac{\sin((l+1/2)\omega)}{\sin(\omega/2)} \quad (8)$$

where ϵ is a scalar variance for parameterizing the $IGSO(3)$ distribution. Torsion of pocket side chains and ligand rotatable bonds lie in the $SO(2)^m$ group and $SO(2)^k$ group respectively, where m and k denote the number of all χ from the pocket side chain and all τ from the ligand. Since each torsion angle coordinate lies in $[0, 2\pi]$, the m torsion angles of a conformer define a hypertorus \mathbb{T}^m . We introduced the diffusion kernel from the work of Torsional Diffusion⁷⁰ to satisfy angle periodicity, and compute its score $\nabla P_{t|0}(\chi(t)|\chi(0))$ as follows:

$$P_{t|0}(\chi(t)|\chi(0)) \propto \sum_{d \in \mathbb{Z}^m} \exp\left(-\frac{\|\chi(0) - \chi(t) + 2\pi d\|^2}{2\sigma_\chi^2(t)}\right) \quad (9)$$

Torsion of ligand rotatable bonds are dealt with the same way as pocket side chains.

Following the equation(3), the loss function is set as follows:

$$J(x) = J(R) + J(T) + \sum_1^k J(\tau) + \sum_1^m J(\chi) \quad (10)$$

The forward diffusion and reverse diffusion are both performed in the product space⁷¹ of $\mathbb{T}(3) \times SO(3) \times SO(2)^k \times SO(2)^m$, corresponding to the aforementioned four kinds of transformation.

During the forward diffusion process, we would sample $t \in [0, 1]$ for each pocket-ligand pair, and then utilize the defined diffusion kernel to sample each transformation. The torsion of ligand and pocket side chains is first applied to the pose, followed by translation and rotation.

The starting point of the denoising stage is a ligand conformation generated by RDKit⁴⁸ and pocket side chains with each type of transformation sampling from their σ_{\max} . According to equation(2), we update complex pose using the predicted score for each type of transformation. After applying the translation and rotation matrix constructed from predicted score, torsion angles get updated. It's noteworthy that there exists entanglement between ligand translation/rotation and its bond torsion, ligand pose need to be re-aligned to the pose before bond torsion, which will lead to model-unaware structural alignment error. With the sampled pocket side chains fixed, we perform fast local energy relaxation on the ligand by Smina¹⁶ for error correction, obtaining the final binding conformations. The number of the denoising steps is defined as 22, and 40 poses are sampled for each pocket-ligand pair, which takes in average 50 s when the batch size is set to 16 on the single 32 GB NVIDIA Tesla V100-SXM2 GPU card.

4.4 Data representation

The complete set of heavy atoms from the ligand molecule and protein pocket is structured into a heterogeneous graph $G = (\mathcal{V}, \mathcal{E})$, where each atom corresponds to a node. For the node representation \mathcal{V}_p of pocket residue atoms, we employ one-hot

encoding encompassing atom type, residue type, and whether the atom is part of the backbone. The ligand node features γ_l include atom type, hybridization type, atomic connectivity, explicit valence, implicit valence, number of rings it belonging to, aromaticity, formal charge, partial charge, chirality type, the number of radical electrons, the number of hydrogens, and whether it is in an N-membered ring (with nitrogen ranging from 3 to 8). Furthermore, pharmacophore features such as hydrogen bond acceptor/donor, aromaticity, hydrophobicity, and positive/negative charge are integrated. The edges e_{ij} , based on the covalent bonds of ligand, incorporate features like bond type, stereochemistry, conjugation, and whether the bond is part of a ring system. Diffusion times t are encoded using a sinusoidal format and are concatenated to the scalar representations of nodes and edges. For ligand atoms, internal edges \mathcal{E}_{ll} connected by covalent bonds are pre-constructed. For pocket atoms, in addition to covalent bonds, edges \mathcal{E}_{pp} are linked between pocket atoms and their own C_α and C_β atoms. During the forward inference of the model, edges are dynamically constructed based on the three-dimensional coordinates of all atoms. Within the ligand molecule, the graph construction uses a cutoff radius of 5 Å, and a similar cutoff is applied for the full atom graph of the pocket and directed edges from pocket to ligand atoms \mathcal{E}_{pl} . These edges, serving as non-covalent interactions, solely encode distances. Given the model's need to predict ligand translational updates, it's essential for the ligand to be aware of the entire pocket atom's position. Therefore, directed edges \mathcal{E}_{lp} from ligand to pocket atoms are dynamically constructed based on the diffusion process, with the translational noise determining the cutoff radius as $0.2\sigma_T + 5$ Å. This ensures that even in high noise scenarios, where the ligand is distant from the pocket, there remains an informational interaction between the ligand and the pocket, thereby pulling the ligand closer. All edges from the heterogeneous graph are $\{\mathcal{E}_{ll}, \mathcal{E}_{pp}, \mathcal{E}_{pl}, \mathcal{E}_{lp}\}$, and their distance features utilize Gaussian radial basis for encoding.

