SUPPLEMENTARY INFORMATION

Three structurally and functionally distinct β-glucuronidases from the human gut microbe *Bacteroides uniformis*

Samuel J. Pellock,[1,6] William G. Walton,[1,6] Kristen A. Biernat,[1] Dariana Torres-Rivera,[1] Benjamin C. Creekmore,[1] Yongmei Xu,[2] Jian Liu,[2] Ashutosh Tripathy,[3] Lance J. Stewart,[4] and Matthew R. Redinbo[1,3,5,*]

From the [1]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [2]Department of Chemical Biology and Medicinal Chemistry, University of North Carolina at Chapel Hill, NC 27599, USA; [3]Departments of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [4]Institute for Protein Design, Department of Biochemistry, University of Washington, Seattle, WA 98195, USA; [5]Departments of Microbiology and Immunology, and Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill; Chapel Hill, NC, 27599, USA; [6]These authors contributed equally

TABLE OF CONTENTS

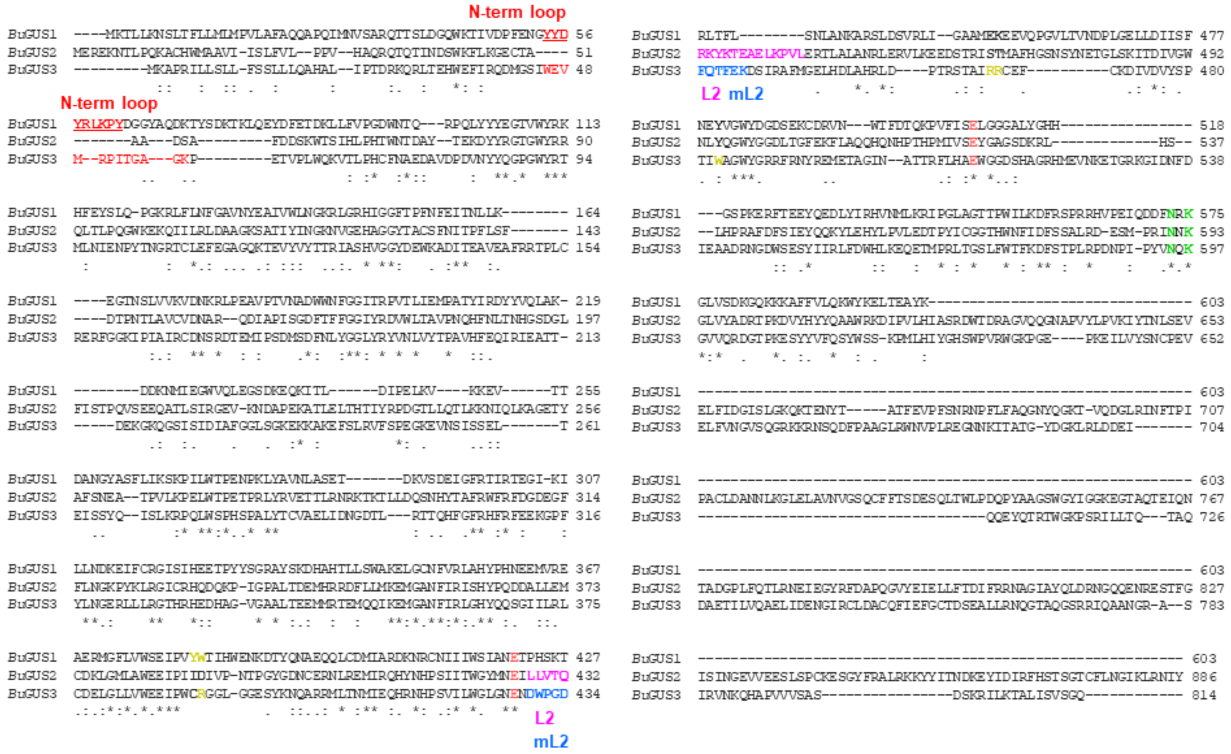


Figure S1. Multiple sequence alignment of *Bu*GUS-1, *Bu*GUS-2, and *Bu*GUS-3. Regions of interest discussed in the text are bolded and highlighted in various colors. Unique active site residues highlighted in yellow, N-terminal loop and catalytic glutamates in red, NxK motif in green, Loop 2 (L2) in magenta, mini-loop 2 (mL2) in blue.

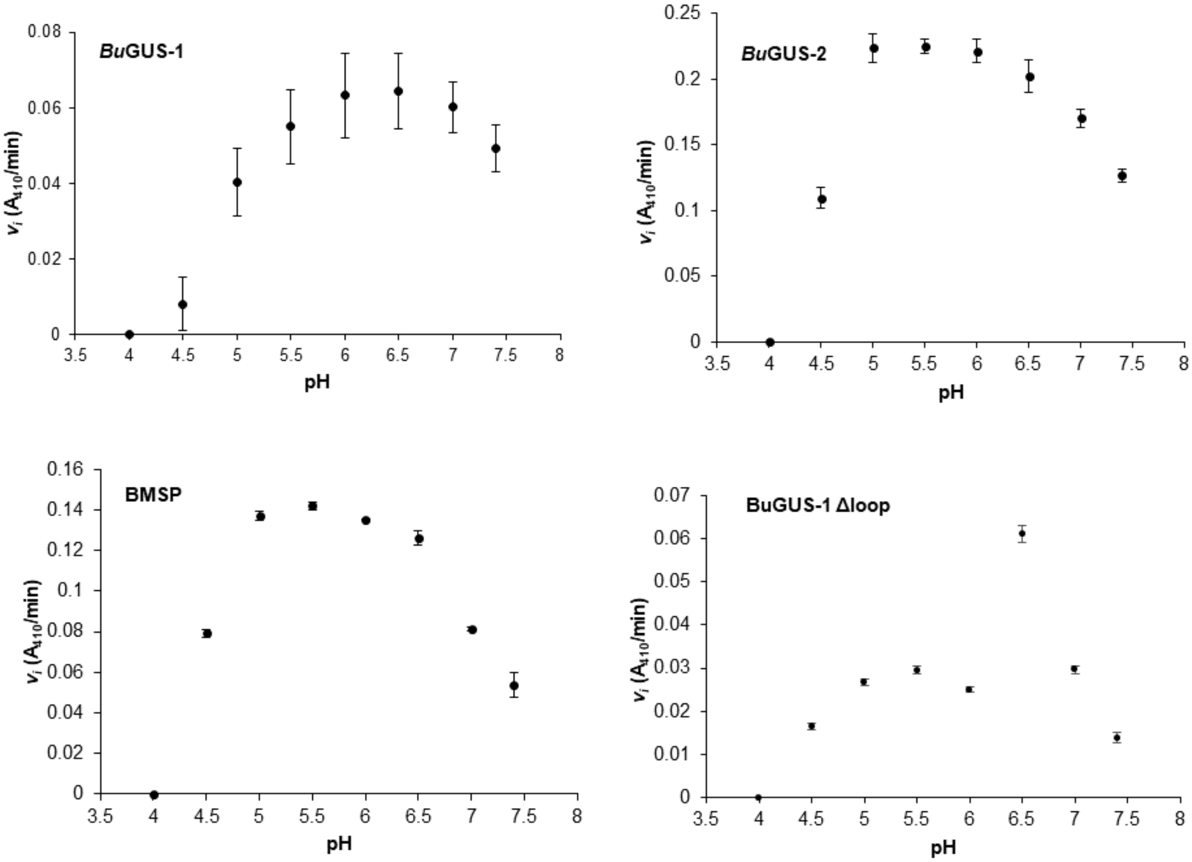


Figure S2. pH screen of PNPG hydrolysis for *Bu*GUS-1, *Bu*GUS-2, and *Bu*GUS-3. Error bars represent SD of n = 3 biological replicates.

