SUPPLEMENTAL INFORMATION

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Supplemental Figures

FIGURE S1. **Protein sequence alignments and phylogenetic relationship of UDP-GlcA decarboxylases and UXNAcS.**
A. Protein sequences of *C. elegans* SQV-1 (NP_501418, amino acid 127 to 467); of *C. neoformans* Uxs1p (AAK59981, from amino acid 77 to 410); of Arabidopsis AtUxs3 (AF387789, amino acid 31 to 357); of Sinorhizobium SmUxs1 (GU062741, from amino acid 19 to 348); of Potato StUaxs (ABC75032, from amino acid 7 to 386); of Ralstonia RsU4kpxs (GQ369438); of ArnA (AY057445, from amino acid 311 to 660); of *Bacillus anthracis* UXNAcS homolog, Ba-UXNAcS (NP_843043) and of UXNAcS were aligned with ClustalX software and the conserved motifs are shown.
B. Phylogenetic relationships of selected UDP-GlcA decarboxylases and UXNAcS. Protein sequences of AtUxs3; of SQV-1; of Uxs1p; of SmUxs1; of ArnA; of *E. tasmaniensis* ArnA homolog, Et-ArnA (YP_001908305, from amino acid 311 to 660); of RsU4kpxs; of StUaxs; of Arabidopsis Uaxs, AtAxs1 (AAC73015) and of UXNAcS were first aligned with ClustalX software. Alignments were subsequently analyzed using *MEGA* version 4 (1) to generate the phylogenetic tree. Percentage bootstrap values of 1,000 replicates are given at each branch point. Branch lengths are shown to scale.

FIGURE S2, **MALDI-MS analysis of UDP-GlcNAcA and UDP-XylNAc, the enzymatic products of UGlcNAcDH and UXNAcS, respectively.**
The enzymatic products of UGlcNAcDH (panel 1) and UXNAcS (panel 2) were collected from the HPLC-column (see Fig. 2 and Fig. 4, respectively) and analyzed by MALDI-MS in the negative-ion model. The ion mass, calculated (abbr. Cal.) or observed (abbr. Obs.), are listed below the spectrum.

FIGURE S3. **Monitoring the combined UGlcNAcDH and UXNAcS activities by Real-time 1D NMR.**
Progression of the enzymatic conversion of UDP-GlcNAc and NAD^+ to UDP-GlcNAcA and NADH; and subsequently to UDP-XylNAc at 0 min, 5 min, 60 min or 120 min are shown. The selected NMR regions pointing to diagnostic peaks for the two protons linked to C-4 of nicotinamide ring of NADH is shown from 2.6 to 2.8 (Panel 3). The region corresponding to the diagnostic anomeric protons of the NDP-sugars, are shown from 5.4 to 5.6 (Panel 2). The region corresponding to other diagnostic peaks for NAD^+ and NADH are shown from 6.0 to 6.2 ppm (Panel 1). The dual-enzyme assay comprised double the amount of UGlcNAcDH compared to the single-enzyme dehydrogenase assay. The labeling for protons belonging to NAD^+ (N) and NADH (H) are as shown in Fig. 6. Peaks labeled as I, II or III indicate the anomeric protons of UDP-GlcNAc, UDP-GlcNAcA or UDP-XylNAc, respectively. For presentation clarity, the vertical scale of panel 1 is six times that of panel 2; and panel 3 scale is three times that of panel 2.

FIGURE S4. **The effects of buffer composition, pH and temperature on UDP-XylNAc synthase activity.**

A. The activity of recombinant UXNAcS was analyzed in different buffers (Tris-HCl and sodium phosphate) at different pH. Each value is the mean of triplicate reactions.
B. The activity of recombinant UXNAcS was analyzed at different temperatures in 50 mM sodium phosphate buffer, pH 8.2. Each value is the mean of triplicate reactions.

FIGURE S5. **Size estimation of active UXNAcS**
Recombinant UXNAcS was fractionated on a Superdex-200 gel filtration column and an aliquot of each fraction was assayed for UXNAcS activity. The amount of UDP-XylNAc formed (indicated by ●) in each assay was plotted against the time. The mass of the calculated monomer protein is 37 kDa and the molecular weight of the enzyme in solution was estimated to be around 70 kDa based on the interpolation from the relative elution time of standard protein markers from the column (indicated by ▲).

