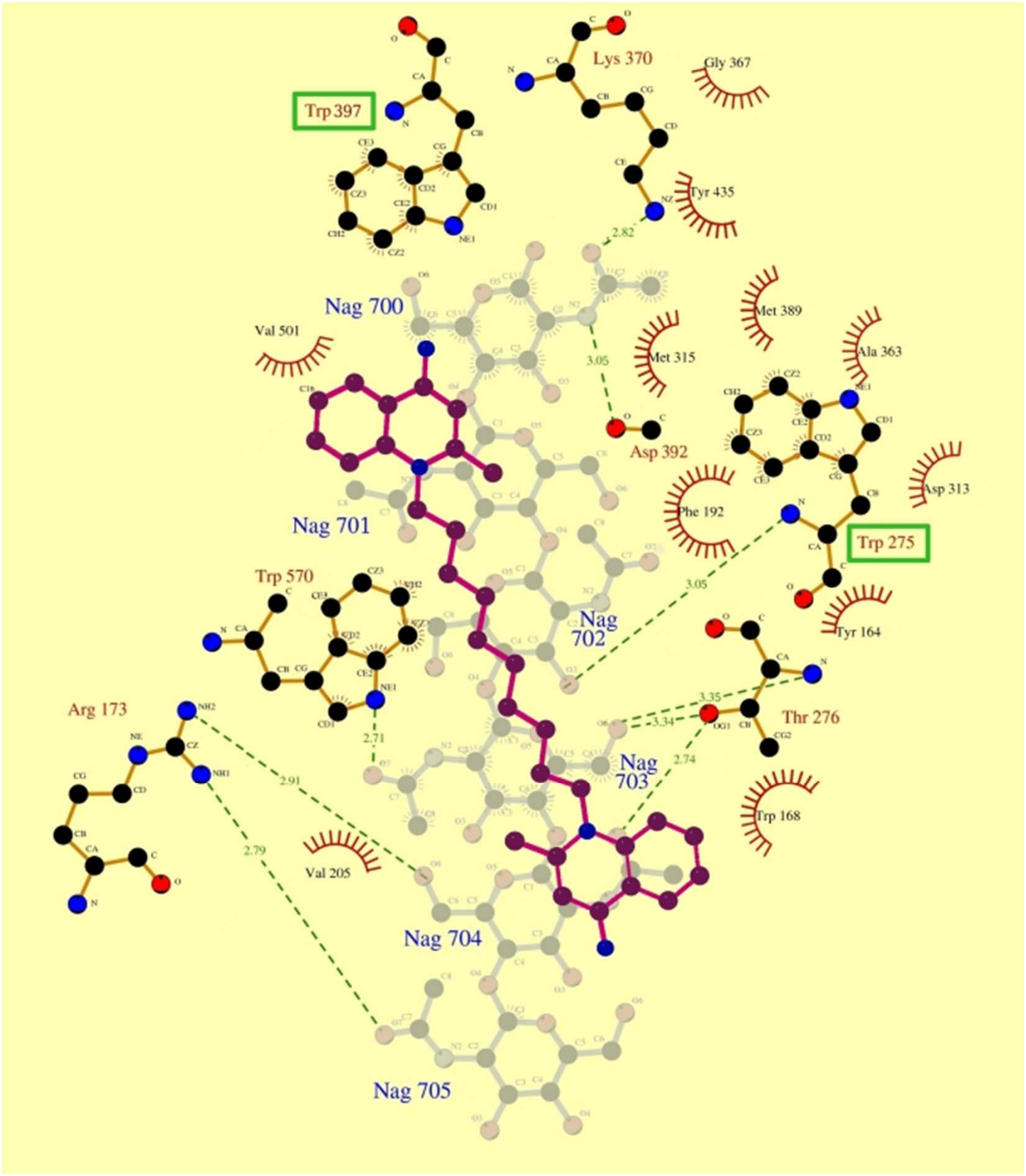
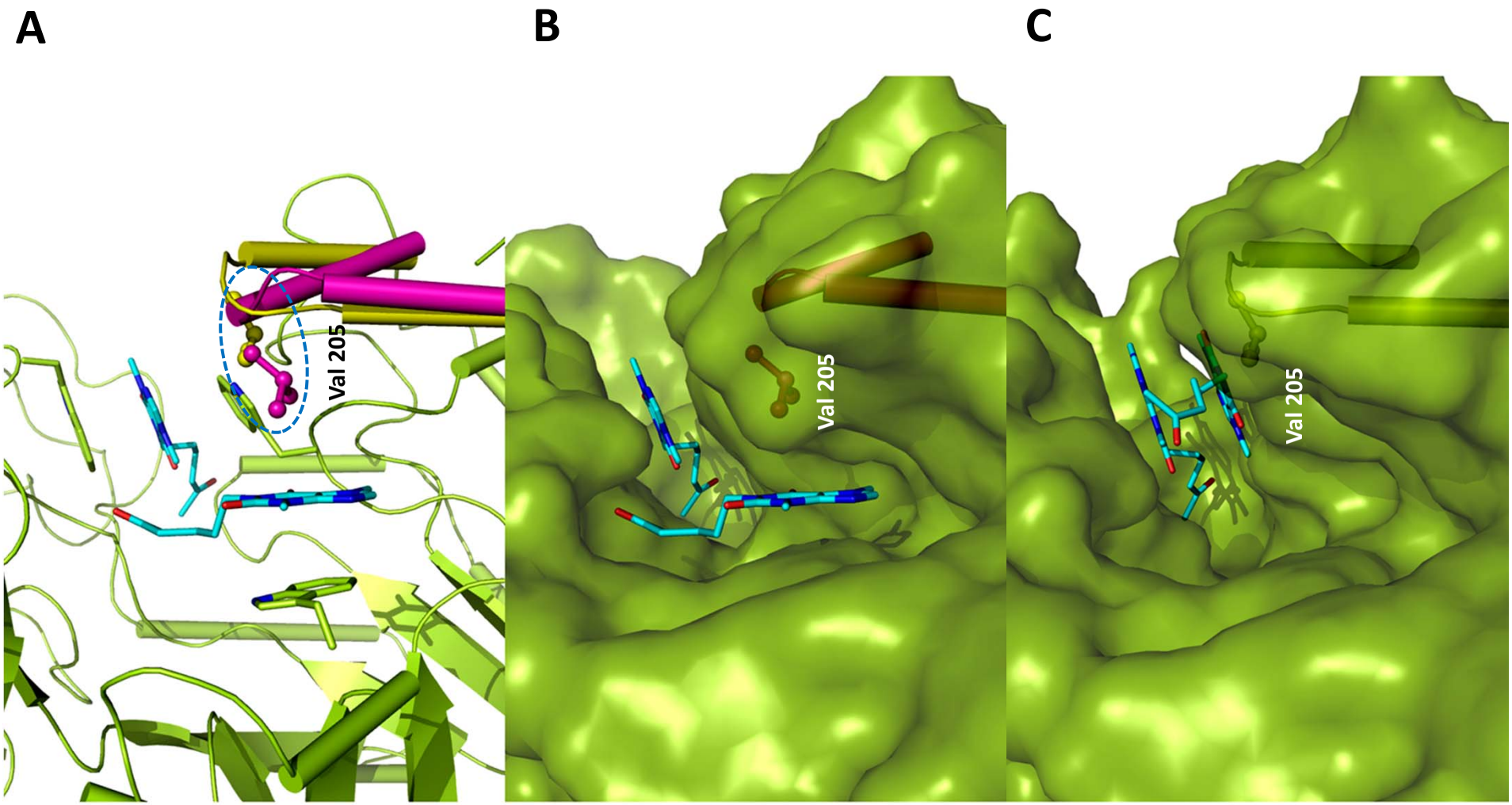
Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Legends and Tables

