Supporting Information

Biocatalytic sulfation of aromatic and aliphatic alcohols catalyzed by arylsulfate sulfotransferases

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Table S1 Summary of the sequences retrieved using BLASTp with ASSTA as protein template

ASST / Source μorganism	**Sequence identity**	**Amino acids**	**NCBI**
ASST *Desulfitobacterium dehalogenans* ATCC 51507*	93.2	629	AFM01510.1
ASST *Desulfitobacterium dichloroeliminans*	87.6	628	WP_015263010.1
ASST *Dehalobacterium formicoaceticum**	86.8	628	WP_089608557.1
ASST *Desulfosporosinus lacus*	85.0	627	WP_073033341
ASST *Desulfosporosinus* sp. HMP52	83.7	628	WP_034601251.1
ASST *Desulfosporosinus meridiei*	84.6	628	WP_014902777.1
ASST *Desulfosporosinus hippei*	83.1	628	WP_092334873.1
ASST *Desulfosporosinus acididurans*	77.3	628	WP_047811757.1
ASST *Desulfosporosinus* sp. FKA	76.5	629	WP_088186734.1
ASST *Desulfitobacterium dehalogenans*	76.1	629	WP_014793958.1
ASST *Desulfitobacterium dichloroeliminans*	75.6	629	WP_015260940.1
ASST *Desulfitobacterium hafniense**	71.2	626	KTE91359.1
ASST *Desulfitobacterium chlororespirans*	71	626	WP_072774114.1
ASST *Clostridiales bacterium*	70	636	PWM49771.1
ASST *Bittarella massiliensis*	64.9	625	WP_059003491.1
Clostridium hypothetical protein	64.7	625	WP_021658034.1
ASST *Desulfohalotomaculum alkaliphilum*	60.4	622	WP_114638903.1
ASST *Lachnoclostridium* sp. An14	57.2	628	WP_087224291.1
ASST *Niameybacter massiliensis*	58.7	619	WP_053984844.1
ASST *Desulfohalotomaculum alkaliphilum*	58.4	618	WP_031517900.1
ASST *Desulfosporosinus orientis**	58.8	621	WP_014184280.1
ASST *Desulfotomaculum ferrireducens*	58.1	618	WP_077713083.1
ASST *Hungatella hathewayi*	59	624	WP_055659810.1
ASST *Desulfosporosinus orientis**	58.3	621	WP_014187168.1
ASST *Niameybacter massiliensis*	56.6	626	WP_053984585.1
ASST *Desulfitobacterium dichloroeliminans*	58.9	623	WP_015263770.1
ASST *Desulfotomaculum ferrireducens*	57	621	WP_077715208.1
ASST *Hungatella effluvia**	58.5	624	WP_110321498.1
ASST *Niameybacter massiliensis*	55.2	622	WP_053983873.1

* Selected DNA sequences on this work

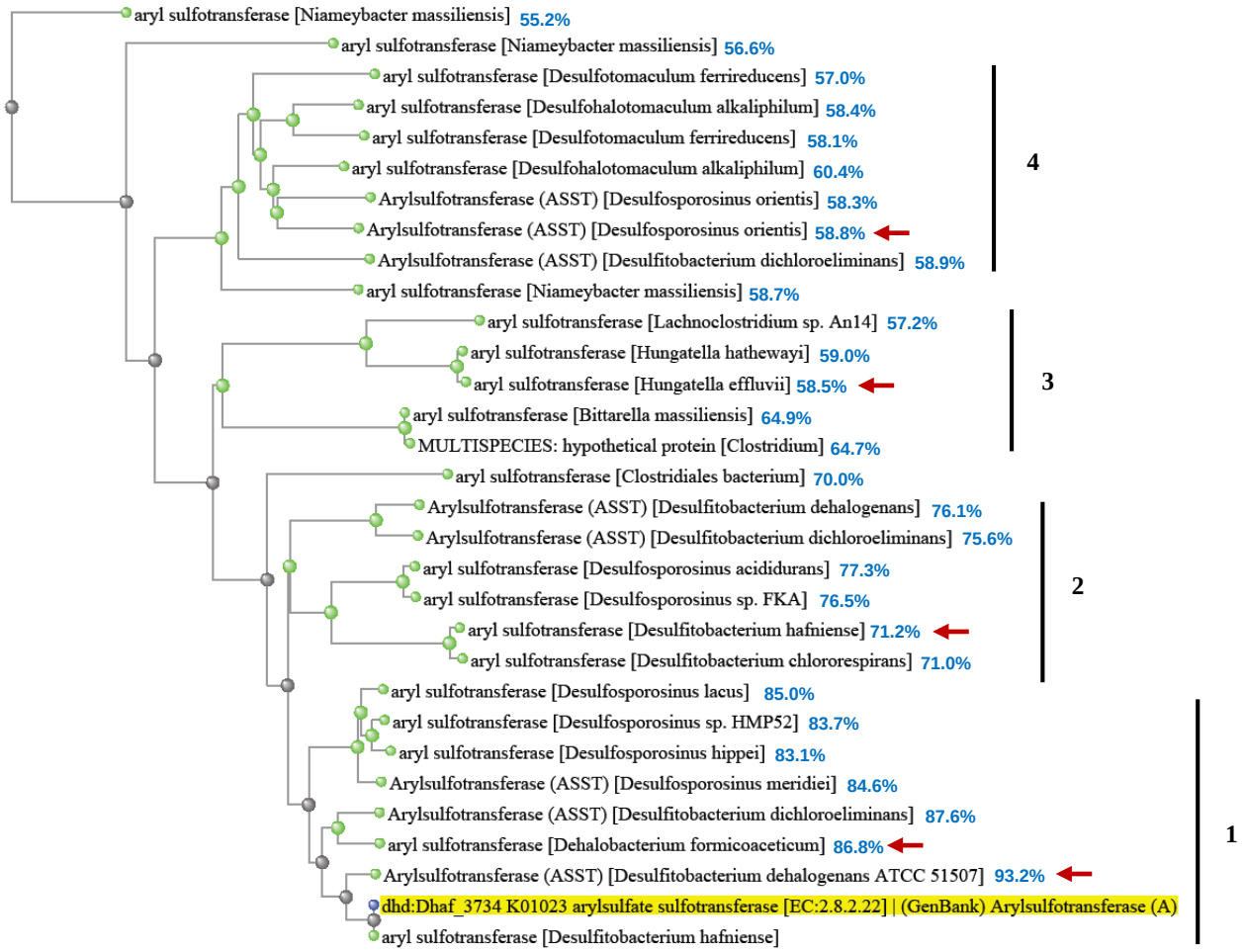
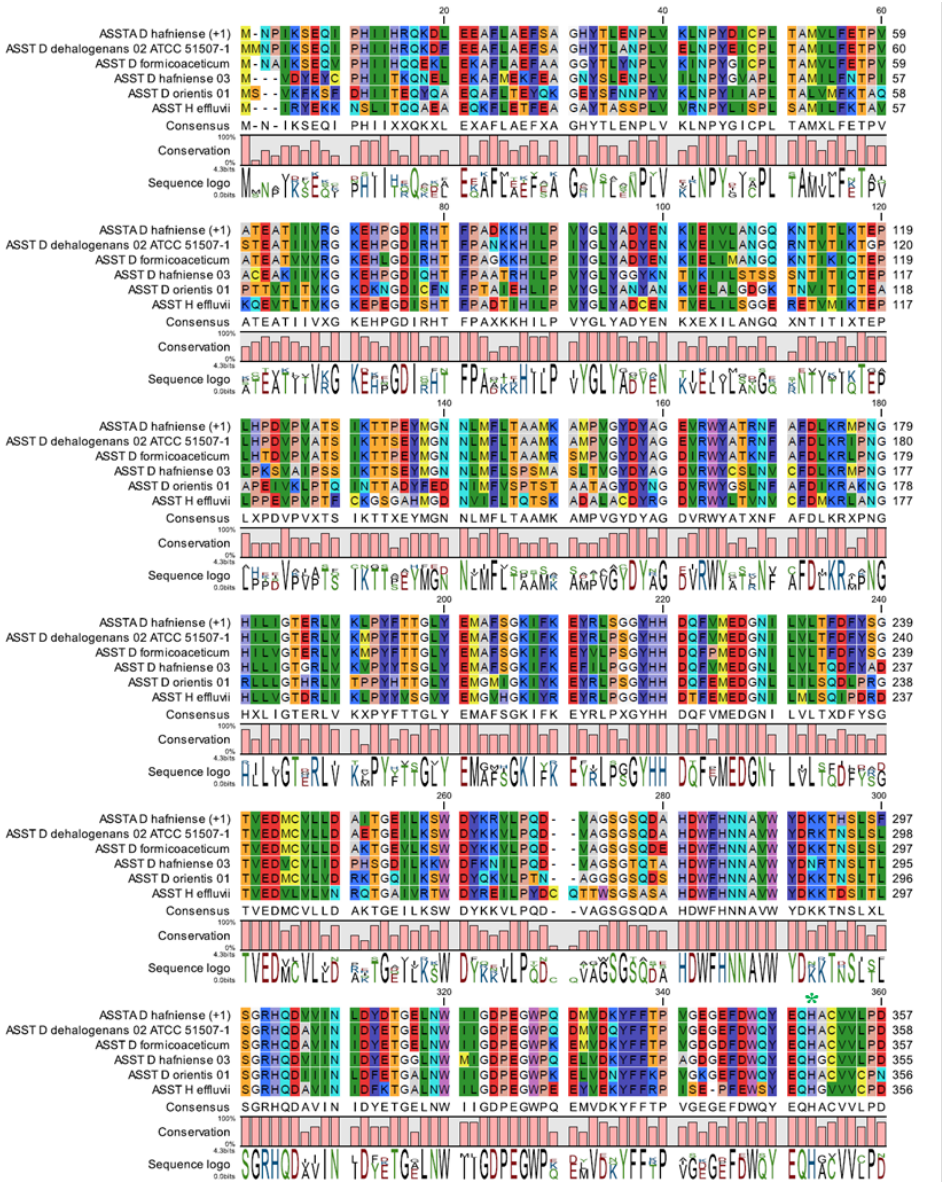


