Supporting Information

Propene Bridged Cyanurate Tetramer Decorated on Carbon Nanosheets with Antibacterial Activity: Insights from Molecular Modeling and In Vitro Studies

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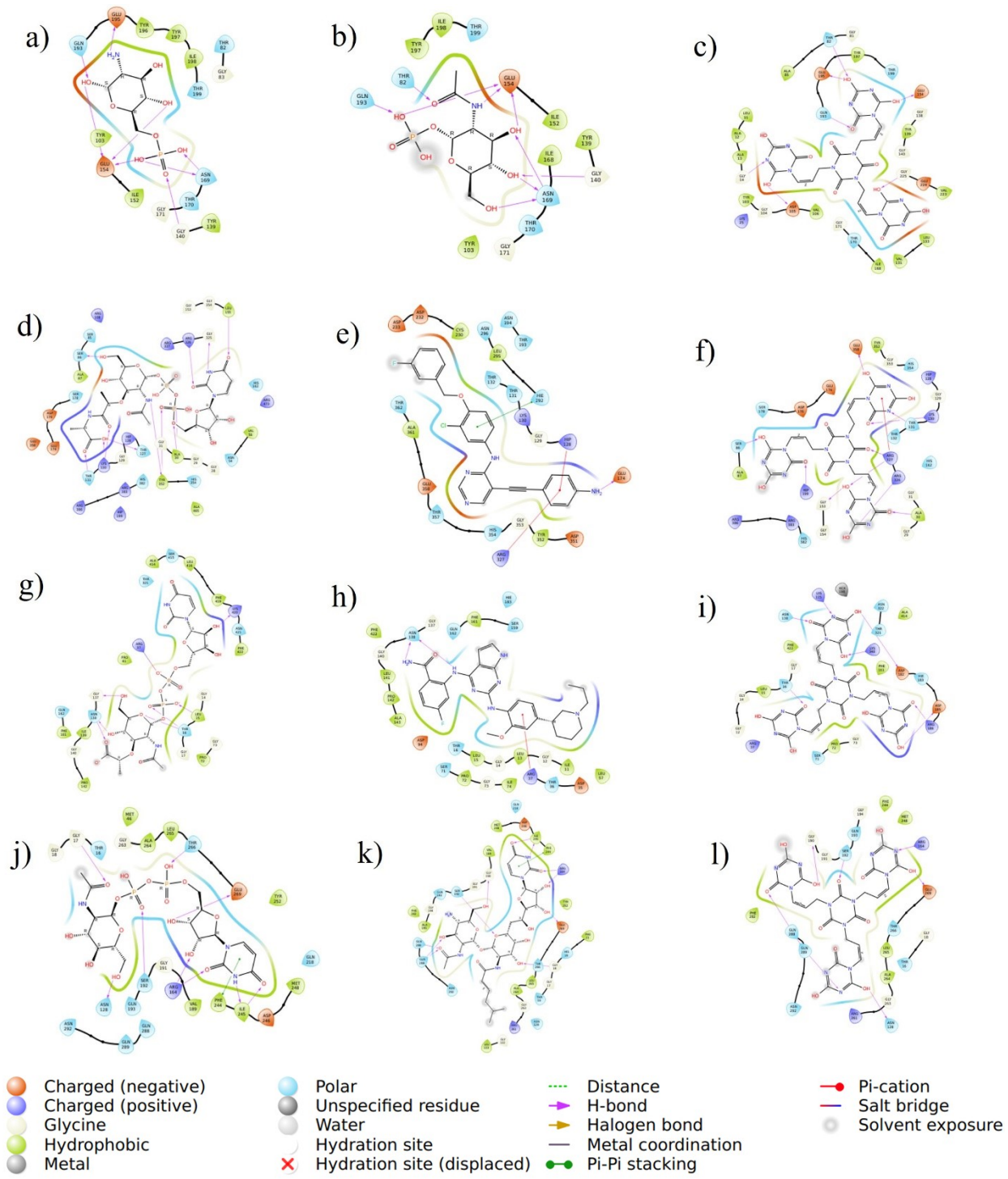

Figure S1. 2D Ligand interaction diagram showing major interaction of ligands in the active sites of protein; (a-c) Substrate, inhibitor and PBCT bound to GlmU, (d-f) Substrate, inhibitor and

PBCT bound to MurC, (g-i) Substrate, inhibitor and PBCT bound to MurD and (j-l) Substrate, inhibitor and PBCT bound to MurG.

