

1 **Electronic Supplementary Information**

2 **Sucrose phosphorylase from *Alteromonas mediterranea*: structural**
3 **insight into the regioselective α -glucosylation of (+)-catechin**

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11 **Table of Contents**

12	Molecular modelling of variants of AmSP-WT.....	3
13	Characteristics of AmSP and its variants.....	4
14	Sequence of AmSP-WT including His-tag on C-term.....	4
15	Structural homology between BaSP and AmSP.....	5
16	Determination of the apparent kinetic parameters.....	9
17	(+)-catechin transglycosylation studies.....	13
18	Synthesis yields for AmSP-Q353F and AmSP-P140D.....	13
19	¹ H and ¹³ C NMR Spectral Data of CAT-4' in DMSO.....	15
20		

21 **Molecular modelling of variants of AmSP-WT**

22 Glucosyl-enzyme intermediate 3D-models were built for AmSP-Q353F and AmSP-P140D using the
23 following procedure and the Rosetta software [22]. Glucosylated-aspartyl 192 residue from chain
24 A of crystal structure of BaSP-WT (PDB: 2GDV-A) was inserted into the crystal structure AmSP-WT
25 (PDB: 7ZNP) that served as initial template for both variants. As this glucosylated aspartyl is a non-
26 standard residue, it was absent from the database of the Rosetta software. Using Pymol3, the
27 initial coordinates of this modified residue were retrieved. While this residue (D192) and the
28 glucose moiety (BGC) are covalently linked in the crystal structure, the Pymol software considered
29 them as two distinct residues. Thus, they were merged them into a single non-standard residue,
30 which was called with a new ID, DGC. Associated charges and rotamers were calculated for this
31 new residue using the Rosetta software. All those data were merged a single file and were added
32 into the Rosetta database (Section 2.7).

33 With the DGC residue ready to be used, glucosyl-intermediates were built for the two variants of
34 AmSP-WT. From the crystal structure (PDB: 7ZNP), using Rosetta, the native aspartyl residue in
35 position 203 was mutated by the glucosylated-aspartyl DGC residue together with either the
36 P140D or Q353F mutation. For each variant (AmSP-Q353F or AmSP-P140D), a sample of 50
37 conformers was generated thanks to the program Backrub from Rosetta suite, with 10 000 tries. In
38 parallel, 12 conformers of (+)-catechin were also generated using the Mercury software (CCDC)
39 [23] from the crystal structure OZIDOR of (+)-catechin.

40

41 Characteristics of AmSP and its variants

42 Sequence of AmSP-WT including His-tag on C-term

43 1 MGSIRNGVQL ITYADRLGDG NIESLTNLLD GPLKGLFKGV HILPFYYPYD GEDAGFDPID
44 61 HTTPVDERLGD WNNIKKLGES VDIMADLIVN HMSGQSEAFD DVLKKGRESE YWPLFLTKED
45 121 VFSGNDQAEI DEQIAKVFRP RPTPFSDYE VGIETDSTET VPFWTTFTSN QIDIDVESEL
46 181 GKEYLSSILQ SFTESNVDLI RLDAAGYAIK RAGSNCFMLE ETFEFIEALS KRARTMGMQC
47 241 LVEIHSYQT QIDIAARCDS VYDFALPPLV LHTLFTK DAS ALAHWLSISP RNCFTVLDTH
48 301 DGIGIVDVG A SGDKPGLISA DAINALVEQI HVNSNGESKK ATGAAANNVD LYQVNCTYYD
49 361 ALGKDDFAYL VARAIQFFSP GIPQVYYGGL LAAHNDMELL ANTNVGRDIN RPYLTTAMVE
50 421 DAIQKPVVKG LMQILITRNE NKAFFGAFDV TYTDNTLVLS WSNLDGDAASL TVDFAAMDAT
51 481 INTVSNGEES TSLIGALLAH HHHHH

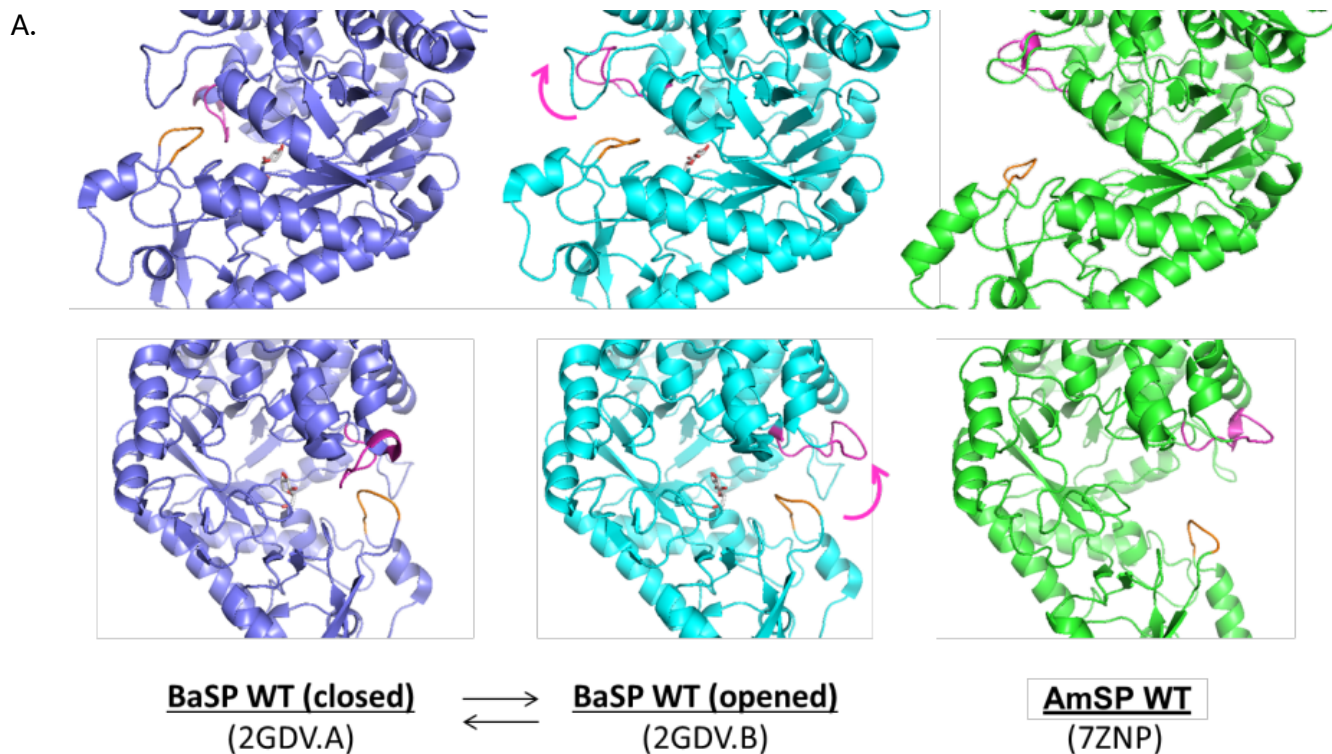
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53 Table S1: List of primers used for quickchange mutagenesis

