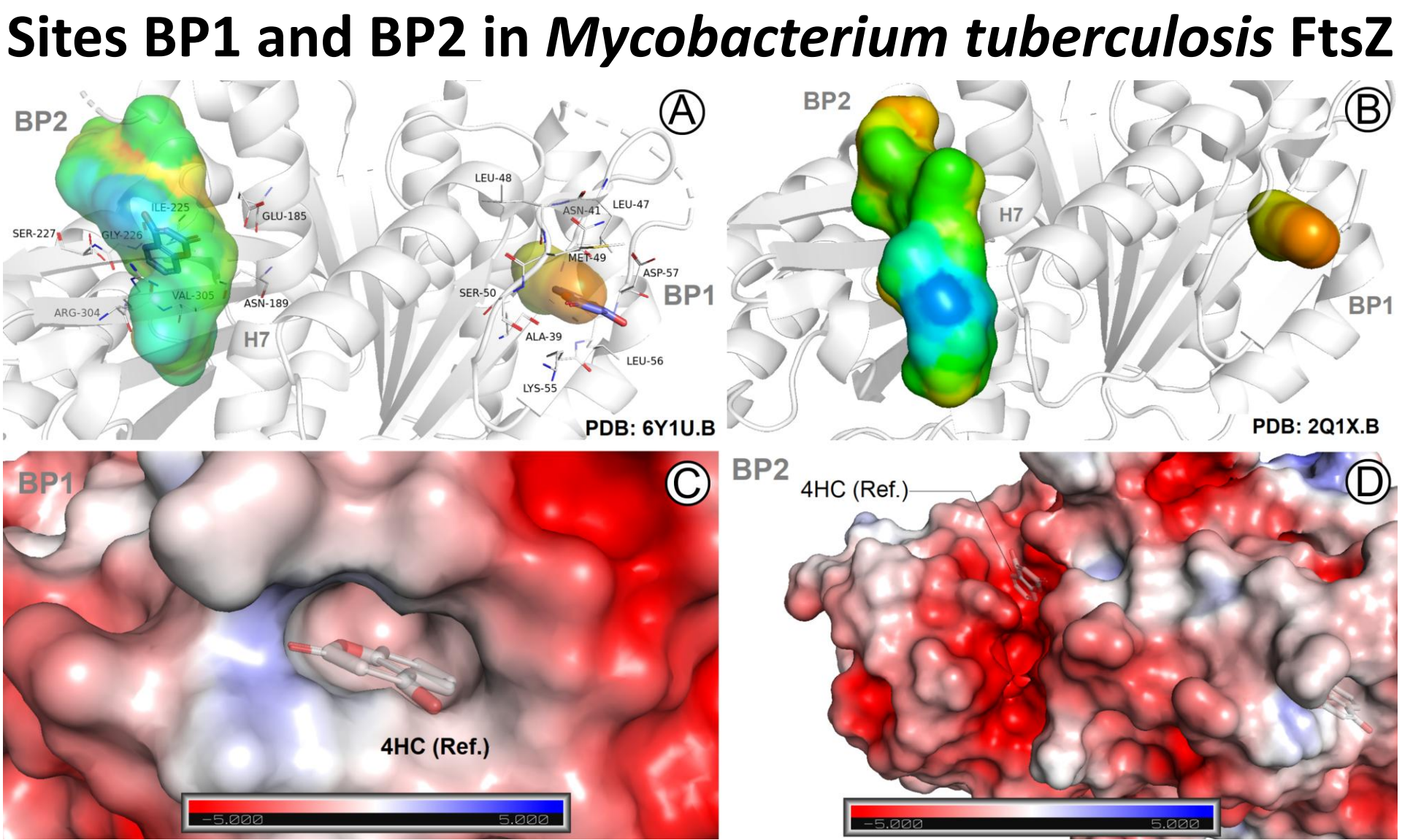


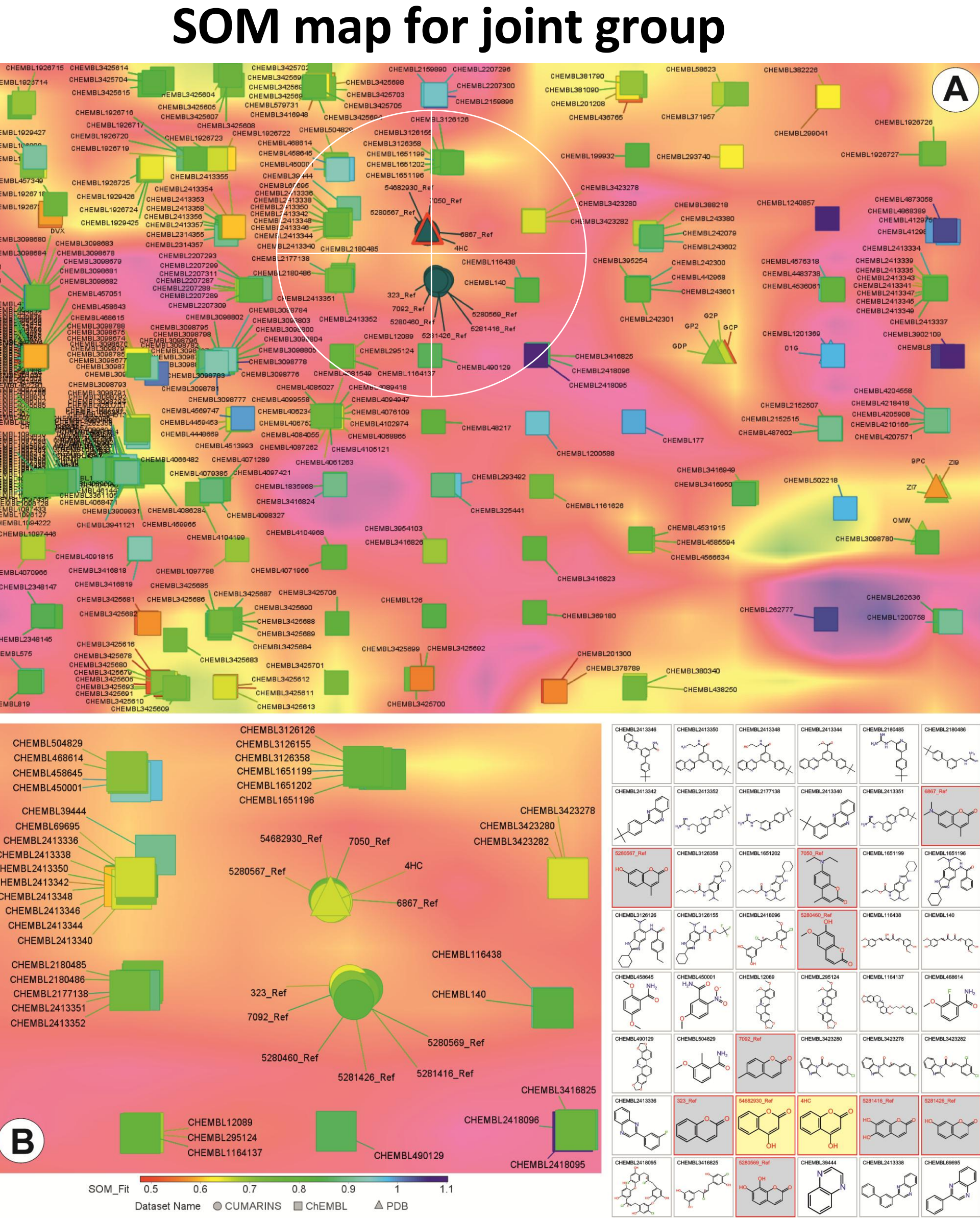
Introduction. In the face of rising bacterial resistance, FtsZ, a bacterial homologue of tubulin, remains a critical target for the inhibition of binary fission. To avoid the toxicity problems associated with GTP-competitive inhibitors targeting the NBS site, current studies have pivoted toward ligands with alternative mechanisms of action. The fungal metabolite 4-hydroxycoumarin (4HC) interacts with FtsZ at two distinct pockets, BP1 and BP2 (PDB: 6Y1V, 6Y1U). Unlike benzamide effectors of the interdomain cleft (IDC), 4HC binds FtsZ exclusively in the nucleotide-free state. This interaction is governed by the conformational flexibility of the H7 helix, where 4HC induces allosteric shifts and a "deformational break" disrupting the NBS pocket, preventing proper protofilament assembly. Notably, the BP2 site of 4HC fits the Taxol-binding site in β -tubulin, reinforcing the evolutionary homology of these proteins. Given that the binding sites for the majority of known FtsZ effectors (379 in the ChEMBL db) remain unknown. This study employs chemoinformatic and pharmacophore screening, molecular docking, AI-modeling, and MD simulations to identify in ChEMBL depositions FtsZ inhibitors associated with the BP1 and BP2 sites of Coumarin Binding (CB).

Results. Using RCSB Protein Data Bank structures, we compared 4HC interactions with BP1 and BP2 sites. Our data confirm Almani et al. (2021) data that 4HC has lower affinity for BP2 than BP1, and binds exclusively to GTP-free FtsZ. The presence of ligands in the BP1 and BP2 pockets caused internal displacements and this mobility predetermine the strategy of subsequent screening protocols. CavitOmiX analysis showed that both sites remain open in the apo-state (Fig. 3).



