Supporting Information

Molecular Insight into glucose induced conformational change to investigate uncompetitive inhibition of GH1 β-glucosidase

Bharat Manna[1] and Amit Ghosh[1, 2*]

[1]*School of Energy Science and Engineering, Indian Institute of Technology Kharagpur, West Bengal, India-721302*

[2]*P.K. Sinha Centre for Bioenergy and Renewables, Indian Institute of Technology Kharagpur, West Bengal, India-721302*

*Corresponding author's Email: amitghosh@iitkgp.ac.in

Number of Pages: 19

Number of Tables: 6

Number of Figures: 10

Table of Contents

Table S1	Details of the simulated systems	S3
Table S2	Average Root-Mean-Square Deviation values of the β-glucosidase	S4
Table S3	Root mean square fluctuation (RMSF) values of some important active site/ tunnel residues along with the residues involved in different communities	S5
Table S4	Average interaction energies between protein and glucose	S6
Table S5	Fractional occupancy of the glucose molecules with the predicted secondary binding sites of β-glucosidase	S7
Table S6	Distance between selected amino acid residues at the SBS and glucose	S9
Figure S1	Structure of glucose molecule in ball and stick model	S10
Figure S2	Snapshot of the energy minimized simulation boxes with the β-glucosidase H0HC94	S11
Figure S3	Structural deviation of the enzyme in the replicate simulations	S12
Figure S4	Plot of the relative cumulative positional fluctuations showing the contribution of different principal components in the overall motion of the β-glucosidase.	S13
Figure S5	Scatter plot of the first two principal components, i.e., PC1 and PC2 showing change in dynamic motion of the β-glucosidase	S14
Figure S6	Porcupine plot of the dominant modes involved in the collective motion of the β-glucosidase H0HC94 in 0 M Glucose system	S15
Figure S7	Plot of the number of hydrogen bonds between glucose and the β-glucosidase in replicate simulations	S16
Figure S8	Plot of the non-bonded interaction energy profiles between glucose and the β-glucosidase in replicate simulations	S17
Figure S9	Plot of change in distances between glucose and selected amino acid residues at the SBS1 and SBS2 in 0.1 M Glucose system	S18
Figure S10	Representation of the predicted secondary binding site on the β-glucosidase H0HC94.	S19

Table S1. Details of the simulated systems.

Sl. No	System Name	System Property	Box Padding (Å)	Total Simulation Time (ns)
1	S1a	Water, pH 7.2, 325 K	10	200
2	S2a	0.1 M Glucose pH 7.2, 325 K	10	200
3	S3a	0.8 M Glucose pH 7.2, 325 K	10	200
4	S1b	Water, pH 7.2, 325 K	10	200
5	S2b	0.1 M Glucose pH 7.2, 325 K	10	200
6	S3b	0.8 M Glucose pH 7.2, 325 K	10	200

Table S2. Average Root-Mean-Square Deviation values of the β-glucosidase H0HC94 in different systems.

	0 M Glucose		**0.1 M Glucose**		**0.8 M Glucose**	
	S1a	**S1b**	**S2a**	**S2b**	**S3a**	**S3b**
RMSD (Å)	1.14 ± 0.09	1.34 ± 0.15	1.11 ± 0.07	1.18 ± 0.14	1.06 ± 0.10	1.13 ± 0.09

Table S3. Root mean square fluctuation (RMSF) values of some important active site/ tunnel residues along with the residues involved in different communities in β-glucosidase H0HC94.

Position	Residue	0 M Glucose (S1b)	0.1 M Glucose (S2b)	0.8 M Glucose (S3b)
Active site	E171	0.6353	0.6387	0.6408
	E359	0.4088	0.5644	0.4453
Tunnel	H126	0.5961	0.5592	0.7669
	W127	0.5887	0.5426	0.5672
	N170	0.5340	0.8784	0.4571
	C174	0.7489	0.7876	0.5740
	N297	0.4369	0.5333	0.3822
	W405	0.4444	0.4698	0.4069
	W413	0.9471	0.7764	1.1076
Gate Keeper	L178	1.0427	1.2941	0.7213
	H185	1.5462	2.2930	0.8583
	N227	1.2469	1.0359	0.5980
	H229	0.5565	0.5643	0.5554
	Y299	0.4689	0.5225	0.4888
	T300	0.4901	0.5066	0.5223
Other residues involved in the communities	F24	0.5653	0.5053	0.7459
	W40	0.6350	0.6209	0.5542
	F43	0.8467	0.7775	0.7550
	R82	0.4081	0.4370	0.4023
	W173	0.7664	0.7842	0.6602
	W177	0.8085	0.9696	0.6361
	V225	0.4509	0.5206	0.3478
	F250	0.5744	0.5540	0.4971
	H251	0.5752	0.6327	0.4997
	M302	0.6079	0.6106	0.5946
	T358	0.3756	0.4132	0.3752

Table S4. Average interaction energies between protein and glucose in different glucose concentration.