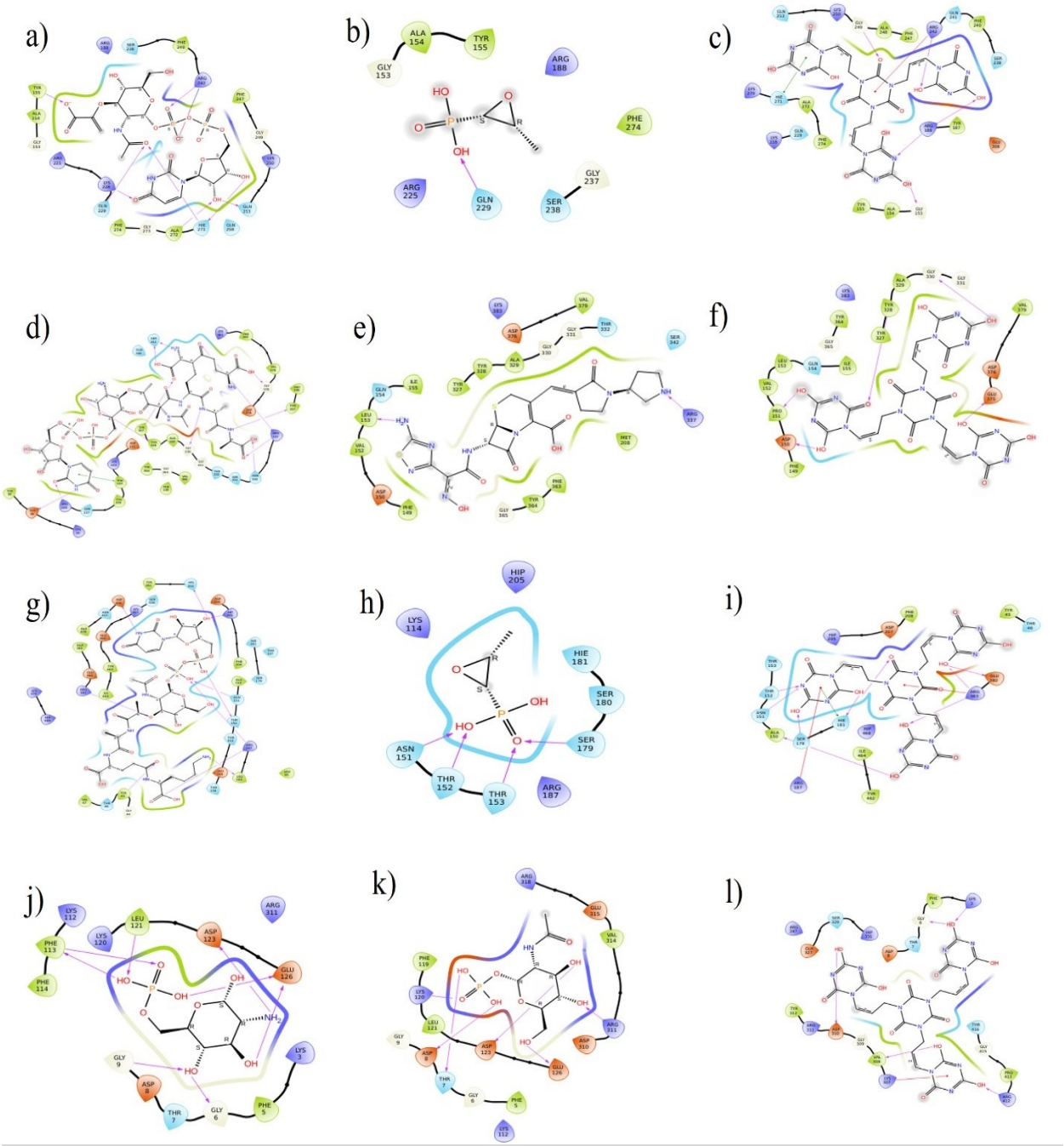

Figure S2. 2D Ligand interaction diagram showing major interaction of ligands in the active sites of protein; (a-c) Substrate, inhibitor and PBCT bound to MurB, (d-f) Substrate, inhibitor and PBCT

bound to FemA, (g-i) Substrate, inhibitor and PBCT bound to MurE and (j-l) Substrate, inhibitor and PBCT bound to GlmM.

Table S1. SP and XP Docking scores of substrate, inhibitor and PBCT bound to *E. coli* proteins

Protein/Enzyme	PDB ID	Material/Inhibitor	SP Score	XP Score
GlmU	1HV9	Glucosamine-6- phosphate	-5.795	-7.178
		N-acetylglucosamine-1-phosphate	-6.774	-8.881
		PBCT	-7.463	-7.025
MurC	2F00	UDP-N-acetyl muramoyl-L-alanine	-7.504	-10.297
		GW659893X	-6.358	-5.351
		PBCT	-6.995	-7.827
MurD	1UAG	UDP-N-acetylmuramic acid	-8.351	-9.709
		GW659893X	-7.184	-6.175
		PBCT	-8.523	-7.217
MurG	1NLM	UDP-N-acetylglucosamine	-9.849	-17.498
		Tunicamycin	-6.987	-13.588
		PBCT	-8.604	-7.702

Table S2. SP and XP Docking scores of substrate, inhibitor and PBCT bound to *S.aureus* proteins

Protein/Enzyme	PDB ID	Substrate/Inhibitor/Material	SP Score	XP Score
GlmM	6GYZ	Glucosamine-6-phosphate	-6.090	-8.968
		N-acetylglucosamine-1-phosphate	-5.366	-6.395
		PBCT	-7.618	-5.933
MurB	1HSK	UDP- N-Acetylglucosamine-enolpyruvate	-6.886	-9.545
		Fosfomycin	-4.205	-3.903
		PBCT	-7.217	-5.976

MurE	4C13	UDP- N-acetyl muramoyl-L-Alanyl-D-Glutamyl-L-Lysine	-7.028	-8.525
		Fosfomycin	-5.001	-5.800
		PBCT	-6.532	-7.255
FemA	1LRZ	UDP-N-acetylmuramic acid pentapeptide	-9.359	-12.144
		Ceftobiprole	-7.972	-4.787
		PBCT	-7.618	-6.990

Table S3. Different interactions of substrate, inhibitor and PBCT bound to GlmM (**PDB ID: 6GYZ)** of *S. aureus*

S. NO	Residues	Substrate	Inhibitor	PBCT
1.	LYS3	-	-	H-Bond (Sidechain, Polar, Donor, Charge)
2.	GLY6	H-Bond (Backbone, Acceptor)	-	H-Bond (Backbone, Acceptor)
3.	THR7	-	H-Bond (Backbone, Acceptor)	-
4.	ASP8	-	H-Bond (Backbone, Sidechain, Polar, Acceptor, Charge)	-
5.	GLY9	H-Bond (Backbone, Donor)	-	-
6.	PHE113	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor, Donor, Aromatic)	-	-
7.	LYS120	-	H-Bond (Backbone, Sidechain, Polar, Donor, Charge)	-
8.	LEU121	H-Bond (Backbone, Sidechain, Hydrophobic, Donor)		-
9.	ASP123	H-Bond (Sidechain, Polar, Acceptor,	H-Bond (Sidechain, Polar, Acceptor,	-

		Charge)	Charge)	
10.	GLU126	H-Bond (Sidechain, Polar, Acceptor, Charge)	H-Bond (Sidechain, Polar, Acceptor, Charge)	-
11.	LYS307	-	-	Pi-Cation (Sidechain, Polar, Charge)
12.	VAL308	-	-	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor)
13.	ASP310	-	-	H-Bond (Sidechain, Polar, Acceptor, Charge)
14.	ARG311	-	H-Bond (Backbone, Sidechain, Polar, Donor, Charge)	-
15.	ARG412	-	-	H-Bond (Sidechain, Polar, Donor, Charge)

Table S4. Different interactions of substrate, inhibitor and PBCT bound to MurB **(PDB ID: 1HSK)** of *S. aureus*

S. No	Residues	Substrate	Inhibitor	PBCT
1.	GLY153			H-Bond (Backbone, Acceptor)
2.	TYR155	H-Bond (Backbone, Sidechain, Hydrophobic, Aromatic)		
3.	ARG188			H-Bond (Sidechain, Polar, Donor, Charged)
4.	GLN229	H-Bond (Sidechain, Polar, Donor)	H-Bond (Sidechain, Polar, Donor)	
5.	ARG242	H-Bond (Sidechain, Polar, Donor, Charged)		H-Bond, P-cation (Backbone, Sidechain, Polar, Charged)
6.	GLY249			H-Bond (Backbone, Donor)
7.	GLN253	H-Bond (Sidechain, Polar)		

8.	HIE271	H-Bond (Backbone, Sidechain, Polar, Acceptor, Donor)		Pi-Pi Stacking (Backbone, Sidechain, Polar)
9.	ALA272	H-Bond (Backbone, Sidechain, Hydrophobic)		

Table S5. Different interactions of substrate, inhibitor and PBCT bound to MurE (**PDB ID: 4C13)** of *S. aureus*

S. No.	Residues	Substrate	Inhibitor	PBCT
1.	TYR45	H-Bond (Backbone, Sidechain, Hydrophobic, Donor, Aromatic)		
2.	ALA150			H-Bond (Backbone, Acceptor)
3.	ASN151	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar, Donor)
4.	THR152	H-Bond (Backbone, Sidechain, Polar, Acceptor)	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar, Donor)
5.	THR153		H-Bond (Backbone)	
6.	SER179			H-Bond (Sidechain, Polar, Donor)
7.	HIE181			H-Bond, Pi-Pi Stacking (Sidechain, Polar, Acceptor, Donor)
8.	LEU185	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor)	H-Bond	
9.	ARG187	H-Bond (Sidechain, Polar, Donor, Charged)		Pi-cation (Sidechain, Polar, Charged)
10.	HIP205	H-Bond (Sidechain, Donor, Charged)		
11.	HIS353	H-Bond (Sidechain, Polar, Acceptor)		

