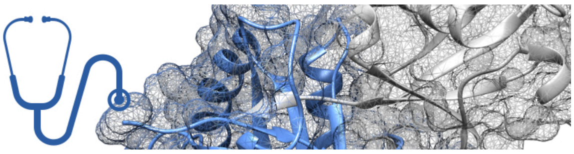
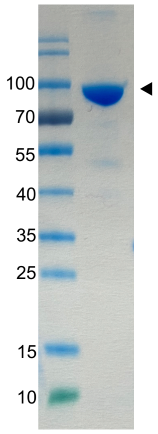
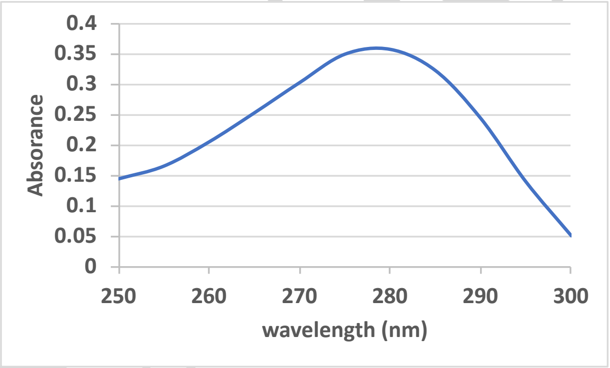
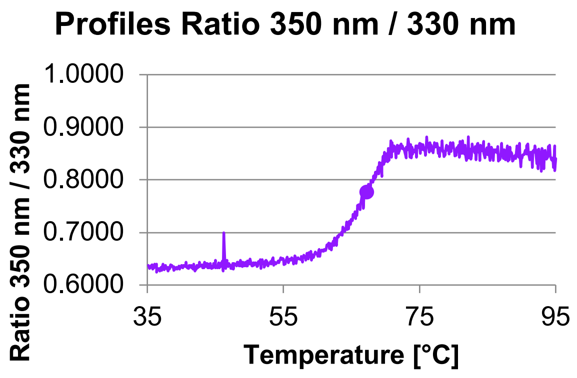
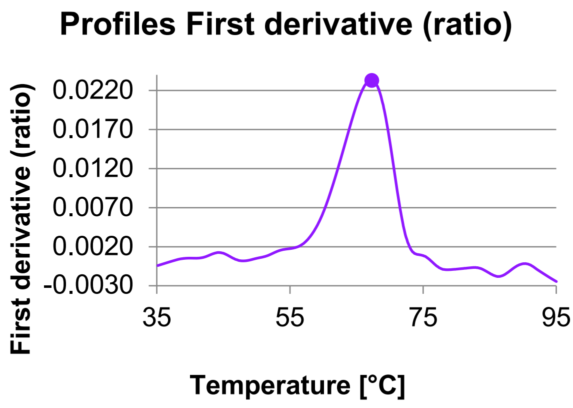