FIGURE S6**. 3D-Structural model of Bacillus UXNAcS with Human Uxs1.**
Three-dimensional structure models for Bacillus UXNAcS were predicted using both the "Protein Homology/analogY Recognition Engine" PHYRE web (sbg.bio.ic.ac.uk/~phyre/) server (25) and the SWISS-MODEL comparative protein-modeling server (swissmodel.expasy.org/). Structural models were superimposed with the unpublished 1.21Å crystal structures of the human Uxs1 that was complexed with UDP and NAD^+ (2b69A1.pdb, deposited at SGC 'Structural, Genomics Consortium'). All models and protein structures were visualized using PyMol. The ribbon represents the superimposed structure of human Uxs1 and UXNAcS. The conserved structure of human Uxs1 and UXNAcS is colored in orange and yellow, respectively. The non-conserved structure between human Uxs1 and UXNAcS are highlighted in red and green respectively. The crystal structures of the human Uxs1 bound NAD^+ and UDP are shown in red and blue respectively.

Supplemental Tables

Table S1. *Proton chemical shifts and coupling constants of purified UDP-GlcNAcA and UDP-XylNAc.*

	^{13}C NMR signals		^{1}H NMR signals			
Position	UDP-XylNAc[a]	Position	UDP-GlcNAcA[b]		UDP-XylNAc[a]	
	δ (ppm)		δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
XylNAc		Sugar				
C1”	97.5	H1”	5.535	$J_{1”,2”}$ 3.3 $J_{1”,P}$ 7.5	5.454	$J_{1”,2”}$ 3.3 $J_{1”,P}$ 6.8
C2”	56.3	H2”	4.014	$J_{2”,3”}$ 9.3	3.962	$J_{2”,3”}$ 9.6 $J_{2”,P}$ 3.0
C3”	73.8	H3”	3.815	$J_{3”,4”}$ 9.3	3.73	$J_{3”,4”}$ 10
C4”	72.5	H4”	3.595	$J_{4”,5”}$ 10.1	3.71	-
C5”	65.3	H5a”	4.161	-	3.806	$J_{5a”,4”}$ 5.5 $J_{5a”,5b”}$ 11.2
		H5b”	-	-	3.760	$J_{5b”,4”}$ 10.2
		NAc	2.069	-	2.064	-
Rib		Rib				
C1’	91.4	H1’	5.963	-	5.971	-
C2’	76.6	H2’	4.362	-	4.365	-
C3’	72.4	H3’	4.327	-	4.350	-
C4’	85.8	H4’	4.272	-	4.280	-
C5’	67.8	H5a’	4.255	-	4.239	-
		H5b’	4.15		4.175	-
Uracil		Uracil				
C5	105.3	H5	5.952	-	5.957	-
C6	153.6	H6	7.905	-	7.944	-

UDP-GlcNAcA was purified from HPLC after reaction of recombinant *Bacillus* UGlcNAcDH with UDP-GlcNAc and NAD^+. UDP-XylNAc was purified from HPLC after reaction of recombinant *Bacillus* UXNAcS with UDP-GlcNAcA. The purified products were lyophilized, re-suspended in D_2O and analyzed by NMR.