Fig. S1 Homology tree showing the sequence similarity displayed by the proteins retrieved from the BLAST search using ASSTA from *D. hafniense* as protein template. The four main branches that constitute the tree are numbered to facilitate their differentiation. The model protein is highlighted in yellow and the homologous proteins chosen are marked with red arrows. Homology percentages with ASSTA are written in blue next to all the protein names



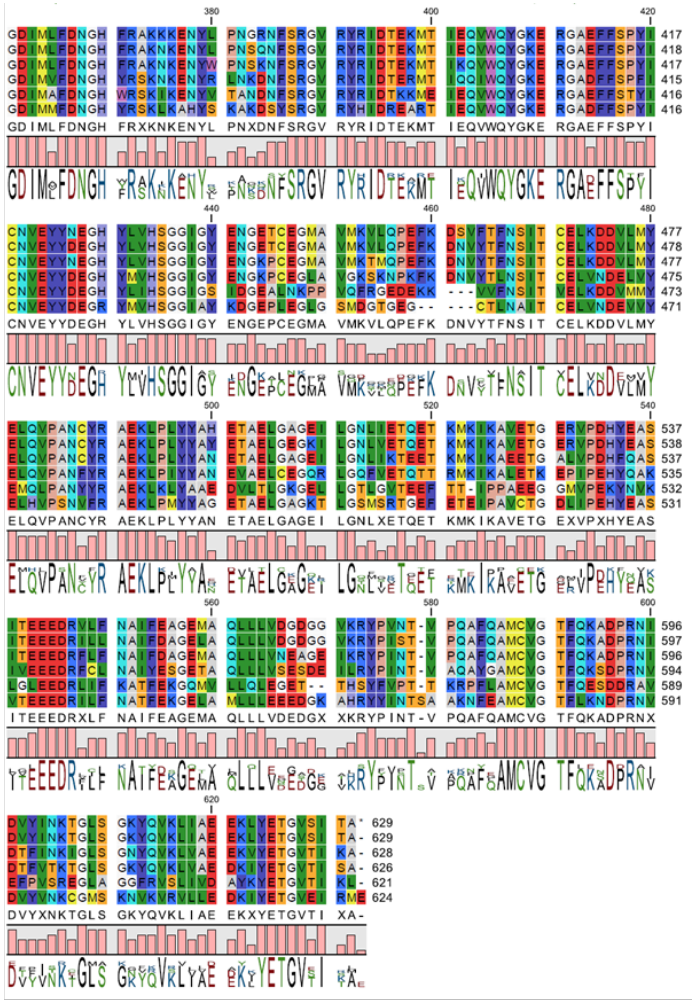


Fig. S2 Alignment of the protein sequences chosen from the ASSTA BLAST analysis. The expected catalytic His of ASSTA in marked with a green asterisk (*)

Table S2 Summary of the strains and culture conditions utilized for the overexpression of the ASSTs

Enzyme	ASSTA	ASSTB	ASST*Heff*	ASST*Dor*	ASST*Dfor*	ASSTC	ASST*Ddeh*
pEG[a]	pEG548	pEG549	pEG559	pEG558	pEG556	pEG557	pEG555
Vector	pET26b(+)	pET28a(+)	pET28a(+)	pET28a(+)	pET28a(+)	pET28a(+)	pET28a(+)
Restriction sites	*Sac*I and *Sal*I	*Nco*I and *Sal*I	*Nco*I and *Sal*I	*Nco*I and *Sal*I	*Nco*I and *Sal*I	*Nco*I and *Sal*I	*Nco*I and *Sal*I
Tag	C-term His-tag	C-term His-tag	C-term His-tag	C-term His-tag	C-term His-tag	C-term His-tag	C-term His-tag
Expression strain	BL21(DE3)	BL21(DE3)	BL21(DE3)	BL21(DE3)	BL21(DE3)	BL21(DE3)	BL21(DE3)
Media	LB	LB	LB	LB	LB	LB	LB
[Antibiotic]$_{cult}$	Kan (50 µg/ml)	Kan (50 µg/ml)	Kan (50 µg/ml)	Kan (50 µg/ml)	Kan (50 µg/ml)	Kan (50 µg/ml)	Kan (50 µg/ml)
O/Nc volume, temperature and shaking	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm	10 ml, 37 °C, 120 rpm
Usual culture volume	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml
Inoculation volume	1 % (5 ml)	1 % (5 ml)	1 % (5 ml)	1 % (5 ml)	1 % (5 ml)	1 % (5 ml)	1 % (5 ml)
Culture temperature and shaking	37 ºC, 140 rpm	37 ºC, 140 rpm	37 ºC, 140 rpm	37 ºC, 140 rpm	37 ºC, 140 rpm	37 ºC, 140 rpm	37 ºC, 140 rpm
Induction OD_{600}	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Inducer	IPTG (1 mM)	IPTG (0.4 mM)	IPTG (1 mM)	IPTG (1 mM)	IPTG (1 mM)	IPTG (1 mM)	IPTG (1 mM)
Induction time, temperature and shaking.	O/N, 20 ºC, 120 rpm	O/N, 30 ºC, 140 rpm	O/N, 20 ºC, 120 rpm	O/N, 20 ºC, 120 rpm	O/N, 20 ºC, 120 rpm	O/N, 20 ºC, 120 rpm	O/N, 20 ºC, 120 rpm
Purification	Ni^{2+}-NTA	Ni^{2+}-NTA	Ni^{2+}-NTA	Ni^{2+}-NTA	Ni^{2+}-NTA	Ni^{2+}-NTA	Ni^{2+}-NTA

[a] pEG refers to the internal plasmid numbering: pEG stand for plasmid of the Elk Group.