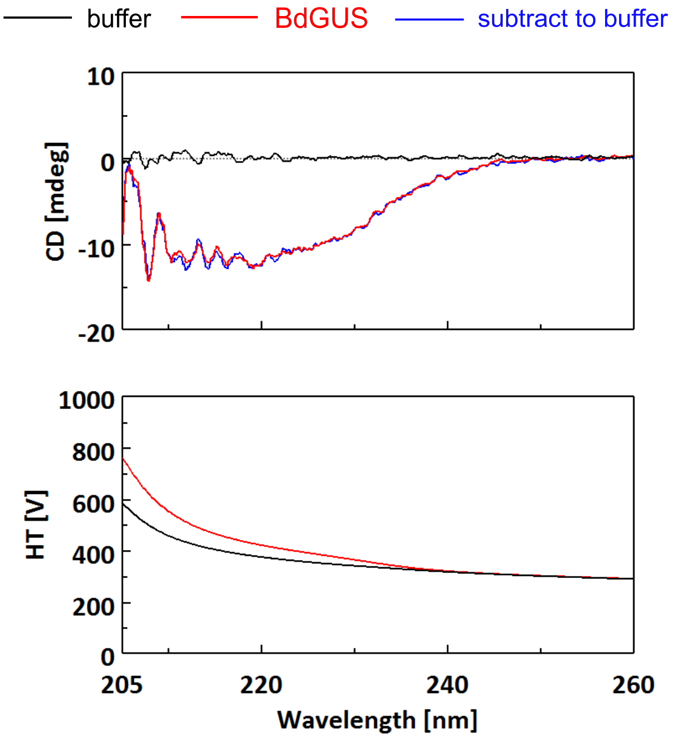
Academia Sinica Protein Clinic Report

Date	2020.01.06
Case number	20200106_test_1
Applicant	Hsien-Ya Lin
Principle investigator	Chun-Hung Hans Lin
Affiliation	Academia Sinica
Institute/department	Institute of Biological Chemistry
ASPC project manager	☒ Hsien-Ya Lin ☐ Yun-Ru Chen
Number of sample(s)	1
Sample 1	
Sample name	*Bifidobacterium dentium* β-glucuronidase
Number of amino acids	692
Molecular weight (Da)	77065
Theoretical pI	4.99
Extinction coefficient @280 nm (M^{-1} cm^{-1})	119180
Buffer condition	150mM NaCl, 50mM Tris-HCl (pH 8.0), 0.02% NaN_3
Package option	☒ Deluxe ☐ Basic ☐ SEC-MALS only
Service items	☒ CF-01 SDS-PAGE
	☒ CF-02 UV-Vis scan & 260/280
	☒ CF-03 Tycho NT. 6
	☒ CF-04 Far-UV CD spectrum
	☒ CF-05 DSF Tm screen
	☒ CF-06 AUC (SV mode)
	☐ CF-07 SEC
	☒ CF-08 SEC-MALS
Supporting information	☒ Tycho NT. 6 raw data
	☒ Far-UV CD raw data
	☒ DSF screen condition details
	☒ AUC raw data
	☒ SEC-MALS Wyatt report

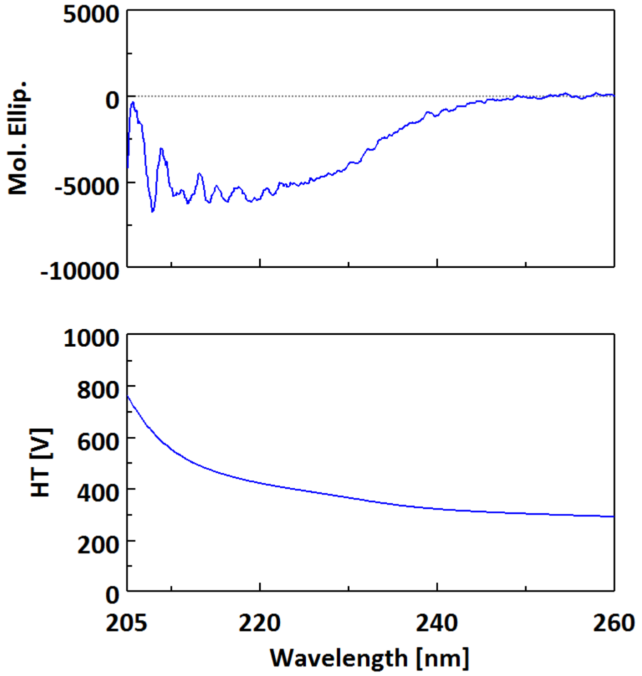
Disclaimer: The content is for research purpose only. Do not use this report for commercial purpose. ASPC only performs the analyses. ASPC is not responsible for the sample preparation.