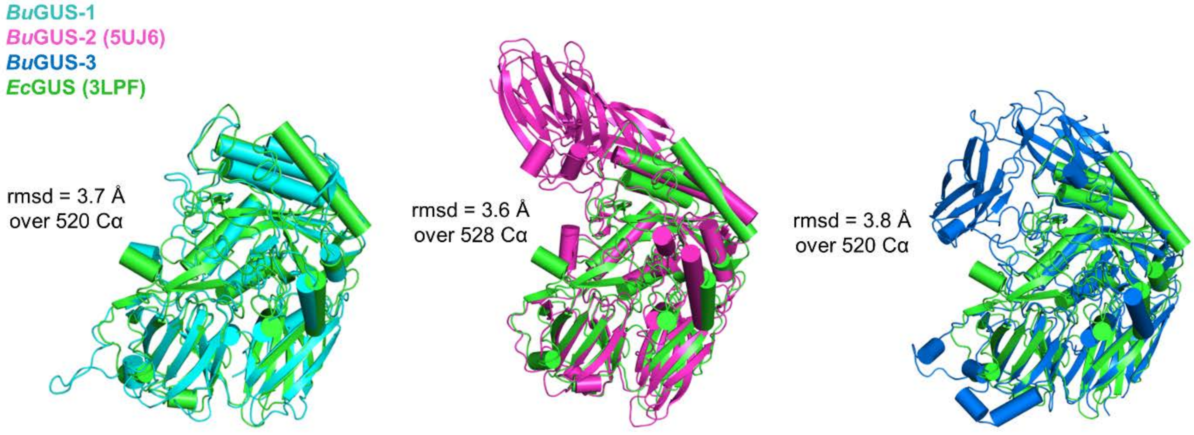


Figure S3. Structural alignments of *Bu*GUS-1 (cyan), *Bu*GUS-2 (magenta), and *Bu*GUS-3 (blue) with previously elucidated structure of *E. coli* GUS (green, *Ec*GUS, PDB: 3LPF).

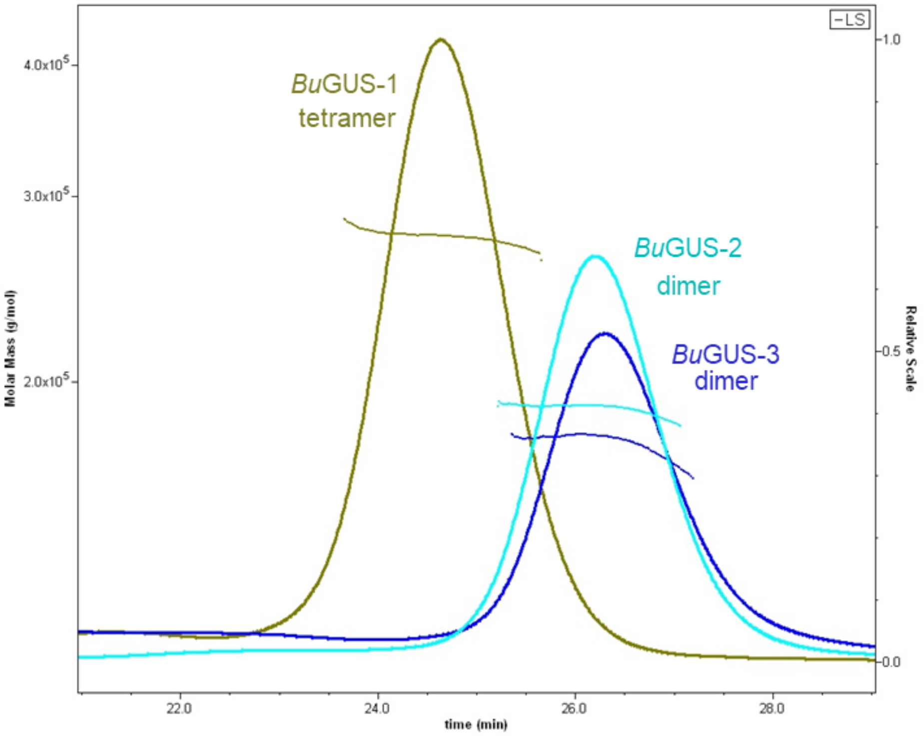


Figure S4. Size-exclusion chromatography multi-angle light scattering analysis (SEC-MALS) of *Bu*GUS-1, *Bu*GUS-2, and *Bu*GUS-3 confirms oligomeric states predicted from crystal structures.

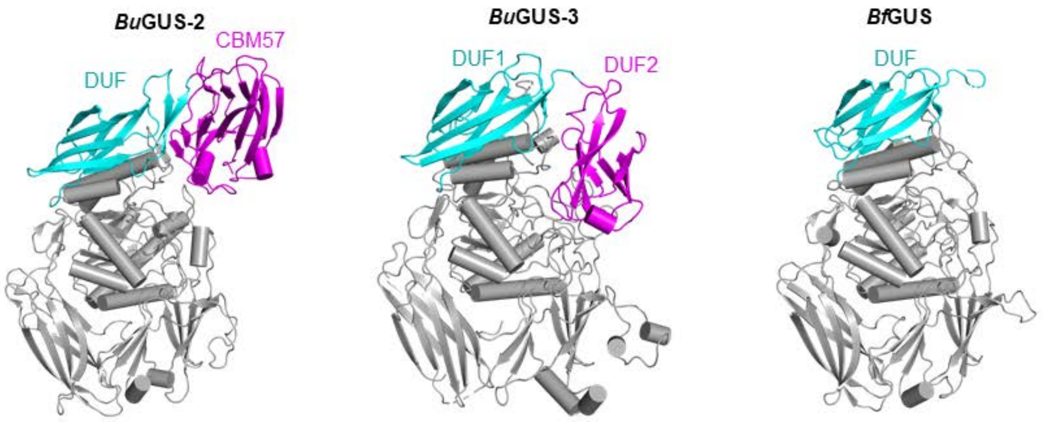


Figure S5. Comparison of CBMs of *Bu*GUS-2, *Bu*GUS-3, and *Bf*GUS. Conserved core fold of GUS shown in grey with DUF-1 and CBM 57 or DUF2 (for *Bu*GUS-2 and *Bu*GUS-3) highlighted in cyan and magenta, respectively.

