ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Molecular Glue-Design-Evaluator (MOLDE): An Advanced Method for In-Silico Molecular Glue Design

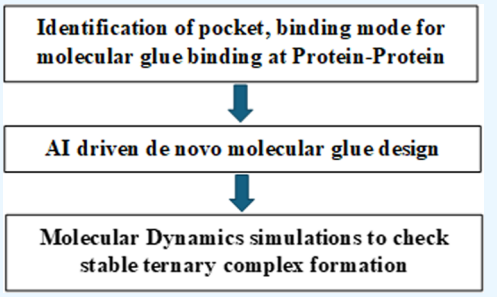
A. S. Ben Geoffrey, Deepak Agrawal,* Nagaraj M. Kulkarni, and Manonmani Gunasekaran

Cite This: https://doi.org/10.1021/acsomega.4c08049

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Protein function modulation using small-molecule binding is an important therapeutic strategy for many diseases. However, many proteins remain undruggable due to the lack of suitable binding pockets for small-molecule binding. Proximity-induced protein degradation using molecular glues has recently been identified as an important strategy to target undruggable proteins. Molecular glues were discovered serendipitously and as such currently lack an established approach for in-silico-driven rationale design. In this work, we aim to establish an in-silico method for designing molecular glues. To achieve this, we leverage known molecular glue-mediated ternary complexes and derive a rationale for the in-silico design of molecular glues. Establishing an in-silico rationale for molecular glue design would significantly contribute to the literature and accelerate the discovery of molecular glues for targeting previously undruggable proteins. Our work presented here and named Molecular Glue-Designer-Evaluator (MOLDE) contributes to the growing literature of in-silico approaches to drug design in-silico literature.



INTRODUCTION

Proteins play important functional roles in the human biological system, and in disease, their function becomes either up or down, requiring them to be modulated in function using some therapeutic means of intervention. However, only about 20% of the human proteome is amenable to modulation of function using small molecules and the remaining proteins are considered "undruggable" due to lack of suitable binding pockets for small molecules.[1] One promising solution is the modulation of protein−protein interactions (PPIs) using small molecules. PPIs are critical for cellular processes, but targeting them with traditional small molecules has proven difficult.[2] Recently, a new class of compounds called molecular glues has emerged. These small molecules can enhance PPIs by bringing proteins into proximity, thereby stabilizing and modulating their interactions.[3] However, designing a molecular glue remains a challenge and there are no established in-silico methods in the literature for rational molecular glue design.[3−6] In this research, we address this gap by using well-established as well as novel in-silico methods for small molecules, including new chemical entity (NCE) generation, NCE optimization, molecular docking, and advanced molecular dynamic simulations for design of molecular glues.

While in-silico approaches for molecular glue design have not been reported in the literature, in-silico approaches to design heterobifunctional molecules such as PROTACs to induce PPI have been reported.[7−11] The heterobifunctional molecule in a PROTAC must have two functional domains that interact with the two different proteins and bring them together to induce the PPI. In contrast, molecular glues are small molecules without a linker that directly "glue" with the two target proteins. Molecular glues offer a few important advantages over the PROTACs. Mainly because molecular glues are small molecules, they do not suffer from the bioavailability and cell permeability issues associated with larger molecules like PROTACs. Consequently, it is more desirable to use molecular glues to induce PPI especially in applications such as protein degradation. Hence, it is important to have a rational in-silico design approach for molecular glues, which can significantly help with designing effective molecular glues using in-silico methods. In our previous work, we have illustrated an in-silico rationale for the design of PROTACs.[11]

Drawing from that experience, we present an in-silico approach for de novo molecular glue design. This research aims to bridge the gap and provide rational methods for designing effective molecular glues.

The in-silico design rationale was developed by studying molecular glue-mediated ternary complexes in RCSB Protein Data Bank (https://www.rcsb.org/) and a retrospective validation of the developed approach was carried out to reproduce experimentally known aspects of molecular glue-mediated ternary complexes. This validation demonstrates the

Received: September 2, 2024
Revised: January 24, 2025
Accepted: January 29, 2025

validity of the in-silico approach. The aspects involved in the retrospective validation of the approach are as follows:

(1) Reproducing the molecular glue binding mode and structure of the ternary complex formed therein.
(2) Reproducing the thermodynamic favorability of molecular glue-mediated ternary complexation through theoretical ΔG calculations.
(3) Differentiating the stability of the molecular glue-mediated ternary complex.

In their review, Rui et al.[12] provide a list of PDB IDs associated with known molecular glue-mediated ternary complexes. In our work, we have used the molecular interactions involved in all of the PDB IDs reported by Rui et al. to understand the types of interactions involved in molecular glue-mediated ternary complexes and identify systems to develop various validations for the proposed design methodology, and they are as follows:

(1) To predict in-silico the binding mode of the molecular glue and structure of the ternary complex formed therein.
Validation System: 6TD3, 8G46, 7BQV.
(2) Free energy calculation to infer molecular glue-mediated thermodynamic favorability of protein−protein interaction.
Validation System: 8G46, 7TE8, 6TD3, 7BQU, 7BQV.
(3) Free energy calculation, QM/MM interaction energy, and thermal titration molecular dynamics (TTMD) to differentiate the stability of the molecular glue-mediated ternary complex.
Validation System: 6H0G, 7BQV, 3SMO, 3SPR.

Using the proposed methodology, validated on the systems as described above, the generative AI-driven approach to design a new molecular glue for the PDB 6TD3 system was carried out as an application of the methodology.

METHODOLOGY

Molecular Glue Binding Mode and Structure of the Ternary Complex Formed Therein. The methodological aspects that were followed to reproduce the molecular glue binding mode and structure of the molecular glue-mediated ternary complex formed therein are reported here. In the chosen system with PDB ID: 6TD3, the molecular glue associated with ID RC8 recruits DNA damage-binding (DDB) protein 1 to tag and degrade cyclin-dependent kinase 12 (CDK12). A protein−protein docking was carried out with the two proteins DDB and CDK; low-energy poses with a pocket formed at the interface were identified; and molecular docking of the molecular glue RC8 was carried out at the pocket formed at the interface of the two proteins DDB and CDK12. The protein−protein docking tool choice was MegaDock4[13] and the protein−ligand (small molecule) docking tool used was AutoDock-Vina.[14] As an additional validation, a similar exercise was carried out for the PDB 8G46, 7BQV systems and the results obtained are discussed in the Results and Discussion section.

Molecular Glue-Mediated Favorability of the Two Proteins to Form a Ternary Complex. The diversity of interactions in the list of PDB IDs associated with the molecular glue-mediated ternary complexes collected by Rui et al.[12] was studied using LigPlot+.[15] Further, to understand the role of the molecular glue inducing the thermodynamic favorability of the protein−protein interaction, biomolecular simulations were carried out using GROMACS[16] molecular dynamics package, and ΔG calculations using gmx_mmpbsa[17] tool were carried out to understand the molecular glue-induced thermodynamic favorability for the protein−protein interaction. The protein residues were parameterized using the "AMBER99SB-ILDN" force field and the ligand was parameterized using acpype.[18] The system was solvated in a cubic solvent box, the TIP3P solvent model was used, and ions were added to physiological pH. To mimic the physiological temperature and pressure, the system was heated to 300 K temperature and 1 bar pressure in 100 ps NVT and NPT runs with the temperature and pressure controlled using the Berendsen thermostat/barostat. A production run of 50 ns was executed, and the stability of the molecular glue-mediated ternary complex was assessed through the RMSD stabilization. The stable portion of the trajectory was used for MMPBSA-based theoretical ΔG calculations to understand the molecular glue-mediated thermodynamic favorability for ternary complexation using the gmx_mmpbsa tool [117].

Furthermore, molecular glues are known to induce protein−protein interaction favorability by inducing conformation change[12] and enhancing the protein−protein interaction. To evaluate the role of molecular glue-induced conformation change and enhanced protein−protein interaction achieved therein, a wide range of conformation ensembles of protein−protein poses were generated using AlphaFlow[19] and the resulting conformation change from the Apo to the Holo form, stabilized by the molecular glue binding, leading to an enhancement of protein−protein interaction was analyzed using interaction energies from the SURFACE tool.[20]

In-Silico Differentiation of the Stability and Ternary Binding Constants (K_d) Associated with the Molecular Glue-Mediated Ternary Complex. Free energy perturbation (FEP) calculations are routinely used in small-molecule drug design to obtain reliable estimates of the absolute binding free energies (ΔG) of small-molecule binding to protein targets. Herein we use FEP binding free energy calculations to differentiate the nanomolar and micromolar ternary binding constants (K_d) associated with a molecular glue mediated ternary complex. The free energy perturbation (FEP) protocol was carried out as per earlier established protocols,[21,22] wherein the ligand is decoupled from the protein in multiple lambda steps where the coulomb and Van Der Waals interactions are turned off in a stepwise manner involving several Lambda steps and the ΔG difference between the coupled and the decoupled state is then used as an estimate of the ΔG of binding. The free energy perturbation (FEP) protocol was implemented in GROMACS[16] biomolecular simulation package.

