Supporting Information

Loop engineering of aryl sulfotransferase B for improving catalytic performance in regioselective sulfation

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Table of content

Table S1. Sequencing results of beneficial ASTB variants from Phase 1 (obtained after the screening of the SeSaM library). All ASTB variants had an activity improvement in the range of 1.3-1.6.

No.	ASTB variant	Substitution
1	ASTB-M1	L225Q
2	ASTB-M2	K533E
3	ASTB-M3	N612D
4	ASTB-M4	F22S; L622Q
5	ASTB-M5	V430A
6	ASTB-M6	Q191R; T393I
7	ASTB-M7	V199A; K371E
8	ASTB-M8	Y218H; E373G
9	ASTB-M9	I71T
10	ASTB-M10	I398V
11	ASTB-M11	R552H
12	ASTB-M12	R600C
13	ASTB-M13	A448V; E542G
14	ASTB-M14	F512L; A582V
15	ASTB-M15	E380G; A410V; F551L

Table S2. Beneficial positions and best two substitutions of each position with improved sulfation activity.

Position	Substitutions	Improvement
Q191	Q191L	1.6
	Q191R	1.1
V199	V199S	2.4
	V199A	1.5
Y218	Y218A	2.2
	Y218H	1.1
L225	L225V	2.4
	L225K	1.8

Table S3. Sequences results of nine randomly picked clones from the OmniChange library.

	Q191	**V199**	**Y218**	**L225**
Clone 1	W (CAG→TGG)	P (GTT→CCT)	G (TAT→GGG)	Q (CTG→CAG)
Clone 2	E (CAG→GAG)	V (GTT→GTT)	F (TAT→TTT)	R (CTG→CGG)
Clone 3	R (CAG→CGG)	L (GTT→CTG)	Stop (TAT→TAG)	N (CTG→AAT)
Clone 4	R (CAG→CGG)	V (GTT→GTG)	L (TAT→TTG)	L (CTG→CTT)
Clone 5	Q (CAG→CAG)	V (GTT→CTT)	M (TAT→ATG)	Q (CTG→CTG)
Clone 6	G (CAG→GGT)	V (GTT→CTG)	Y (TAT→TAT)	L (CTG→TTA)
Clone 7	H (CAG→CAT)	C (GTT→TGT)	L (TAT→TTG)	L (CTG→CTG)
Clone 8	M (CAG→ATG)	W (GTT→TGG)	Y (TAT→TAT)	L (CTG→CTG)
Clone 9	Q (CAG→CAG)	V (GTT→CTT)	Y (TAT→TAT)	L (CTG→CTT)

Table S4. Kinetic characterization of 3-chlorocatechol by ASTB-WT and variants.

ASTB variant	k_{cat} [s^{-1}]	K_M [μM]	k_{cat}/K_M [M^{-1} s^{-1}]
ASTB-WT	17 ± 2	95 ± 2	1.8 x 10^5
ASTB-OM1	136 ± 11	257 ± 63	5.3 x 10^5
ASTB-OM2	179 ± 7	217 ± 32	8.2 x 10^5
ASTB-OM3	202 ± 6	570 ± 53	3.5 x 10^5

Table S5. Sequences and T_m value of primers used for the OmniChange library generation.

PTO primer	Sequence (5′→3′)	T_m (°C)*
Q191/V199Fw	catcgtaccgtgNNKAAACCGTATTACAACGTGGGCNNKATGGAAATGG	68.2
Q191/V199Rv	accgcccggcagACGGTATTCTTTGTAG	65.3
Y218/L225Fw	ctgccgggcggtNNKCATCACGACGCGGTTGAANNKGAAAACGGCAAT	73.5
Y218/L225Rv	ggcgaaacccgaCAGGACTATAAAGATACC	61.4
2148Fw	tcgggtttcgccACCTCTGACTTGAGC	65.8
2148Rv	cacggtacgatgCGACGTATACAGCAGGCGACCATT	68.1

*T_m for degenerate primers have been reported as minimum, mean and maximum value; capital letter: normal nucleotide, small letter: phosphorothioated nucleotide; N=A, C, T, G; K=G, T.

