

Table S1. Purification scheme of GH13 α -amylase variants based on the activity resulted in purification steps. The α -amylase activity was measured toward (A.1-4.) 2-Chloro-4-nitrophenyl- β -D-maltotrioxide (CNP3); (B.1-4.) Soluble starch; (C.1-4.) Rice starch; (D.1-4.) Corn starch; (E.1-4.) Wheat starch; (F.1-4.) Potato starch.

(A)^a

(A.1) CBM20_GH13	Volume (mL)	Nitrophenol release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)
Spent medium	300	0.8	15.1	4534	0.17	51.1	89	100
Unbound fraction	300	0.3	6.1	1834	0.15	45.8	40	40 *Unbound protein:40%
Washed fraction	300	0.003	0.1	17	0.006	1.9	8.5	0 *Bound protein: 60%
Eluted protein	15	4.8	96.2	1443	0.02	0.3	4815	32
Rest activity (likely remained in starch)				1241				27
(A.2) GH13_CBM20	Volume (mL)	Nitrophenol release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)
Spent medium	300	0.9	18.4	5513	0.21	64.5	85	100
Unbound fraction	300	0.5	10.1	3022	0.18	52.7	57	55 *Unbound protein: 56%
Washed fraction	300	0.01	0.2	48	0.01	2.6	18	1 *Bound protein: 44%
Eluted protein	15	5.7	113.1	1697	0.03	0.44	3886	31
Rest activity (likely remained in starch)				746				14
(A.3) GH13	Volume (mL)	Nitrophenol release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)
Spent medium	300	0.3	6.1	1839	0.17	52.5	35	100
Unbound	300	0.3	5.6	1684	0.17	51.3	33	92 *Unbound protein: 92%
Washed	300	0.001	0.012	3	0.01	2.0	2	0 *Bound protein: 8%
Eluted protein	15	0.4	8.3	124	0.005	0.1	1732	7
Rest Activity (likely remained in starch)				28				2
(A.4) MGG029 (parental strain with empty vector)	Volume (mL)	Nitrophenol release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)
Spent medium	300	0.1	2.8	827.6	0.16	49.5	17	100
Unbound	300	0.1	2.7	809.4	0.16	49.4	16	97.8 *Unbound protein: 98%
Washed	300	0.0	0.0	0.08	0.00	1.0	0.0	0.01 *Bound protein: 2%
Eluted protein	15	0.1	1.2	17.8	0.003	0.04	423	2.15
Rest Activity (likely remained in starch)				0.3				0.04

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein: [Total activity of spent medium (in 100%) – total activity of unbound (%)].

^aThese activities were measured based on the corn-starch binding purification

(B)^a

(B.1) CBM20_GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.2	3.3	997.4	0.17	51.1	19.5	100	
Unbound	300	0.1	1.4	420.4	0.15	45.8	9.2	42	*Unbound protein: 42%
Washed	300	0.0	0.0	0.2	0.006	1.9	0.1	0.0	*Bound protein: 58%
Eluted protein	15	1.0	21.0	314.7	0.02	0.3	1125.6	32	
Rest Activity (likely remained in starch)				262.1				26	

(B.2) GH13_CBM20	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.2	4.3	1278.0	0.22	67.4	19.0	100	
Unbound	300	0.1	2.3	693.7	0.18	53.0	13.1	54	*Unbound protein: 56%
Washed	300	0.0	0.1	27.5	0.01	2.6	10.5	2.2	*Bound protein: 44%
Eluted protein	15	1.2	23.6	353.3	0.03	0.4	902.0	28	
Rest Activity (likely remained in starch)				203.6				16	

(B.3) GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	1.6	486.3	0.17	52	9.3	100	
Unbound	300	0.1	1.5	444.9	0.17	51	8.7	91	*Unbound protein: 92%
Washed	300	0.0	0.0	4.5	0.01	2	2.3	0.9	*Bound protein: 8%
Eluted protein	15	0.1	2.4	35.6	0.005	0.1	432.5	7	
Rest Activity (likely remained in starch)				1.3				0	

