

**Supplementary material**

**with**

**HYDROPHOBIC INTERACTIONS CONTRIBUTE TO CONFORMATIONAL  
STABILIZATION OF ENDO-GLYCOSYLCERAMIDASE II (*EGCII*) BY MECHANISM-  
BASED PROBES**

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# Lenght:      602
# Identity:    88/602 (14.6%)
# Similarity:  161/602 (26.7%)
# Gaps:       238/602 (39.5%)

EGCII  1  -----MSG
GBA    1  EFARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELSMGPTQA

EGCII  4  SSGSGTALTTPSYLKDDDGRLILRGENTASSAKSAPDGMPOFTEADLAREYADMGTNFV
GBA   61  NHTGICLLLT-----QPEQKFQVKGEGGAMDAALNILAL-----

EGCII  64  RFLISWRSVEPAPGVYDQYLDREVDRVGNVYAEFGYKVMLDMHQDVYSGAITPEGNS---
GBA   99  -----SPAQNLLKSYFS--EEGTGMNIIRVPMASCDFSIRTYTYADTPDFQLHN

EGCII 121  -----GNGAGATGNGAPAWAT
GBA  149  FSLPEEDTKLKIPLIHRALQLAQRVSLLASPWTSPTWLKTNGAVNGKCSI-----

EGCII 137  YMDGLEVEP-QPRWELYIIPGVMRAFDNFWNTTGKHPELVEHYAKAWRAVADRFDNDA
GBA  200  --KQEGDIYHQTWARYFVKF-----LDAY-----AEHKLQFW-----

EGCII 196  VVAYDLMNEPFGGSLOGPAFEAGPLAAMYQRTTD-----ATROVDQDTWVC
GBA  231  --AVTAENEPSAGLLSGYPEQCLGFTPEHQDFIARDLGPTLANSTHNVRLMLDDQRL

EGCII 242  VAEQAIGVNOGLPSGLTKIDDPRAQOQRIA-----YCPHLYPLPLDIGDGEGLARTIT-
GBA  289  ILPHWAKV-----VLTDEPAKYVHCIAVHWYLDPLAPAKATLGETHRLFPNTMLF

EGCII 296  -----DVTIDAMRANTA-----HTARVL
GBA  340  ASEACVGSKFWEQSVRLGSDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNNVNF

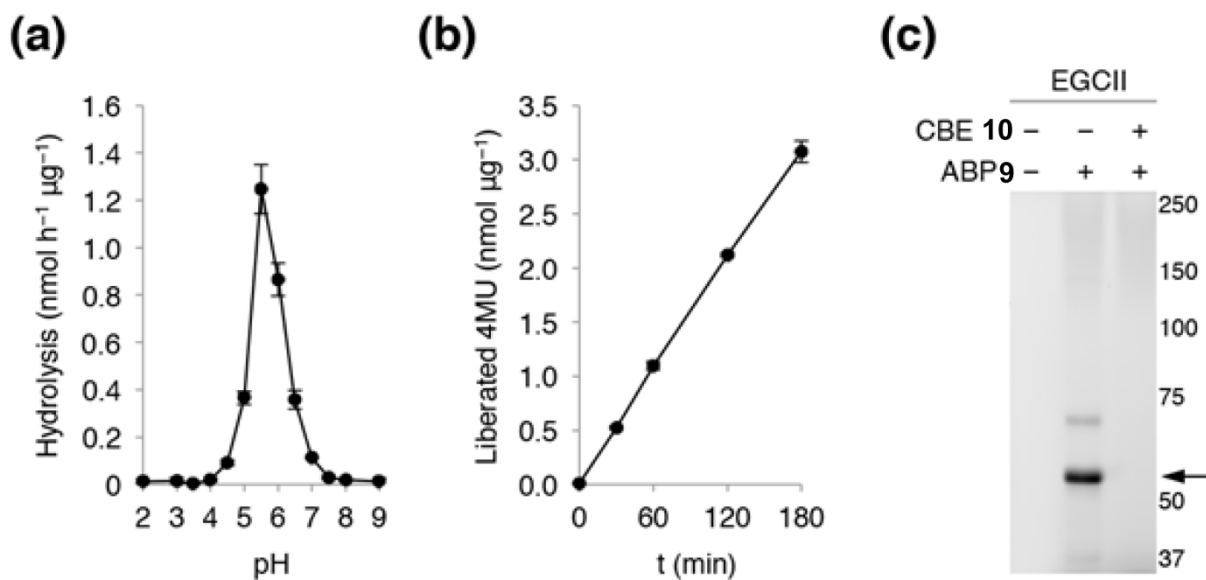
EGCII 314  GVPPIILGEFGLDTLPGARDYIERVYGTAREMGAGVSYWSSDPGPWGPYLPDGTQTLIV
GBA  400  VDSPIIV-DITKDTFYKQPMF-----YHLGHFSKFIPEGSQRVGL