CF-01 SDS-PAGE		**CF-02 UV-Vis scan and 260/280 ratio**	
SDS-PAGE	4-20% gradient	Instrument	Beckman DU® 730
Protein marker	Thermo Scientific PageRuler™ Prestained Protein Ladder, 10 to 180 kDa	Sample concentration (µM)	3
Protein sample Protein:4x loading dye=3.1	5 µL	OD_{260}/OD_{280} *Ration < 0.7: no nucleic acid contamination	0.57
			
CF-03 Tycho NT.6			
The system measures the fluorescence of intrinsic tryptophan and tyrosine residues detected at both 350 nm and 330 nm as a 30°C/min. temperature ramp is applied from 35–95°C.			
Profiles Ratio 350 nm / 330 nm		Profiles First derivative (ratio)	
		Inflection point (ºC)	**67.2**

CF-04 Far-UV CD spectrum	
Data collection parameters:	
Digital integration time: (sec)	1
Data interval (nm)	0.5
Measure range (nm)	200-260
Sample temperature (ºC)	20



CD signal expressed in molar ellipticity (ME)



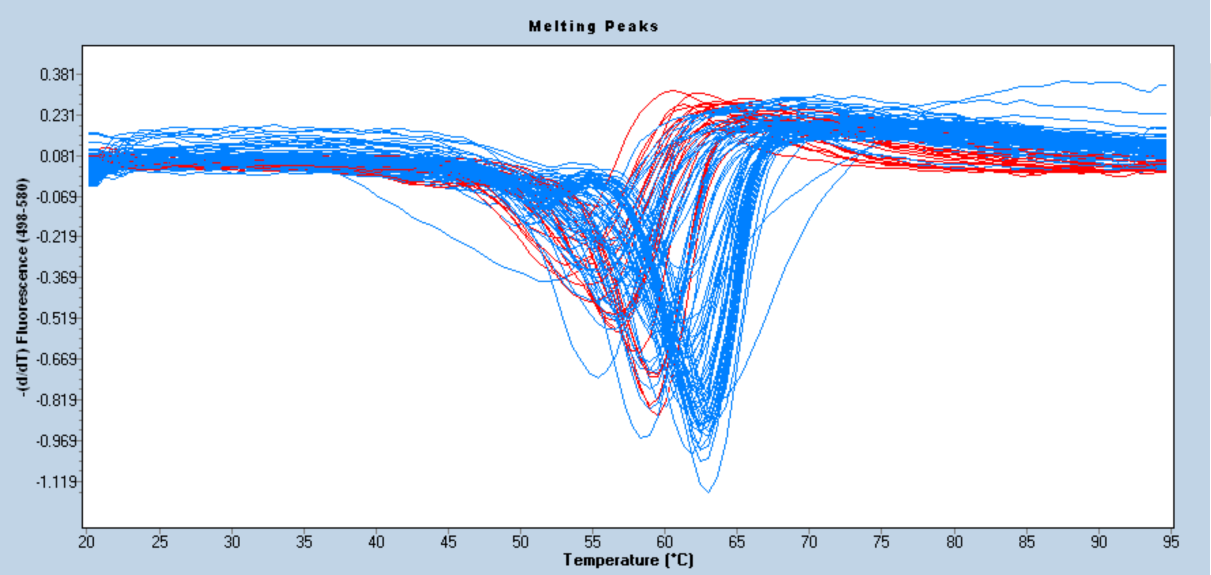
Note: The chloride ion content in the sample buffer (Tris-HCl) limits reliable data analysis in the short wavelength range (195-200 nm) due to the high dynode voltage

CF-05 DSF T_m screen

Data collection parameters:

Temp. (°C)	Cycles	Hold (sec.)	Ramp Rate (°C/sec.)	Acquisitions (per °C)
20	1	15	4.40	
95	1		0.02	30
20	1	15	2.20	