Table S3 Expected molecular weights and theoretical molar extinction coefficients for the different overexpressed ASSTs

ASST	Molecular weight (kDa)	Theoretical molar extinction coefficient [M^{-1} cm^{-1}]
ASSTA	76.0	93360
ASSTB	74.0	110310
ASST*Heff*	70.7	99290
ASST*Dor*	70.5	97650
ASST*Dfor*	71.4	97770
ASSTC	71.0	96160
ASST*Ddeh*	71.6	94640

Table S4 Determined *p*NP molar extinction coefficient under the different reaction conditions employed

Buffer	pH	Wavelength (nm)	Co-solvent (10%, v/v)	Molar extinction coefficient (ε) mM^{-1} cm^{-1}
Bis Tris	5.5	350 nm	—	5.0
	6.0		—	5.0
	7.0	410 nm	—	8.4
Na^+ phosphate	7.0	410 nm	—	11.1
	8.0		—	15.7
Tris / HCl	8.0	410 nm	—	15.6
	9.0		—	16.7
Na^+ carbonate	9.0	410 nm	—	16.9
	10.0		—	16.9
	10.5		—	16.8
AMP	9.0	410 nm	—	17.4
	10		—	17.4
	10.5		—	17.7
Tris / HCl	8.0	410	DMSO	18.7
			Acetone	18.3
			Methanol	17.8
			2-Propanol	19.0
			DMF	19.2
			1,2-$(MeO)_2$Et	18.8

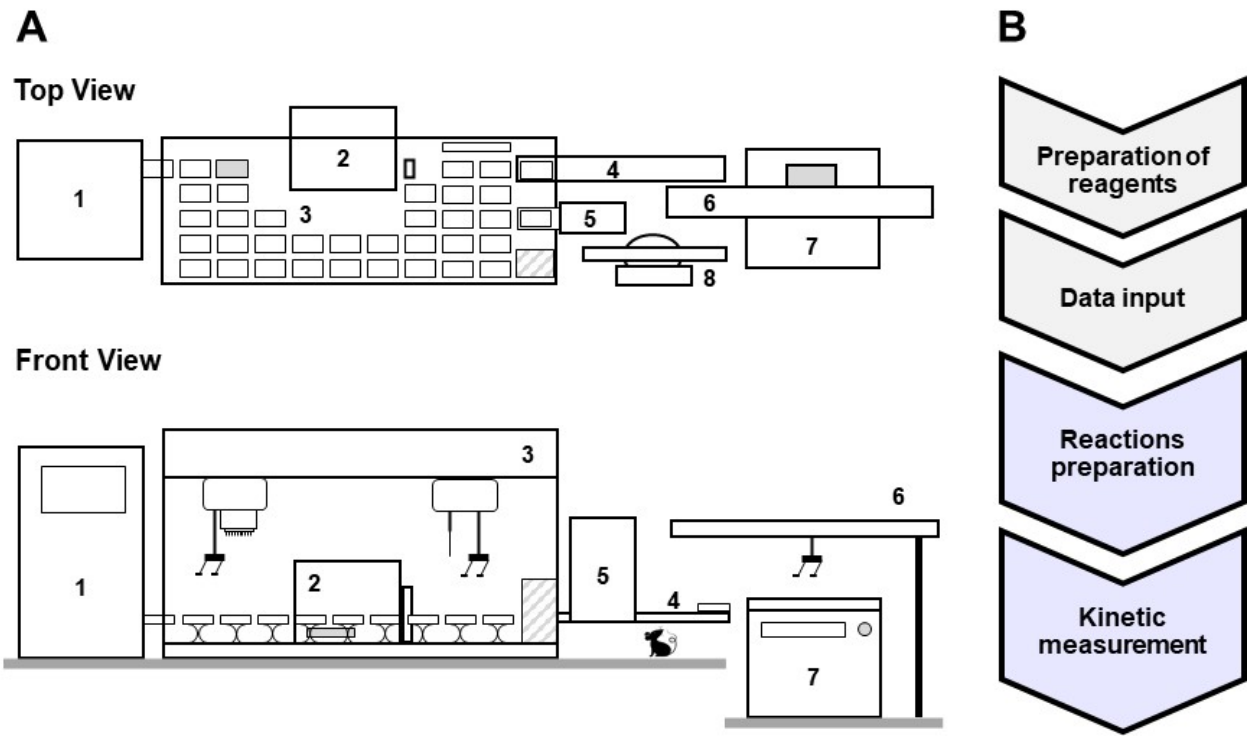


Fig. S3 (**A**) Schematic representation of the high throughput robotic platform (Xavier), including (**1**) an SBS-format plates incubator (Cytomat 2C450), (**2**) an UV/vis and fluorescence microtiter plate (MTP) reader (FLUOstar Omega, BMG), (**3**) a liquid handling robot (Biomek i7 Hybrid, Beckman Coulter), (**4**) MTP shuttle (Servo Shuttle, Beckman Coulter), (**5**) colony detection module (Visual ALP, Amplius), (**6**) BRT II and (**7**) refrigerated centrifuge (Rotanta 460, Hettich). These different devices are controlled and integrated through (**8**) Biomek and DART 2.0 software (representation of the PC on the front view was avoided for clarity). (**B**) Workflow for measuring the ASSTs Michaelis-Menten kinetics (grey background: steps performed manually; blue background: automated steps performed by the robotic platform)

Table S5 FIA-MS method applied for the detection of mono-, di- and trisulfated compounds synthesized by ASSTs

Column:	no column, SecurityGuard™ C18 cartridge
Column temperature:	not controlled
Eluent composition:	H_2O/MeOH 50:50, isocratic
Eluent flow rate:	0.5 mL/min
Run time:	1.4 min
Injection volume:	5 µl
Ionization mode:	API-ES, negative polarity
Drying gas temperature:	300 ºC
Drying gas flow:	6.0 L/min
Nebulizer pressure:	15 psig (2.0 bar)
Capillary voltage:	3000 V
Signal 1 (scan):	100% cycle time
	Mass range 30-500
	Fragmentor 70 V

Note: All samples were analyzed in scan mode and the chromatograms of the target masses were extracted in post-processing

Table S6 Target masses and internal standards used for the detection of the sulfated compounds synthesized in the biotransformations with substrates 2-7

