Supplementary Materials for

Dodecyl maltopyranoside enabled purification of active human GABA type A receptors for deep and direct proteomic sequencing, by Xi Zhang and Keith W. Miller

< 1. Pdf for Supplementary Figures and Note >

Supplementary Fig. S1. Chemical structures of detergents and additives relevant to this study.

Supplementary Fig. S2. Protein purification method development: screening of detergent and filter size.

Supplementary Fig. S3. Reaction condition screening for PNGase F digestion identified enzyme concentration as a key parameter to complete $GABA_AR$ deglycosylation.

Supplementary Fig. S4. Representative MS/MS spectra for the identification of deamidation from in-gel trypsin-chymotrypsin digestion of PNGase F-treated $GABA_AR$.

Supplementary Note 1. Detailed methods of [^{3}H]radioligand binding assays.

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Supplementary Table S1. Proteins identified from deep in-gel analysis of DDM/CHS-based affinity-purified $GABA_AR$ by searching against the human proteome and CRAPome.

Supplementary Table S2. Proteins identified from deep in-gel analysis of PNGase F-treated DDM/CHS-based affinity-purified $GABA_AR$ by searching against the human proteome and CRAPome.

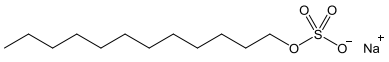
Supplementary Table S3. Proteins identified from direct pepsin digestion and analysis of DDM/CHS-based affinity-purified $GABA_AR$ by searching against the human proteome (PD 1.3, peptide FDR<1%) and CRAPome.

< 3. Excel for Supplementary Table S4 >

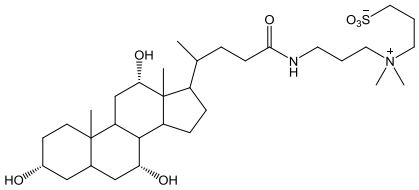
Supplementary Table S4. Peptide identification lists. (**a**) Peptides identified from in-gel trypsin-chymotrypsin digestion of the whole 40-72 kDa band, searched against $GABA_AR$ sequences, supplementing **Fig. 4a** and Expts. 8-9 in **Fig. 3a**. (**b**) Deamidated peptides identified from in-gel trypsin-chymotrypsin digestion of PNGase F-treated GABAAR bands at 50-55 kDa (Expt. 10, band e' in **Fig. 3**) and 40-55 kDa (Expt. 11, band f' in **Fig. 3**), prior to PNGase F-treatment decoy filtering. (**c**) Peptides identified from direct analysis of gel-free pepsin-column digestion of DDM/CHS-purified $GABA_AR$ solution, searched against $GABA_AR$ sequences (PD 1.3, peptide FDR<1% by Percolator, mass variation tolerance 10 ppm and 0.8 Da for MS and MS/MS respectively; dynamic M oxidation), supplementing **Fig. 5c**.

Supplementary Figure S1.

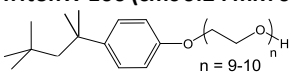
SDS (cmc 6-8 mM, 0.17-0.23%, mw 288.4, Pierce)



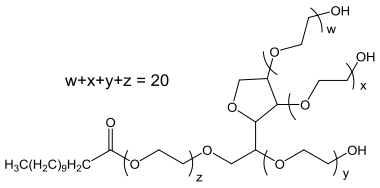
CHAPS (cmc 8-10 mM, 0.49-0.62%, mw 614.9, Pierce)



Triton X-100 (cmc 0.24 mM 0.0155%, ave. mw 647, Pierce)

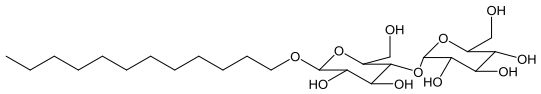


Tween 20 (cmc 0.059 mM, 0.0072%, ave. mw 1228, Anatrace)

