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Supporting information for article:

Substrate-induced dimerization of elaiophylin glycosyltransferase reveals a novel self-activating form of glycosyltransferase for symmetric glycosylation

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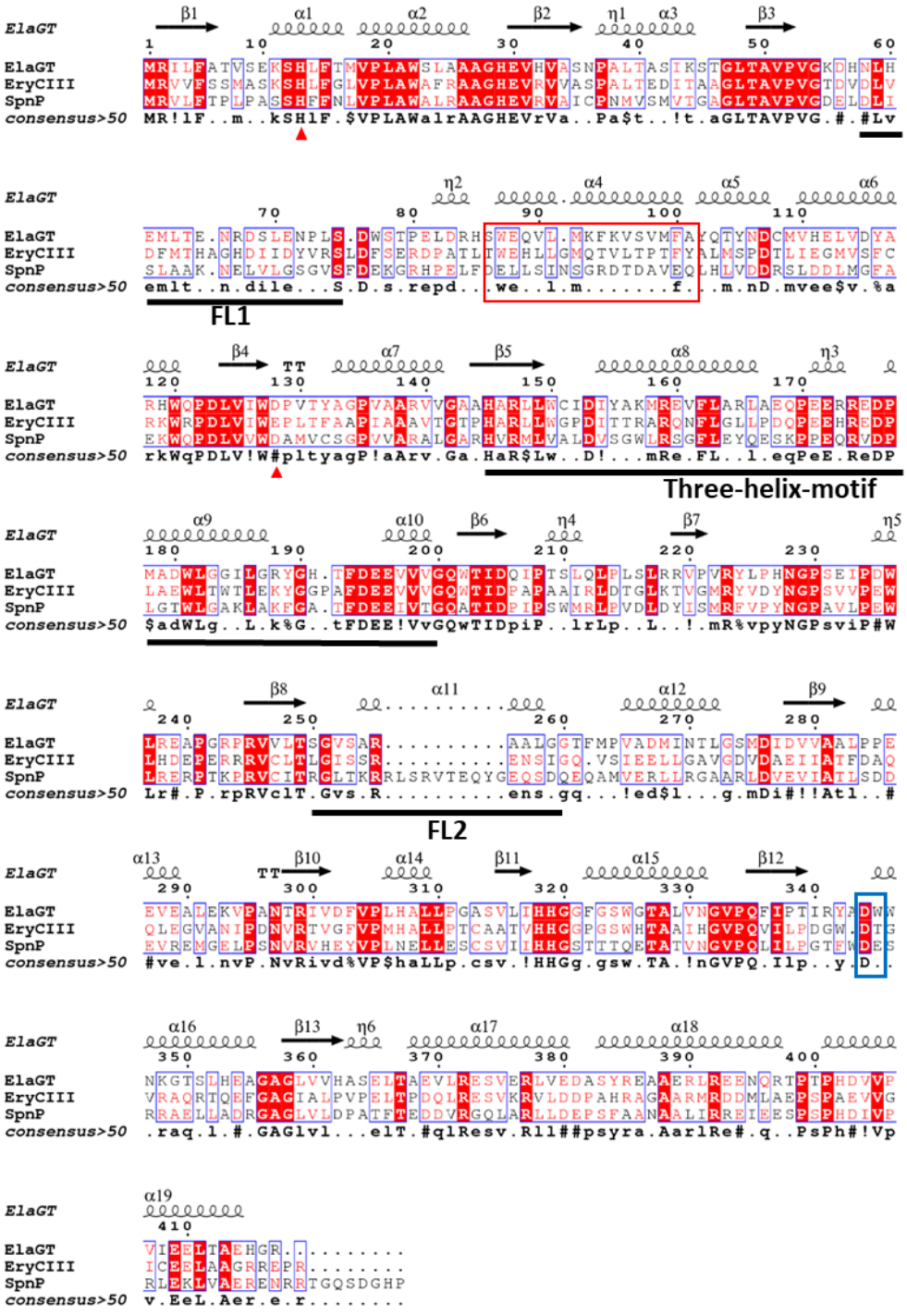
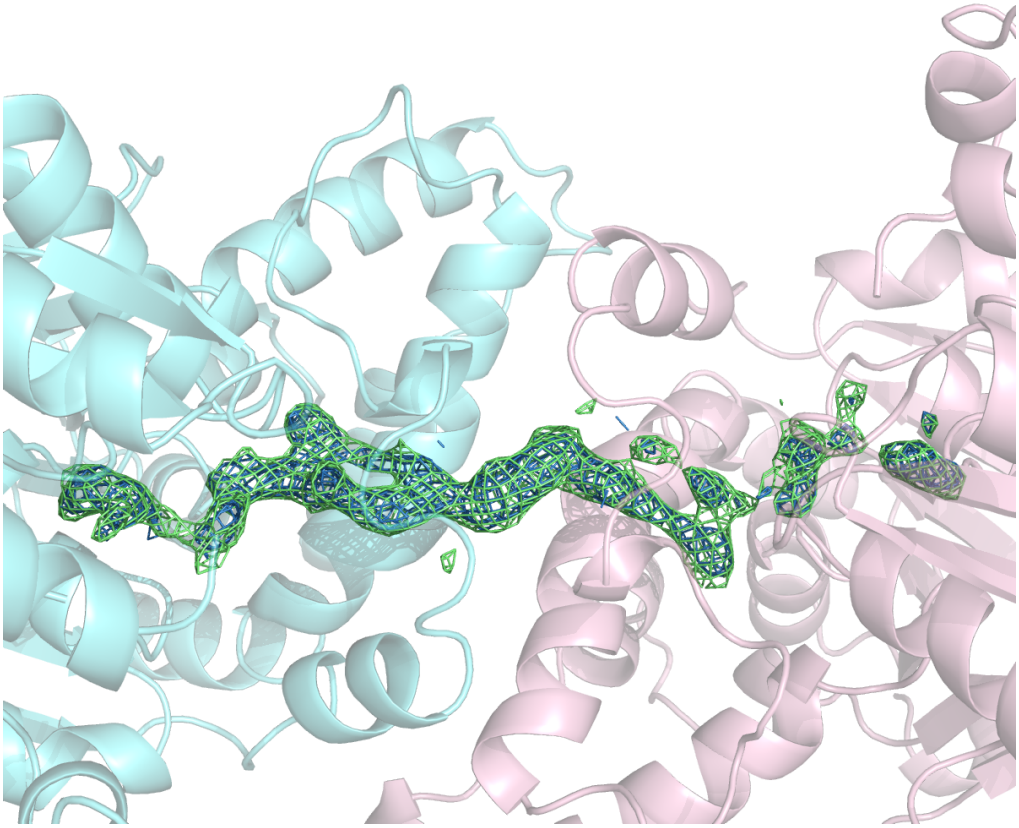
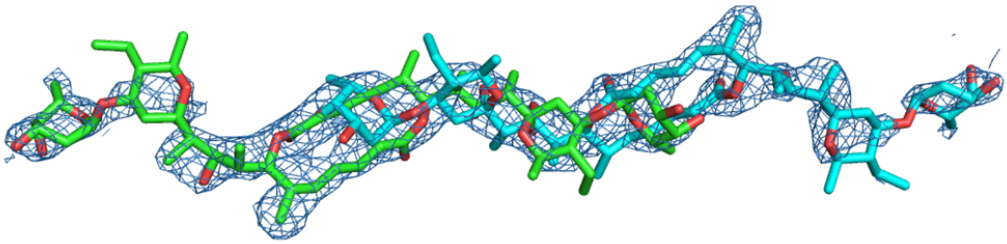