Systems		Electrostatic (kcal/mol)	van der Waals (kcal/mol)	Total Energy (kcal/mol)
0.1 M Glucose	**S2a**	-530.10±114.29	-147.94±30.39	-678.03±130.87
	S2b	-464.97±110.93	-129.83±31.89	-594.81±131.94
0.8 M Glucose	**S3a**	-3000.02±303.56	-760.82±77.68	-3760.83±364.19
	S3b	-3122.21±328.47	-764.21±83.16	-3886.42±391.42

Table S5. Fractional occupancy (cutoff of 0.5) of the glucose molecules with the predicted secondary binding sites of β-glucosidase H0HC94.

0.1 M Glucose (Set 2a)			**0.8 M Glucose (Set 3a)**		
No. of Binding Site	**Residue**	**Fractional occupancy**	**No. of Binding Site**	**Residue**	**Fractional occupancy**
1. **ΔG = -4.06 kcal/mol**	ASN 55 ASP 57 VAL 58 ALA 59 TYR 417 TYR 428	0.57800 0.67000 0.60300 0.57000 0.61900 0.50700	1. **ΔG = -4.51 kcal/mol**	HIS 229 SER 230 VAL 231 PHE 248 GLN 249 ASP 257 LYS 261 PRO 265 ALA 266 SER 341 LEU 342 THR 345 ARG 349 TYR 350	0.92300 1.00000 0.99900 0.99200 0.79400 0.98400 0.81600 0.75300 0.67800 0.99600 0.99600 0.98700 0.85700 0.55200
2. **ΔG = -4.50 kcal/mol**	GLN 25 TYR 299 TRP 332 TRP 405 SER 406 ASN 410 GLU 412 TRP 413 PHE 421	0.98100 0.98800 0.92400 0.99300 0.92300 0.98200 0.98000 0.98700 0.98100	2. **ΔG = -3.05 kcal/mol**	ASP 3 HIS 4 ALA 6 LEU 7 ARG 10 GLN 376 LEU 379 ASP 380 ALA 383 GLU 384 GLY 387 ILE 388 ASP 391 TRP 441	0.98200 0.93000 0.69100 0.88700 0.98900 0.80600 0.96700 0.58000 0.87300 0.69700 0.89000 0.68800 0.99400 0.53100
			3. **ΔG =-3.82 kcal/mol**	SER 324 ASP 325 VAL 326 VAL 334 TYR 335 ALA 336	0.79500 0.80400 0.73700 0.78200 0.77500 0.77700

				PRO 337 ALA 338	0.77000 0.94300
			4. **ΔG =-3.91 kcal/mol**	ARG 142 PRO 277 VAL 278 VAL 279 GLU 280 GLU 283	0.59300 0.62200 0.64000 0.64000 0.61000 0.60600
			5. **ΔG =-4.18 kcal/mol**	GLY 28 ALA 29 THR 30 ASP 57 VAL 58 ASP 61 ASN 64	0.56400 0.60200 0.56200 0.72500 0.51400 0.99600 0.93800
			6. **ΔG =-4.50 kcal/mol**	GLU 313 ALA 316 THR 317	0.54100 0.53300 0.51300

Table S6. Distance between selected amino acid residues at the SBS and glucose. The average distances are calculated after the freely moving glucose binds and remains trapped to the SBS.

SBS	0.1 M Glucose (Set 2a)		0.8 M Glucose (Set 3a)	
1	ASP 57	3.48±0.23 Å	PHE 248	3.54±0.39 Å
2	TRP 405	3.45±0.32 Å	ARG10	3.49±0.32 Å
3			ASP 325	3.73±0.24 Å
4			VAL279	3.68±0.55 Å
5			ASP 61	2.69±0.25 Å
6			GLU313	2.86±0.40 Å

Figure S1. Structure of glucose molecule in ball and stick model. Carbon, oxygen, and hydrogen are colored in cyan, red, and white respectively.

