Supplementary Material

for

Structural and Functional Analysis of *Campylobacter jejuni* PseG: A UDP-Sugar Hydrolase from the Pseudaminic Acid Biosynthetic Pathway

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Table S1. Mutagenic oligonucleotides used in this study.

Oligonucleotide	Sequence (5'→3')
H17F-F	GCTCCAGTCAAATAGGCTTTGGA**TT**CATTAAACGCGATCTAGTCTTAGC
H17F-R	GCTAAGACTAGATCGCGTTTAATG**AA**TCCAAAGCCTATTTGACTGGAGC
H17L-F	GCTCCAGTCAAATAGGCTTTGGAC**T**CATTAAACGCGATCTAGTCTTAGC
H17L-R	GCTAAGACTAGATCGCGTTTAATG**A**GTCCAAAGCCTATTTGACTGGAGC
H17N-F	GCTCCAGTCAAATAGGCTTTGGA**A**ACATTAAACGCGATCTAGTCTTAGC
H17N-R	GCTAAGACTAGATCGCGTTTAATGT**T**TCCAAAGCCTATTTGACTGGAGC
Y78F-F	GAACTTCTTATCATTGATCACT**TC**GGCATCAGCGTAGATGATGAAAAACTG
Y78F-R	CAGTTTTTCATCATCTACGCTGATGCC**GA**AGTGATCAATGATAAGAAGTTC
N255A-F	GGCAAATTTCAAAGCTATTTGTTATGTTAAA**GC**TCAAGAATCTACAGCAACTTGGCTTGC
N255A-R	GCAAGCCAAGTTGCTGTAGATTCTTGA**GC**TTTAACATAACAAATAGCTTTGAAATTTGCC

Table S2. α-helical parameters for PseG variants obtained from CD experiments (see Fig. S2 for further details).

PseG or variant	MW	[c] mg/ml	ABS_{280}	$-[\theta] \times 10^{-3}$ (λ_{min}, nm)	$-[\theta]_{209} \times 10^{-3}$	$-[\theta]_{222} \times 10^{-3}$	$[\theta]_{222}/[\theta]_{209}$
17.1	32383.3	0.099	0.082	11.22 (213.6)	9.55	8.69	0.91
133.1	32384.2	0.102	0.084	12.20 (212.2)	11.01	9.32	0.85
N255A	32340.3	0.100	0.083	11.61 (212.8)	10.43	8.94	0.86
17.1(2)	32383.3	0.098	0.081	10.94 (213.2)	9.61	8.37	0.87
H17L	32359.3	0.099	0.082	10.84 (210.8)	10.56	8.22	0.78
H17N	32360.2	0.098	0.081	11.32 (212.0)	10.29	8.04	0.78
H17F	32393.3	0.101	0.083	11.72 (213.4)	9.52	8.81	0.93
Y78F	32367.3	0.104	0.081	9.57 (212.0)	8.87	7.01	0.79

ε_{280}=26735 $M^{-1}cm^{-1}$ (for all compounds except only Y78F)
ε_{280}=25245 $M^{-1}cm^{-1}$ (for Y78F)
cell length: 0.05 cm

Table S3. PseG-substrate solvated interaction energy (SIE) and PseG solvated conformational energy (SCE) for predicted models for PseG-substrate complexes corresponding to $^5S_1$ and $^1C_4$ sugar ring conformations of the substrate.

Energy (kcal/mol)	PseG-UDP-($^5\mathbf{S_1}$)-6-deoxy-AltdiNAc	PseG-UDP-($^1\mathbf{C_4}$)-6-deoxy-AltdiNAc
$\langle E_C \rangle$ [a]	−61.51 ± 0.51	−58.12 ± 0.52
$\langle E_{vdw} \rangle$	−75.07 ± 0.33	−72.84 ± 0.27
$\langle \gamma \cdot \Delta MSA \rangle$	−13.84 ± 0.02	−12.93 ± 0.03
$\langle \Delta G^R_{bind} \rangle$	46.99 ± 0.32	56.77 ± 0.32
$\langle SIE \rangle_{PseG\text{-}substrate}$ [b]	−13.72 ± 0.04	−12.02 ± 0.05
$\langle SCE \rangle_{PseG}$ [c]	116.05 ± 0.38	127.28 ± 0.37

[a] <…> denotes the average over 160 snapshots at 0.5 ps intervals from the last 80 ps QM/MM MD trajectory of the complex after solvent and ions were stripped off. The uncertainties are mean standard errors.

[b] $SIE(\rho, D_{in}, \alpha, \gamma, C) = \alpha \cdot [E_C(D_{in}) + \Delta G^R_{bind}(\rho, D_{in}) + E_{vdw} + \gamma \cdot \Delta MSA(\rho)] + C$

represents intermolecular (protein-ligand) solvated interaction energy, where E_C and E_{vdw} are the intermolecular Coulomb and van der Waals interaction energies in the bound state, respectively, calculated using the AMBER molecular mechanics force field with an optimized dielectric constant. The ΔG^R_{bind} is the change in the reaction field energy between the bound and free states, calculated by solving the Poisson equation with the boundary element method program, BRI BEM (1,2) and using a molecular surface generated with a variable-radius solvent probe (3). The free (uncomplexed) state is approximated by rigid separation from the complex. The ΔMSA term is the change in molecular surface area upon binding. The AMBER van der Waals radii linear scaling coefficient (ρ), the solute interior dielectric constant (D_{in}), the molecular surface area coefficient (γ), the global proportionality coefficient relating to the loss of configurational entropy upon binding (α), and a constant (C), are parameters calibrated by fitting

to the absolute binding free energies for a set of 99 protein-ligand complexes (4). The optimized values of these parameters are $\alpha = 0.1048$, $D_{in} = 2.25$, $\rho = 1.1$, $\gamma = 0.0129$ kcal/mol/Å^2, and $C = -2.89$ kcal/mol.

$$^{c}\ \mathrm{SCE}(\rho, D_{in}, \alpha, \gamma) = \alpha \cdot [E_{bond} + E_{angle} + E_{torsion} + E_{improper} + E_{vdW14} + E_{vdW15} + E_{elec14} + E_{elec15} + G^{R}(\rho, D_{in}) + \gamma \cdot \Delta MSA(\rho)]$$

represents the solvated conformational energy of a solute, where, E_{bond}, E_{angle}, $E_{torsion}$, $E_{improper}$, E_{vdW14}, E_{vdW15}, E_{elec14}, and E_{elec15} are intramolecular energy terms calculated with the AMBER force field, $G^R(\rho,D_{in})$ is the reaction field energy calculated with BRI BEM (1,2) using a molecular surface generated with a variable-radius solvent probe (3), and *MSA* is the molecular surface area. The parameters α, ρ, D_{in} and γ take the same values as for the SIE calculation. Here, SCE of the protein (PseG) was calculated for its substrate-bound conformation after rigid extraction from the complex.

References

1. Purisima, E. O. (1998) *J. Comput. Chem.* **19**, 1494-1504.

2. Purisima, E. O., and Nilar, S. H. (1995) *J. Comput. Chem.* **16**, 681-689.

3. Bhat, S., and Purisima, E. O. (2006) *Proteins* **62**, 244-261.

4. Naim, M., Bhat, S., Rankin, K. N., Dennis, S., Chowdhury, S. F., Siddiqi, I., Drabik, P., Sulea, T., Bayly, C. I., Jakalian, A., and Purisima, E. O. (2007) *J. Chem. Inf. Model.* **47**, 122-133.