Primer 1	CAAATTGCGAAAGTTTTTCGTGATCGTCCGACCCCGTTCTTTAGC
Primer 2	GCTAAAGAACGGGGTCGGACGATCACGAAAACTTTTCGCAATTTG
Primer 3	CGAACAACGTGGACCTGTACTTTGTTAACTGCACCTACTATGATG
Primer 4	CATCATAGTAGGTGCAGTTAACAAAAGTACAGGTCCACGTTGTTCG

54

55 **Structural homology between BaSP and AmSP**



B.

<i>BaSP</i> -WT	336	A	A	A	S	N	L	D	L	Y	Q
<i>AmSP</i> -WT	344	A	A	A	S	N	L	D	L	Y	Q

C.

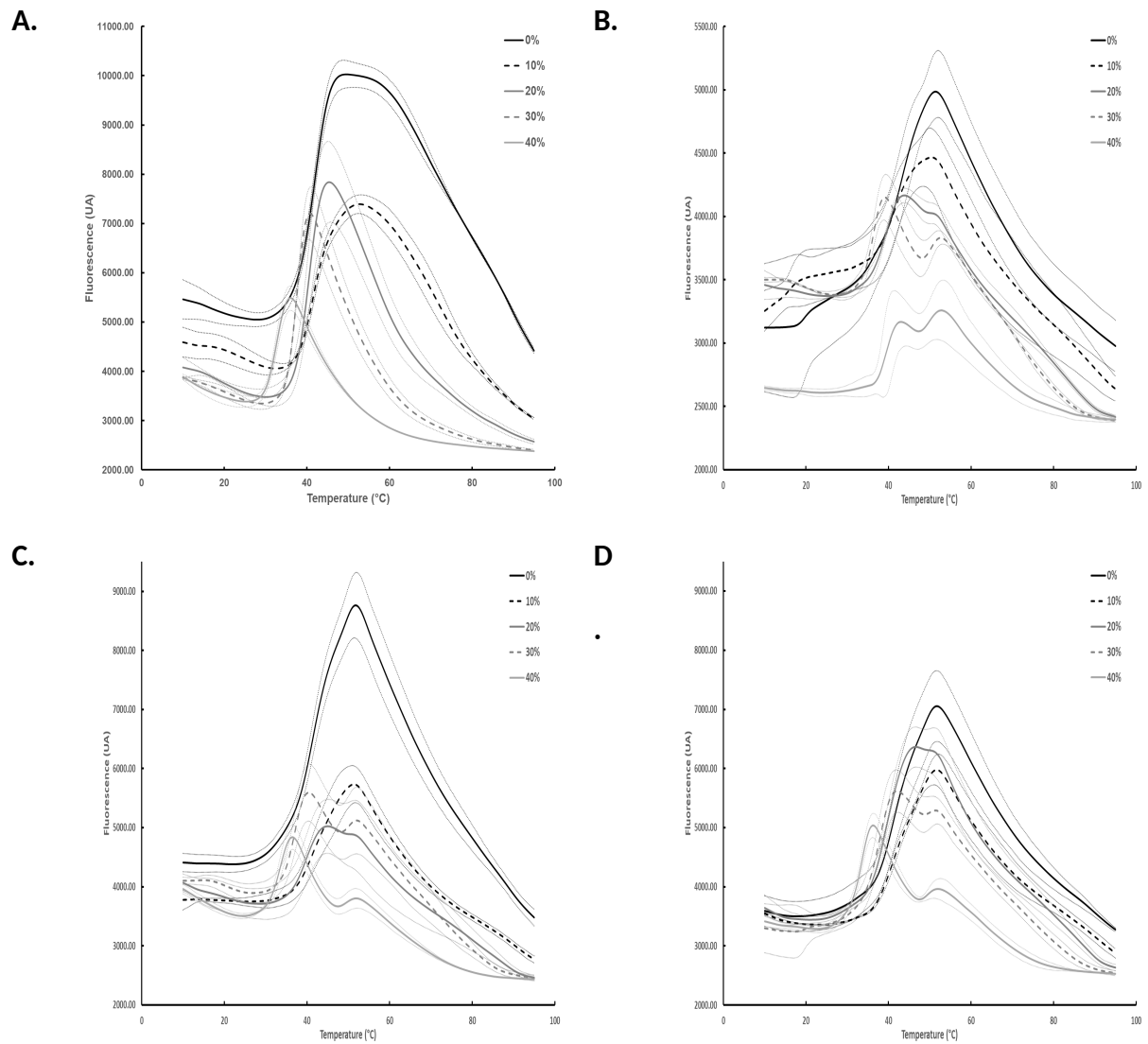
<i>BaSP</i> -WT	132	Y	R	P	R	P
<i>AmSP</i> -WT	138	F	R	P	R	P

56 **Figure S1: Structural homology between BaSP-WT and AmSP-WT.** (A) Comparison of closed
 57 (purple, PDB: 2GDV.A) and opened (cyan, PDB: 2GDV.B) conformations of *BaSP*-WT and structure
 58 of *AmSP*-WT (green, PDB: 7ZNP). The functional loops are featured in magenta for loop A (involved
 59 in sucrose binding) and in orange for loop B (involved with polyphenol binding). (B) Sequence
 60 comparison for loop A between *BaSP*-WT and *AmSP*-WT. (C) Sequence comparison for loop B
 61 between *BaSP*-WT and *AmSP*-WT. *: these residues are involved in the binding of phosphate.

62

63 **Table S2: Conserved residues and potential substrate interaction of AmSP.** Potential substrate
64 interaction and function of conserved residues were determined using structural homology
65 between crystal structures of apoenzyme AmSP-WT (PDB: 7ZNP), apoenzyme BaSP-WT (1R7A) and
66 open conformation BaSP-WT (2GDV.B). Residue numbering of AmSP derived from crystal structure
67 7ZNP. Residue numbering of BaSP came from crystal structures 1R7A and 2GDV.B. Numbered OH-
68 groups and C-atoms address to sucrose (apostrophe for fructosyl moiety), unless stated otherwise.
69 ^a: residues from the -1 subsite, ^b: residues from the Loop A/+1 subsite, ^c: residues from the Loop
70 B/+1 subsite. Approx.: Approximately the same position.