12.	GLU382			H-Bond, Pi-cation (Backbone, Sidechain, Polar, Acceptor, Charged)
13.	ARG383	H-Bond (Sidechain, Polar, Charged)		H-Bond (Sidechain, Polar, Donor, Charged)
14.	ASP406	H-Bond Sidechain, Polar, Acceptor, Charged)		

Table S6. Different interactions of substrate, inhibitor and PBCT bound to FemA (**PDB ID: 1LRZ)** of *S. aureus*

S. No.	Residues	Substrate	Inhibitor	PBCT
1.	GLU36	H-Bond (Sidechain, Polar, Acceptor, Charged)		
2.	ASP150			H-Bond (Backbone, Acceptor)
3.	Pro151			H-Bond (Backbone)
4.	LEU153		H-Bond (Backbone, Acceptor)	
5.	PHE207	H-Bond (Backbone, Acceptor)		
6.	PHE224	Pi-Pi Stacking (Backbone, Sidechain, Hydrophobic, Aromatic)		
7.	ARG228	H-Bond (Sidechain, Polar, Donor, Charged)		
8.	TYR327	H-Bond (Sidechain, Hydrophobic, Donor, Aromatic)		
9.	TYR328	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor, Aromatic)		H-Bond (Backbone, Sidechain, Hydrophobic, Donor, Aromatic)

10.	GLY330	H-Bond (Backbone, Acceptor)		H-Bond (Backbone, Acceptor)
11.	ARG337	H-Bond (Sidechain, Polar, Donor, Charged)	H-Bond (Sidechain, Polar, Donor, Charged)	
12.	SER342	H-Bond (Sidechain, Polar, Acceptor)		
13.	GLU375			H-Bond (Backbone, Sidechain, Polar, Acceptor, Charged)
14.	GLY378	H-Bond (Backbone, Acceptor)		

Table S7. Different interactions of substrate, inhibitor and PBCT bound to GlmU (**PDB ID: 1HV9)** of *E.coli*

S. NO	Residues	Substrate	Inhibitor	PBCT
1.	GLY14			H-Bond (Backbone)
2.	THR82		H-Bond (Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar)
3.	ASP105			H-Bond (Sidechain, Polar, Acceptor, Charged)
4.	GLY140	H-Bond (Backbone, Donor)	H-Bond (Backbone, Donor)	
5.	GLU154	H-Bond (Sidechain, Polar, Acceptor, Charged)	H-Bond (Sidechain, Polar, Acceptor, Charged)	H-Bond (Sidechain, Polar, Acceptor, Charged)
6.	ASN169	H-Bond (Backbone, Sidechain, Polar, Acceptor, Donor)	H-Bond (Backbone, Sidechain, Polar, Acceptor, Donor)	
7.	GLN193	H-Bond (Sidechain, Polar)	H-Bond (Sidechain, Polar, Donor)	H-Bond (Sidechain, Polar, Donor)
8.	GLU195	H-Bond (Sidechain, Polar, Acceptor, Charged)		H-Bond (Sidechain, Polar, Acceptor, Charged)
9.	GLY225			H-Bond (Backbone,

				Donor)

Table S8. Different interactions of substrate, inhibitor and PBCT bound to MurC (**PDB ID: 2F00)** of *E.coli*

S. NO	Residues	Substrate	Inhibitor	PBCT
1.	ALA30	H-Bond (Backbone, Sidechain, Hydrophobic)		H-Bond (Backbone)
2.	SER86	H-Bond (Backbone, Sidechain, Polar, Acceptor)		H-Bond (Sidechain, Polar, Donor)
3.	THR127	H-Bond (Backbone, Acceptor)		
4.	HIP128		Pi-cation (Backbone, Sidechain, Charged)	
5.	LYS130	H-Bond (Backbone, Sidechain, Polar, Donor, Charged)		H-Bond (Backbone, Sidechain, Polar, Charged)
6.	THR131	H-Bond (Backbone, Sidechain, Polar)		
7.	GLY153			H-Bond (Backbone, Acceptor)
8.	LEU155	H-Bond (Backbone, Sidechain, Hydrophobic, Donor)		
9.	GLU174		H-Bond (Sidechain, Polar, Acceptor, Charged)	
10.	HIS199			H-Bond (Sidechain, Donor, Charged)
11.	HIE292		Pi-Pi Stacking (Backbone, Sidechain, Polar)	
12.	GLY325	H-Bond (Backbone, Acceptor)		
13.	ARG326	H-Bond (Sidechain, Polar, Donor, Charged)		H-Bond, Pi-cation (Sidechain, Polar, Charged)
14.	ARG327		Pi-cation (Sidechain, Polar, Charged)	H-Bond (Sidechain, Polar, Donor, Charged)
15.	TYR352	H-Bond (Sidechain, Hydrophobic, Donor, Aromatic)		
16.	GLU358			H-Bond (Sidechain, Polar, Acceptor, Charged)

Table S9. Different interactions of substrate, inhibitor and PBCT bound to MurD (**PDB ID: 1UAG)** of *E.coli*

S. NO	Residues	Substrate	Inhibitor	PBCT
1.	LEU15	H-Bond (Backbone, Sidechain, Hydrophobic, Donor)		
2.	THR16	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar, Donor)
3.	THR36		H-Bond (Backbone, Sidechain, Polar, Acceptor)	
4.	ARG37	Salt bridge (Sidechain, Polar, Donor, Charged)		
5.	LYS115			H-Bond (Sidechain, Polar, Donor, Charged)
6.	GLY137	H-Bond (Backbone, Acceptor)		
7.	ASN138	H-Bond (Backbone, Sidechain, Polar, Acceptor, Donor)		H-Bond (Sidechain, Polar, Donor)
8.	ASP182			H-Bond (Sidechain, Polar, Acceptor, Charged)
9.	ASP185			H-Bond (Sidechain, Polar, Acceptor, Charged)
10.	ARG186			H-Bond (Sidechain, Polar, Donor, Charged)
11.	LYS348			H-Bond (Sidechain, Polar, Donor, Charged)
12.	LYS420	H-Bond (Backbone, Acceptor)		

Table S10. Different interactions of substrate, inhibitor and PBCT bound to MurG (**PDB ID: 1NLM)** of *E.coli*

S. NO	Residues	Substrate	Inhibitor	PBCT
1.	GLY17	H-Bond (Backbone, Donor)		
2.	GLY18		H-Bond (Backbone, Donor)	
3.	ASN128			H-Bond (Sidechain, Polar)
4.	ARG164	H-Bond (Sidechain, Polar, Donor, Charged)	H-Bond (Sidechain, Polar, Donor, Charged)	H-Bond (Sidechain, Polar, Charged)
5.	GLY190		H-Bond (Backbone, Acceptor)	H-Bond (Backbone, Acceptor)
6.	SER192	H-Bond (Backbone, Sidechain, Polar, Donor)	H-Bond (Backbone, Sidechain, Polar)	H-Bond (Backbone, Sidechain, Polar, Donor)
7.	PHE244	Pi-Pi Stacking (Backbone, Sidechain, Hydrophobic, Aromatic)	Pi-Pi Stacking (Backbone, Sidechain, Hydrophobic, Aromatic)	
8.	ILE245	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor, Donor)	H-Bond (Backbone, Sidechain, Hydrophobic, Acceptor, Donor)	
9.	THR266	H-Bond (Backbone, Sidechain, Polar, Donor)		
10.	GLU269	H-Bond (Sidechain, Polar, Acceptor, Charged)	H-Bond (Sidechain, Polar, Acceptor, Charged)	H-Bond (Sidechain, Polar, Acceptor, Charged)
11.	GLN288		H-Bond (Sidechain, Polar, Donor)	
12.	GLN289		H-Bond (Sidechain, Polar, Donor)	H-Bond (Sidechain, Polar, Donor)