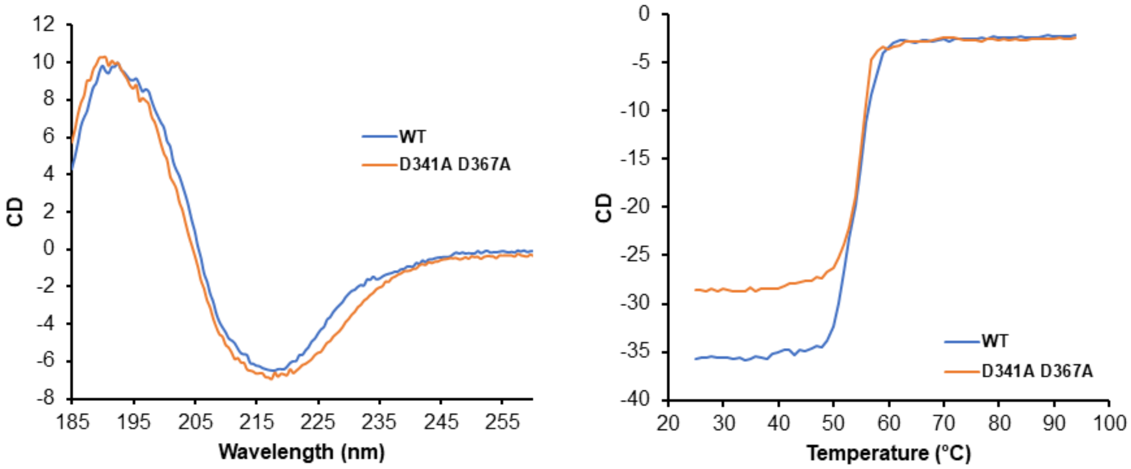
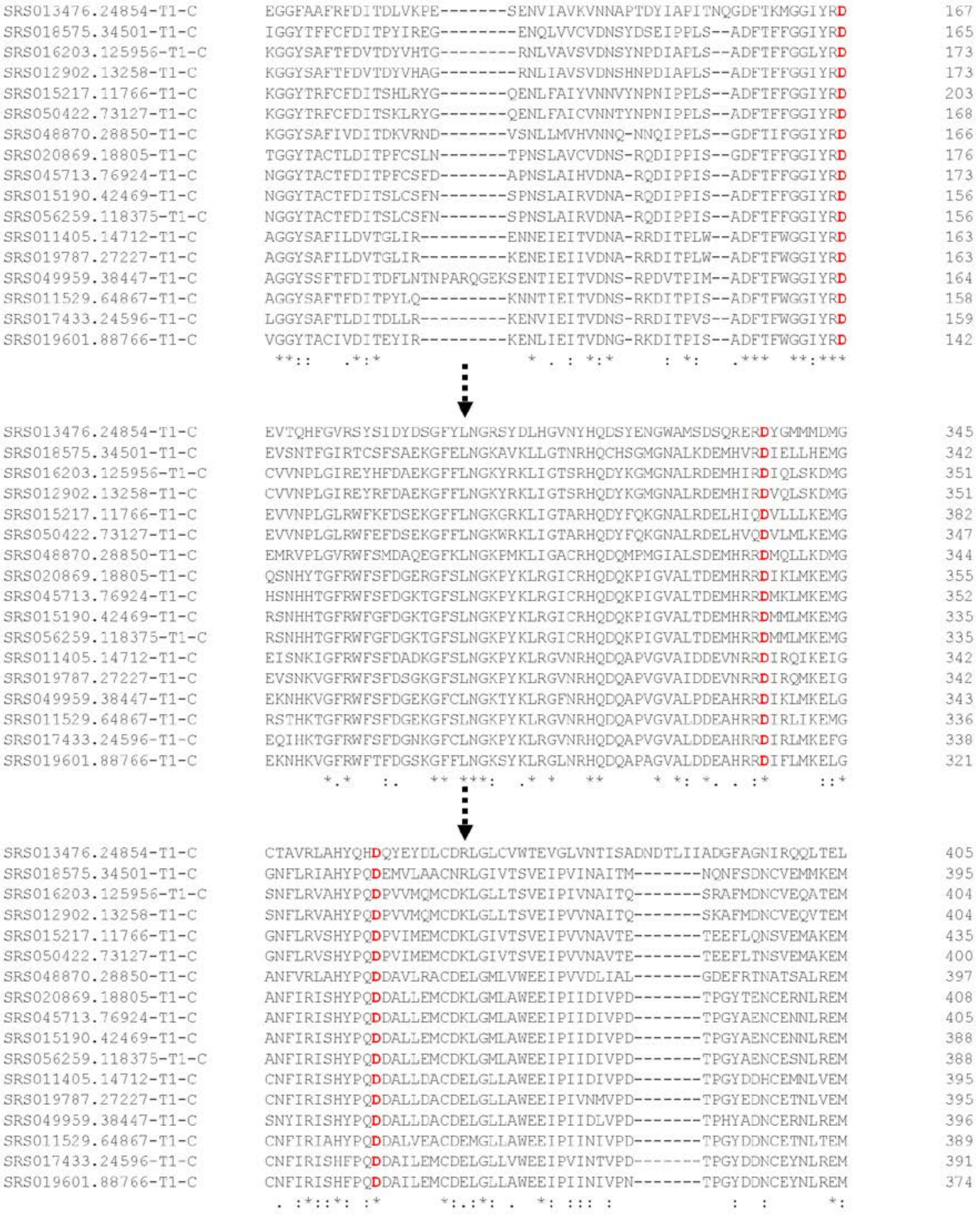