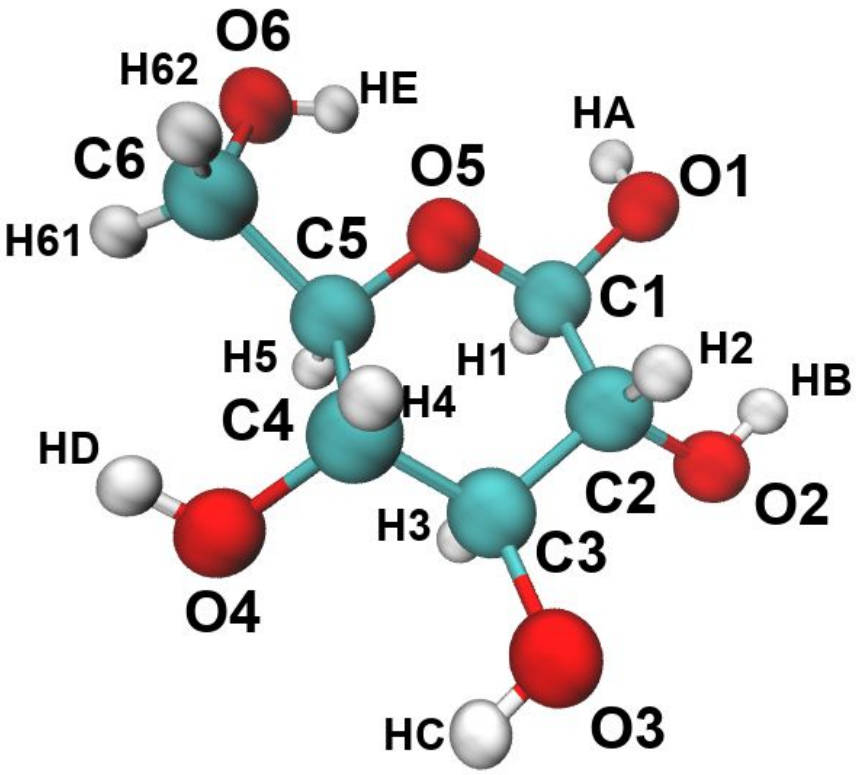

Figure S2. Snapshot of the energy minimized simulation boxes with the β-glucosidase H0HC94 showing the glucose crowding in (a) 0 M, (b) 0.1 M and (c) 0.8 M Glucose. The protein, water and glucose molecules are shown in cartoon, line and van der Waals sphere representation, respectively.

