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Supplementary Materials

Simultaneous Optimal Production of Flavonol Aglycones and Degalloylated Catechins from Green Tea Using a Multi-Function Food–Grade Enzyme

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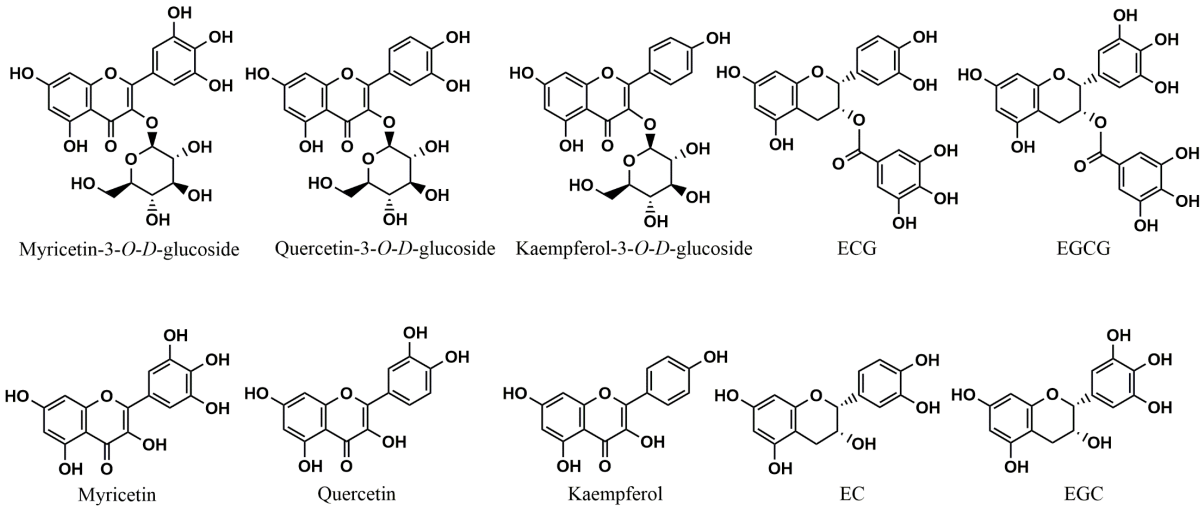

Figure S1. Chemical structures of catechins and flavonols in GTE. The three mono-glycosides of flavonols depicted in this figure are an example of flavonol glycosides in green tea.

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Table S1. Changes of catechins by hydrolysis of glycoside hydrolase (GH) enzymes (numerical values).

Treatments [a]	Gallic acid	Caffeine	EGC	EC	EGCG	ECG	Total catechins [b]
1-AT	N/D [c]	39.65 ± 0.49 a	128.73 ± 1.35 b	29.67 ± 1.06 b	199.52 ± 2.54 a	34.25 ± 0.39 a	392.17 ± 5.07 a
2-50	N/D	38.51 ± 0.93 a	130.62 ± 1.35 b	28.60 ± 1.00 b	198.67 ± 3.48 a	34.76 ± 0.52 a	396.54 ± 5.27 a
AC	N/D	39.85 ± 2.76 a	123.69 ± 6.81 b	30.36 ± 1.77 b	181.10 ± 11.19 a	31.57 ± 1.96 a	366.72 ± 21.72 ab
AN	N/D	38.48 ± 2.18 a	123.63 ± 6.46 b	30.25 ± 1.58 b	186.35 ± 10.78 a	32.76 ± 1.82 a	372.99 ± 20.64 a
BT	N/D	37.41 ± 2.92 a	120.16 ± 8.09 b	28.20 ± 2.33 b	186.56 ± 13.31 a	32.26 ± 2.22 a	367.18 ± 25.94 ab
CF	78.13 ± 3.77 b	41.28 ± 2.72 a	223.89 ± 13.26 a	51.55 ± 3.10 a	26.65 ± 1.97 b	4.53 ± 0.20 b	306.63 ± 18.50 ab
KN	N/D	38.53 ± 2.11 a	124.53 ± 6.45 b	28.99 ± 1.96 b	194.24 ± 10.95 a	33.34 ± 1.79 a	381.10 ± 21.15 a
NS	N/D	38.29 ± 3.18 a	124.73 ± 9.61 b	28.98 ± 2.20 b	193.50 ± 15.00 a	33.01 ± 2.60 a	380.22 ± 29.42 a
TN	93.56 ± 0.26 a	32.87 ± 0.42 a	222.47 ± 1.36 a	44.03 ± 0.23 a	0.89 ± 0.10 b	1.38 ± 0.20 b	268.76 ± 1.43 b
UF	N/D	38.38 ± 3.82 a	125.82 ± 11.02 b	29.72 ± 2.69 b	184.73 ± 15.60 a	31.76 ± 2.78 a	372.03 ± 32.06 ab

Data shown: mean ± SEM (mg/g), the different letters after the values are statistically different by column. [a] Abbreviations: 1-AT, control-ambient temp.; 1–50, control-50 °C; AC, Sumizyme-AC; AN, Cellulyve-AN; BT, Glucosidase-BT; CF, Plantase CF; NS, Peclyve ARA-NS; KN, Cellulase-KN; UF, Plantase-UF; and TN, Tannase KTFHR. [b] Total catechins: sum of EGC, EC, EGCG, and ECG. [c] N/D: not detected.

