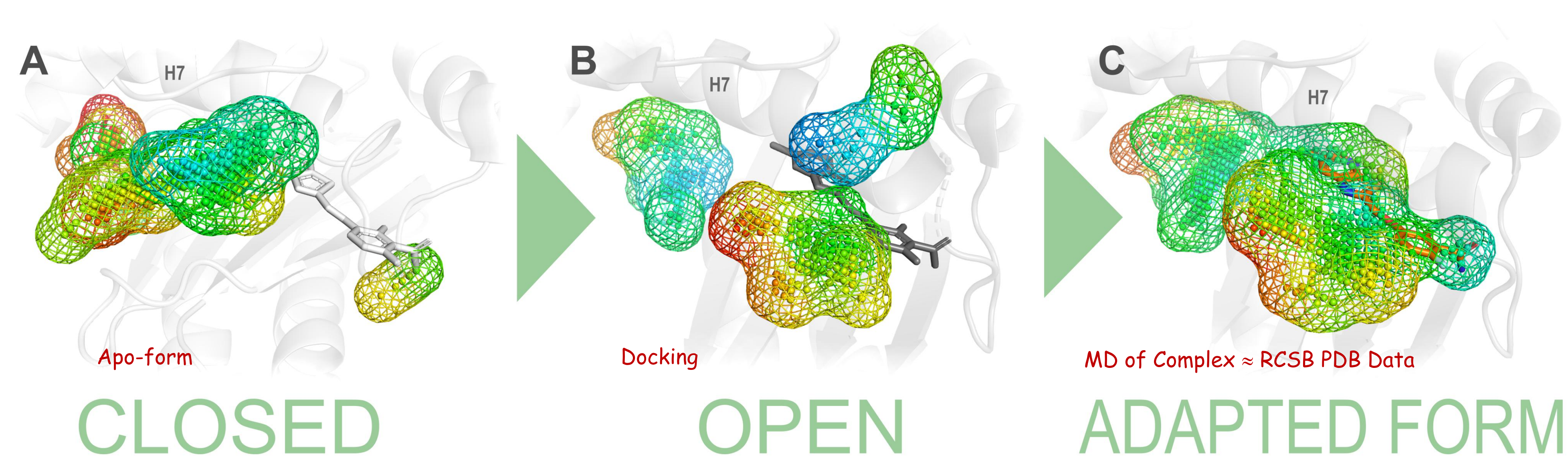


Introduction. The bacterial binary fission apparatus consists of a complex protein ensemble within the divisome. Among these, Filamenting temperature-sensitive mutant Z (FtsZ) is the key component of the Z-ring, responsible for recruiting other proteins to the complex (Wang et al., 2020). This makes FtsZ a promising molecular target for inhibiting bacterial cell division (Karpov et al., 2016; Pradhan et al., 2021). While a significant number of FtsZ inhibitors have been biochemically validated, their specific binding sites often remain poorly defined. A search of the ChEMBL database (www.ebi.ac.uk/chembl/) identified 379 FtsZ ligands; however, the target sites for most of these compounds are either only predicted or unknown. Currently, 85 FtsZ protein structures in complex with 61 unique ligands are available in the RCSB Protein Data Bank. In the present study, potential inhibitors targeting the IDC site were identified through pharmacophore screening and molecular docking.

