Supporting Information

Substrate Binding in the Allosteric Site Mimics Homotropic Cooperativity in the SIS-fold Glucosamine-6-phosphate Deaminases

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Figures

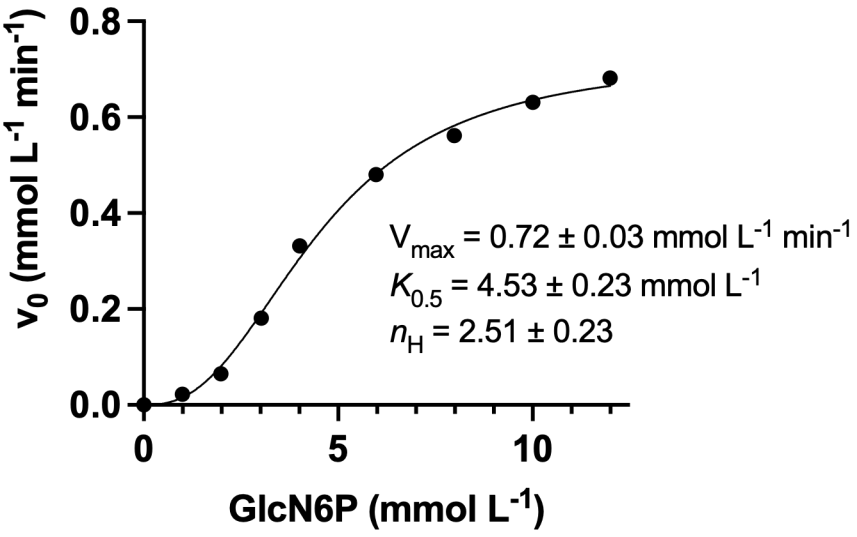

Fig. S1. Kinetics of SoNagBII in absence of GlcNAc6P at 30 ºC and pH 7.5. Data was fitted to Hill equation (Eq. 1). These data confirm the data previously reported by Yang et al. 2006 for the same enzyme. Uncertainties are the standard errors of the fitted parameters.