Thermal titration molecular dynamics (TTMD) originally developed within the context of differentiating tight and weak small binders of protein targets[23,24] was adapted for the molecular glue problem to differentiate the stability of the molecular glue-mediated ternary complex. A classical molecular dynamics simulation was carried out for 5 ns in an increasing temperature ramp, and the Tanimoto similarity is computed between the interaction fingerprint vector of the first frame and the subsequent frames. The similarity index measures the retention of interaction, which is expected to be more for a strong binder as compared to a weak binder.

And last, quantum mechanical/molecular mechanics (QM/MM) calculations were carried out to estimate the QM level

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

interaction energy between the molecular glue and the pocket residues and thereby develop QM/M- based interaction energy as a score to differentiate the ternary binding constants (K_d) of different magnitudes associated with a strongly binding and weakly binding molecular glue-mediated ternary complex. The QM/MM methodology is adapted from Wang et al.,[25] where the QM-based interaction energy for a small molecule bound to a protein pocket is given by

QM-based interaction energy = QM energy of the complex − QM energy of the ligand (molecular glue) − QM energy of pocket residues of the protein

The use of QM/MM methods serves to provide extra validation to the molecular glue design, similar to the MMPBSA and TTMD methods.

The in-silico molecular glue design method (MOLDE, which is an acronym for Molecular Glue Design & Evaluator) is summarized as a flow diagram in Figure 1.

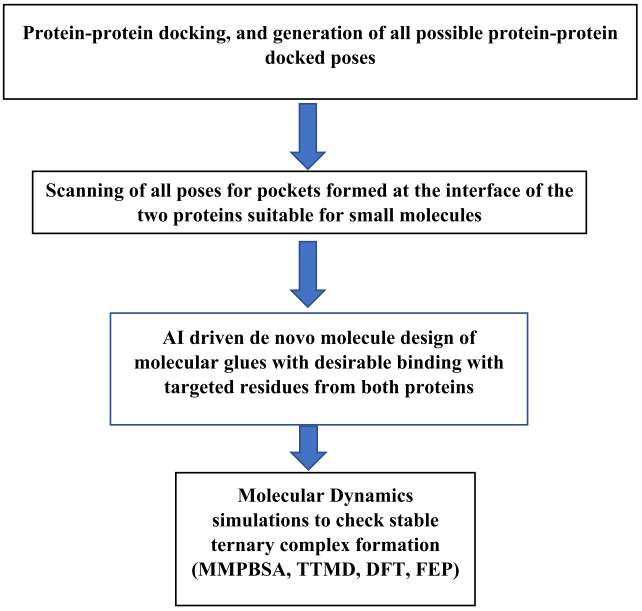


Figure 1. In-silico-driven molecular glue design method (MOLDE: Molecular Glue Design & Evaluator).

AI-Driven De Novo Molecular Glue Design Demonstration. Having thus developed the methodology and demonstrated its ability to reproduce the known aspects of experimentally reported molecular glue-mediated ternary complexes in the RCSB PDB, we apply the validated methodology to design a novel molecular glue using Generative AI for the PDB ID: 6TD3 system. We chose the ligand-based approach to de novo design using Generative AI[26,27] and implemented the ligand-based de novo design model reported by Chen et al.[28] The generated designs were evaluated as per the proposed methodology and the results obtained are discussed below.

RESULTS AND DISCUSSION

Molecular Glue Binding Mode and Structure of the Ternary Complex Formed Therein. In the system (PDB ID: 6TD3), the molecular glue RC8 mediates the ternary complex between the two proteins DDB1 and CDK12. Protein−protein docking was carried out using MegaDock for the proteins DDB1 and CDK12 and, among the low-energy poses, a pose with pocket formed at the interface that a molecular glue molecule can bind was identified as shown below. Among the low-energy poses as tabulated in Table 1, the lowest-energy pose with pocket comparability at the interface was shortlisted for the next phase and is shown graphically in Figures 2 and 3.

Table 1. Protein−Protein Docking and Interface Pocket Compatibility for Molecular Glue Binding for the PDB 6TD3 System

pose ID	protein−protein interaction (PPI) score from Megadock	interface pocket compatibility
PPI_Pose_1	4441	no
PPI_Pose_2	4001	yes
PPI_Pose_3	3883	no
PPI_Pose_4	3779	no
PPI_Pose_5	3523	no
PPI_Pose_6	3479	no
PPI_Pose_7	3421	no
PPI_Pose_8	3397	no
PPI_Pose_9	3319	no
PPI_Pose_10	3307	no

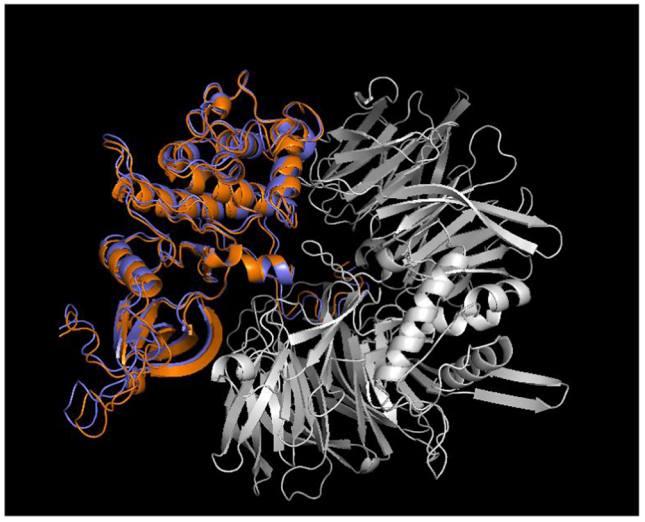


Figure 2. Experimental pose reproduced in protein−protein docking.

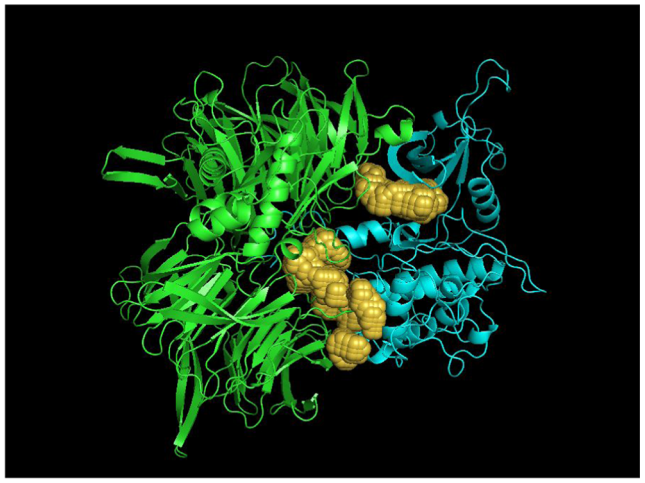


Figure 3. Analysis of the pocket formation at the interface of the two proteins for PPI_Pose_2.

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

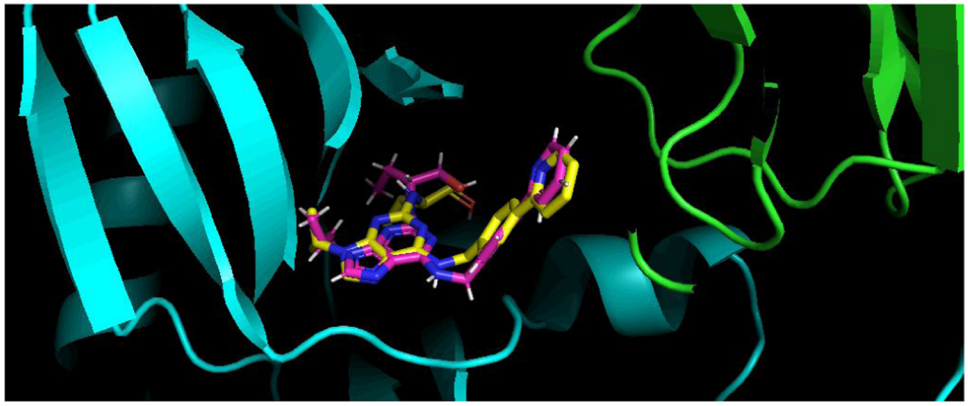


Figure 4. Pose of RC8 reproduced in DDB1-CDK12 and the resulting ternary complex.