EGCII 374  DTINKPYPRAVAGTPTLWSSTSDRLQLTTEPDAAITAPTEIYLPAGFGDVHVE-GADV
GBA  439  VASOKNDLDAVALMHPDGSVVVVLT-----NRSSKDVELTIKDPVGFLETISPGYSIHT

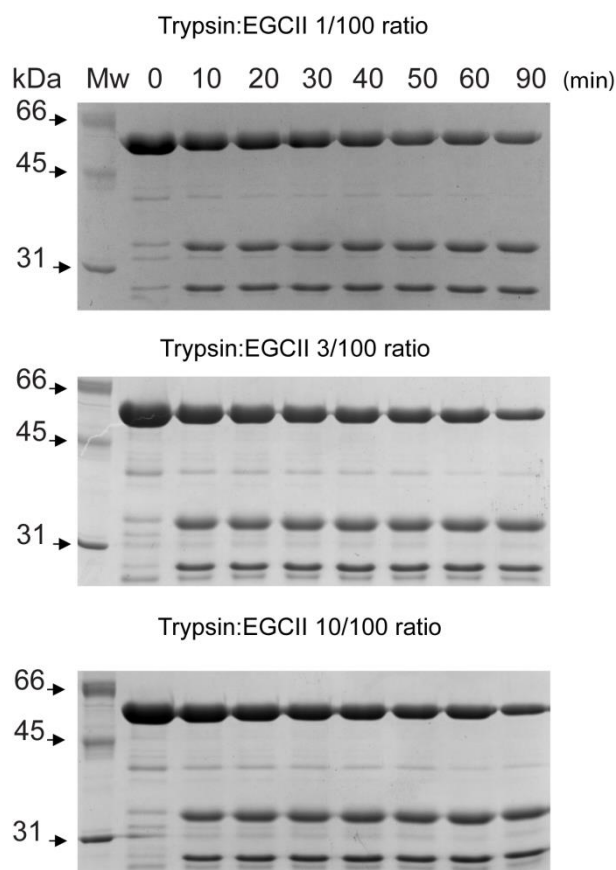
EGCII 433  VGWDRQSRLITVTPADSGNVTVTVTPAA
GBA  494  YLWHRQLLVDTM-----

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**Figure S1:** EGCII and GBA primary sequences alignment using Needleman-Wunsch algorithm from EMBOSS Needle server ([emboss.open-bio.org](http://emboss.open-bio.org)). Identical residue are highlighted on black background, conservative changes are highlighted in grey. The acid /base and nucleophile catalytic residues are marked with stars.



