Supplementary Information for

Structural basis for the substrate binding and catalytic mechanism of *Se*-glycosyltransferase SenB in the biosynthesis of selenoneine

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Supplementary Table 1. Data collection and refinement statistics of SenB in complex with different sugar donors.

	SenB/UDP-GlcNAc	**SenB/UDP-GalNAc**	**SenB/UDP-Glc/PO_4^{3-}**	**SenB/UDP-Rha**
PDB Entry	8JJT	8JJQ	8JJN	8JK7
Data collection				
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
a, *b*, *c* (Å)	108.22, 56.38, 108.45	108.11, 56.38, 108.13	107.83, 55.79, 108.08	109.10, 56.56, 109.69
α, β, γ (°)	90, 91.11, 90	90, 91.01, 90	90, 90.94, 90	90, 90.60, 90
Resolution (Å)	36.55-1.88 (1.95-1.88)	48.68- 1.64 (1.70-1.64)	47.99- 1.98 (2.05-1.98)	37.05-1.93 (2.00-1.93)
Unique reflections	106607 (10530)	157919 (14143)	89964 (8920)	98802 (7956)
Redundancy	6.6 (6.6)	6.2 (3.9)	6.6 (5.9)	6.2 (6.5)
Completeness (%)	99.75 (99.57)	98.72 (88.63)	99.91 (99.94)	97.62 (79. 23)
I/σ(I)	15.60 (2.77)	13.44 (2.09)	13.76 (1.98)	14.37 (6.77)
R_{merge}	0.08 (0.71)	0.08 (0.50)	0.11 (0.92)	0.11 (0.24)
$CC_{1/2}$	0.998 (0.856)	0.997 (0.716)	0.998 (0.66)	0.991 (0.95)
Refinement				
R_{work}/R_{free}	0.201/0.227	0.189/0.207	0.195/0.221	0.259/0.286
No. atoms				
Protein	7223	7218	7273	7255
Ligands	117	117	123	105
***B*-factor (Å^2)**				
Protein	26.03	18.86	29.97	34.48
Ligands	20.67	14.19	32.56	35.42
R.m.s deviations				
Bond length (Å)	0.008	0.007	0.009	0.158
Bond angle (°)	1.05	1.02	0.96	4.28
Ramachandran Plot				
Favored (%)	96.85	97.27	97.60	89.36
Allowed (%)	2.73	2.10	1.88	8.45
Outliers (%)	0.42	0.63	0.52	2.19

*Values in parentheses refer to the highest resolution shell.

Supplementary Table 2. Detailed interactions between SenB and UDP.

Residue	Protein atom	Ligand atom	Distance (Å)
Hydrogen bond			
R155	NH2	O2B	2.7
K158	NZ	O2B	3.2
G19	N	O1B	3.2
H235	ND1/N	O1A	2.8/2.8
V236	N	O2A	3.3
R22	NH1/NE	O3C	3.0/4.0
V236	OE2	O3C	2.6
E239	OE1	O2C	2.9
N17	ND2	O2	2.8
L209	O/N	N3/O4	3.2/3.5
T214	OG1	N3	3.3
hydrophobic interaction			
V151	CG1	C5	3.7

Supplementary Table 3. DNA coding sequences and amino acid sequences of *Cb*SenB and *Rs*SenB.

***Cb*SenB (from *C. bacterium*, DNA coding sequences)**
atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcagccatatgagccgtagcagtag
tgcagtgattgtgagtccggccctggccgatgcaaataatggtaattggcgcaccgcccagcgttggcagcagatgctg
gccccgcagcgccctgtgcgtattgttcgcgaatggccggatgcacaggcagccagcgatggtgtgatgctggcactg
catgcccgtcgcagtgcagcagcagtgcgtgcctggagtgaagcacatccgggccgtggcctggccgttgtgctgac
aggtaccgatctgtatcaggatctggcaaccagcgcagaagcacgtcatagtgtggccgtggcccagcgcctggttgt
gctgcaggaacgcggcgttgaagcactggatgcaccgctgcgtccgaaagcccgtgtgatttatcagagtggtccggc
ctggccgccgctggcaaaacctcgtgatggcctgggcgcagtgagtgttggtcatctgcgtgcagtgaaaaccccgca
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gctgctggcacagcaggcacgtgatacccagcgcgattgcccgggttatgaatggctgggtgccctgccgcatgatgc
aaccctgcagcgcattcgcgaagcacatgtgctggttcataccagtgccatggaaggtggcgcccatgtgattctggaa
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ttttccgcatggcgatgcagcagcactggcacgcctgctgcaggattgccgtgcaacccagagtgataatccggcccc
gggtctgctggatcgcctggcagcacagtgtgcactgcgcgccccgctgtttgatgccgatgccgaacgtcgtgcact
gctgaatctgctgcaggaactggaaccgaccccgtaa