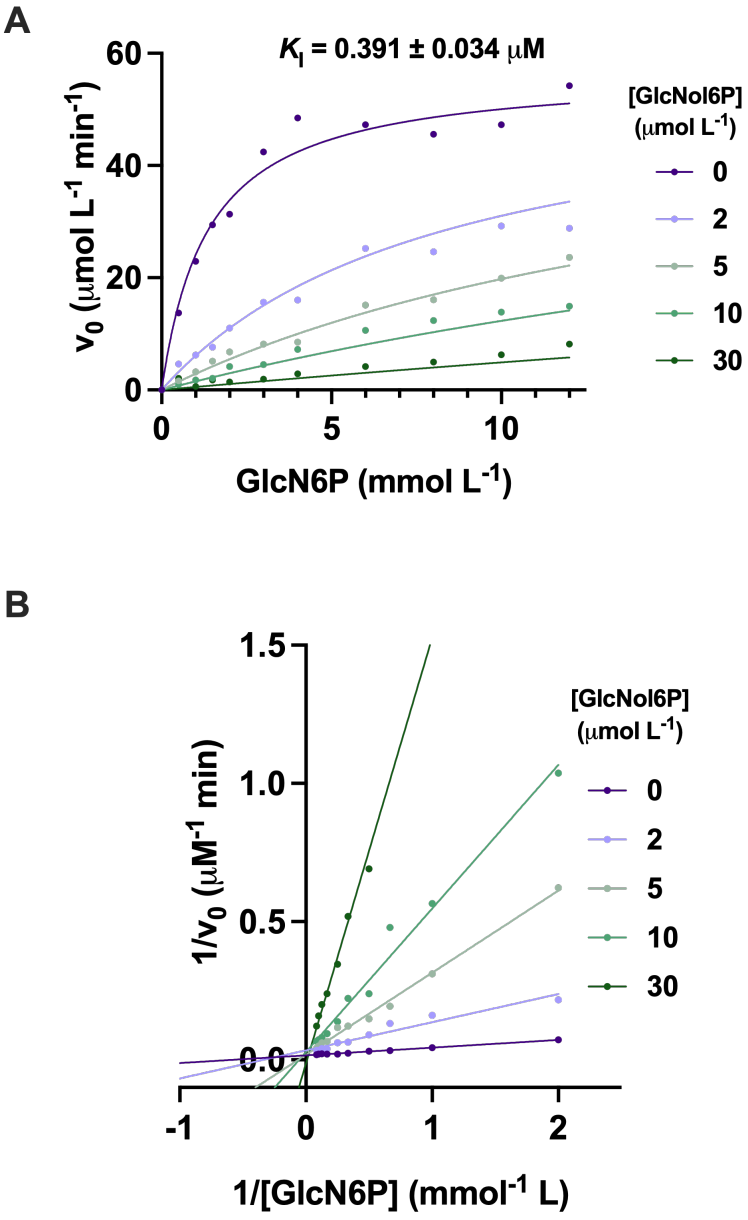

Fig. S2. Global fitting of the GlcNAc6P-saturated SdNagBII kinetic data (Fig 1 B) to the classic competitive inhibition equation with GraphPad Prism 5.0. A) Non-linear regression fitting; V_{max} = 56.86 ± 1.52 μM min^{-1}, K_m = 1.36 ± 0.14 mM, and K_I = 0.391 ± 0.034 μM. Uncertainties are the standard errors of the fitted parameters. B) Double reciprocal plot of data in panel A.