Figure S6. Circular dichroism wavelength scan and melting temperature for WT and D341A D367A calcium binding mutant of *Bu*GUS-2.



```
SRS013476.24854-T1-C        EGGFAAFRFDITDLVKPE-------SENVIAVKVNNAPTDYIAPITNQGDFTKMGGIYRD      167
SRS018575.34501-T1-C        IGGYTFFCFDITPYIREG--------ENQLVVCVDNSYDSEIPPLS--ADFTFFGGIYRD      165
SRS016203.125956-T1-C       KGGYSAFTFDVTDYVHTG--------RNLVAVSVDNSYNPDIAPLS--ADFTFFGGLYRD      173
SRS012902.13258-T1-C        KGGYSAFTFDVTDYVHAG--------RNLIAVSVDNSHNPDIAPLS--ADFTFFGGIYRD      173
SRS015217.11766-T1-C        KGGYTRFCFDITSHLRYG-------QENLFAIYVNNVYNPNIPPLS--ADFTFFGGIYRD      203
SRS050422.73127-T1-C        KGGYTRFCFDITSKLRYG-------QENLFAICVNNTYNPNIPPLS--ADFTFFGGIYRD      168
SRS048870.28850-T1-C        KGGYSAFIVDITDKVRND-------VSNLLMVHVNNQ-NNQIPPLS--GDFTIFGGIYRD      166
SRS020869.18805-T1-C        TGGYTACTLDITPFCSLN-------TPNSLAVCVDNS-RQDIPPIS--GDFTFFGGIYRD      176
SRS045713.76924-T1-C        NGGYTACTFDITPFCSFD-------APNSLAIHVDNA-RQDIPPIS--ADFTFFGGIYRD      173
SRS015190.42469-T1-C        NGGYTACTFDITSLCSFN-------SPNSLAIRVDNA-RQDIPPIS--ADFTFFGGIYRD      156
SRS056259.118375-T1-C       NGGYTACTFDITSLCSFN-------SPNSLAIRVDNA-RQDIPPIS--ADFTFFGGIYRD      156
SRS011405.14712-T1-C        AGGYSAFILDVTGLIR---------ENNEIEITVDNA-RRDITPLW--ADFTFWGGIYRD      163
SRS019787.27227-T1-C        AGGYSAFILDVTGLIR---------KENEIEIIVDNA-RRDITPLW--ADFTFWGGIYRD      163
SRS049959.38447-T1-C        AGGYSSFTFDITDFLNTNPARQGEKSENTIEITVDNS-RPDVTPIM--ADFTFWGGIYRD      164
SRS011529.64867-T1-C        AGGYSAFTFDITPYLQ---------KNNTIEITVDNS-RKDITPIS--ADFTFWGGIYRD      158
SRS017433.24596-T1-C        LGGYSAFTLDITDLLR---------KENVIEITVDNS-RRDITPVS--ADFTFWGGIYRD      159
SRS019601.88766-T1-C        VGGYTACIVDITEYIR---------KENLIEITVDNG-RKDITPIS--ADFTFWGGIYRD      142
                             **::   .*:*               * . : *:*     : *:   .***  **:***

SRS013476.24854-T1-C        EVTQHFGVRSYSIDYDSGFYLNGRSYDLHGVNYHQDSYENGWAMSDSQRERDYGMMMDMG      345
SRS018575.34501-T1-C        EVSNTFGIRTCSFSAEKGFELNGKAVKLLGTNRHQCHSGMGNALKDEMHVRDIELLHEMG      342
SRS016203.125956-T1-C       CVVNPLGIREYHFDAEKGFFLNGKYRKLIGTSRHQDYKGMGNALRDEMHIRDIQLSKDMG      351
SRS012902.13258-T1-C        CVVNPLGIREYRFDAEKGFFLNGKYRKLIGTSRHQDYKGMGNALRDEMHIRDVQLSKDMG      351
SRS015217.11766-T1-C        EVVNPLGLRWFKFDSEKGFFLNGKGRKLIGTARHQDYFQKGNALRDELHIQDVLLLKEMG      382
SRS050422.73127-T1-C        EVVNPLGLRWFEFDSEKGFFLNGKWRKLIGTARHQDYFQKGNALRDELHVQDVLMLKEMG      347
SRS048870.28850-T1-C        EMRVPLGVRWFSMDAQEGFKLNGKPMKLIGACRHQDQMPMGIALSDEMHRRDMQLLKDMG      344
SRS020869.18805-T1-C        QSNHYTGFRWFSFDGERGFSLNGKPYKLRGICRHQDQKPIGVALTDEMHRRDIKLMKEMG      355
SRS045713.76924-T1-C        HSNHHTGFRWFSFDGKTGFSLNGKPYKLRGICRHQDQKPIGVALTDEMHRRDMKLMKEMG      352
SRS015190.42469-T1-C        RSNHHTGFRWFGFDGKTGFSLNGKPYKLRGICRHQDQKPIGVALTDEMHRRDMMLMKEMG      335
SRS056259.118375-T1-C       RSNHHTGFRWFGFDGKTGFSLNGKPYKLRGICRHQDQKPIGVALTDEMHRRDMMLMKEMG      335
SRS011405.14712-T1-C        EISNKIGFRWFSFDADKGFSLNGKPYKLRGVNRHQDQAPVGVAIDDEVNRRDIRQIKEIG      342
SRS019787.27227-T1-C        EVSNKVGFRWFSFDSGKGFSLNGKPYKLRGVNRHQDQAPVGVAIDDEVNRRDIRQMKEIG      342
SRS049959.38447-T1-C        EKNHKVGFRWFSFDGEKGFCLNGKTYKLRGFNRHQDQAPVGVALPDEAHRRDIKLMKELG      343
SRS011529.64867-T1-C        RSTHKTGFRWFSFDGEKGFSLNGKPYKLRGVNRHQDQAPVGVALDDEAHRRDIRLIKEMG      336
SRS017433.24596-T1-C        EQIHKTGFRWFSFDGNKGFCLNGKPYKLRGVNRHQDQAPVGVALDDEAHRRDIRLMKEFG      338
SRS019601.88766-T1-C        EKNHKVGFRWFTFDGSKGFFLNGKSYKLRGLNRHQDQAPAGVALDDEAHRRDIFLMKELG      321
                                  *.*   :.   ** ***:  .* *   **     * *: *. . :*     ::*

SRS013476.24854-T1-C        CTAVRLAHYQHDQYEYDLCDRLGLCVWTEVGLVNTISADNDTLIIADGFAGNIRQQLTEL      405
SRS018575.34501-T1-C        GNFLRIAHYPQDEMVLAACNRLGIVTSVEIPVINAITM-------NQNFSDNCVEMMKEM      395
SRS016203.125956-T1-C       SNFLRVAHYPQDPVVMQMCDKLGLLTSVEIPIVNAITQ-------SRAFMDNCVEQATEM      404
SRS012902.13258-T1-C        SNFLRVAHYPQDPVVMQMCDKLGLLTSVEIPVVNAITQ-------SKAFMDNCVEQVTEM      404
SRS015217.11766-T1-C        GNFLRVSHYPQDPVIMEMCDKLGIVTSVEIPVVNAVTE-------TEEFLQNSVEMAKEM      435
SRS050422.73127-T1-C        GNFLRVSHYPQDPVIMEMCDKLGIVTSVEIPVVNAVTE-------TEEFLTNSVEMAKEM      400
SRS048870.28850-T1-C        ANFVRLAHYPQDDAVLRACDELGMLVWEEIPVVDLIAL-------GDEFRTNATSALREM      397
SRS020869.18805-T1-C        ANFIRISHYPQDDALLEMCDKLGMLAWEEIPIIDIVPD-------TPGYTENCERNLREM      408
SRS045713.76924-T1-C        ANFIRISHYPQDDALLEMCDKLGMLAWEEIPIIDIVPD-------TPGYAENCENNLREM      405
SRS015190.42469-T1-C        ANFIRISHYPQDDALLEMCDKLGMLAWEEIPIIDIVPD-------TPGYAENCENNLREM      388
SRS056259.118375-T1-C       ANFIRISHYPQDDALLEMCDKLGMLAWEEIPIIDIVPD-------TPGYAENCESNLREM      388
SRS011405.14712-T1-C        CNFIRISHYPQDDALLDACDELGLLAWEEIPIIDIVPD-------TPGYDDHCEMNLVEM      395
SRS019787.27227-T1-C        CNFIRISHYPQDDALLDACDELGLLAWEEIPIVNMVPD-------TPGYEDNCETNLVEM      395
SRS049959.38447-T1-C        SNYIRISHYPQDDALLDACDELGLLAWEEIPIIDLVPD-------TPHYADNCERNLREM      396
SRS011529.64867-T1-C        CNFIRIAHYPQDDALVEACDEMGLLAWEEIPIINIVPD-------TPGYDDNCETNLTEM      389
SRS017433.24596-T1-C        CNFIRISHFPQDDAILEMCDELGLLVWEEIPVINTVPD-------TPGYDDNCEYNLREM      391
SRS019601.88766-T1-C        CNFIRISHFPQDDAILEMCDELGLLAWEEIPIINIVPN-------TPGYDDNCEYNLREM      374
                             . :*::*: :*      *:.:*: .  *: ::: :            :  :      *:
```

Figure S7. Sequence alignment of putative calcium binding GUS enzymes from the Human Microbiome Project stool sample database. Related to Figure 5.