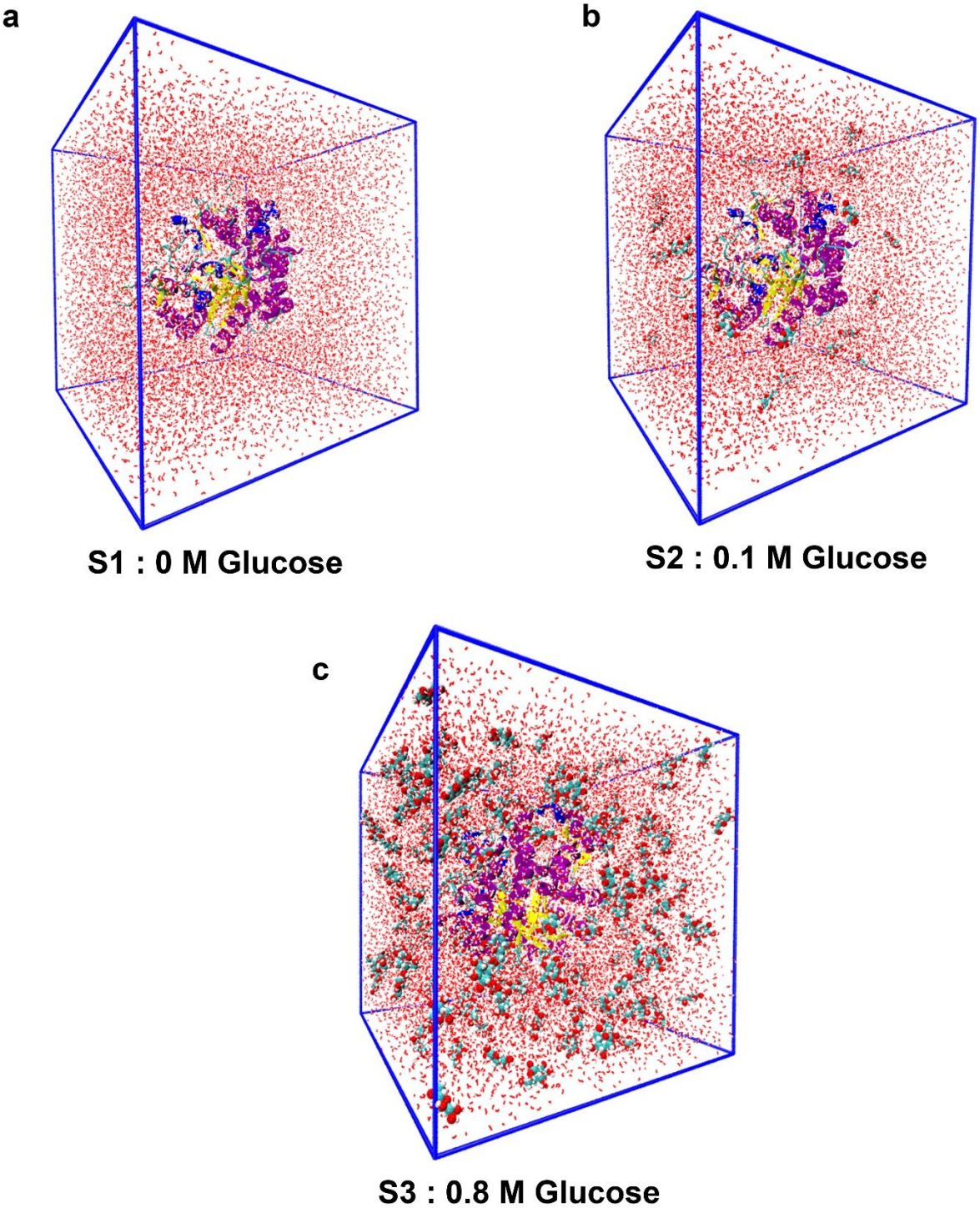

Figure S3. Structural deviation of the enzyme in the replicate simulations. (A) root mean square deviation and (B) root mean square fluctuation of the β-glucosidase H0HC94 for MD trajectories in 0 M Glucose (black), 0.1 M Glucose (red), and 0.8 M Glucose (green) systems.

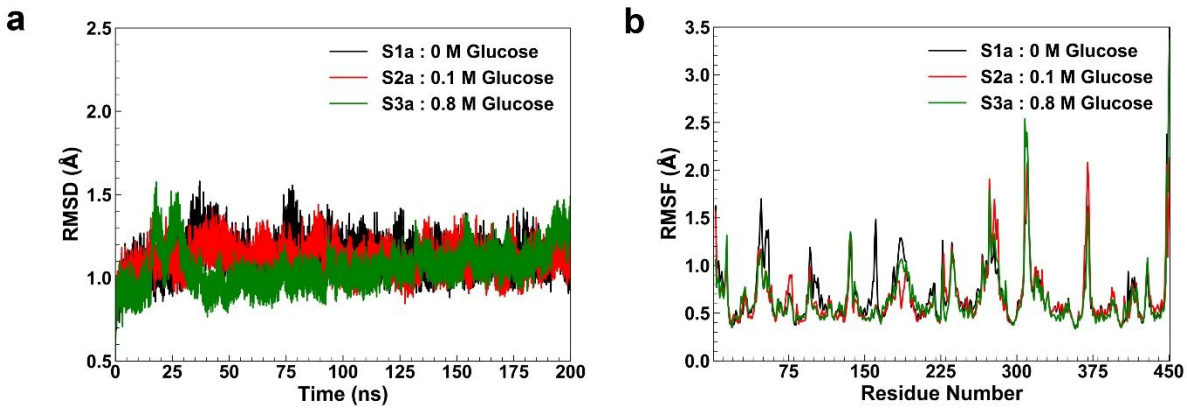

Figure S4. Plot of the relative cumulative positional fluctuations showing the contribution of different principal components in the overall motion of the β-glucosidase H0HC94 in S1a: 0 M Glucose (black), S2a: 0.1 M Glucose (red), and S3a: 0.8 M Glucose (green) systems.