Table 2. Protein−Protein Docking and Interface Pocket Compatibility for Molecular Glue Binding for the PDB 7BQV System

pose ID	protein−protein interaction (PPI) score from Megadock	interface pocket compatibility
PPI_Pose_1	2081	no
PPI_Pose_2	1978	no
PPI_Pose_3	1779	no
PPI_Pose_4	1657	yes
PPI_Pose_5	1534	no
PPI_Pose_6	1511	no
PPI_Pose_7	1491	no
PPI_Pose_8	1378	no
PPI_Pose_9	1354	no
PPI_Pose_10	1311	no

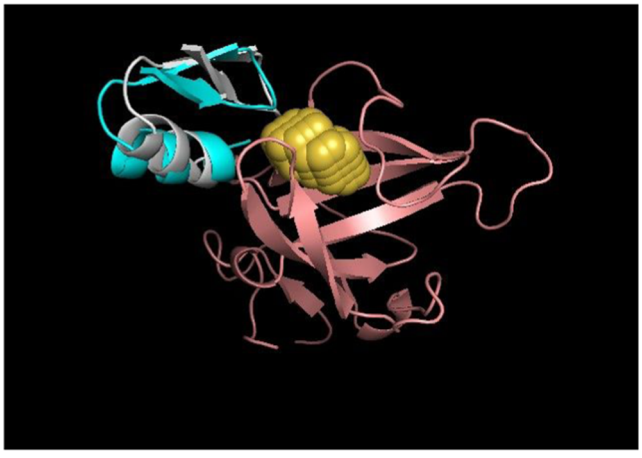


Figure 5. Identification pocket suitable for molecular glue binding formed at the interface of cereblon-SALL4 through protein−protein docking.

Further, at the pocket formed at the interface of these two proteins, molecular docking was carried out using AutoDock-Vina, which reproduced the experimental pose of the molecular glue (RC8) in PDB ID: 6TD3 as shown with the docking-derived pose in yellow and experimental pose in cyan in Figure 4.

Similar attempts to reproduce the binding mode of the molecular glue and structure of the ternary complex formed therein were carried out for the molecular glue-mediated ternary system as in PDB ID: 7BQV and 8G46.

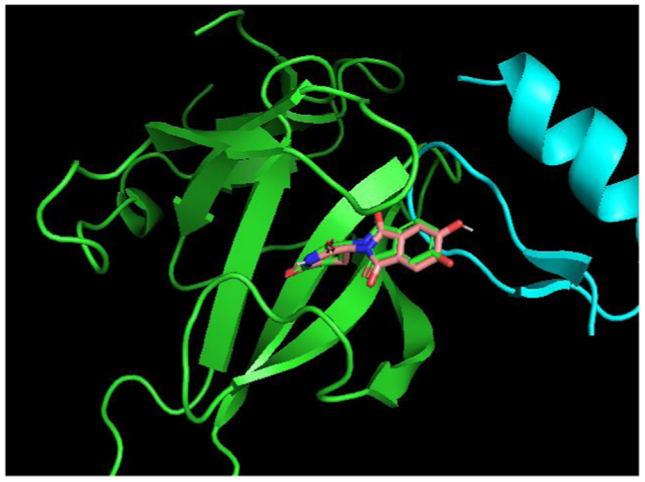


Figure 6. Pose of the molecular glue in the PDB entry 7BQV system.

Table 3. Protein−Protein Docking and Interface Pocket Compatibility for Molecular Glue Binding for the PDB 8G46 System

pose ID	protein−protein interaction (PPI) score from Megadock	interface pocket compatibility
PPI_Pose_1	3022	no
PPI_Pose_2	2878	no
PPI_Pose_3	2814	no
PPI_Pose_4	2771	no
PPI_Pose_5	2658	yes
PPI_Pose_6	2614	no
PPI_Pose_7	2578	no
PPI_Pose_8	2481	no
PPI_Pose_9	2393	no
PPI_Pose_10	2313	no

The protein−protein interaction poses between cereblon and SALL4 as in the PDB 7BQV system was reproduced as one of the top scoring protein−protein poses from Megadock with interface pocket compatibility for molecular glue binding. The protein−protein docking score obtained for the top poses from Megadock and their interface pocket compatibility evaluated as per the criteria that the pocket identification tool CAVIAR identifies a pocket suitable for small -molecule binding at the interface are tabulated in Table 2.

The in-silico-identified protein−protein pose as per the proposed criteria of choosing a top scoring pose with pocket

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

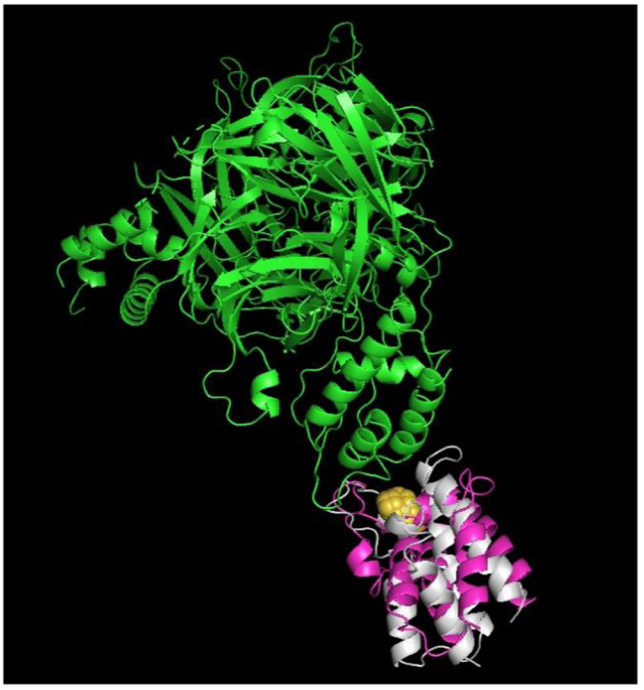


Figure 7. Identification pocket suitable for molecular glue binding formed at the interface of DDB1_Assembly-BRD4 through protein−protein docking.

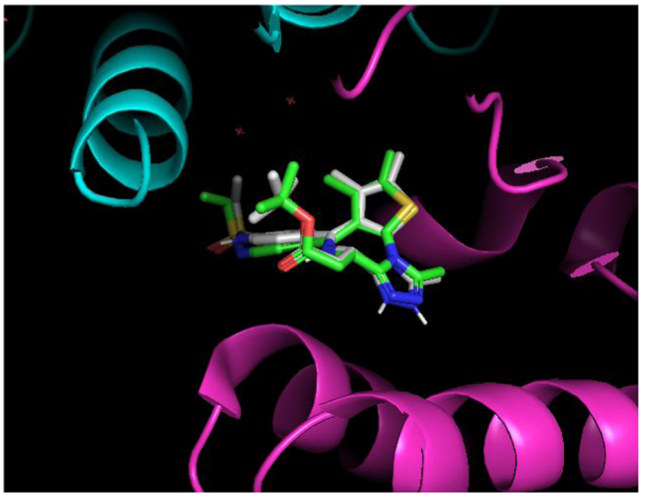


Figure 8. Reproducing the pose of the molecular glue in the PDB entry 8G46 system.

compatibility at the interface was compared to the experimental pose in PDB 7BQV shown in Figure 5. In the protein−protein docking, cereblon was defined as the rigid receptor, shown in brown, and SALL4 as the ligand that was docked; the docked pose is shown in turquoise blue, which is superimposed on the crystallographic pose, shown in gray, and the pocket identified at the interface of the two proteins using CAVIAR is shown in golden yellow.

Following this, the binding mode of the molecular glue at the pocket formed at the interface of the protein−protein pose was reproduced using Autodock-Vina and the in-silico-derived pose as compared to the experimental one is shown in Figure 6 for the PDB 7BQV system. The theoretical pose achieved

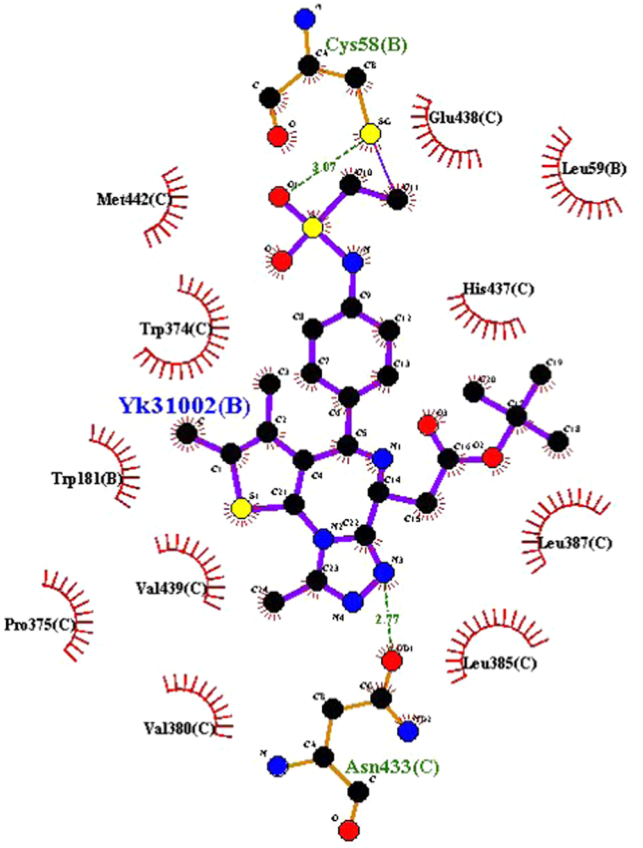


Figure 9. YK3 as a representative example for molecular glues that work through covalent interactions.