Analyte	**Mass (*m*/*z*)**
PIPES	301 $[M\text{-H}]^-$
*p*NPS	218 $[M\text{-H}]^-$
*p*NP	138 $[M\text{-H}]^-$
Cathechol (**2**)	109 $[M\text{-H}]^-$
Monosulfated **2**	189 $[M\text{-H}]^-$
Disulfated **2**	269 $[M\text{-H}]^-$, 134 $[M\text{-2H}]^{2-}$
Resorcinol (**3**)	109 $[M\text{-H}]^-$
Monosulfated **3**	189 $[M\text{-H}]^-$
Disulfated **3**	269 $[M\text{-H}]^-$, 134 $[M\text{-2H}]^{2-}$
Hydroquinone (**4**)	109 $[M\text{-H}]^-$
Monosulfated **4**	189 $[M\text{-H}]^-$
Disulfated **4**	269 $[M\text{-H}]^-$, 134 $[M\text{-2H}]^{2-}$
1,2,4-benzenetriol (**5**),	125 $[M\text{-H}]^-$
Monosulfated **5**	205 $[M\text{-H}]^-$
Disulfated **5**	285 $[M\text{-H}]^-$, 142 $[M\text{-2H}]^{2-}$
Trisulfated **5**	365 $[M\text{-H}]^-$
1,3,5-benzenetriol (**6**)	125 $[M\text{-H}]^-$
Monosulfated **6**	205 $[M\text{-H}]^-$
Disulfated **6**	285 $[M\text{-H}]^-$, 142 $[M\text{-2H}]^{2-}$
Trisulfated **6**	365 $[M\text{-H}]^-$
4,4′-dihydroxybiphenyl (**7**)	185 $[M\text{-H}]^-$
Monosulfated **7**	265 $[M\text{-H}]^-$
Disulfated **7**	345 $[M\text{-H}]^-$, 172 $[M\text{-2H}]^{2-}$

Table S7 HPLC-MS method applied for the detection of mono-, di- and trisulfated compounds synthesized by ASSTs

Column:	*Phenomenex* Luna C18 column, SecurityGuard™ C18 cartridge
Column temperature:	not controlled
Eluent composition:	H_2O/MeOH 50:50, isocratic
Eluent flow rate:	1.0 mL/min
Run time:	15 min
Injection volume:	5 µl
Ionization mode:	API-ES, negative polarity
Drying gas temperature:	350 ºC
Drying gas flow:	10.0 L/min
Nebulizer pressure:	35 psig (2.4 bar)
Capillary voltage:	3000 V
Signal 1 (scan):	100% cycle time
	Mass range 35-500
	Fragmentor 40 V

Note: All samples were analysed in scan mode and the chromatograms of the target masses were extracted in post-processing

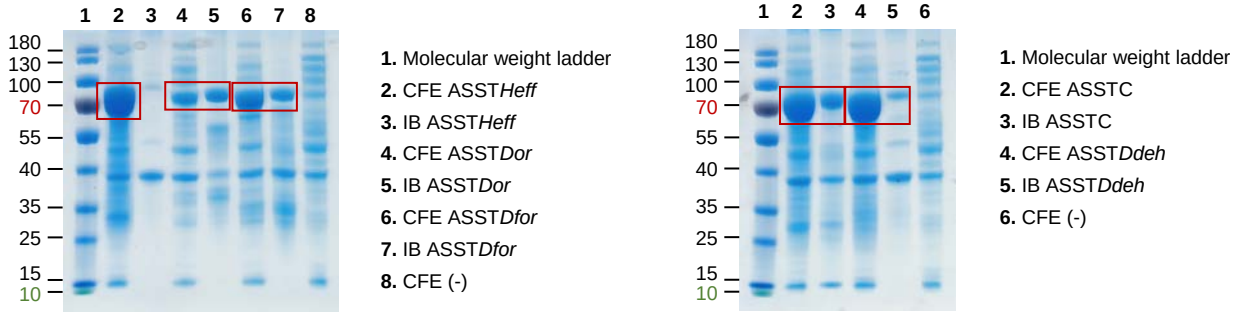


Fig. S4 Overexpression of the ASSTs displaying sequence homology with ASSTA. **CFE**: cell free extract; **IB**: inclusion bodies; **(-)**: negative control. The expected molecular weights are described in **Table S3**

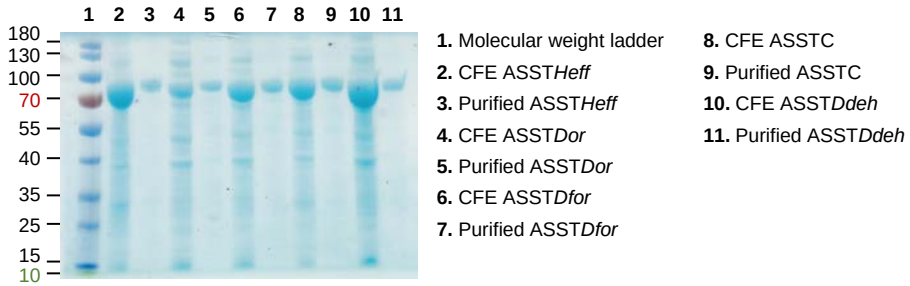
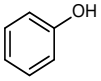
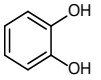
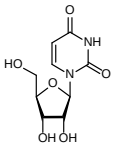
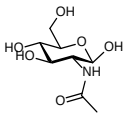


Fig. S5 IMAC purification of the ASSTs displaying sequence homology with ASSTA. **CFE**: cell free extract; **(-)**: negative control. The expected molecular weights are described in **Table S3**

Table S8 Activity measured for the different ASSTs

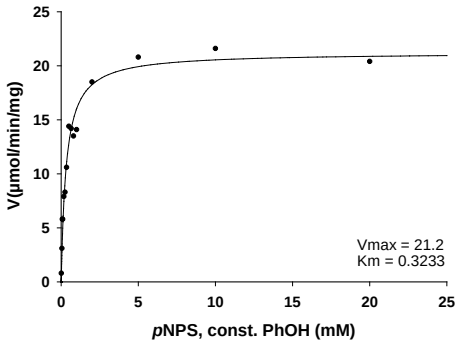
	Activity U/ml$_{CFE}$			
	Substrate			
ASST	 **Phenol**	 **Catechol**	 **Uridine**	 **GlcNAc**
ASST*Heff*	1.7	20.0	n.d.	n.d.
ASST*Dor*	0.1	2.0	n.d.	n.d.
ASST*Dfor*	7.4	26.0	n.d.	n.d.
ASSTC	0.2	1.5	n.d.	n.d.
ASST*Ddeh*	22.3	135.9	n.d.	n.d.
ASSTA	0.7	3.4	n.d.	n.d.
ASSTB	11.6	16.6	n.d.	n.d.

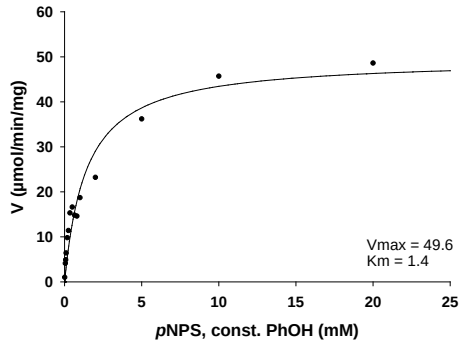
n.d: no activity detected

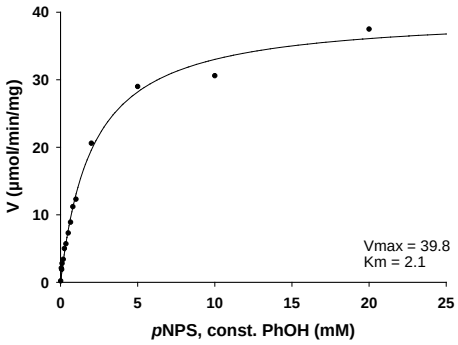
Reactions conditions: Tris/HCl buffer (50 mM, pH 8.0), *p*NPS (1 mM), acceptor substrate (1 mM) and the ASST-containing CFE. Reaction progress was followed spectrophotometrically at 410 nm