***Cb*SenB (from *C. bacterium*, amino acid sequences)**
MGSSHHHHHHSSGLVPRGSHMSRSSSAVIVSPALADANNGNWRTAQRWQQ
MLAPQRPVRIVREWPDAQAASDGVMLALHARRSAAAVRAWSEAHPGRGL
AVVLTGTDLYQDLATSAEARHSVAVAQRLVVLQERGVEALDAPLRPKARVI
YQSGPAWPPLAKPRDGLGAVSVGHLRAVKTPQTLFEAARLMRDRADIRITHI
GDGGGEPLLAQQARDTQRDCPGYEWLGALPHDATLQRIREAHVLVHTSAM
EGGAHVILEAVRCGTPVLASRVDGNVGMLGADYEGYFPHGDAAALARLLQ
DCRATQSDNPAPGLLDRLAAQCALRAPLFDADAERRALLNLLQELEPTP

***Rs*SenB (from *Ramlibacter sp.*, DNA coding sequences)**
atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcagccatatgagtcgcccgagcgt
ggcaattgtgagtccggccgtggcaagcgccaataatggcaattggcagaccgcccgccgttggcaggaatttctgga
tggcacctgcaatgttcgtatgacccagcgctggccggatgatggcagtcaggatgatgttgttatgctggccctgcatg

cacgtcgcagtgcagatagcattgaagcatgggccagtgtgcatggcgatcgtggtctggcagttgttctgaccggtac

cgatctgtatcaggatattgtggtggatccgcgtgcccgccatagtctggaactggccggtcagctggttgttctgcagg

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gcagccagcaaaccggataccgtgctgcaggcactgatggtgggccatctgcgtgaagttaaaagtccgcagaccct

gtttcaggcagcccgcctgctggccggtcatgatgatattcgcattgatcatattggtgaagccctggatccggttctggg

cgaacaggccctggcaacccagcgcgattgcccgaattatcgctggctgggtgcactgccgcatgatggtacccgcg

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cagtggcaccccggttctggccagccgtattccgggtaatgtgggcatgctgggcgccgattatgcaggttattttaccc

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atccgctgctggcacgcctgggtgcacagtgcgccctgcgtgcaccgctgtttgccccggaagcagaacgcgcagcc

ctgctgcgtctggtggccgatctgatgtaa

***Rs*SenB (from *Ramlibacter sp.*, amino acid sequences)**

MGSSHHHHHHSSGLVPRGSHMSRPSVAIVSPAVASANNGNWQTARRWQEFL

DGTCNVRMTQRWPDDGSQDDVVMLALHARRSADSIEAWASVHGDRGLAV

VLTGTDLYQDIVVDPRARHSLELAGQLVVLQDLGAEALPPALRGKTRVIYQ

STPSQAAASKPDTVLQALMVGHLREVKSPQTLFQAARLLAGHDDIRIDHIG

EALDPVLGEQALATQRDCPNYRWLGALPHDGTRERIRCAHLLVHASAMEG

GAHVIMEAVCSGTPVLASRIPGNVGMLGADYAGYFTHGDAAALAALLVRC

RQGQAASGDVPADPLLARLGAQCALRAP LFAPEAERAALLRLVADLM*

Supplementary Table 4. Data collection and refinement statistics of *Rs*SenB.