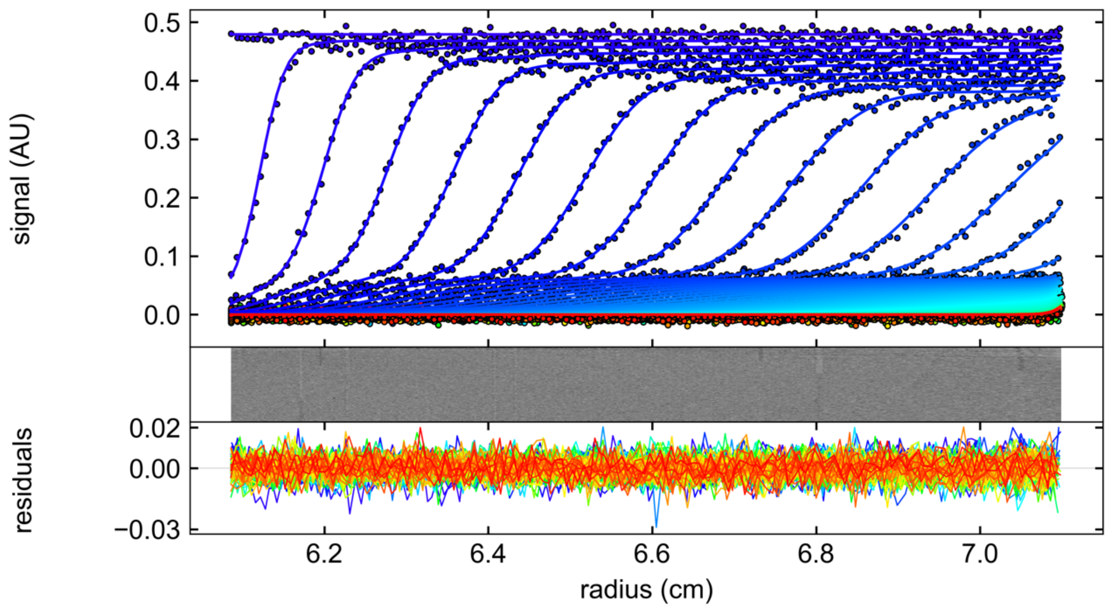
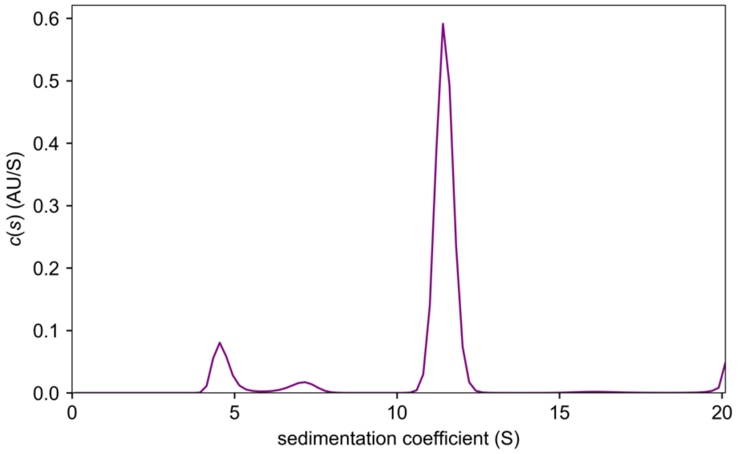
First derivative of the raw thermograms