Legends to supplementary figures

Fig. S1 (related to Figure 2)
Schematic protein-ligand interactions of dequalinium and chitihexaose substrate ($GlcNAc_6$) calculated with LIGPLOT. Dequalinium shows exclusively hydrophobic contacts whereas the $GlcNAc_6$ has a number of potential hydrogen bonds to the chitinase.

Fig. S2 (related to Figures 2&3)
Comparison of the loop containing Val205 in wild type and the mutant W275G: Opening of the location 2 in the mutant due to changes in the backbone conformation leads to loss of potential interactions and thus an empty binding site in the mutant.

Table S1. Details of data collection and structure refinement.
Unit cell for space group "$P2_1$": 65.1 50.9 93.2 90 99.5 90, for "$P2_1$-A": 60.0 85.3 63.0 90 112.9 90

Crystal	WT-APO 3ARO	WT-DEQ 3ARP	WT-IDA 3ARQ	WT-SAN 3ARV	WT-CHE 3ARW	WT-PRO 3ARX	WT-IMI 3ARY	WT-PEN 3ARR	WT-IMI 3ARZ	W275G-APO 3ARS	W275G-DEQ 3ART	W275G-SAN 3AS0	W275G-CHE 3AS1	W275G-PRO 3AS2	W275G-IMI 3AS3	W275G – PEN 3ARU
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$-A	$P2_1$-A	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Resolution range (Å)	19.82-2.22 (2.28-2.22)	19.96-1.55 (1.59-1.55)	19.90-1.5 (1.54-1.5)	38.04-1.50 (1.54-1.50)	39.81-1.50 (1.54-1.50)	40.66-1.16 (1.19-1.16)	19.97-1.35 (1.39-1.35)	19.95-1.65 (1.69-1.65)	19.94-1.82 (1.87-1.82)	19.78-2.45 (2.51-2.45)	19.9-2.23 (2.29-2.23)	33.43-2.0 (2.05-2.0)	19.96-2.0 (2.05-2.0)	36.49-1.80 (1.85-1.80)	46.58-2.4 (2.5-2.4)	40.62-1.90 (1.95-1.90)
No of observed reflections	68816	291154	698077	344568	268054	786080	482805	211087	158556	67399	119424	311975	268938	210243	90927	343052
No of unique reflections	28030	82238	95004	95064	90870	199166	123615	66052	49568	19696	27041	38365	39084	51578	22493	45190
Redundancy	2.46	3.54	7.3	3.62	2.95	3.78	3.90	3.20	3.20	3.42	4.16	8.13	6.88	4.08	4.04	7.59
I/σ(I)	15.91(6.34)	19.14(3.50)	23.76(7.19)	16.79(8.33)	14.86(4.04)	12.88(3.76)	16.94(3.29)	12.80(4.21)	13.72(4.16)	14.47(4.08)	18.45(6.09)	19.21(6.45)	13.27(5.43)	16.78(4.38)	13.54(6.54)	18.32(8.14)
Completeness (%)	94.7(85.8)	93.4 (77.0)	98.3(95.1)	98.0 (91.8)	94.0 (74.8)	95.8 (79.1)	93.2(85.0)	93.8 (88.0)	94.2 (89.5)	91.0 (67.5)	94.1 (67.4)	97.4 (94.1)	96.9 (93.5)	96.1 (89.5)	97.1 (93.7)	98.3 (95.8)
R_{merge} (%)	5.7(15.3)	4.2(28.5)	7.1(47.5)	6.3(15.0)	5.1(27.0)	5.9(27.6)	5.1(44.0)	6.9(42.0)	6.0(40.8)	8.2(30.3)	7.8(29.6)	8.3(38.2)	13.2(48.5)	6.1(35.1)	11.2(44.7)	11.9(34.9)
R_{cryst}, R_{free}	14.3,21.6	14.6,18.7	14.5, 18.4	14.4,17.7	15.3,19.0	16.0,18.5	16.3,19.4	14.3,18.8	14.8,19.1	19.8,25.9	16.2,22.9	14.5,19.7	15.3,20.5	15.0,19.6	15.4,23.0	14.4,19.6
Rmsd from ideal geometry Bonds (Å) Angles (°)	0.021 1.828	0.029 2.391	0.029 2.335	0.029 2.437	0.029 2.427	0.034 2.597	0.032 2.542	0.029 2.368	0.027 2.109	0.006 0.865	0.021 1.765	0.024 1.903	0.025 1.862	0.025 2.019	0.019 1.748	0.024 1.981
B factor (Å^2) Bonded, mainchain protein[a] ligands[a] water[a]	14.1 11.6 12.4 - 25.5	16.8 12.9 14.1 17.8 30.7	15.0 10.8 12.2 22.0 29.7	15.2 10.6 12.1 46.6 29.4	17.7 12.4 14.1 29.7 34.4	17.4 11.9 13.3 26.4 33.9	18.1 12.8 14.3 38.2 35.1	19.2 14.4 16.5 35.8 35.4	25.1 20.8 23.3 79.9 38.9	19.6 19.6 19.6 - 20.7	16.7 14.5 14.9 61.6 23.6	21.6 18.9 20.2 51.6 32.2	19.7 16.5 17.8 76.3 30.7	18.8 15.1 16.6 52.4 30.2	22.9 20.9 22.2 75.8 26.4	15.3 11.6 13.0 39.6 28.2