	***Rs*SenB**
PDB Entry	8K5U
Space group	$P2_1$
a, *b*, *c* (Å)	80.83, 73.79, 81.28
α, β, γ (°)	90, 116.11, 90
Resolution (Å)	35.39-2.15 (2.23-2.15)
Unique reflections	46812 (4638)
Redundancy	6.6 (5.9)
Completeness (%)	99.84 (99.98)
I/σ(I)	10.21 (2.47)
R_{merge}	0.15 (0.69)
$CC_{1/2}$	0.994 (0.813)
R_{work}/R_{free}	0.206/0.250
No. atoms	
Protein	4808
Ligands	0
***B*-factor (Å^2)**	
Protein	24.90
Ligands	0
Bond length (Å)	0.008
Bond angle (°)	0.91
Favored (%)	96.68
Allowed (%)	3.01
Outliers (%)	0.32

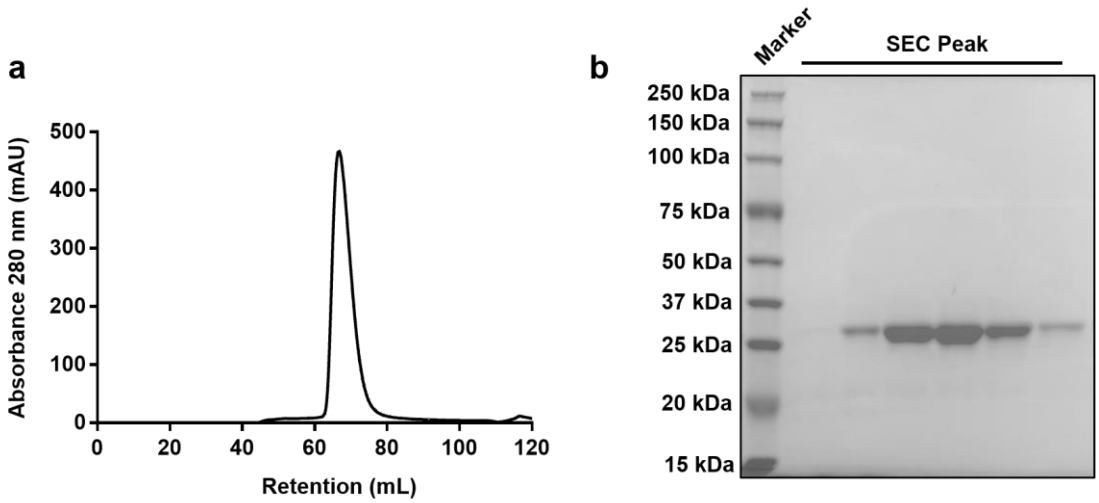
*Values in parentheses refer to the highest resolution shell.

Supplementary Table 5. Primers used in this work.