AmSP	BaSP	Conserved Conformation 1R7A/2GDV.B	Potential substrate interaction/remarks	Potential function
Asp203 ^a	Asp192	Yes	Hydrogen bond with OH6	catalytic nucleophile
Glu243 ^a	Glu232	Yes	Hydrogen bond with OH1 and OH1'	general acid/base catalyst
Asp301 ^a	Asp290	Yes	Hydrogen bond with OH2	transition state stabiliser
Phe56 ^a	Phe53	Yes	hydrophobic/ π interaction with C3-C4-C5; Cation- π interaction with oxocarbenium ion-like transition state	hydrophobic platform
Phe167 ^a	Phe156	Yes	hydrophobic/ π interaction with C6 and C1'	hydrophobic platform
His91 ^a	His88	Approx.	Hydrogen bond with OH6	binding of glycosyl moiety
Arg201 ^a	Arg190	Yes	Hydrogen bond with OH2	binding of glycosyl moiety
His300 ^a	His289	Yes	Hydrogen bond with OH2 and OH3	binding of glycosyl moiety
Asp53 ^a	Asp50	Yes	Hydrogen bond with OH4	binding of glycosyl moiety
Arg407 ^a	Arg399	Yes	Hydrogen bond with OH3 and OH4	binding of glycosyl moiety
Gln171	Gln160	Approx.	Hydrogen bond with OH6	binding of glycosyl moiety
Ala204	Ala193	Yes	hydrophobic interaction with C6 and C1'	binding of glycosyl/fructosyl moiety
Leu351 ^b	Leu341	No	hydrophobic interaction with C6 and C6'	binding of glycosyl/fructosyl moiety
Tyr207	Tyr196	Approx.	hydrophobic/ π interaction with C1'	involved in fructose binding
Asp350 ^b	Asp342	No/Approx.	Hydrogen bond with OH4'	involved in fructose binding
Gln353 ^b	Gln345	Yes	Hydrogen bond with O3' and O6'	involved in fructose binding
Phe138 ^c	Tyr132	Approx.	at the entrance of active site; hydrophobic surroundings	Specificity for fructose
Pro140 ^c	Pro134	Approx.	in vicinity of C4-OH of fructose	involved in the binding of the fructose-bound phosphate group
Tyr207	Tyr196	Approx.	CE2-atom close to C1 of fructose	Specificity for fructose/phosphate
His245	His234	Yes	in vicinity of C3 and C3-OH of fructose	Specificity for fructose/phosphate
Arg141 ^c	Arg135	No/Approx.	Hydrogen bond with phosphate O; Specificity for fructose/phosphate	involved in phosphate binding
Leu351 ^b	Leu343	No/Yes	in between important residues Asp342 and Tyr344	Specificity for phosphate
Tyr352 ^b	Tyr344	No/Yes	Hydrogen bond with phosphate O	involved in phosphate binding
Ile244	Val233	Yes	next to the acid/base catalyst; side chain turned away from active site	unknown

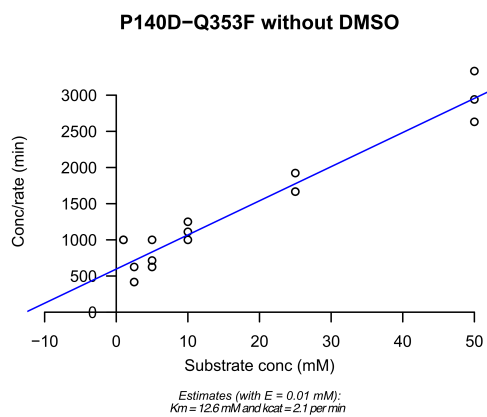
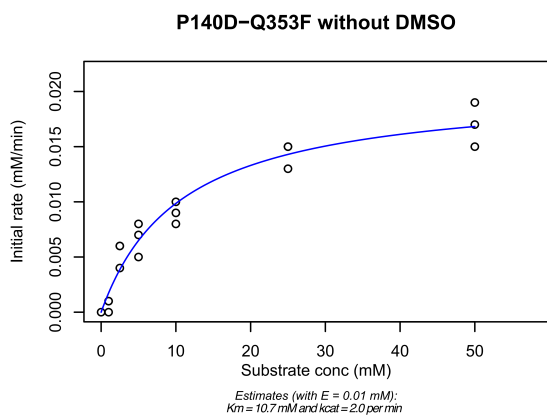
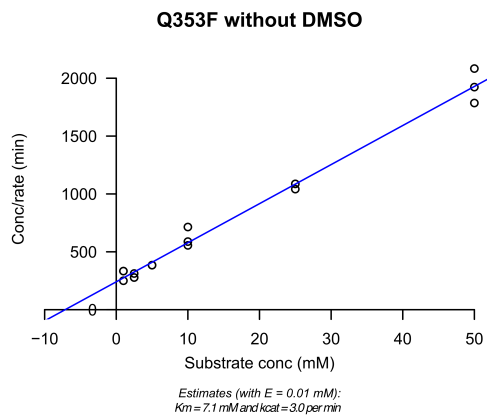
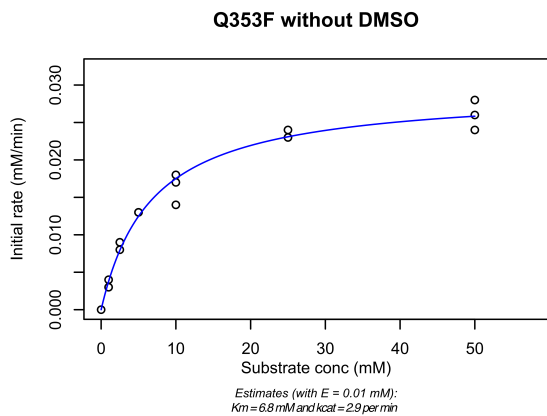
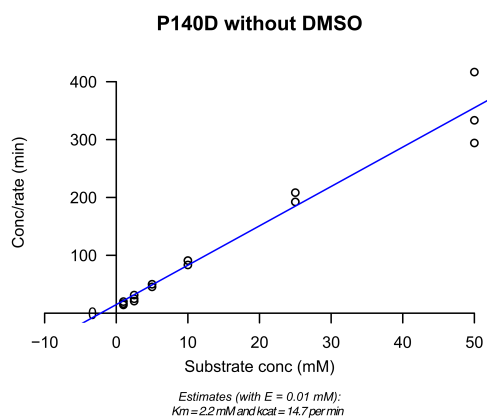
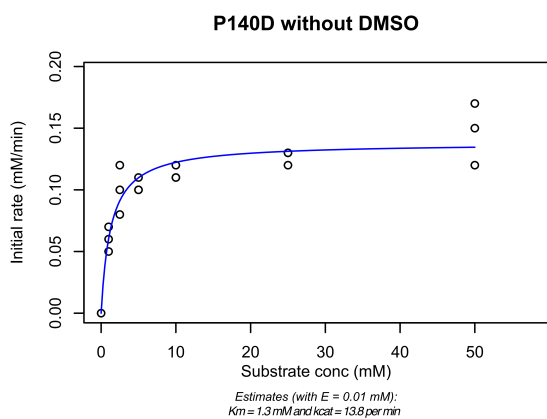
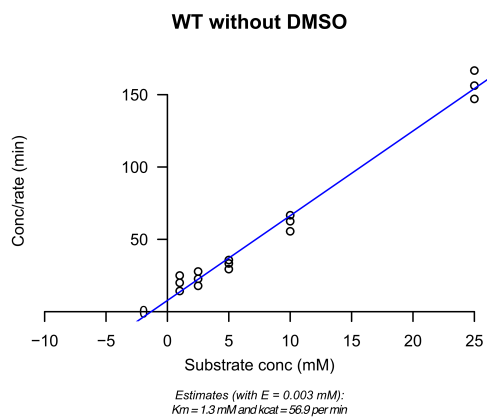
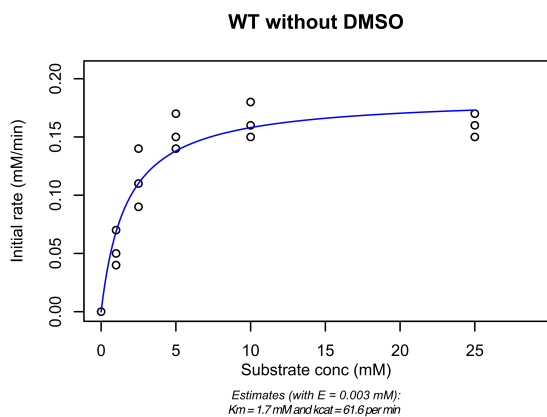