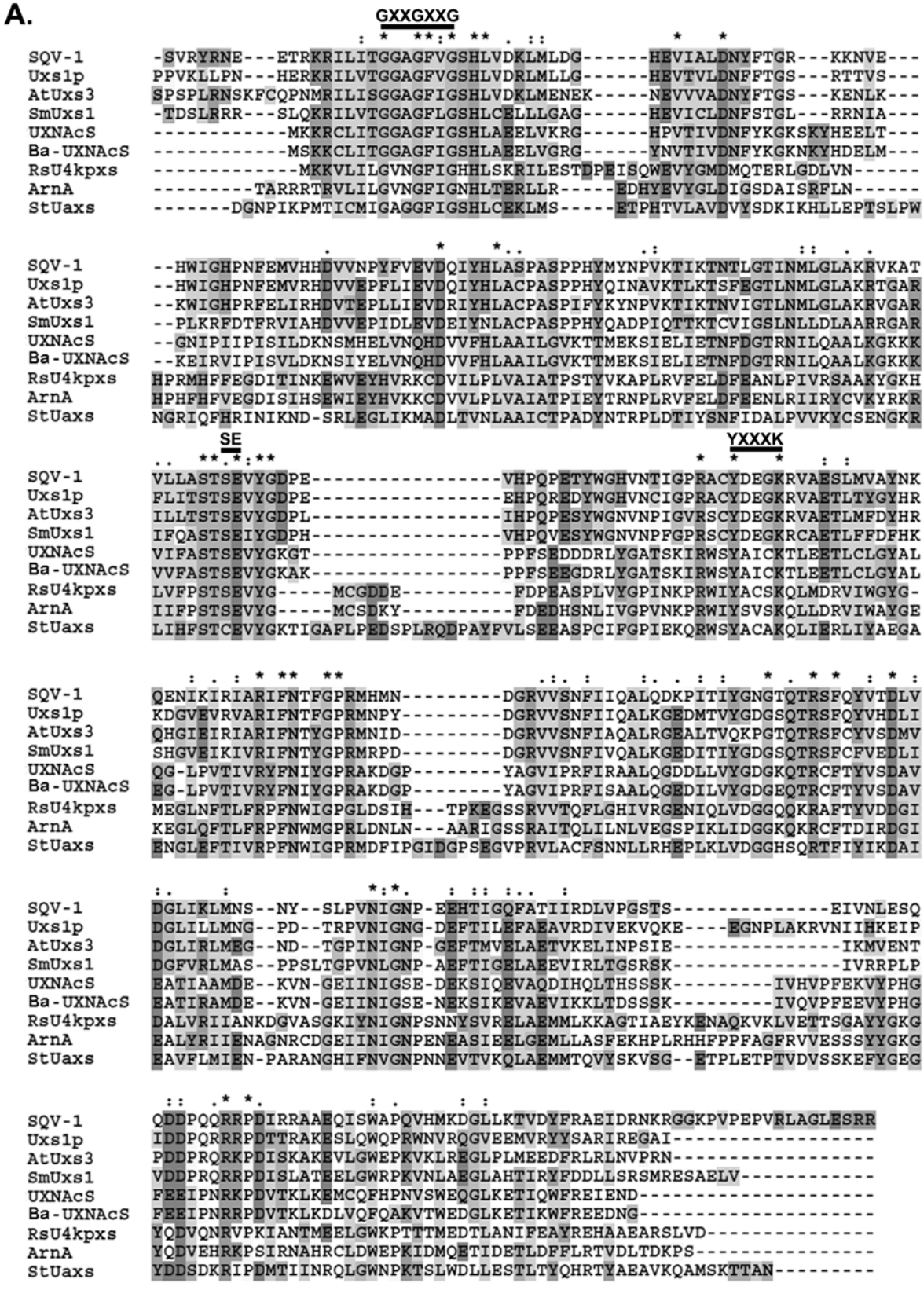
[a] Chemical shifts were determined at 25 °C using 900 MHz NMR and referenced to 2,2-dimethyl-2-silapentane-5-sulphonate.

[b] Chemical shifts were determined at 37 °C using 600 MHz and referenced to 2,2-dimethyl-2-silapentane-5-sulphonate.

REFERENCE

1. Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) *Mol Biol Evol* **24**, 1596-1599