Table S2. Changes of flavonols by hydrolysis of GH enzymes (numerical values).

Treatments [a]	Myricetin	Quercetin	Kaempferol	Total flavonols [b]
1-AT	N/D [c]	N/D	N/D	N/D
2-50	N/D	N/D	N/D	N/D
AC	0.10 ± 0.01 c	0.08 ± 0.00 de	N/D	0.18 ± 0.01 d
AN	0.10 ± 0.01 c	0.05 ± 0.00 e	N/D	0.15 ± 0.01 d
BT	0.11 ± 0.01 c	0.04 ± 0.02 e	N/D	0.16 ± 0.02 d
CF	3.50 ± 0.22 a	3.95 ± 0.22 a	2.34 ± 0.15 a	9.78 ± 0.59 a
KN	0.34 ± 0.00 c	0.24 ± 0.03 de	N/D	0.58 ± 0.04 d
NS	1.90 ± 0.14 b	0.55 ± 0.04 cd	N/D	2.45 ± 0.18 c
TN	1.46 ± 0.01 b	2.18 ± 0.00 b	1.17 ± 0.00 b	4.81 ± 0.01 b
UF	1.61 ± 0.11 b	0.72 ± 0.04 cd	0.19 ± 0.02 c	2.52 ± 0.17 c

Data shown: mean ± SEM (mg/g), the different letters after the values are statistically different by column. [a] Abbreviations: 1-AT, control-ambient temp.; 1-50, control-50 °C; AC, Sumizyme-AC; AN, Cellulyve-AN; BT, Glucosidase-BT; CF, Plantase CF; NS, Peclyve ARA-NS; KN, Cellulase-KN; UF, Plantase-UF; and TN, Tannase KTFHR. [b] Total flavonols: sum of myricetin, quercetin, and kaempferol. [c] N/D: not detected.

Table S3. Correlation result for producing flavonol aglycones by multivariate analysis.

Variable [a]	by Variable	Correlation	Significant probability
Abf	Mean(F-Sum)	0.9878	0.0122
Xyl	Mean(F-Sum)	0.9197	0.0803
Man	Mean(F-Sum)	0.5711	0.4289
Gal	Mean(F-Sum)	0.4564	0.6983
Tan	Mean(F-Sum)	0.2491	0.6861
Abp	Mean(F-Sum)	0.0743	0.9257

[a] Abbreviations mean activities of enzymes; Abf, α-arabinofuranosidase; Abp, α-arabinopyranosidase; Gal, β-galactosidase; Man, β-mannosidase; Tan, tannin acyl hydrolase; Xyl, β-xylosidase, and F-Sum, sum of flavonol aglycone produced by enzyme.