72 **Figure S2: Effect of DMSO on thermal stability of AmSP and its variants: (A) AmSP-WT, (B) AmSP-**
 73 **P140D, (C) AmSP-Q353F, (D) AmSP-P140D/Q353F.** Representative melting curve from 0% to 40%
 74 of DMSO (v/v) were obtained in a final volume of 25 μ L in MOPS-NaOH 50 mM pH 8.0 with 3 μ M
 75 of enzyme and Sypro orange 5x (n=3). The multiphase curve in presence of DMSO suggests the
 76 presence of a multi-domain protein or protein aggregation. Compared to the single curve obtained
 77 without DMSO, increasing DMSO concentration is shown to increase the destabilization of the
 78 enzymes.

79 **Table S3: Melting temperature of AmSP and its variants.** Values, given in °C, were obtained by
 80 calculating the first derivative $-(dRFU)/dT$ of the melting curves.

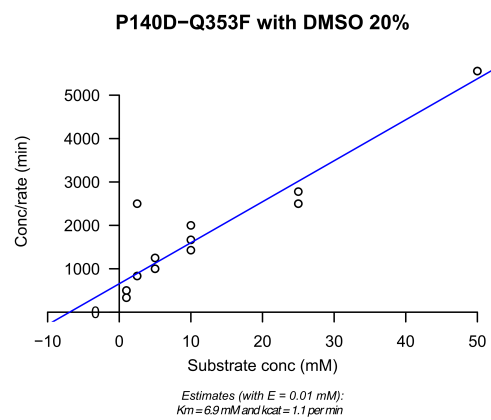
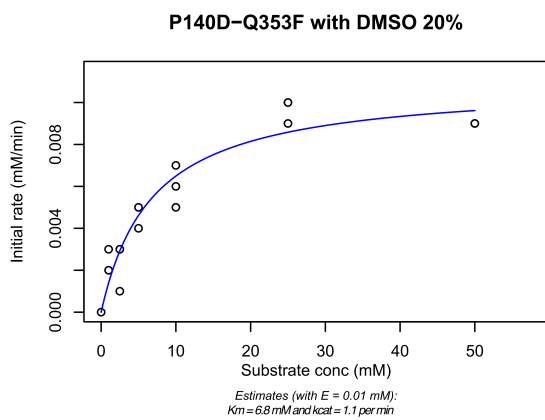
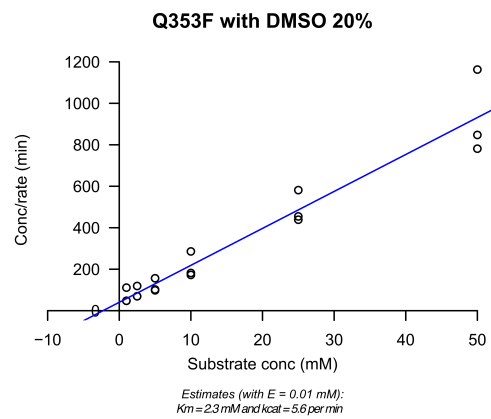
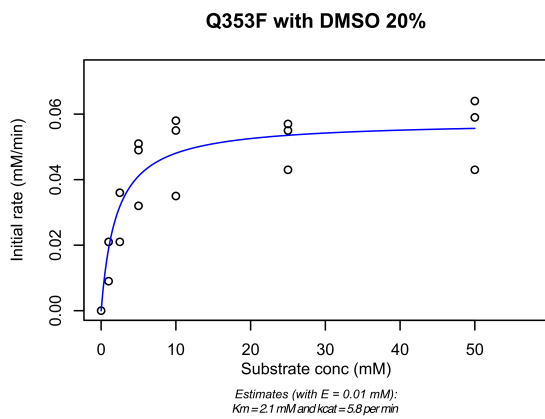
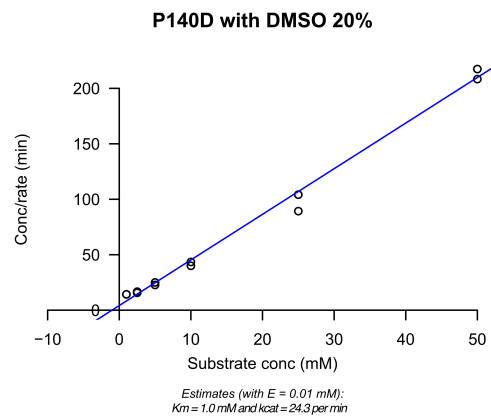
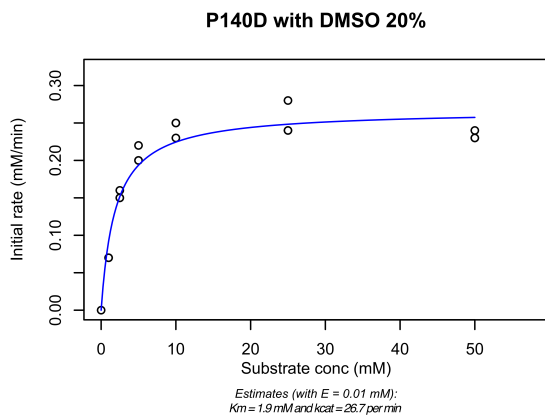
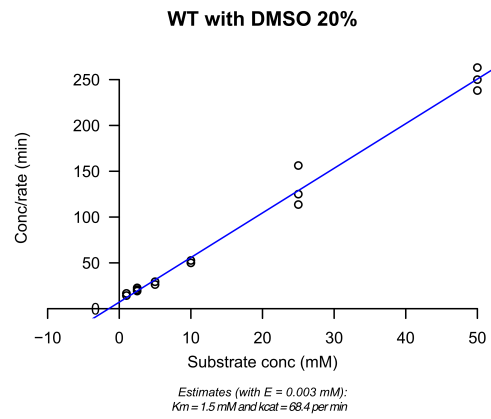
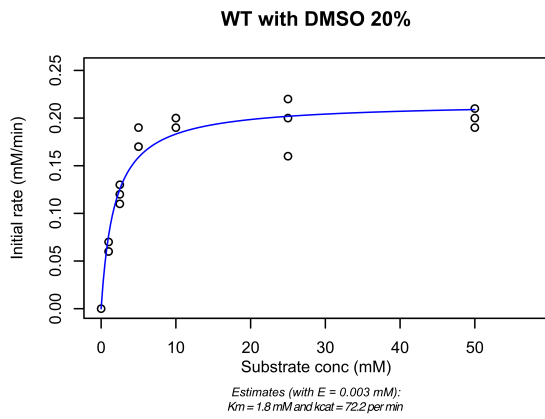
	WT	P140D	Q353F	P140D/Q353F
H₂O	42.0 ± 0.0	42.8 ± 0.3	42.0 ± 0.9	42.2 ± 0.3
NPI-5	42.7 ± 0.3	42.5 ± 0.0	42.2 ± 0.3	41.5 ± 0.0
NPI-250	38.7 ± 0.3	38.2 ± 0.3	38.3 ± 0.3	37.3 ± 0.3
MOPS pH 7.0	44.8 ± 0.6	45.2 ± 0.3	44.0 ± 0.0	43.7 ± 0.3
pH 7.0 10%D	42.5 ± 0.0	42.2 ± 0.3	41.8 ± 0.3	42.3 ± 0.3
pH 7.0 20%D	39.5 ± 0.0	39.0 ± 0.0	39.2 ± 0.3	39.5 ± 0.0
pH 7.0 30%D	36.3 ± 1.0	36.3 ± 0.3	36.5 ± 0.5	36.7 ± 0.3
pH 7.0 40%D	31.3 ± 0.3	32.5 ± 0.0	32.7 ± 0.3	33.2 ± 0.3
MOPS pH 8.0	42.0 ± 0.0	43.0 ± 0.0	41.5 ± 0.0	41.2 ± 0.3
pH 8.0 10%D	41.3 ± 0.3	41.3 ± 0.4	41.5 ± 0.5	40.8 ± 1.0
pH 8.0 20%D	40.5 ± 0.0	39.3 ± 0.6	39.8 ± 0.3	39.5 ± 0.5
pH 8.0 30%D	37.5 ± 0.5	39.0 ± 0.7	37.0 ± 0.0	38.0 ± 0.5
pH 8.0 40%D	32.7 ± 0.3	39.5 ± 1.4	33.7 ± 0.3	33.7 ± 0.3
Citrate	45.5 ± 0.0	43.8 ± 0.4	43.5 ± 0.0	43.3 ± 0.3
HEPES	43.5 ± 0.3	44.0 ± 0.0	42.2 ± 0.6	42.2 ± 0.6