[a]<B> = average B factor. Values in parentheses are for the highest resolution shell.

Table S2. (related to Schemes 1-3 and Figure 6)

Parameters used for the calculation of theoretical curves in Figure 6.
Number of binding sites (N), equilibrium dissociation constants K_D, enthalpy changes ΔH, IC_{50} values, and Hill coefficients n_H, which have been used for the calculation of the theoretical curves, are shown for the two inhibitors DEQ and SAN and *Vh*ChiA wild-type (WT) or mutant W275G in Figure 6. All ITC data were first fitted to a single-site binding model, yielding N, K_D, and ΔH values upon binding. If the single-site binding model was unsatisfactory, then the data were fitted with a fixed number of N=2 using a two-independent-site model or a two-sequential-site model. Based on such multi-parameter fits, the resultant K_D1, K_D2, ΔH1 and ΔH2 values were correlated, and hence could not be derived unambiguously. The corresponding parameters for two PEN molecules bound to W275G were evaluated, but are not shown in Figure 6. The reaction scheme, to which the ITC data were fitted, is indicated as: 1 for one-site binding; 2 for independent two-site binding; or 3 for sequential two-site binding.

Enzyme-inhibitor complex	Binding to one site (Black lines in Figure 6)					Binding to two independent sites or sequential sites											
	N	K_D (μM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1})	Scheme	N	K_D1 (μM)	K_D2 (μM)	ΔG1 (kcal mol^{-1})	ΔG2 (kcal mol^{-1})	ΔH1 (kcal mol^{-1})	ΔH2 (kcal mol^{-1})	ΔS1 (cal mol^{-1})	ΔS2 (cal mol^{-1})	IC_{50} (μM)	n_H
DEQ WT[a]	1.1	0.07	-9.8	-8.7	3.5	1	-	-	-	-	-	-	-	-	-	0.4	1.4
SAN WT	1.3	2.3	-7.7	-7.9	-0.7	2	2	0.3	0.8	-8.7	-8.1	-4.5	-8.3	14.4	-0.6	2.3	0.9
PEN WT	1.2	4.0	-7.4	13	68.3	2	2	2	10	-7.6	-6.7	-16	-1	-28.9	19.4	3.5	1
DEQ W275G[b]	1.3	37	-6.0	-3.0	10.2	2	2	11	500	-6.6	-4.4	-2.7	-1.5	13.4	9.9	68	0.7
SAN W275G	3.9	28	-6.2	-4.0	7.4	3	2	214	66	-4.9	-5.6	-9.2	-6.4	-14.8	-2.9	71	1.4
PEN W275G	1.8	600	-4.4	-11	-22.2	3	2	93	690	-5.4	-4.2	-2.6	-4.1	9.5	0.4	260	1.9

[a]The ITC experiments of WT were carried out at 25°C (298 K).
[b]The ITC experiments of W275G were carried out at 18°C (291 K).