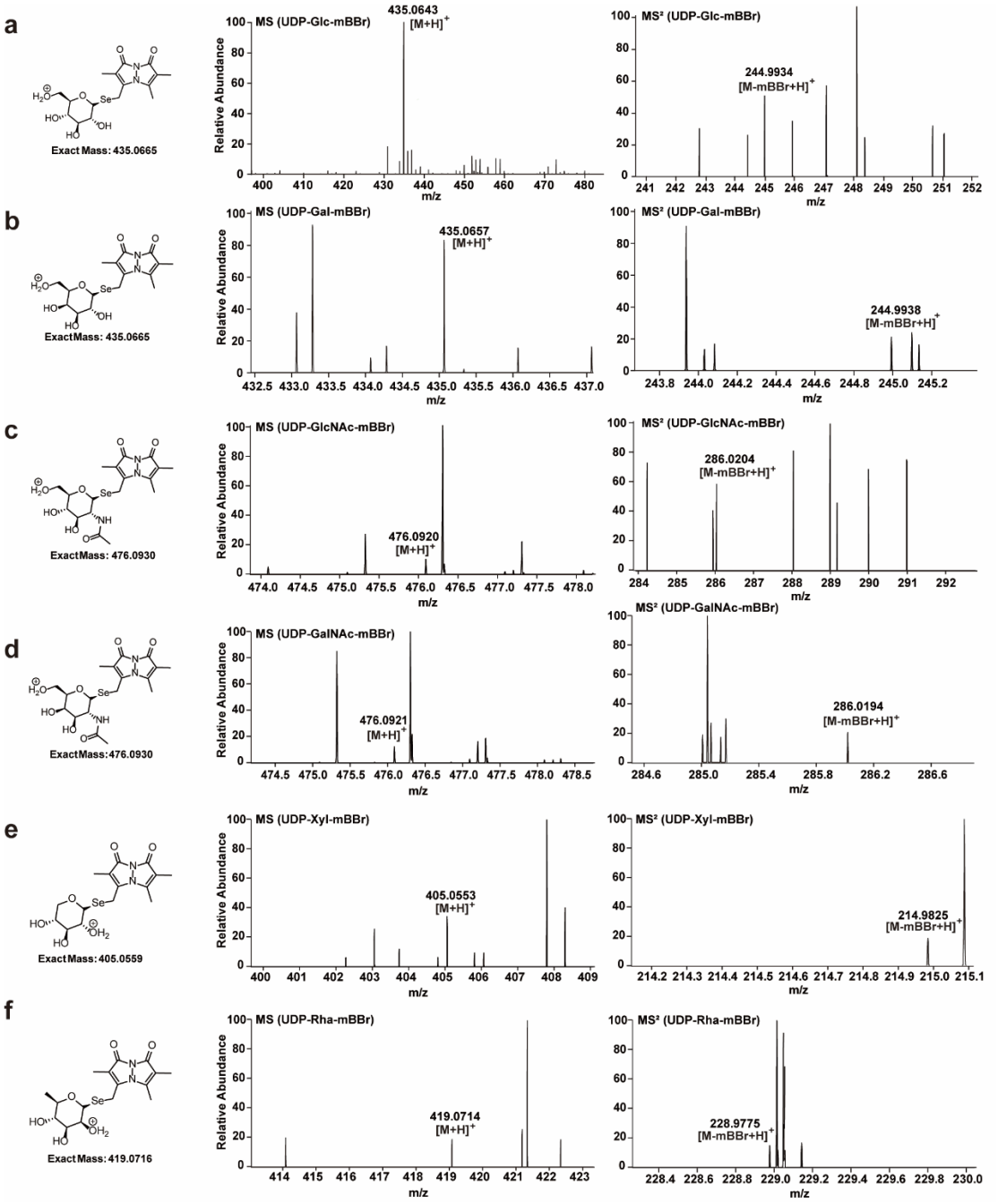
Primer name	Sequence (5′-3′)
N17A-F	GCGCTGCCGGGCGCGGCAAACGGCAACTGGCGC
N17A-R	GCGCCAGTTGCCGTTTGCCGCGCCCGGCAGCGC
G19A-F	CCGGGCGCGAACAACGCAAACTGGCGCACCGCT
G19A-R	AGCGGTGCGCCAGTTTGCGTTGTTCGCGCCCGG
N20A-F	GGCGCGAACAACGGCGCATGGCGCACCGCTCAA
N20A-R	TTGAGCGGTGCGCCATGCGCCGTTGTTCGCGCC
R22A-F	AACAACGGCAACTGGGCAACCGCTCAACGCTGG
R22A-R	CCAGCGTTGAGCGGTTGCCCAGTTGCCGTTGTT
T23A-F	AACGGCAACTGGCGCGCAGCTCAACGCTGGAAA
T23A-R	TTTCCAGCGTTGAGCTGCGCGCCAGTTGCCGTT
H58A-F	GTGATGCTGGCGCTGGCAGCGCGCCGCAGCGCG
H58A-R	CGCGCTGCGGCGCGCTGCCAGCGCCAGCATCAC
H58K-F	GTGATGCTGGCGCTGAAAGCGCGCCGCAGCGCG
H58K-R	CGCGCTGCGGCGCGCTTTCAGCGCCAGCATCAC
H58Q-F	GTGATGCTGGCGCTGCAGGCGCGCCGCAGCGCG
H58Q-R	CGCGCTGCGGCGCGCCTGCAGCGCCAGCATCAC
H58F-F	GTGATGCTGGCGCTGTTTGCGCGCCGCAGCGCG
H58F-R	CGCGCTGCGGCGCGCAAACAGCGCCAGCATCAC
H58D-F	GTGATGCTGGCGCTGGATGCGCGCCGCAGCGCG
H58D-R	CGCGCTGCGGCGCGCATCCAGCGCCAGCATCAC
R61A-F	GCGCTGCATGCGCGCGCAAGCGCGGAAAGCATT
R61A-R	AATGCTTTCCGCGCTTGCGCGCGCATGCAGCGC
T83A-F	TGGGCGTGGTGCTGGCAGGCACCGATCTGTATC
T83A-R	ATACAGATCGGTGCCTGCCAGCACCACGCCCAG
T85A-F	GTGGTGCTGACCGGCGCAGATCTGTATCAAGAT
T85A-R	ATCTTGATACAGATCTGCGCCGGTCAGCACCAC
D86A-F	GCTGACCGGCACCGCACTGTATCAAGATATCGG

