

Supplementary Materials: Crystal Structure of the Substrate-Binding Domain from *Listeria monocytogenes* Bile-Resistance Determinant Bile

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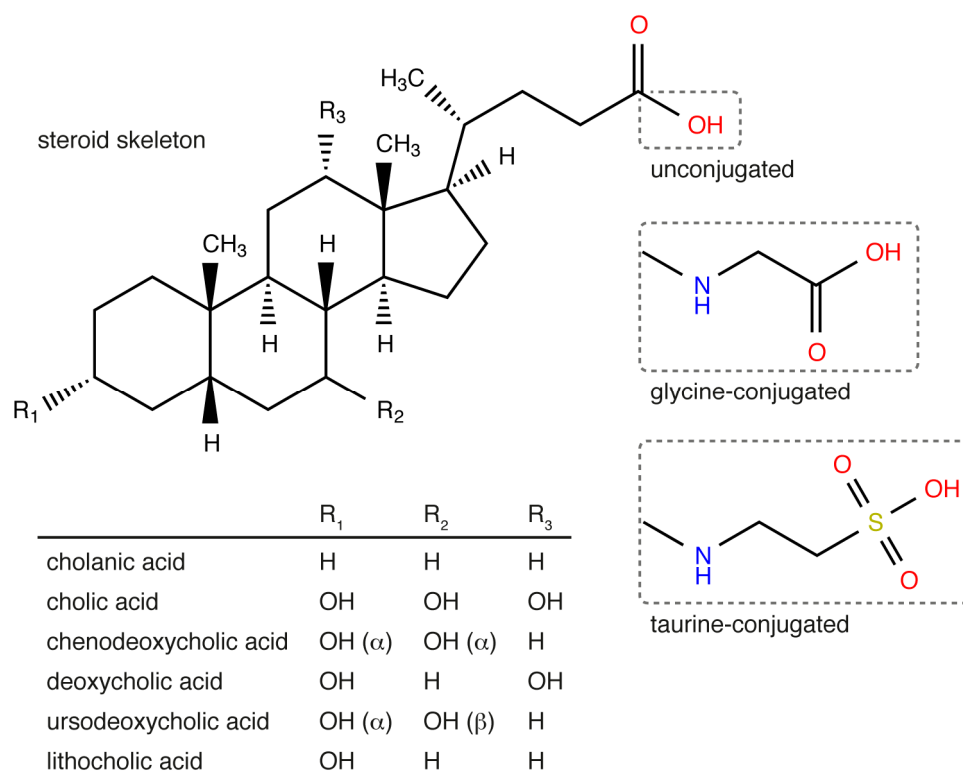


Figure S1. Chemical structures of the most abundant bile acids in humans. Modified from [14], with corrections.