Supplemental Figure Legends

Fig. S1. SDS-PAGE (12.5%) analyses of *C. jejuni* PseGHis$_6$ derivatives after nickel-nitrilotriacetic acid purification, 1 μg each. *Lane 1*, WT; *lane 2*, H17F; *lane* 3, H17L; *lane* 4, H17N; *lane* 5, Y78F; *lane* 6, N255A. Molecular mass standards are shown on the left in kDa.

Fig. S2. CD spectra for PseGHis$_6$ and variants. (A) 17.1 (WT E155K), 133.1 (WT) and N255A (E155K) as well as (B) 17.1 (WT E155K), H17L (E155K), H17N (E155K), H17F (E155K) and Y78F (E155K) in 25 mM sodium phosphate pH 7.3, 25 mM NaCl. Protein concentrations were 0.098 - 0.104 mg/ml. CD α-helix parameters are shown in Table S2.

Fig. S3. Optimized conformations of the free 6-deoxy-AltdiNAc sugar (O1-methylated) from DFT QM calculations. Assigned ring conformations according to Berces *et al.* (Berces, A., Nukada, T., and Whitfield, D. M. (2001) *Tetrahedron* **57,** 477-491).

Fig. S4. Identification of critical residues in the putative PseG hydrolase family. Multiple sequence alignment of several PseG representatives carried out using MUSCLE (Edgar, R. C. (2004) *BMC Bioinformatics* **5,** 113). The PseG and related sequences used are from *Campylobacter jejuni* (gi15792635), *Helicobacter pylori* (HP0326B), *Aeromonas punctata* (gi5616173), *Aeromonas hydrophila* (gi74273852), *Pseudomonas aeruginosa* (gi20560143 and gi20560095), *Rhizobium* species NGR234 (gi86772969) and *Sinorhizobium meliloti* (gi6523008). The sequence of the structurally related *Escherichia coli* MurG glycosyltransferase is also aligned based on structural superpositions of its substrate-bound crystal structure (PDB code 1NLM, molecule A) onto the UDP-bound *C. jejuni* PseG crystal structure (this work). Invariant residues within the PseG hydrolase family are highlighted on a red background, including those that are conserved in MurG. Observed secondary structure elements (arrows: β-strands; cylinders: α-helices) are indicated above the *C. jejuni* PseG sequence and below the *E. coli* MurG sequence. Residues within 4 Å from the UDP moiety in the two

structures are indicated with green dots, those within 4 Å from the respective donor sugar moieties are marked by blue triangles. These residues are further labeled according to their participation in interactions with the substrate *via* their main-chain (m) side-chain (s), or both (b). Note that residue numbering of *E.coli* MurG differs by 1 in its crystal structure (PDB code 1NLM) relative to the Swiss-Prot entry (P17443), due to an additional methionine at the N-terminus in the crystal structure. In order to provide a streamlined structural comparison, we adopted the crystal structure numbering of *E.coli* MurG throughout this paper.

Fig. S5. Ligand binding in the inter-domain tunnel of PseG. (A) Overall view of PseG represented as a Z-sliced molecular surface (magenta) on the left and as a main-chain ribbon with secondary structure elements (spectrum colored from N-terminus – blue to C-terminus – red) on the right. Close-up views in the tunnel reveal (B) the bound UDP, glycerol and water molecules from the crystal structure, and (C) the UDP-6-deoxy-AltdiNAc substrate from the modeled structure (the 2N acetyl group is cut-out from the view by Z-slicing).

Fig. S6. Analysis of QM/MM MD trajectories for PseG-substrate complexes with the sugar ring in the $^{1}C_{4}$ (red curve) and $^{5}S_{1}$ (black curve) conformations. (A) Sugar ring stability expressed as the τ_5 dihedral angle (C2-C1-O5-C5), (B) distance between the anomeric C1 atom of the sugar ring and the NE atom of His17, (C) partial charge on the anomeric C1 atom, (D) partial charge on the anomeric O_{gly} atom, (E) substrate-induced RMS deviations in the PseG active site region (Q12-H17, K19, R20, Y78, D101, N115, R143, F146, C163-I169, A189, T190, I219, S232-L236, E239, Y252, V253, N255, Q256, T259) from the crystal structure of the PseG-UDP complex, and (F) flexibility of the substrate-complexed PseG expressed as time- and residue-averaged RMS fluctuations, with residues in the active site region (indicated above) highlighted by filled circles.

Fig. S7. Water structure around the PseG-substrate complex calculated from the QM/MM MD simulations. The model with the sugar ring of the substrate in $^{5}S_{1}$ conformation is

shown in (A), and the model in $^1C_4$ conformation in shown in (B). Water occupancy is indicated as density contours represented by solid isosurfaces colored red for water oxygen atoms and white for water hydrogen atoms. Contours are drawn at 25% of the maximal density values for the water O and H atoms. The density plots are calculated on a 0.2 Å spaced Cartesian cubic grid (40 x 40 x 40 Å) centered on the center of mass of the substrate. The calculations were done on the last 120 ps and 90 ps of QM/MM MD simulations for the models corresponding to the $^5S_1$ and $^1C_4$ sugar ring conformations, respectively. The shown coordinates of the solutes (PseG and substrate) are averaged (not energy minimized) over the same simulation window. An arrow indicates the calculated location of the putative nucleophilic water O atom. Select PseG side-chains are shown and labeled (for Ile13 the main-chain carbonyl group is rendered). The viewing orientations are similar to those in Fig. 4*AB* of the main paper. Figure produced using VMD 1.8.5 (Humphrey, W., Dalke, A., and Schulten, K. (1996) *J. Mol. Graphics* **14,** 33-38).

Fig. S8. Kinetic analysis of PseGHis$_6$ and respective substitution derivatives. Michaelis-Menten and Eadie-Hofstee (*insets*) analysis of PseG (A), PseG Y78F (B), PseG H17N (C) and PseG N255A (D) reactions with the substrate UDP-2,4-diacetamido-2,4,6-trideoxy-β-L-altropyranose.

Fig. S9. Structural comparison of PseG-UDP-6-deoxy-AltdiNAc (yellow) and MurG-UDP-GlcNAc (magenta) complexes based on (A) an RMS fit of protein Cα atoms, and (B) an RMS fit of the UDP moiety of the substrates plus Cα atoms from select residues from the core β-sheet of the C-terminal domain of the proteins. The side chains of the putative catalytic residue His17 of PseG and of the corresponding conserved residue in MurG (His19) are highlighted.