D86A-R	ATATCTTGATACAGTGCGGTGCCGGTCAGCACC
Q131A-F	GCCCGCGTGGTGTATGCAAGCACGAGCGCGCGT
Q131A-R	ACGCGCGCTCGTGCTTGCATACACCACGCGGGC
V151A-F	AGCTGCGCGCGGTGATGGCAGGCCATCTGCGCC
V151A-R	AGATGGCCTGCCATCACCGCGCGCAGCTGACG
H153A-F	TGATGGTGGGCGCACTGCGCCAAGTGAAAAGCCCGC
H153A-R	TTCACTTGGCGCAGTGCGCCCACCATCACCGCGCGC
R155A-F	ATGGTGGGCCATCTGGCACAAGTGAAAAGCCCG
R155A-R	CGGGCTTTTCACTTGTGCCAGATGGCCCACCAT
K158A-F	CATCTGCGCCAAGTGGCAAGCCCGCAGACCCTG
K158A-R	CAGGGTCTGCGGGCTTGCCACTTGGCGCAGATG
K158R-F	CATCTGCGCCAAGTGCGTAGCCCGCAGACCCTG
K158R-R	CAGGGTCTGCGGGCTACGCACTTGGCGCAGATG
K158H-F	CATCTGCGCCAAGTGCATAGCCCGCAGACCCTG
K158H-R	CAGGGTCTGCGGGCTATGCACTTGGCGCAGATG
K158E-F	CATCTGCGCCAAGTGGAAAGCCCGCAGACCCTG
K158E-R	CAGGGTCTGCGGGCTTTCCACTTGGCGCAGATG
K158N-F	CATCTGCGCCAAGTGAATAGCCCGCAGACCCTG
K158N-R	CAGGGTCTGCGGGCTATTCACTTGGCGCAGATG
K158G-F	CATCTGCGCCAAGTGGGTAGCCCGCAGACCCTG
K158G-R	CAGGGTCTGCGGGCTACCCACTTGGCGCAGATG
K158V-F	CATCTGCGCCAAGTGGTTAGCCCGCAGACCCTG
K158V-R	CAGGGTCTGCGGGCTAACCACTTGGCGCAGATG
I181A-F	AAGATATTCGCATTGATCATGCAGGGGACGCG
I181A-R	CGCGTCCCCTGCATGATCAATGCGAATATCTTCGC
L209A-F	ATGGTTGGGCGCGGCACCGCATGCGCAGACCCG
L209A-R	TGCGCATGCGGTGCCGCGCCCAACCATCTGTACCC
H211A-F	TTGGGCGCGCTGCCGGCAGCGCAGACCCGTC
H211A-R	ACGGGTCTGCGCTGCCGGCAGCGCGCCCAACC