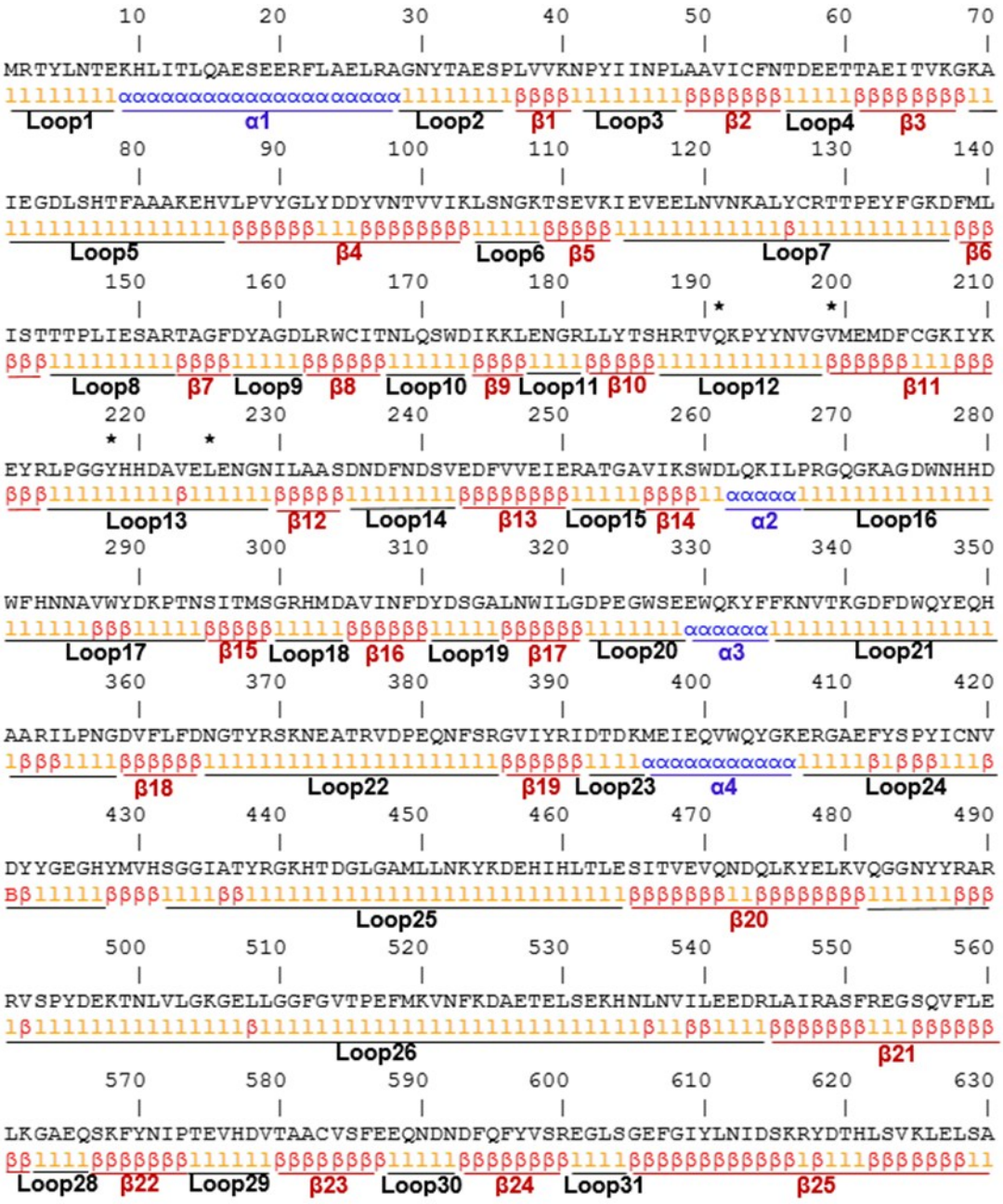

Figure S1. ASTB secondary structure prediction. The secondary structure of the ASTB was predicted using the NPS@ consensus secondary structure prediction webserver with PHD method for secondary structure prediction based on amino acid sequences. The secondary structure elements are given as α-helices (α), β-sheets (β), loop (l). The sequence number of α-helices , β-sheets, and loop are named by continuous letter more than 3.