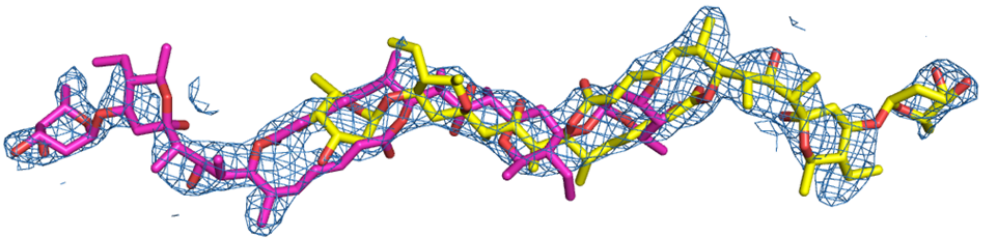
Supplementary Figure S1. Sequence alignment between ElaGT and its homologues EryCIII and SpnP. Sequence alignment was performed using the programs MultAlin and ESPript [1, 2]. Secondary structure elements of ElaGT are shown on the top. Flexible loop region 1 and 2 (FL1, FL2) and three-helix-motif are underlined. Putative catalytic residues His13 and Asp128 are marked with red triangle. Helix4 is marked with a red box and the sugar-recognition motif is marked with blue box.