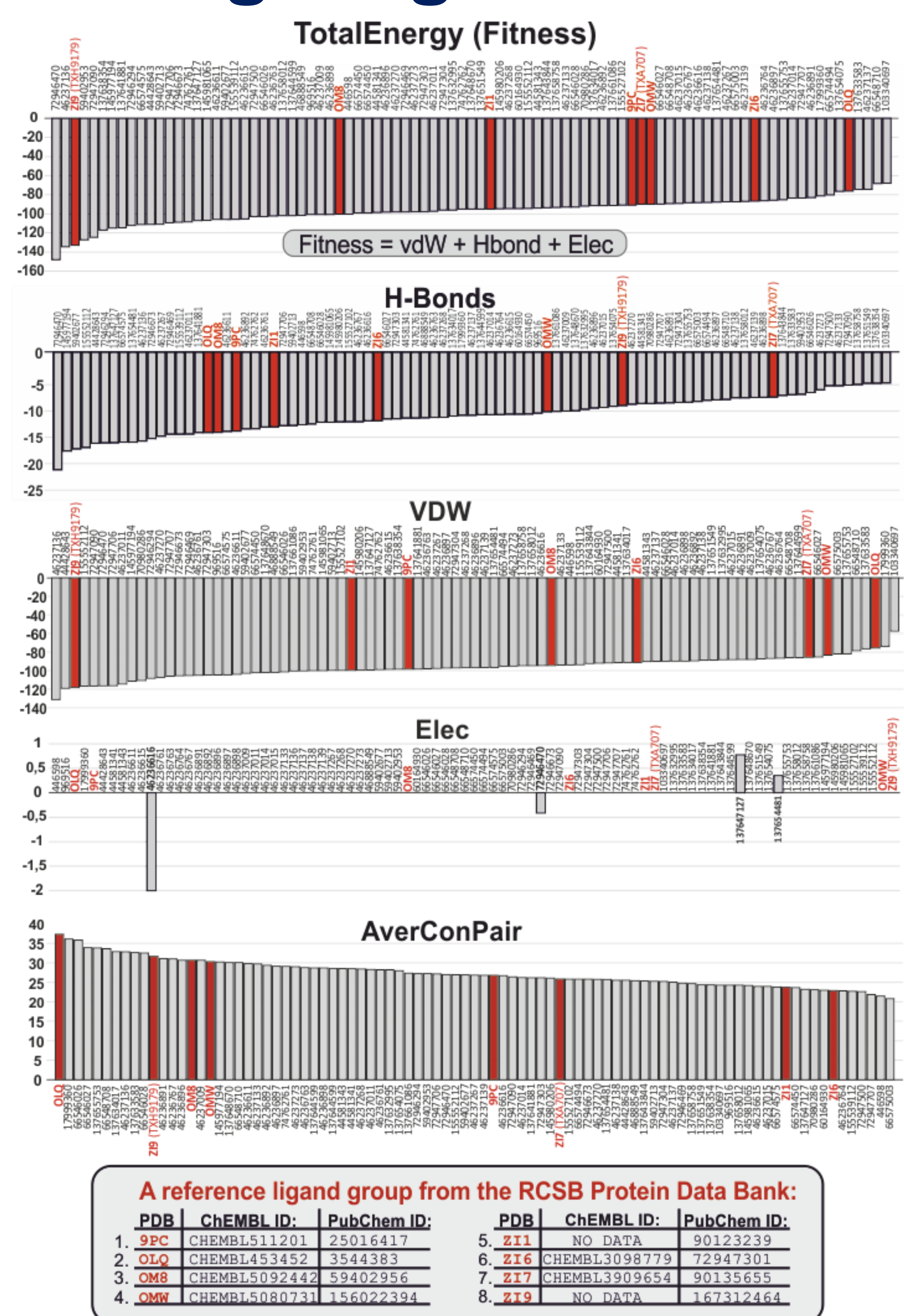
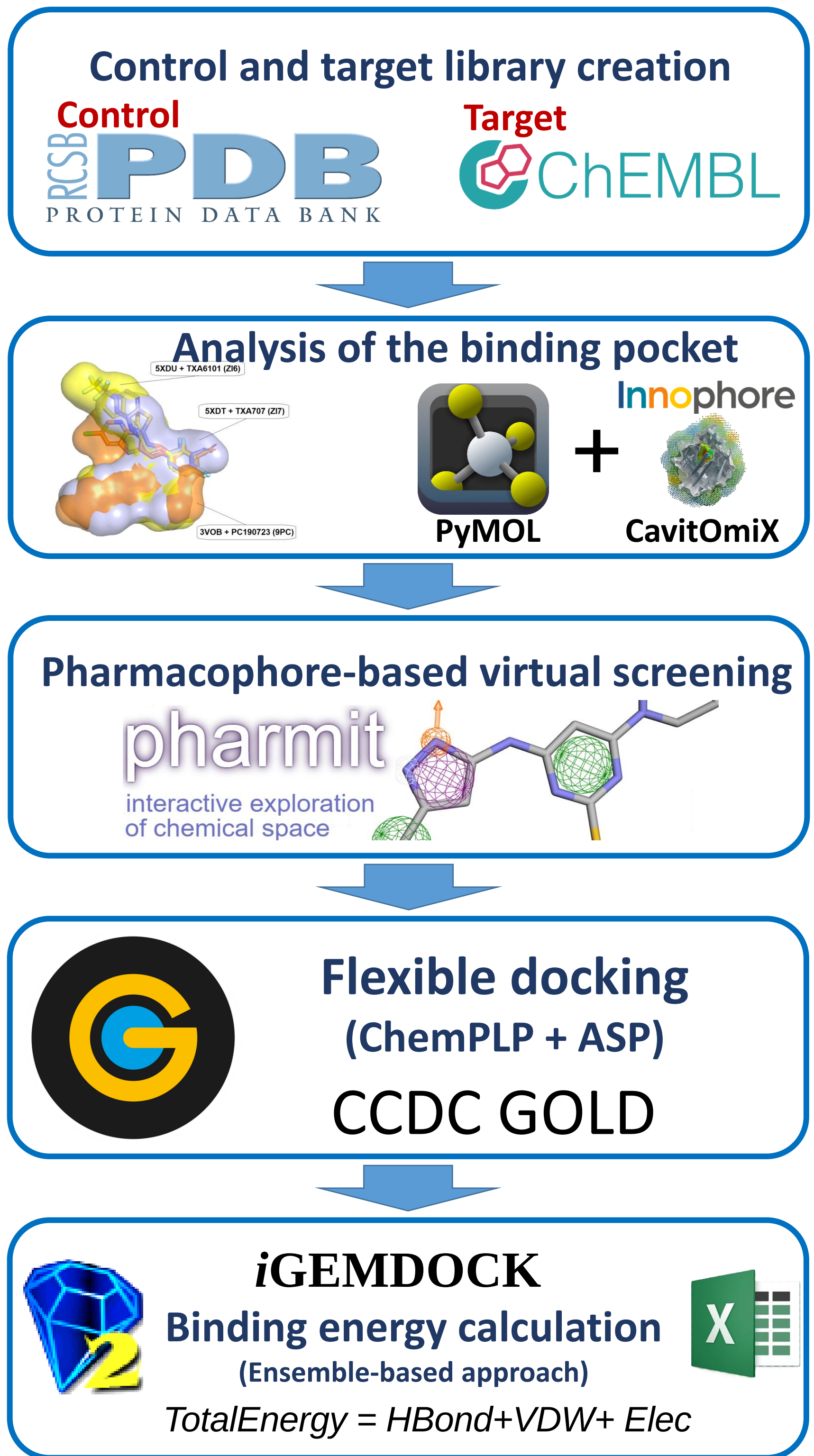
Conformational plasticity of the FtsZ IDC site upon ligand binding