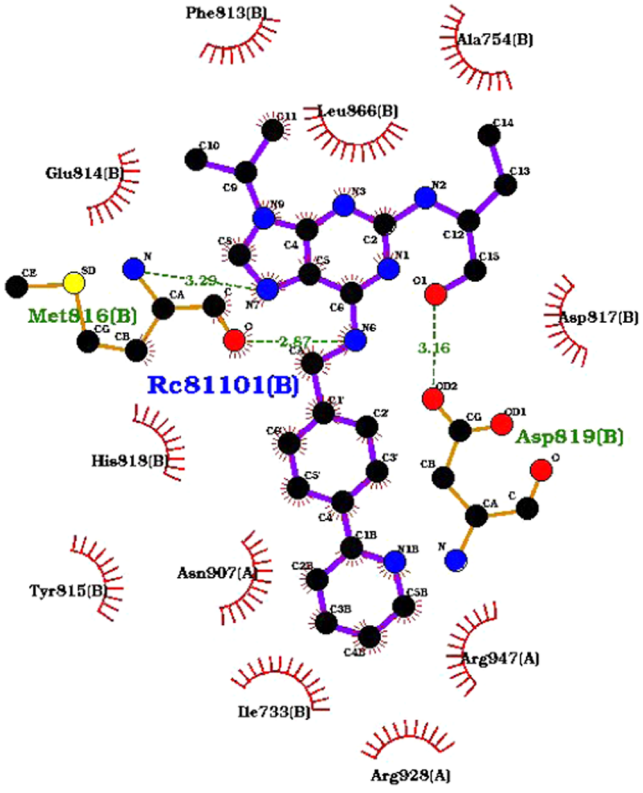


Figure 10. RC8 as a representative example for molecular glues that work through noncovalent interaction.

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

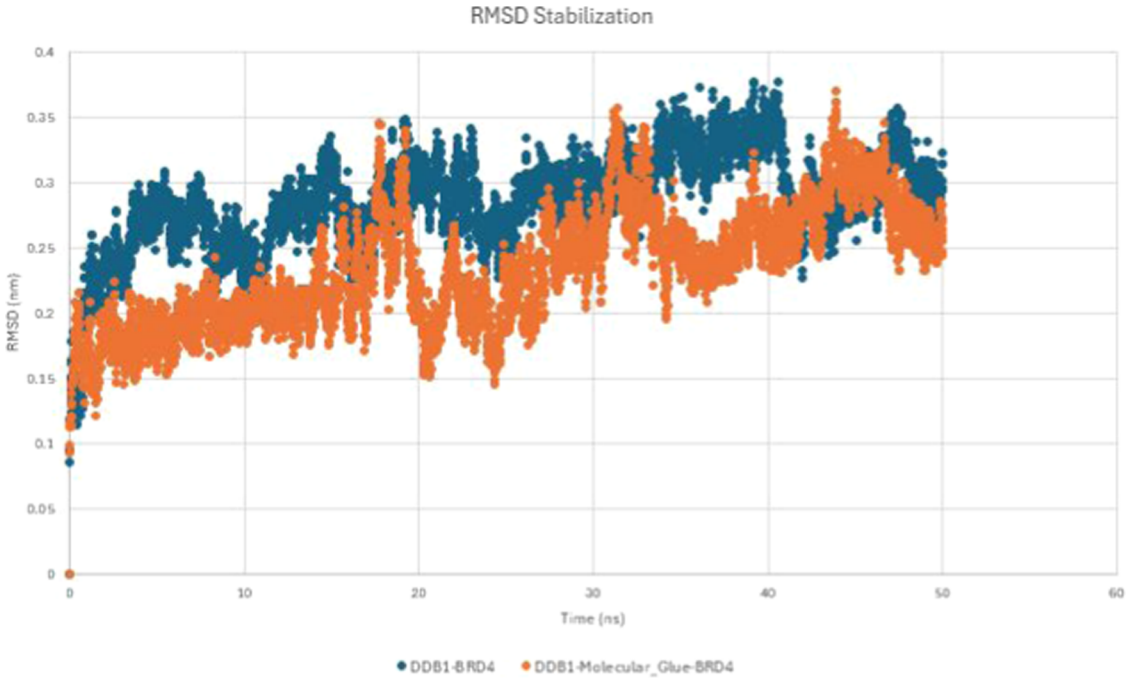


Figure 11. RMSD stabilization of the PDB 8G46 system with and without the molecular glue.

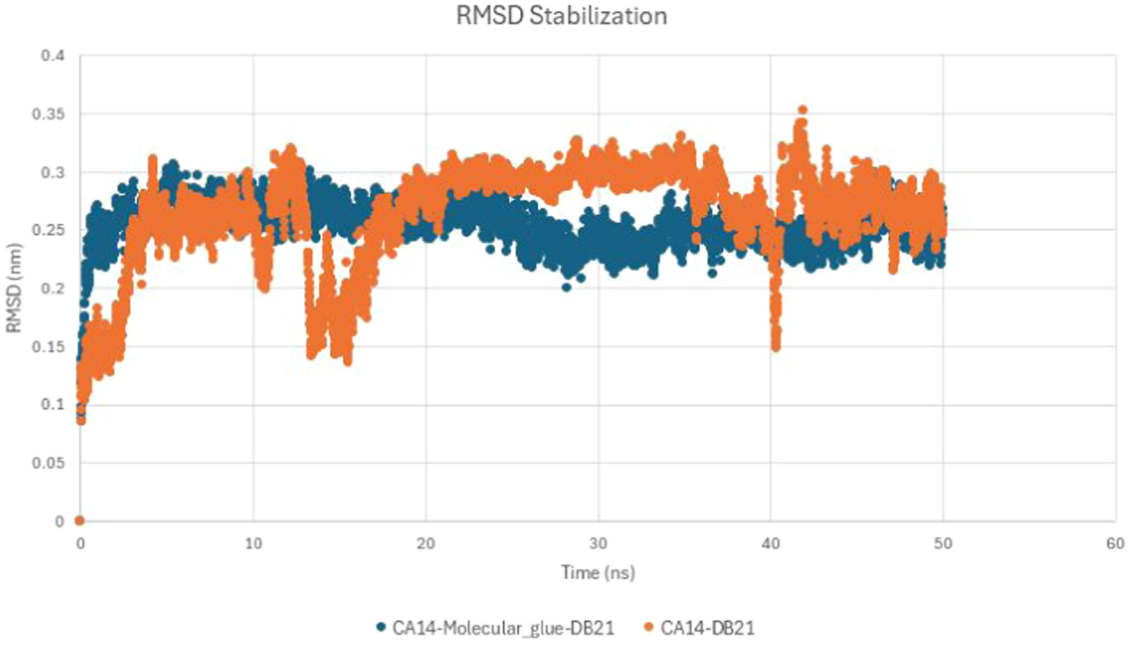


Figure 12. RMSD stabilization of the PDB 7TE8 system with and without the molecular glue.

through the molecular docking shown in brown is superimposed on the experimental pose shown in green.

Further, to add the additional validation, a similar process was carried out for the PDB 8G46 system and the results are as follows. The protein−protein interaction poses between CUL4 DCAF16 ligase and BRD4 as in the PDB 8G46 system was reproduced as one of the top-scoring protein−protein poses from Megadock with interface pocket compatibility for molecular glue binding. The protein−protein docking score obtained for the top poses from Megadock and their interface pocket compatibility evaluated as per the criteria that pocket identification tool CAVIAR identifies a pocket suitable for small-molecule binding at the interface is tabulated in Table 3.

The in-silico-identified protein−protein pose as per the proposed criteria of choosing a top scoring pose with pocket compatibility at the interface was compared to the experimental pose in PDB 8G46 as shown in Figure 7. In the protein−protein docking, DNA damage-binding protein assembly was treated as the rigid receptor shown in green and BRD4 as the ligand that was docked; the docked pose of BRD4 is shown in cyan, which is superimposed on the crystallographic pose shown in gray, and the pocket identified at the interface of the two proteins using CAVIAR is shown in golden yellow.