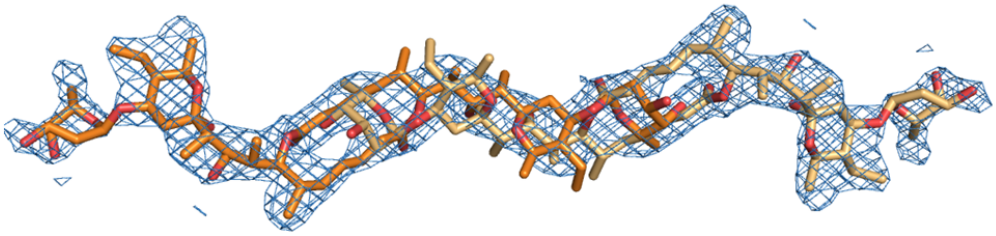
Supplementary Figure S2. Fo-Fc map and 2Fo-Fc map in the cleft before placement of Ela. The Fo-Fc map is countered at 2 σ and colored green. The 2Fo-Fc map is countered at 1 σ and colored blue.



Chain B/C

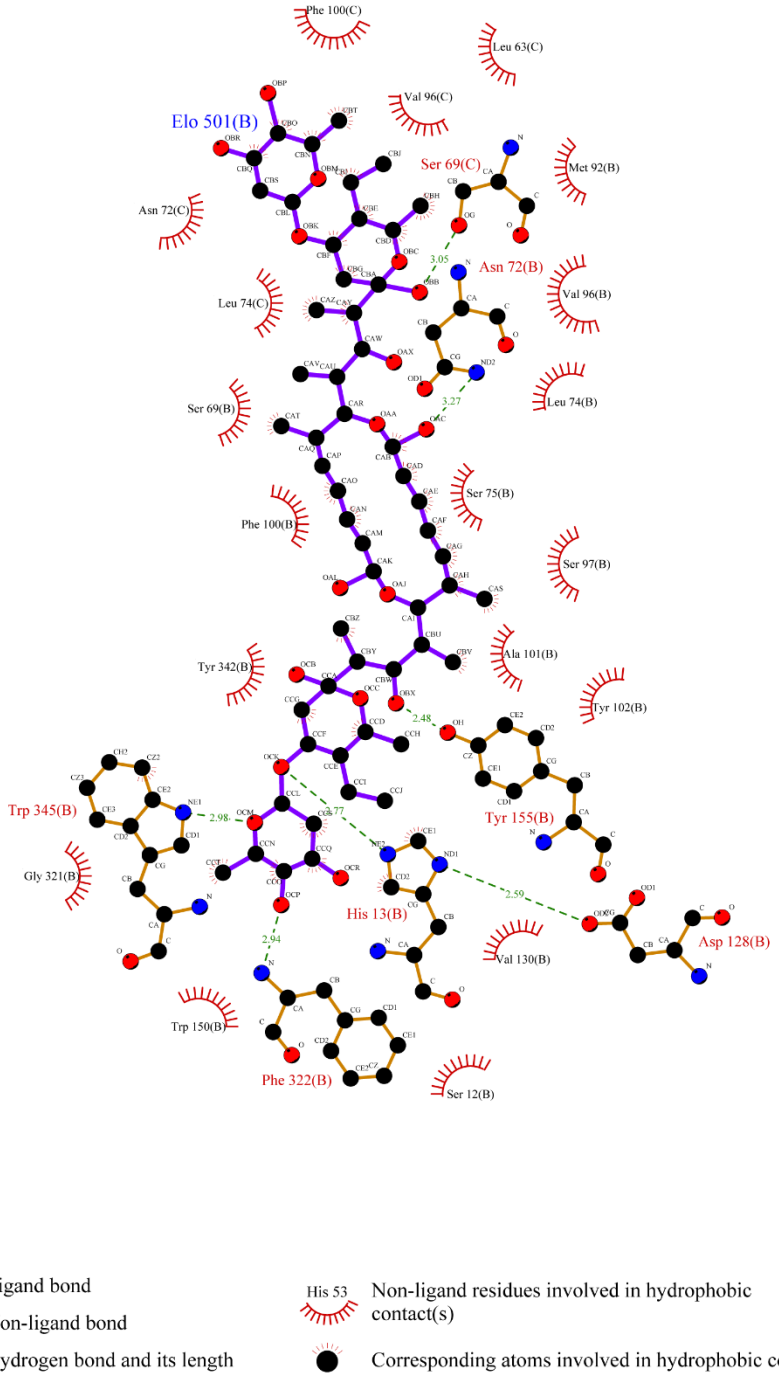


Chain D/E



Chain F/F’

Supplementary Figure S3. 2*Fo*-*Fc* map of Ela pairs in chain B/C, D/E, and F/ F’. The map is countered at 0.9 σ and colored blue.



Key

Ligand bond

Non-ligand bond

3.0 Hydrogen bond and its length

His 53 Non-ligand residues involved in hydrophobic contact(s)

Corresponding atoms involved in hydrophobic contact(s)