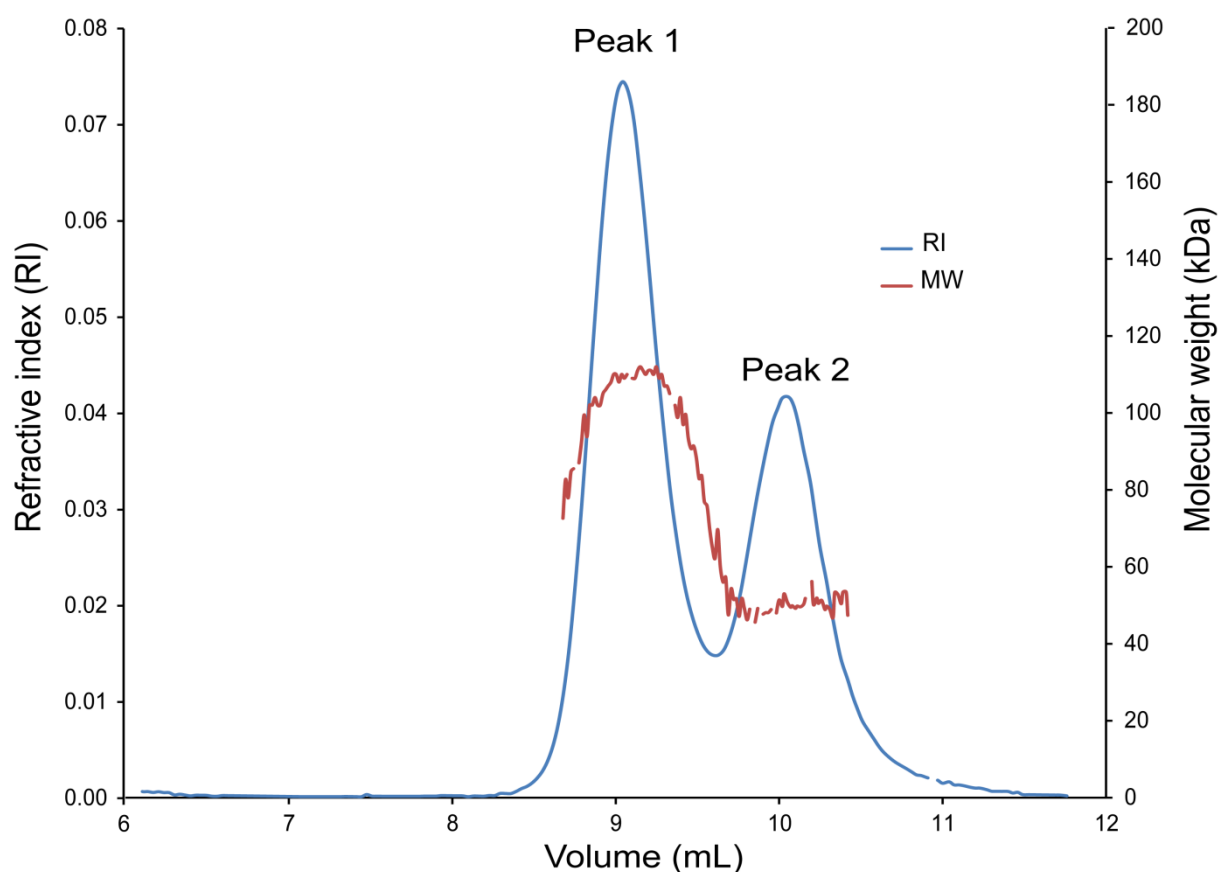
**Figure S2. Read-outs of active EGCII molecules.** (a) Hydrolysis of 4MU-Lac by EGCII across a pH range, at 17 °C in 150 mM McIlvaine's buffer. (b) Time-dependent liberation of 4MU from 4MU-Lac by EGCII at 17 °C and in 150 mM McIlvaine's buffer pH 5.5 (c) Labeling of EGCII with cyclophellitol-aziridine ABP 9 at pH 5.5 and 17 °C and its competition by pre-incubation with CBE 10 in 150 mM McIlvaine's buffer pH 5.5. The main EGCII protein band is marked with an arrow.



**Figure S3: Tryptic digestion of EGCII at different ratios:** Tryptic digestion of EGCII with Trypsin (Sigma) with Trypsin:EGCII ratios 1:100, 3:100, 10:100 (by weight) in 150 mM McIlvaine's(citrate/phosphate) buffer pH 5.5 at 25°C.

**Tryptic digestion of EGCII ratios:** Tryptic digestion of trypsin from bovine pancreas

(by weight) in 150 mM



**Figure S4.** EGCII size exclusion chromatogram analyzed by Multi Angle Laser Light Scattering.

**Results:**

	Peak 1	Peak 2
Volume (mL)	8.75-9.42	9.64-10.42
Injection Mass (g)	$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$
Calc. Mass (g)	$3.82 \cdot 10^{-5}$	$2.47 \cdot 10^{-5}$
dn/dc (mL/g)	0.186	0.186
Polydispersity (Mw/Mn)	1.003 +/- 0.033	1.002 +/- 0.086
Molar Mass Moments (g/mol)	$1.062 \cdot 10^{+5}$	$5.0 \cdot 10^{+4}$

$^1\text{H}$ -NMR spectra of compound **8** in  $\text{CD}_3\text{OD}$