(B.4) MGG029 (parental strain with empty vector)	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.7	203.6	0.16	49	4.1	100.0	
Unbound	300	0.0	0.7	197.9	0.16	49	4.0	97.2	*Unbound protein: 97%
Washed	300	0.0	0.0	0.0	0.00	1	0.0	0.0	*Bound protein: 3%
Eluted protein	15	0.0	0.4	5.4	0.003	0.0	133.1	2.7	
Rest Activity (likely remained in starch)				0.3				0.1	

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein: [Total activity of spent medium (in 100%) – total activity of unbound (%)]

^aThese activities were measured based on the corn-starch binding purification

(C)^a

(C.1) CBM20_GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.3	5.6	1670.1	0.17	51.1	32.7	100	
Unbound	300	0.1	2.5	748.4	0.15	45.8	16.3	45	*Unbound protein: 45%
Washed	300	0.0	0.0	7.5	0.006	1.9	3.9	0	*Bound protein: 55%
Eluted protein	15	1.63	32.6	489.7	0.02	0.28	1751.6	29	
Rest Activity (likely remained in starch)				424.5				25	

(C.2) GH13_CBM20	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.4	7.0	2113.1	0.22	67.4	31.4	100	
Unbound	300	0.2	4.1	1219.6	0.18	53.0	23.0	58	*Unbound protein: 60%
Washed	300	0.0	0.2	56.6	0.01	2.6	21.6	2.7	*Bound protein: 40%
Eluted protein	15	1.9	37.2	558.5	0.03	0.4	1426.1	26	
Rest Activity (likely remained in starch)				278.4				13	

(C.3) GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.2	4.2	1264.8	0.17	52	24.1	100	
Unbound	300	0.2	3.9	1165.7	0.17	51	22.7	92	*Unbound protein: 92%
Washed	300	0.0	0.0	3.2	0.01	2	1.6	0.3	*Bound protein: 8%
Eluted protein	15	0.3	6.0	90.7	0.005	0.1	1217.6	7	
Rest Activity (likely remained in starch)				5.3				0	

(C.4) MGG029 (parental strain with empty vector)	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	2.7	808.7	0.16	49	16.3	100.0	
Unbound	300	0.1	2.6	793.6	0.16	49	16.1	98.1	*Unbound protein: 98%
Washed	300	0.0	0.0	0.0	0.00	1	0.0	0.0	*Bound protein: 2%
Eluted protein	15	0.0	1.0	14.5	0.003	0.0	318.3	1.8	
Rest Activity (likely remained in starch)				0.6				0.1	

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein: [Total activity of spent medium (in 100%) – total activity of unbound (%)]

^aThese activities were measured based on the corn-starch binding purification

(D)^a

(D.1) CBM20_GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	2.0	613.2	0.17	51.1	12.0	100	
Unbound	300	0.0	0.8	248.8	0.15	45.8	5.4	41	*Unbound protein: 41%
Washed	300	0.0	0.0	2.5	0.006	1.9	1.3	0	*Bound protein: 59%
Eluted protein	15	0.6	12.7	191.2	0.02	0.3	684.0	31	
Rest activity (likely remained in starch)				170.7				28	

(D.2) GH13_CBM20	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	2.2	663.5	0.22	67.4	9.8	100	
Unbound	300	0.1	1.3	384.5	0.18	53.0	7.3	58	*Unbound protein: 58%
Washed	300	0.0	0.0	0.0	0.01	2.6	0.0	0.0	*Bound protein: 42%
Eluted protein	15	0.7	14.0	210.6	0.03	0.4	537.8	32	
Rest activity (likely remained in starch)				68.3				10	

(D.3) GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.8	248.8	0.17	52	4.7	100.0	
Unbound	300	0.0	0.8	231.9	0.17	51	4.5	93.2	*Unbound protein: 93%
Washed	300	0.0	0.0	0.0	0.01	2	0.0	0.0	*Bound protein: 7%
Eluted protein	15	0.1	1.0	15.7	0.005	0.1	207.1	6.3	
Rest activity (likely remained in starch)				1.2				0.5	