LmGbuA	1	MSKIKVEELTKIFGKKASKASSLLSQGKSKTDILKETG-ATIGV	60
LmOpuCA	1	--MLKFEHVTK-----TYKGG-K-KAVNDTLNIDKGEFFVCI	35
BIEA	1	--MIRFDNVSK-----KYSD-D-KTAAVNVTLIDKGEFFVFI	36
Ybt1_yeast_NBD1	694	---FAFENSTI-----SWDKDNQDFKIDNIEFKTGKLNVI	729
Ybt1_yeast_NBD2	1381	---TEVNDLSL-----RYAPNL-PRVIKNVSFVDAQSKI	1415
ABCB11_mouse_NBD2	1078	---IDFIDCKF-----TYPSPRDIQVLNGLSVSDPGOTLAFV	1113
ABCB11_rat_NBD2	1078	---IDFIDCKF-----TYPSPRDIQVLNGLSVSVNPGOTLAFV	1113
ABCB11_human_NBD2	1078	---IDFVDCKF-----TYPSPRDSQVLNGLSVSISPGOTLAFV	1113
ABCB11_rabbit_NBD2	1078	---IDFVDCKF-----TYPSPRDIQVLNGLSVSMSRQTLAFV	1113
ABCB11_rabbit_NBD1	420	---TEFHNVTF-----HYPSRPEVKILNLSMVIKPGEMTALV	455
ABCB11_human_NBD1	420	---TEFHNVTF-----HYPSRPEVKILNLSMVIKPGEMTALV	455
ABCB11_mouse_NBD1	420	---TEFHNVTF-----HYPSRPEVKILNLSMVIKPGETTALV	455
ABCB11_rat_NBD1	420	---TEFHNVTF-----HYPSRPOVKILNLSMVIKPGETTALV	455
LmGbuA	61	LSGGGKSTLVRLLNRLIEPTSGKIWLQKELSSLNKELLEVRKSM	121
LmOpuCA	36	PSGGGKSTLTKMINRLIEPTGKIFINDKIDMAEDPVK----LR	92
BIEA	37	PSGGGKSTLTKMINRLIPTTGTIYINEKRISDYDTH----LR	93
Ybt1_yeast_NBD1	730	PTSGGKSTLMLALGEMHYLLNGKVVPALEPRQELVD-ANGT	788
Ybt1_yeast_NBD2	1416	RTGAGKSTIITALLFRLEPETGHKIDNIDSGVDLQ----LR	1471
ABCB11_mouse_NBD2	1114	SSGGGKSTSIQLLRFYDPDQGTVMIDGHDSKKVNVQF----	1169
ABCB11_rat_NBD2	1114	SSGGGKSTSIQLLRFYDPDQGTVMIDGHDSKKVNIQF----	1169
ABCB11_human_NBD2	1114	SSGGGKSTSIQLLRFYDPDQGTVMIDGHDSKKVNVQF----	1169
ABCB11_rabbit_NBD2	1114	SSGGGKSTSIQLLRFYDPDQGTVMIDGHDSKKVNVQF----	1169
ABCB11_rabbit_NBD1	456	PSGAGKSTALQLIHRFYGPTEGMVTVESHDIRSSHTQW----	511
ABCB11_human_NBD1	456	PSGAGKSTALQLIHRFYDPCEGMVTVDGHDIRSLNIQW----	511
ABCB11_mouse_NBD1	456	SSGAGKSTALQLIQRFYDPCEGMVTVDGHDIRSLNIQW----	511
ABCB11_rat_NBD1	456	SSGAGKSTALQLIQRFYDPCEGMVTVDGHDIRSLNIQW----	511
LmGbuA	122	INRNVEYGLIEIQGMDKEER-----EKNAAE-----SLALVGL	155
LmOpuCA	93	IRENTVLVPLKLKWKSEKK-----QERAKE-----LKLVDL	127
BIEA	94	IENTAIVPELKKWSKEKI-----HDRITE-----LDSVGLDPE	129
Ybt1_yeast_NBD1	789	VKNLLFNSPFNEARYKAVVEACGL-----KR-----DIEI	826
Ybt1_yeast_NBD2	1472	IKTNLDPYDEFSDRQIFEALKRVNLISEEQQQGATRETSNEAS	1530
ABCB11_mouse_NBD2	1170	IMDNIKYGDNTKEISVER-----AIAAAQ-----AQLHDFV	1210
ABCB11_rat_NBD2	1170	IMDNIKYGDNTKEISVER-----AIAAAQ-----AQLHDFV	1210
ABCB11_human_NBD2	1170	IMDNIKYGDNTKEIPMER-----VIAAAQ-----AQLHDFV	1210
ABCB11_rabbit_NBD2	1170	IKDNIKYGDNTKEIPMER-----IIAAAK-----AQVHDFV	1210
ABCB11_rabbit_NBD1	512	IAEKIRYGRD--ATMED-----LIQAAE-----ANAYNFI	550