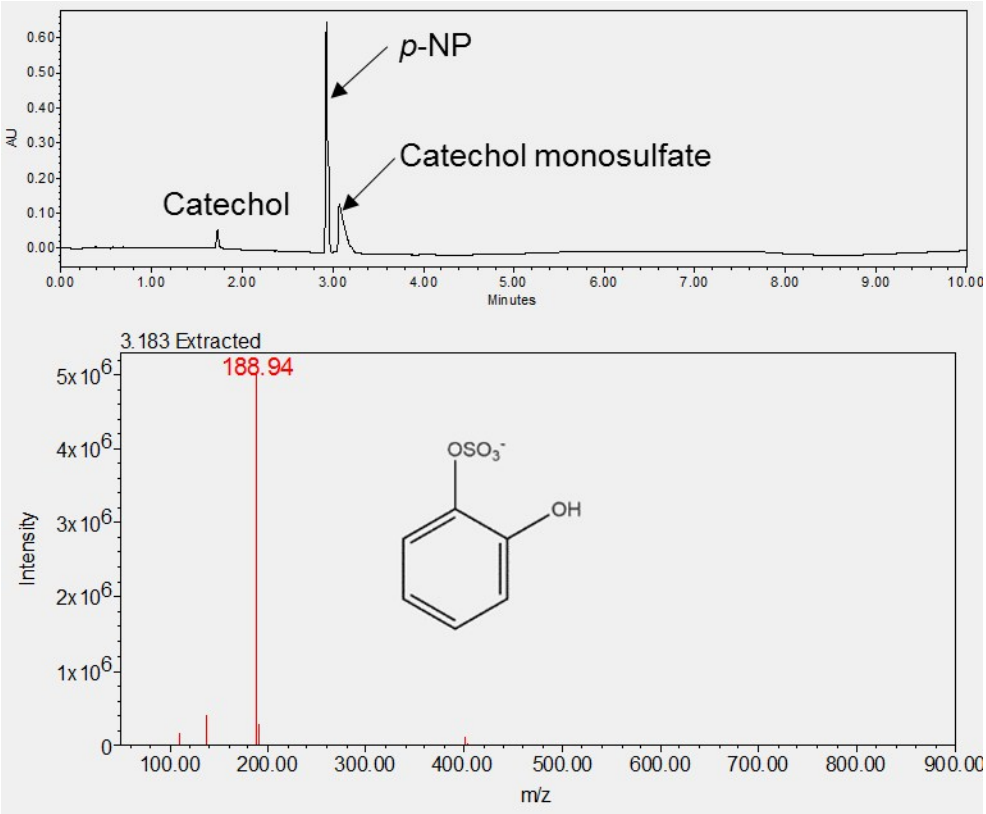

Figure S2. HPLC-MS analysis of enzymatic sulfation of catechol by ASTB-OM2 (negative mode: catechol 1-monosulfate: m/z= 188.94; theoretical mass: m/z= 189.00).