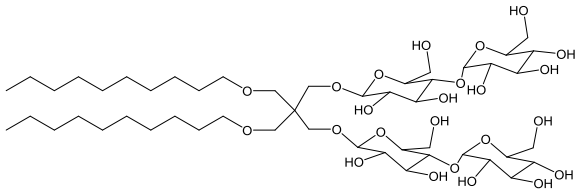


SDS, sodium dodecyl sulfate
CHAPS, 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate
DDM, n-dodecyl-β-D-maltoside
MNG-2, maltose–neopentyl glycol type 2
OG, n-octyl-β-D-glucoside
CHS, cholesteryl hemisuccinate Trizma salt

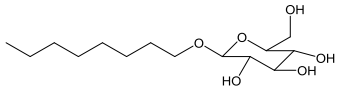
DDM (cmc ~ 0.12 mM 0.006% in 0.2 M NaCl, 0.17 mM 0.0087% in H_2O, mw 510.6, Anatrace)



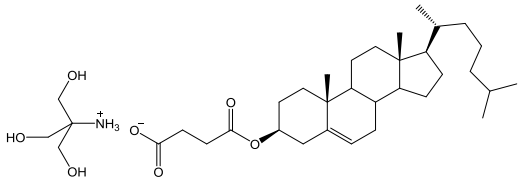
MNG-2 (cmc ~ 9 µM, 0.0010%, mw 1005.2, Ref. 44)



OG (cmc 23.4 mM 0.684% in 0.1 M NaCl, 18-20 mM 0.526-0.585% in H_2O, mw 292.4, Anatrace)