Figure S1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ArnA
StUaxs
B.
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SQV-1
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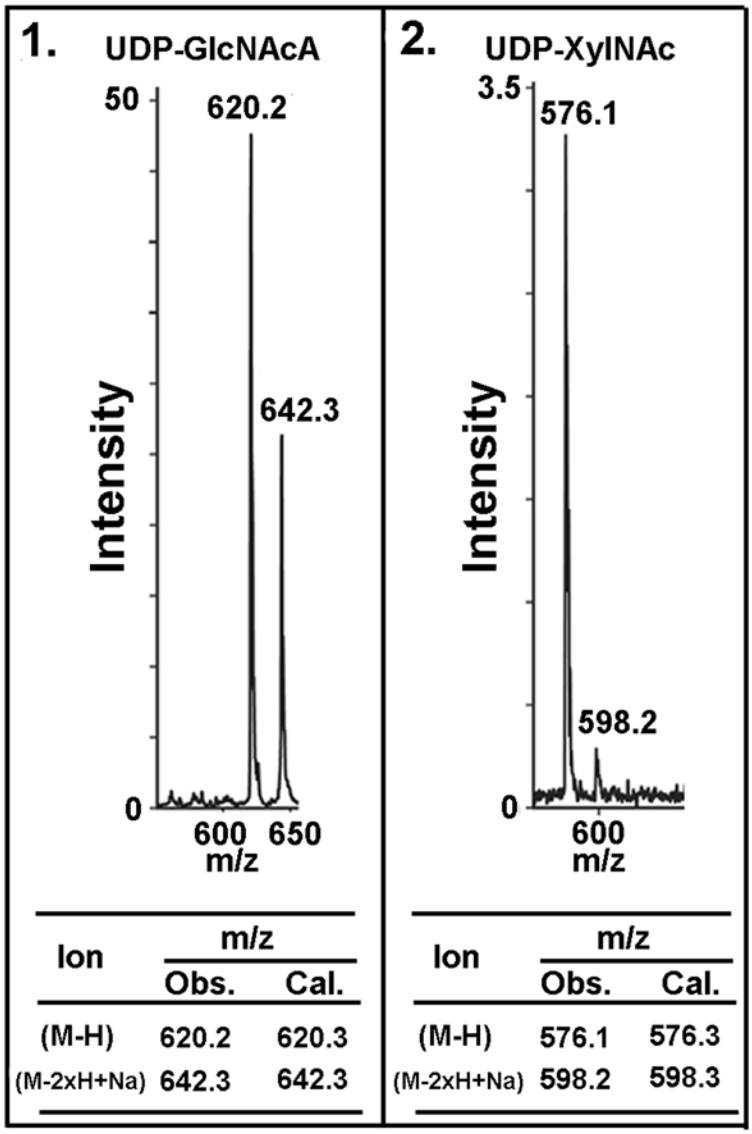
Figure S2