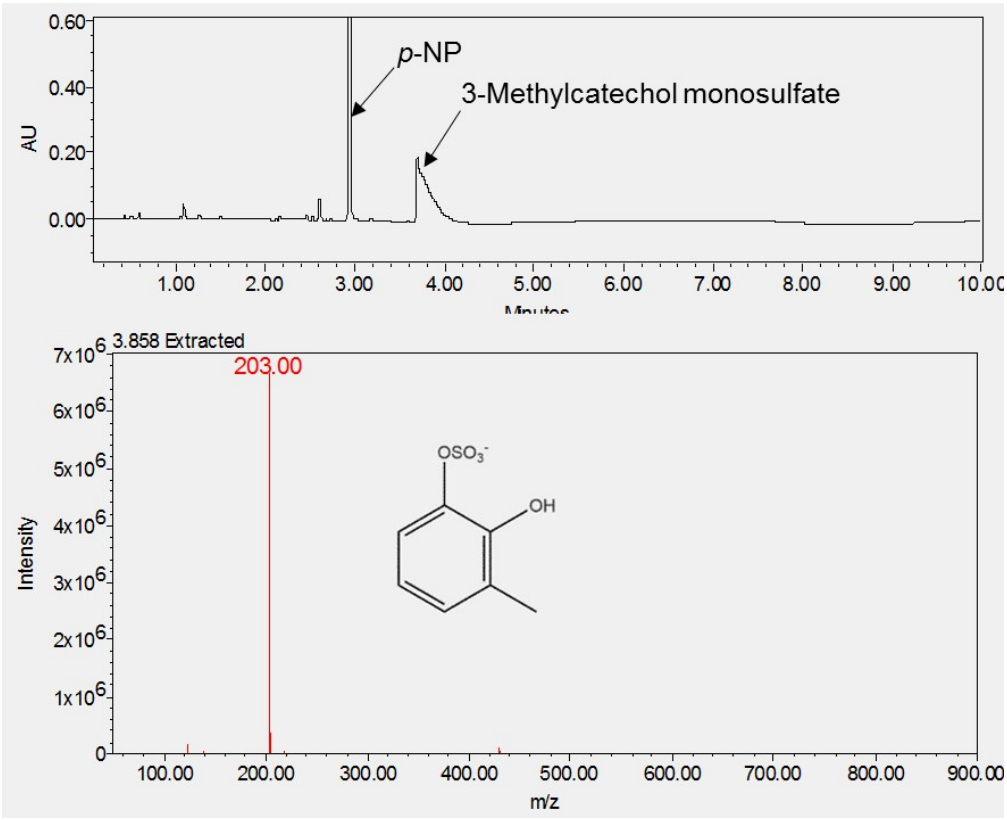

Figure S3. HPLC-MS analysis of enzymatic sulfation of 3-methylcatechol by ASTB-OM2 (negative mode: 3-methylcatechol monosulfate: m/z= 203.00; theoretical mass: m/z= 203.00).