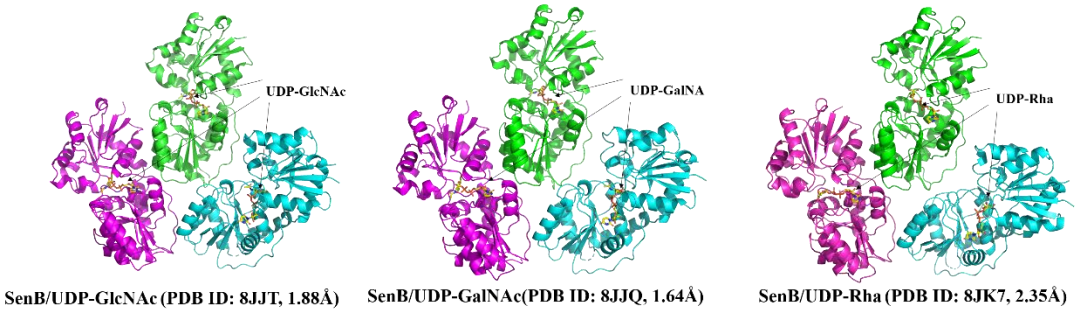
T214A-F	CTGCCGCATGCGCAGGCACGTCAGCGCATTCAG
T214A-R	CTGAATGCGCTGACGTGCCTGCGCATGCGGCAG
E231A-F	CATACGAGCGCGCTGGCAGGCGGCGCGCATGTG
E231A-R	CACATGCGCGCCGCCTGCCAGCGCGCTCGTATG
H235A-F	CTGGAAGGCGGCGCGGCAGTGATTATGGAAGCG
H235A-R	CGCTTCCATAATCACTGCCGCGCCGCCTTCCAG
V236A-F	GAAGGCGGCGCGCATGCAATTATGGAAGCGGTG
V236A-R	CACCGCTTCCATAATTGCATGCGCGCCGCCTTC
E239A-F	GCGCATGTGATTATGGCAGCGGTGCGCAGCGGC
E239A-R	GCCGCTGCGCACCGCTGCCATAATCACATGCGC
N20A/T23A-F	AACGGCGCATGGCGCGCAGCTCAACGCTGGAAA
N20A/T23A-R	TTTCCAGCGTTGAGCTGCGCGCCATGCGCCGTT
T83A/T85A-F	GTGGTGCTGGCAGGCGCAGATCTGTATCAAGAT
T83A/T85A-R	ATCTTGATACAGATCTGCGCCTGCCAGCACCAC



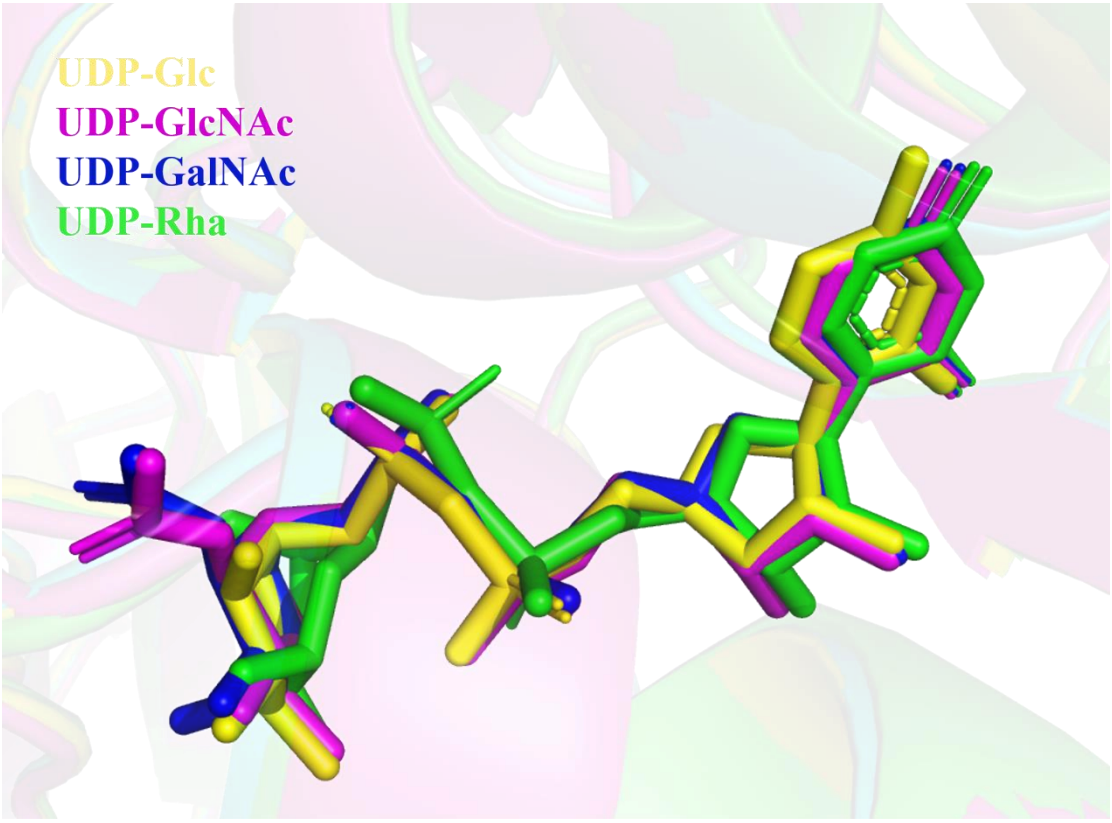
Supplementary Fig 1. Profiles of the size exclusion chromatography and SDS-PAGE analysis of SenB. a, Size exclusion chromatography of SenB. **b,** SDS-PAGE analysis of the purified SenB.