Followingly, the binding mode of the molecular glue at the pocket formed at the interface of the protein−protein pose was

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

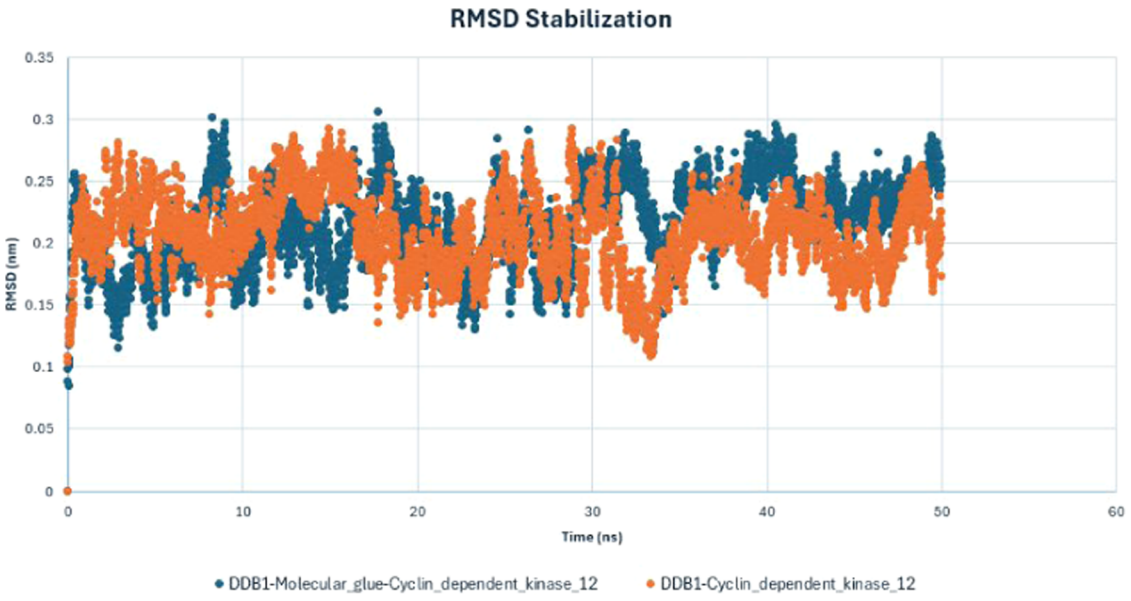


Figure 13. RMSD stabilization of the PDB 6TD3 system with and without the molecular glue.

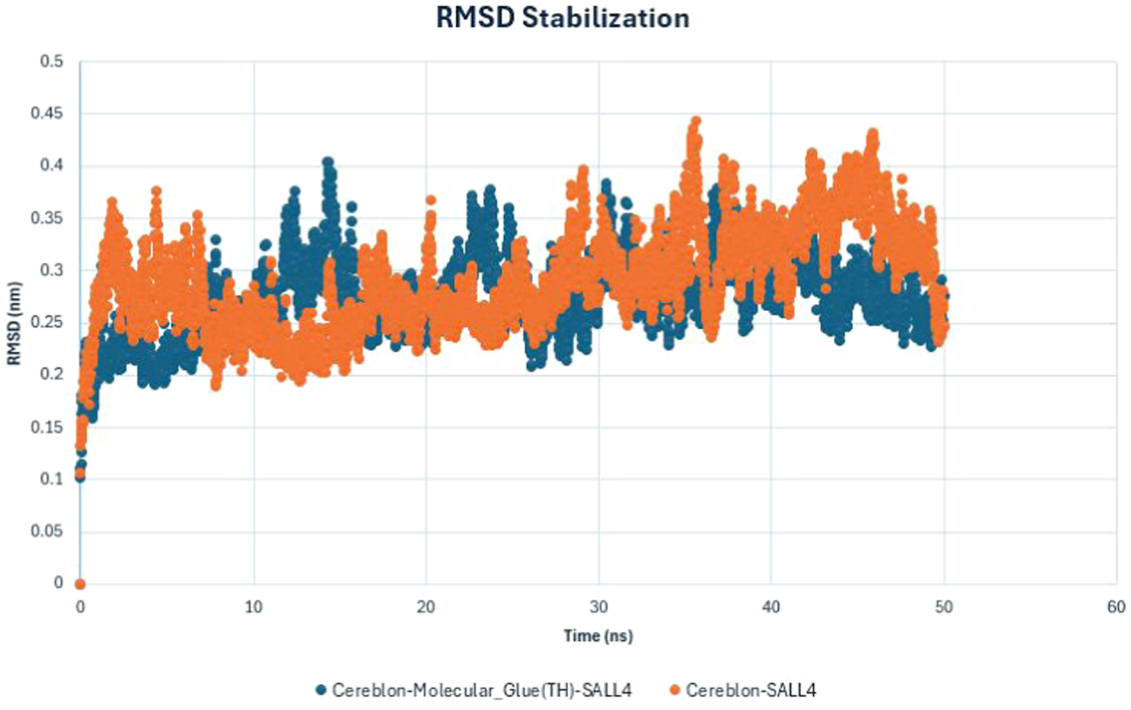


Figure 14. RMSD stabilization of the PDB 7BQU system with and without the molecular glue.

reproduced using Autodock-Vina, and the in-silico-derived pose as compared to the experimental pose is shown in Figure 8 for the PDB 8G46 system. The theoretical docking-derived pose in gray has been superimposed on the experimental pose shown in green in Figure 8.

Molecular Glue-Mediated Favorability of the Two Proteins to Form a Ternary Complex. The diversity of interactions mediated by molecular glues in ternary complexes reported in RCSB PDBs in the list collected by Rui et al.[12] was studied using LigPlot+[15] and it was found that molecular glues can mediate either covalent or noncovalent interactions. A representative example for each case is discussed below along with the interactions from LigPlot. For the covalent case, PDB ID: 8G46 was chosen as the representative example for the class and for noncovalent case PDB ID: 6TD3 was chosen as the representative example for the class.

As seen in Figure 9, covalent interactions are manifest in the PDB ID: 8G46 system, wherein the cysteine residue (Cys58), a usual participant of covalent bonding interaction, is observed to interact with the molecular glue (YK3).

Only noncovalent interactions are observed in the PDB ID: 6TD3 system, where molecular glue (RC8) has hydrophobic interactions with the hydrophobic residues in the pocket and hydrogen bonding with MET816 and ASP819, as shown in Figure 10.

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

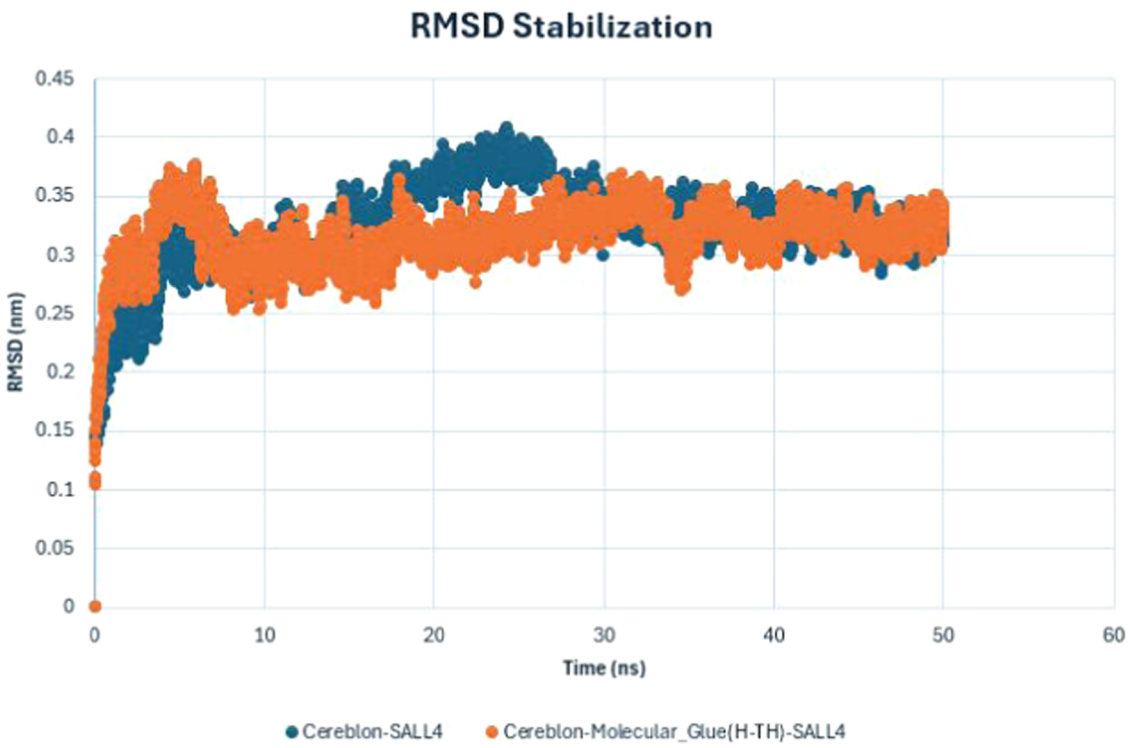


Figure 15. RMSD stabilization of the PDB 7BQV system with and without the molecular glue.