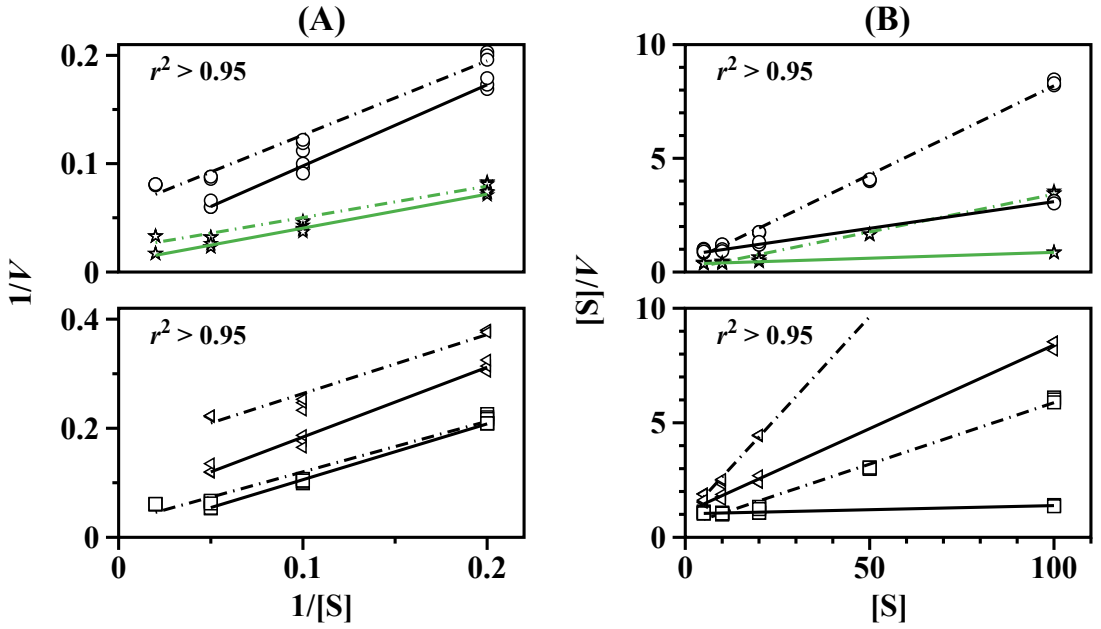

Figure S2. Kinetics of enzyme CF. (**A**) Lineweaver-Burk plot, (**B**) Hanes-Woof plot of enzyme CF reaction with GTE. [S]: substrate concentration (mg/mL), *V*: velocity of producing flavonols ($mg \cdot mL^{-1} \cdot h^{-1}$). Symbols and lines indicate: □, myricetin; ○, quercetin; ◁, kaempferol; ☆, flavonols; dotted line, 35 °C; solid line, 50 °C. Lines were fitted by first order-regression ($r^2 > 0.95$) using Datagraph (Visual Data Tools, Inc., NC, USA).

Table S4. Parameters and result of docking run.

PDB code: 1NHC	PDB code: 4J0C
--- --- GOLD DOCKING RUN (GOLD v5.7.2) --- --- GOLD is Genetic Optimisation for Ligand Docking. GOLD is the result of a collaborative project between Sheffield University, the CCDC and Glaxo Wellcome. GOLD is a proprietary product for which the copyright is held by the Cambridge Crystallographic Data Centre (CCDC) Please forward any comments, bugs, or queries to CCDC Software Support: Cambridge Crystallographic Data Centre 12 Union Road Cambridge CB2 1EZ tel: (44) (1223) 336022 email: support@ccdc.cam.ac.uk --- User: chans Invocation Time: Sat Aug 17 18:23:47 2019 --- --- Parameter setting overview (user-defined or automatic) --- --- maxops automatic setting popsiz automatic setting select_pressure automatic setting n_islands automatic setting niche_siz automatic setting pt_crosswt automatic setting allele_mutatewt automatic setting migratewt automatic setting start_vdw_linear_cutoff defined by user initial_virtual_pt_match_max defined by user --- --- ChemScore parameters--- --- * ChemScore coefficients ZERO_COEFFICIENT: -5.480 HBOND_COEFFICIENT: -3.000 INTERNAL_HBOND_COEFFICIENT: -3.340 METAL_COEFFICIENT: -6.000 LIPO_COEFFICIENT: -0.117 ROT_COEFFICIENT: 2.560 INTRA_COEFFICIENT: 1.000 LINK_BEND_COEFFICIENT: 0.250 PROTEIN_ENERGY_COEFFICIENT: 1.000 CHARGED_HBOND_SCALING: 2.000 CHARGED_METAL_SCALING: 2.000 * Hydrogen-bond term HBOND_FUNCTION: CHEMSCORE_DOCKING R_IDEAL: 1.850 DELTA_R_IDEAL: 0.250 DELTA_R_MAX: 0.650 HBOND_R_SIGMA: 0.000	--- --- GOLD DOCKING RUN (GOLD v5.7.2) --- --- GOLD is Genetic Optimisation for Ligand Docking. GOLD is the result of a collaborative project between Sheffield University, the CCDC and Glaxo Wellcome. GOLD is a proprietary product for which the copyright is held by the Cambridge Crystallographic Data Centre (CCDC) Please forward any comments, bugs, or queries to CCDC Software Support: Cambridge Crystallographic Data Centre 12 Union Road Cambridge CB2 1EZ tel: (44) (1223) 336022 email: support@ccdc.cam.ac.uk --- User: chans Invocation Time: Sat Aug 17 22:12:51 2019 --- --- Parameter setting overview (user-defined or automatic) --- --- maxops automatic setting popsiz automatic setting select_pressure automatic setting n_islands automatic setting niche_siz automatic setting pt_crosswt automatic setting allele_mutatewt automatic setting migratewt automatic setting start_vdw_linear_cutoff defined by user initial_virtual_pt_match_max defined by user --- --- ChemScore parameters--- --- * ChemScore coefficients ZERO_COEFFICIENT: -5.480 HBOND_COEFFICIENT: -3.000 INTERNAL_HBOND_COEFFICIENT: -3.340 METAL_COEFFICIENT: -6.000 LIPO_COEFFICIENT: -0.117 ROT_COEFFICIENT: 2.560 INTRA_COEFFICIENT: 1.000 LINK_BEND_COEFFICIENT: 0.250 PROTEIN_ENERGY_COEFFICIENT: 1.000 CHARGED_HBOND_SCALING: 2.000 CHARGED_METAL_SCALING: 2.000 * Hydrogen-bond term HBOND_FUNCTION: CHEMSCORE_DOCKING R_IDEAL: 1.850 DELTA_R_IDEAL: 0.250 DELTA_R_MAX: 0.650 HBOND_R_SIGMA: 0.000