HEPES NaCl DTT	44.8 ± 0.6	45.0 ± 0.0	43.7 ± 0.8	44.3 ± 0.3

81 **Determination of the apparent kinetic parameters**



83 **Figure S3: Michaelis-Menten and Hanes-Woolf plots of sucrose hydrolysis in absence of DMSO**
84 **obtained with AmSP-WT and its variants (P140D, Q353F and P140D/Q353F).** Data were obtained
85 by glucose titration using GOD/POD method of the reaction medium that reacted for 3h at 25°C
86 and that contained the SP enzyme (10 μ M for variants and 3 μ M for WT) and 1mM to 50 mM of
87 sucrose, in MOPS-NaOH 50 mM pH 8.0 (n=3). Kinetic data were fitted to Michaelis-Menten non-
88 linear model and Hanes-Woolf linear model using respectively *nlm* and *lm* functions implemented
89 in R to estimate the kinetic parameters.

90



91

92 **Figure S4: Michaelis-Menten and Hanes-Woolf plots of sucrose hydrolysis obtained with AmSP-**

93 **WT and its variants (P140D, Q353F and P140D/Q353F) in presence of 20% DMSO (v/v). Data**

94 were obtained by glucose titration using GOD/POD method of the reaction medium that reacted
95 for 3h at 25°C and that contained the SP enzyme (10 μ M for variants and 3 μ M for WT), 1mM to
96 50 mM of sucrose and DMSO 20% (v/v), in MOPS-NaOH 50 mM pH 8.0 (n=3). Kinetic data were
97 fitted to Michaelis-Menten non-linear model and Hanes-Woolf linear model using respectively *nlm*
98 and *lm* functions implemented in R to estimate the kinetic parameters

99 **(+)-catechin transglycosylation studies**

100 **Synthesis yields for AmSP-Q353F and AmSP-P140D**

101 **Table S4: Compounds concentration obtained during (+)-catechin glucosylation by AmSP-Q353F**
 102 **and AmSP-P140D.** Compound concentrations were calculated from the area under the curves
 103 obtained by analytical HPLC on a C-18 column for 7 h and at 24 h of incubation at 25°C of the
 104 reaction mixture in a final volume of 1 mL in MOPS-NaOH 50 mM pH 8.0 with 10 µM of enzyme,
 105 80 mM sucrose and 20% DMSO (v/v) (n=3).