(D.4) MGG029 (parental strain with empty vector)	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.4	105.6	0.16	49	2.1	100.0	
Unbound	300	0.0	0.3	101.8	0.16	49	2.1	96.4	*Unbound protein: 96%
Washed	300	0.0	0.0	0.0	0.00	1	0.0	0.0	*Bound protein: 4%
Eluted protein	15	0.0	0.2	3.7	0.003	0.1	71.6	3.5	
Rest activity (likely remained in starch)				0.1				0.1	

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein: [Total activity of spent medium (in 100%) – total activity of unbound (%)]

^aThese activities were measured based on the corn-starch binding purification

(E)^a

(E.1) CBM20_GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	1.2	348.7	0.17	51.1	6.8	100	
Unbound	300	0.0	0.5	156.6	0.15	45.8	3.4	45	*Unbound protein: 45%
Washed	300	0.0	0.0	0.0	0.006	1.9	0.0	0	*Bound protein: 55%
Eluted protein	15	0.3	6.9	103.6	0.02	0.3	370.4	30	
Rest Activity (likely remained in starch)				88.5				25	

(E.2) GH13_CBM20	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.1	1.3	394.0	0.22	67.4	5.8	100	
Unbound	300	0.0	0.7	218.7	0.18	53.0	4.1	56	*Unbound protein: 57%
Washed	300	0.0	0.0	3.8	0.01	2.6	1.4	1.0	*Bound protein: 43%
Eluted protein	15	0.4	7.2	107.8	0.03	0.4	275.3	27	
Rest Activity (likely remained in starch)				63.7				16	

(E.3) GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.4	132.0	0.17	52.5	2.5	100.0	
Unbound	300	0.0	0.4	122.5	0.17	51.3	2.4	92.9	*Unbound protein: 93%
Washed	300	0.0	0.0	0.0	0.01	2	0.0	0.0	*Bound protein: 7%
Eluted protein	15	0.0	0.6	9.2	0.005	0.1	129.2	7.0	
Rest Activity (likely remained in starch)				0.2				0.1	

(E.4) MGG029 (parental strain with empty vector)	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.2	71.6	0.16	49	1.4	100	
Unbound	300	0.0	0.2	68.8	0.16	49	1.4	96	*Unbound protein: 96%
Washed	300	0.0	0.0	0.2	0.00	1	0.2	0.3	*Bound protein: 4%
Eluted protein	15	0.0	0.2	2.5	0.003	0.0	54.1	4	
Rest Activity (likely remained in starch)				0				0	

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein : [Total activity of spent medium (in 100%) – total activity of unbound (%)]

^aThese activities were measured based on the corn-starch binding purification

(F)^a

(F.1) CBM20_GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.6	167.8	0.17	51.1	3.3	100	
Unbound	300	0.0	0.3	77.3	0.15	45.8	1.7	46	*Unbound protein: 46%
Washed	300	0.0	0.0	0.02	0.006	1.9	0.0	0	*Bound protein: 54%
Eluted protein	15	0.16	3.3	49.5	0.02	0.3	177.0	29	
Rest Activity (likely remained in starch)				41.0				24	

(F.2) GH13_CBM20	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.4	126.3	0.22	67.4	1.9	100	
Unbound	300	0.0	0.2	71.6	0.18	53.0	1.4	57	*Unbound protein: 57%
Washed	300	0.0	0.0	0.1	0.01	2.6	0.0	0.07	*Bound protein: 43%
Eluted protein	15	0.1	2.2	32.7	0.03	0.4	83.5	26	
Rest Activity (likely remained in starch)				21.9				17	

(F.3) GH13	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.2	56.6	0.17	52	1.1	100	
Unbound	300	0.0	0.2	54.7	0.17	51	1.1	97	*Unbound protein: 97%
Washed	300	0.0	0.0	0.0	0.01	2	0.0	0.0	*Bound protein: 3%
Eluted protein	15	0.0	0.1	1.8	0.005	0.1	25.7	3	
Rest Activity (likely remained in starch)				0.1				0	