ALPHA_IDEAL: 180.000	ALPHA_IDEAL: 180.000
DELTA_ALPHA_IDEAL: 30.000	DELTA_ALPHA_IDEAL: 30.000
DELTA_ALPHA_MAX: 80.000	DELTA_ALPHA_MAX: 80.000
HBOND_ALPHA_SIGMA: 0.000	HBOND_ALPHA_SIGMA: 0.000
BETA_IDEAL: 180.000	BETA_IDEAL: 180.000
DELTA_BETA_IDEAL: 80.000	DELTA_BETA_IDEAL: 80.000
DELTA_BETA_MAX: 100.000	DELTA_BETA_MAX: 100.000
HBOND_BETA_SIGMA: 0.000	HBOND_BETA_SIGMA: 0.000
USE_HBOND_STRENGTHS = OFF	USE_HBOND_STRENGTHS = OFF
CH... O term: SPECIAL	CH... O term: SPECIAL
CHO_COEFFICIENT (kJ/mol): -3.000	CHO_COEFFICIENT (kJ/mol): -3.000
CHO_CLASH_RADIUS (Angstrom): 2.400	CHO_CLASH_RADIUS (Angstrom): 2.400
CHO_R_IDEAL (Angstrom): 2.350	CHO_R_IDEAL (Angstrom): 2.350
CHO_DELTA_R_IDEAL (Angstrom): 0.250	CHO_DELTA_R_IDEAL (Angstrom): 0.250
CHO_DELTA_R_MAX (Angstrom): 0.650	CHO_DELTA_R_MAX (Angstrom): 0.650
CHO_ALPHA_IDEAL (degrees): 180.000	CHO_ALPHA_IDEAL (degrees): 180.000
CHO_DELTA_ALPHA_IDEAL (degrees): 50.000	CHO_DELTA_ALPHA_IDEAL (degrees): 50.000
CHO_DELTA_ALPHA_MAX (degrees): 100.000	CHO_DELTA_ALPHA_MAX (degrees): 100.000
CHO_BETA_IDEAL (degrees): 180.000	CHO_BETA_IDEAL (degrees): 180.000
CHO_DELTA_BETA_IDEAL (degrees): 80.000	CHO_DELTA_BETA_IDEAL (degrees): 80.000
CHO_DELTA_BETA_MAX (degrees): 100.000	CHO_DELTA_BETA_MAX (degrees): 100.000
Sulfur...acceptor term:	Sulfur...acceptor term:
* Metal term	* Metal term
METAL_R1: 2.600	METAL_R1: 2.600
METAL_R2: 3.000	METAL_R2: 3.000
METAL_R_SIGMA: 0.100	METAL_R_SIGMA: 0.100
* Specific metal-coordination types	* Specific metal-coordination types
* Lipophilic term	* Lipophilic term
MAKE_PLANAR_N_LIPO: 0	MAKE_PLANAR_N_LIPO: 0
LIPO_R1: 4.100	LIPO_R1: 4.100
LIPO_R2: 7.100	LIPO_R2: 7.100
LIPO_R_SIGMA: 0.100	LIPO_R_SIGMA: 0.100
* Clash term	* Clash term
CLASH_FUNCTION: CHEMSCORE_DOCKING	CLASH_FUNCTION: CHEMSCORE_DOCKING
CLASH_RADIUS_GENERAL: 3.100	CLASH_RADIUS_GENERAL: 3.100
CLASH_RADIUS_SULPHUR: 3.350	CLASH_RADIUS_SULPHUR: 3.350
CLASH_RADIUS_HBOND: 1.600	CLASH_RADIUS_HBOND: 1.600
CLASH_RADIUS_METAL: 1.380	CLASH_RADIUS_METAL: 1.380
CLASH_RADIUS_SIGMA: 0.000	CLASH_RADIUS_SIGMA: 0.000
* Torsion term	* Torsion term
SP3_SP3_BOND A = 0.18750 (kJ/mol); n = 3.0; f0 = 3.14159 (rad)	SP3_SP3_BOND A = 0.18750 (kJ/mol); n = 3.0; f0 = 3.14159 (rad)
SP3_SP2_BOND A = 0.09375 (kJ/mol); n = 6.0; f0 = 0.00000 (rad)	SP3_SP2_BOND A = 0.09375 (kJ/mol); n = 6.0; f0 = 0.00000 (rad)
SP2_SP2_BOND A = 0.18750 (kJ/mol); n = 2.0; f0 = 0.00000 (rad)	SP2_SP2_BOND A = 0.18750 (kJ/mol); n = 2.0; f0 = 0.00000 (rad)
UNKNOWN_BOND A = 0.00000 (kJ/mol); n = 0.0; f0 = 0.00000 (rad)	UNKNOWN_BOND A = 0.00000 (kJ/mol); n = 0.0; f0 = 0.00000 (rad)
EXOCYCLIC_BOND A = 0.50000 (kJ/mol); n = 1.0; f0 = 3.14159 (rad)	EXOCYCLIC_BOND A = 0.50000 (kJ/mol); n = 1.0; f0 = 3.14159 (rad)
USE_EXOCYCLIC_TORSION_TERM = 1	USE_EXOCYCLIC_TORSION_TERM = 1
* Scaling	* Scaling
HBOND_SCALING: 0	HBOND_SCALING: 0
HBOND_SCALING_SAMPLES: 1	HBOND_SCALING_SAMPLES: 1
HBOND_SCALING_END: 75.000	HBOND_SCALING_END: 75.000
DELTA_R_MAX_START: 1.150	DELTA_R_MAX_START: 1.150