ABCB11_human_NBD1	512	IAENIRYGRD--ATMED-----IVQAAE-----ANAYNFI	550
ABCB11_mouse_NBD1	512	IAENIRLGRD--ATMED-----IVQAAK-----ANAYNFI	550
ABCB11_rat_NBD1	512	IAENIRFGRD--ATMED-----IVQAAK-----ANAYNFI	550
LmGbuA	156	YGDQYPSQLSGGQQRVGLARALANNPDILLMDFAFSALDPLNR	216
LmOpuCA	128	FLDRYPYELSGGQQRIGVLRALAAEQNLIMDEPFPGALDPITR	188
BIEA	130	YRHRKPAELSGGQQRGVVLRALAADPGIILMDPEFSALDPTSR	190
Ybt1_yeast_NBD1	827	EIEKIGITLSGGQQRVSLARALYSNARHVLDDCLSAVDSHTA	887
Ybt1_yeast_NBD2	1531	EISEGGSNLSGGQQRVLMCLARSLRSPKILLDEATASIDYS	1589
ABCB11_mouse_NBD2	1211	NVGISGQSLSRGKQRIATARAIVRDPKILLDEATSALDTESEK	1269
ABCB11_rat_NBD2	1211	NVGISGQSLSRGKQRIATARAIVRDPKILLDEATSALDTESEK	1269
ABCB11_human_NBD2	1211	NVGISGQSLSRGKQRIATARAIVRDPKILLDEATSALDTESEK	1269
ABCB11_rabbit_NBD2	1211	NVGISGQSLSRGKQRIATARAIVRDPKILLDEATSALDTESEK	1269
ABCB11_rabbit_NBD1	551	LVGEGGGMGGQQRVAIARALIRNPKILLDMATSALDNESAMV	609
ABCB11_human_NBD1	551	LVGEGGGMGGQQRVAIARALIRNPKILLDMATSALDNESAMV	609
ABCB11_mouse_NBD1	551	LVGEGGGMGGQQRVAIARALIRKPKILLDMATSALDNESAKV	609
ABCB11_rat_NBD1	551	LVGEGGGMGGQQRVAIARALIRNPKILLDMATSALDNESARV	609
LmGbuA	217	IIFITHMDAELRIGHIMIRDSVSVQTSPEIILAHPANVEKFI	277
LmOpuCA	189	IIFVTHMDAELKADRIVIMKDEIVQFDPDEILRNPAASFVED	249
BIEA	191	IIFVTHMQEALALGDRICVMHGGEIVQVAPQIMKNPENDFVK	251
Ybt1_yeast_NBD1	888	CLLVSHNIALTLRNAELVLLDGRVKDQDPIDMOK--GLFGE	935
Ybt1_yeast_NBD2	1590	ILTIARHLRS-VIDYDKILVMDAGEVKEYDHPYSLLNKQSAF	1636
ABCB11_mouse_NBD2	1270	CIIVIAHRLST-IQNSDIHAVMSQGVVIEKGTHEKLM-AQK	1316
ABCB11_rat_NBD2	1270	CIIVIAHRLST-IQNSDIHAVMSQGVVIEKGTHEKLM-AQK	1316
ABCB11_human_NBD2	1270	CIIVIAHRLST-IQNSDIHAVMSQGVVIEKGTHEELM-AQK	1316
ABCB11_rabbit_NBD2	1270	CIIVIAHRLST-IQNSDIHAVMSQGVVIEKGTHEELM-VQK	1316
ABCB11_rabbit_NBD1	610	IISVAHRLPAT-IRTAADVIGCEHCAAVRGTEELL-ERKGV	656
ABCB11_human_NBD1	610	IISVAHRLST-VRAADTVIGFEHGTAVRGTEELL-ERKGV	656
ABCB11_mouse_NBD1	610	IISVAHRLST-VRSADVIGFEHGTAVRGTEELL-ERKGV	656
ABCB11_rat_NBD1	610	IISVAHRLST-VRAADTVIGFEHGTAVRGTEELL-ERKGV	656
LmGbuA	278	---VMIRPEIVNFE-KDGPVALKRMREAGTSSVFVKRNREL	334
LmOpuCA	250	QVAQIMNTNPVSITADKSLQAATVMKEKRVDTLLVVDG	309
BIEA	252	NF-----PTTGL--DTPLAEIMDTI--STTTIPIAVTED	253
LmGbuA	335	SLETALHRDV-----PTTGL--DTPLAEIMDTI--STTTI	382
LmOpuCA	310	SVMDIEIKNVFYVEDTLL-----RDTVQRILKRGYKIP	359
BIEA	254	TVNDLIEADLFYSYQTSDGLTGISSTPEPVNLVRRIA--	311
LmGbuA	383	SVLAALSGNEVNV-----NA-----	397
LmOpuCA	360	SLVDIVYDSIWGTLEDATENQEEQADSKTTEPEMKQEG	397
BIEA	312	HVMQFLARHLE---SSGELV-----	328