(F.4) MGG029 (parental strain with empty vector)	Volume (mL)	Reducing sugar release (mM)	Amylase activity (U/mL,hr)	Total activity (U)	Protein (mg/mL)	Total Protein (mg)	Specific activity (U/mg,hr)	Enzyme recovery (%)	
Spent medium	300	0.0	0.1	29.2	0.16	49	0.6	100.0	
Unbound	300	0.0	0.1	28.8	0.16	49	0.6	98.5	*Unbound protein: 98%
Washed	300	0.0	0.0	0.00	0.00	1	0.0	0.0	*Bound protein: 2%
Eluted protein	15	0.0	0.0	0.4	0.003	0.1	8.0	1.4	
Rest Activity (likely remained in starch)				0.0				0.0	

*Unbound protein (%) is the total of unbound fraction and washed protein; *Bound protein : [Total activity of spent medium (in 100%) – total activity of unbound (%)]

^aThese activities were measured based on the corn-starch binding purification

Figure S1. Amino acid sequence of α -amylase variants used in this study

>Chimeric CBM20_GH13 (New design)

MSFRSLLALSGLVCTGLA TVLHRHGHQGRHIHARQDCATVAVTFDLTATTTYGENIYLVGSI SQLGDWETS DGIAL
SADKYTSSDPLWYVTVTL PAGESFEYKFI RIESDDSV EWESDPNREYTV PQACGTSTATVTD TWVSQEQWWCSEDD
PAAVAASQAARVYMDCHPKPRHPRKPIPVFVPD WRTQSIYFLLTDRFGRTDNSTTATCDTGDQI YCGGSWQGI INH
LDYIQGMGFTA IWISPI TEQLPQDTADGEAYHGYWQQKI YDVNSNFGTADDLKSLSDALHARGMYLMVDVVPNHMG
YAGNGNDVDYSVFD PFDSSSYFHPYCLITDWDNLTMVQDCWEGDTIVSLPDLN TTETAVRTIWYDWVADLVS NYSV
DGLRIDS VLEVEP DFFPGYQEAAGVYCVGEVDNNGNPALDCPYQKVL DGVNLNPIYWQLLYAFESSSGSISNLYNMI
KSVASDCSDPTLLGNFIENHDNPRFASYTSDYSQAKNVLSYIFLSDGIPIVYAGEEQHYSGGKVPYNREATWLSGY
DTSAELYTWIATTNAIRKLAISADSAYITYANDAFYTDSENTIAMRKGTSGSQVITVLSNKGSSGSSYTLTSLGSGY
TSGTKLIEAYTCTSVTVDSSGDI PVPMASGLPRVLLPASVVDSSSLCGGS

>GH13 (Native_ ANI_1_460094)

MRLSTSSFLSVSLLGKLALGLSAAE WRTQSIYFLLTDRFGRTDNSTTATCDTGDQI YCGGSWQGI INHLDYIQGM
GFTA IWISPI TEQLPQDTADGEAYHGYWQQKI YDVNSNFGTADDLKSLSDALHARGMYLMVDVVPNHMGYAGNGND
VDYSVFD PFDSSSYFHPYCLITDWDNLTMVQDCWEGDTIVSLPDLN TTETAVRTIWYDWVADLVS NYSV DGLRIDS
VLEVEP DFFPGYQEAAGVYCVGEVDNNGNPALDCPYQKVL DGVNLNPIYWQLLYAFESSSGSISNLYNMIKSVASDC
SDPTLLGNFIENHDNPRFASYTSDYSQAKNVLSYIFLSDGIPIVYAGEEQHYSGGKVPYNREATWLSGYDTSAELY
TWIATTNAIRKLAISADSAYITYANDAFYTDSENTIAMRKGTSGSQVITVLSNKGSSGSSYTLTSLGSGYTSGTKLI
EAYTCTSVTVDSSGDI PVPMASGLPRVLLPASVVDSSSLCGGS

>GH13_CBM20 (Native, as reference_ KAI3001921.1)