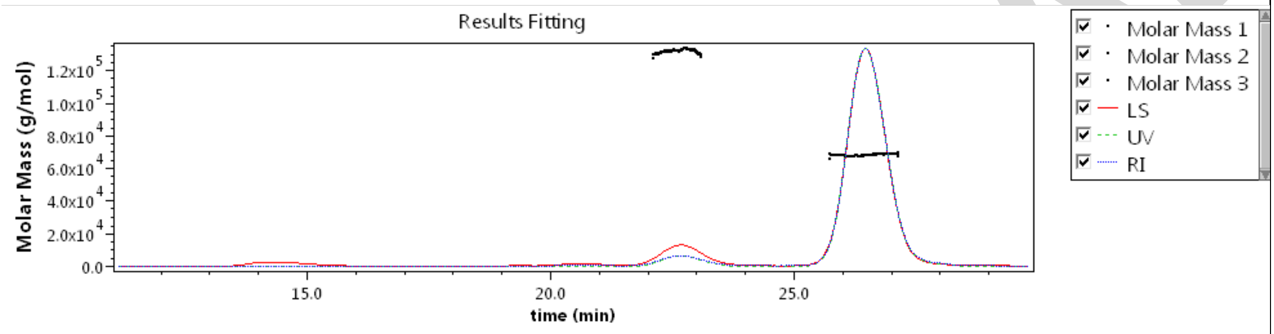


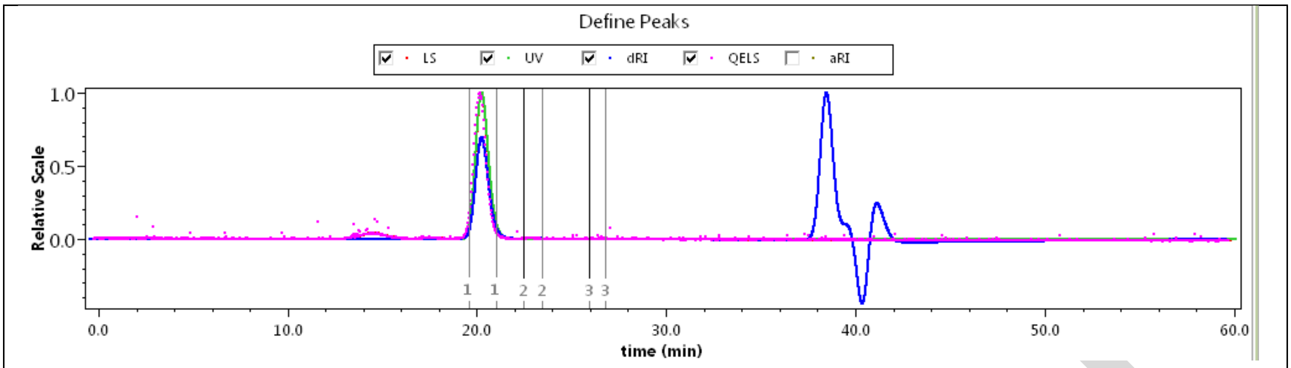
Melting temperatures of individual screening conditions

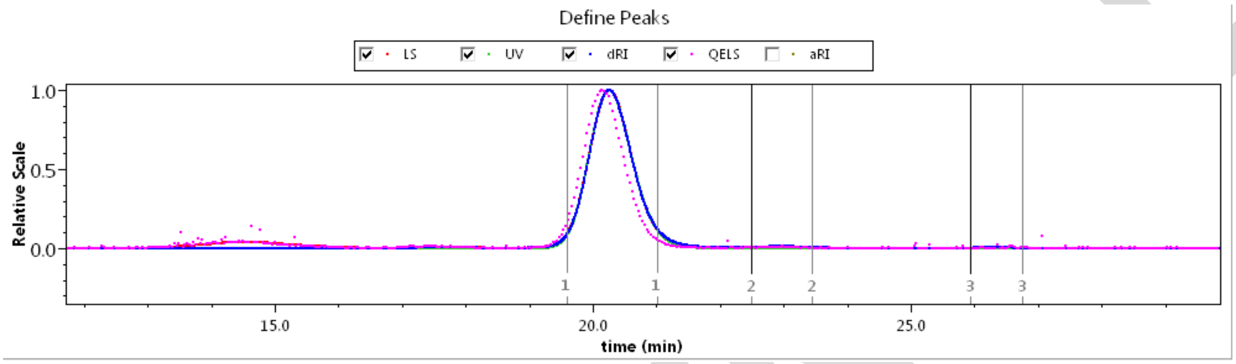
	pH		4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5
Salt		1	2	3	4	5	6	7	8	9	10	11	12
0	A	58.91	63.61	62.43	63.02	63.61	63.02	60.08	57.15	56.56	55.38	56.56	55.38
0.05M	B	59.50	63.02	62.43	63.02	63.02	62.43	59.50	57.15	56.56	55.38	58.91	55.38
0.1 M	C	60.67	61.84	62.43	62.43	63.02	62.43	58.91	56.56	56.56	54.21	53.04	55.38
0.15 M	D	59.50	61.84	62.43	62.43	62.43	61.84	58.91	56.56	55.97	54.80	53.04	55.38
0.2 M	E	59.50	61.26	62.43	62.43	62.43	58.32	55.38	57.15	58.32	55.38	53.04	54.80
0.25 M	F	51.27	61.26	62.43	62.43	62.43	61.26	58.91	57.15	56.56	55.38	53.04	54.80
0.5 M	G	58.32	60.08	62.43	61.84	61.26	61.26	58.91	58.32	57.15	55.97	53.62	54.21
1.0 M	H	61.26	61.26	61.84	61.26	61.26	61.26	59.50	59.50	58.32	56.56	57.15	54.21

