

SUPPLEMENTAL DATA

Biochemical and structural characterization of a five-domain GH115- α -glucuronidase from the marine bacterium *Saccharophagus degradans* 2-40^T

Weijun Wang^{1,¶}, Ruoyu Yan^{1,¶}, Boguslaw P. Nocek², Thu V. Vuong¹, Rosa Di Leo¹, Xiaohui Xu¹, Hong Cui¹, Paul Gatenholm³, Guillermo Toriz^{3,4}, Maija Tenkanen⁵, Alexei Savchenko^{1,*}, Emma R. Master^{1,*}

¹Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada.

²The Structure Biology Center, Biosciences Division Argonne National Laboratory, Argonne IL, USA

³Department of Chemistry and Chemical Engineering, Wallenberg Wood Science Center and Biopolymer Technology, Chalmers University of Technology, Kemivägen 4, Gothenburg, 412 96, Sweden

⁴Department of Wood, Cellulose and Paper Research, University of Guadalajara, Guadalajara 44100, Mexico

⁵ Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, Helsinki 00014, Finland

[¶]: These authors contributed equally to this work.

Running title: A five-domain GH115 α -glucuronidase

*To whom correspondence should be addressed:

Emma R. Master

Telephone: 416-946-7861

Fax: 416-978-8605

Email: emma.master@utoronto.ca

Alexei Savchenko

Telephone: 416-978-3925

Fax: 416- 978-8605

Email: alexei.savchenko@utoronto.ca

SUPPLEMENTAL TABLES

Table S1. Protein representatives structurally related to SdeAgu115A and specific sub-domains.

PDB code	Z score	rmsd	lali (number of aligned residues)	nres (total number of residues)	% sequence identity in structurally consd. Regs.	Protein	Family	Organism
Whole enzyme								
4C90	53.1	2.4	656	822	41	α -Glucuronidase	GH115	<i>Bacteroides ovatus</i>
5BV3	38.3	2.1	713	759	31	α -Glucuronidase	GH115	<i>Bacteroides thetaiotaomicron</i>
2VUR	24.0	6.2	471	584	13	O-GlcNAcase	GH84	<i>Clostridium perfringens</i>
1K9E	22.6	4.7	469	671	14	α -Glucuronidase	GH67	<i>Bacillus stearothermophilus</i>
4H04	10.7	2.6	117	633	19	Lacto- <i>N</i> -biosidase	GH20	<i>Bifidobacterium bifidum</i>
Domain A								
4C90	22.8	1.5	160	811	36	α -Glucuronidase	GH115	<i>Bacteroides ovatus</i>
5BV3	19.0	1.4	140	759	25	α -Glucuronidase	GH115	<i>Bacteroides thetaiotaomicron</i>
1H41	14.0	2.2	124	700	19	α -Glucuronidase	GH67	<i>Cellvibrio japonicus</i>
1K9D	12.4	2.5	118	670	15	α -Glucuronidase	GH67	<i>Geobacillus stearothermophilus</i>
3GH7	11.0	2.5	117	506	17	Beta-N-acetylhexosaminidase	GH20	<i>Paenibacillus sp.</i>
4H04	10.7	2.2	1.0	633	19	Lacto- <i>N</i> -biosidase	GH20	<i>Bifidobacterium bifidum</i>
2YDQ	10.3	2.5	114	579	20	O-GlcNAcase	GH84	<i>Clostridium perfringens</i>

PDB code	Z score	rmsd	lali (number of aligned residues)	nres (total number of residues)	% sequence identity in structurally consd. Regs.	Protein	Family	Organism
Domain B (Catalytic domain)								
4C90	37.4	1.3	285	822	54	α -Glucuronidase	GH115	<i>Bacteroides ovatus</i>
2EPK	15.2	3.7	239	613	9	N-acetyl-beta-D-glucosaminidase	GH20	<i>Streptococcus gordonii</i>
3S6T	15.0	3.4	242	575	12	N-acetyl-beta-D-glucosaminidase	GH20	<i>Ostrinia furnacalis</i>
2CBI	14.9	3.2	231	584	10	O-GlcNAcase (OGA),	GH84	<i>Clostridium perfringens</i>
1K9D	13.8	4	242	670	13	α -Glucuronidase	GH67	<i>Geobacillus stearothermophilus</i>
Domain C								
4C90	25.0	0.9	174	811	29	α -Glucuronidase	GH115	<i>Bacteroides ovatus</i>
5BV3	14.9	1.8	154	759	25	α -Glucuronidase	GH115	<i>Bacteroides thetaiotaomicron</i>
4P3F	7.2	3.4	112	193	5	SRP68 RNA-binding domain	-	<i>Homo sapiens</i>
2EPN	7.1	3.9	146	623	5	N-acetyl-beta-D-glucosaminidase	GH20	<i>Streptococcus gordonii</i>
1YWT	6.8	3.7	111	222	5	14-3-3 σ protein	-	<i>Homo sapiens</i>
Domain C⁺								
1L4I	10.3	2.6	95	189	12	S pilus periplasmic chaperone SfaE	-	<i>Escherichia coli</i>
3BWU	10.3	2.5	95	200	12	Chaperone protein fimC	-	<i>Escherichia coli</i>
3ZMR	10.1	2.8	87	469	17	Bacon-domain in BoGH5A(endo-xyloglucanase)	-	<i>Bacteroides ovatus</i>