MRLSTSSLLLSVSVSLLGKLALGLSAAE WRTQSIYFLLTDRFGRTDNSTTATCNTGDQI YCGGSWQGI INHLDYIQGM
GFTA IWISPI TEQLPQDTADGEAYHGYWQQKI YDVNSNFGTADDLKSLSDALHARGMYLMVDVVPNHMGYAGNGND
VDYSVFD PFDSSSYFHPYCLITDWDNLTMVQDCWEGDTIVSLPDLN TTETAVRTIWYDWVADLVS NYSV DGLRIDS
VLEVEP DFFPGYQEAAGVYCVGEVDNNGNPALDCPYQYLDGVNLNPIYWQLLYAFESSSGSISDLYNMIKSVASDC
SDPTLLGNFIENHDNPRFASYTSDYSQAKNVLSYIFLSDGIPIVYAGEEQHYSGGKVPYNREATWLSGYDTSAELY
TWIATTNAIRKLAISADSAYITYANDAFYTDSENTIAMRKGTSGSQVITVLSNKGSSGSSYTLTSLGSGYTSGTKLI
EAYTCTSVTVDSSGDI PVPMASGLPRVLLPASVVDSSSLCGGS GSNSSTTTT TATSSSTATSKSASTSSTSTACT
ATSTSLAVT FEELV TTTYGEEI YLSGSI SQLGDWDTSDAVKMSADDTSSNPEWSVTVLTPVGTTFEYKFIKVESD
GTVTWE SDPNREYTVPECGSGETVVD TWR

Yellow: Signal sequence

Grey: linker

Purple: CBM20 chimeric and native

Green: GH13

Figure S2. Multiple alignment of protein sequence. **(a).** Multiple sequence alignment of modular GH13 α -amylase variants from the new and native design. Both N- and C-terminal CBM20 domain are marked with purple font. GH13 α -amylase domain are marked with green font. Sequence identity is indicated by asterisks, while sequence similarity is marked with a dot (PDB code 2AAA). **(b).** Sequence alignment of CBM20 domains from the new CBM20_GH13 and native GH13_CBM20 design. Sequence identities are indicated by asterisks and black font. Non-identical sequences are marked with dot and red font. The amino acid residues involved in substrate binding are highlighted in soft blue and all are conserved for both N and C-terminus CBM20 used in this study. These four essential binding sites were predicted based on the crystal structure of CBM20 from *A. niger* glucoamylase that was also applied for the CBM20_GH13 design (PDB code 1AC0)

(a.)

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CBM20_GH13 (new)   QDCATVAVTFDLTATTYGENIYLVGSISQLGDWETSDGIALSADKYTSSDPLWYVTTL
GH13_CBM20 (native) -----
GH13 (native)     -----

CBM20_GH13 (new)   PAGESFEYKFIRESDDSVEWESDPNREYTPQACGTSTATVTDTWVSQEQWCSDDPA
GH13_CBM20 (native) -----
GH13 (native)     -----

CBM20_GH13 (new)   AVAASQAARVYMDCHPKPRHPRKPIPVFVPDWR TQSIYFLLTDRFGRD NSTTATCDTGD
GH13_CBM20 (native) -----WR TQSIYFLLTDRFGRD NSTTATCNTGD
GH13 (native)     -----WR TQSIYFLLTDRFGRD NSTTATCDTGD
                                     *****

CBM20_GH13 (new)   QIYCGGSWQGIINHLDIYIQMGFTAIWISPITEQLPQDTADGEAYHGYWQQKIYDVNSNF
GH13_CBM20 (native) QIYCGGSWQGIINHLDIYIQMGFTAIWISPITEQLPQDTADGEAYHGYWQQKIYDVNSNF
GH13 (native)     QIYCGGSWQGIINHLDIYIQMGFTAIWISPITEQLPQDTADGEAYHGYWQQKIYDVNSNF
                                     *****

CBM20_GH13 (new)   GTADDLKSLSDALHARGMYLMVDVVPNHMGYAGNGNDVDYSVDFPFDSSSYFHPYCLITD
GH13_CBM20 (native) GTADDLKSLSDALHARGMYLMVDVVPNHMGYAGNGNDVDYSVDFPFDSSSYFHPYCLITD
GH13 (native)     GTADDLKSLSDALHARGMYLMVDVVPNHMGYAGNGNDVDYSVDFPFDSSSYFHPYCLITD
                                     *****