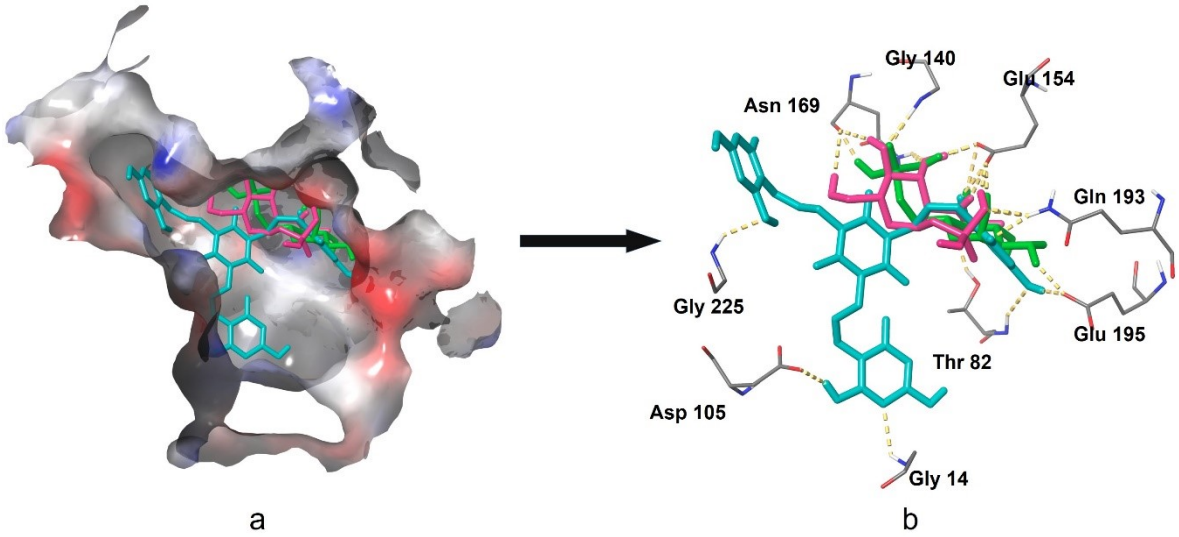

Figure S3. Orientation of docked ligands in the binding site of GlmU. The binding site of the GlmU cavity is represented by electrostatic potential surface (a). Ligands are shown in different colours; PBCT (cyan), Substrate (green) and inhibitor (pink). The residues of binding site and ligands are represented by thin and thick tubes, respectively. H-bonds are represented by yellow dotted lines (b).

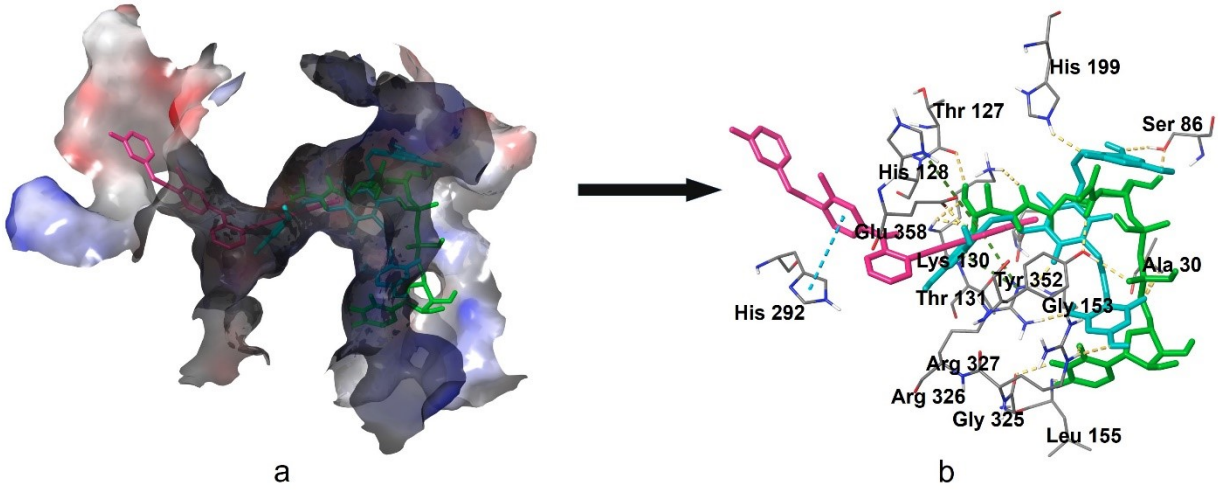

Figure S4. Orientation of docked ligands in the binding site of MurC. The binding site of the MurC cavity is represented by electrostatic potential surface (a). Ligands are shown in different colours; PBCT (cyan), Substrate (green) and inhibitor (pink). The residues of binding site and ligands are represented by thin and thick tubes, respectively. H-bonds and π–π interactions are represented by yellow and cyan dotted lines, respectively and cation-π interactions are shown with green dotted lines (b).

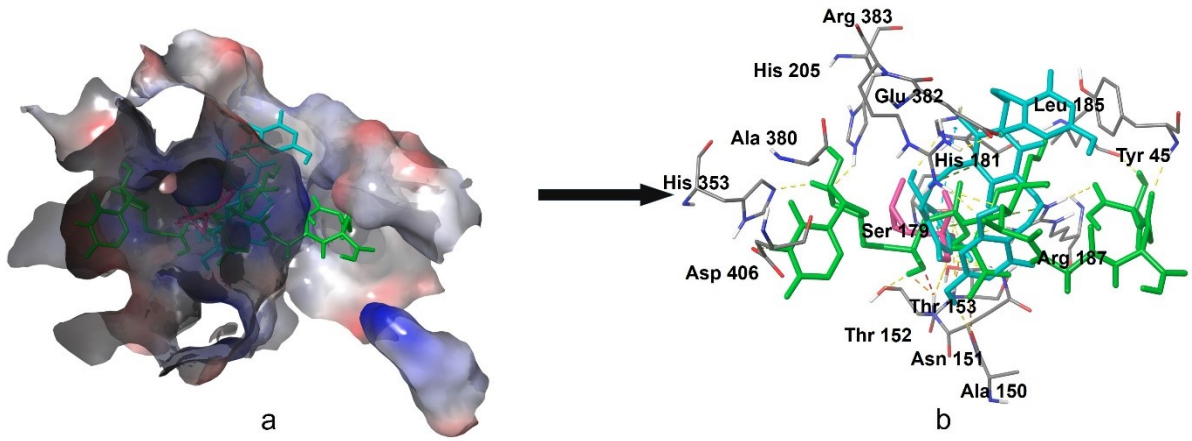

Figure S5. Orientation of docked ligands in the binding site of MurE. The binding site of the MurE cavity is represented by electrostatic potential surface (a). Ligands are shown in different colours; PBCT (cyan), Substrate (green) and inhibitor (pink). The residues of binding site and ligands are represented by thin and thick tubes, respectively. H-bonds and π–π interactions are represented by yellow and cyan dotted lines, respectively and cation-π interactions are shown with green dotted lines (b).