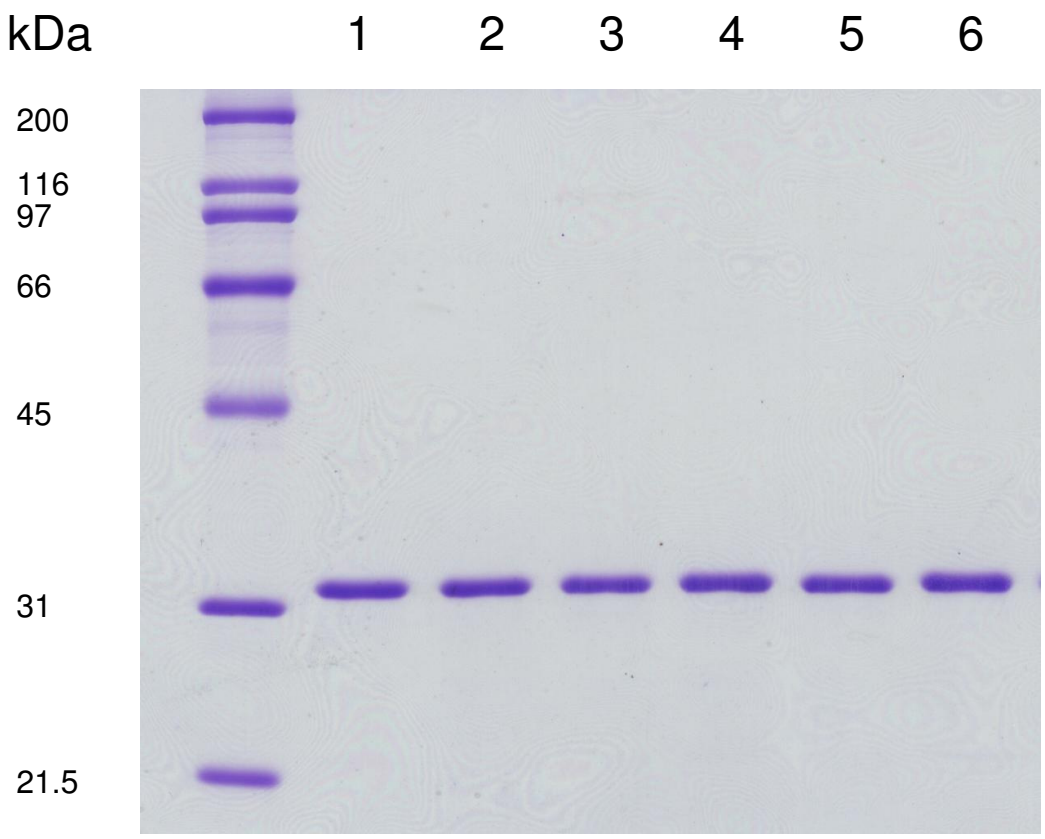
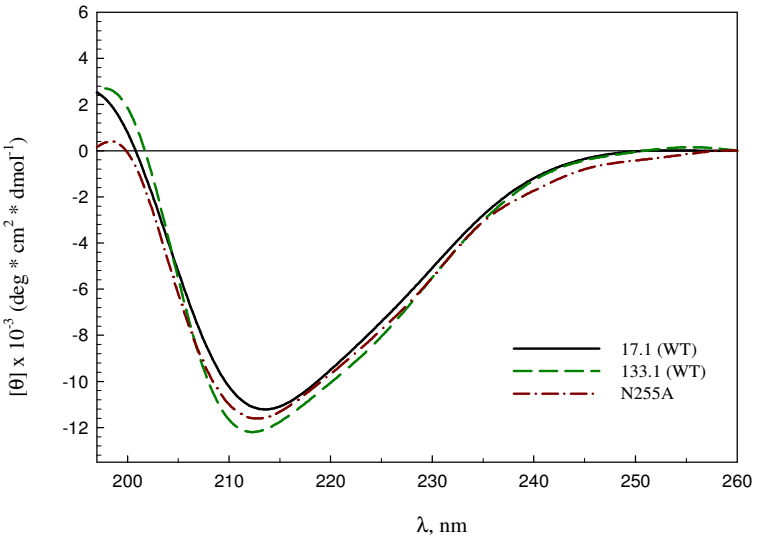


Figure S1

A

CD spectra of WT (17.1 and 133.1) and their mutant analog



B

CD spectra of WT 17.1 and its mutant analogs

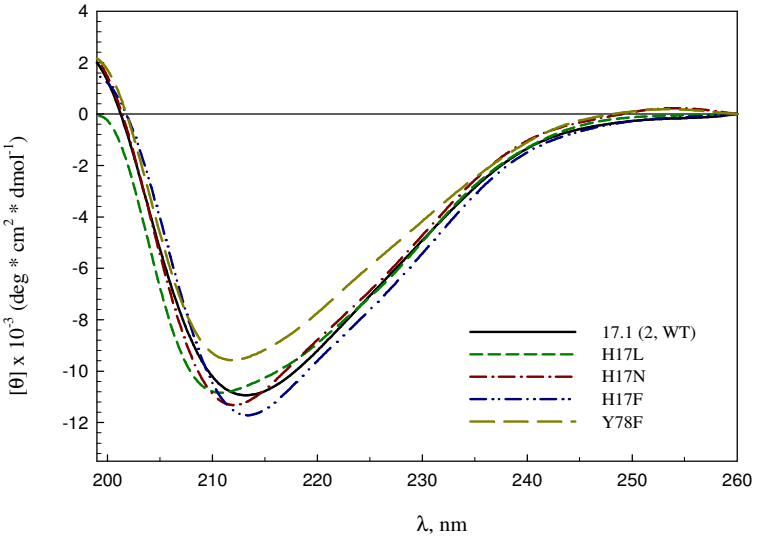


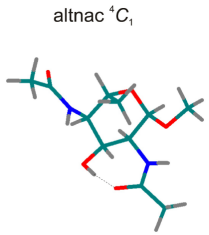
Figure S2

Altnac $^4C_1$ Conformer

Bond Energy LDA -8.80496866 a.u. Zero-Point Energy : 0.310610 a.u.
1 a.u. = 2625.4985 kJ mol^{-1} = 627.5095 kcal mol^{-1}

Ring Torsion Angles τ_1, τ_2, τ_3 etc Where τ_1 = C-1-C-2--C-3-C4, τ_2 = C2-C-3--C-4-C-5 etc.

-48.50 49.80 -51.17 53.97 -52.78 49.29
Ring Descriptors[1]
$^4C_1$ 0.849 $B_{1,4}$ 0.005 $^OS_2$ 0.044



$^4C_1$ 0.849 $B_{1,4}$ 0.005 $^OS_2$ 0.044

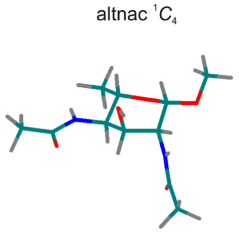
Cartesian Coordinates

Atom	X	Y	Z	(Angstrom)
1 O	0.002366	-0.001222	-0.000915	
2 O	6.782206	0.001557	0.000876	
3 C	2.866857	3.120294	-0.000004	
4 C	2.157558	1.785451	0.146132	
5 C	2.814012	0.884273	1.176397	
6 C	4.315936	0.813284	0.954004	
7 C	4.932218	2.192441	0.812522	
8 C	5.088406	2.889487	2.138859	
9 H	2.207612	1.271206	-0.832836	
10 H	2.632695	1.317122	2.181212	
11 H	4.781478	0.337872	1.839559	
12 O	4.238260	2.980612	-0.162193	
13 H	4.130405	2.993469	2.671677	
14 O	2.486731	3.894635	1.107290	
15 N	0.774273	2.068607	0.444180	
16 O	2.292562	-0.420296	1.078347	
17 N	4.541816	-0.016710	-0.197568	
18 H	5.929110	2.039175	0.369946	
19 H	5.507942	3.895770	1.985294	
20 H	5.792581	2.322156	2.768793	
21 H	2.524920	3.630917	-0.925578	
22 C	2.913640	5.236120	0.993052	
23 C	5.762380	-0.432809	-0.548634	
24 H	3.731196	-0.596705	-0.447605	
25 H	4.015116	5.303813	1.013302	
26 H	2.498170	5.788243	1.846174	
27 H	2.544778	5.683268	0.049984	
28 H	0.591948	3.010752	0.807414	
29 C	-0.220682	1.193736	0.294219	
30 H	1.381832	-0.352630	0.618767	
31 C	-1.603604	1.693325	0.482381	
32 C	5.827036	-1.430648	-1.655074	
33 H	-2.139052	1.608749	-0.476792	
34 H	-1.642316	2.738264	0.820377	
35 H	-2.125998	1.047183	1.203119	
36 H	6.333711	-0.969838	-2.517538	
37 H	4.838903	-1.791974	-1.975442	
38 H	6.445480	-2.280835	-1.331032	

Altnac $^1C_4$ Conformer

Bond Energy LDA -8.79841355 a.u. Zero-Point Energy : 0.309077 a.u.