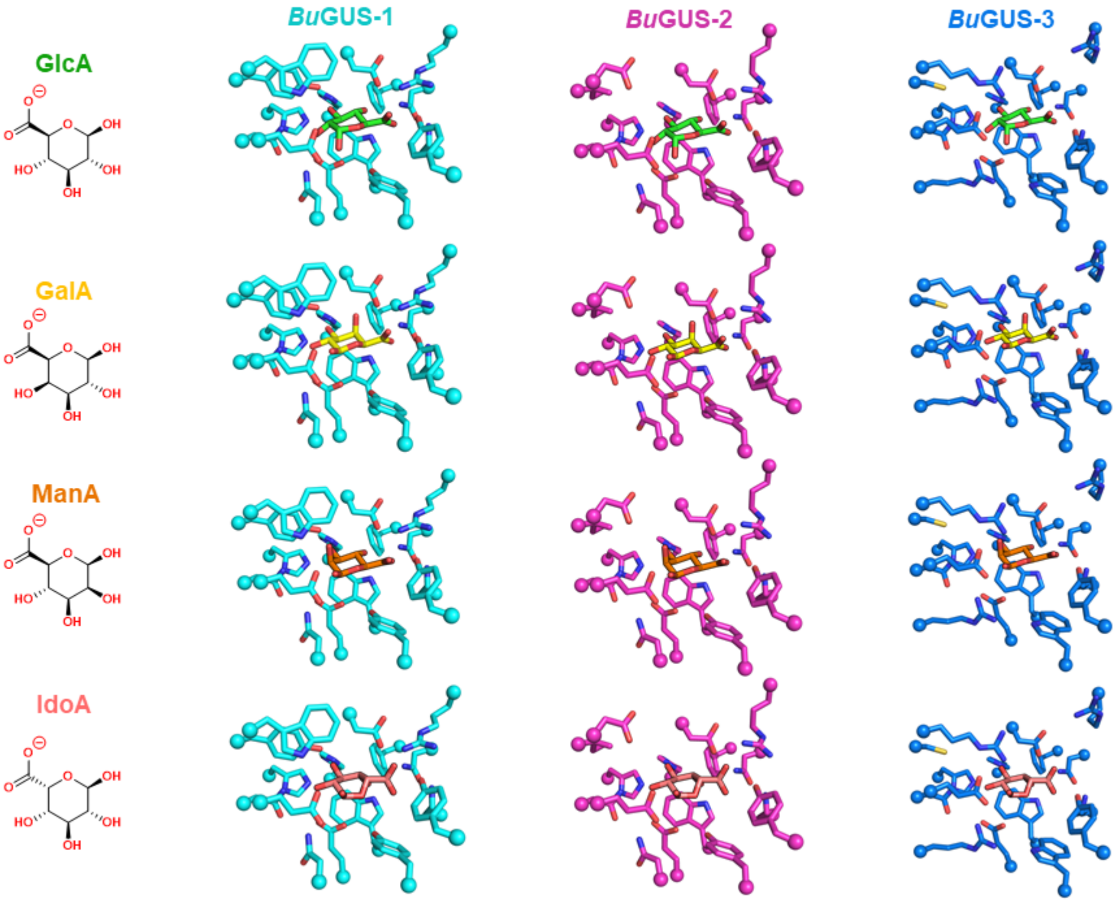
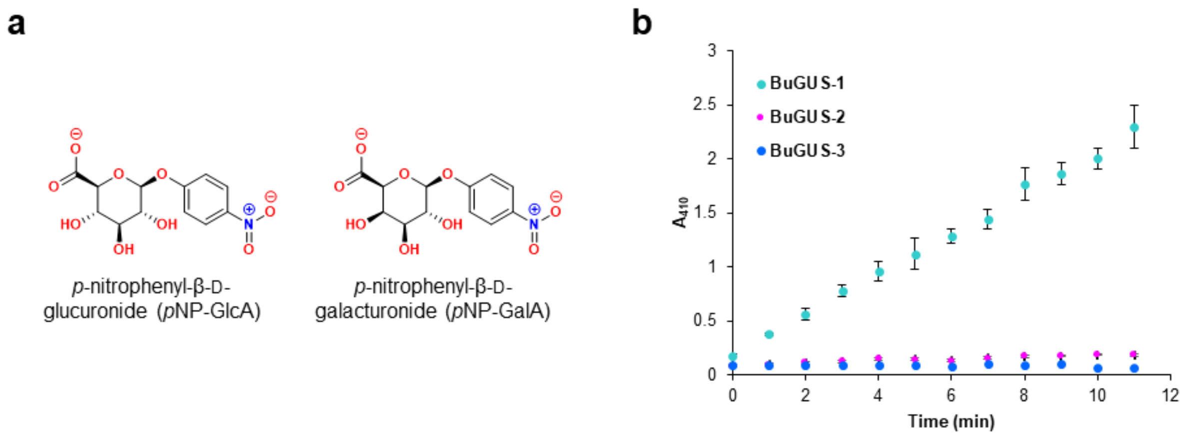


Figure S8. Modeling of sugar monosaccharides of glucuronate (GlcA), galacturonate (GalA), mannuronate (ManA), and iduronate (IdoA) in the active sites of *Bu*GUS-1, *Bu*GUS-2, and *Bu*GUS-3. Related to Figure 6.



c

Substrate	k_{cat} (s^{-1})	K_m (µM)	k_{cat}/K_m (s^{-1} M^{-1})
pNP-GlcA	12 ± 1	57 ± 9	2.2×10^5
pNP-GalA	14.7 ± 0.5	380 ± 10	3.9×10^4

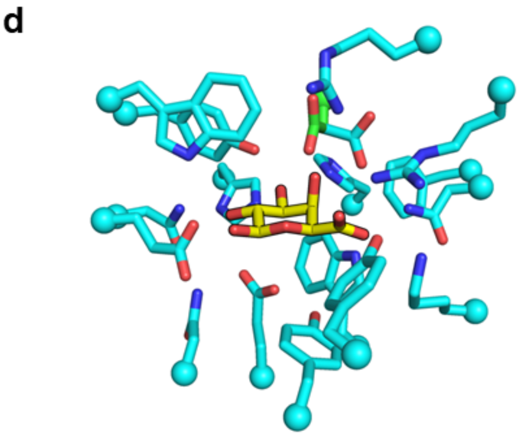


Figure S9. *Bu*GUS-1, but not *Bu*GUS-2 or *Bu*GUS-3, can process both glucuronides and galacturonides. Related to Table 1. *a*, chemical structures of *p*NP-GlcA and *p*NP-GalA. *b*, progress curves for *Bu*GUS-1, *Bu*GUS-2, and *Bu*GUS-3 with *p*NP-GalA. *c*, kinetic parameters of *p*NP-GlcA and *p*NP-GalA hydrolysis by *Bu*GUS-1. *d*, active site of BuGUS-1 with GalA manually docked and potentially important aspartic acid residue highlighted in green. This aspartic acid likely undergoes a rotamer shift, shown in cyan, to accommodate the GalA sugar.