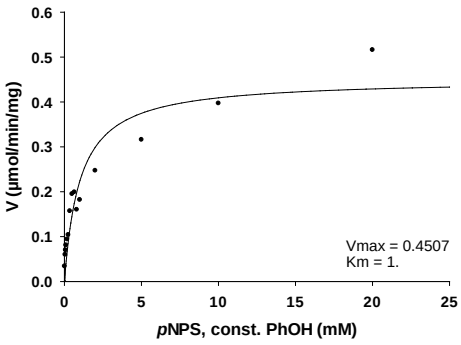
Table S9 Melting temperature values measured by thermofluor assay. Each sample contained Sypro® Orange as fluorescent dye (2.5 µl in 50 mM MES buffer pH 7.0) and the corresponding ASST (1 mg/ml final conc.) dissolved in buffer (Tris/HCl 50 mM at pH 8.0 or 9.0, 25 µl final volume)

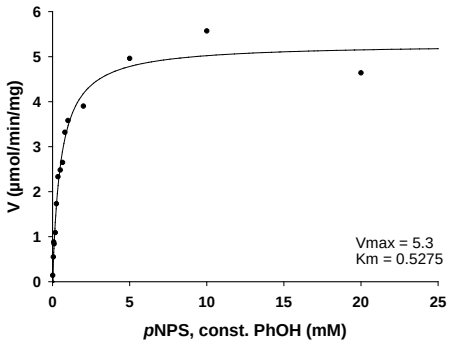
	T_m (°C)	
	pH 8.0	**pH 9.0**
ASSTA	44.5 ± 0.7	40.0 ± 0.0
ASSTB	43.0 ± 0.0	38.3 ± 0.6
ASSTC	39.3 ± 0.6	35.7 ± 0.6
ASST*Heff*	38.7 ± 0.6	35.7 ± 2.1
ASST*Dfor*	40.7 ± 0.6	38.0 ± 0.0
ASST*Dor*	38.0 ± 1.0	38.0 ± 0.0
ASST*Ddeh*	39.0 ± 0.0	36.0 ± 0.0

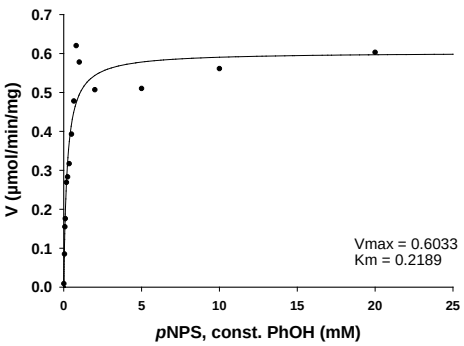












ASST*Ddeh*

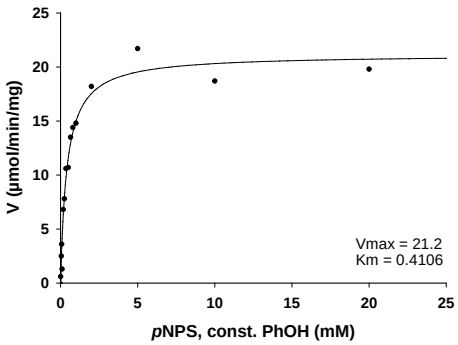
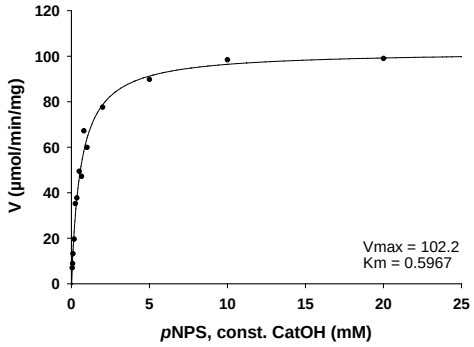
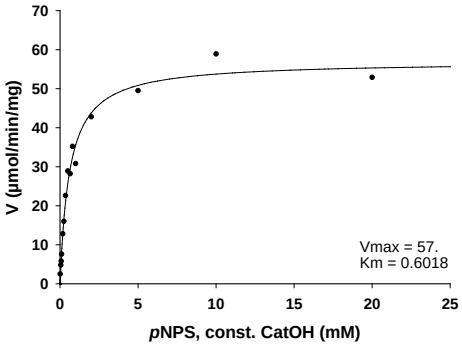
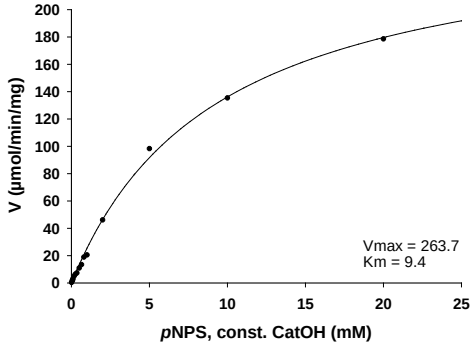
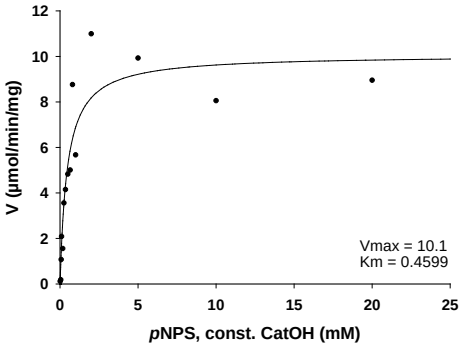


Fig. S6 Apparent kinetic parameters of ASSTA, ASSTB, ASST*Heff*, ASST*Dor*, ASST*Dfor*, ASSTC, and ASST*Ddeh* for *p*NPS. Reaction conditions: Tris/HCl buffer (50 mM, pH 8.0), *p*NPS (0-20 mM), phenol (**1**) (100 mM) and the purified ASST (50 nM)

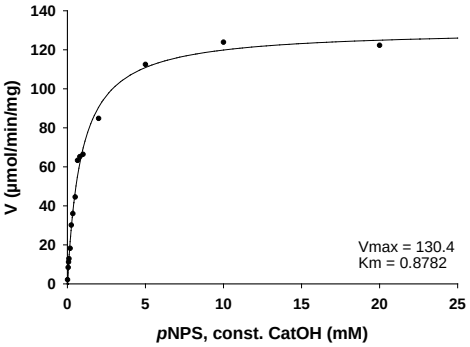


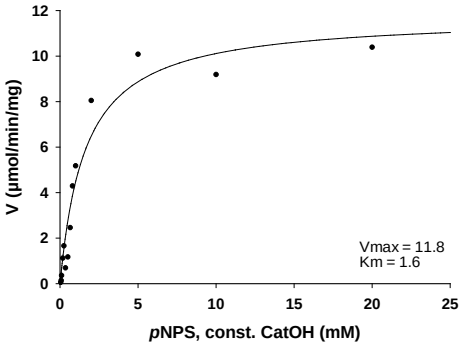






ASSTC





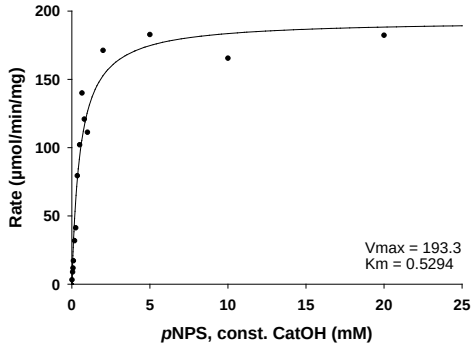


Fig. S7. Apparent kinetic parameters of ASSTA, ASSTB, ASST*Heff*, ASST*Dor*, ASST*Dfor*, ASSTC, and ASST*Ddeh* for *p*NPS. Reaction conditions: Tris/HCl buffer (50 mM, pH 8.0), *p*NPS (0-20 mM), catechol (**2**) (100 mM) and the purified ASST (50 nM)