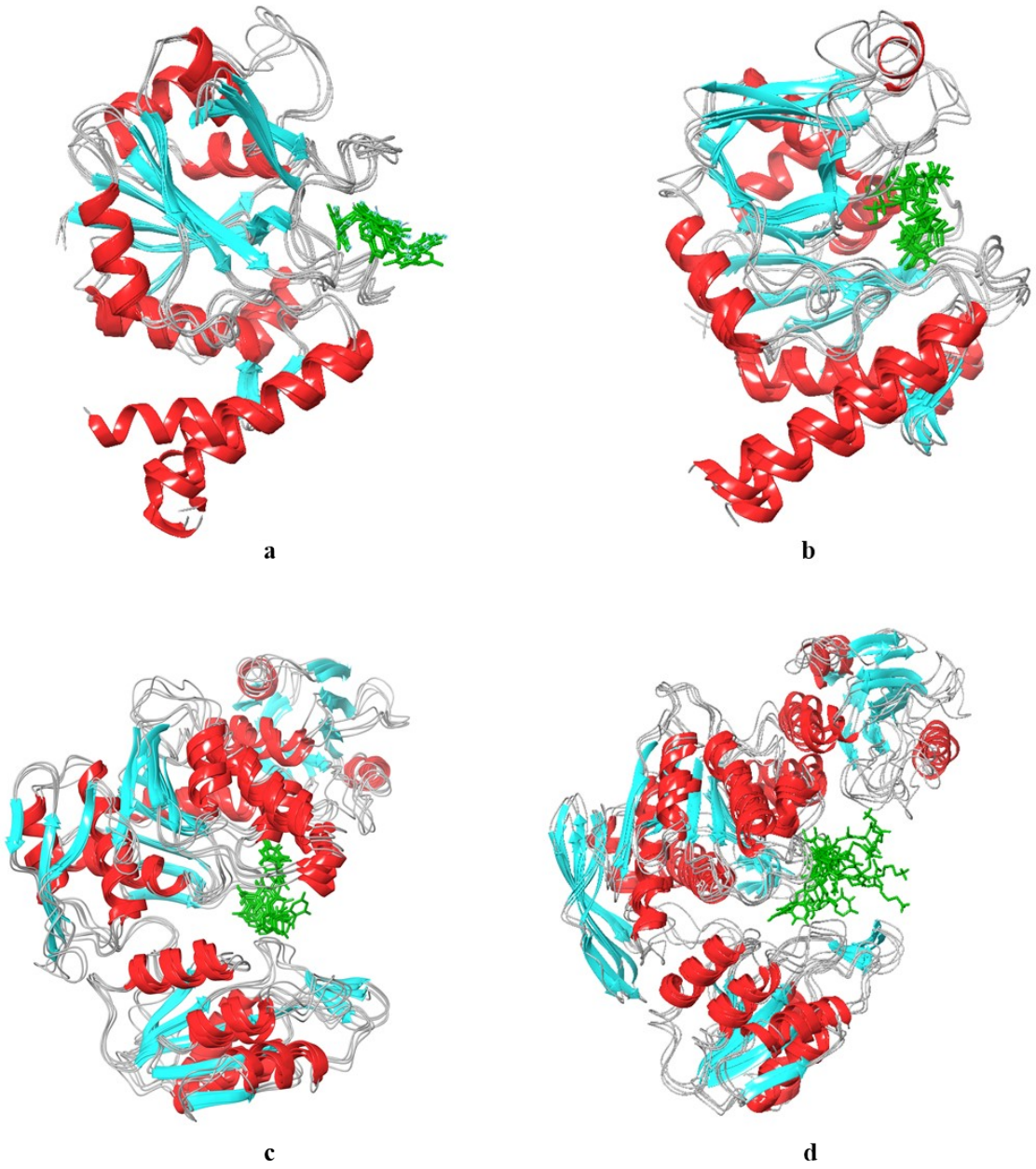

Figure S6. Conformational ensemble consisting of 4 conformations obtained after RMSD clustering of 500ns trajectory (a) GlmU with PBCT and (b) GlmU with substrate (c) MurE with PBCT and (d) MurE with substrate.

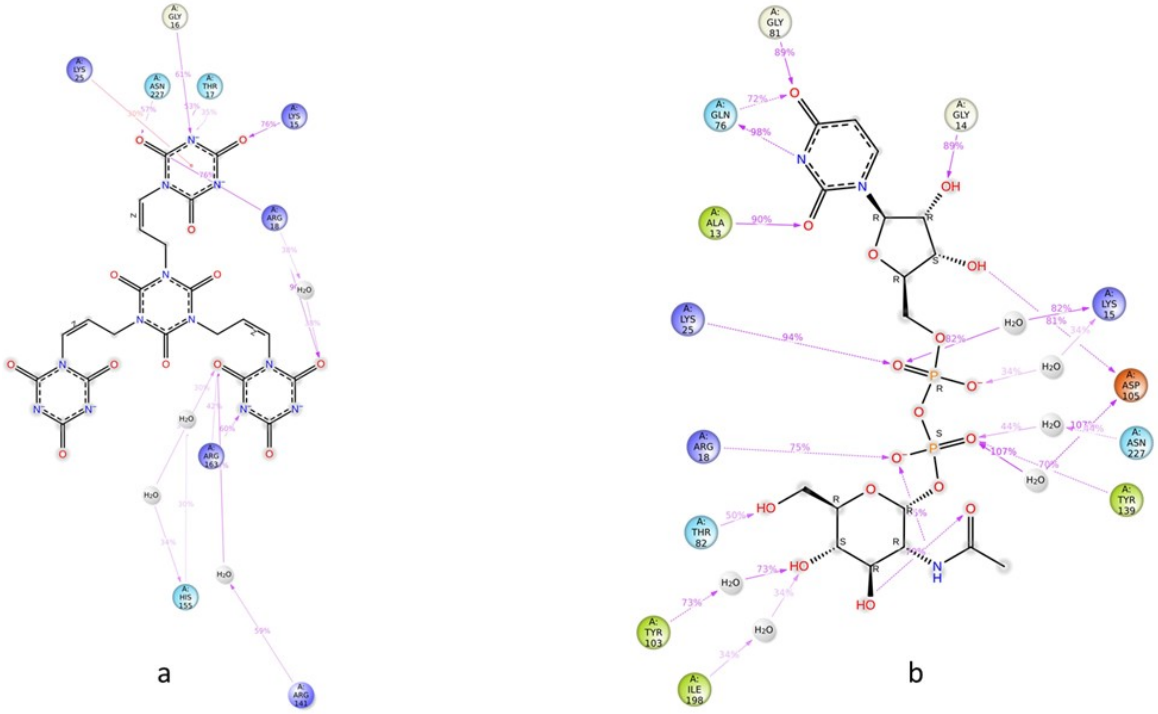

Figure S7. 2D intermolecular interactions between *E. coli* protein (PDB ID: 1HV9) and (a) PBCT and (b) substrate.