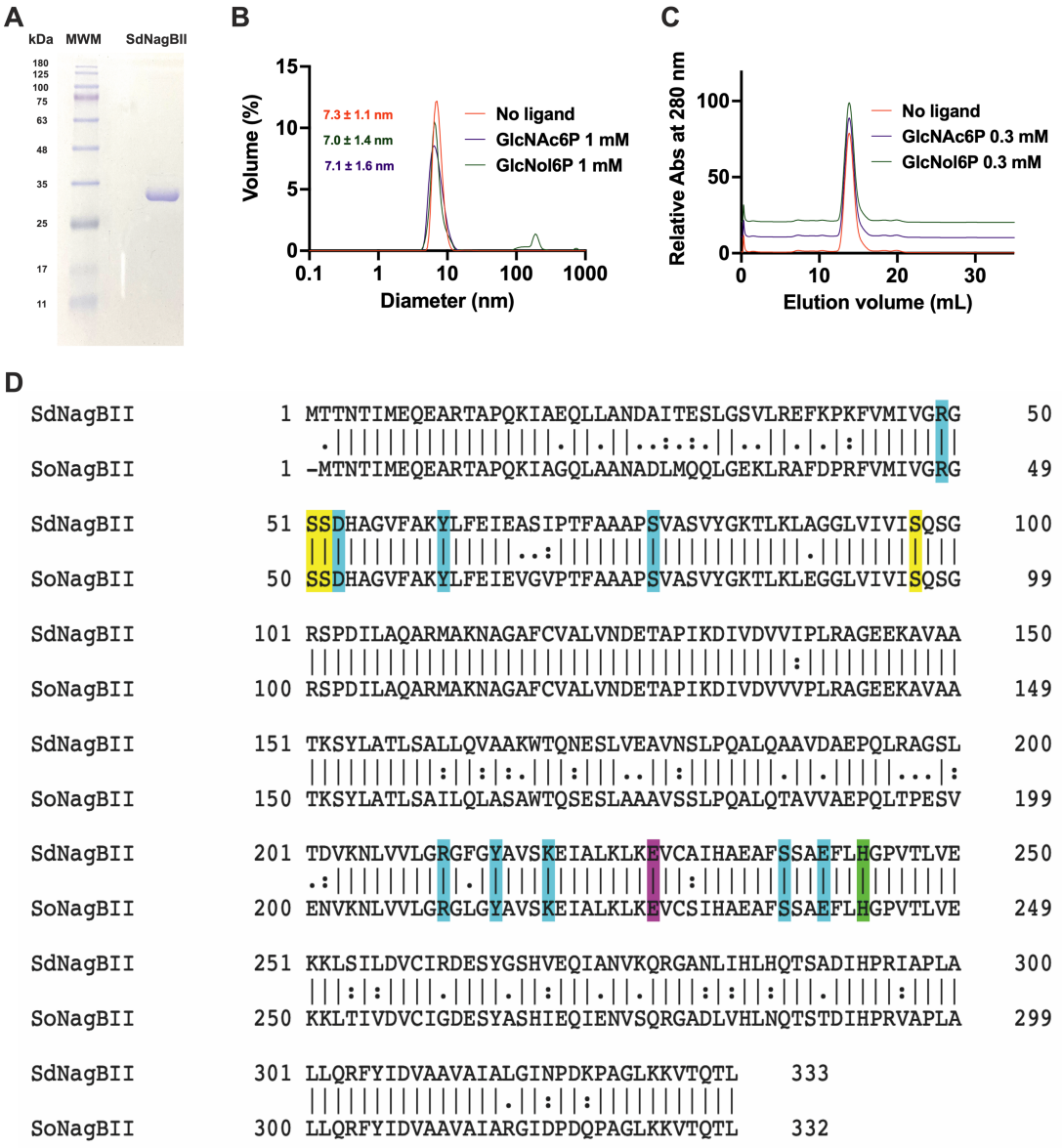

Fig. S3. A) SDS-PAGE analysis of the purified SdNagBII. B) DLS analysis of the hydrodynamic diameter of SdNagBII 0.2 mg/mL without ligands (orange), with 1 mM GlcNol6P (green), and with 1 mM GlcNAc6P (purple). The analysis was carried out in Tris-HCl 100 mM, pH 8.4, 300 mM NaCl, 1 mM DTT at 30 ºC. The diameter of the protein species is congruent with the diameter of the dimer measured in the crystallographic structures. C) SEC analysis of the hydrodynamic diameter of SdNagBII without ligands (orange), with 0.3 mM GlcNol6P (green), and with 0.3 mM GlcNAc6P (purple). D) Alignment of the protein sequences of the NagBII from *S. denitrificans* (GenBank ABE55984) and *S. oendensis* (GenBank AAN56497). Residues involved in active and allosteric site binding are shown in yellow and cyan respectively; H243, catalytic His, and E227, residue responsible for enolization of substrate, are shown in green and magenta respectively. Mutating His242Gln in SoNagBII reduced k_{cat} from 6.02 ± 0.25 s^{-1} to 0.005 ± 0.001 s^{-1}.