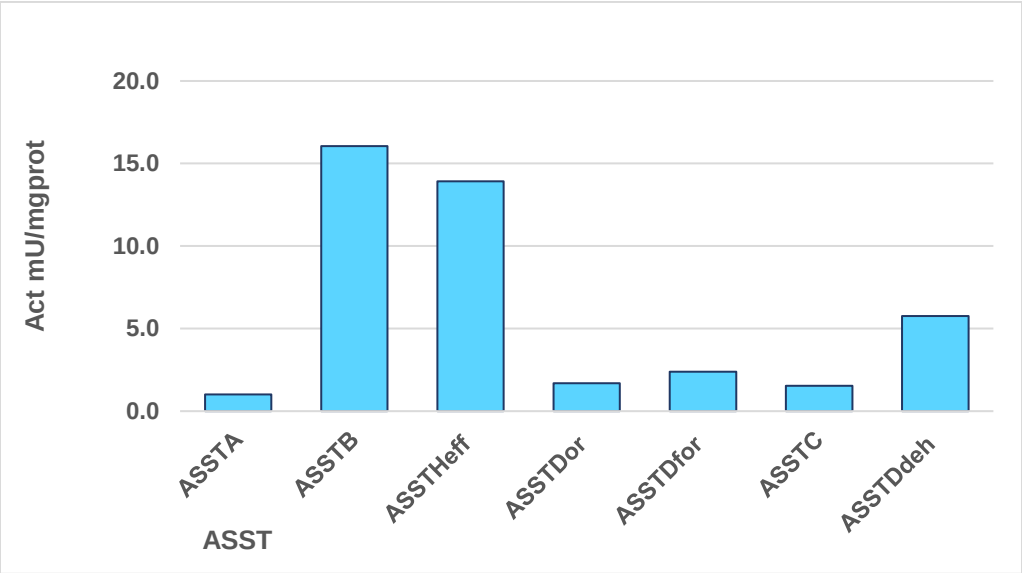


Fig. S8 Activity displayed by each enzyme in presence of 10% 2-propanol in the absence any other acceptor substrate. Reactions conditions: Tris/HCl buffer (50 mM, pH 8.0), *p*NPS (1 mM), 2-propanol (10%, v/v) and the purified ASST

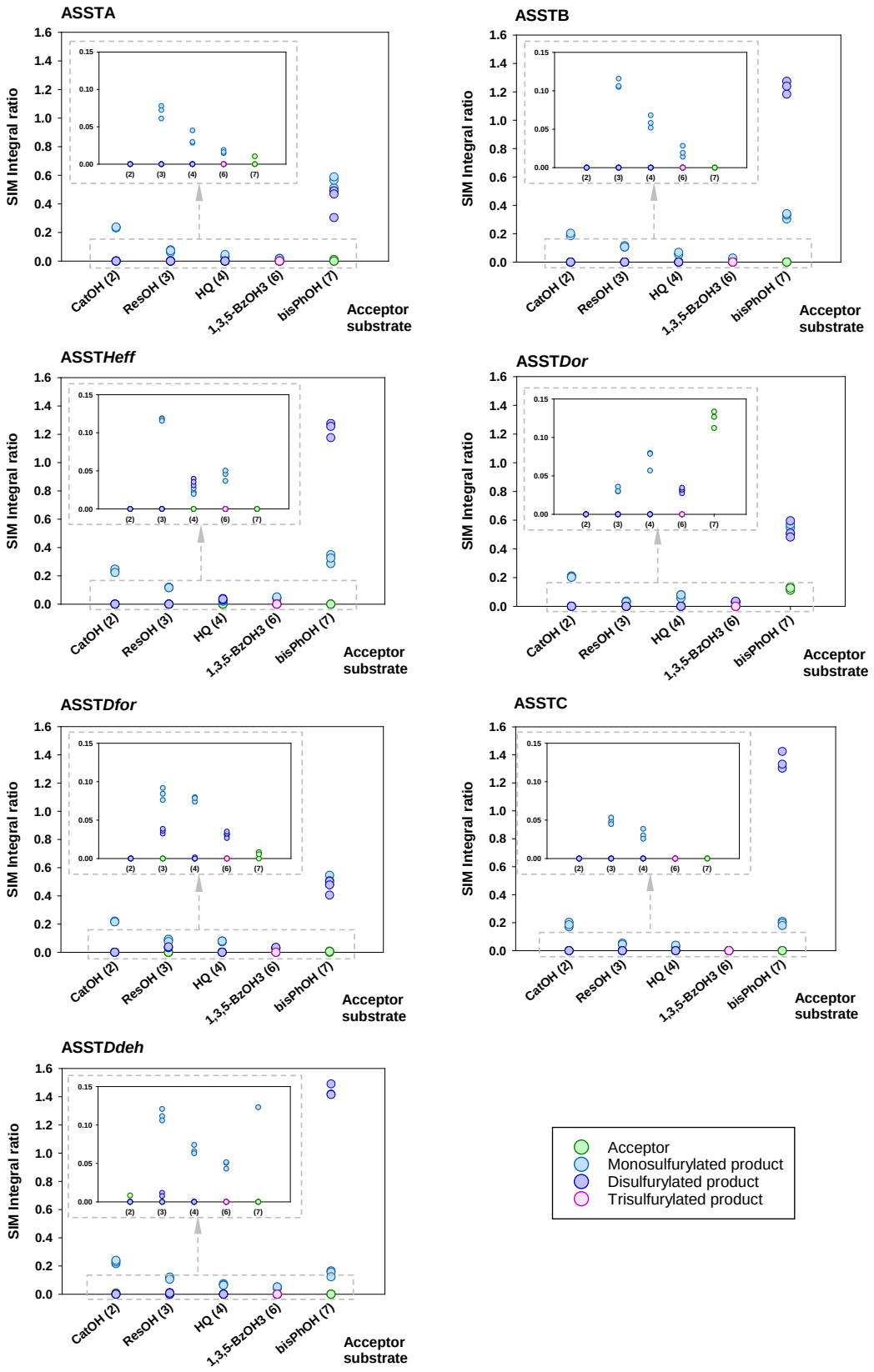


Fig. S9 Polysulfation screening of di- and trihydroxy aromatic compounds catalyzed by ASSTs. Coloured dots show the SIM integral ratios (target/buffer) of the substrate and the possible products in the biotransformations. Reactions were carried out in buffer PIPES 20 mM, pH 8.0; acceptor (2, 3, 4, 6, or 7) 0.5 mM; *p*NPS 1.5 mM; and purified ASST.