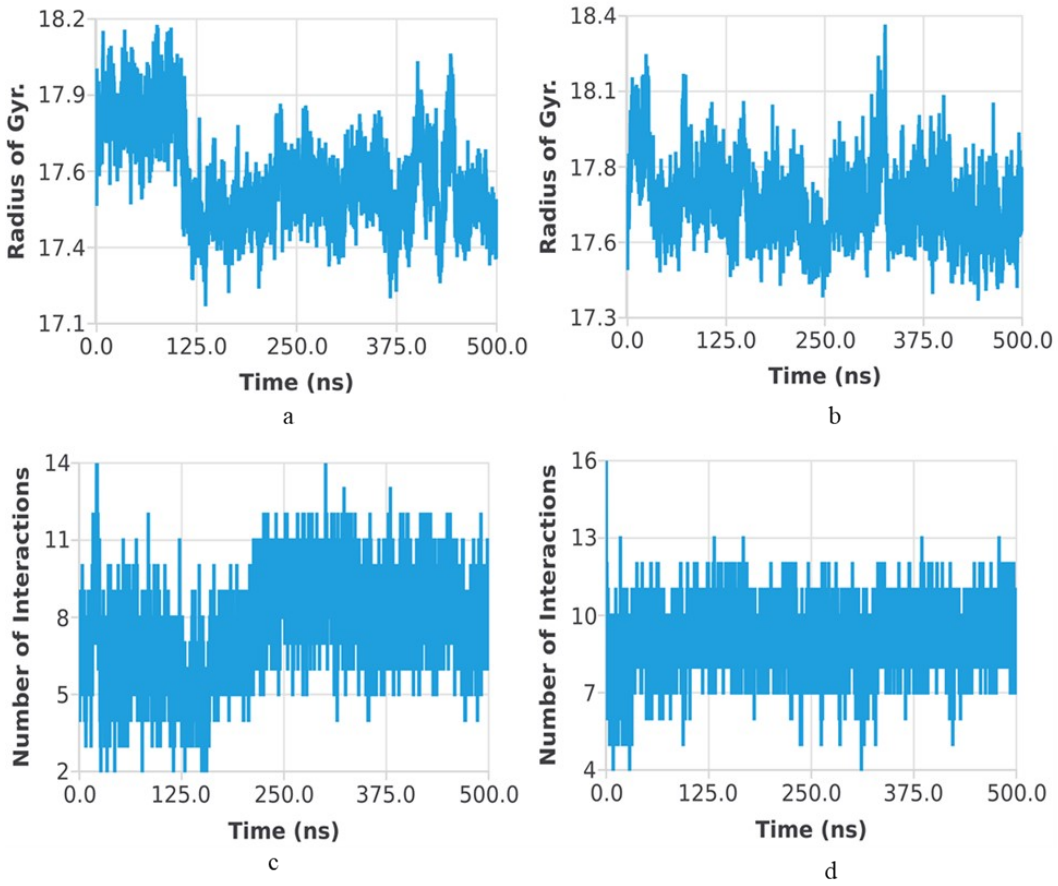

Figure S8. Plot of the Radius of Gyration of *E. coli* protein (PDB ID: 1HV9) with (a) PBCT and (b) substrate. Plot of number of hydrogen bonds observed in the complex of GlmU and (c) PBCT and (d) substrate during 500ns MD Simulation

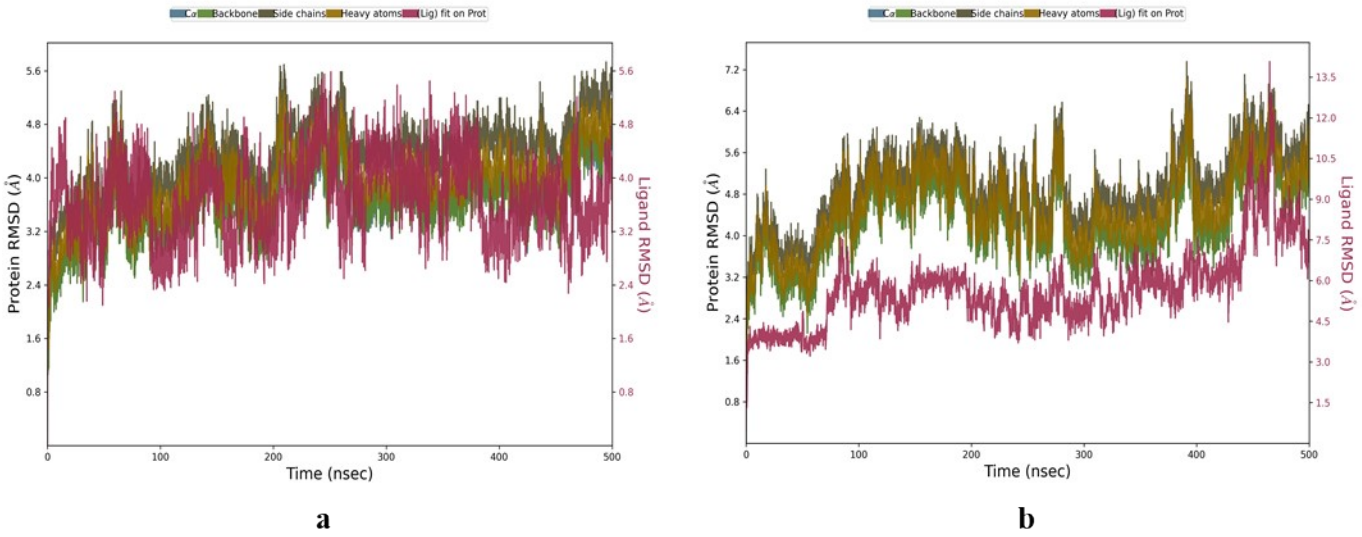

Figure S9. Structure and interaction analysis of MD simulation trajectories obtained for *E. coli*

protein (PDB ID:2F00) (a) The RMSD plot of MurC with PBCT and (b) with substrate. The simulation time of 500 ns showing the formation of stable complex without any significant conformational changes in protein structure.