Table 4. MMPBSA based on Calculations

system	complex	MMPBSA based ΔG (kcal/mol)	receptor (R) and Ligand (L) definitions for MMPBSA calculations
PDB ID: 8G46	DDB1-BRD4	−46.3	R = BRD4
			L = DDB1
	DDB1-Molecular_glue-BRD4	−64.7	R = BRD4
			L = DDB1-Molecular_glue
PDB ID: 7TE8	CA14-DB21	−27.8	R = CA14
			L = DB21
			L = DB21-Molecular_glue
	CA14-Molecular_glue-DB21	−119.1	R = CA14
			L = DB21-Molecular_glue
PDB ID: 6TD3	DDB1-Cyclin_dependent_kinase_12	−47.1	R = DDB1
			L = Cyclin_dependent_kinase_12
	DDB1-Molecular_Glue-Cyclin_dependent_kinase_12	−73.19	R = DDB1
			L = Cyclin_dependent_kinase_12- Molecular_Glue
PDB ID: 7BQU	Cereblon-SALL4	−39.1	R = Cereblon
			L = SALL4
	Cereblon-Molecular_Glue (Thalidomide)-SALL4	−61.9	R = Cereblon
			L = Molecular_Glue-SALL4
PDB ID: 7BQV	Cereblon-SALL4	−39.3	R = Cereblon
			L = SALL4
	Cereblon-Molecular_Glue (Hydroxy-Thalidomide)-SALL4	−67.7	R = Cereblon
			L = Molecular_Glue-SALL4

The molecular glue-induced thermodynamic favorability to induce the protein−protein interaction in the systems associated with PDB IDs 8G46, 7TE8, 6TD3, 7BQU, and 7BQV was assessed using theoretical ΔG calculations carried out using the MMPBSA method. A classical molecular dynamics simulation was carried out for 50 ns for the protein−protein system associated with PDB IDs 8G46, 7TE8, 6TD3, 7BQU, and 7BQV with and without the molecular glue. The stabilization of the protein−protein complex was observed using the RMSD stabilization graphs shown in Figures 11−15 and the stable portion of the trajectory was used for MMPBSA-based ΔG calculations. Results of the MMPBSA calculations are listed in Table 4.

From the results of the MMPBSA calculations shown in Table 4, it can be inferred that the molecular glue enhanced the protein−protein interaction in all 5 cases.

Furthermore, molecular glues are also known to enhance protein−protein interaction through molecular glue-mediated conformation change, as in the thalidomide-mediated conformation change of CRBN that enhances the interaction

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

Table 5. In-Silico Investigation of CRBN-CK1α Allostery Mediated by Molecular Glue (Thalidomide)

PPI poses of CRBN-CK1α	RMSD from alignment of PPI pose with CRBN open (apo) form PDB	RMSD from alignment of PPI pose with CRBN closed (holo) form	PPI interaction of CRBN-CK1α from surface	protein−ligand interaction score of molecular glue (thalidomide) and CRBN
pose 1	**3.570**	**0.874**	**−33.89**	**−7.89**
pose 2	2.234	1.113	−23.89	−6.15
pose 3	2.781	1.783	−25.41	−6.31
pose 4	1.435	2.187	−25.78	−6.81
pose 5	1.133	2.257	−26.11	−6.07
pose 6	**0.759**	**4.112**	**−21.17**	**−5.77**
pose 7	1.738	3.071	−17.88	−5.89
pose 8	2.178	4.311	−16.81	−6.07
pose 9	3.119	2.788	−20.11	−5.68
pose 10	4.148	3.791	−18.81	−5.57

Table 6. In-Silico Investigation of CRBN-CK1α Allostery Mediated by Molecular Glue (Thalidomide) through MMPBSA-based Calculations

system		ΔG (kcal/mol)
CRBN-CK1α	CRBN closed form	−67.89
	CRBN open form	−44.13

between CRBN and casein kinase 1α (CK1α) interaction, leading to subsequent degradation of CK1α.[29,30] To investigate this mechanism in-silico, an ensemble protein−protein interaction pose was generated using AlphaFlow and the molecular glue (thalidomide) stabilizing the closed (Holo) form of CRBN as opposed to its open (Apo) form, which resulted in increase in interaction between CRBN and CK1α, was rationalized through the scores obtained, which are tabulated in Table 5. The generated ensembles of conformation were compared against the experimentally known Open (Apo) form of CRBN as in PDB ID: 8D7X and closed (Holo) form as in PDB ID: 5FQD.

It can be inferred from Table 5 that the molecular glue thalidomide binds to CRBN and stabilizes the closed form of CRBN and the closed form of CRBN has an enhanced interaction with CK1α as compared to the open form. The MMPBSA scores shown in Table 6 further support the same inference.

The open and closed form of CRBN bound to CK1α is shown in Figure 16 with the open form in green and the closed form (in purple) stabilized by the molecular glue (in cyan) having an enhanced interaction with CK1α (in yellow).

Table 7. FEP and QM/MM Calculations to Differentiate the Stability of the Molecular Glue-Mediated Ternary Complex

system	experimental ternary binding constants (K_d), μM	ΔG (kcal/mol) from FEP calculations	interaction energy (kcal/mol) from QM/MM calculations
PDB ID: 6H0G	2.3[12]	−11.37	−76.96
PDB ID: 7BQV	0.76[12]	−18.72	−92.16

In-Silico Differentiation of the Stability and Ternary Binding Constants (K_d) Associated with the Molecular Glue-Mediated Ternary Complex. Free energy perturbation and QM/MM calculations were carried out on two systems with PDB ID: 6H0G and 7BQV, which have a micromolar ternary binding constant (K_d) of 2.3 μM for 6H0G and a nanomolar ternary binding constant (K_d) of 0.76 μM for 7BQV. Free energy perturbation calculations and QM/MM calculations show that it is possible to differentiate the ternary systems of different orders of ternary binding constant values as shown in Table 7.

Further, thermal titration molecular dynamics (TTMD) was also carried out to estimate the retention of interaction in an increasing temperature ramp. The results obtained are presented in Table 8.

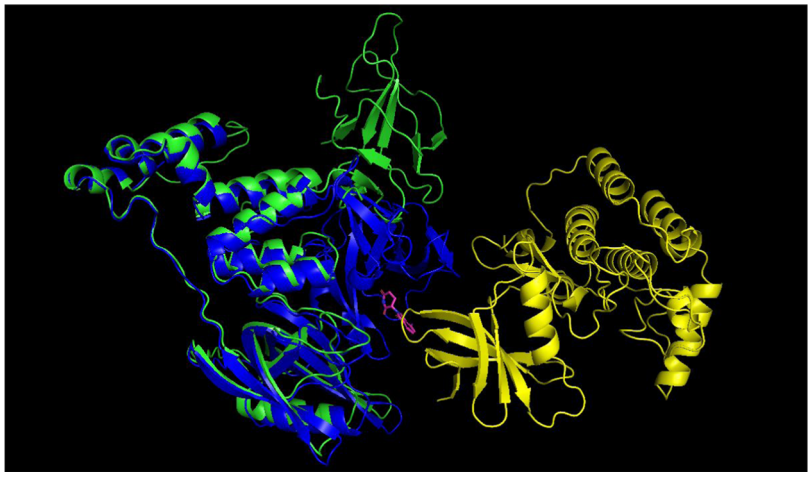


Figure 16. Molecular glue allostery-mediated CRBN-CK1α interaction.

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

Table 8. TTMD Calculations to Differentiate the Stability of the Molecular Glue-Mediated Ternary Complex

	temperature ramp				
	300 K		450 K		
	K_d (ternary) = 2.3 μM, PDB ID: 6H0G	K_d (ternary) = 0.76 μM, PDB ID: 7BQV	K_d (ternary) = 2.3 μM, PDB ID: 6H0G	K_d (ternary) = 0.76 μM, PDB ID: 7BQV	remarks and interpretation
average interaction retained	0.71	0.83	0.07	0.29	should be higher for tight binder than weak binder

Table 9. FEP and QM/MM Calculations to Differentiate the Stability of the Molecular Glue-Mediated Ternary Complex

system	experimental ternary binding constants (K_d), μM	ΔG (kcal/mol) from FEP calculations	interaction energy (kcal/mol) from QM/MM calculations
PDB ID: 3SMO	0. 56[12]	−12.78	−59.38
PDB ID: 3SPR	0. 0.08[12]	−19.61	−83.70

The obtained TTMD profile indicates that the ternary system mediated by the molecular glue in 7BQV is more stable as compared to 6H0G, which is consistent with the experimentally known K_d (ternary) for 7BQV and 6H0G.

Similarly, fusicoccin is known to be a protein−protein interaction stabilizer between 14 and 3-3 protein and the human potassium channel TASK-3. The ability of the proposed free energy and QM/MM calculations to differentiate the stability of the molecular glue-mediated ternary complex mediated by fusicoccin and its derivative between 14 and 3-3 protein and the human potassium channel TASK-3 has been demonstrated. The free energy and QM/MM interaction energy as proposed was implemented for the PDB 3SMO, with the associated ternary K_d = 0.56 μM, and for PDB 3SPR, with the associated K_d = 0.08 μM, and the obtained free energy and QM/MM profiles tabulated in Table 9 were found to differentiate the different orders of K_d values associated with the PDB 3SPR and 3SMO systems.

The thermal titration molecular dynamics technique as proposed was also implemented to differentiate the different orders of ternary K_d values associated with the PDB 3SPR and 3SMO systems and the obtained TTMD profile has been tabulated in Table 10.

AI-Driven De Novo Design of Molecular Glues for the PDB ID: 6TD3 System. AI-driven de novo molecular design approaches[22,,27] were employed to design new molecular glues for the PDB ID: 6TD3 system around the chemical space of the base scaffold derived from RC8. RC8 is a potent nanomolar compound, so we aimed to design a molecular glue that will be comparable to RC8, if not better. The newly designed molecular glues were screened through the developed approach and the results are tabulated below. The newly designed molecular glues were docked in the pocket of RC8 and the interactions of RC8 and the new designs are tabulated in Table 11. The interactions conserved among RC8 and the new designs are highlighted in green, and based on the SURFACE protein−ligand interaction score, the candidate "SAIT_MG_26121" was shortlisted for further consideration.