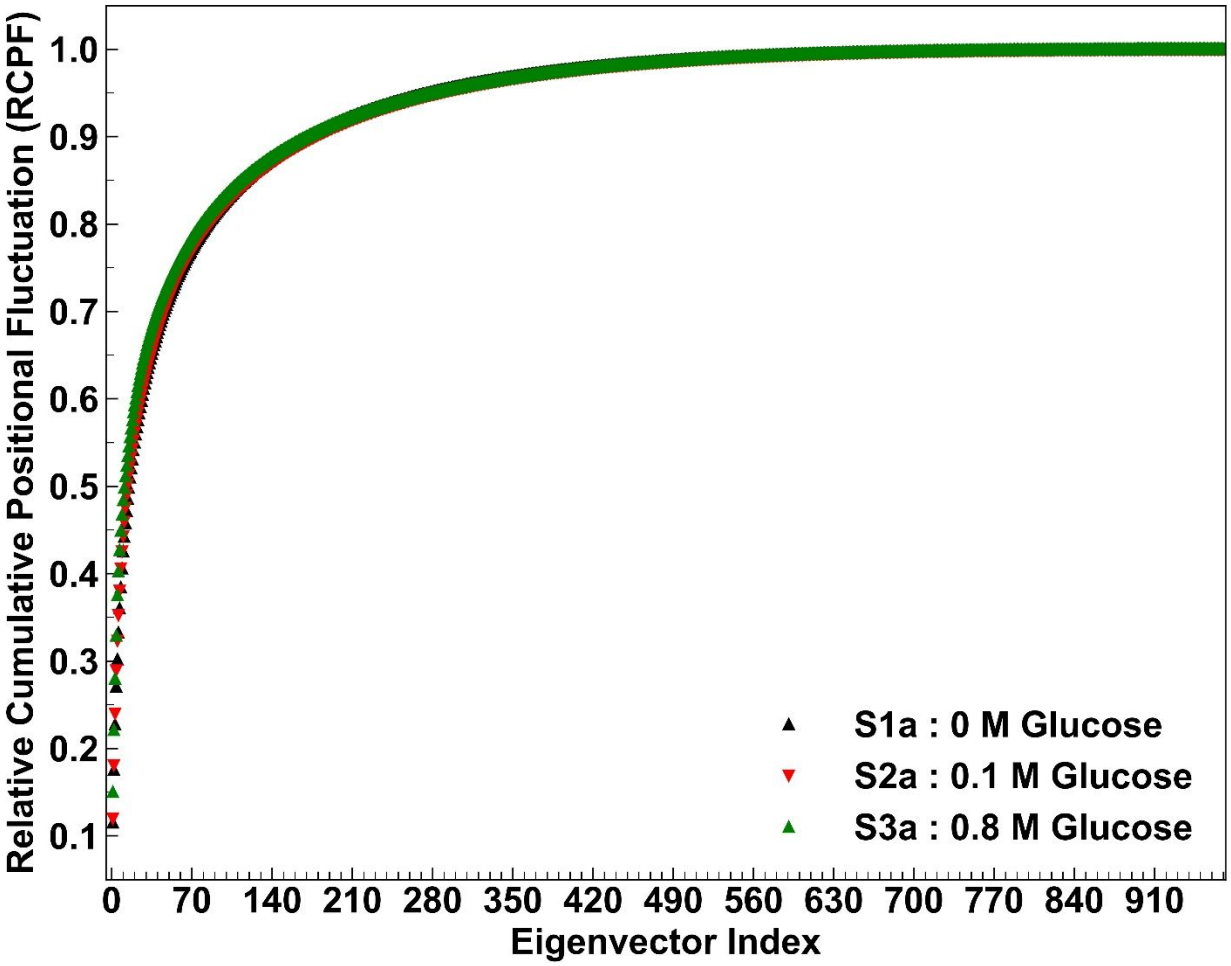

Figure S5. Scatter plot of the first two principal components, *i.e.,* PC1 and PC2 showing change in dynamic motion of the β-glucosidase H0HC94 across the replicate simulations (a) S1b: 0 M Glucose (black), (b) S2b: 0.1 M Glucose (red), and (c) S3b: 0.8 M Glucose (green) systems.