PDB code	Z score	rmsd	lali (number of aligned residues)	nres (total number of residues)	% sequence identity in structurally consd. Regs.	Protein	Family	Organism
Domain D								
4C90	21.4	2.1	167	811	26	α -Glucuronidase	GH115	<i>Bacteroides ovatus</i>
5BV3	13.9	2.0	132	759	17	α -Glucuronidase	GH115	<i>Bacteroides thetaiotaomicron</i>
4CRR	13.1	1.8	112	124	16	C-terminal CBM6 in a GH16 laminariase	CBM6	<i>Zobellia galactanivorans</i>
2CDO	12.7	2.2	117	137	12	CBM6 in a beta-agarase	CBM6	<i>Saccharophagus degradans</i>
3ZM8	12.3	2.3	119	444	11	C-CBM35 in GH26 endo- β -1,4-mannanase (PaMan26A)	CBM35	<i>Podospora anserina</i>

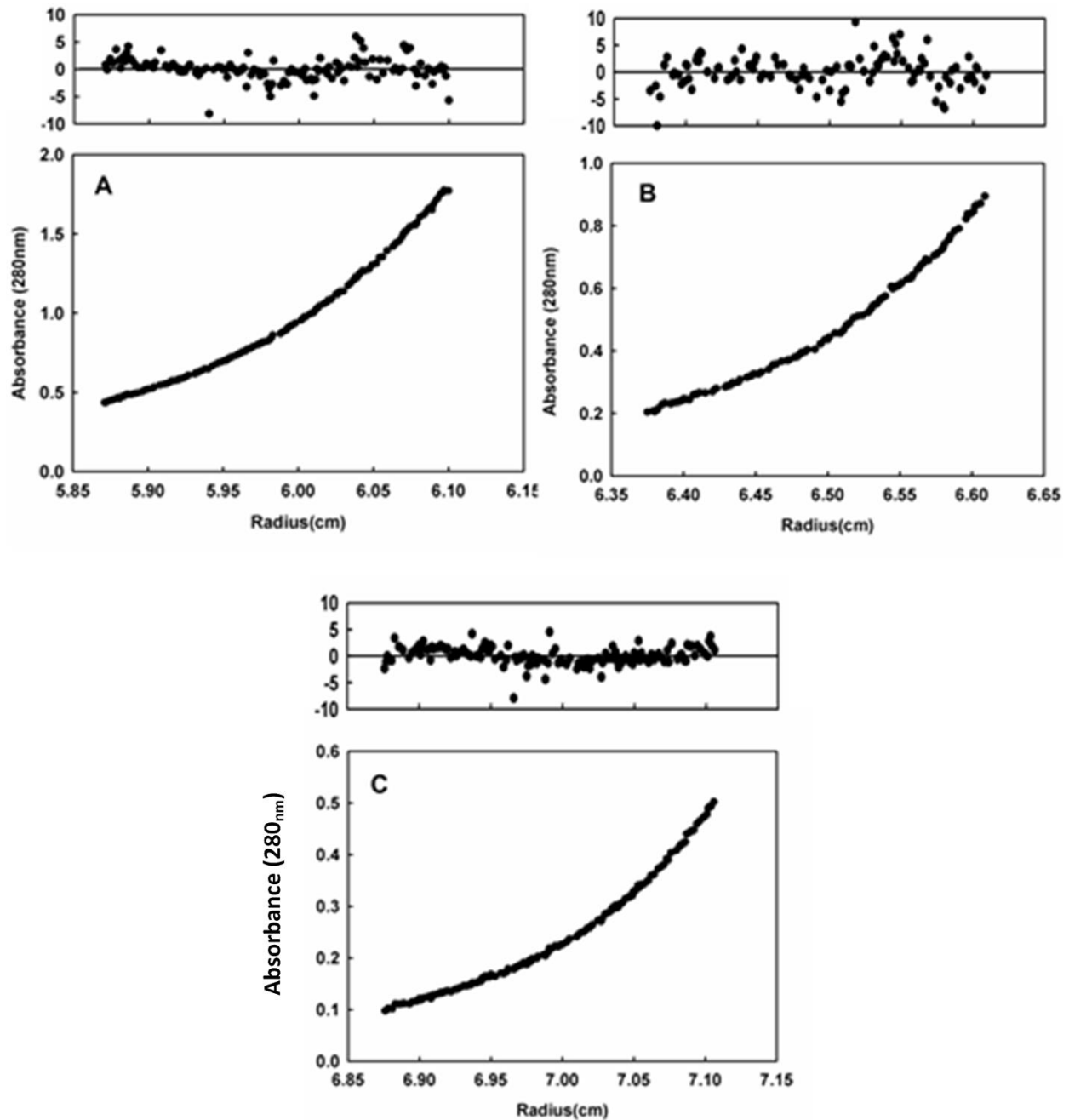


Fig. S1 Equilibrium analytical ultracentrifugation analysis to probe the oligomeric state of SdeAgu115A. The equilibrium profile and the residuals of the fit for the data obtained at 20°C for SdeAgu115A at concentration of 5.9 μM (A); 3.0 μM (B); 1.5 μM (C) in 20 mM HEPES buffer pH7.0 containing 300 mM NaCl. The rotor speed for the displayed data was 7000 rpm in a Beckman Optima XL-A analytical ultracentrifuge. The weight-average molecular weight of native SdeAgu115A was determined to be 189 ± 3.9 kD.