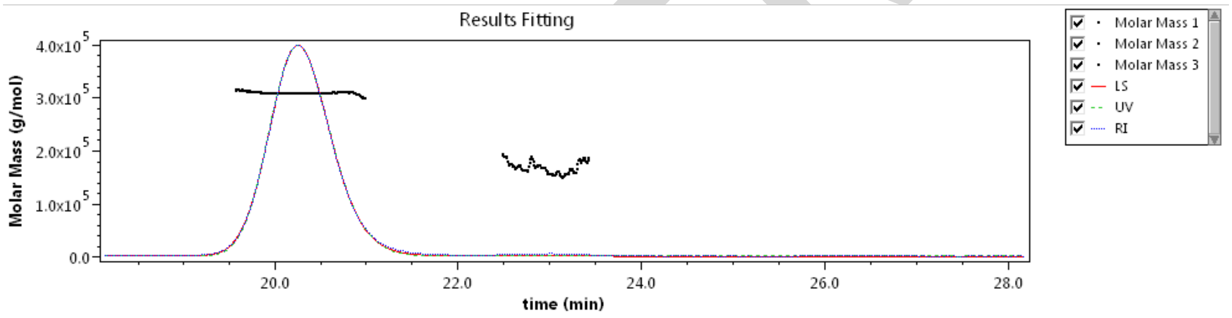
Note: pH 4.5-6.5, salt concentration 0-1M is the recommended buffer condition

CF-06 AUC SV mode	
Data collection parameters:	
Sample buffer	150mM NaCl, 50mM Tris pH8.0, 0.02% NaN$_3$
Detection mode	UV absorption at 280nm
Rotation speed (rpm)	40 000
Duration (hour)	5
Sample temperature (°C)	20
Buffer density (g/ml)*	1.0367
Buffer viscosity (η)*	0.0105373
* calculated by Sednterp based on the buffer composition	
Raw data fitting	
	
SV distribution	
	
Molecular weight estimate (kDa)	84.5 (monomer), 150 (dimer) and 391 (tetramer)

CF-07 SEC-MALS	
Data collection parameters:	
Sample buffer	150mM NaCl, 50mM Tris pH8.0, 0.02% NaN_3
SEC column	GE 10/300 Superdex 200 increase
Flow rate	0.5 ml/min
Sample temperature (°C)	ambient
Control sample (BSA)	1.0 mg/ml
Calculated MW of BSA (kDa) Theoretical value 66.5 kDa	131.9 ± 0.82 (dimer) 68.09 ± 0.32 (monomer)
	
Sample 1	1.0 mg/ml
Calculated MW (kDa)	(peak 1) 308.5 ± 0.10 (tetramer) (peak 2) 167.6 ± 1.43 (dimer) (peak 3) 82.13 ± 10.84 (monomer)
Polydispersity	(peak 1) Mw/Mn: 1.000 (± 0.529%), Mz/Mn: 1.000 (± 0.917%) (peak 2) Mw/Mn: 1.009 (± 15.108%), Mz/Mn: 1.019 (± 27.180%) (peak 3) Mw/Mn: 1.865 (± 92.616%), Mz/Mn: 3.163 (± 175.173%)







Note: Buffer mismatch is very significant. Running buffer and sample buffer are not well-matched

Hydrodynamic radius (nm) derived from dynamic light scattering (DLS)	5.889 (± 0.343%)