CBM20_GH13 (new)   WDLNLTMVQDCWEGDTIVSLPDLNLTETAVRTIWDWVADLVSNSV DGLRI DSVLEVEPD
GH13_CBM20 (native) WDLNLTMVQDCWEGDTIVSLPDLNLTETAVRTIWDWVADLVSNSV DGLRI DSVLEVEPD
GH13 (native)     WDLNLTMVQDCWEGDTIVSLPDLNLTETAVRTIWDWVADLVSNSV DGLRI DSVLEVEPD
                                     *****

CBM20_GH13 (new)   FFPGYQEAAAGVYCVGEVDNGNPALDCPYQKVL DGVLNYPYIYWQLLYAFESSSGSISNLYN
GH13_CBM20 (native) FFPGYQEAAAGVYCVGEVDNGNPALDCPYQEYLDGVLNYPYIYWQLLYAFESSSGSISDLYN
GH13 (native)     FFPGYQEAAAGVYCVGEVDNGNPALDCPYQKVL DGVLNYPYIYWQLLYAFESSSGSISNLYN
                                     *****

CBM20_GH13 (new)   MIKSVASDCSDPTLLGNFIENH D N P R F A S Y T S D Y S Q A K N V L S Y I F L S D G I P I V Y A G E E Q H
GH13_CBM20 (native) MIKSVASDCSDPTLLGNFIENH D N P R F A S Y T S D Y S Q A K N V L S Y I F L S D G I P I V Y A G E E Q H
GH13 (native)     MIKSVASDCSDPTLLGNFIENH D N P R F A S Y T S D Y S Q A K N V L S Y I F L S D G I P I V Y A G E E Q H
                                     *****

CBM20_GH13 (new)   YSGGKVPYNREATWLSGYD TSAELYTWIATTN AIRKLAI SADSAYIT YAN DAFY TDSNTI
GH13_CBM20 (native) YSGGKVPYNREATWLSGYD TSAELYTWIATTN AIRKLAI SADSAYIT YAN DAFY TDSNTI
GH13 (native)     YSGGKVPYNREATWLSGYD TSAELYTWIATTN AIRKLAI SADSAYIT YAN DAFY TDSNTI
                                     *****

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Figure S4. SDS-PAGE of 0.5 ug purified α -amylase obtained by starch binding purification. Gels were stained with Sypro Ruby and the α -amylase bands were identified because of the absence of bands in these positions in the parental strain transformed with the empty vector. Lane 1: molecular weight marker, Lane2: CBM20_GH13, Lane3: GH13_CBM20.

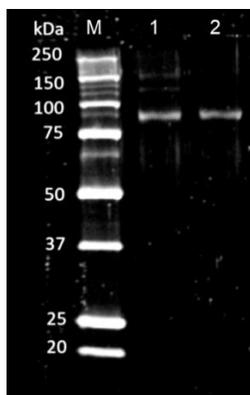
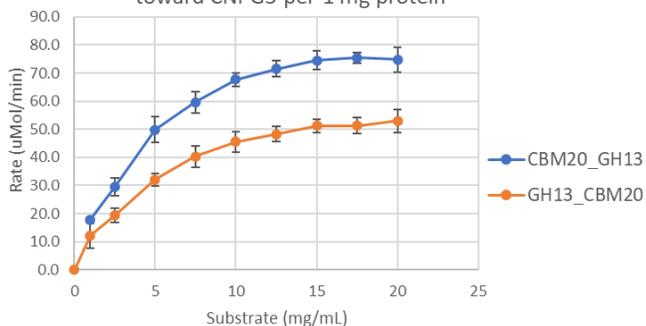
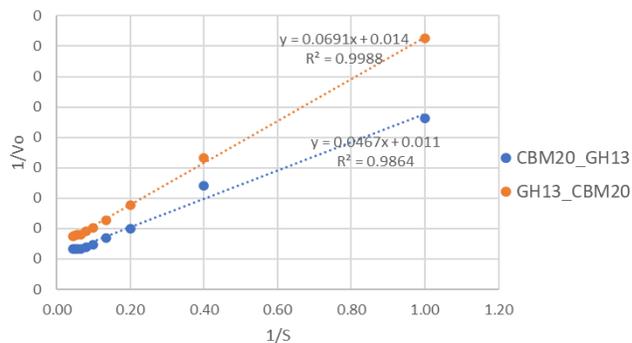


Figure S5. Kinetic Rate of Enzyme on Various Substrates. (A) 2-Chloro-4-nitrophenyl- β -D-maltotriose (CNP3), (B) Soluble Starch, (C) Rice starch, (D) Corn starch, (E) Wheat starch, (F) Potato starch.