DELTA_ALPHA_MAX_START (deg): 110.000	DELTA_ALPHA_MAX_START (deg): 110.000
---	---
---	---
--- CHEMPLP parameters---	--- CHEMPLP parameters---
---	---
PLP_COEFFICIENT: 1.000	PLP_COEFFICIENT: 1.000
PLP_LIGAND_CLASH_COEFFICIENT: 1.000	PLP_LIGAND_CLASH_COEFFICIENT: 1.000
PLP_LIGAND_TORSION_COEFFICIENT: 2.000	PLP_LIGAND_TORSION_COEFFICIENT: 2.000
PLP_PROTEIN_ENERGY_COEFFICIENT: 1.000	PLP_PROTEIN_ENERGY_COEFFICIENT: 1.000
PLP_CONSTRAINT_COEFFICIENT: 1.000	PLP_CONSTRAINT_COEFFICIENT: 1.000
PLP_GRID_SPACING: 0.300	PLP_GRID_SPACING: 0.300
PLP_LIGAND_TORSION_FUNCTION: CHEMSCORE	PLP_LIGAND_TORSION_FUNCTION: CHEMSCORE
PLP_LIGAND_CLASH_FUNCTION: PLP	PLP_LIGAND_CLASH_FUNCTION: PLP
PLP_HBOND_METAL_FUNCTION: CHEMSCORE	PLP_HBOND_METAL_FUNCTION: CHEMSCORE
PLP_WATER_BARRIER: 3.000	PLP_WATER_BARRIER: 3.000
HBOND_A: 2.300	HBOND_A: 2.300
HBOND_B: 2.600	HBOND_B: 2.600
HBOND_C: 3.100	HBOND_C: 3.100
HBOND_D: 3.400	HBOND_D: 3.400
HBOND_E: -1.000	HBOND_E: -1.000
HBOND_F: 20.000	HBOND_F: 20.000
BURIED_A: 3.400	BURIED_A: 3.400
BURIED_B: 3.600	BURIED_B: 3.600
BURIED_C: 4.500	BURIED_C: 4.500
BURIED_D: 5.500	BURIED_D: 5.500
BURIED_E: -0.100	BURIED_E: -0.100
BURIED_F: 20.000	BURIED_F: 20.000
METAL_A: 1.400	METAL_A: 1.400
METAL_B: 2.200	METAL_B: 2.200
METAL_C: 2.600	METAL_C: 2.600
METAL_D: 2.800	METAL_D: 2.800
METAL_E: -1.000	METAL_E: -1.000
METAL_F: 20.000	METAL_F: 20.000
NONPOLAR_A: 3.400	NONPOLAR_A: 3.400
NONPOLAR_B: 3.600	NONPOLAR_B: 3.600
NONPOLAR_C: 4.500	NONPOLAR_C: 4.500
NONPOLAR_D: 5.500	NONPOLAR_D: 5.500
NONPOLAR_E: -0.400	NONPOLAR_E: -0.400
NONPOLAR_F: 20.000	NONPOLAR_F: 20.000
REPULSIVE_A: 3.200	REPULSIVE_A: 3.200
REPULSIVE_B: 5.000	REPULSIVE_B: 5.000
REPULSIVE_C: 0.100	REPULSIVE_C: 0.100
REPULSIVE_D: 20.000	REPULSIVE_D: 20.000
---	---
---	---
--- Parameter settings---	--- Parameter settings---
---	---
* Population settings	* Population settings
maxops: auto	maxops: auto
popsiz: auto	popsiz: auto
select_pressure: auto	select_pressure: auto
n_islands: auto	n_islands: auto
niche_siz: auto	niche_siz: auto
* GA settings	* GA settings