Further, MMPBSA calculations based on binding free energy calculations were carried out to understand the molecular glue (SAIT_MG_26121)-induced favorability for ternary complex formation for the DDB1-Cyclin_dependent_kinase_12 system. A 50 ns classical molecular dynamics simulation was carried out to estimate the stability of the ternary complex mediated by SAIT_MG_26121. RMSD stabilization is shown in Figure 17. The stable portion of the trajectory was chosen for MMPBSA-based binding energy calculations, which indicate the molecular glue (SAIT_MG_26121)-induced ternary complexation, and the results are tabulated in Table 12.

Finally, the stability of the molecular glue-mediated ternary complex mediated by RC8 (reference) and the de novo designed candidate "SAIT_MD_26121" was compared through FEP and QM/MM scores, which are tabulated in Table 13, and their respective TTMD profiles are tabulated in Table 14.

The results of the FEP, QM/MM, and TTMD calculations indicate that the newly designed candidate "SAIT_MD_26121" has a similar binding profile as that of the reference RC8, which is a nanomolar molecular glue.

CONCLUSIONS, LIMITATIONS, AND FUTURE SCOPE

Molecular glues, which more closely resemble traditional small molecules, offer a promising alternative to heterobifunctional PROTACs for target protein degradation. The PROTAC field faces challenges related to permeability and bioavailability due to the large size of the PROTAC molecules. However, for the serendipitously discovered molecular glues, a rational design approach remains elusive.

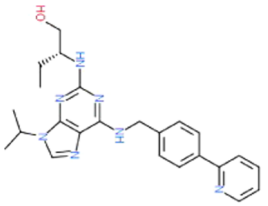
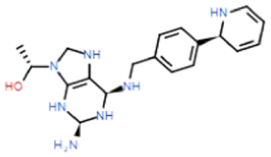
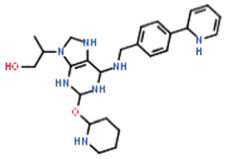
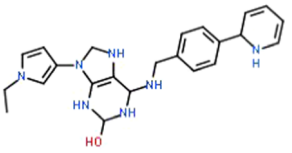
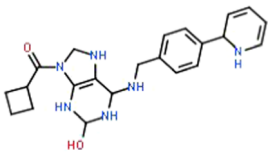
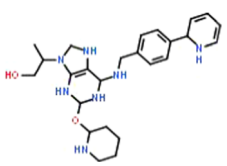
To establish the in-silico rationale for molecular glue design, we take known molecular glue mediated ternary complexes reported in the RCSB PDB and develop in-silico methods that are able to do the following: (1) reproduce experimentally known binding modes of molecular glues and the ternary complex formed therein; (2) rationalize the thermodynamic

Table 10. TTMD Calculations to Differentiate the Stability of the Molecular Glue-Mediated Ternary Complex

	temperature ramp				
	300 K		450 K		
	K_d (ternary) = 0.56 μM, PDB ID: 3SMO	K_d (ternary) = 0.08 μM, PDB ID: 3SPR	K_d (ternary) = 0.56 μM, PDB ID: 3SMO	K_d (ternary) = 0.08 μM, PDB ID: 3SPR	remarks and interpretation
average interaction retained	0.67	0.77	0.08	0.33	should be higher for tight binder than weak binder

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

Table 11. De Novo Design of Molecular Glues for the 6TD3 System

ID	2D Structure of the Molecular Glue molecule	Interactions	SURFACE protein-ligand interaction score
RC8		ILE25(CRBN), VAL33(CRBN), ALA46(ALA), PHE105(CRBN), TYR107(CRBN), MET108(CRBN), ARG628(CK1α)	-7.81
SAIT_MG_26121		ILE25(CRBN), VAL33(CRBN), LEU158(CRBN) VAL75(CRBN), MET108(CRBN), ASP111(CRBN) ARG628(CK1α)	-8.21
SAIT_MG_425		ILE25(CRBN), VAL33(CRBN), VAL75(CRBN), LEU158(CRBN) ALA168(CRBN) MET108(CRBN), ASP111(CRBN) ARG628(CK1α)	-8.01
SAIT_MG_10536		ILE25(CRBN), VAL33(CRBN), VAL79(CRBN), LEU158(CRBN) ALA168(CRBN) MET108(CRBN), ASP111(CRBN) ASP169(CRBN) ARG628(CK1α)	-8.23
SAIT_MG_ 26460		ILE25(CRBN), VAL33(CRBN), LEU158(CRBN), MET108(CRBN), ASP111(CRBN) ARG628(CK1α)	-8.17
SAIT_MG_8507		ILE25(CRBN), PHE105(CRBN), LEU158(CRBN) MET108(CRBN), ASP111(CRBN) ILE609(CK1α) ASP625(CK1α)	-8.11

favorability induced by molecular glues for the ternary complex through theoretical ΔG calculations; and (3) differentiate the stability of molecular glue-mediated ternary complexes. After carrying out a retrospective validation for the developed

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

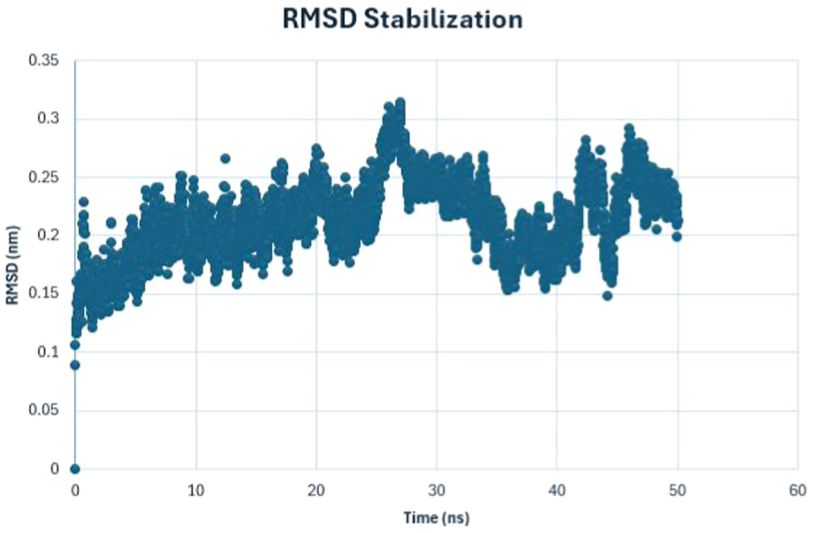


Figure 17. RMSD stabilization of DDB1-Cyclin_dependent_kinase_12 ternary complex stabilized by SAIT_MG_26121.

Table 12. MMPBSA Calculations for "SAIT_MD_26121"

system		MMPBSA ΔG (kcal/mol)
PDB ID: 6TD3	DDB1-Cyclin_dependent_kinase_12	−47.1
SAIT_MD_26121	DDB1-Molecular_Glue-Cyclin_dependent_kinase_12	−74.21

Table 13. FEP and QM/MM Calculations for SAIT_MD_262121

system	experimental ternary binding constants (K_d), nM	ΔG (kcal/mol) from FEP calculations	interaction energy (kcal/mol) from QM/MM calculations
PDB ID: 6TD3 (RC8)	<100[12]	−17.11	−105.22
SAIT_MD_21621		−17.89	−103.81

approach, we used the developed approach to design a new molecular glue as a demonstrative case. The generalizability of the proposed and retrospectively validated methodology endows it with the potential to be applied and validated for systems other than the molecular glue-mediated ternary system from RCSB PDB, as is presently known and tabulated by Rui et al.[12] While the systems outside this class remain unaddressed by our work, the methods used have generalizability, in principle. Further, the synthesis and biotesting of the generative AI-driven de novo-designed molecular glues for the PDB 6TD3 system, which was chosen as the system for demonstrative application of the proposed and retrospectively validated methodology, remains a part of the future scope of the work.

■ ASSOCIATED CONTENT

Data Availability Statement

All results can be reproduced as per the methodology reported in the Methodology section and the use of publicly available research data and software packages which are as follows: Data: [1] All molecular glue ternary complexes used as a part of developing the methodology were downloaded from RCSB PDB (https://www.rcsb.org/). Software packages: [2] MEGADOCK for protein−protein docking (https://github.com/akiyamalab/MEGADOCK). [3] Autodock-Vina for protein−ligand (small-molecule) docking (https://github.com/ccsb-scripps/AutoDock-Vina). [4] CAVIAR for pocket identification CAVIAR for pocket identification (https://github.com/jr-marchand/caviar). [5] RDKIT for cheminformatics tasks (https://github.com/rdkit/rdkit). [6] For molecular dynamics simulations involving ternary complex modeling, TTMD, and free energy calculations, we used GROMACS 2023 software (https://github.com/gromacs/gromacs). [7] For MMPBSA-based free energy calculations, we used GMX_MMPBSA software (https://github.com/Valdes-Tresanco-MS/gmx_MMPBSA). [8] For interaction fingerprinting in TTMD, we used PLIP (https://github.com/pharmai/plip). [9] ORCA for QM calculations (https://www.faccts.de/orca/).