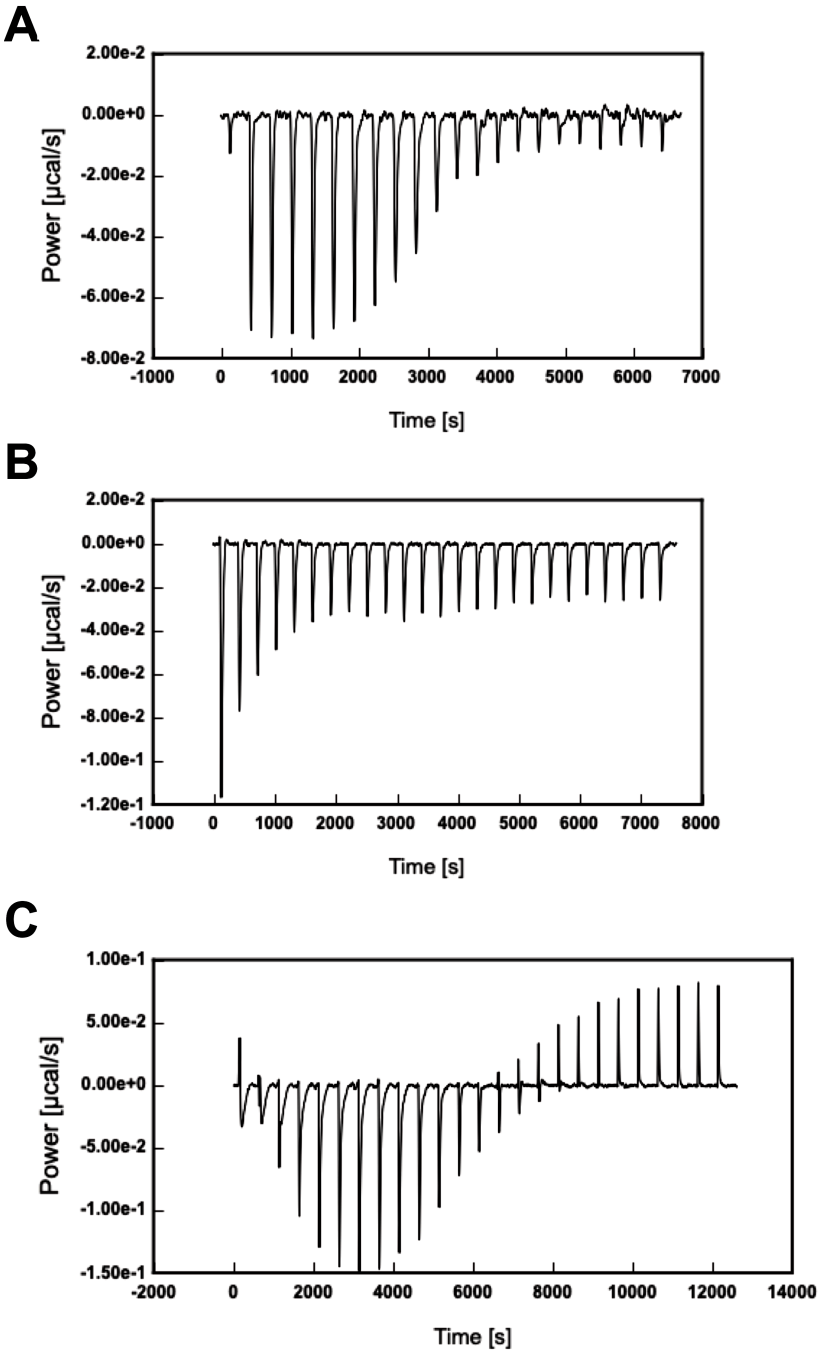

Fig. S4. Representative baseline-corrected ITC thermograms of samples shown in Figure 1 D-F. A) titration of SdNagBII with GlcNAc6P; B) titration of the GlcNAc6P-saturated SdNagBII with GlcNol6P; C) titration of SdNagBII with GlcNol6P.

Tables

Table S1. List of protein-GlcNol6P interactions at the novel allosteric site (1).

PDB code: 8FDB chain A

Hydrogen bonds

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	62	OG	SER	237	B	---	12	O3P	AGP	601	A	2.836
2	56	NH2	ARG	211	B	---	10	O2P	AGP	601	A	3.168
3	34	OH	TYR	61	B	---	9	O1P	AGP	601	A	2.933
4	22	OG	SER	77	A	---	9	O1P	AGP	601	A	2.582
5	17	N	SER	77	A	---	10	O2P	AGP	601	A	3.041
6	45	NH2	ARG	49	A	---	10	O2P	AGP	601	A	2.709

Non-bonded contacts

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	85	CZ	TYR	215	B	---	13	O4	AGP	601	A	3.284
2	84	CE2	TYR	215	B	---	13	O4	AGP	601	A	3.499
3	61	CB	SER	237	B	---	12	O3P	AGP	601	A	3.479
4	52	CD	ARG	211	B	---	12	O3P	AGP	601	A	3.879
5	101	CD	GLU	240	B	---	11	O3	AGP	601	A	3.859
6	100	CG	GLU	240	B	---	11	O3	AGP	601	A	3.797
7	54	CZ	ARG	211	B	---	10	O2P	AGP	601	A	3.730
8	33	CZ	TYR	61	B	---	9	O1P	AGP	601	A	3.798
9	31	CE1	TYR	61	B	---	9	O1P	AGP	601	A	3.745
10	34	OH	TYR	61	B	---	6	C6	AGP	601	A	3.704
11	86	OH	TYR	215	B	---	1	C1	AGP	601	A	3.397
12	16	P	AGP	601	A	---	21	CB	SER	77	A	3.589
13	10	O2P	AGP	601	A	---	21	CB	SER	77	A	3.186
14	9	O1P	AGP	601	A	---	21	CB	SER	77	A	3.276
15	10	O2P	AGP	601	A	---	18	CA	SER	77	A	3.597
16	9	O1P	AGP	601	A	---	18	CA	SER	77	A	3.878
17	15	O6	AGP	601	A	---	69	CD	PRO	76	A	3.470
18	14	O5	AGP	601	A	---	69	CD	PRO	76	A	3.344
19	9	O1P	AGP	601	A	---	69	CD	PRO	76	A	3.786
20	10	O2P	AGP	601	A	---	67	CB	PRO	76	A	3.748
21	9	O1P	AGP	601	A	---	74	CB	ALA	75	A	3.456
22	9	O1P	AGP	601	A	---	72	C	ALA	75	A	3.614
23	9	O1P	AGP	601	A	---	71	CA	ALA	75	A	3.658
24	3	C3	AGP	601	A	---	94	OD2	ASP	53	A	3.608
25	2	C2	AGP	601	A	---	93	OD1	ASP	53	A	3.621
26	5	C5	AGP	601	A	---	44	NH1	ARG	49	A	3.859
27	15	O6	AGP	601	A	---	43	CZ	ARG	49	A	3.860
28	10	O2P	AGP	601	A	---	43	CZ	ARG	49	A	3.886

Table S1. Continuation.