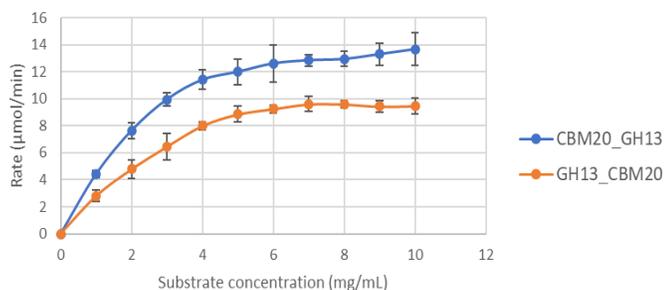
(A) Specific rate of CBM20_GH13 and GH13_CBM20 toward CNPG3 per 1 mg protein



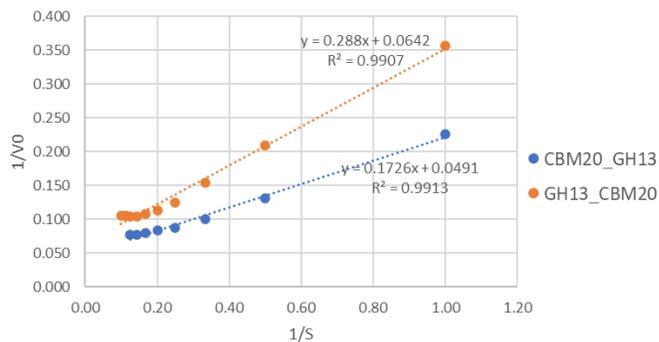
Lineweaver-burk Plot of CBM20_GH13 and GH13_CBM20 toward CNPG3



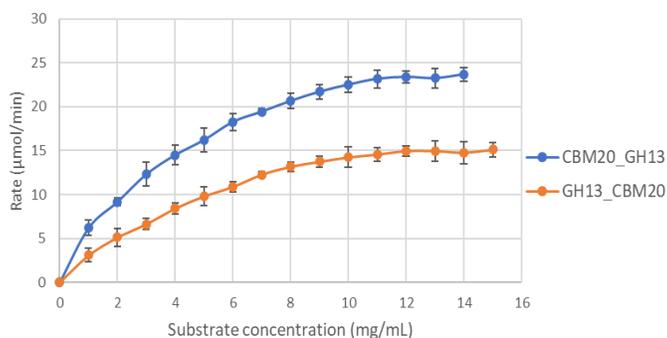
(B) Specific rate of CBM20_GH13 and GH13_CBM20 toward soluble starch per 1 mg protein



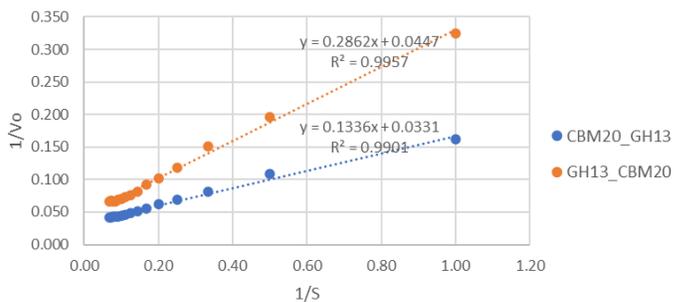
Lineweaver-burk Plot of CBM20_GH13 and GH13_CBM20 toward soluble starch