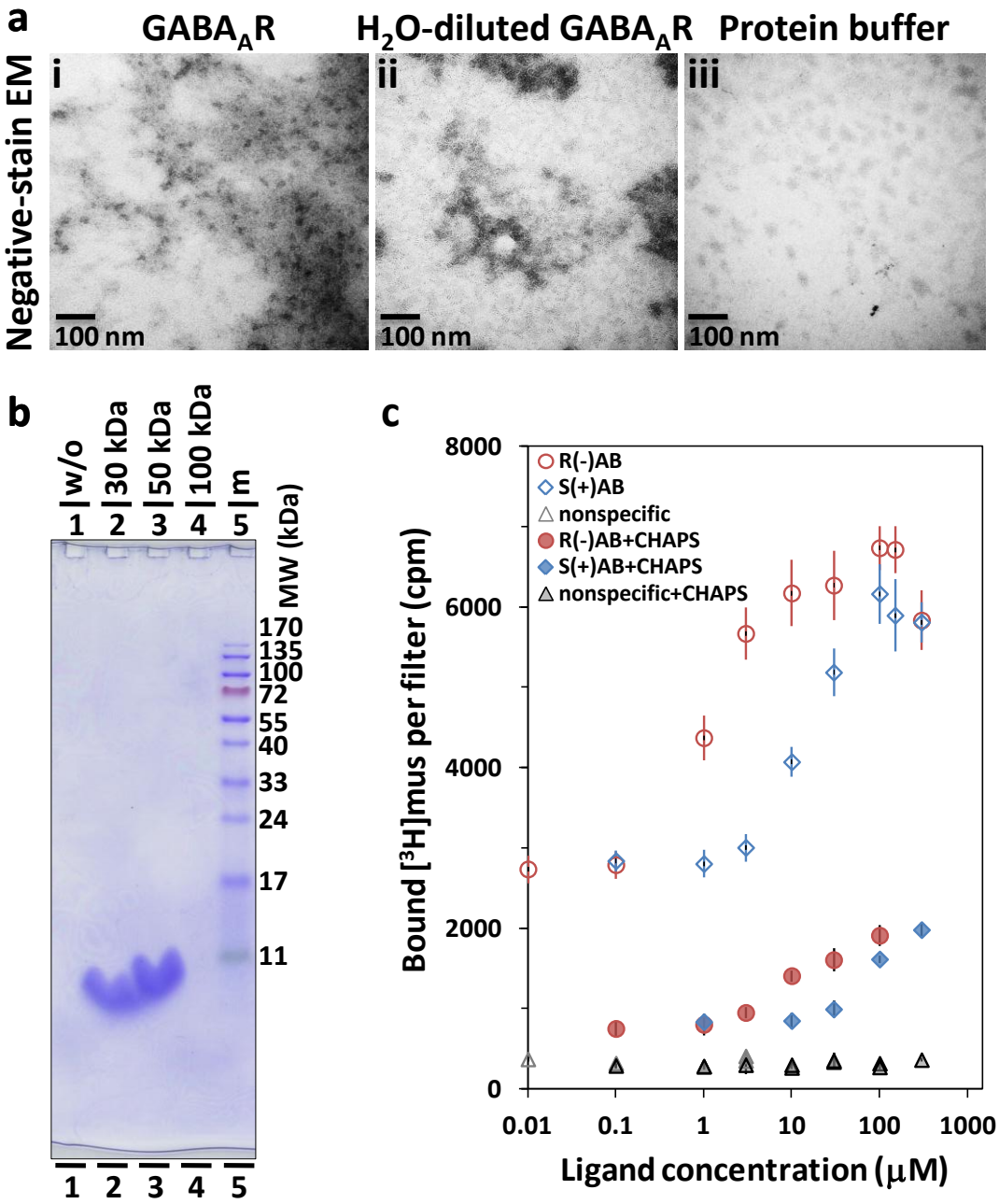


CHS (Trizma salt, total mw 607.9, Anatrace)



Supplementary Figure S1. Chemical structures of detergents and additives relevant to this study. Cmc, critical micelle concentration; %, m/v; mw, molecular weight. Sources of the cmc values were Anatrace or Pierce (Thermo Fisher Scientific) product information sheets, unless specified otherwise.

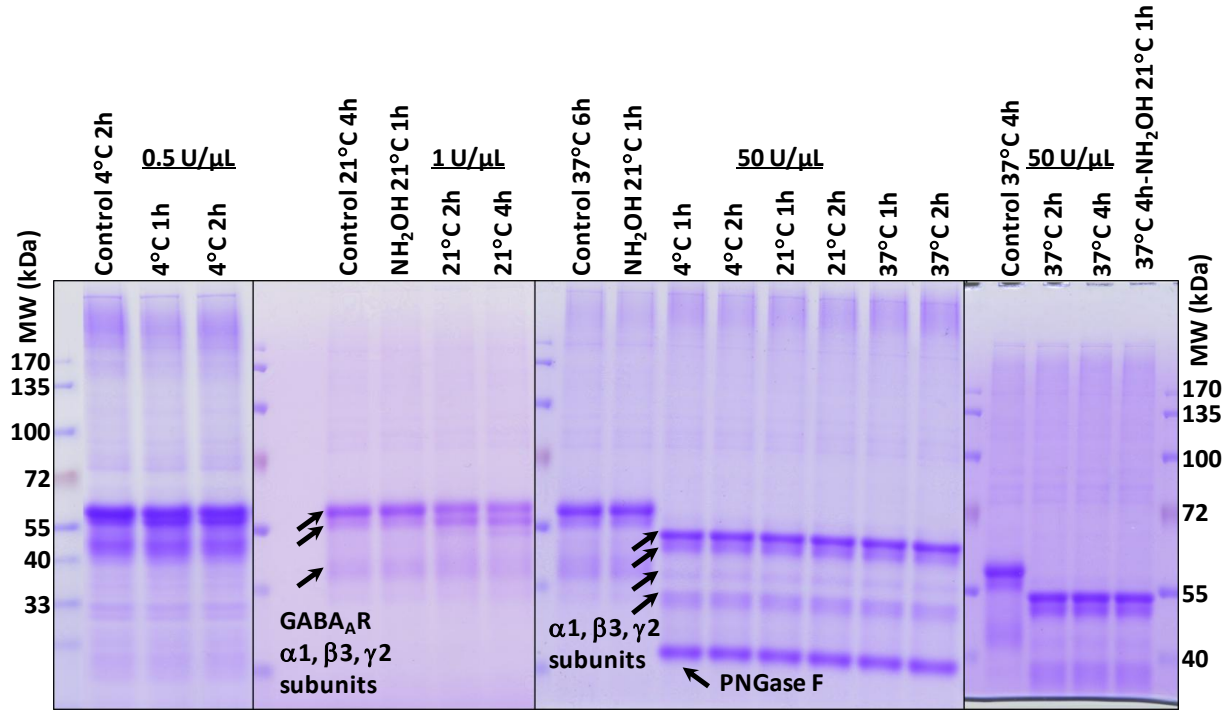
Supplementary Figure S2.