pt_crosswt: auto	pt_crosswt: auto
allele_mutatewt: auto	allele_mutatewt: auto
migratewt: auto	migratewt: auto
* Search settings	* Search settings
start_vdw_linear_cutoff: 4.000	start_vdw_linear_cutoff: 4.000
initial_virtual_pt_match_max: 3.000	initial_virtual_pt_match_max: 3.000
* Cavity detection	* Cavity detection
Cavity detection algorithm: Ligsite	Cavity detection algorithm: Ligsite
Cavity radius: 10.000	Cavity radius: 10.000
Cavity origin: 25.00 38.00 -15.00	Cavity origin: 20.00 95.00 72.00
* Solvent Accessibility	* Solvent Accessibility
Solvate all H bond donors and acceptors: on	Solvate all H bond donors and acceptors: on
* Ligand treatment	* Ligand treatment
Internal ligand hydrogen bonds: ignored	Internal ligand hydrogen bonds: ignored
Flexible ligand ring corners: fixed	Flexible ligand ring corners: fixed
Ligand carboxylic OH groups: flip	Ligand carboxylic OH groups: flip
Ligand amide bonds: fixed	Ligand amide bonds: fixed
Ligand pyramidal nitrogen groups: fixed	Ligand pyramidal nitrogen groups: fixed
Ligand bond to planar nitrogen groups: allow 180 degrees flip	Ligand bond to planar nitrogen groups: allow 180 degrees flip
Ligand planar nitrogen bonds (Ring-NH-R): can flip	Ligand planar nitrogen bonds (Ring-NH-R): can flip
Ligand planar nitrogen bonds (Ring-NRR'): can flip	Ligand planar nitrogen bonds (Ring-NRR'): can flip
Flatten amide and trigonal nitrogens: yes	Flatten amide and trigonal nitrogens: yes
Torsional distributions: are used	Torsional distributions: are used
Calculating torsional energy of ligand: yes	Calculating torsional energy of ligand: yes
Ligand energy: relative wrt best tors+vdw+hbond	Ligand energy: relative wrt best tors+vdw+hbond
: reported fitness is adjusted	: reported fitness is adjusted
Post-process ligand bonds: yes	Post-process ligand bonds: yes
* Protein treatment:	* Protein treatment:
Protein carboxylic OH groups: flip	Protein carboxylic OH groups: flip
* Algorithm Settings (from parameter file):	* Algorithm Settings (from parameter file):
VDW potential (external): 4-8 LJ potential (no. 1)	VDW potential (external): 4-8 LJ potential (no. 1)
VDW potential (external): 4-8 2-4 split potential (no. 2)	VDW potential (external): 4-8 2-4 split potential (no. 2)
VDW potential (external): 4-8 1-2 split potential (no. 3)	VDW potential (external): 4-8 1-2 split potential (no. 3)
VDW potential (internal): 6-12 LJ potential (no. 1)	VDW potential (internal): 6-12 LJ potential (no. 1)
VDW potential (internal): 4-8 LJ potential (no. 2)	VDW potential (internal): 4-8 LJ potential (no. 2)
VDW potential (internal): slot 3 not used	VDW potential (internal): slot 3 not used
Weighting for distance matching: squared	Weighting for distance matching: squared
Weighting for angle matching: squared	Weighting for angle matching: squared
Mapping, second pass results: taking best three	Mapping, second pass results: taking best three
Internal energy: on absolute scale	Internal energy: on absolute scale
External energy weight: 1.375	External energy weight: 1.375
Internal energy weight: 1.000	Internal energy weight: 1.000
Length of H bond: 2.900	Length of H bond: 2.900
Angle coding: binary coded	Angle coding: binary coded
Angle bit length: 8 bits	Angle bit length: 8 bits
Allow duplicates in chromosome mappings: yes	Allow duplicates in chromosome mappings: yes
Parameter scaling: on	Parameter scaling: on
Simplex minimisation after every GA run: yes	Simplex minimisation after every GA run: yes
Infer hetatm types from residue names: yes	Infer hetatm types from residue names: yes
Ligand relaxation: on	Ligand relaxation: on
Maximum relax distance: 2.000	Maximum relax distance: 2.000
Maximum relax angle: 60.000	Maximum relax angle: 60.000
Ionisation dispersion term: external	Ionisation dispersion term: external
* Geometric parameters for Fitness Function (from parameter file)	* Geometric parameters for Fitness Function (from parameter file)