MD simulations confirmed that transient ligand binding in BP2 is the condition for correct formation of a stable 4HC complex with the BP1 site (Almani et al., 2021, Ozheredov et al., 2024).

It was selected ten compounds with proven FtsZ interaction based on PDB and literature data. SOM and UMAP analyses of the joint group of control and 379 ChEMBL effectors identified a isolated Coumarin cluster. Similar compounds were identified within shared 'valleys' and isolated from other clusters by chemical landscape 'ridges' and structural distance:



Structures 6Y1U.B and 6Y1V.B were used for pharmacophore construction. Subsequent Pharmit screening and flexible docking with CCDC GOLD yielded 13 hits, leading to a final selection of 47 compounds: 10 from the control group and 37 from chemoinformatic analysis. (Fig. 6b). Flexible docking in CCDC GOLD showed that 41 of the 47 selected compounds demonstrated correct poses in the BP1 pocket. For the BP2 pocket, docking predicted complexes for 45 ligands, with the compound CID: 103961572 being the only exception. Notably, five ligands that did not fit the BP1 pocket showed reliable docking poses in BP2. Subsequent AI reconstruction of the complexes using the Protenix Server (<https://protenix-server.com>) reduced the number of candidates to 40.

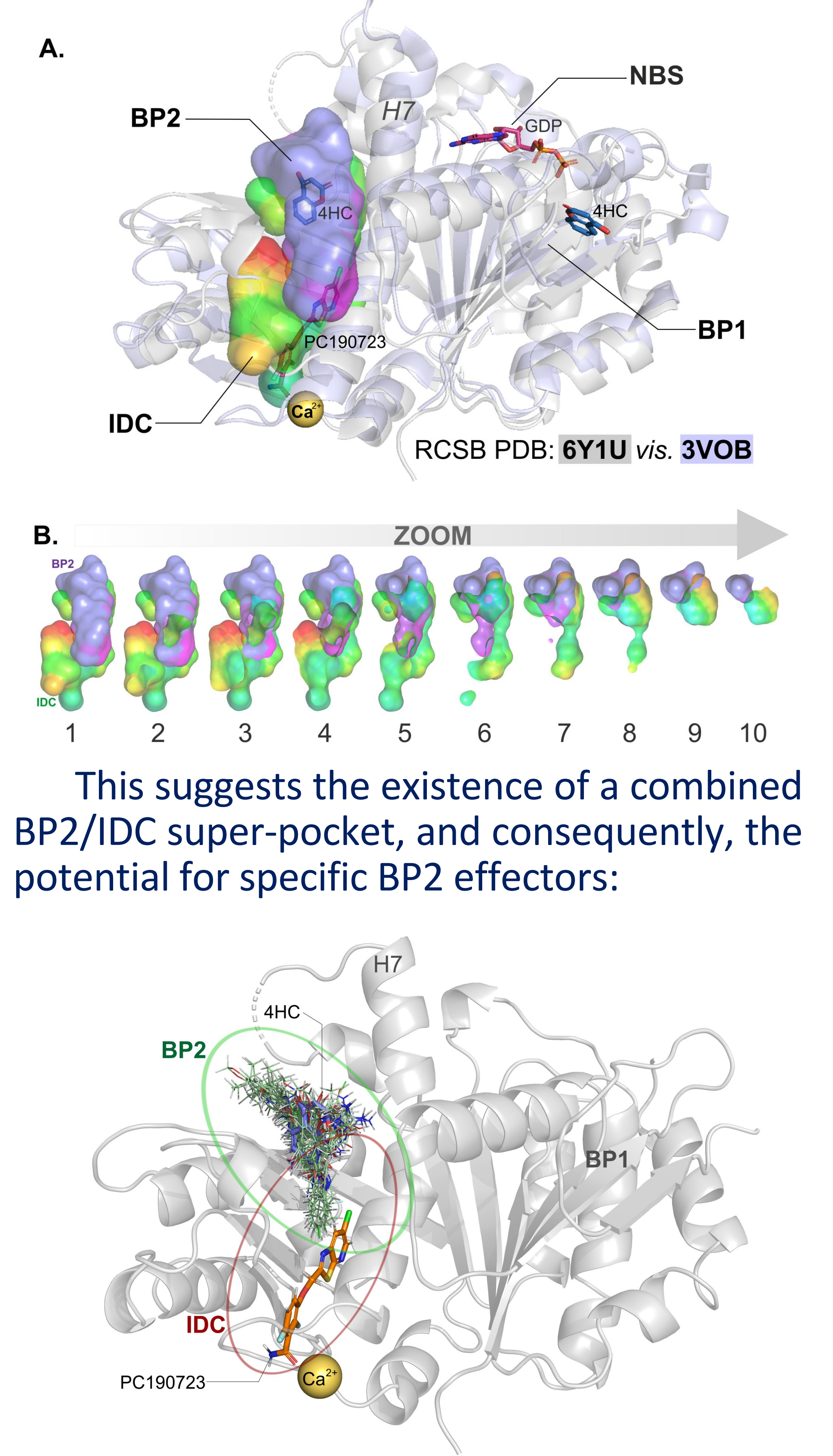
To estimate the potential binding energy of the selected compounds to BP1 and BP2, we performed redocking using iGEMDOCK to quantitatively assess the total energy of ligand–protein interactions, taking into account the contributions of hydrogen bonds (H-bonds), van der Waals forces (vdW), and electrostatics (Elec):