Supplementary Figure S2. Protein purification method development: screening of detergent and filter size. (**a**) Standard negative-stain EM of DDM/CHS-purified $GABA_AR$ analyzed in (**i**) 0.05% DDM/0.0125% CHS protein buffer, or in (**ii**)10-fold DDM/CHS-depleted buffer (10-fold dilution with H_2O), showed that solubilized $GABA_AR$ aggregated upon the decrease in detergent

concentration, illustrating the necessity of supplying sufficient detergents to keep membrane proteins soluble; (**iii**) protein buffer without protein was used as control. (**b**) SDS-PAGE of the protein-free DDM/CHS protein buffer (lane 1), dia-filtrated with filters at MWCO of 30 kDa (lane 2), 50 kDa (lane 3) or 100 kDa (lane 4), indicated that 100 kDa MWCO is required to avoid detergent micelle accumulation during dia-filtration. (**c**) Effects of CHAPS on $GABA_AR$ radioligand binding and barbiturate modulation activities. The [^{3}H]muscimol (2 nM) binding and modulations by R (red circle) or S (blue diamond)-AB in the absence (open markers) and presence (solid markers) of 5 mM CHAPS were measured using $GABA_AR$/HEK293 membrane suspensions at 0.15 mg protein /mL. Nonspecific binding (gray triangle) was measured with 1 mM GABA. Abundance was presented in the original cpm readings of filters. Error bars represented the standard deviations from 4 filters per incubation and one or two reaction incubations at each ligand concentration.

Supplementary Figure S3.



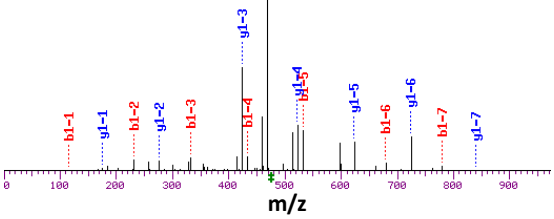
Supplementary Figure S3. Reaction condition screening for PNGase F digestion identified enzyme concentration as a key parameter to complete $GABA_AR$ deglycosylation. Reaction time (1, 2 and 4 h), temperature (4, 21 and 37 °C) and enzyme concentration (0.5, 1 and 50 U/µL) were compared. NH_2OH treatment at 0.7 M under 21 °C for 1 h before or after deglycosylation didn't affect the appearance of the major gel bands.

Supplementary Figure S4.

a α1-NTT: K.DN#TTVFTR.I

seq	b#	b	y	y#
D	1	116.0	-	8
*N	2	231.0	839.4	7
T	3	332.1	724.4	6
T	4	433.1	623.3	5
V	5	532.2	522.3	4
F	6	679.3	423.2	3
T	7	780.3	276.1	2
R	8	-	175.1	1