Initial Virtual pt dist max: 3.000	Initial Virtual pt dist max: 3.000
Final Virtual pt dist max: 2.000	Final Virtual pt dist max: 2.000
Virtual point distance min: 0.500	Virtual point distance min: 0.500
Virtual point angle min: 60.000	Virtual point angle min: 60.000
Virtual point angle max: 160.000	Virtual point angle max: 160.000
Cone virtual point angle min: 120.000	Cone virtual point angle min: 120.000
Cone virtual point angle max: 160.000	Cone virtual point angle max: 160.000
Contact distance: 10.000	Contact distance: 10.000
D/A vdw contact weighting: 1.000	D/A vdw contact weighting: 1.000
Minimum neighbour weighting: 0.200	Minimum neighbour weighting: 0.200
Mapping second pass distance: 2.000	Mapping second pass distance: 2.000
* Lone pair parameters	* Lone pair parameters
Planar LP: minimum angle with plane: 20.000	Planar LP: minimum angle with plane: 20.000
Planar LP: maximum angle with plane: 90.000	Planar LP: maximum angle with plane: 90.000
Protein planar acceptors: need only one lp solvated	Protein planar acceptors: need only one lp solvated
Solvated point distance: -0.200	Solvated point distance: -0.200
* VDW parameters	* VDW parameters
Border round vdw grid: 4.000	Border round vdw grid: 4.000
VDW cutoff fraction: 1.500	VDW cutoff fraction: 1.500
VDW cutoff distance: 20.000	VDW cutoff distance: 20.000
Number of bins in VDW lookup: 10000	Number of bins in VDW lookup: 10000
* Specific metal-coordination types	* Specific metal-coordination types
* Other settings	* Other settings
Write statistics: off	Write statistics: off
Current parameters file:	Current parameters file:
C:\Program Files (x86)\CCDC/Discovery_2019\GOLD\gold\gold.params	C:\Program Files (x86)\CCDC/Discovery_2019\GOLD\gold\gold.params
---	---
Processing protein 1: C:/Users/chans/Dropbox/0PaperWork/MD-Simul/Gold/Tannase/1NHC/1NHC_protein.mol2 DE(Protein): 0.00	Processing protein 1: C:/Users/chans/Dropbox/0PaperWork/MD-Simul/Gold/Tannase/4J0C/4J0C_protein.mol2 DE(Protein): 0.00
---	---
--- Loading protein---	--- Loading protein---
---	---
Protein file: C:/Users/chans/Dropbox/0PaperWork/MD-Simul/Gold/Tannase/1NHC/1NHC_protein.mol2	Protein file: C:/Users/chans/Dropbox/0PaperWork/MD-Simul/Gold/Tannase/4J0C/4J0C_protein.mol2
Substructure list check (mol2 format): ok	Substructure list check (mol2 format): ok
N.plc donors:	N.plc donors:
arg: 461 arg: 463 arg: 464 arg: 612 arg: 614 arg: 615	arg: 36 arg: 38 arg: 39 arg: 246 arg: 248 arg: 249
arg: 1501 arg: 1503 arg: 1504 arg: 1667 arg: 1669	arg: 520 arg: 522 arg: 523 arg: 691 arg: 693
arg: 1670	arg: 694 arg: 816 arg: 818 arg: 819 arg: 886
	arg: 888 arg: 889 arg: 901 arg: 903 arg: 904
Acidic nitrogen acceptors: none found	arg: 969 arg: 971 arg: 972 arg: 1086 arg: 1088
---	arg: 1089 arg: 1158 arg: 1160 arg: 1161 arg: 1209
--- Active site---	arg: 1211 arg: 1212 arg: 1737 arg: 1739 arg: 1740
---	arg: 2100 arg: 2102 arg: 2103 arg: 2133 arg: 2135
Coordinates: 25.00 38.00 -15.00	arg: 2136 arg: 2470 arg: 2472 arg: 2473 arg: 2758
Cavity radius: 10.000	arg: 2760 arg: 2761 arg: 3019 arg: 3021 arg: 3022
Ligsite cavity detection, MIN_PSP: 4	arg: 3038 arg: 3040 arg: 3041 arg: 3081 arg: 3083
* Cavity atoms	arg: 3084
1438 1439 1441 3195 3199 4476 3410 3384 1296 1121	
1119 1120 4477 1129 4040 4478 685 4041 1295 1297	Acidic nitrogen acceptors: none found
3417 1949 3656 3682 1947 1948 3694 1970 1976 3678	---
1669 1304 3672 1687 3419 1144 1303 4656 1143 3418	--- Active site---
1142 4464 684 683 1969 3684 4033 4644 4300 676	---