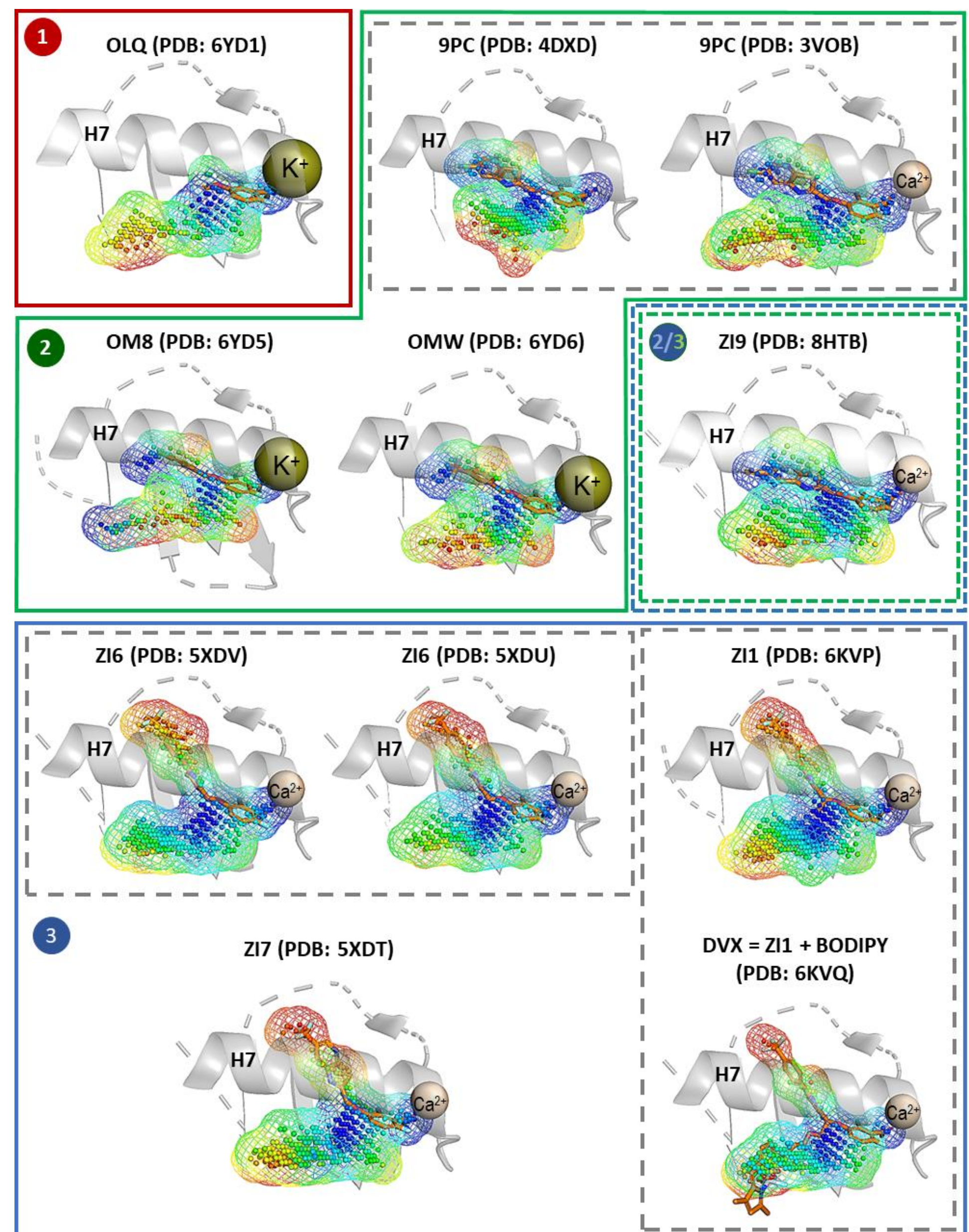
The variability of the FtsZ protein IDC pocket was investigated. Point-cloud models of the IDC pockets were constructed based on 11 structures of *S. aureus* FtsZ in complex with 2,6-difluorobenzamides (including ligands OLQ, 9PC, OM8, OMW, ZI1, ZI6, ZI7, and ZI9) currently deposited in the RCSB PDB. Significant variability in the volume and shape of the IDC pocket, resulting from adaptation to the ligand, was confirmed. Four main conformational states of the pocket, arising from induced ligand-protein fit, were identified. This indicates that docking ligands into the IDC site of a single 3D model of the FtsZ protein is ineffective. It was concluded that virtual screening efficiency can be significantly improved by using an ensemble of molecular targets that accounts for the conformational variability of the site.

