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Supporting information for article:

Structural insight into the hydrolase and synthase activities of an alkaline α -galactosidase from *Arabidopsis* from complexes with substrate/product

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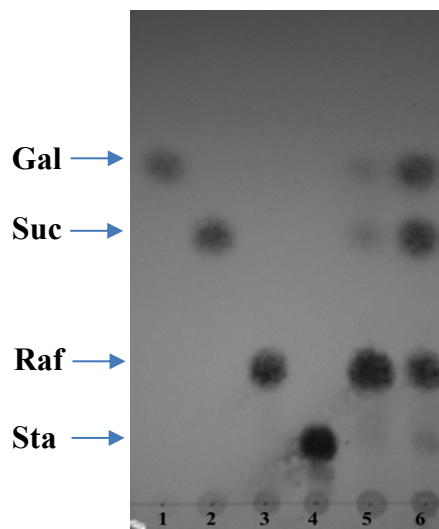


Figure S1 Thin-layer chromatography (TLC) analysis. Hydrolysis reaction of AtAk α Gal3, compared with rice raffinose synthase, toward raffinose as a substrate. Lane 1: standard (std.) galactose (Gal); lane 2: std. sucrose (Suc); lane 3: std. raffinose (Raf); lane 4: std. stachyose (Sta); lane 5: raffinose incubated with rice raffinose synthase; lane 6: raffinose incubated with AtAk α Gal3.

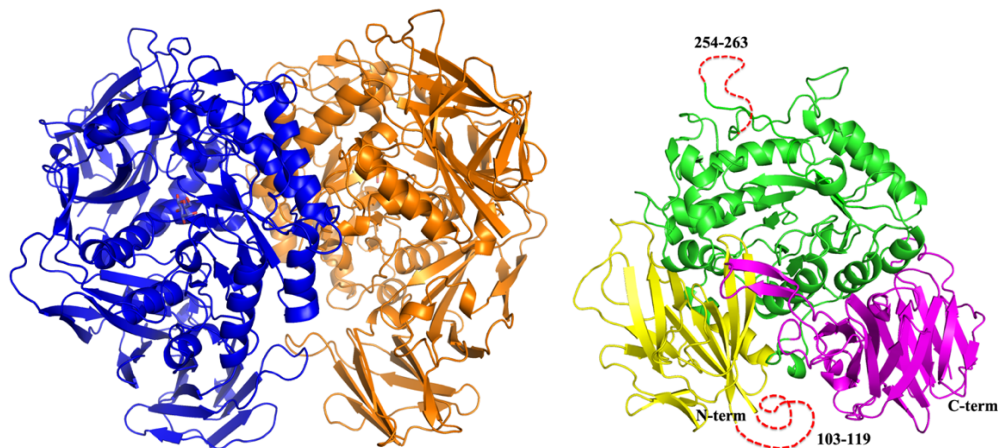


Figure S2 Crystal structure of AtAkαGal3. (A) Two AtAkαGal3 molecules (blue and orange) exist in one asymmetric unit. (B) The overall structure of a functional monomer is composed of three domains: the N-terminal domain (yellow), the catalytic domain (green) and the C-terminal domain (magenta). Disordered residues 103-119 and 254-263 without defined electron density are shown in red dashed lines.



Figure S3 Alignment of the catalytic region of acidic α-galactosidases with known structures. PDB 1UAS: α-galactosidase from *Oryza sativa*, 1R46: human α-galactosidase, 1KTB: chicken α-N-acetylgalactosaminidase, 6F4C: α-galactosidase from *Nicotiana benthamiana*, 4NFJ: α-galactosidase from *Bacteroides fragilis*, 3LRK: α-galactosidase from *Saccharomyces cerevisiae*, 1T00: α-galactosidase from *Trichoderma reesei*.

	380	390	440	450
AtAkaGal3	GVDGVKVDV	QC VLE T L	QA AV I RA S	DD F Y P
Alk_Oryza_sativa	GIDGVKVDV	QN ILE T L	RS AV V RA S	DD F W P
Alk_I_Melon	GVDGVKVDV	QN ILE T L	RN AV I RA S	DD F W P
Alk_Tomato	GIDGVKVDV	QN ILE T L	RS AV I RA S	DD F W P
A.thaliana_seed_imbibition	GVDGVKVDV	QN ILE T L	KT AV I RA S	DD F W P
Rice_seed_imbibition	GVDGVKVDV	QN IIE T L	QT AV V RA S	DD F Y P
Raf_II_Melon	GIDGVKVDV	QN IIE T L	QT AV V RA S	DD Y Y P
Raf_Soybean	GVDGVKVDV	QN IIE T L	QT AIV RA S	DD F Y P
Raf_Oryza_sativa	GIDGVKVDV	I H LLE M V	AV AL GRV G	DD F W C
Raf_Cucumis	GIDGVKIDV	I H LLE M L	AI S L GRV G	DD F W C
STA_Prunus	GITGVKVDV	I H TLE Y V	QI S I GRV G	DD F W F
STA_Citrus	GITGVKVDV	I H TLE Y V	QI S M GRV G	DD F W F

K X D R x x D D

Figure S4 Alignment of the catalytic region of alkaline α -galactosidase and RFO synthases from different plants. There are AtAkaGal3, alkaline α -galactosidases from rice (AF251068) (Alk_Oryza_sativa), melon (AAM75139) (Alk_I_Melon), tomato (NP_001234763) (Alk_Tamato), and seed imbibition protein from AtAkaGal1 (Atlg55740) (A.thaliana_seed_imbibition), and rice (ABF99470) (Rice_seed_imbibition), RFO synthases from melon (NP_001284472) (Raf_II_Melon), soybean (XP_006576826) (Raf_Soybean), rice (XP_015621501) (Raf_Oryza_sativa), cucumber (AAD02832) (Raf_cucumis), yoshino cherry (PQQ02596) (STA_Prunus) and clementine (XP_006444535) (STA_Citrus), showed two conserved motifs of nucleophile: 381KVD383 and 443RxxDD447. The residue numbers are based on the AtAkaGal3 sequence.

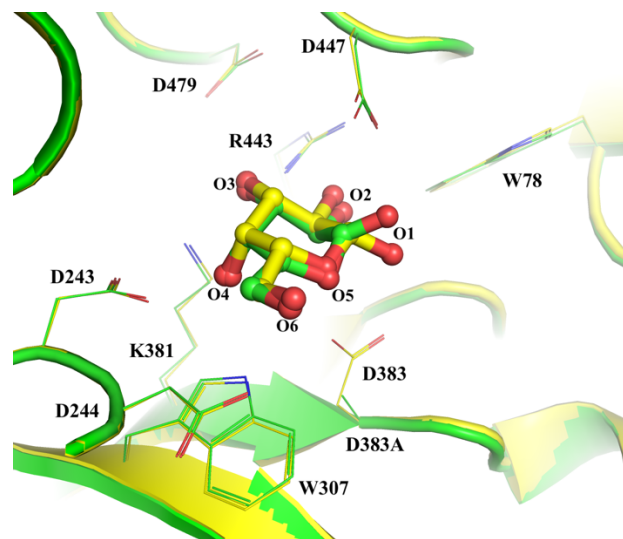


Figure S5 Structural comparison of the mutant D383 and wild-type in complex with galactose. Superimposed two galactose moieties of the D383A mutant (green stick) and wild-type AtAkaGal3 (yellow stick) show that the O1 hydroxyl group at the C1-carbon are in α - and β -anomeric forms, respectively.