PDB code: 8FDB chain B

Hydrogen bonds

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	86	N	GLY	212	A	---	46	MG	MG	2	C	3.191
2	46	MG	MG	2	C	---	28	OH	TYR	61	A	2.898
3	46	MG	MG	2	C	---	12	O3P	AGP	401	B	2.544
4	85	NZ	LYS	219	B	---	8	O1	AGP	401	B	3.030
5	34	OG	SER	77	B	---	9	O1P	AGP	401	B	2.537
6	29	N	SER	77	B	---	10	O2P	AGP	401	B	2.921
7	69	NH2	ARG	49	B	---	10	O2P	AGP	401	B	2.780
8	58	OH	TYR	215	A	---	13	O4	AGP	401	B	2.929
9	45	NH2	ARG	211	A	---	10	O2P	AGP	401	B	3.057
10	44	NH1	ARG	211	A	---	10	O2P	AGP	401	B	2.822
11	28	OH	TYR	61	A	---	9	O1P	AGP	401	B	2.708

Non-bonded contacts

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	16	P	AGP	401	B	---	33	CB	SER	77	B	3.758
2	10	O2P	AGP	401	B	---	33	CB	SER	77	B	3.389
3	9	O1P	AGP	401	B	---	33	CB	SER	77	B	3.320
4	10	O2P	AGP	401	B	---	30	CA	SER	77	B	3.632
5	15	O6	AGP	401	B	---	76	CD	PRO	76	B	3.361
6	14	O5	AGP	401	B	---	76	CD	PRO	76	B	3.458
7	10	O2P	AGP	401	B	---	76	CD	PRO	76	B	3.651
8	10	O2P	AGP	401	B	---	74	CB	PRO	76	B	3.532
9	10	O2P	AGP	401	B	---	72	C	PRO	76	B	3.802
10	10	O2P	AGP	401	B	---	71	CA	PRO	76	B	3.894
11	9	O1P	AGP	401	B	---	94	CB	ALA	75	B	3.342
12	9	O1P	AGP	401	B	---	92	C	ALA	75	B	3.719
13	9	O1P	AGP	401	B	---	91	CA	ALA	75	B	3.760
14	2	C2	AGP	401	B	---	111	OD2	ASP	53	B	3.606
15	2	C2	AGP	401	B	---	110	OD1	ASP	53	B	3.843
16	10	O2P	AGP	401	B	---	67	CZ	ARG	49	B	3.853
17	11	O3	AGP	401	B	---	101	CD	GLU	240	A	3.853
18	11	O3	AGP	401	B	---	100	CG	GLU	240	A	3.742
19	12	O3P	AGP	401	B	---	116	CB	SER	237	A	3.144
20	1	C1	AGP	401	B	---	58	OH	TYR	215	A	3.245
21	13	O4	AGP	401	B	---	57	CZ	TYR	215	A	3.134
22	13	O4	AGP	401	B	---	56	CE2	TYR	215	A	3.171
23	12	O3P	AGP	401	B	---	43	CZ	ARG	211	A	3.664
24	10	O2P	AGP	401	B	---	43	CZ	ARG	211	A	3.265
25	6	C6	AGP	401	B	---	28	OH	TYR	61	A	3.595
26	9	O1P	AGP	401	B	---	27	CZ	TYR	61	A	3.614
27	9	O1P	AGP	401	B	---	25	CE1	TYR	61	A	3.630

Table S2. List of protein-GlcNAc6P interactions at the novel allosteric site (1).