4.5 DiffBind score network

The architecture of DiffBind is meticulously crafted upon the foundation provided by the e3nn library⁴⁶. It primarily comprises the following pivotal components: a module for input embedding, modules for intra-molecular interaction encoding, and modules dedicated to inter-molecular interaction encoding. The network ingests a geometric heterogeneous graph, encompassing invariant scalar representations of both ligand heavy atom nodes γ_l and pocket residue atom nodes γ_p . We harnessed the irreducible representations (Irreps) to encode features by spherical harmonics. As the depth of feature encoding advances, the scalar inputs evolve towards higher-order physical quantity representations. Every interaction module is constructed using the Tensor Product Layer (TPL), establishing SE(3) equivariant message-passing functions. The tensor products are realized by encoding edge vectors with spherical harmonic functions and then doing spherical tensor product of irreps with path weights. The weights of these tensor products are derived from a transformation of node representations constituting the edge and the edge representation itself through a layer of Multi-Layer Perceptrons (MLP);

these weights also constitute the primary learnable parameters at each layer. For any given submodule, the general formula for message passing to node a is:

$$h_a \leftarrow h_a \bigoplus_{z \in \{l, p\}} LN^{(z_a, z)} \left(\frac{1}{|N_a^{(z)}|} \sum_{b \in N_a^{(z)}} Y(\hat{r}_{ab}) \otimes \psi_{ab} h_b \right) \quad (11)$$

$h_a = (h_a^0, \vec{h}_a)$ represents Irreps of node a , which is the concatenation of scalar representation h_a^0 and vector representation \vec{h}_a . z_a is the node type of node a , and z can be any node type from the pocket node or the ligand node. $N_a^{(z)}$ denotes the neighbour nodes of node a . Y are the spherical harmonics up to $l = 2$. LN is the equivariant layer normalization. \bigoplus refers to normal vector addition, and $\otimes \psi_{ab}$ refers to the spherical tensor product of Irreps with path weights, with $\psi_{ab} = MLP^{(z_a, z)}(e_{ab}, h_a^0, h_b^0)$ following the graph message passing paradigm.

For predicting the scores of ligand translation and rotation, we construct a node o for each ligand center and gather the message from other ligand nodes to the center. We output the final single odd and single even vectors through layer normalization for translational and rotational score prediction as follows:

$$[h_l^{(10)}, h_l^{(1e)}] \leftarrow \frac{1}{|\gamma_l|} \sum_{a \in \gamma_l} Y(\hat{r}_{oa}) \otimes \psi_{oa} h_a, \quad (12)$$

with $\psi_{oa} = MLP(\mu_{oa}, h_a^0)$

μ_{oa} denotes the Gaussian radial embeddings for distance r_{oa} . $h_l^{(10)}$ is the predicted score for translation. $h_l^{(1e)}$ is the predicted score for rotation axis ω .

For both the rotatable bonds of ligands and the dihedral angles of protein residue side chains, updates for each angle are anticipated based on a consistent paradigm. We define the central axis of the rotatable bond or dihedral angle as $B = (i, j)$, represented by a bond formed by atoms i and j . Further, the center of the rotatable bond is denoted as c . A radius graph of ligand nodes with a 4 Å cutoff is constructed to predict the torsion score of the ligand rotatable bonds.

$$h_c \leftarrow \frac{1}{N_c} \sum_{a \in N_c} Y^2(\hat{r}_{ab}) \otimes Y(\hat{r}_{ca}) \otimes \psi_{ca} h_a, \quad (13)$$

with $\psi_{ca} = MLP(\mu_{ca}, h_a, h_i + h_j)$

To satisfy the parity of dihedral angles, spherical harmonics Y^2 up to $l = 2$ is utilized. We will employ the scalar features derived from the final obtained h_c to predict the torsion angles. An analogous procedure is adopted for the torsion of pocket side chains.