Ring Torsion Angles τ_1, τ_2, τ_3 etc
51.22 -50.35 55.67 -64.87 67.63 -58.90
Ring Descriptors



$^1C_4$ 0.968 $B_{0,2}$ 0.129 $^1S_5$ 0.028

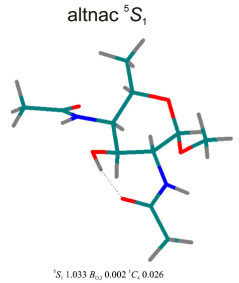
Cartesian Coordinates

Atom	X	Y	Z	(Angstrom)
1.O	0.070018	-0.039373	-0.016208	
2.O	6.213779	-0.028125	0.022546	
3.C	2.095308	3.579243	-0.009673	
4.C	1.639517	2.173432	-0.301310	
5.C	2.671557	1.486981	-1.187159	
6.C	4.061002	1.621352	-0.602331	
7.C	4.367892	3.071688	-0.246846	
8.C	5.687413	3.231252	0.436933	
9.H	0.675711	2.186236	-0.840335	
10.H	2.404731	0.414978	-1.253498	
11.H	4.135891	1.026883	0.326876	
12.O	3.354684	3.543358	0.632669	
13.H	5.699975	2.652050	1.374740	
14.O	1.192095	4.193064	0.824558	
15.N	1.462872	1.456201	0.924271	
16.O	2.710164	2.077680	-2.472877	
17.N	5.001382	1.076687	-1.531094	
18.H	4.338817	3.674730	-1.181684	
19.H	5.878430	4.291670	0.660588	
20.H	6.497141	2.853044	-0.206338	
21.H	2.204226	4.136085	-0.971030	
22.C	1.486537	5.565191	1.011861	
23.C	6.003745	0.256558	-1.160396	
24.H	4.811753	1.248703	-2.522064	
25.H	2.463340	5.696120	1.506603	
26.H	0.692630	5.985411	1.642772	
27.H	1.499920	6.092931	0.039331	
28.H	1.926456	1.815130	1.762095	
29.C	0.659739	0.379733	0.987181	
30.H	1.950791	1.747721	-2.992212	
31.C	0.526645	-0.272144	2.320310	
32.C	6.847299	-0.282421	-2.265552	
33.H	1.114893	0.220753	3.108074	
34.H	0.844098	-1.322584	2.234556	
35.H	-0.535533	-0.276278	2.608849	
36.H	7.891981	0.018996	-2.094490	
37.H	6.531500	0.056313	-3.263193	
38.H	6.817139	-1.382069	-2.228743	

Altnac $^5S_1$ Conformer

Bond Energy LDA -8.80274347 a.u. Zero-Point Energy : 0.309753 a.u.

Ring Torsion Angles τ_1, τ_2, τ_3 etc
-27.84 -30.93 64.31 -32.52 -28.90 61.50
Ring Descriptors
$^5S_1$ 1.033 $B_{0,3}$ 0.002 $^1C_4$ 0.026



$^5S_1$ 1.033 $B_{0,3}$ 0.002 $^1C_4$ 0.026

Cartesian Coordinates

Atom	X	Y	Z	(Angstrom)
1.O	0.290764	-0.136407	-0.454159	
2.O	7.147550	0.772904	-0.573867	
3.C	2.802110	3.178211	0.384314	
4.C	2.090093	1.956874	-0.133606	
5.C	3.087804	0.831148	-0.429526	
6.C	4.450171	1.404800	-0.783658	
7.C	4.289387	2.750036	-1.467066	
8.C	5.584671	3.338114	-1.927545	
9.H	1.618809	2.220988	-1.098647	
10.H	3.187143	0.209020	0.483282	
11.H	5.043374	1.573410	0.132563	
12.O	3.704228	3.684611	-0.551451	
13.H	6.034409	2.699803	-2.703910	
14.O	3.402280	2.800808	1.593076	
15.N	1.066357	1.573265	0.799942	
16.O	2.623700	0.058123	-1.512318	
17.N	5.149178	0.449480	-1.582952	
18.H	3.600273	2.625030	-2.330397	
19.H	6.290131	3.406259	-1.083397	
20.H	5.419423	4.340655	-2.351009	
21.H	2.089173	4.007602	0.586047	
22.C	4.021167	3.892656	2.241692	
23.C	6.448835	0.157096	-1.385254	
24.H	4.555614	-0.153531	-2.161797	
25.H	3.292891	4.708893	2.414193	
26.H	4.860309	4.283598	1.640595	
27.H	4.397307	3.531195	3.207626	
28.H	1.075819	2.020053	1.720170	
29.C	0.226148	0.559548	0.581369	
30.H	1.652420	-0.114852	-1.260960	
31.C	-0.803194	0.296587	1.616689	
32.C	6.997859	-0.953715	-2.215905	
33.H	-1.798277	0.429725	1.163912	
34.H	-0.716281	0.957318	2.490623	
35.H	-0.730064	-0.753533	1.936919	
36.H	7.826803	-0.564573	-2.826761	
37.H	6.249954	-1.417065	-2.875924	
38.H	7.419743	-1.719862	-1.547688	