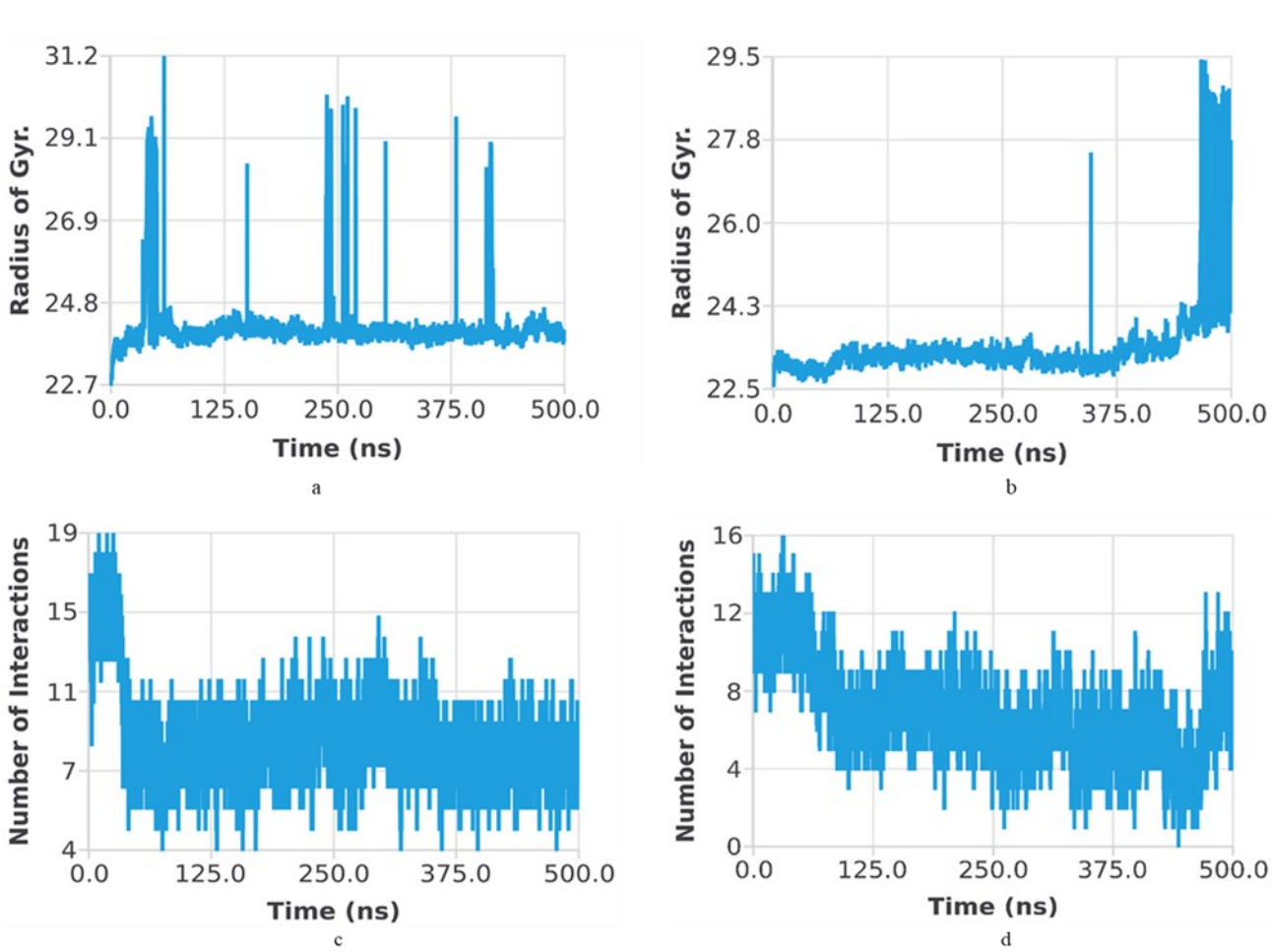

Figure S10. Plot of the Radius of Gyration of *E. coli* protein (PDB ID: 2F00) with (a) PBCT and (b) substrate. Plot of number of hydrogen bonds observed in the complex of MurC and (c) PBCT and (d) substrate during 500ns MD Simulation.

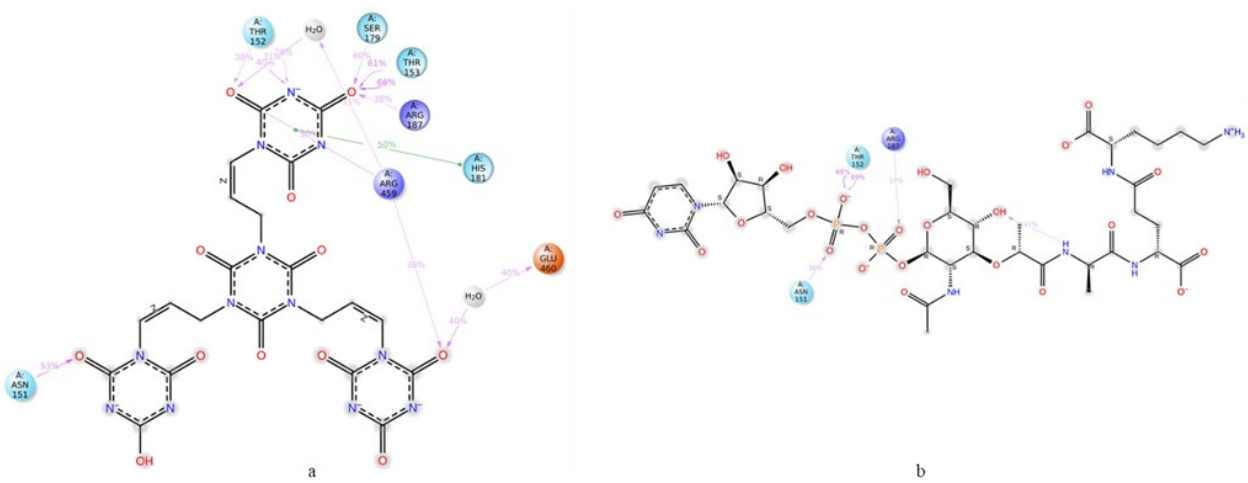

Figure S11. 2D intermolecular interactions between *S. aureus* protein (PDB ID: 4C13) and (a) PBCT and (b) substrate.