PDB code: 8EYM chain A

Hydrogen bonds

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	61	N	SER	237	B	---	82	O	HOH	25	S	2.944
2	88	N	GLY	212	B	---	31	O	HOH	110	S	2.967
3	49	OH	TYR	61	B	---	31	O	HOH	110	S	2.762
4	82	O	HOH	25	S	---	15	O4	16G	400	A	2.615
5	31	O	HOH	110	S	---	14	O3P	16G	400	A	2.718
6	66	OG	SER	237	B	---	14	O3P	16G	400	A	2.676
7	57	NE	ARG	211	B	---	14	O3P	16G	400	A	2.802
8	60	NH2	ARG	211	B	---	12	O2P	16G	400	A	3.346
9	31	O	HOH	110	S	---	11	O1P	16G	400	A	2.651
10	49	OH	TYR	61	B	---	11	O1P	16G	400	A	2.753
11	37	OG	SER	77	A	---	11	O1P	16G	400	A	2.698
12	32	N	SER	77	A	---	12	O2P	16G	400	A	2.482
13	9	N2	16G	400	A	---	74	OD2	ASP	53	A	3.017
14	30	NH2	ARG	49	A	---	12	O2P	16G	400	A	2.889
15	29	NH1	ARG	49	A	---	17	O6	16G	400	A	3.049

Non-bonded contacts

	<----- A T O M 1 ----->					<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	103	CD	GLU	240	B	---	15	O4	16G	400	A	3.580
2	102	CG	GLU	240	B	---	15	O4	16G	400	A	3.304
3	101	CB	GLU	240	B	---	15	O4	16G	400	A	3.104
4	65	CB	SER	237	B	---	15	O4	16G	400	A	3.764
5	65	CB	SER	237	B	---	14	O3P	16G	400	A	3.497
6	58	CZ	ARG	211	B	---	14	O3P	16G	400	A	3.598
7	56	CD	ARG	211	B	---	14	O3P	16G	400	A	3.656
8	48	CZ	TYR	61	B	---	11	O1P	16G	400	A	3.636
9	46	CE1	TYR	61	B	---	11	O1P	16G	400	A	3.625
10	135	O7	16G	400	B	---	8	C8	16G	400	A	3.895
11	65	CB	SER	237	B	---	6	C6	16G	400	A	3.751
12	19	P	16G	400	A	---	36	CB	SER	77	A	3.603
13	12	O2P	16G	400	A	---	36	CB	SER	77	A	3.328
14	11	O1P	16G	400	A	---	36	CB	SER	77	A	3.280
15	12	O2P	16G	400	A	---	33	CA	SER	77	A	3.389
16	19	P	16G	400	A	---	81	CD	PRO	76	A	3.750
17	17	O6	16G	400	A	---	81	CD	PRO	76	A	3.220
18	12	O2P	16G	400	A	---	81	CD	PRO	76	A	3.120
19	12	O2P	16G	400	A	---	80	CG	PRO	76	A	3.493
20	12	O2P	16G	400	A	---	79	CB	PRO	76	A	3.207
21	12	O2P	16G	400	A	---	77	C	PRO	76	A	3.228
22	12	O2P	16G	400	A	---	76	CA	PRO	76	A	3.330
23	11	O1P	16G	400	A	---	96	CB	ALA	75	A	3.775
24	12	O2P	16G	400	A	---	94	C	ALA	75	A	3.799
25	11	O1P	16G	400	A	---	94	C	ALA	75	A	3.885
26	1	C1	16G	400	A	---	109	O	ALA	74	A	3.490
27	10	O1	16G	400	A	---	108	C	ALA	74	A	3.414
28	18	O7	16G	400	A	---	87	CB	ALA	73	A	3.545
29	10	O1	16G	400	A	---	87	CB	ALA	73	A	3.549
30	9	N2	16G	400	A	---	87	CB	ALA	73	A	3.730
31	7	C7	16G	400	A	---	87	CB	ALA	73	A	3.578
32	18	O7	16G	400	A	---	117	CG2	VAL	57	A	3.868
33	7	C7	16G	400	A	---	74	OD2	ASP	53	A	3.637
34	7	C7	16G	400	A	---	73	OD1	ASP	53	A	3.897
35	18	O7	16G	400	A	---	72	CG	ASP	53	A	3.845
36	9	N2	16G	400	A	---	72	CG	ASP	53	A	3.632
37	6	C6	16G	400	A	---	29	NH1	ARG	49	A	3.541
38	5	C5	16G	400	A	---	29	NH1	ARG	49	A	3.739
39	17	O6	16G	400	A	---	28	CZ	ARG	49	A	3.807
40	12	O2P	16G	400	A	---	28	CZ	ARG	49	A	3.829

Table S2. Continuation.