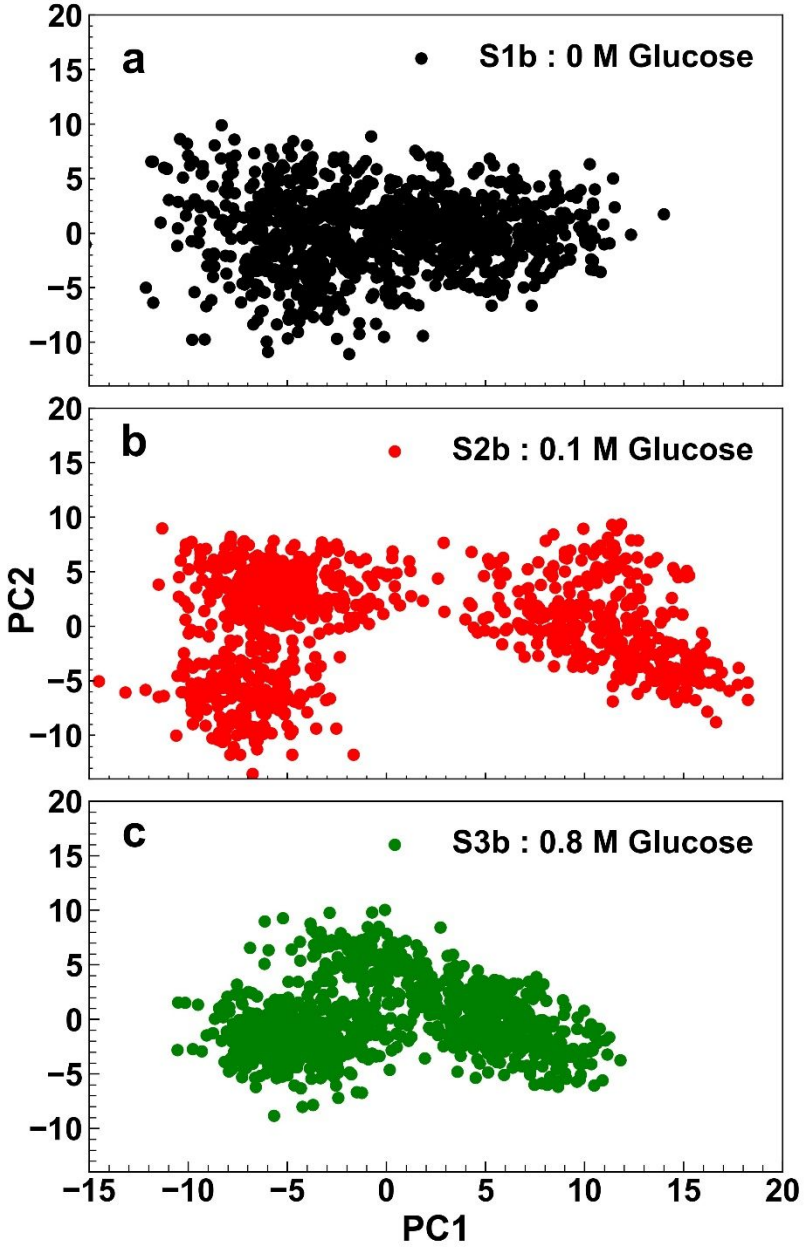

Figure S6. Porcupine plot of the dominant modes (a) PC1 and (b) PC2 involved in the collective motion of the β-glucosidase H0HC94 in 0 M Glucose (S1a) system. The A, B, C and D loops are colored in blue, yellow, cyan and green, respectively. The two active site catalytic residues are shown in violet.