Ion	m/z	
	Obs.	Cal.
(M-H)	620.2	620.3
(M-2xH+Na)	642.3	642.3

Ion	m/z	
	Obs.	Cal.
(M-H)	576.1	576.3
(M-2xH+Na)	598.2	598.3

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Figure S3

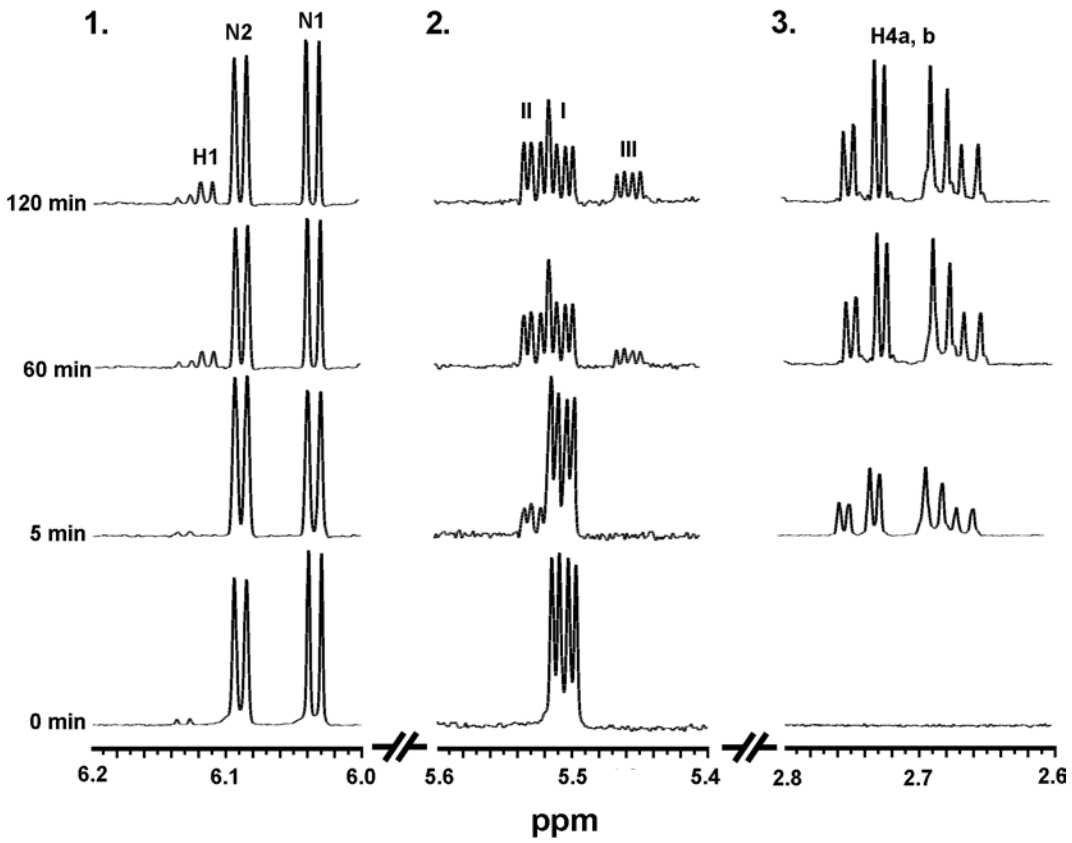

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Figure S4