Figure S2. Multiple sequence alignment of selected NBDs. The sequences included are the same as in Sleator et al. [18], with the addition of proteins from the *L. monocytogenes* Gbu and OpuC systems. UniProt Accession Numbers: LmGbuA, Q7AP76; LmOpuCA, Q7AP65; BIEA, Q7AP69; yeast YBT1, P32386; mouse ABCB11, Q9QY30; rat ABCB11, O70127; human ABCB11, O95342; rabbit ABCB11, Q9N0V3. The eukaryotic proteins have the domain organization TMD1-NBD1-TMD2-NBD2. The two regions corresponding to NBD1 and NBD2 (based on annotation in UniProt) were included as separate sequences in the alignment. Residues are shaded according to percentage sequence identity.

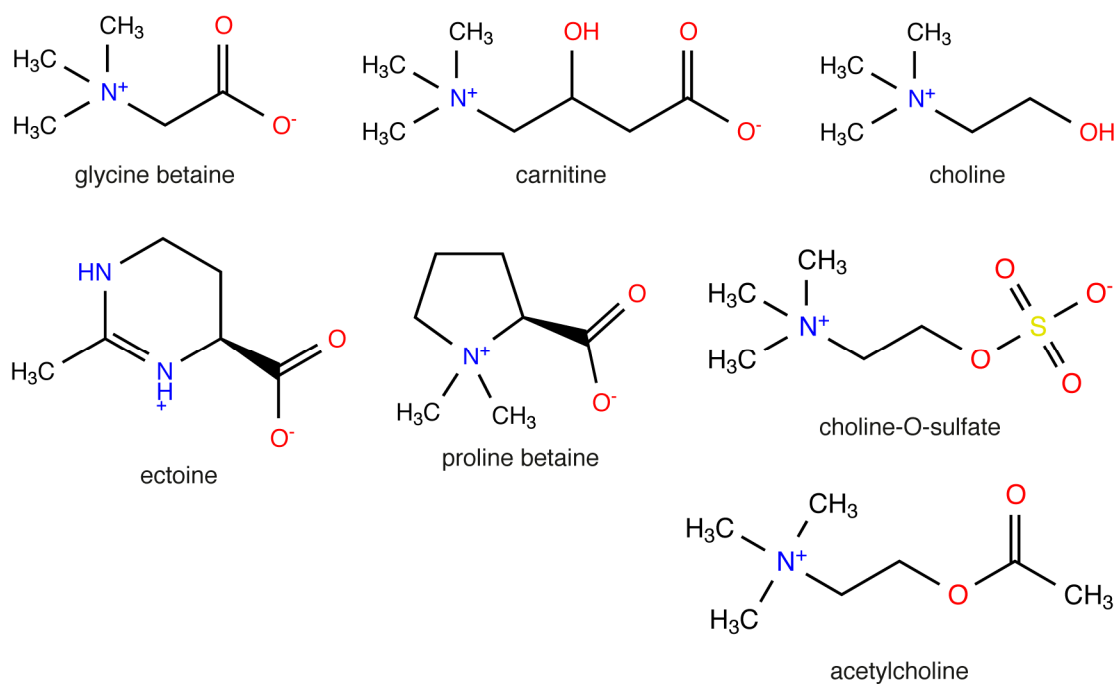


Figure S3. Ligands of substrate-binding proteins homologous to BileB_{SBD}.