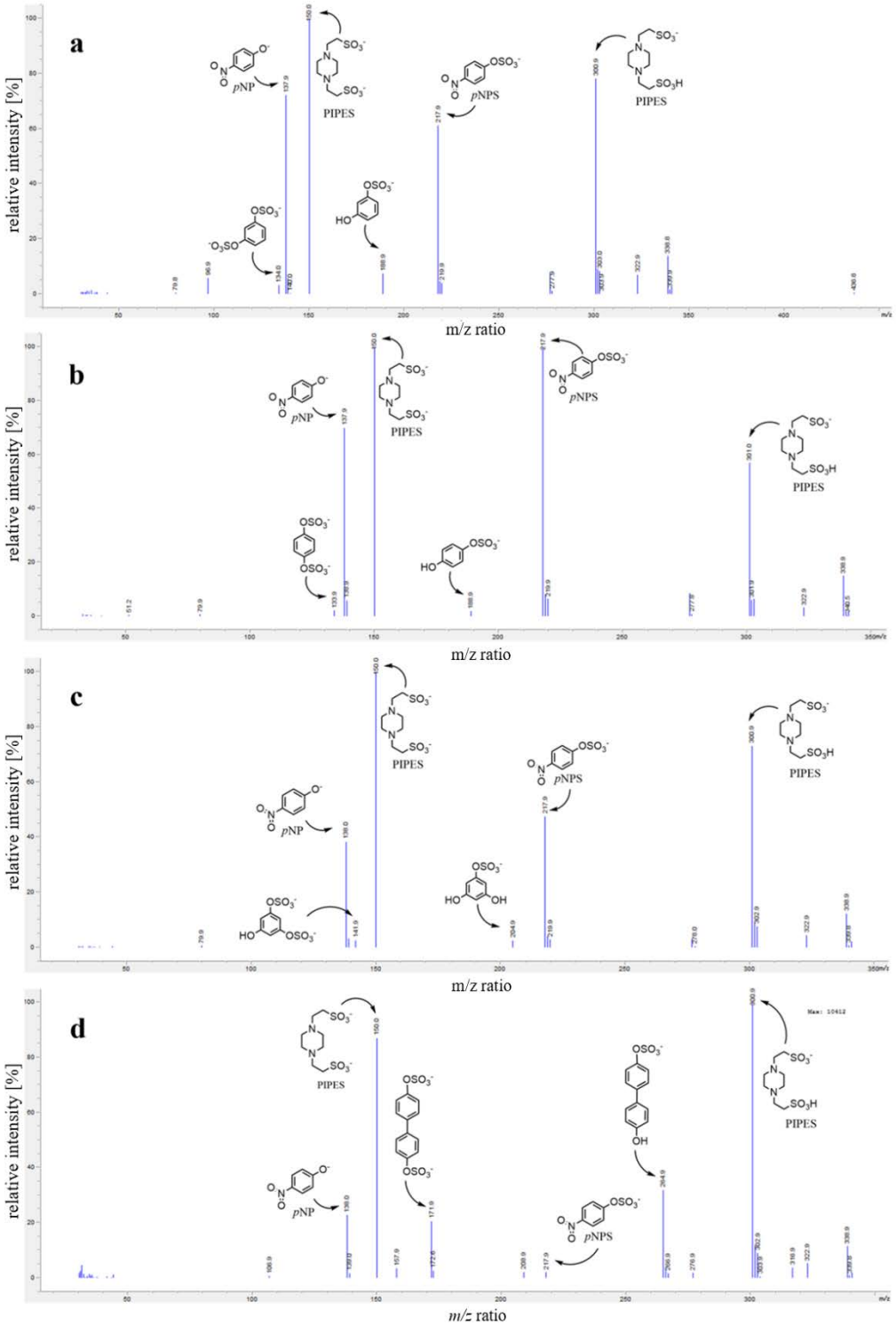


Fig. S10 Examples of mass spectra obtained in FIA-MS analysis of disulfation reactions with ASST. (**a**) Conversion of resorcinol (**3**) to the mono- and disulfate derivative (*m/z*: 188.9 and 134.0 respectively). (**b**) Conversion of hydroquinone (**4**) to the mono- and disulfate (*m/z*: 188.9 and 134.0 respectively). (**c**) Conversion of 1,3,5-benzenetriol (**6**) to the mono- and disulfate (*m/z*: 204.9 and 141.9 respectively). (**d**) Conversion of 4,4‘-dihydroxybiphenyl (**7**) to the mono- and disulfate (*m/z*: 264.9 and 171.9 respectively).

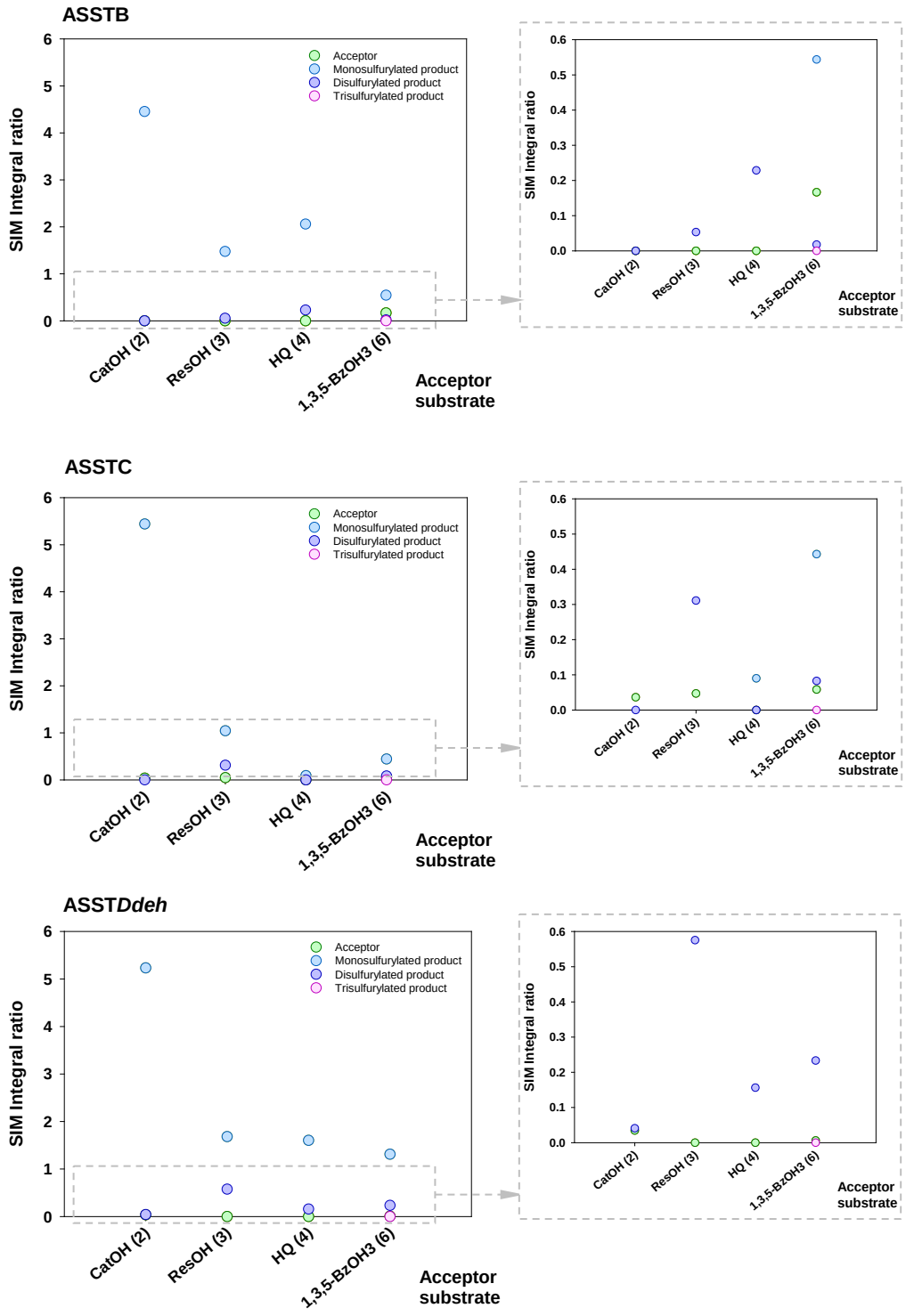


Fig. S11 Polysulfation screening of di- and trihydroxy aromatic compounds catalyzed by ASSTB, ASSTC and ASST*Ddeh* during a long period of time. Colored dots show the SIM integral ratios (target/buffer) of the substrate and the possible products in the biotransformations. Reactions were carried out in buffer PIPES 20 mM, pH 8.0; acceptor (**2**, **3**, **4**, or **6**) 10.0 mM; *p*NPS 30.0 mM; and purified ASST. Reactions were shaken at 650 rpm during 16 h.