AmSP-Q353F

Time (h)	CAT-3' (mM)	CAT-4' (mM)	CAT-5 (mM)	CAT-3',5 (mM)	(+)-catechin (mM)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00
1	1.12 ± 0.12	0.04 ± 0.00	0.04 ± 0.00	0.00 ± 0.00	8.80 ± 0.13
2	2.26 ± 0.22	0.08 ± 0.01	0.08 ± 0.01	0.00 ± 0.00	7.58 ± 0.23
3	3.23 ± 0.16	0.12 ± 0.01	0.11 ± 0.01	0.01 ± 0.00	6.53 ± 0.18
4	4.00 ± 0.14	0.15 ± 0.01	0.13 ± 0.01	0.01 ± 0.00	5.70 ± 0.16
5	4.74 ± 0.25	0.18 ± 0.02	0.16 ± 0.01	0.02 ± 0.00	4.90 ± 0.28
6	5.37 ± 0.21	0.20 ± 0.01	0.18 ± 0.01	0.03 ± 0.00	4.22 ± 0.23
24	8.36 ± 0.13	0.36 ± 0.01	0.21 ± 0.02	0.20 ± 0.02	0.88 ± 0.15

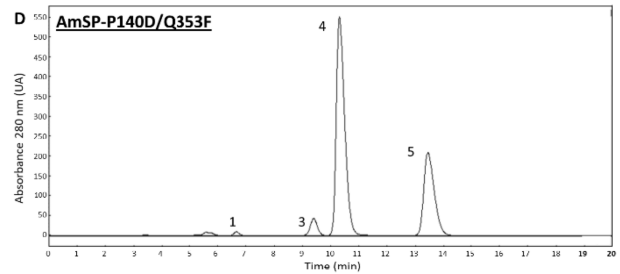
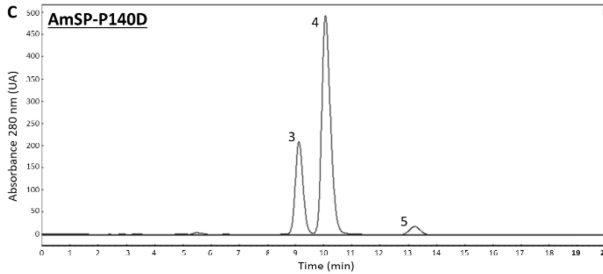
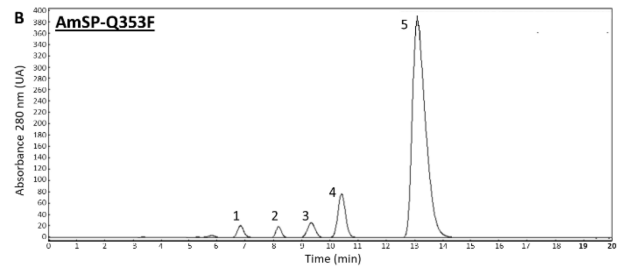
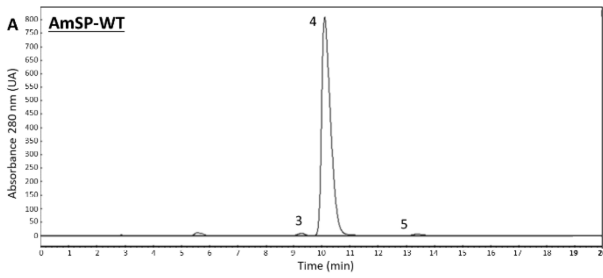
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AmSP-P140D

Time (h)	CAT-3' (mM)	CAT-4' (mM)	(+)-catechin (mM)
0	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00
1	0.05 ± 0.00	0.40 ± 0.02	9.55 ± 0.02
2	0.10 ± 0.00	0.74 ± 0.02	9.17 ± 0.02
3	0.12 ± 0.00	0.99 ± 0.02	8.88 ± 0.02
4	0.15 ± 0.00	1.19 ± 0.03	8.65 ± 0.03
5	0.17 ± 0.00	1.36 ± 0.06	8.47 ± 0.06
6	0.19 ± 0.01	1.49 ± 0.07	8.29 ± 0.03
24	0.31 ± 0.02	2.50 ± 0.19	7.19 ± 0.21

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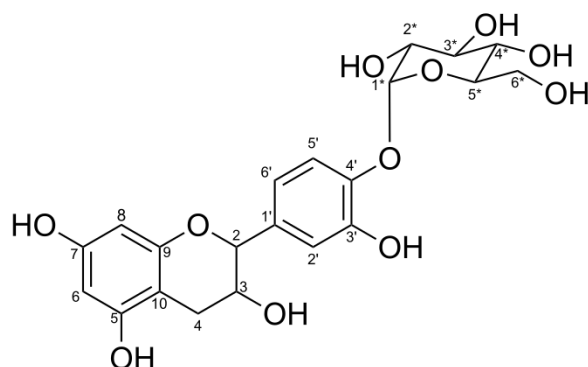
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111 **Figure S5: HPLC chromatogram of a 24 h reaction medium of (+)-catechin glucosylation by AmSP-**
 112 **WT and its variants. Peak 1: CAT-5, peak 2: CAT-3',5, peak 3: CAT-4', peak 4: (+)-catechin, peak 5:**
 113 **CAT-3'. Unassigned peaks correspond to impurities and degradation of (+)-catechin. (10 μ M**
 114 **enzyme, 10 mM (+)-catechin, 80 mM sucrose, 20% DMSO (v/v) in MOPS 50 mM pH 8.0 at 25°C for**
 115 **24 h). HPLC conditions: isocratic mode at 80% H₂O (v/v), 0.1% formic acid (v/v) and 20% MeOH**
 116 **(v/v), 0.1% formic acid (v/v).**