Identification of ligands targeting the IDC

Based on a revision of the RCSB PDB and ChEMBL databases, a library of 379 FtsZ protein effectors was compiled. Nine 2,6-difluorobenzamide derivatives served as positive controls, while six compounds targeting alternative sites were used as negative controls.



iGEMDOCK docking results: Average results of independent docking sessions performed using four PDB structures: 3VOB, 5XDT, 6YD1, and 5XDU. (a) Total Energy; (b) H-Bonds – contribution of hydrogen bonds; (c) vdW – contribution of van der Waals forces; (d) Elec – contribution of electrostatic interactions; (e) AverConPair – average energy of the conformational pair (ligand–protein induced-fit potential).



Conformational types of IDC pocket (1, 2, 3, and 2/3) identified via the 'point-cloud' Catalophore method."

To identify IDC site effectors, the prepared library with pre-generated conformers was uploaded to Pharmit. Due to variations in ligand-protein fitting, the screening strategy relied on 11 individual sessions, one for each PDB complex. To accurately define the pharmacophores, hydrogen bonding, hydrophobicity, electrostatics, and interaction maps were analyzed using BIOVIA Discovery Studio. To minimize false-positive results, constraints were applied to the target pocket shape. Following 11 screening sessions, a set of 89 unique compounds was selected based on scoring and duplicate filtration.

The binding potential of the selected compounds was further examined via molecular docking in CCDC GOLD and iGEMDOCK programs. An ensemble of targets (PDB: 3VOB, 5XDT, 6YD1, and 5XDU) was used for both platforms to account previously identified conformational flexibility of the IDC pocket. Visual assessment in PyMOL and analysis using the ChemPLP and ASP scoring functions in CCDC GOLD confirmed the correct binding poses for all compounds in the selected group. Final evaluation in iGEMDOCK using the same ensemble of targets enabled the quantification of the binding energy (TotalEnergy = Fitness) of the selected ligands with the IDC site. This analysis accounted for the contributions of hydrogen bonds (H-Bonds), electrostatics (Elec), van der Waals (VDW) forces, and the steric potential of ligand-protein adaptation (AverConPair) to the stability of the resulting complexes.

CONCLUSIONS: By combining pharmacophore screening (Pharmit) and molecular docking (CCDC GOLD, iGEMDOCK) of 379 biochemically active compounds from PDB and ChEMBL, we identified 88 definitive the Inter-Domain Cleft site effectors and one potential curcumin-based lead. The selected compounds will be subjected to further biochemical validation and combinatorial design of new inhibitors of bacterial FtsZ.