PDB code: 8EYM chain B

Hydrogen bonds

	<----- A T O M 1 ----->						<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	61	N	SER	237	A	---	82	O	HOH	27	S	2.974
2	95	OH	TYR	215	A	---	83	O	HOH	132	S	3.167
3	110	N	GLY	212	A	---	20	O	HOH	9	S	2.514
4	20	O	HOH	9	S	---	60	OH	TYR	61	A	3.199
5	83	O	HOH	132	S	---	18	O7	16G	400	B	3.185
6	82	O	HOH	27	S	---	15	O4	16G	400	B	2.778
7	20	O	HOH	9	S	---	14	O3P	16G	400	B	3.012
8	20	O	HOH	9	S	---	11	O1P	16G	400	B	3.335
9	48	OG	SER	77	B	---	11	O1P	16G	400	B	2.754
10	43	N	SER	77	B	---	12	O2P	16G	400	B	2.728
11	9	N2	16G	400	B	---	73	OD1	ASP	53	B	3.349
12	42	NH2	ARG	49	B	---	12	O2P	16G	400	B	2.768
13	41	NH1	ARG	49	B	---	17	O6	16G	400	B	3.065
14	66	OG	SER	237	A	---	14	O3P	16G	400	B	2.699
15	31	NH2	ARG	211	A	---	12	O2P	16G	400	B	2.912
16	28	NE	ARG	211	A	---	14	O3P	16G	400	B	2.789
17	60	OH	TYR	61	A	---	11	O1P	16G	400	B	2.645

Non-bonded contacts

	<----- A T O M 1 ----->						<----- A T O M 2 ----->					
	Atom no	Atom name	Res name	Res no	Chain		Atom no	Atom name	Res name	Res no	Chain	Distance
1	19	P	16G	401	B	---	47	CB	SER	77	B	3.692
2	12	O2P	16G	400	B	---	47	CB	SER	77	B	3.341
3	11	O1P	16G	400	B	---	47	CB	SER	77	B	3.304
4	12	O2P	16G	400	B	---	44	CA	SER	77	B	3.529
5	17	O6	16G	400	B	---	81	CD	PRO	76	B	3.282
6	12	O2P	16G	400	B	---	81	CD	PRO	76	B	3.364
7	12	O2P	16G	400	B	---	80	CG	PRO	76	B	3.827
8	12	O2P	16G	400	B	---	79	CB	PRO	76	B	3.587
9	12	O2P	16G	400	B	---	77	C	PRO	76	B	3.559
10	12	O2P	16G	400	B	---	76	CA	PRO	76	B	3.700
11	11	O1P	16G	400	B	---	123	CB	ALA	75	B	3.592
12	16	O5	16G	400	B	---	120	CA	ALA	75	B	3.850
13	1	C1	16G	400	B	---	117	O	ALA	74	B	3.552
14	10	O1	16G	400	B	---	116	C	ALA	74	B	3.449
15	10	O1	16G	400	B	---	109	CB	ALA	73	B	3.168
16	9	N2	16G	400	B	---	109	CB	ALA	73	B	3.629
17	8	C8	16G	400	B	---	109	CB	ALA	73	B	3.800
18	7	C7	16G	400	B	---	109	CB	ALA	73	B	3.850
19	10	O1	16G	400	B	---	107	C	ALA	73	B	3.843
20	8	C8	16G	400	B	---	149	CG2	VAL	57	B	3.534
21	8	C8	16G	400	B	---	74	OD2	ASP	53	B	3.798
22	3	C3	16G	400	B	---	74	OD2	ASP	53	B	3.807
23	9	N2	16G	400	B	---	72	CG	ASP	53	B	3.818
24	6	C6	16G	400	B	---	41	NH1	ARG	49	B	3.507
25	5	C5	16G	400	B	---	41	NH1	ARG	49	B	3.775
26	12	O2P	16G	400	B	---	40	CZ	ARG	49	B	3.642
27	18	O7	16G	400	B	---	131	C8	16G	400	A	3.895
28	4	C4	16G	400	B	---	103	OE1	GLU	240	A	3.615
29	3	C3	16G	400	B	---	103	OE1	GLU	240	A	3.659
30	15	O4	16G	400	B	---	102	CD	GLU	240	A	3.496
31	15	O4	16G	400	B	---	101	CG	GLU	240	A	3.596
32	15	O4	16G	400	B	---	100	CB	GLU	240	A	3.426
33	14	O3P	16G	400	B	---	65	CB	SER	237	A	3.312
34	6	C6	16G	400	B	---	65	CB	SER	237	A	3.834
35	14	O3P	16G	400	B	---	29	CZ	ARG	211	A	3.665
36	12	O2P	16G	400	B	---	29	CZ	ARG	211	A	3.712
37	14	O3P	16G	400	B	---	27	CD	ARG	211	A	3.562
38	11	O1P	16G	400	B	---	59	CZ	TYR	61	A	3.571
39	11	O1P	16G	400	B	---	57	CE1	TYR	61	A	3.603