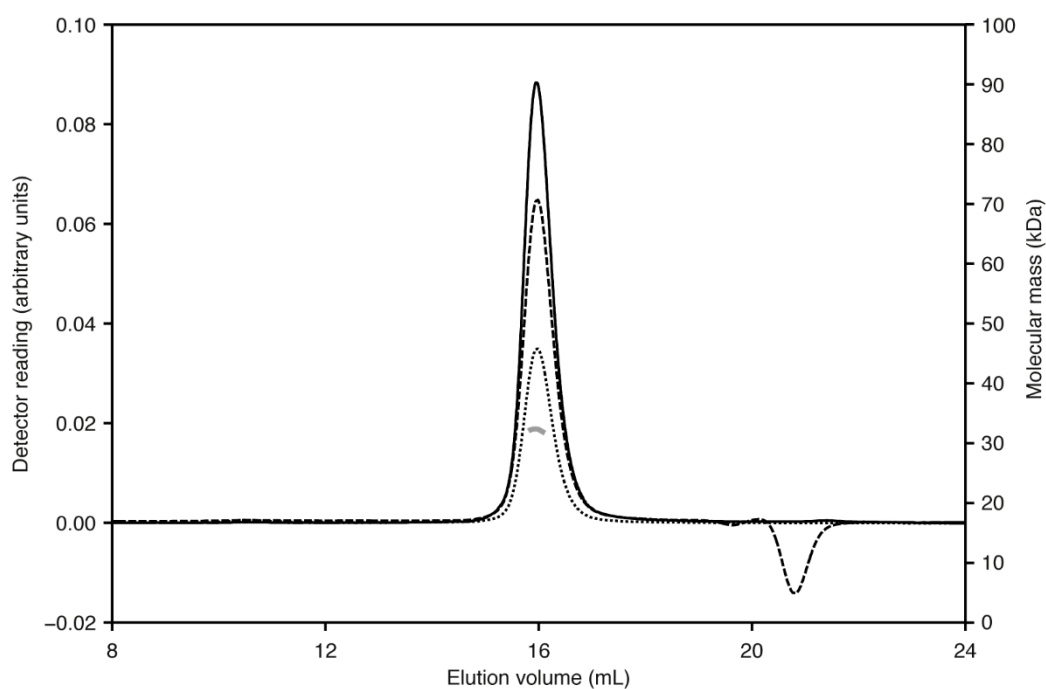


Figure S4. Determination of the oligomeric state of BileB_{SBD}. A sample of purified protein was passed over a gel filtration column coupled to detectors for absorbance at 280 nm (solid black line), refractive index (dashed line), and static light scattering (dotted line). The solid gray line shows the calculated molecular weight of the eluted protein (32.4 kDa), which closely matches the molecular weight of a BileB_{SBD} monomer predicted on the basis of the amino acid sequence (33.4 kDa).

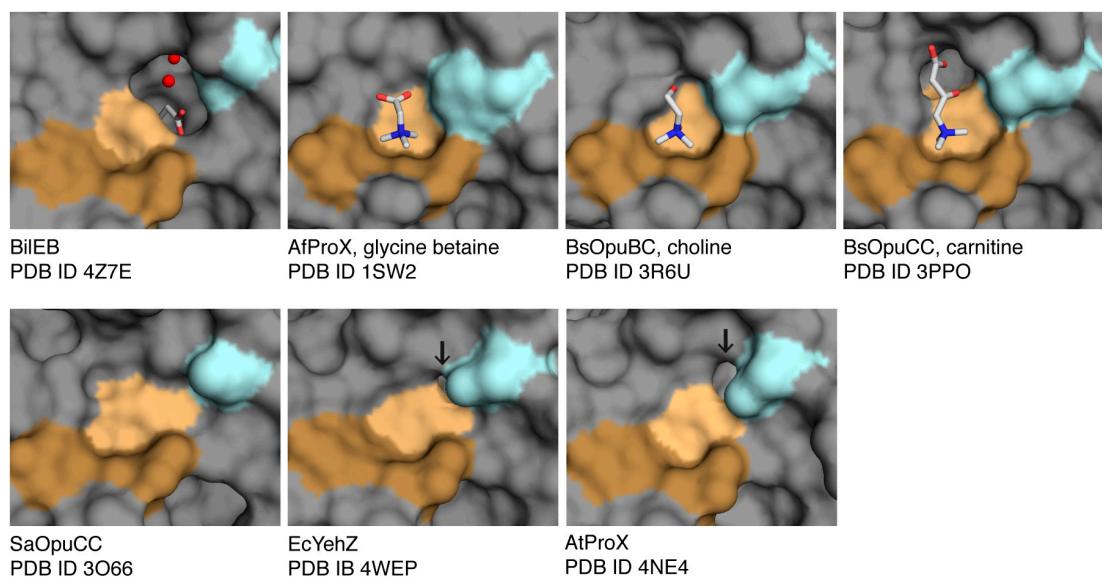


Figure S5. The binding site on the face of domain A. Proteins are shown in surface representation with residues of domain B omitted for clarity. Ligands are shown in stick representation with oxygens colored red, nitrogens colored blue and carbons colored white. Water molecules are shown as red spheres. Residues forming a cage around the quaternary ammonium are dark gold except for the position corresponding to Phe²⁹² of BileB, which is pale gold. Residue at positions corresponding to Gly²⁴³ and Pro²⁴⁶ of BileB are colored pale blue. Images were prepared in PyMOL [52].