118 ^1H and ^{13}C NMR Spectral Data of CAT-4' in DMSO

119



120

121 **MS (ESI positive):**

122 Ion Formula: $\text{C}_{21}\text{H}_{23}\text{O}_{11}$

123 m/z calculated: 451.1239

124 m/z experimental: 451.1240

125 error [ppm]: -0.2

126

127 ^1H NMR (DMSO- d_6 , δ) : 7.14 (d, $^3J_{5-6} = 8.3$ Hz, 1H, H_5), 6.80 (d, $^3J_{2-6} = 2.0$ Hz, 1H, H_2), 6.72 (dd, $^3J_{6-5} = 8.3$ Hz, 1H, H_6), 5.91 (d, $^3J_{6-8} = 2.2$ Hz, 1H, H_6), 5.71 (d, $^3J_{8-6} = 2.2$ Hz, 1H, H_8),
128 5.17 (d, $^3J_{1*-2*} = 3.6$ Hz, 1H, H_{1*}), 4.55 (d, $^3J_{2-3} = 7.4$ Hz, 1H, H_2), 3.85 (td, $^3J_{3-2} = 7.4$ Hz, $^3J_{3-4a} = 5.3$
129 Hz, $^3J_{3-4b} = 8.4$ Hz, 1H, H_3), 3.71-3.65 (m, $^3J_{3*-2*} = 9.7$ Hz, , 1H, H_{3*}), 3.65-3.62 (m, 1H, H_{6a*}), 3.55-
130 3.49 (m, 1H, H_{5*}), 3.55-3.49 (m, 1H, H_{6b*}), 3.33 (dd, $^3J_{2*-1*} = 3.6$ Hz, $^3J_{2*-3*} = 9.7$ Hz, 1H, H_{2*}), 3.21-
131 3.17 (m, 1H, H_{4*}), 2.65 (dd, $^3J_{4a-3} = 5.3$ Hz, $^2J_{4a-4b} = 16.0$ Hz, 1H, H_{4a}), 2.36 (dd, $^3J_{4b-3} = 8.4$ Hz, $^2J_{4b-4a} = 16.3$ Hz, 1H, H_{4b}).

134

135 ^{13}C NMR (DMSO- d_6 , δ) : 156.5 (C_7), 156.2 (C_5), 155.2 (C_{10}), 147.1 (C_4), 144.7 (C_3), 134.7 (C_1), 118.2
136 (C_6), 117.3 (C_5), 114.8 (C_2), 100.4 (C_{1*}), 99.0 (C_9), 95.2 (C_6), 93.8 (C_8), 80.7 (C_2), 73.8 (C_{5*}), 73.1
137 (C_{3*}), 72.0 (C_{2*}), 70.0 (C_{4*}), 66.3 (C_3), 60.7 (C_{6*}), 27.8 (C_4).

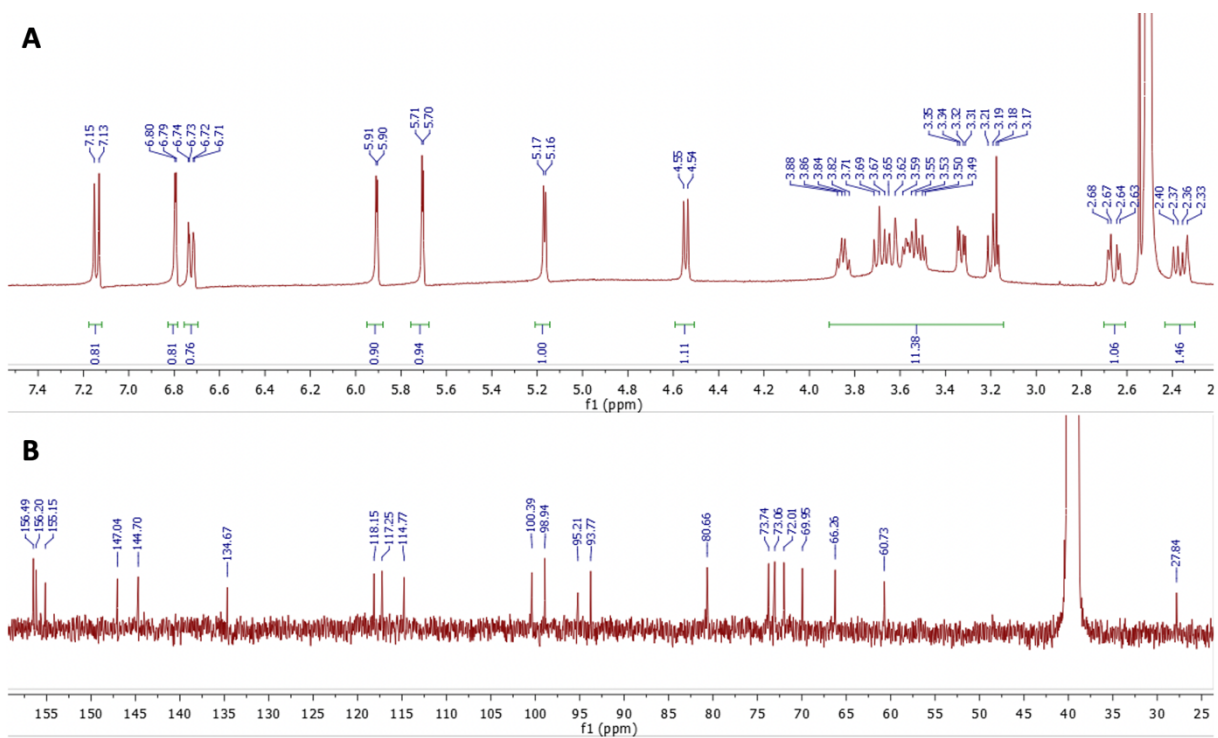
138 **Table S5: Comparison of ¹H and ¹³C NMR Spectrum data of CAT-4' and (+)-catechin in DMSO.**

139 Multiplicity abbreviations for ¹H NMR: d = doublet, dd = doublet of doublets, td = pseudo triplet of

140 doublets, m = multiplet. For characterization of CAT-3', CAT-5 and CAT-3',5 please see [10].