Figure S3

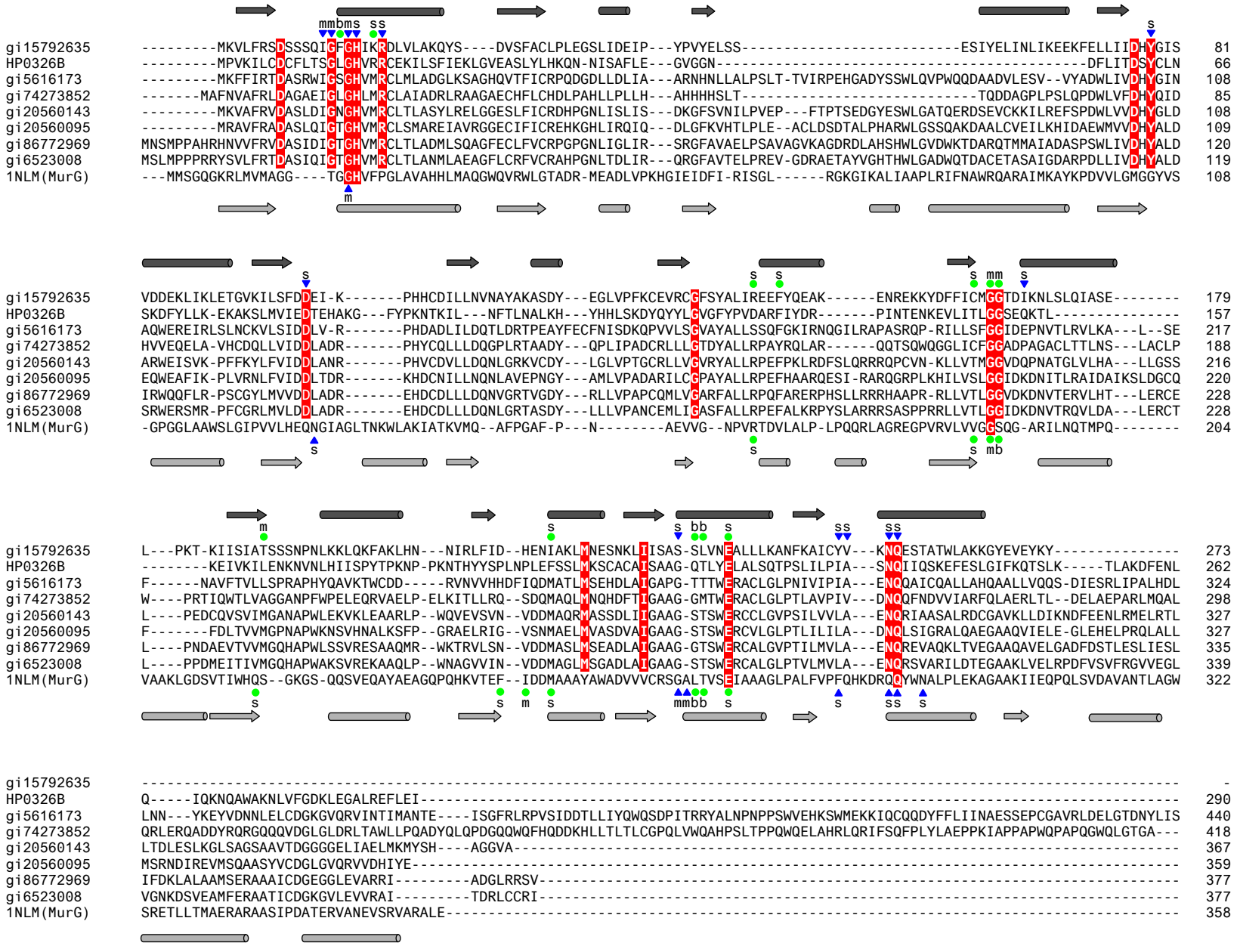

gi15792635 MKVLFRSDSSSQIGFGHIKRDLVLAKQYS----DVSFACLPLEGSLIDEIP---YPVYELSS----------ESIYELINLIKEEKFELLIIDHYGIS 81
HP0326B MPVKILCDCFLTSGLGHVRRCEKILSFIEKLGVEASLYLHKQN-NISAFLE---GVGGN----------DFLITDSYCLN 66
gi5616173 MKFFIRTDASRWIGSGHVMRCLMLADGLKSAGHQVTFICRPQDGDLLDLIA---ARNHNLLALPSLT-TVIRPEHGADYSSWLQVPWQQDAADVLESV--VYADWLIVDHYGIN 108
gi74273852 MAFNVAFRLDAGAEIGLGHLMRCLAIADRLRAAGAECHFLCHDLPAHLLPLLH---AHHHHSLT----------TQDDAGPLPSLQPDWLVFDHYQID 85
gi20560143 MKVAFRVDASLDIGNGHVMRCLTLASYLRELGGESLFICRDHPGNLISLIS---DKGFSVNILPVEP---FTPTSEDGYESWLGATQERDSEVCKKILREFSPDWLVVDHYGLD 108
gi20560095 MRAVFRADASLQIGTGHVMRCLSMAREIAVRGGECIFICREHKGHLIRQIQ---DLGFKVHTLPLE--ACLDSDTALPHARWLGSSQAKDAALCVEILKHIDAEWMVVDHYALD 109
gi86772969 MNSMPPAHRHNVVFRVDASIDIGTGHVMRCLTLADMLSQAGFECLFVCRPGPGNLIGLIR---SRGFAVAELPSAVAGVKAGDRDLAHSHWLGVDWKTDARQTMMAIADASPSWLIVDHYALD 120
gi6523008 MSLMPPPRRYSVLFRTDASIQIGTGHVMRCLTLANMLAEAGFLCRFVCRAHPGNLTDLIR---QRGFAVTELPREV-GDRAETAYVGHTHWLGADWQTDACETASAIGDARPDLLIVDHYALD 119
1NLM(MurG) ---MMSGQGKRLMVMAGG----TGGHVFPGLAVAHHLMAQGWQVRWLGTADR-MEADLVPKHGIEIDFI-RISGL------RGKGIKALIAAPLRIFNAWRQARAIMKAYKPDVVLGMGGYVS 108
gi15792635 VDDEKLIKLETGVKILSFDDEI-K-------PHHCDILLNVNAYAKASDY---EGLVPFKCEVRCGFSYALIREEFYQEAK------ENREKKYDFFICMGGTDIKNLSLQIASE-------- 179
HP0326B SKDFYLLK-EKAKSLMVIEDTEHAKG---FYPKNTKIL---NFTLNALKH---YHHLSKDYQYYLGVGFYPVDARFIYDR-------PINTENKEVLITLGGSEQKTL---------- 157
gi5616173 AQWEREIRLSLNCKVLSIDDLV-R-------PHDADLILDQTLDRTPEAYFECFNISDKQPVVLSGVAYALLSSQFGKIRNQGILRAPASRQP-RILLSFGGIDEPNVTLRVLKA---L--SE 217
gi74273852 HVVEQELA-VHCDQLLVIDDLADR-------PHYCQLLLDQGPLRTAADY---QPLIPADCRLLLGTDYALLRPAYRQLAR------QQTSQWQGGLICFGGADPAGACLTTLNS---LACLP 188
gi20560143 ARWEISVK-PFFKYLFVIDDLANR-------PHVCDVLLDQNLGRKVCDY---LGLVPTGCRLLVGVRYALLRPEFPKLRDFSLQRRRQPCVN-KLLVTMGGVDQPNATGLVLHA---LLGSS 216
gi20560095 EQWEAFIK-PLVRNLFVIDDLTDR-------KHDCNILLNQNLAVEPNGY---AMLVPADARILCGPAYALLRPEFHAARQESI-RARQGRPLKHILVSLGGIDKDNITLRAIDAIKSLDGCQ 220
gi86772969 IRWQQFLR-PSCGYLMVVDDLADR-------EHDCDLLLDQNVGRTVGDY---RLLVPAPCQMLVGARFALLRPQFARERPHSLLRRRHAAPR-RLLVTLGGVDKDNVTERVLHT---LERCE 228