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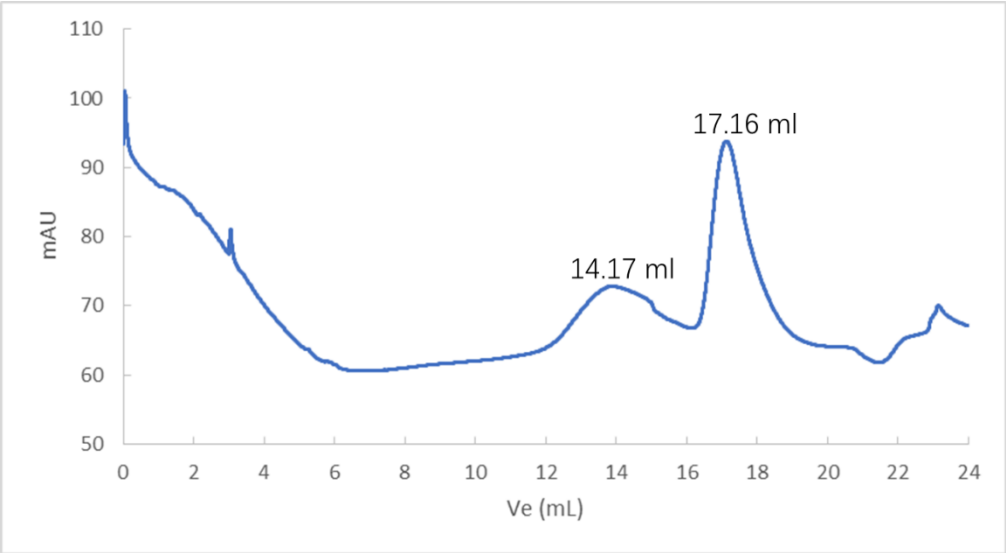
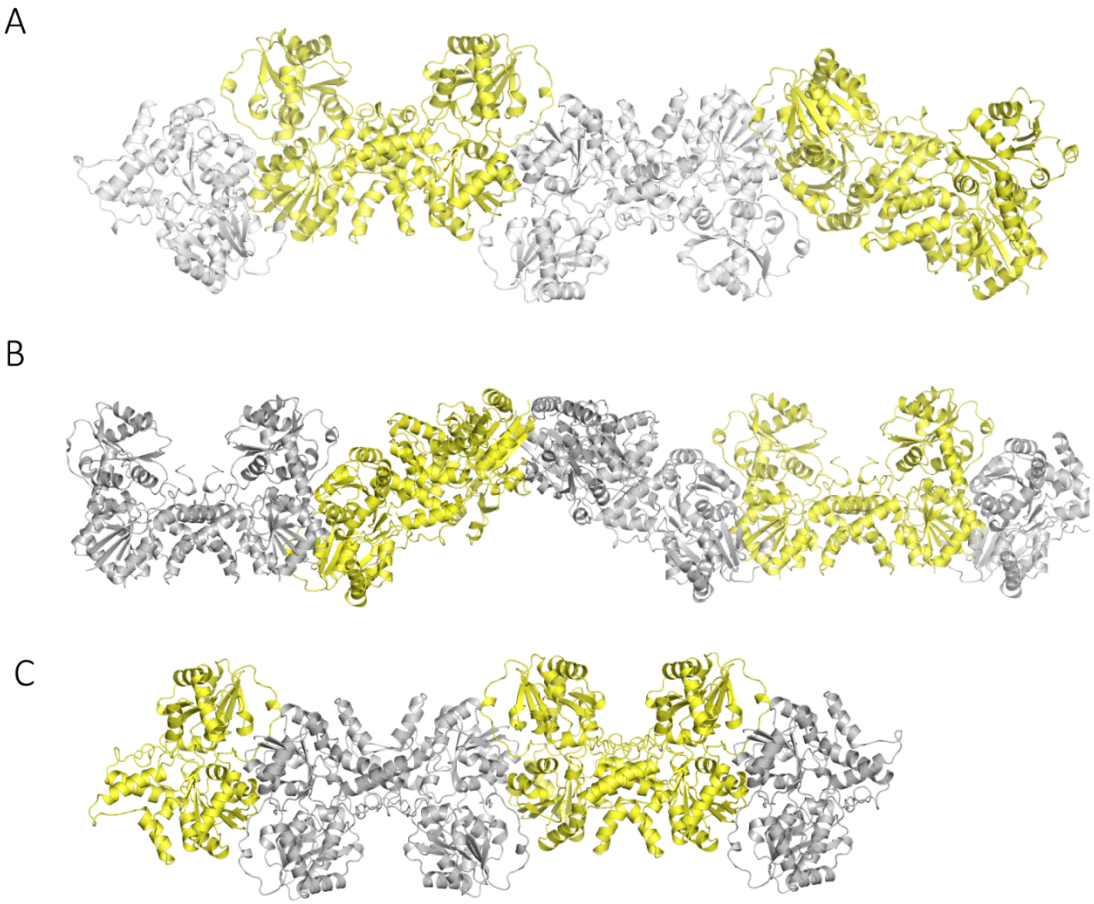
Supplementary Figure S4. Interactions between Ela and ElaGT.

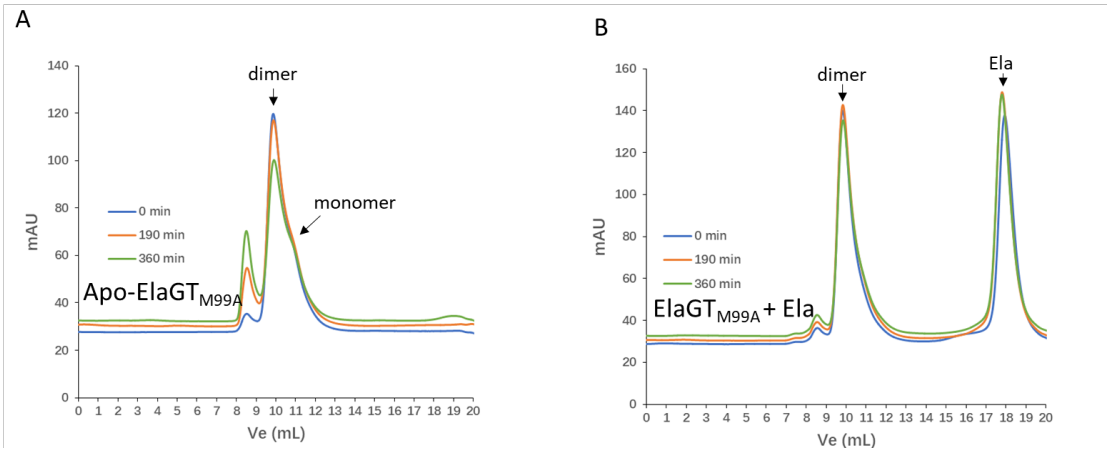
Interactions between Ela and chain B of ElaGT was shown as representative. Analysis was performed by LIGPLOT [3].