1968 4643 1324 3685 923 4036 1472 4299 677 1962
1471 3218 4642 1166 3229 675 1165 4307 4055 673
674 1473 1167 1478 4668 921 920 4296 1339 1945
3646 3654 4126 4133 4124 1338 1340 3231 1477 3671
3246 3431 3230 1967 734 4293 4304 4491 1504 3247
1961 3668 1164 4490 1700 3445 3683 1960 1702 1959
1162 4669 3253 3444 3241 3457 1501 1502 1180 1181
4528 1337 4662 4661 1500 3242 1489 3446 3243 3227
1503 1499 3205 3228 1491 1492 1493
Number of active atoms: 137
* Buried lysines
Lysine 734 is fixed by an hbond (acceptor atno 922 hbond angle 21.6)

--- Fitting points summary---

Protein donor atoms: 22
1439 1129 685 1949 1976 1669 1687 676 923 1473
1478 1340 1967 734 1504 1959 1501 1181 1503 1122
1670 942

Protein acceptor atoms: 21
1296 1121 1297 1949 1970 1976 1304 1144 1143 684
676 1472 1962 1166 674 1167 1478 1339 1181 1489
1493

Hydrogens: (n = 34)
3195 4477 4478 4040 4041 3682 3678 3384 3385 3417
3418 3419 4033 4299 4300 3218 3229 4668 4669 3671
4123 4124 4125 3246 3247 3683 3241 4509 3253 3254
4476 3410 3411 4304

Lone pairs added to molecule: 41
Numbering: from 4724 to 4764

Protein TRP residues: none found in active site

Protein rotatable bonds: [1949 1948] [1976 1975] [1687 1686] [676 675]
[1478 1477] [1181 1180] [942 941]

--- Shape grid---

Maximum length of gridpt list: 127
Average list length: 66

--- Receptor Density Scaling---

Minimum Protein Density: 0
Maximum Protein Density: 143
Receptor density scaling is OFF
Length of shape fitting list: 1829

--- Interaction Motif---

Number of interactions: 0

Coordinates: 20.00 95.00 72.00
Cavity radius: 10.000
Ligsite cavity detection, MIN_PSP: 4
* Cavity atoms
6000 6001 2480 2481 3098 3100 3096 5284 6002 4382
4380 6762 6776 3332 1740 5283 4295 627 4296 6021
3097 6093 6111 2591 2592 6092 4581 4582 717 904
6110 2489 6010 3336 6771 4302 4416 720 6018 2490
3331 6104 606 4283 727 4392 4281 6009 3338 3340
4947 4303 605 603 604 2513 3334 3339 6101 3095
3099 4954 4301 602 4417 728 4948 2600 1260 4950
1254 1259 601 4282 590 6058 1528 6105 4953 4951
4284 3484 2599 2601 1252 4395 1253 4945 597 596
589 3487 4938 1250 1251 4260 588 4304 4936 4940
594 3508 616 582 4941 585 4249
Number of active atoms: 107
* Buried lysines
Lysine 2490 is fixed by an hbond (acceptor atno 3092 hbond angle 0.6)

--- Fitting points summary---

Protein donor atoms: 13
1740 717 904 2490 605 3339 1260 601 1253 594
744 1261 1248

Protein acceptor atoms: 17
3098 3100 3332 3097 3338 604 2513 3099 2600 1260
590 2601 1253 597 1251 582 585

Hydrogens: (n = 18)
5283 5284 4382 4581 4582 6009 6010 6011 4301 6764
4950 4282 4945 4249 4395 4396 4938 4940

Lone pairs added to molecule: 33
Numbering: from 6886 to 6918

Protein TRP residues: TRP91

Protein rotatable bonds: [1260 1259] [1253 1252]

--- Shape grid---

Maximum length of gridpt list: 133
Average list length: 74

--- Receptor Density Scaling---

Minimum Protein Density: 0
Maximum Protein Density: 141
Receptor density scaling is OFF
Length of shape fitting list: 3548

--- Interaction Motif---

Number of motifs: 0 Weight: 50.000 Minimum Include Score: 0.000001 Ignore Zero Bits?: 0 * Generating PLP maps for types DONOR ACCEPTOR DONACC NONPOLAR --- * Protein initialisation time: total 5.0009 user 5.0001 sys 0.0008 --- --- --- Active Molecule Summary--- ---	--- Number of interactions: 0 Number of motifs: 0 Weight: 50.000 Minimum Include Score: 0.000001 Ignore Zero Bits?: 0 * Generating PLP maps for types DONOR ACCEPTOR DONACC NONPOLAR --- * Protein initialisation time: total 5.0008 user 5.0003 sys 0.0005 --- --- --- Active Molecule Summary--- ---