Table 14. TTMD Profile for SAIT_MD_26121

	temperature ramp				
	300 K		450 K		
	RC8	SAIT_MD_26121	RC8	SAIT_MD_26121	remarks and interpretation
average interaction retained	0.73	0.69	0.17	0.19	SAIT_MD_26121 retains interactions similar to reference RC8 at higher temperature indicating a strong binding profile

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX

AUTHOR INFORMATION

Corresponding Author

Deepak Agrawal − *Sravathi AI Technology Pvt. Ltd., Bengaluru 560099 Karnataka, India;* orcid.org/0009-0005-1722-0864; Email: deepak.a@sravathi.ai

Authors

A. S. Ben Geoffrey − *Sravathi AI Technology Pvt. Ltd., Bengaluru 560099 Karnataka, India*

Nagaraj M. Kulkarni − *Sravathi AI Technology Pvt. Ltd., Bengaluru 560099 Karnataka, India*

Manonmani Gunasekaran − *Sravathi AI Technology Pvt. Ltd., Bengaluru 560099 Karnataka, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c08049

Notes

The authors declare no competing financial interest.
All authors are employees of Sravathi AI Technology Private Limited, Bengaluru, India.

ACKNOWLEDGMENTS

The authors are grateful for the various useful discussions they have had with their colleagues at Sravathi AI Technology Pvt Ltd. In particular, the authors would like to thank Raghu Bhagavat, Srinivasan Krishnaswami, Nivedita Bharti, and Rajesh Kondabala for their valuable input and insights.

REFERENCES

(1) Duran-Frigola, M.; Cigler, M.; Winter, G. E. Advancing targeted protein degradation via multiomics profiling and artificial intelligence. *J. Am. Chem. Soc.* **2023**, *145* (5), 2711−2732.

(2) Qiu, Y.; Li, X.; He, X.; et al. Computational methods-guided design of modulators targeting protein-protein interactions (PPIs). *Eur. J. Med. Chem.* **2020**, *207*, No. 112764.

(3) Domostegui, A.; Nieto-Barrado, L.; Perez-Lopez, C.; et al. Chasing molecular glue degraders: screening approaches. *Chem. Soc. Rev.* **2022**, *51*, 5498−5517, DOI: 10.1039/D2CS00197G.

(4) Sasso, J. M.; et al. Molecular glues: The adhesive connecting targeted protein degradation to the clinic. *Biochemistry* **2022**, *62*, 601−623, DOI: 10.1021/acs.biochem.2c00245.

(5) Geiger, T. M.; Schäfer, S. C.; Dreizler, J. K.; et al. Clues to molecular glues. *Curr. Res. Chem. Biol.* **2022**, *2*, No. 100018.

(6) Maneiro, M.; De Vita, E.; Conole, D.; et al. PROTACs, molecular glues and bifunctionals from bench to bedside: Unlocking the clinical potential of catalytic drugs. *Prog. Med. Chem.* **2021**, *60*, 67−190.

(7) Drummond, M. L.; Christopher, I. W. In silico modeling of PROTAC-mediated ternary complexes: validation and application. *J. Chem. Inf. Model.* **2019**, *59* (4), 1634−1644.

(8) Weng, G.; Li, D.; Kang, Y.; et al. Integrative modeling of PROTAC-mediated ternary complexes. *J. Med. Chem.* **2021**, *64* (21), 16271−16281.

(9) Zaidman, D.; Prilusky, J.; London, N. PRosettaC: Rosetta based modeling of PROTAC mediated ternary complexes. *J. Chem. Inf. Model.* **2020**, *60* (10), 4894−4903.

(10) Liao, J.; Nie, X.; Unarta, I. C.; et al. In Silico Modeling and Scoring of PROTAC-Mediated Ternary Complex Poses. *J. Med. Chem.* **2022**, *65* (8), 6116−6132.

(11) Ben Geoffrey, A. S.; Agrawal, D.; Kulkarni, N. M.; Vetrivel, R.; Gurram, K. PROTAC-Design-Evaluator (PRODE): An Advanced Method for In-Silico PROTAC Design. *ACS Omega* **2024**, *9* (11), 12611−12621.

(12) Rui, H.; Kate, S. A.; Min, J.; Wang, C.; Potts, P. R. Protein−protein interfaces in molecular glue-induced ternary complexes: classification, characterization, and prediction. *RSC Chem. Biol.* **2023**, *4* (3), 192−215.

(13) Ohue, M.; Shimoda, T.; Suzuki, S.; Matsuzaki, Y.; Ishida, T.; Akiyama, Y. MEGADOCK 4.0: an ultra−high-performance protein−protein docking software for heterogeneous supercomputers. *Bioinformatics* **2014**, *30* (22), 3281−3283.

(14) Eberhardt, J.; Santos-Martins, D.; Tillack, A. F.; Forli, S. AutoDock Vina 1.2. 0: New docking methods, expanded force field, and python bindings. *J. Chem. Inf. Model.* **2021**, *61* (8), 3891−3898.

(15) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng., Des., Sel.* **1995**, *8* (2), 127−134.

(16) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. GROMACS: fast, flexible, and free. *J. Comput. Chem.* **2005**, *26* (16), 1701−1718.

(17) Valdés-Tresanco, M. S.; Valdés-Tresanco, M. E.; Valiente, P. A.; Moreno, E. gmx_MMPBSA: a new tool to perform end-state free energy calculations with GROMACS. *J. Chem. Theory Comput.* **2021**, *17* (10), 6281−6291.

(18) da Silva, A. W. S.; Vranken, W. F. ACPYPE-Antechamber python parser interface. *BMC Res. Notes* **2012**, *5*, 367.

(19) Jing, B.; Berger, B.; Jaakkola, T.AlphaFold Meets Flow Matching for Generating Protein Ensembles, 2024. arXiv:2402.04845. https://arxiv.org/abs/2402.04845.

(20) Teruel, N.; Borges, V. M.; Najmanovich, R. Surfaces: a software to quantify and visualize interactions within and between proteins and ligands. *Bioinformatics* **2023**, *39* (10), No. btad608.

(21) Aldeghi, M.; Heifetz, A.; Bodkin, M. J.; Knapp, S.; Biggin, P. C. Accurate calculation of the absolute free energy of binding for drug molecules. *Chem. Sci.* **2016**, *7* (1), 207−218.

(22) Weiss, D. R.; Bortolato, A.; Sun, Y.; Cai, X.; Lai, C.; Guo, S.; Shi, L.; Shanmugasundaram, V. On ternary complex stability in protein degradation: In silico molecular glue binding affinity calculations. *J. Chem. Inf. Model.* **2023**, *63* (8), 2382−2392.

(23) Pavan, M.; Menin, S.; Bassani, D.; Sturlese, M.; Moro, S. Qualitative estimation of protein−ligand complex stability through thermal titration molecular dynamics simulations. *J. Chem. Inf. Model.* **2022**, *62* (22), 5715−5728.

(24) Menin, S.; Pavan, M.; Salmaso, V.; Sturlese, M.; Moro, S. Thermal titration molecular dynamics (TTMD): not your usual post-docking refinement. *Int. J. Mol. Sci.* **2023**, *24* (4), No. 3596.

(25) Wang, Y.; Murlidaran, S.; Pearlman, D. A. Quantum simulations of SARS-CoV-2 main protease Mpro enable high-quality scoring of diverse ligands. *J. Comput.-Aided Mol. Des.* **2021**, *35* (9), 963−971.

(26) Tong, X.; Liu, X.; Tan, X.; Li, X.; Jiang, J.; Xiong, Z.; Xu, T.; Jiang, H.; Qiao, N.; Zheng, M. Generative models for de novo drug design. *J. Med. Chem.* **2021**, *64* (19), 14011−14027.

(27) Palazzesi, F.; Pozzan, A. Deep learning applied to ligand-based de novo drug design. *Methods Mol. Biol.* **2022**, *2390*, 273−299.

(28) Chen, Z.; Min, M. R.; Parthasarathy, S.; Ning, X. A deep generative model for molecule optimization via one fragment modification. *Nat. Mach. Intell.* **2021**, *3* (12), 1040−1049.

(29) Watson, E. R.; Novick, S.; Matyskiela, M. E.; Chamberlain, P. P.; de la Peña, A. H.; Zhu, J.; Tran, E.; Griffin, P. R.; Wertz, I. E.; Lander, G. C. Molecular glue CELMoD compounds are regulators of cereblon conformation. *Science* **2022**, *378* (6619), 549−553.

(30) Petzold, G.; Fischer, E. S.; Thomä, N. H. Structural basis of lenalidomide-induced CK1α degradation by the CRL4CRBN ubiquitin ligase. *Nature* **2016**, *532* (7597), 127−130.

https://doi.org/10.1021/acsomega.4c08049
ACS Omega XXXX, XXX, XXX−XXX