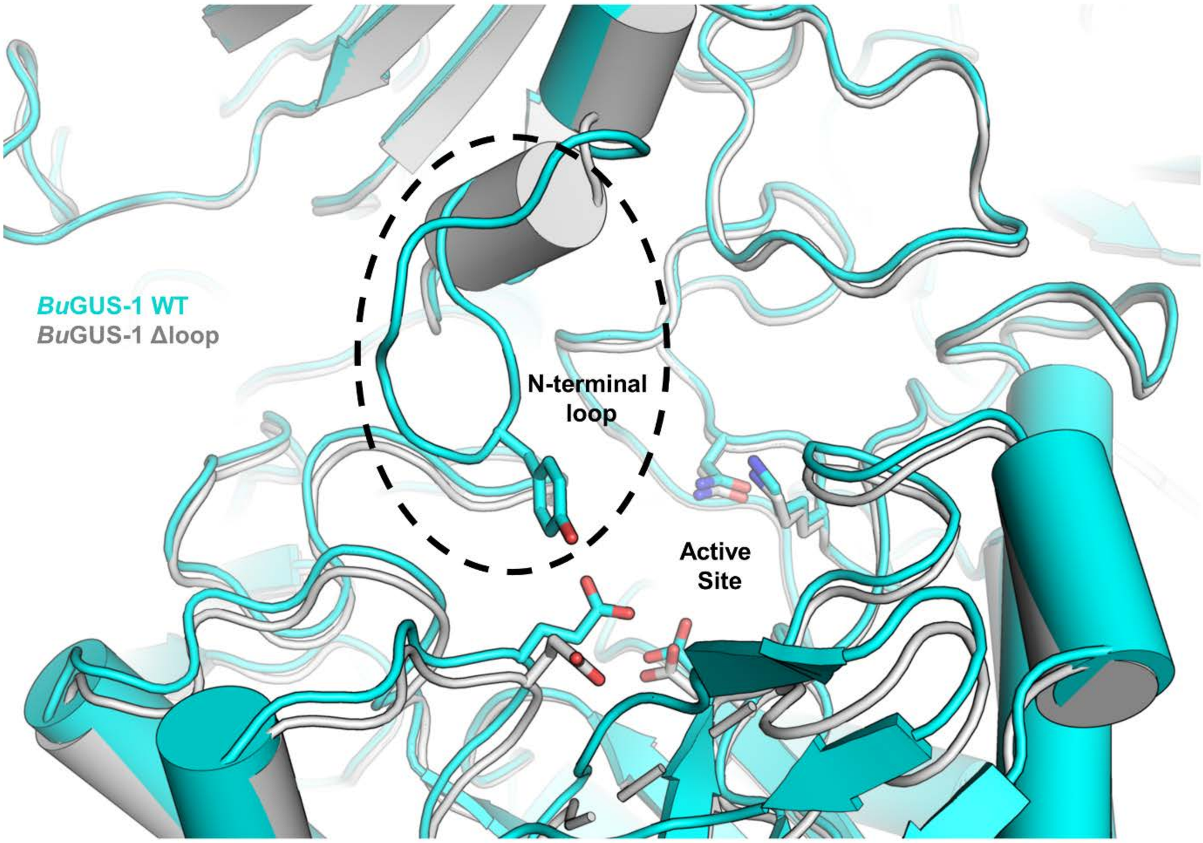
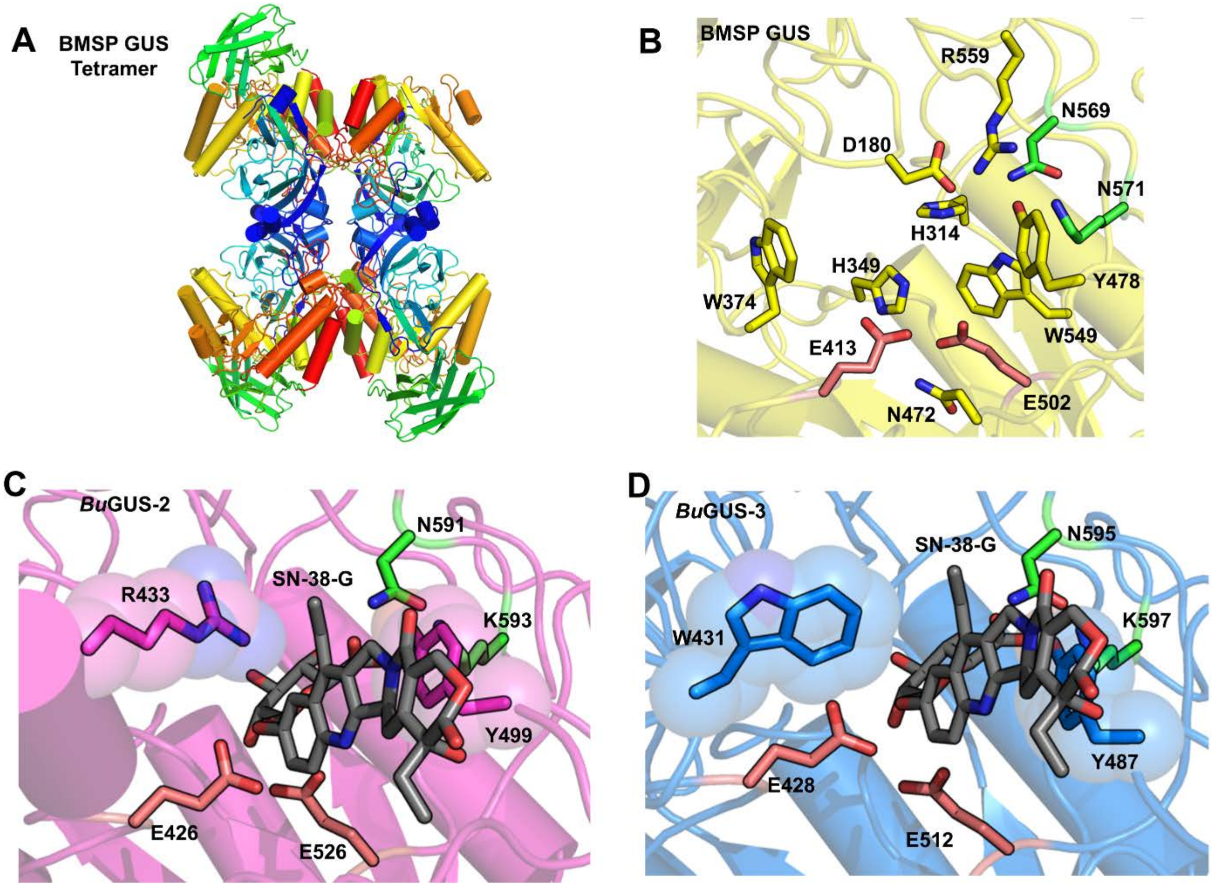


Figure S10. Structural overlay of *Bu*GUS-1 WT (cyan) and BuGUS-1 Δloop (grey) reveals absence of N-terminal loop necessary for efficient processing of SN-38-G. Related to Figure 8.



Supplementary Figure 11.

Figure S11. Structural analysis of BMSP GUS and docking studies of SN-38-G in the active sites of *Bu*GUS-2 and *Bu*GUS-3. Related to Figure 1, Figure 6. *a*, tetrameric biological assembly of BMSP GUS. *b*, active site of BMSP GUS with NxK motif in green and catalytic glutamates in deep salmon. Manually docked SN-38-G in active site of *c*, *Bu*GUS-2 and *d*, *Bu*GUS-3 based on the PTG-bound structure of *Bu*GUS-1.

Inhibitor 1

UNC10201652

Figure S12. Chemical structures of Inhibitor 1 and UNC10201652. Related to Table 3.