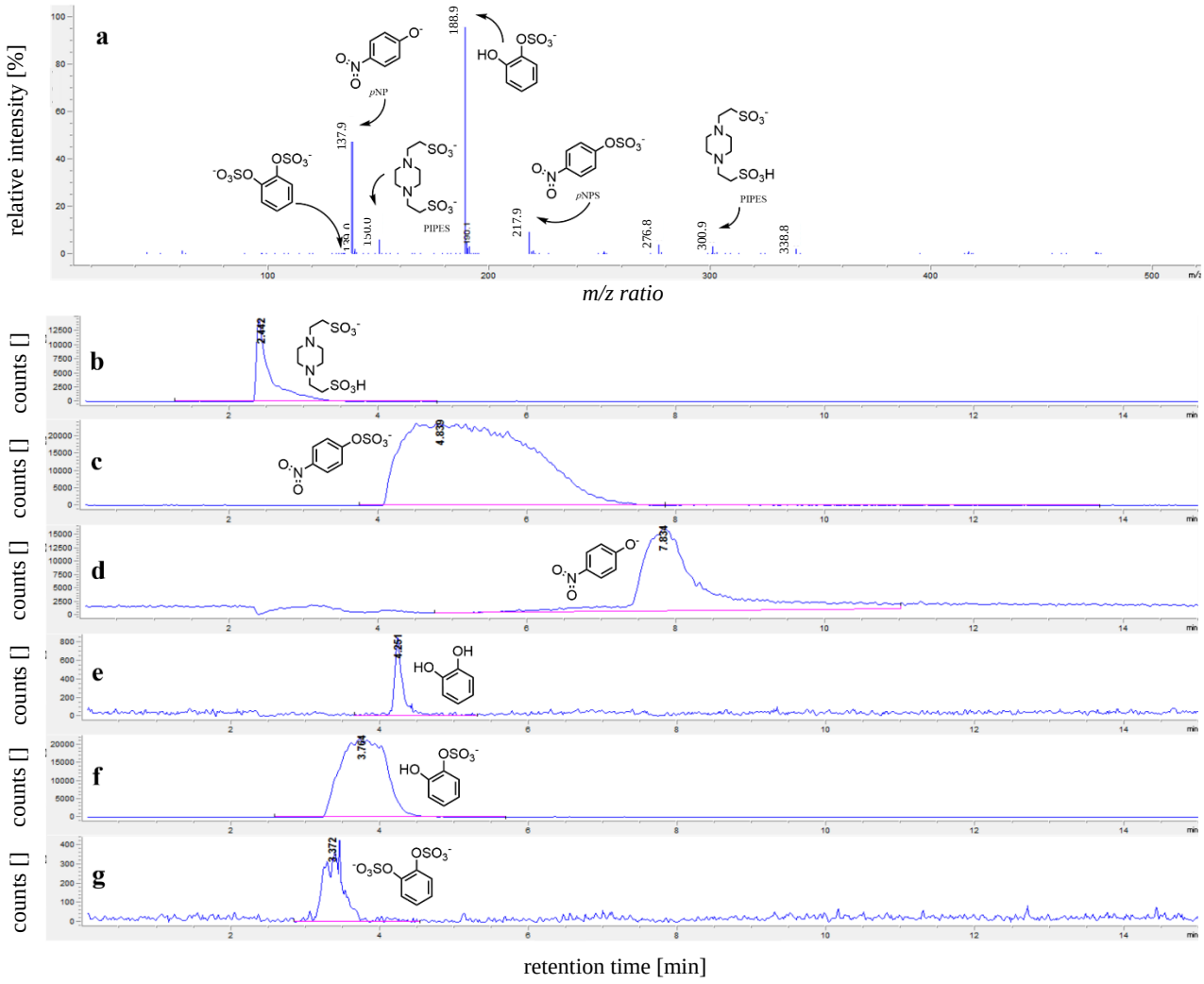


Fig. S12 (**a**) Mass spectrum obtained in 16 h disulfation reactions catalyzed by ASST*Ddeh* with catechol (2) as acceptor substrate, identifying important observed species. (**b**-**g**) Extracted ion chromatograms showing the elution peaks of different species, i.e., (**b**) PIPES (*m*/*z* 301), (**c**) *p*NPS (*m*/*z* 218), (**d**) *p*NP (*m*/*z* 138), (**e**) catechol (2, *m*/*z* 109), (**f**) catechol monosulfate (*m*/*z* 189) and (**g**) catechol disulfate (*m*/*z* 134).

Plasmid sequences

ASSTA *D. hafniense* as cloned in pET26b *in silico*

Name: pET26b-PelB- ASSTA-His

Restriction Enzymes

5': *Sac*I

3': *Sal*I

5' Flanking: A

3' Flanking: None

Gene sequence ordered

ATGAATCCGATCAAAAGTGAACAGATTCCCCATATTATCCATAGACAAAAGGATTTGGAAGAAGCTTTTCTGGCTG
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ASSTB *D. hafniense* as cloned in pET28a *in silico*

Name: pET28a-ASSTB-His

Restriction Enzymes

5': *Nco*I

3': *Sac*I

5' Flanking: CA

3' Flanking: AA

Gene sequence ordered (optimized for *E. coli*)

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ASST *D. dehalogenans* as cloned in pET28a *in silico*

Name: pET28a-ASST*Ddeh*-His

Restriction Enzymes

5': *Nco*I

3': *Sal*I

5' Flanking: GA

3' Flanking: -

Gene sequence ordered (to be optimized for *E. coli*)

ATGATGAATCCGATTAAAAGCGAACAGATTCCGCATATTATTCATCGTCAGAAAGATTTTGAAGAAGCATTTCTGG
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ASST *D. formicoaceticum* as cloned in pET28a *in silico*

Name: pET28a-ASST*Dfor*-His

Restriction Enzymes

5': *Nco*I

3': *Sal*I

5' Flanking: GA

3' Flanking: -

Gene sequence ordered (to be optimized for *E. coli*)

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ASSTC *D. hafniense* as cloned in pET28a *in silico*

Name: pET28a-ASST*Dhaf*03-His

Restriction Enzymes

5': *Nco*I

3': *Sal*I

5' Flanking: GA

3' Flanking: -

Gene sequence (to be optimize for *E. coli*)

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ASST *D. orientis* as cloned in pET28a *in silico*

Name: pET28a-ASST*Dori*-His

Restriction Enzymes

5': *Nco*I

3': *Sal*I

5' Flanking: GA

3' Flanking: -

Gene sequence ordered (to be optimize for *E. coli*)

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ASST *H. effluvii* as cloned in pET28a *in silico*

Name: pET28a-ASSTHeff-His

Restriction Enzymes

5': *Nco*I

3': *Sal*I

5' Flanking: GA

3' Flanking: -

Gene sequence ordered (to be optimize for *E. coli*)

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TCTGTTAGAAGATAAAATCTACGAAACGGGTGTAGAGATCCGTATGGAA