4.6 Confidence model

We have explored two approaches to rank the poses generated by DiffBind. First, the traditional scoring function Smina is utilized to quickly score the generated full-atom pocket-ligand poses. Second, a deep learning-based scoring model based on mixture density network (MDN) is trained to fit the distance distribution between ligand atoms and pocket residues. The architecture of our

MDN model is similar to the scoring module of KarmaDock, and it shares the same input representations with DiffBind. To better cater for the full-atom complex system, we set the distance pairs as each ligand atom with their nearest atoms from each pocket residues.

4.7 Model Training

DiffBind neural network is trained using the AdamW optimizer⁷² with a learning rate of 0.0005 and a batch size of 64 for 1000 epochs on eight 80 GB NVIDIA A800 TENSOR CORE GPUs. MDN confidence model is trained using the Adam optimizer⁷³ with a batch size of 256 for 1000 epochs on four 32 GB NVIDIA Tesla V100-SXM2 GPUs.

4.8 Evaluation Metrics

We utilize the Ligand Root Mean Square Deviation (L-RMSD) and centroid distance to assess the predictive quality of ligand conformations. Meanwhile, the evaluation of side chain conformations' predictive quality is based on the side chain Root Mean Square Deviation (sc-RMSD) and the angular discrepancy of the first dihedral angle χ_1 . Let X_l^{pred} represents the generated ligand pose, and X_l^{gt} denotes the native ligand pose.

4.8.1 L-RMSD

We take into account Ligand Root Mean Square Deviation (L-RMSD) corrected for symmetry. The precise calculation formula is given below. Herein, N represents the number of heavy atoms in the ligand, and isom denotes the isomers of the ligand molecular graph.

$$L\text{-RMSD} = \operatorname{argmin}_{X_l^{\text{isom}} \sim \text{isom}(X_l^{gt})} \sqrt{\frac{1}{N} \sum_{i=1}^N (X_l^{\text{isom}}(i) - X_l^{\text{pred}}(i))^2} \quad (14)$$

4.8.2 Success rate

$L\text{-RMSD} < 2 \text{ \AA}$ is widely recognized as a benchmark indication of successful docking⁷⁴. In fact, for cross-docking experiments, the threshold for determining docking success can be relaxed to 2.5 \AA . Nonetheless, to ensure equitable comparison, we adhere to the stricter threshold in this context.

4.8.3 PB-success rate

The PoseBusters test suite serves as a rigorous validation tool, assessing both the chemical and geometric consistency of a ligand, inclusive of its stereochemistry. Moreover, it evaluates the physical plausibility of intra-molecular and intermolecular measurements, focusing on factors like the planarity of aromatic rings, canonical bond lengths, and potential protein-ligand clashes. Therefore, the PoseBusters suite provides us a more accurate and realistic estimation of the success rate, PB-success rate, through further checking the physical plausibility of poses with $L\text{-RMSD} < 2 \text{ \AA}$.

Centroid distance

We compute the distance between the geometric center of the generated conformation and that of the native pose. A distance

less than 1 \AA is considered indicative of successfully identifying the binding site.

4.8.4 sc-RMSD

Given that the pocket backbone remains fixed, we compute the RMSD for the side chains of each residue individually and subsequently average the results. Furthermore, in consideration of the symmetrical topology inherent in side chain structures, symmetry corrections have been implemented for the ASP, GLU, PHE, and TYP residues. We regard an sc-RMSD value of less than 1 \AA as indicative of success.

4.8.5 Angular discrepancy of χ_1

For each residue within the binding pocket, we compute the angular disparity between the generated and native side chain conformations. For residues that exhibit symmetry, we select the smallest angular difference as the final value. While sampling solely based on χ_1 may not fully encapsulate the entire flexibility of the side chains, χ_1 exerts the most significant influence on side chain conformational changes. As such, observations derived from χ_1 can reveal the most pronounced discrepancies in side chain conformations. Moreover, we have already characterized the overall conformational differences using sc-RMSD. An angular deviation of less than 15° is deemed a successful recovery of the side chain conformation.