Supplementary Figure S5. Gel filtration results of ElaGT$_{WT}$ by Superose 6 increase 10/300.

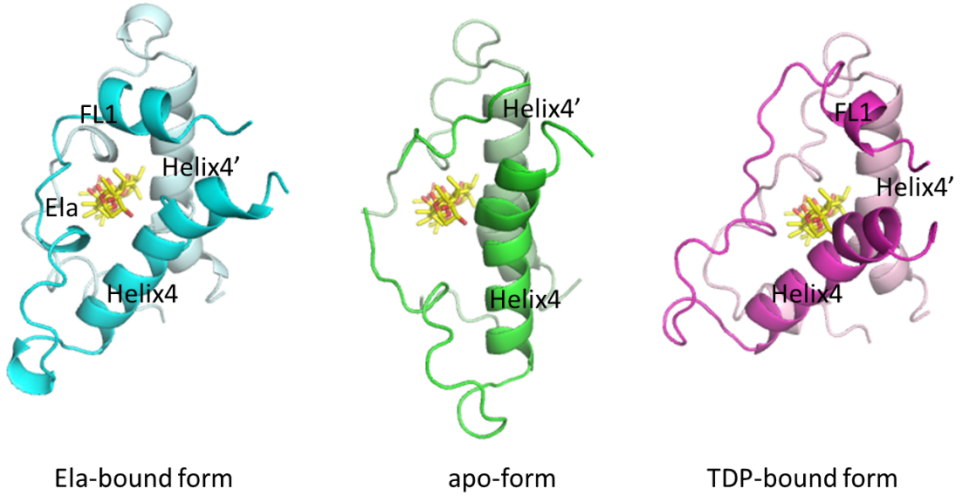


Supplementary Figure S6. The linear packing profiles of ElaGT in different structures.
A) The arrangement profile of Ela-binding form; B) The arrangement profile of apo-form; C) The arrangement profile of TDP-binding form. The face-to-face dimers are marked in the same color alternatively with yellow and gray.



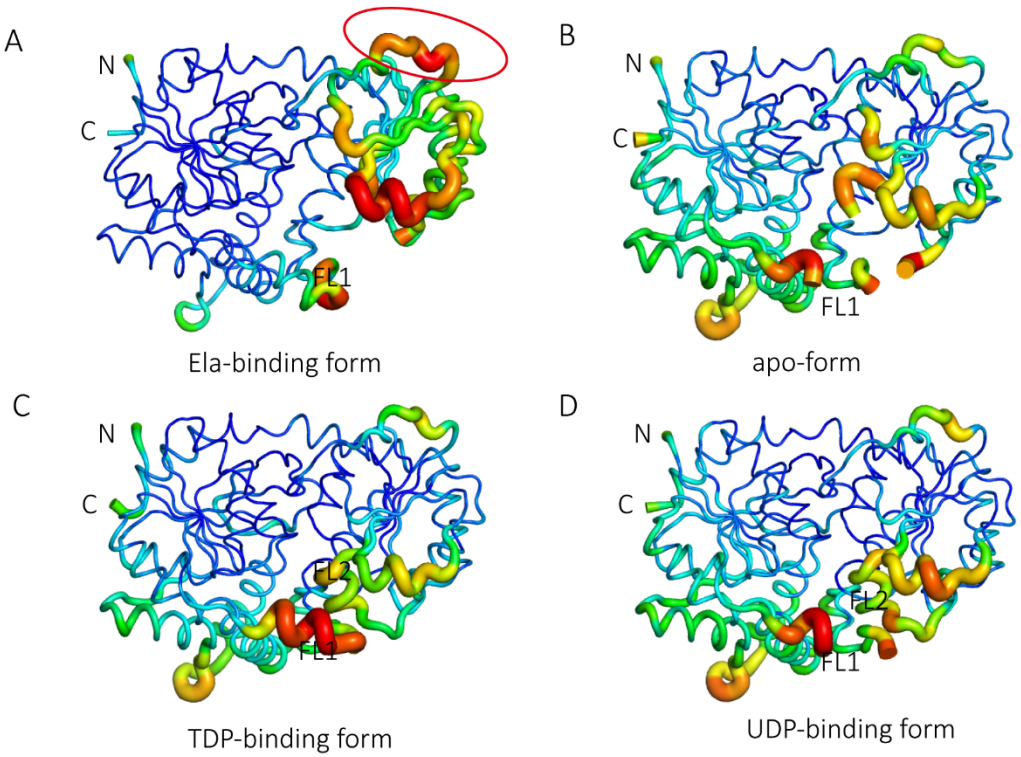
Supplementary Figure S7. Gel filtration by Superdex 75 increase.