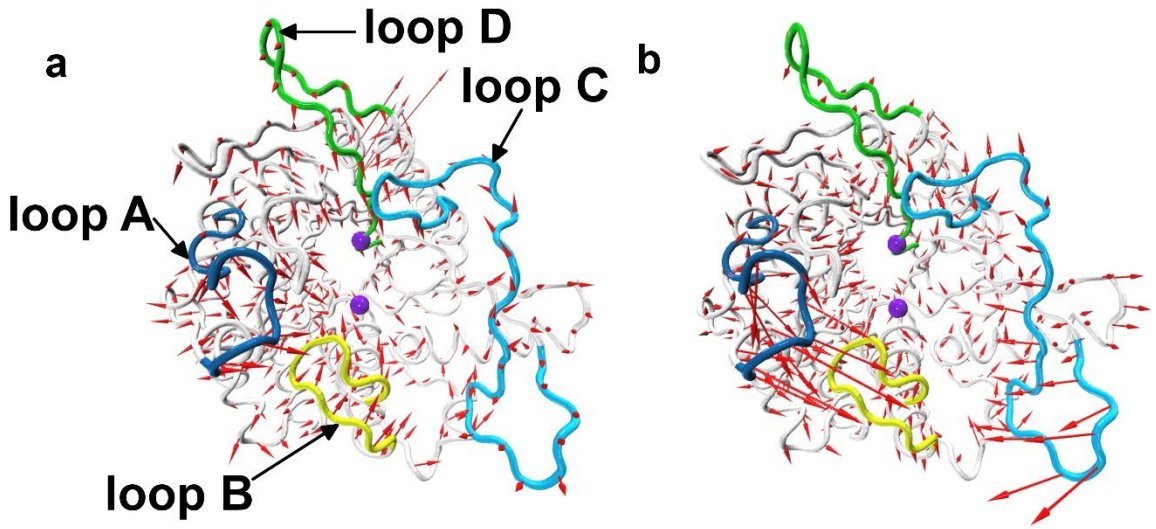

Figure S7. Plot of the number of hydrogen bonds between glucose and the β-glucosidase H0HC94 in the simulation trajectories of replicate simulations S2b: 0.1 M Glucose (red), and S3b: 0.8 M Glucose (green) systems.

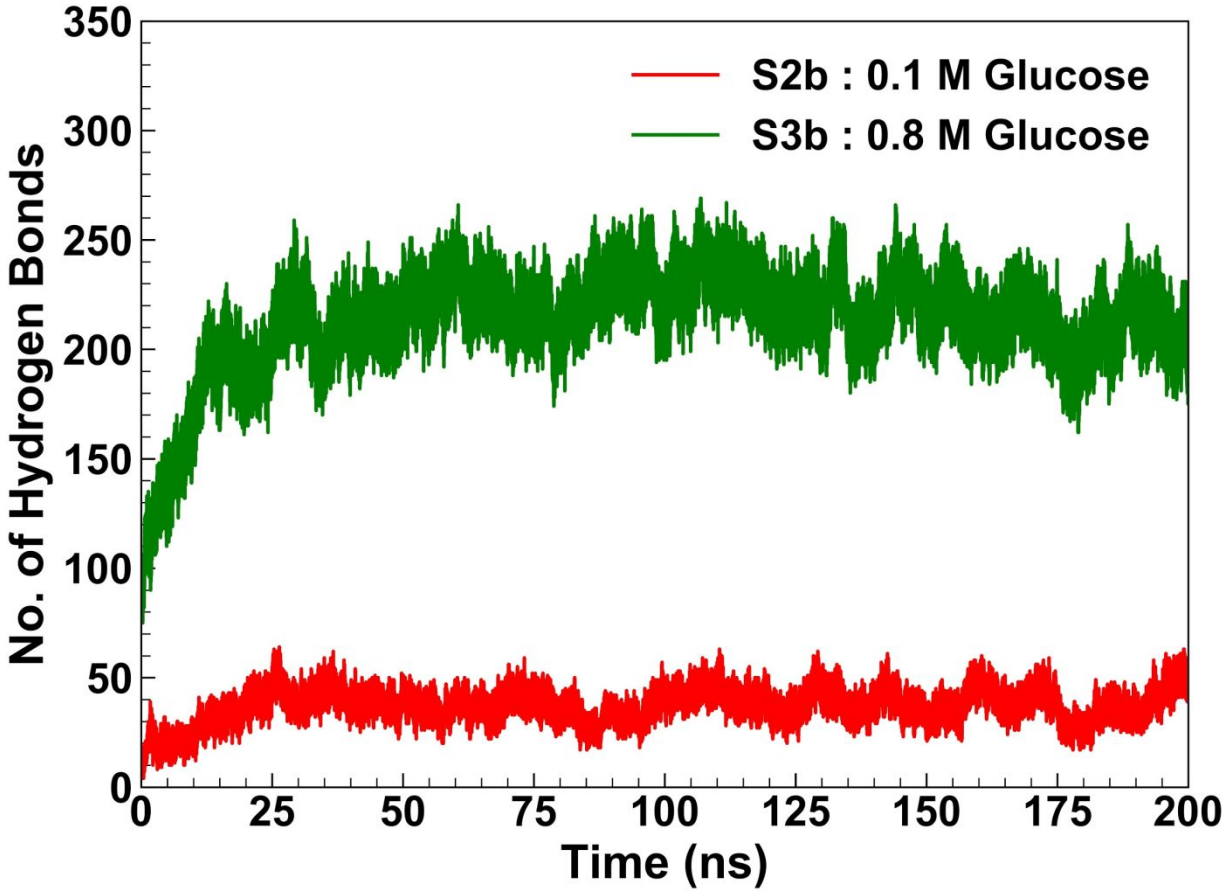

Figure S8. Plot of the non-bonded interaction energy profiles between glucose and the β-glucosidase H0HC94 in the simulation trajectories of replicate simulations S2b: 0.1 M Glucose (red), and S3b: 0.8 M Glucose (green) systems.