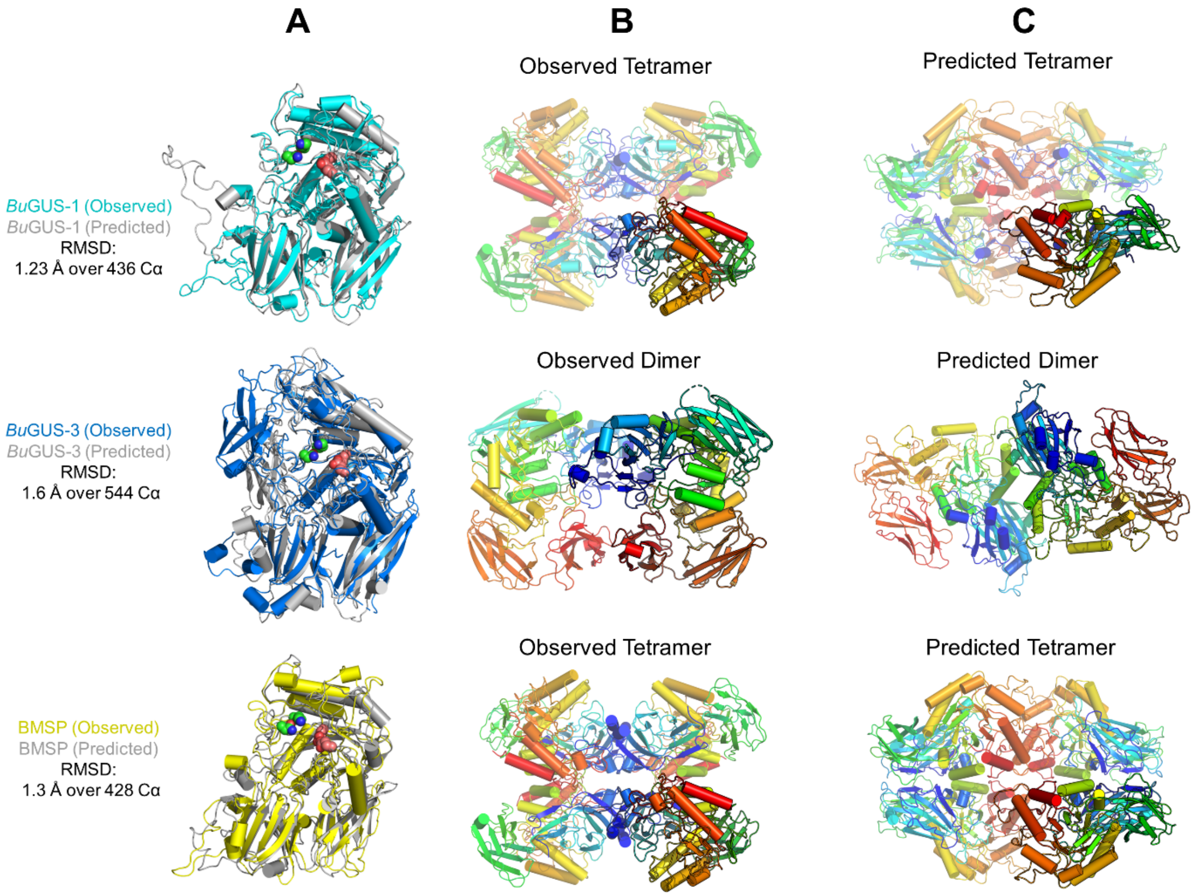


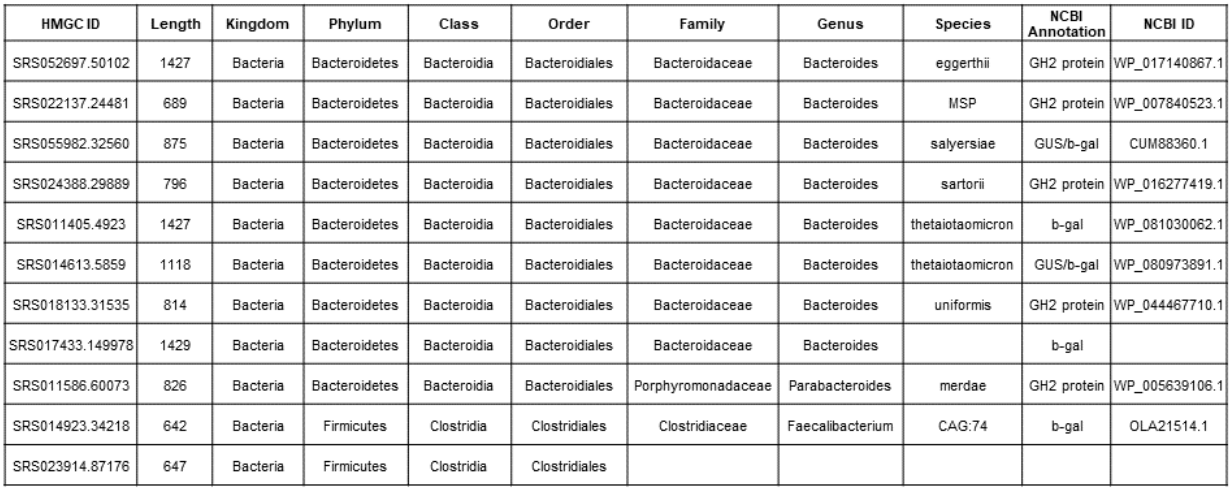
Figure S13. Predicted structures by Rosetta-based modeling of unique tertiary and quaternary structures discovered by crystallography. (A) Overlay of observed and Rosetta-predicted tertiary structure for *Bu*GUS-1, *Bu*GUS-3, and BMSP GUS. (B) Observed quaternary structures for *Bu*GUS-1, *Bu*GUS-3, and BMSP. (C) Rosetta prediction of quaternary structure for *Bu*GUS-1, *Bu*GUS-3, and BMSP.

Protein	*Bu*GUS-1	*Bu*GUS-3	BMSP	*Bu*GUS-1 + G-1,5-L	*Bu*GUS-2 + G-1,5-L	*Bu*GUS-1 + GlcA	*Bu*GUS-1 Δloop	*Bu*GUS-2 ΔCa^{2+}	*Bu*GUS-1 + TPG
Resolution (Å)	29.6 – 2.20	29.48 – 2.35	29.43 – 2.65	29.93-1.90	29.48-2.50	29.98-1.80	29.34-2.00	29.55-3.00	29.30-2.40
Resolution in highest shell (Å)	2.25 – 2.20	2.39 – 2.35	2.71 – 2.65	1.93 -1.90	2.56-2.50	1.83-1.80	2.03-2.00	3.12-3.00	2.49-2.40
Space group	C2	C2	$I4_1$	C2	$P2_12_12_1$	C2	$P12_11$	$P2_12_12_1$	$P3_221$
Unit cell dimensions a,b,c (Å); α,β,γ (°)	125.8,130.2, 94.2; 90, 100.3, 90	209.7, 68.2, 167.4; 90, 122.5, 90	213.5, 213.5, 112.6; 90, 90, 90	126.1, 130.2, 94.5; 90, 100.2, 90	74.5, 141.9, 180.8; 90, 90, 90	134.2, 133.9, 163.4; 90,100.3, 90	76.7,199.2, 84.6; 90,102.8, 90	75.0,142.0, 181.2; 90, 90, 90	252.2, 252.2, 107.3 90, 90, 120
Total reflections (F>0)	517245	568681	305377	797212	446802	1761568	995295	525999	3085366
Unique reflections	75592	83443	73245	114979	67106	261960	163378	39506	152030
Multiplicity	6.8 (7.0)	6.8 (6.9)	4.2 (4.2)	6.9 (6.9)	6.7 (6.8)	6.7 (6.9)	6.1 (5.8)	13.3 (14.0)	20.3 (21.0)
Completeness, %(highest shell)	99.9 (100)	99.9 (99.9)	99.7 (100)	97.7 (96.3)	99.9 (100.0)	100 (100)	98.4 (97.7)	99.8 (100)	99.8 (99.7)
Mean I/sigma (I) (highest shell)	18.6 (4.7)	21.0 (4.9)	16.1 (2.6)	16.6 (4.4)	15.9 (4.1)	11.8 (4.3)	12.1 (3.7)	20.9 (3.9)	23.4 (7.0)
Wilson B-factor ($Å^2$)	21.4	31.9	61.8	14.9	36.5	8.3	25.6	67.7	33.1
R_{merge} (highest shell)	0.078 (0.387)	0.073 (0.445)	0.060 (0.50)	0.081 (0.351)	0.084 (0.438)	0.126 (0.471)	0.118 (0.496)	0.120 (0.69)	0.111 (0.511)
R_{work}	0.1494	0.1755	0.2069	0.1377	0.1703	0.18	0.176	0.1670	0.1739
R_{free}	0.1841	0.2224	0.2827	0.1694	0.2277	0.229	0.23	0.2409	0.2467
Number of molecules in asymmetric unit	2	2	4	2	2	4	4	2	6
Number of water molecules in asymmetric unit	1075	815	110	1549	535	4087	1808	0	1561
rms bonds (Å)	0.003	0.009	0.008	0.012	0.004	0.006	0.007	0.009	0.007
rms angles (°)	0.631	1.24	1.000	1.072	0.669	0.874	0.849	1.030	0.94
Ramachandran favored (%)	96.53	95.34	92.85	96.79	96.23	96.71	96.30	92.91	92.23
Ramachandran outliers (%)	0.00	0.39	0.19	0.00	0.24	0.00	0.09	0.18	0.20
Clash score	3.04	6.87	13	1.96	2.12	7.05	5	8.33	16.84
Average B-factor ($Å^2$)	28.3	35.1	67.0	21.2	41.0	14.6	30.0	57.9	37.9
RCSB ID	6D1N	6D1P	6D8K	6D41	6D5O	6D6W	6D89	6D8G	6D7F

Table S1. X-ray data collection and refinement statistics.