A-B) gel filtration results of ElaGT variant M99A in absence and in presence of Ela over the time.



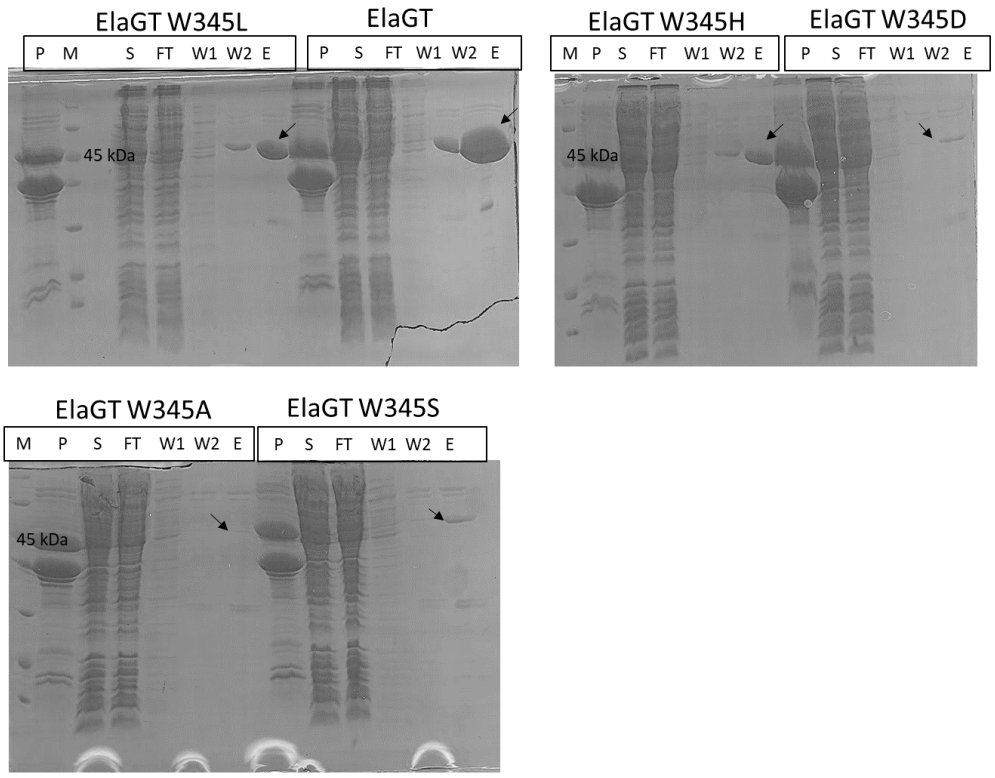
Supplementary Figure S8. The channel gates of face-to-face dimer in different forms of ElaGT structure.