gi6523008 SRWERSMR-PFCGRLMVLDDLADR-------EHDCDLLLDQNLGRTASDY---LLLVPANCEMLIGASFALLRPEFALKRPYSLARRRSASPPRRLLVTLGGIDKDNVTRQVLDA---LERCT 228
1NLM(MurG) -GPGGLAAWSLGIPVVLHEQNGIAGLTNKWLAKIATKVMQ--AFPGAF-P---N--------AEVVG--NPVRTDVLALP-LPQQRLAGREGPVRVLVVGGSQG-ARILNQTMPQ-------- 204
gi15792635 L---PKT-KIISIATSSSNPNLKKLQKFAKLHN---NIRLFID--HENIAKLMNESNKLIISAS-SLVNEALLLKANFKAICYV---KNQESTATWLAKKGYEVEYKY---------- 273
HP0326B --------KEIVKILENKNVNLHIISPYTPKNP-PKNTHYYSPLNPLEFSSLMKSCACAISAAG-QTLYELALSQTPSLILPIA---SNQIIQSKEFESLGIFKQTSLK-----TLAKDFENL 262
gi5616173 F------NAVFTVLLSPRAPHYQAVKTWCDD-----RVNVVHHDFIQDMATLMSEHDLAIGAPG-TTTWERACLGLPNIVIPIA---ENQQAICQALLAHQAALLVQQS-DIESRLIPALHDL 324
gi74273852 W----PRTIQWTLVAGGANPFWPELEQRVAELP-ELKITLLRQ--SDQMAQLMNQHDFTIGAAG-GMTWERACLGLPTLAVPIV---DNQQFNDVVIARFQLAERLTL--DELAEPARLMQAL 298
gi20560143 L----PEDCQVSVIMGANAPWLEKVKLEAARLP--WQVEVSVN--VDDMAQRMASSDLIIGAAG-STSWERCCLGVPSILVVLA---ENQRIAASALRDCGAVKLLDIKNDFEENLRMELRTL 327
gi20560095 F-------FDLTVVMGPNAPWKNSVHNALKSFP--GRAELRIG--VSNMAELMVASDVAIGAAG-STSWERCVLGLPTLILILA---DNQLSIGRALQAEGAAQVIELE-GLEHELPRQLALL 327
gi86772969 L----PNDAEVTVVMGQHAPWLSSVRESAAQMR--WKTRVLSN--VDDMASLMSEADLAIGAAG-GTSWERCALGVPTILMVLA---ENQREVAQKLTVEGAAQAVELGADFDSTLESLIESL 335
gi6523008 L----PPDMEITIVMGQHAPWAKSVREKAAQLP--WNAGVVIN--VDDMAGLMSGADLAIGAAG-STSWERCALGLPTVLMVLA---ENQRSVARILDTEGAAKLVELRPDFVSVFRGVVEGL 339
1NLM(MurG) VAAKLGDSVTIWHQS--GKGS-QQSVEQAYAEAGQPQHKVTEF--IDDMAAAYAWADVVVCRSGALTVSEIAAAGLPALFVPFQHKDRQQYWNALPLEKAGAAKIIEQPQLSVDAVANTLAGW 322
gi15792635 ---------- -
HP0326B Q-----IQKNQAWAKNLVFGDKLEGALREFLEI---------- 290
gi5616173 LNN---YKEYVDNNLELCDGKGVQRVINTIMANTE----ISGFRLRPVSIDDTLLIYQWQSDPITRRYALNPNPPSWVEHKSWMEKKIQCQQDYFFLIINAESSEPCGAVRLDELGTDNYLIS 440
gi74273852 QRLERQADDYRQRGQQQVDGLGLDRLTAWLLPQADYQLQPDGQQWQFHQDDKHLLTLTLCGPQLVWQAHPSLTPPQWQELAHRLQRIFSQFPLYLAEPPKIAPPAPWQPAPQGWQLGTGA--- 418
gi20560143 LTDLESLKGLSAGSAAVTDGGGGELIAELMKMYSH----AGGVA---------- 367
gi20560095 MSRNDIREVMSQAASYVCDGLGVQRVVDHIYE---------- 359
gi86772969 IFDKLALAAMSERAAAICDGEGGLEVARRI---------ADGLRRSV---------- 377
gi6523008 VGNKDSVEAMFERAATICDGKGVLEVVRAI---------TDRLCCRI---------- 377
1NLM(MurG) SRETLLTMAERARAASIPDATERVANEVSRVARALE---------- 358