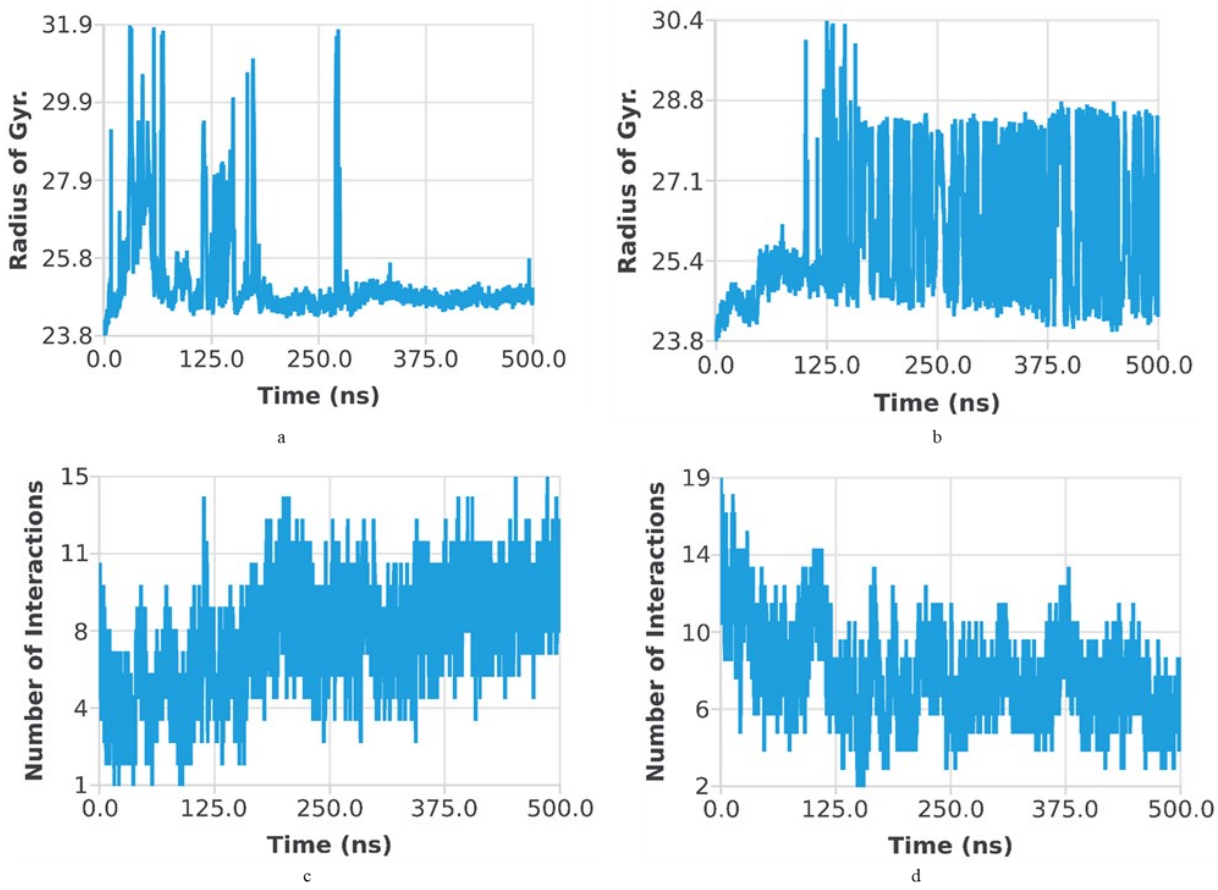

Figure S12. Plot of the Radius of Gyration of *S. aureus* protein (PDB ID: 4C13) with (a) PBCT and (b) substrate. Plot of number of hydrogen bonds observed in the complex of MurE and (c) PBCT and (d) substrate during 500ns MD Simulation.

Characterization of Mesenchymal Stem Cells

The presences of MSCs were further confirmed via flow cytometry against the antibodies CD19 FITC (negative marker) and CD44 PE (positive marker). From the scattered plots, the auto showed no cells in either MSC negative or positive marker's quadrants (Q1 and Q4 respectively) (**Figure S13a**). In positive sample (PS), labeled with CD44 PE showed more than 80% population of cells in Q1 (**Figure S13b**), while less than 3% were labeled with CD19 FITC in Q4 the negative sample (NS) (**Figure 3c**). Moreover, in the test group (**Figure S13d**), a significant (p-value<0.001) (**Figure S13e**) population of PS labeled with CD44 PE (Q1) was observed, suggesting that the isolated cells are MSCs only and exclusively expressing CD44 marker.

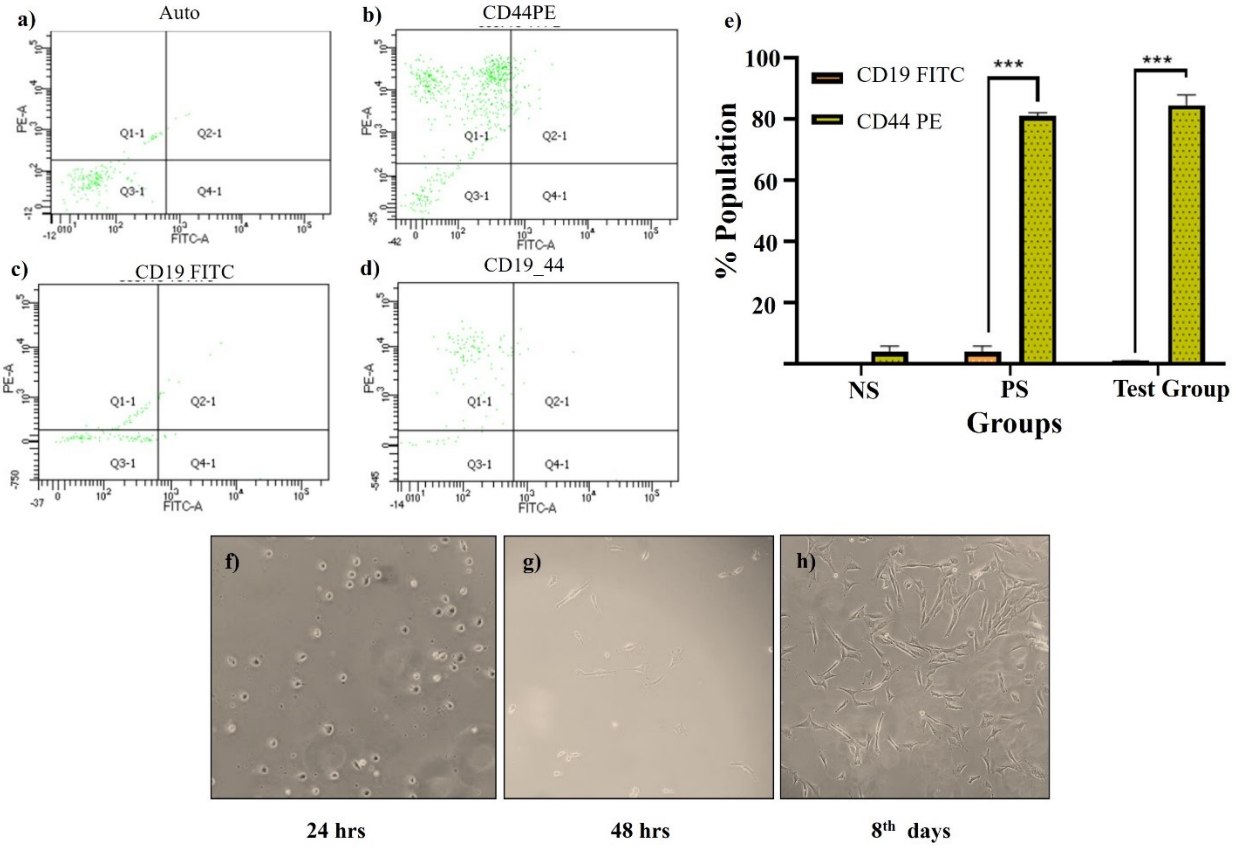

Figure S13 (a-d) Flow cytometry characterization of MSCs. (a) Auto, (b) CD44PE (positive marker), (c) CD19 FITC (negative marker), (d) Dual (CD19/CD44), (f) PE-FITC mean bar graph (***<0.001). Microscopic images of the growth of MSCs from day 1 to 8. The development of elongated spindle shaped adhered MSCs can be observed over the course of 14 days, which eventually took a month to reach ~70% confluency.

Table S11: Reduction in cell viability of *S. aureus* upon the exposure CNH-4 and CNH-24.

	Time (h)	**Control**	**½ MIC**	**MIC**	**2MIC**	**4MIC**
Cell viability in Log10 CFU/mL with CNH-4	0	5.47	5.62	5.52	5.48	5.27
	4	6.27	5.29	5.21	5.02	4.38
	8	7.4	4.81	4.62	4.3	3.3
	16	9.1	4.32	4.1	3.6	2.3
	24	10.24	4.13	3.94	3.59	2
Cell viability in Log10 CFU/mL with CNH-24	0	5.22	5.13	4.99	4.95	4.87
	4	5.97	5.16	4.93	4.72	3.69
	8	8.35	4.05	3.82	3.38	2.47
	16	9.3	3.76	3.54	3.14	1.3
	24	10.19	3.69	3.3	3	1
Cell viability in Log10 CFU/mL with CNH-48	0	6.42	6.33	6.04	5.96	5.93
	4	7.26	6.08	5.76	5.05	4.7
	8	8.33	5.44	4.66	4.03	3.8
	16	10.96	4.78	3.58	3.13	2.6
	24	12.03	4.08	2.95	2.08	1