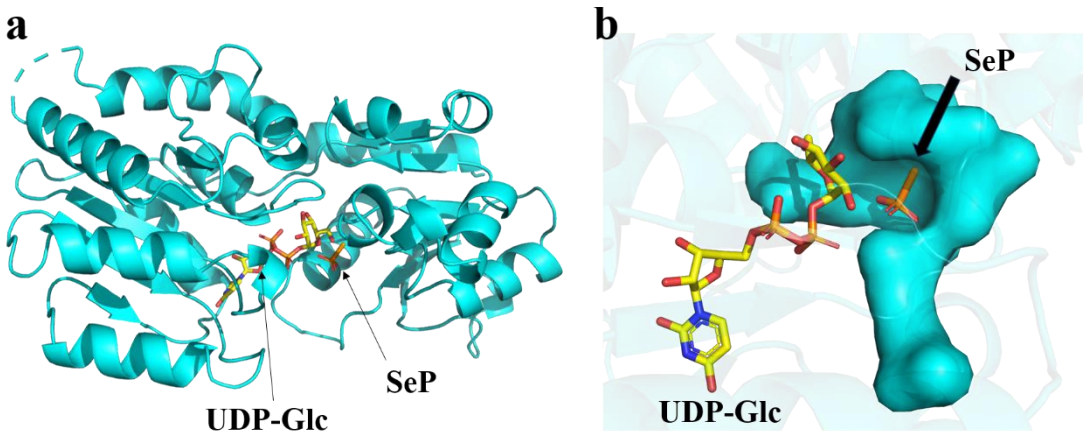
Supplementary Fig 2. LC-MS analysis of the mBBr derivatives of the SenB-catalysed products using different UDP-sugars as the sugar donors. a, (+) ESI MS and MS/MS spectra for UDP-Glc-mBBr. **b,** (+) ESI MS and MS/MS spectra for UDP-Gal-mBBr. **c,** (+) ESI MS and MS/MS spectra for UDP-GlcNAc-mBBr. **d,** (+) ESI MS and MS/MS spectra for UDP-GalNAc-mBBr. **e,** (+) ESI MS and MS/MS spectra for UDP-Xyl-mBBr. **f,** (+) ESI MS and MS/MS spectra for UDP-Rha-mBBr.