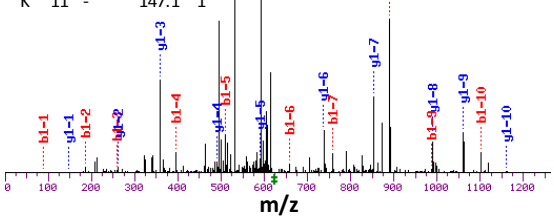
XCorr 2.384
m/z 477.7
z=2
MS/MS



b α1-NMT: K.SVAHN#M*TMPNK.L

seq	b#	b	y	y#
S	1	88.0	-	11
V	2	187.1	1159.5	10
A	3	258.1	1060.4	9
H	4	395.2	989.4	8
*N	5	510.2	852.3	7
*M	6	657.2	737.3	6
T	7	758.3	590.3	5
M	8	889.3	489.2	4
P	9	986.4	358.2	3
N	10	1100.4	261.1	2
K	11	-	147.1	1

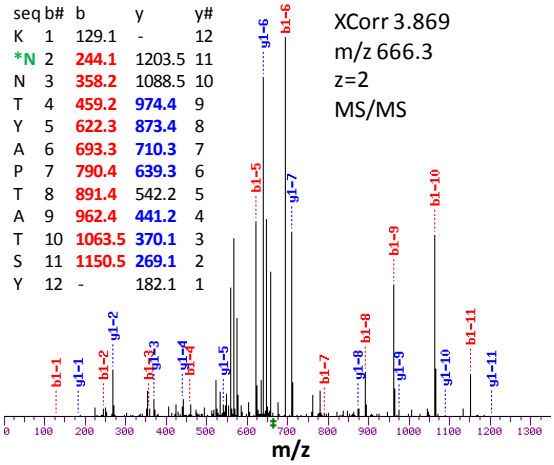
XCorr 3.002
m/z 623.8
z=2
MS/MS



c α1-ICL2 (NNT): K.KN#NTYAPTATSY.T

seq	b#	b	y	y#
K	1	129.1	-	12
*N	2	244.1	1203.5	11
N	3	358.2	1088.5	10
T	4	459.2	974.4	9
Y	5	622.3	873.4	8
A	6	693.3	710.3	7
P	7	790.4	639.3	6
T	8	891.4	542.2	5
A	9	962.4	441.2	4
T	10	1063.5	370.1	3
S	11	1150.5	269.1	2
Y	12	-	182.1	1

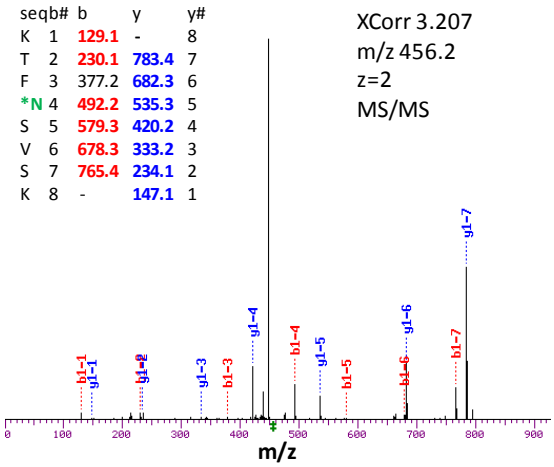
XCorr 3.869
m/z 666.3
z=2
MS/MS



d α1-ICL2 (NSV): K.KTFN#SVSK.I

seq	b#	b	y	y#
K	1	129.1	-	8
T	2	230.1	783.4	7
F	3	377.2	682.3	6
*N	4	492.2	535.3	5
S	5	579.3	420.2	4
V	6	678.3	333.2	3
S	7	765.4	234.1	2
K	8	-	147.1	1