The two gates on the face-to-face homodimer interface of Ela-bound ElaGT are colored with cyan and palecyan, respectively, and one Ela was shown and colored by yellow. The two gates in the apo-ElaGT are colored with green and palegreen, and in the TDP-bound ElaGT they are colored with magenta and pink. All the Helix4' are aligned and viewed in the same direction and the Ela is also shown in apo-ElaGT and TDP-bound structure.



Supplementary Figure S9. B-factor putty representation of the overall structure of ElaGT in different crystal forms.

A) structure of ElaGT in complex with Ela; the loop involved in the back-to-back interaction on CTD is marked with red circle. B) structure of ElaGT in apo-form; C), structure of ElaGT in complex with TDP; D), structure of ElaGT in complex with UDP. Two highly variant regions FL1 and FL2 are marked. FL1 are missing from the electron density map in the apo-form and UDP-binding form. FL2 are missing from the electron density map in the Ela-binding and apo-form structures. The N-/C- terminal ends both are marked. The structures are colored from blue to red, and the width of the tube indicates B-factors from low to high.



Supplementary Figure S10. Ni-affinity purification results of ElaGT and the mutants. The mutations of W345 to Ala, Ser and Asp, totally abolish the protein soluble expression, and Leu or His replacement at this site reduced the expression yield greatly. M, P, S, FT, W1, W2, E are short for marker, precipitant, supernatant, flow through, wash1, wash2, and eluted.

Supplementary Table S1. The RMSD values between the individual molecules in the asymmetric unit.

Ela-binding form

RMSD (Å)	A	B	C	D	E	F	G
A	0	0.2	0.24	0.12	0.16	0.13	0.13
B		0	0.08	0.17	0.16	0.16	0.24
C			0	0.12	0.12	0.11	0.22
D				0	0.08	0.08	0.14
E					0	0.08	0.13
F						0	0.16
G							0

apo-form

RMSD (Å)	A	B	C
A	0	0.1	0.09
B		0	0.1
C			0

TDP-binding form

RMSD (Å)	B
A	0.09

UDP-binding form

RMSD (Å)	A	B	C
A	0	0.11	0.14
B		0	0.17
C			0

Supplementary Table S2. Kinetic parameters of ElaGT protein binding with Ela.

	K_{D1} (μM)	K_{D2} (μM)	Full χ^2	R^2
WT 1:1 model	3.13	-	0.0334	0.9832
	3.27	-	0.0664	0.9177
	3.41	-	0.0494	0.9
	3.27±0.14[a]			
WT 2:1 HL model	3.55	2.98	0.0193	0.9903
	4.99	2.78	0.0524	0.9351
	7.00	1.46	0.0342	0.9306
	5.18±3.65[a]	2.41±1.57[a]		
M99A 1:1 model	1.63	-	0.0159	0.9548
	0.67	-	0.0163	0.9542
	1.87	-	0.0369	0.9394
	1.39±0.64[a]			

[a]Errors are standard deviation from three independent experiments.

Method

The equations used for the fitting in the BLI

For 1:1 model, the full fitting solution for a 1:1 binding is:

Association phase:

$$y = R_{max}\frac{1}{1+\frac{k_d}{k_{a*[\text{Analyte}]}}}(1-e^{-(k_a*[\text{Analyte}]+k_d)x})$$

Dissociation phase:

$$y = y_0 e^{-kd(x-x0)}$$

$$y_0 = R_{max}\frac{1}{1+\frac{k_d}{k_{a*[\text{Analyte}]}}}(1-e^{-(k_a*[\text{Analyte}]+k_d)x_0})$$

For 2:1 Heterogeneous Ligand Model, the equation used to fit a 2:1 binding interaction is a combination of two 1:1 curve fits, with an additional parameter to account for percentage of binding contributed by each interaction.

References:

[1] Corpet, F., 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16, 10881–10890

[2] Gouet, P., Courcelle, E., Stuart, D.I., Metoz, F., 1999. ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics 15, 305–308

[3] Wallace, A. C., Laskowski, R. A., and Thornton, J. M., 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. 8, 127–134