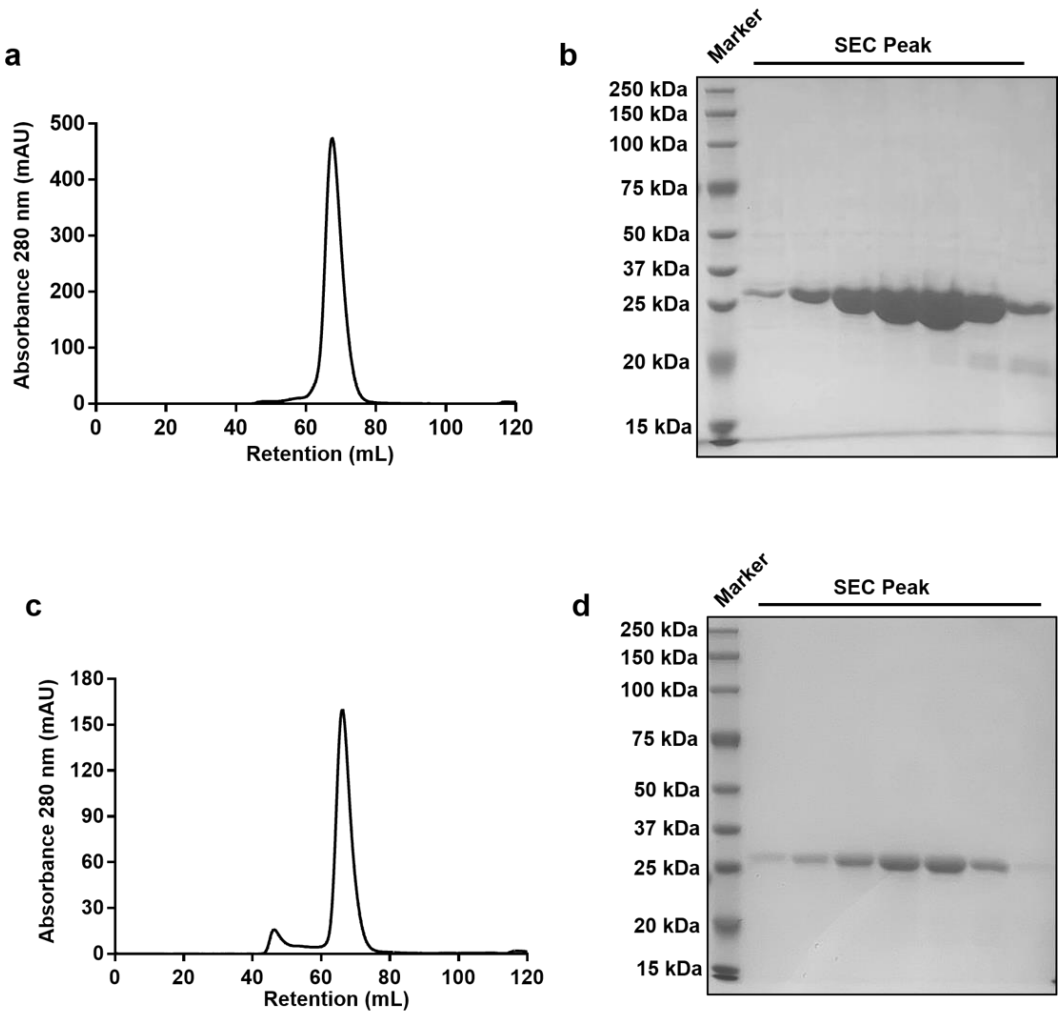
Supplementary Fig 3. The structures of SenB complexed with various sugar donors.



Supplementary Fig 4. Structural superposition of four complex structures of SenB.



Supplementary Fig 5. The ternary complex structure of SenB/UDP-Glc/SeP and the narrow SeP binding pocket of SenB. **a,** The ternary complex structure of SenB/UDP-Glc/SeP. **b,** The narrow SeP binding pocket of SenB.



Supplementary Fig 6. Profiles of the size exclusion chromatography and SDS-PAGE analysis of *Cb*SenB and *Rs*SenB. a, Size exclusion chromatography of *Cb*SenB. **b,** SDS-PAGE analysis of the purified *Cb*SenB. **c,** Size exclusion chromatography of *Rs*SenB. **d,** SDS-PAGE analysis of the purified *Rs*SenB.