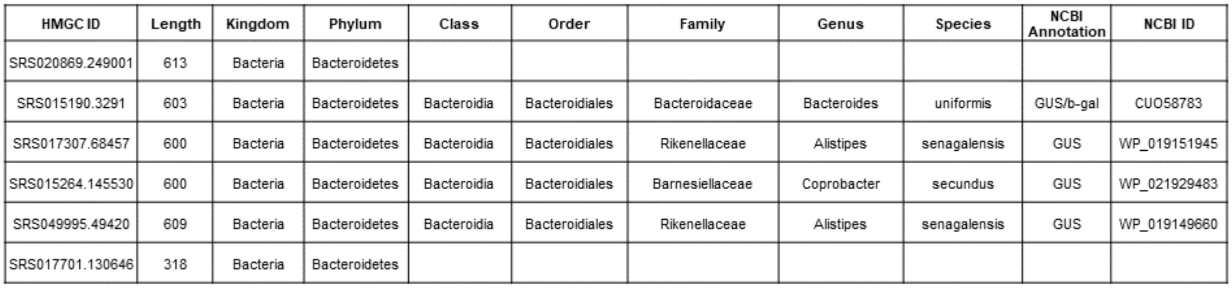
HMGC ID	Length	Kingdom	Phylum	Class	Order	Family	Genus	Species	NCBI ID
SRS011529.64867	855	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella		
SRS049959.38447	872	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri	CUO96334.1
SRS018575.34501	885	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	clarus	WP_009122035.1
SRS050422.73127	891	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	finegoldii	OKZ23312.1
SRS020869.18805	891	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	intestinalis	WP_007661425.1
SRS011405.14712	861	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	intestinalis	CCY84748.1
SRS015217.11766	926	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	ovatus	WP_004304963.1
SRS019601.88766	840	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	vulgatus	WP_011965330.1
SRS012902.13258	891	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS016203.125956	891	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS045713.76924	888	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS048870.28850	883	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS015190.42469	871	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS056259.118375	871	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS019787.27227	861	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS017433.24596	858	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		
SRS013476.24854	693	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium		

Table S2. Bioinformatic analysis of the HMP GUS enzymes identified 18 GUS enzymes with calcium binding sites.



HMGC ID	Length	Kingdom	Phylum	Class	Order	Family	Genus	Species	NCBI Annotation	NCBI ID
SRS052697.50102	1427	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	eggerthii	GH2 protein	WP_017140867.1
SRS022137.24481	689	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	MSP	GH2 protein	WP_007840523.1
SRS055982.32560	875	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	salyersiae	GUS/b-gal	CUM88360.1
SRS024388.29889	796	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	sartorii	GH2 protein	WP_016277419.1
SRS011405.4923	1427	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	thetaiotaomicron	b-gal	WP_081030062.1
SRS014613.5859	1118	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	thetaiotaomicron	GUS/b-gal	WP_080973891.1
SRS018133.31535	814	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	uniformis	GH2 protein	WP_044467710.1
SRS017433.149978	1429	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides		b-gal	
SRS011586.60073	826	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Porphyromonadaceae	Parabacteroides	merdae	GH2 protein	WP_005639106.1
SRS014923.34218	642	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Faecalibacterium	CAG:74	b-gal	OLA21514.1
SRS023914.87176	647	Bacteria	Firmicutes	Clostridia	Clostridiales					

Table S3. Bioinformatic analysis of the HMP identified 11 GUS enzymes containing the variable active site tryptophan observed in *Bu*GUS-3.



HMGC ID	Length	Kingdom	Phylum	Class	Order	Family	Genus	Species	NCBI Annotation	NCBI ID
SRS020869.249001	613	Bacteria	Bacteroidetes							
SRS015190.3291	603	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Bacteroidaceae	Bacteroides	uniformis	GUS/b-gal	CUO58783
SRS017307.68457	600	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Rikenellaceae	Alistipes	senagalensis	GUS	WP_019151945
SRS015264.145530	600	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Barnesiellaceae	Coprobacter	secundus	GUS	WP_021929483
SRS049995.49420	609	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidiales	Rikenellaceae	Alistipes	senagalensis	GUS	WP_019149660
SRS017701.130646	318	Bacteria	Bacteroidetes							

Table S4. Bioinformatics analysis of the HMP identified 6 GUS enzymes containing an N-terminal loop like that observed in *Bu*GUS-1.

Primer Name	**Sequence**
*Bu*GUS-1 Fwd	TACTTCCAATCCAATGCGTTCGCACAGCAGGCACCGCAGATTATGAAC
*Bu*GUS-1 Rev	TTATCCACTTCCAATGCGCTATTTATAGGCTTCAGTCAGTTCTTTATACCATTTCTG
*Bu*GUS-3 Fwd	TACTTCCAATCCAATGCGCTGATCCCGACCGATCGTAAACAGCGTC
*Bu*GUS-3 Rev	TTATCCACTTCCAATGCGCTACTGGCCGCTCACAGAGATCAGCGC
BMSP Fwd	TACTTCCAATCCAATGCGCAGAACCTGATTACCAATGTTTACGGTCGTGACATCC
BMSP Rev	TTATCCACTTCCAATGCGCTATTCACCGAATTTGGTTTTGTAGTACTCACGCATCAGGTA
*Bu*GUS-1 ΔN-term loop Fwd	GTTGATCCGTTCGAAAACGGTCGTGGCATGAAAGTTTACCAGGATAAAACCTATAGTGAT
*Bu*GUS-1 ΔN-term loop Rev	ATCACTATAGGTTTTATCCTGGTAAACTTTCATGCCACGACCGTTTTCGAACGGATCAAC
*Bu*GUS-2 D341A Fwd	GTCCGGCTCTGACGGCTGAAATGCATCGTCG
*Bu*GUS-2 D341A Rev	CGACGATGCATTTCAGCCGTCAGAGCCGGAC
*Bu*GUS-2 D367A Fwd	CACTACCCGCAGGCTGACGCTCTGCTG
*Bu*GUS-2 D367A Rev	CAGCAGAGCGTCAGCCTGCGGGTAGTG
BuGUS-2 N591A/K593A Fwd	CTGCGTGATGAAAGCATGCCGCGTATTGCCAACGCAGGCCTGGTTTAC
BuGUS-2 N591A/K593A Rev	CGTAAACCAGGCCTGCGTTGGCAATACGCGGCATGCTTTCATCACGCAG

Table S5. Primer sequences used in this study.