Figure S4

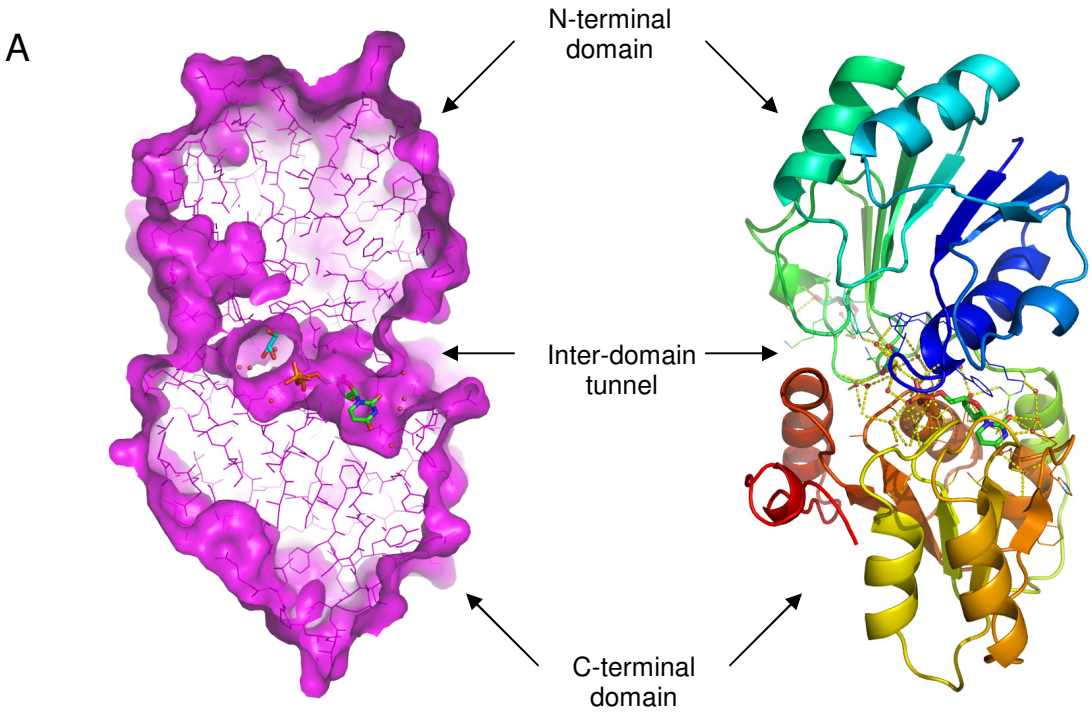

A
N-terminal domain
Inter-domain tunnel
C-terminal domain
B
C

Figure S5

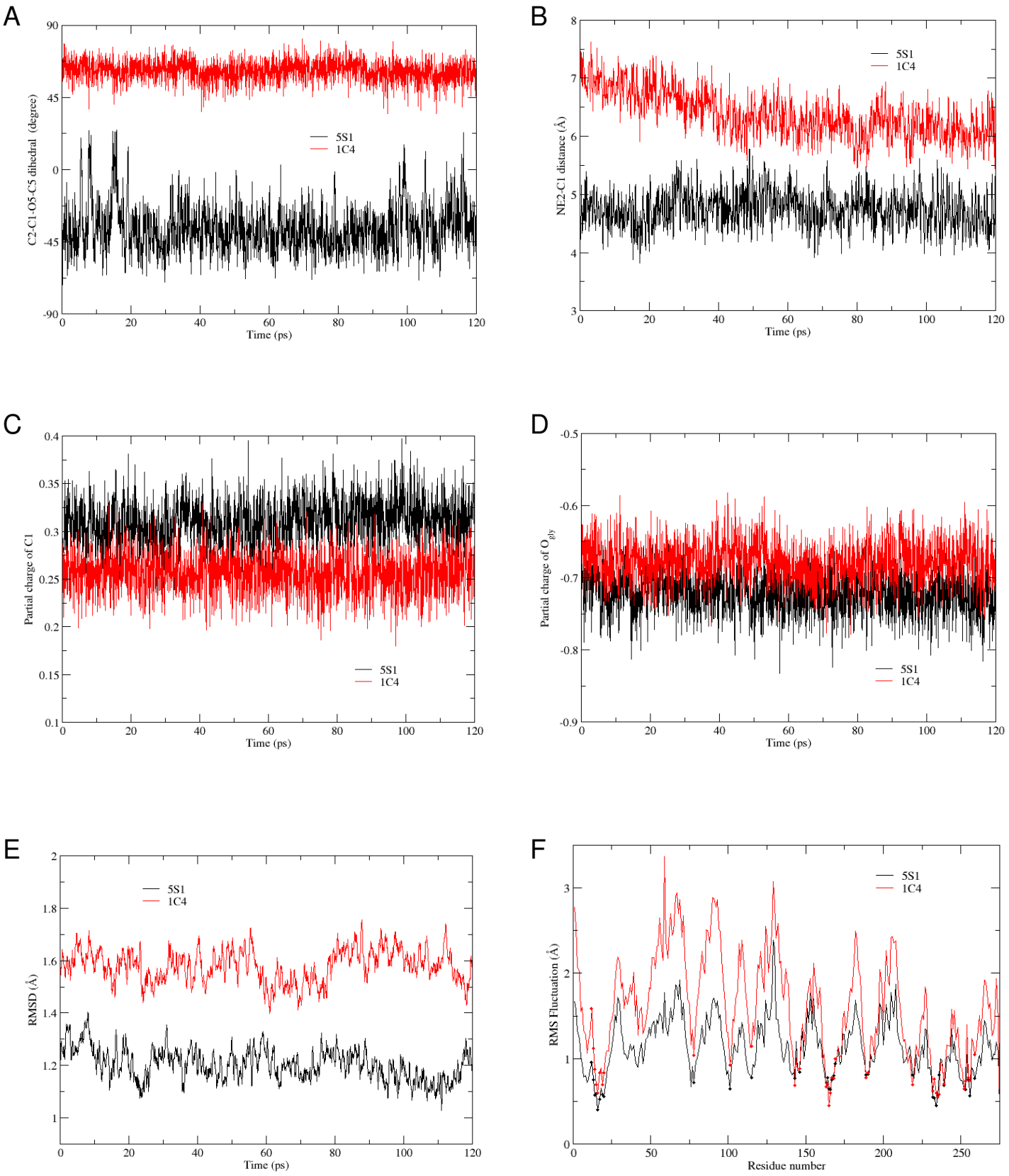

A
C2-C1-O5-C5 dihedral (degree)
Time (ps)
5S1
1C4
B
NE2-C1 distance (Å)
Time (ps)
5S1
1C4
C
Partial charge of C1
Time (ps)
5S1
1C4
D
Partial charge of O$_{gly}$
Time (ps)
5S1
1C4
E
RMSD (Å)
Time (ps)
5S1
1C4
F
RMS Fluctuation (Å)
Residue number
5S1
1C4