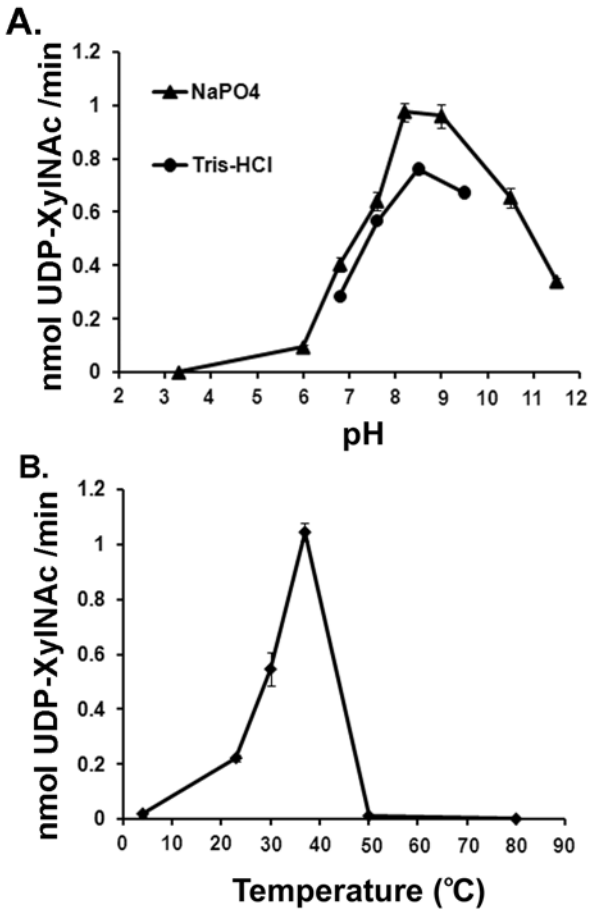

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Figure S5

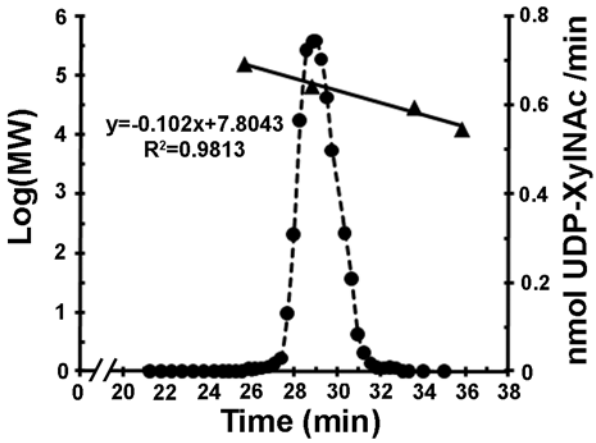

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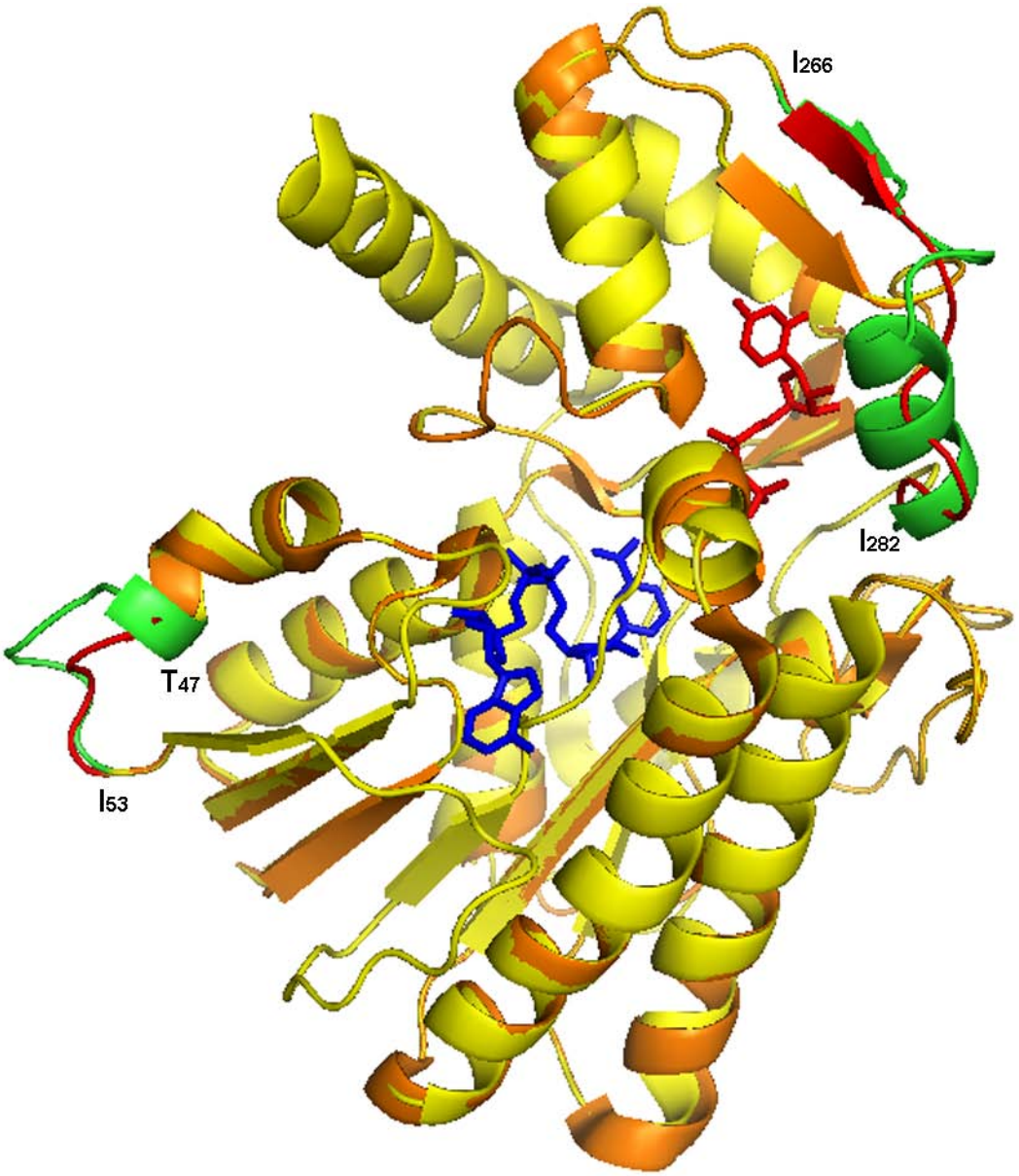
Figure S6



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