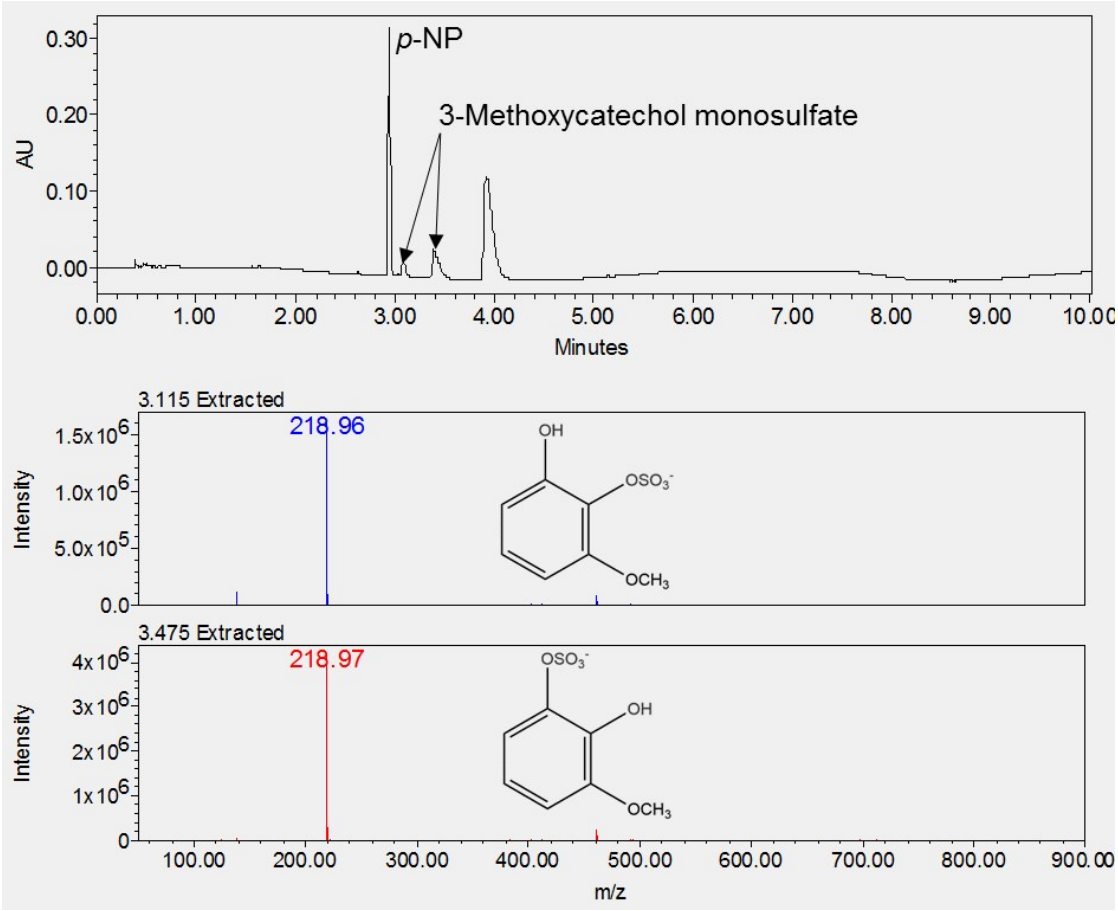

Figure S4. HPLC-MS analysis of enzymatic sulfation of 3-methoxycatechol by ASTB-OM2 (negative mode: 3-methoxycatechol monosulfate: m/z= 218.97/218.96; theoretical mass: m/z= 219.00).