Figure S6

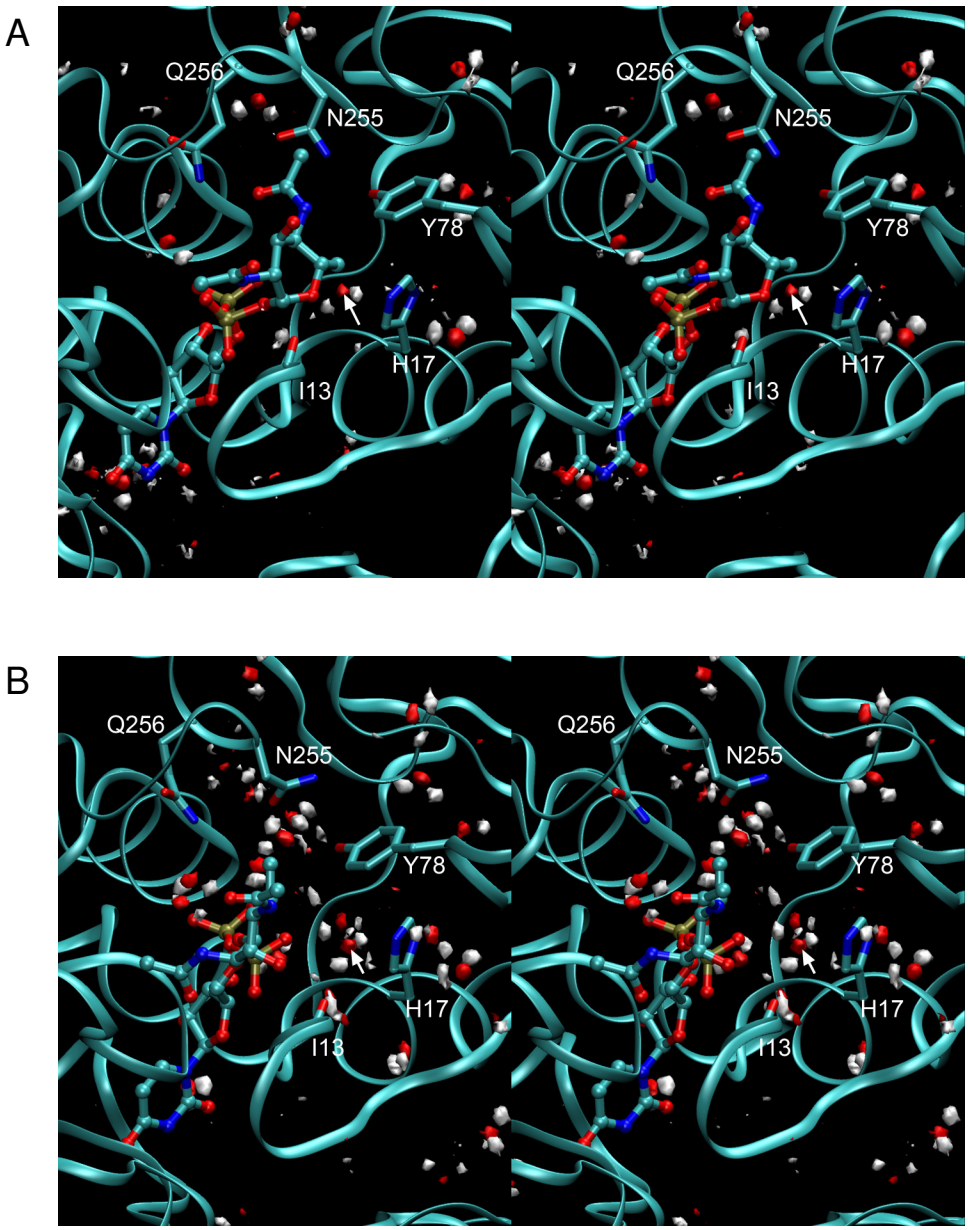

A
Q256
N255
Y78
H17
I13
Q256
N255
Y78
H17
I13
B
Q256
N255
Y78
H17
I13
Q256
N255
Y78
H17
I13

Figure S7

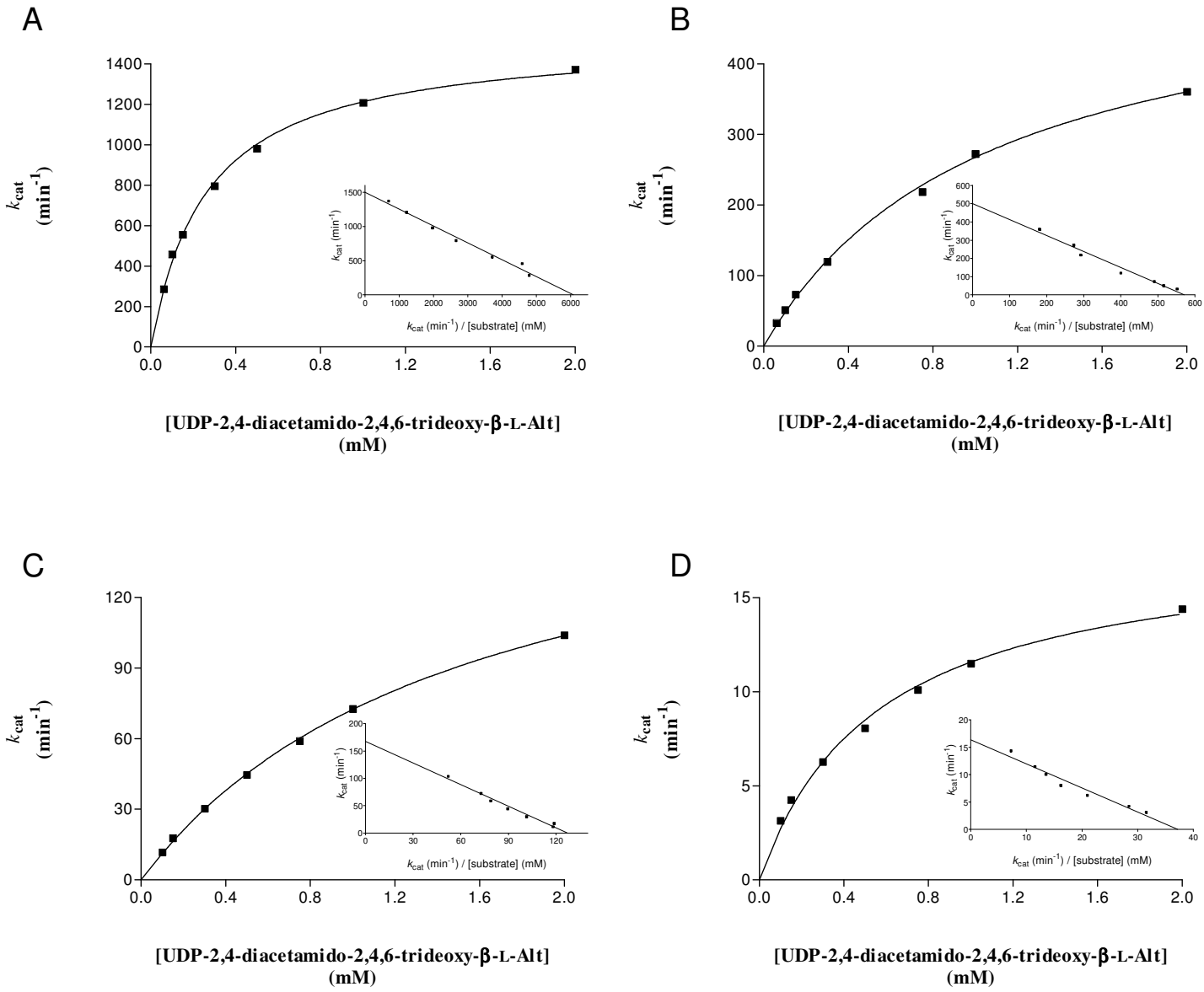

A
B
C
D
k_{cat} (min^{-1})
k_{cat} (min^{-1}) / [substrate] (mM)
[UDP-2,4-diacetamido-2,4,6-trideoxy-β-L-Alt] (mM)

Figure S8

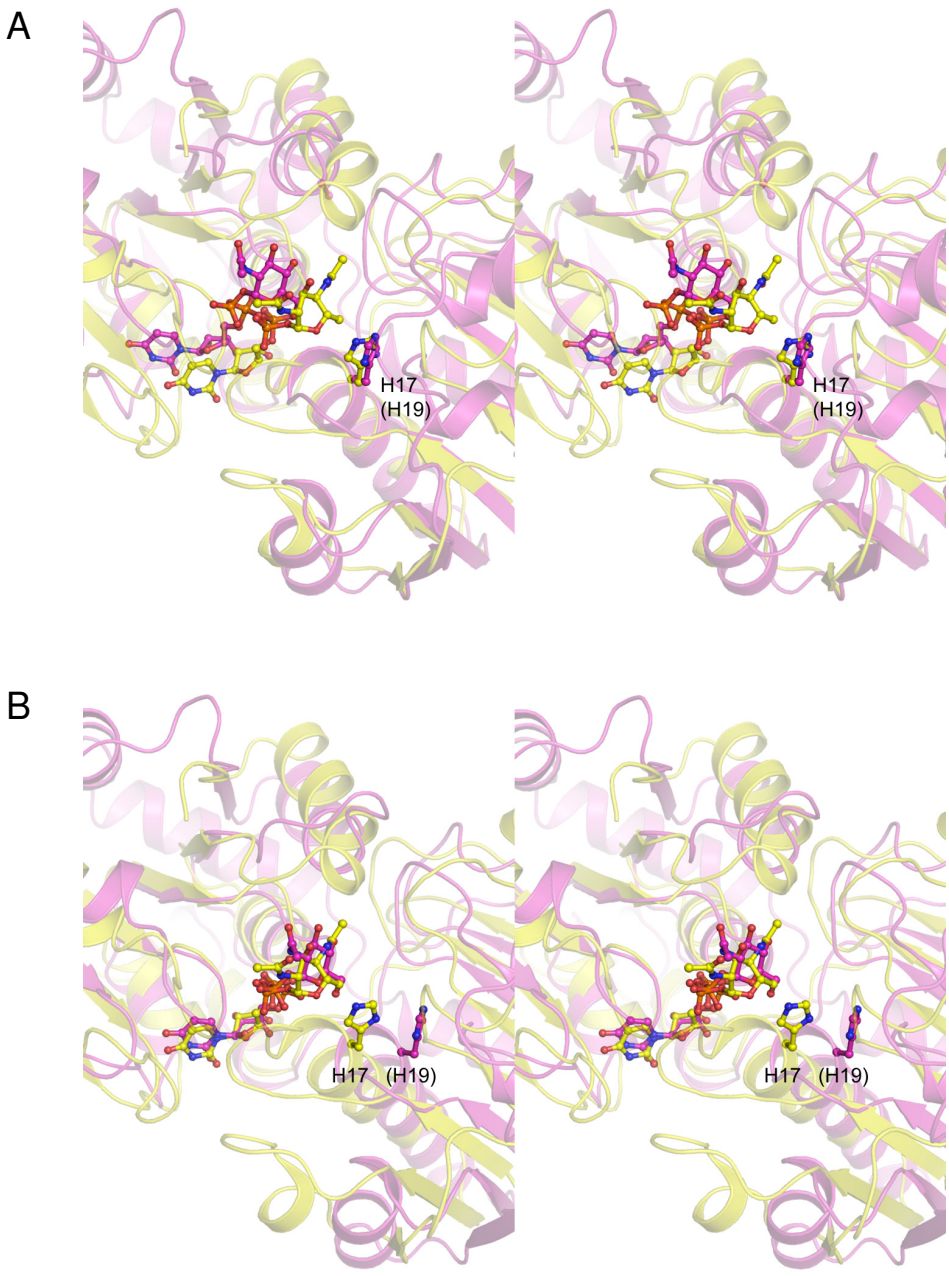


Figure S9