POSITION	CATECHIN δ ¹ H; J (HZ)	CAT-4' δ ¹ H; J (HZ)	CAT-4' δ ¹³ C
1	X	X	X
2	4.48 (d 7.4)	4.55 (d 7.4)	80.67
3	3.82 (td 8.1, 7.4, 5.2)	3.85 (td 8.4, 7.4, 5.33)	66.26
4	2.67 (dd 16.1, 5.2) 2.35 (dd 16.0, 8.1)	2.65 (dd 16.0, 5.3) 2.36 (dd 16.3, 8.4)	27.84
5	X	X	156.21
6	5.89 (d 2.2)	5.91 (d 2.2)	95.22
7	X	X	156.51
8	5.69 (d 2.2)	5.71 (d 2.2)	93.78
9	X	X	98.95
10	X	X	155.17
1'	X	X	134.68
2'	6.73 (d 1.9)	6.80 (d 2.0)	114.79
3'	X	X	144.71
4'	X	X	147.06
5'	6.69 (d 8.1)	7.14 (d 8.3)	117.26
6'	6.60 (dd 8.1, 1.9)	6.72 (dd 8.3, 2.0)	118.16
1*	X	5.17 (d 3.6)	100.40
2*	X	3.33 (dd 9.7, 3.6)	72.02
3*	X	3.71-3.65 (m)	73.06
4*	X	3.21-3.17 (m)	69.96
5*	X	3.59-3.55 (m)	73.75
6*	X	3.65-3.62 (m) 3.55-3.49 (m)	60.73

141

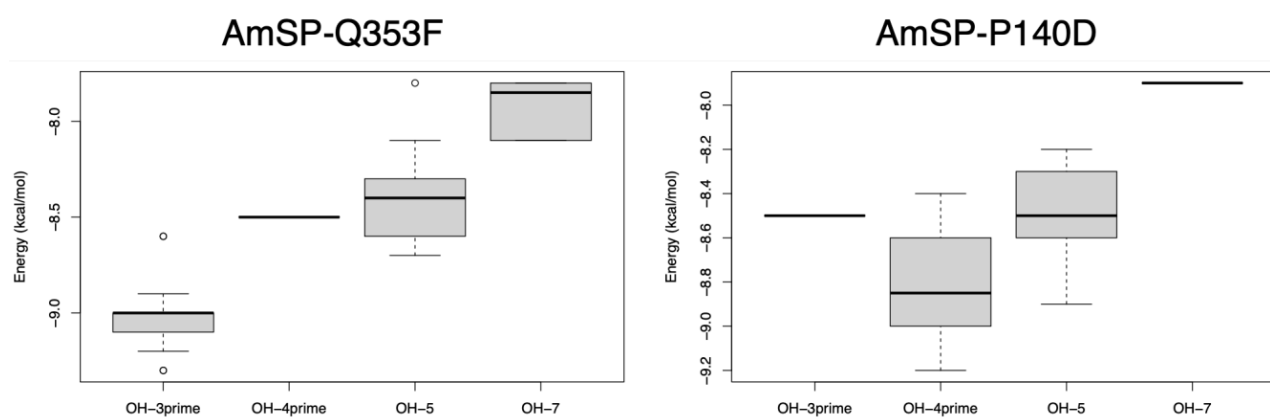


142

143 **Figure S6: NMR spectrum of (+)-catechin-4'-O- α -D-glucoside. (A) ¹H NMR and (B) ¹³C NMR in**

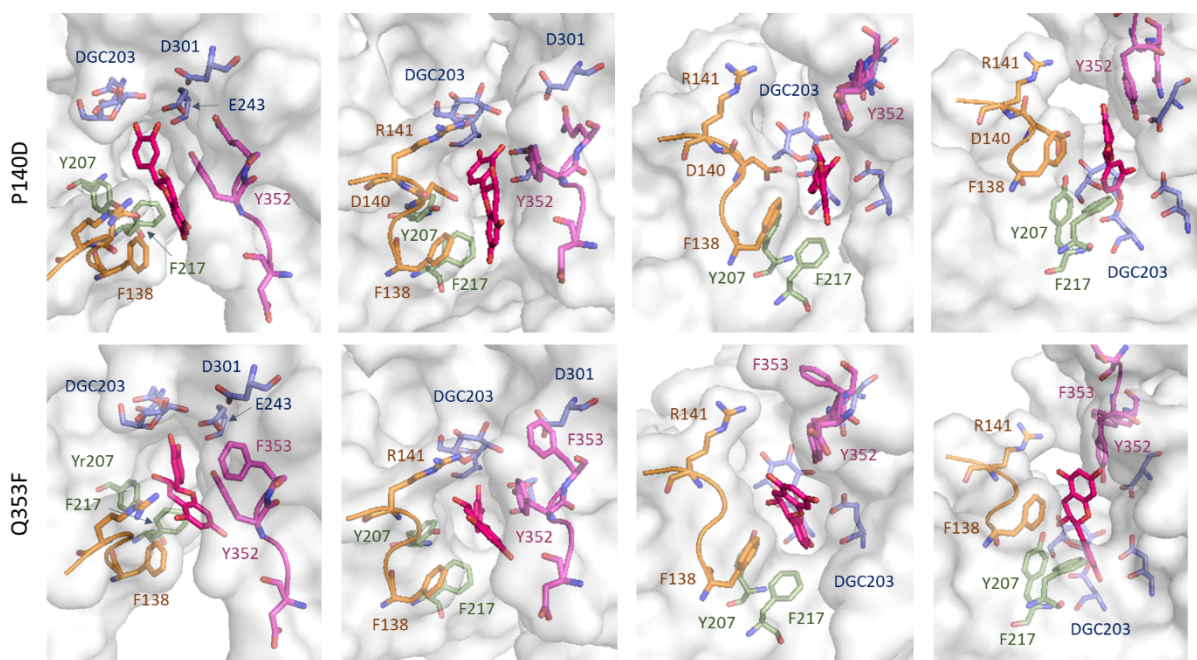
144 DMSO- d_6 (400 MHz).

145



146

147 **Figure S7: Statistical analysis of the binding energies of productive poses for glucosylation of (+)**
 148 **catechin in OH-3', OH-4', OH-5 and OH-7 positions with Q353F and P140D.** Molecular docking
 149 poses were filtered by considering those with an oxygen of (+)-catechin within 3 Å of the C1 atom
 150 of the glucosyl moiety as productive (see sections 2.4, 2.5 and 3.4 in main text for additional
 151 details).



152

153 **Figure S8: Different views of the position of the best productive poses of (+)-catechin in the**
 154 **active site of AmSP-P140D and AmSP-Q353F.** In magenta: Loop A with in sticks
 155 Y352/F350/Q(F)353 residues; orange: Loop B with in sticks R141/F138 and D140 for AmSP-P140D;
 156 blue: residues of the catalytic triad with in sticks DGC203/E243/D301; green: aromatic residues
 157 involved in steric clash with in sticks Y207 and F217; and pink: (+)-catechin.

158