4.9 Baseline method

4.9.1 Vina

Autodock Vina¹⁵ is a widely-used traditional docking method. We define the box using the center of the ligand present in the crystal structure, setting the box dimensions to 24×24×24 \AA^3 . The 'exhaustiveness' parameter in Vina is set to 32, producing up to 10 poses for each docking run. Docking is repeated running 40 times with different random seeds to get the top-ranked pose.

4.9.2 Smina

Smina¹⁶ improves Autodock Vina with a new scoring function and is more easy-to-use. The box construction and the sampling strategy are the same from the aforementioned baseline method AutoDock Vina.

4.9.3 LinF9

LinF9⁶² improves Autodock Vina with a new scoring function and is more user-friendly. The box construction and the sampling strategy are the same from the aforementioned baseline method AutoDock Vina.

4.9.4 Gold

Gold¹⁸ is another widely-used traditional docking method. The binding sites are defined as pocket residues within radius 12.5 \AA around the crystal ligand. The settings used are rescore function 'plp', autoscale 10, and early termination off. The docking performance is taken from Buttenschoen et al. reported⁴⁷.

4.9.5 VinaFlex

AutoDock Vina also supports semi-flexible docking with movable side chains³². However, it requires the explicit designation of

the side chains allowed to move and can support up to 14 flexible residues. Before each docking attempt, we randomly select up to 14 residues within the defined $24 \times 24 \times 24$ Å³ box to act as the flexible residues. The 'exhaustiveness' parameter is set to 16. Each docking run can generate up to 10 poses, and this docking process is repeated running 40 times using different random seeds to get the top-ranked pose.

4.9.6 rDock

rDock³¹ is another traditional docking method. The box construction and the sampling strategy are the same from the aforementioned baseline method AutoDock Vina. Otherwise, functional groups, specifically -OH and -NH3+, located within 3 Å of the ligand on the pocket residues are allowed to move. Docking is repeated running 40 times with different random seeds to get the top-ranked pose.

4.9.7 Glide

Glide¹⁷ is a powerful commercial docking method. The rigid docking was executed using the Glide-SP docking method in the Schrodinger software suite. The system was protonated at pH=7.0 and energy minimization was performed on hydrogen atoms using the OPLS 2005 force field. For the generation of grid files, the parameter 'INNERBOX' was set to 15 and 'UTERBOX' was set to 30, with all other parameters as default. Each docking run produced a maximum of 10 poses, and the docking was repeated running 40 times to get the top-ranked pose..

4.9.8 TankBind

TankBind³⁵ is a recently developed deep learning-based method. Instead of using the P2Rank prediction for pocket localization, the model utilizes the center of the ligand from the crystal structure, with all other parameters set to their default values. Since this method reconstructs ligand coordinates from the predicted distance matrix of complex, it can only generate a single pose for the ligand.

4.9.9 EDM-Dock

EDM-Dock³⁶ is a deep learning-based method sharing similar algorithm with TankBind. The box is defined as a $22.5 \times 22.5 \times 22.5$ Å³ cube. Extra energy minimization was performed for the single ligand pose predicted by EDM-Dock.

4.9.10 KarmaDock

KarmaDock⁴³ is a recently developed deep learning-based regression model which predict the ligand coordinates directly in the euclidean. We retrained the network in PDBBind time-split training set following the protocol from KarmaDock article. Docking is run under default parameters from their codes.

Additionally, we augmented the KarmaDock model with a ResNet module to predict the side-chain torsion angles of the binding pocket, resulting in a refined model named KarmaDock-sc.

4.9.11 DiffDock

DiffDock⁴⁴ is a blind-docking method based on diffusion generative model. Although it's not fair to compare DiffDock with

pocket-docking methods, we still evaluate its performance to reflect the defect of ignoring physical plausibility of these deep learning-based methods. Each generation of ligand poses was repeated running 40 times, and the generated poses are ranked by DiffDock confidence model. Again, the docking performance is taken from Buttenschoen et al. reported⁴⁷.

5 Author Contributions

J.Z and Z.G. designed the research, wrote source code and performed the experiments. J.P. and L.L. designed and supervised the project. J.Z analyzed the experimental results. Z.G. and J.Z wrote the manuscript. J.P. and L.L. revised the manuscript. All authors read and approved the final manuscript.

6 Conflicts of interest

There are no conflicts to declare.

7 Acknowledgements

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