Rate equation

Considering that the active site is not formed until the novel allosteric site is formed (ES_A), the main forms of the dimeric enzyme bound to the substrate will be those shown in Scheme 1.

For this Scheme the rate equation is

$v_0 = k_p[E_2S_AS_S] + 2k_p[E_2S_{A2}S_S] + k_p[E_2S_{A2}S_{S2}]$ ... Eq. S1

From the definition of the dissociation constants of each complex

$[E_2S_A] = \frac{[E_2][S]}{K_{A1}}$ ... Eq. S2

$[E_2S_AS_S] = \frac{[E_2S_A][S]}{K_{S1}}$ ... Eq. S3

By substituting Eq. 2 in Eq. 3, $[E_2S_AS_S] = \frac{[E_2][S]^2}{K_{A1}K_{S1}}$ ... Eq. S6

$[E_2S_{A2}S_S] = \frac{[E_2S_AS_S][S]}{K_{A2}}$ ... Eq. S4

By substituting Eq. 6 in Eq. 4, $[E_2S_AS_S] = \frac{[E_2][S]^3}{K_{A1}K_{S1}K_{A2}}$ ... Eq. S7

$[E_2S_{A2}S_{S2}] = \frac{[E_2S_{A2}S_S][S]}{K_{S2}}$ ... Eq. S5

By substituting Eq. 7 in Eq. 5, $[E_2S_AS_S] = \frac{[E_2][S]^4}{K_{A1}K_{S1}K_{A2}K_{S2}}$ ... Eq. S8

The mass balance for the enzyme forms is

$[E_2]_T = [E_2] + [E_2S_A] + [E_2S_AS_S] + [E_2S_{A2}S_S] + [E_2S_{A2}S_{S2}]$ ... Eq. S9

where $[E_2]_T$ is the total amount of enzyme.

Dividing Eq. S1 by Eq. S9

$\frac{v_0}{[E_2]_T} = \frac{k_p[E_2S_AS_S]+2k_p[E_2S_{A2}S_S]+k_p[E_2S_{A2}S_{S2}]}{[E_2]+[E_2S_A]+[E_2S_AS_S]+[E_2S_{A2}S_S]+[E_2S_{A2}S_{S2}]}$ ... Eq. S10

Substituting Eqs. S2, S6, S7 and S8 in Eq. S10, reordering and considering that $V_{max} = k_p [E_2]_T$

$$v_0 = \frac{V_{max}\left(\frac{[S]^2}{K_{S1}} + 2\frac{[S]^3}{K_{S1}K_{A2}} + \frac{[S]^4}{K_{S1}K_{A2}K_{S2}}\right)}{K_{A1} + [S] + \frac{[S]^2}{K_{S1}} + \frac{[S]^3}{K_{S1}K_{A2}} + \frac{[S]^4}{K_{S1}K_{A2}K_{S2}}}$$ ... Eq. S11 (Eq. 2 in paper)

Equation S11 (Eq. 2 in the main body of the paper) resembles the Adair equation for a tetramer, which has the substrate concentration raised at the fourth power. Here, the substrate concentration is also raised at the fourth power, even when it has only two active sites. This explains the intense sigmoidicity ($n_H \geq 2$) of the rate *vs*. substrate concentration curve in the absence of the allosteric activator GlcNAc6P (Fig. 5).

References

1. R. A. Laskowski, M. B. Swindells, LigPlot+: Multiple Ligand–Protein Interaction Diagrams for Drug Discovery. *J. Chem. Inf. Model.* **51**, 2778–2786 (2011).