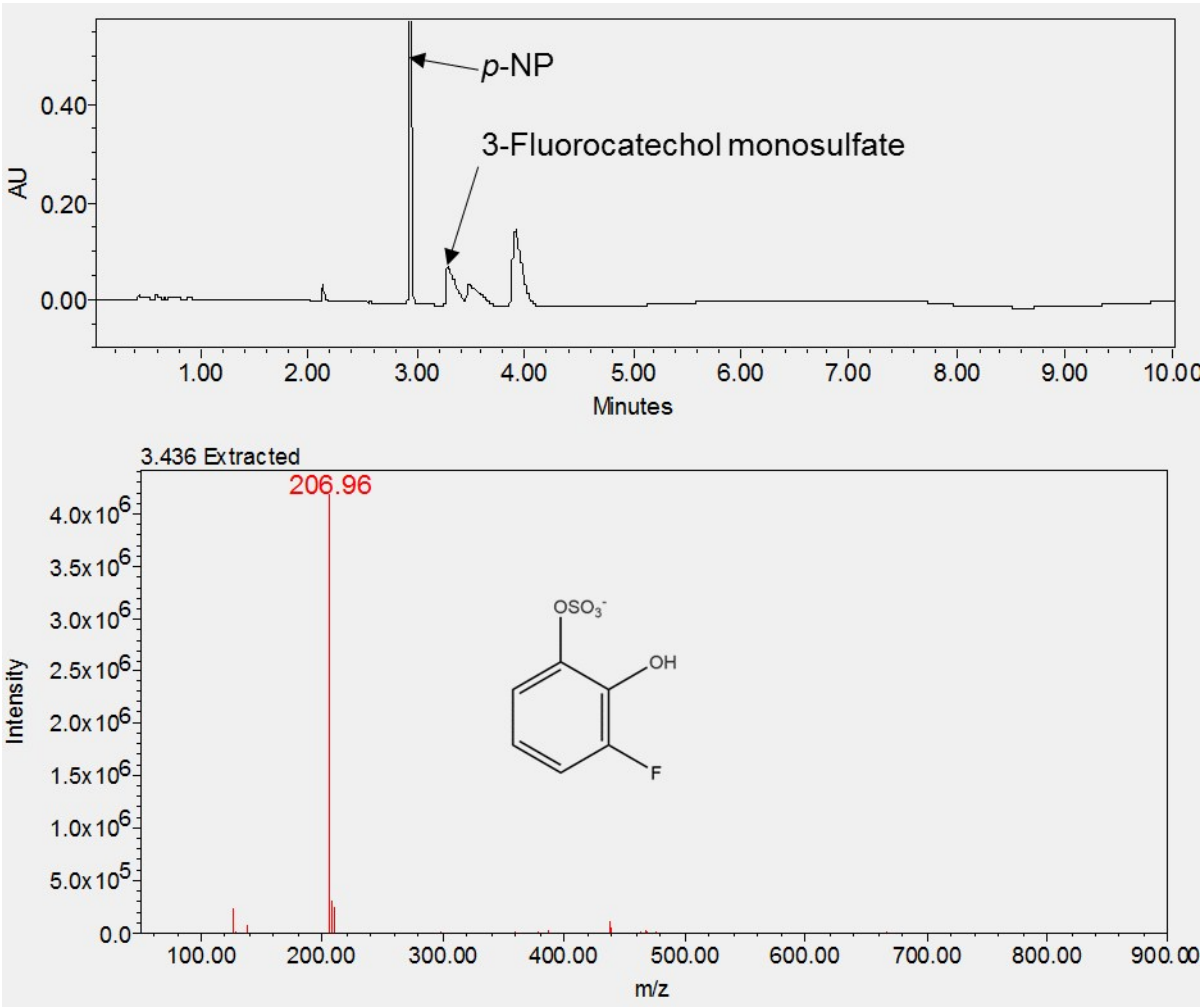

Figure S5. HPLC-MS analysis of enzymatic sulfation of 3-fluorocatechol by ASTB-OM2 (negative mode: 3-fluorocatechol monosulfate: m/z= 206.96; theoretical mass: m/z= 207.00).

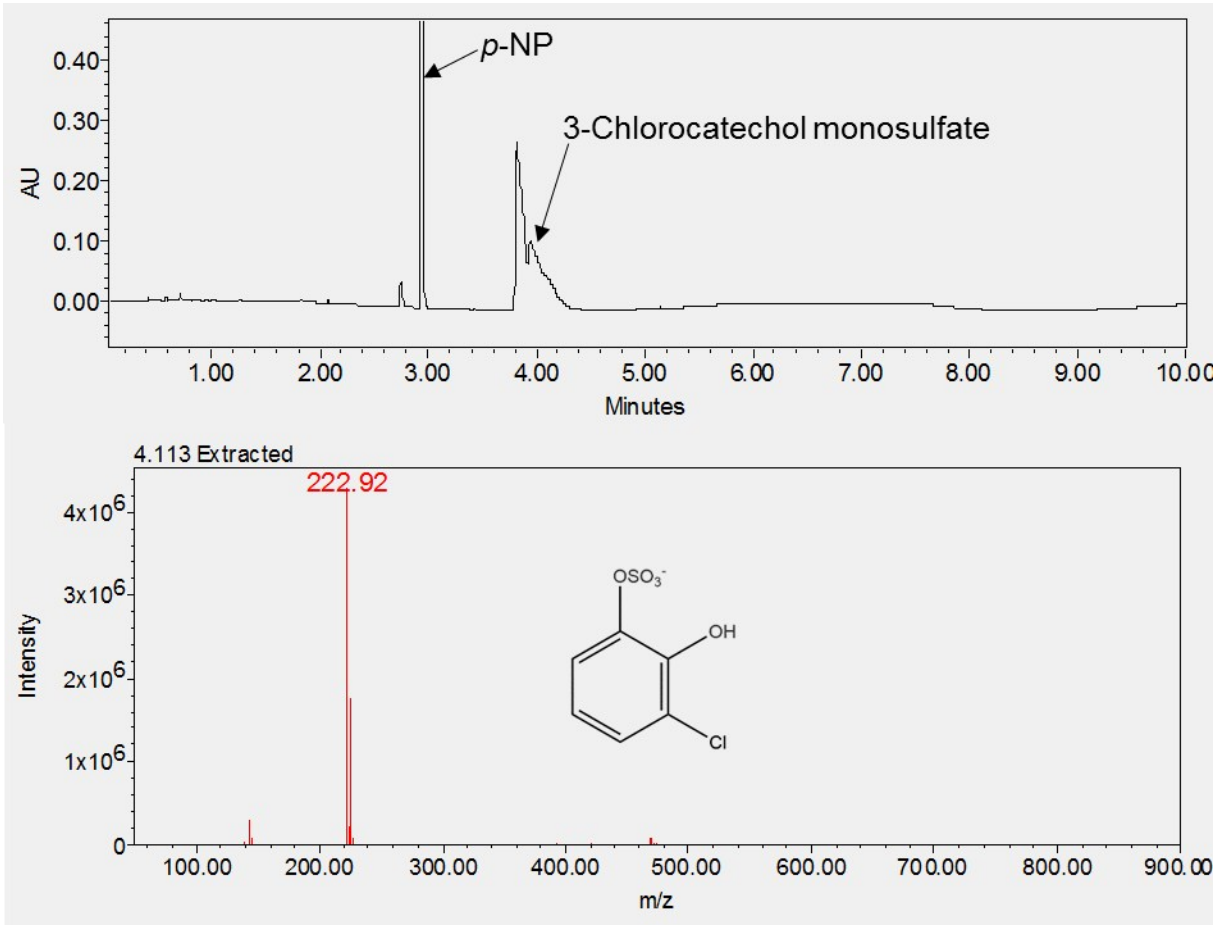

Figure S6. HPLC-MS analysis of enzymatic sulfation of 3-chlorocatechol by ASTB-OM2 (negative mode: 3-chlorocatechol monosulfate: m/z= 222.92, theoretical mass: m/z= 223.00).