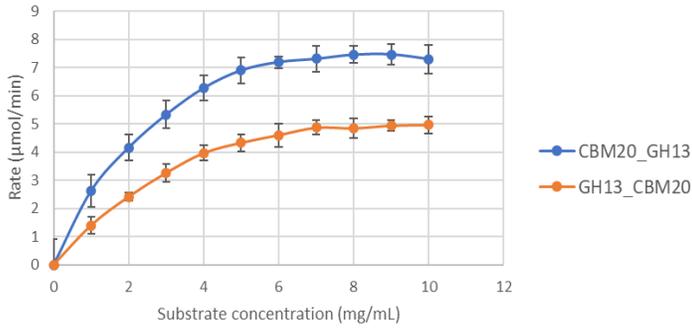
(C) Specific rate of CBM20_GH13 and GH13_CBM20 toward rice starch per 1 mg protein



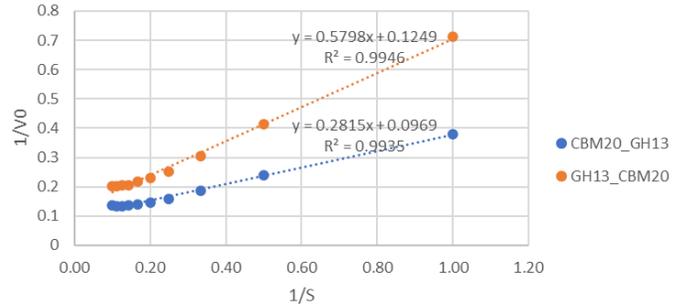
Lineweaver-burk plot of CBM20_GH13 and GH13_CBM20 toward rice starch



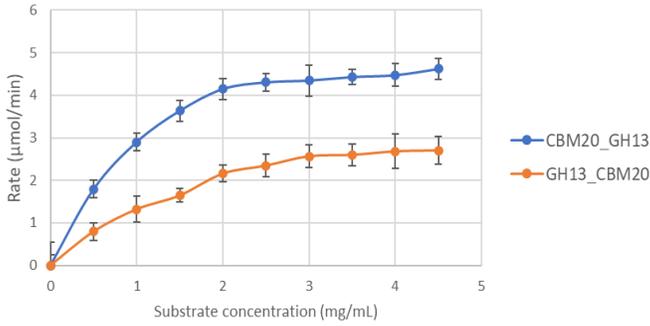
(D) Specific rate of CBM20_GH13 and GH13_CBM20 toward corn starch per 1 mg protein



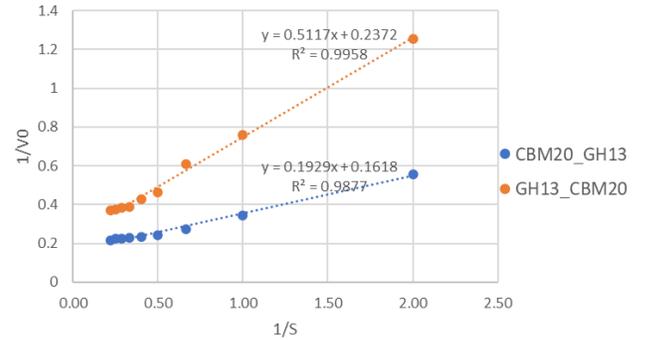
Lineweaver-burk Plot of CBM20_GH13 and GH13_CBM20 toward corn starch



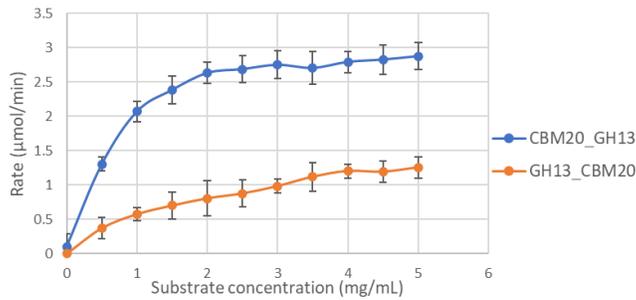
(E) Specific rate of CBM20_GH13 and GH13_CBM20 toward wheat starch per 1 mg protein



Lineweaver-burk plot of CBM20_GH13 and GH13_CBM20 toward wheat starch



(F) Specific rate of CBM20_GH13 and GH13_CBM20 toward raw potato starch per 1 mg protein



Lineweaver-burk Plot of CBM20_GH13 and GH13_CBM20 toward raw potato starch