Total Energy = H-bonds + vdW + Elec,
(Yang and Chen, 2004; Bellaver et al., 2004):

Ranking of effectors of the sites BP1 and BP2 based on the TotalEnergy index		TotalEnergy	
ChEMBL	PubChem	BP1	BP2
CHEMBL2413346	71817840	-98,2845	-96,4274
CHEMBL2413352	71657194	-95,3787	-85,7701
CHEMBL2413351	71657195	-91,7359	-96,1009
CHEMBL3423282	84377541	-90,6111	-92,9133
CHEMBL3126358	71738318	-90,522	-85,8665
CHEMBL1164137	46907295	-87,9809	-101,575
CHEMBL3423278	84377669	-87,8915	-92,2812
●CHEMBL244743	5281416	-87,7507	-86,0041
●CHEMBL244948	5280569	-87,1474	-87,1891
CHEMBL417799	5154	-86,4036	-83,0937
CHEMBL1651202	25101148	-85,7027	-99,6606
CHEMBL3416825	60171588	-84,8938	-95,3958
●CHEMBL12208	5280567	-83,5027	-77,0225
CHEMBL2180485	71460998	-83,3847	-98,8649
CHEMBL2180486	71462731	-83,272	-93,4459
●CHEMBL71851	5280460	-83,0955	-82,7345
CHEMBL3126155	72793845	-82,9066	-80,6849
CHEMBL2177138	71450208	-82,4729	-99,8866
CHEMBL458645	320829	-81,2833	-88,8945
●CHEMBL51628	5281426	-80,5638	-75,2703
CHEMBL2413342	73347578	-79,4185	-83,2567
CHEMBL442942	72878	-79,4062	-80,4199
CHEMBL2413336	71817836	-78,887	-79,2981
CHEMBL450001	18403949	-77,3247	-76,7892
CHEMBL453452	3544383	-76,0296	-77,2169
●CHEMBL443436	7092	-75,2762	-65,797
CHEMBL2413340	71817838	-74,6649	-74,7481
CHEMBL22425	11461	-74,5238	-78,5835
CHEMBL4102611	45120136	-73,9581	-77,9664
●CHEMBL301141	54682930	-73,9442	-74,197
●CHEMBL3186529	6867	-73,4485	-73,4607
CHEMBL4079927	137652240	-71,8853	-81,6065
●CHEMBL6466	323	-71,7765	63,9019
CHEMBL468614	10630958	-71,4733	-76,9443
CHEMBL504829	16094695	-70,6492	-70,3514
CHEMBL39444	7045	-68,2111	-64,6405
CHEMBL453180	820515	-67,7091	-72,4443
CHEMBL468616	17999355	-66,9474	-64,5336
CHEMBL1885859	162162	-64,229	-67,3576
CHEMBL69695	78725	-1,00845	-21,1283

Further ranking was performed based on the 'Total Energy' values calculated for BP1, as this site is associated with the inhibitory effect of 4HC (Almani et al., 2021). All compounds of the control group demonstrate relatively similar binding energy values with BP1 and BP2 with a slight advantage in favor of BP1 (see Tabl.).

Since BP2 is significantly larger than BP1 and yields more favorable binding energies, we suggest it may overlap with the IDC pocket. Also, some compounds showed superior 'Total Energy' for the BP2 pocket. We suggest it may share the space with the IDC pocket. Essentially, these two sites represent opposite ends of a single 'super-pocket'. CavitOmiX catalophoric casts for *M. tuberculosis* FtsZ (in complex with 4HC, PDB: 6Y1U.B) and *S. aureus* FtsZ (in complex with PC190723) showed significant mutual overlap:



However, despite possessing a Coumarin scaffold, certain structural features—particularly molecular size—may hinder interaction of this compounds with the BP1 pocket.

CONCLUSIONS: The selected effectors can be categorized into two subgroups: (1) 'true' 4HC-like compounds primarily targeting the BP1 pocket, and (2) BP2/IDC super-pocket effectors. The latter include bulky derivatives unable to bind BP1. Since their binding is centered on the BP2 site, their mechanism likely involves allosteric shifts distinct from those of canonical IDC effectors.