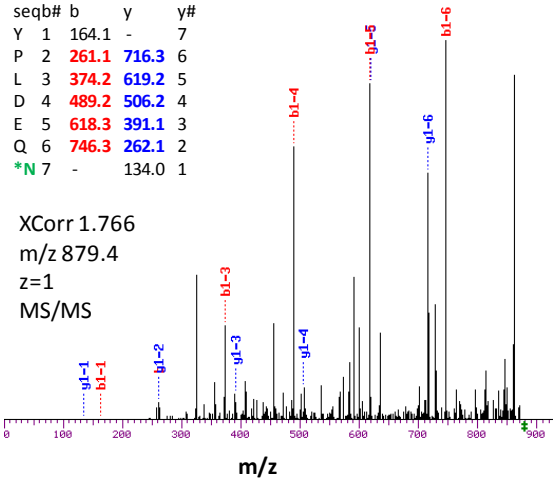
XCorr 3.207
m/z 456.2
z=2
MS/MS



e β3-NCT: R.YPLDEQN#.C

seq	b#	b	y	y#
Y	1	164.1	-	7
P	2	261.1	716.3	6
L	3	374.2	619.2	5
D	4	489.2	506.2	4
E	5	618.3	391.1	3
Q	6	746.3	262.1	2
*N	7	-	134.0	1

XCorr 1.766
m/z 879.4
z=1
MS/MS



Supplementary Figure S4. Representative MS/MS spectra for the identification of deamidation from in-gel trypsin-chymotrypsin digestion of PNGase F-treated $GABA_AR$. (**a**) α1- NTT, (**b**) α1-NMT, (**c**) tentative α1-ICL2 NNT, (**d**) tentative α1-ICL2 NSV, and (**e**) β3-NCT. N#, N deamidation; M*, Mox. Peptide identification details are shown in **Supplementary Table S4b**.

Supplementary Note 1. Detailed methods of [^{3}H]radioligand binding assays.

To determine the effects of CHAPS on the modulation activity of R or S enantiomer of 5-allyl-1-methyl-5-(m-trifluoromethyl-diazirynylphenyl)barbituric acid (AB) on $GABA_AR$ [^{3}H]muscimol binding, the α1β3γ2L $GABA_AR$/HEK293 membrane suspensions were incubated at final concentration of 0.15 mg membrane protein/mL with 2 nM [^{3}H]muscimol and R or S AB (0, 0.01, 0.1, 1, 3, 10, 30, 100, 150 or 300 μM) in the presence and absence of 5 mM CHAPS at 21˚C for 60 min, in the assay buffer composed of 1x PBS pH 7.4. The reactions without CHAPS were quenched by filtration through Whatman GF/B glass fiber filters pre-soaked in 0.5% (m/v) polyethyleneimine using a Millipore manifold (model 1225, Billerica, MA). The reactions with CHAPS were quenched by adding a protein precipitating buffer (2.25 mg/mL bovine albumin IgG and 35 % (m/v) PEG 400), to help trapping protein during filtration. The amounts of protein applied to each filter with and without CHAPS were comparable. Each filter was washed once with 7 mL ice-cold assay buffer or buffer supplemented with 7% (m/v) PEG 400, dried under a lamp, soaked in 5 mL Liquiscint (National Diagnostics, Atlanta, CA) and measured on Perkin-Elmer Tri-Carb 1900 scintillation counter (Waltham, MA).

The ligand binding activity and etomidate modulation sensitivity of DDM/CHS-purified $GABA_AR$ were measured by incubating purified $GABA_AR$ at final concentration of 0.03 μM with 2 nM [^{3}H]muscimol in the assay buffer supplemented with 0.05% DDM/0.0125% CHS in the absence and presence of 10 μM etomidate. The reaction was ended after 60 min incubation by adding the PEG/IgG protein precipitating buffer. Each filter was washed twice with 5 mL assay buffer containing 7% (m/v) PEG 400. The effects of various detergents and additives on the ligand binding activity and etomidate modulation sensitivity of receptors were measured using diluted DDM/CHS-solubilized membranes following the same procedures, except that 5

mM CHAPS, 0.1 mM soy bean asolectin, 4 mM (0.0125%) CHS, or nothing was added for the reaction. Non-specific [^{3}H]muscimol binding was measured with 1 mM GABA, and subtracted from total binding to obtain specific binding. Etomidate modulation of [^{3}H]muscimol binding was presented either as original cpm readings, or as the percentage of specific [^{3}H]ligand binding with etomidate over that without it.