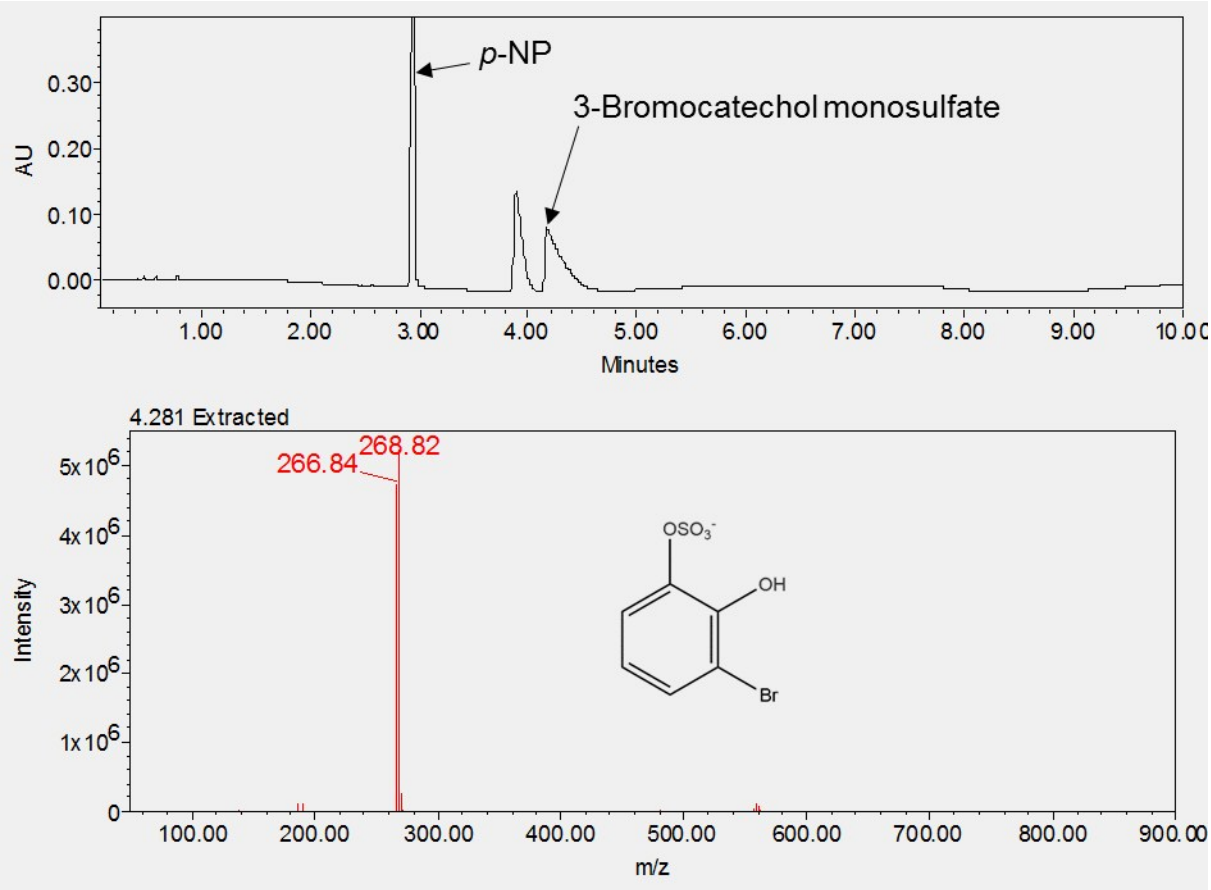

Figure S7. HPLC-MS analysis of enzymatic sulfation of 3-bromocatechol by ASTB-OM2 (negative mode: 3-bromocatechol monosulfate: m/z= 266.84/268.82; theoretical mass: m/z= 267.00/269.00).