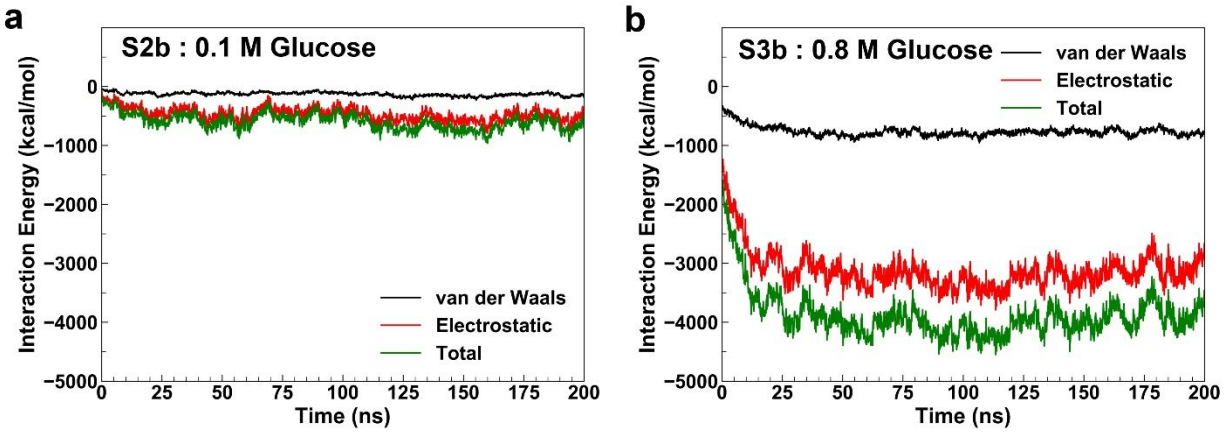

Figure S9. Plot of change in distances between glucose and selected amino acid residues at the (a) SBS1 and (b) SBS2 in S2a: 0.1 M Glucose system.

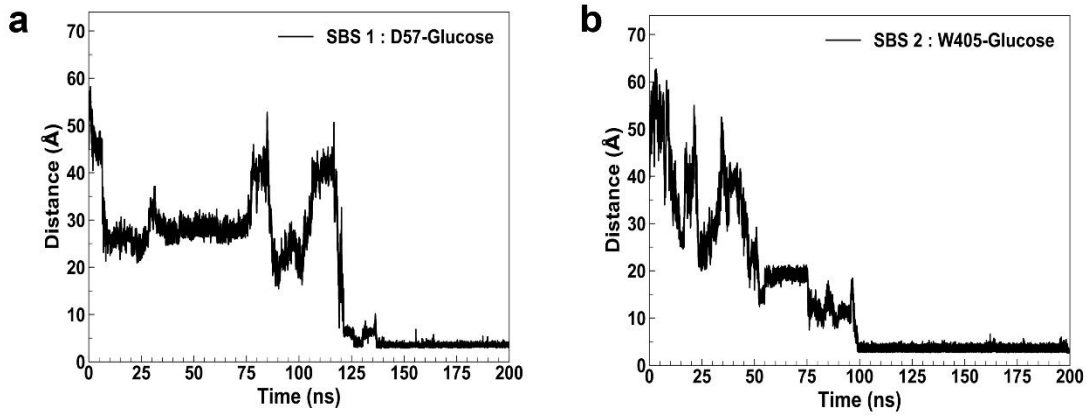

Figure S10. Representation of the predicted secondary binding site on the β-glucosidase H0HC94. (a) SBS2 shows the glucose binding interactions in S2a: 0.1 M Glucose, whereas (b) SBS2, (c) SBS3, (d) SBS4, (e) SBS5, (f) SBS6 belong to the S3a: 0.8 M Glucose systems. The glucose molecule is represented in ball and stick model whereas the amino acid residues are shown in licorice. Carbon, oxygen, nitrogen and hydrogen are colored in grey, red, blue and white respectively.