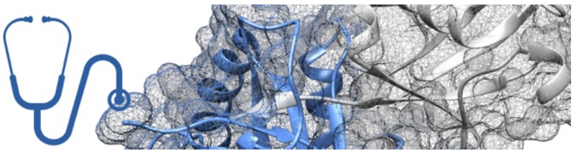
整合型蛋白質結構與功能分析核心設施
ASPC Academia Sinica Protein Clinic



Academia Sinica Protein Clinic Report

Date	2020.01.06
Case number	20200106_test_2
Applicant	Yun-Ru Chen
Principle investigator	Shang-Te Danny Hsu
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	BAP1
Number of amino acids	238
Molecular weight (Da)	26949.72
Theoretical pI	5.63
Extinction coefficient @280 nm (M^{-1} cm^{-1})	30940
Buffer condition	PBS pH7.4, 10% glycerol, 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

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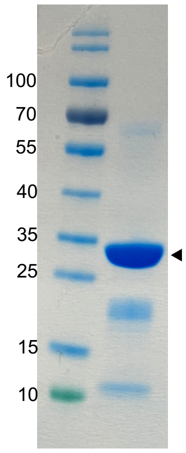
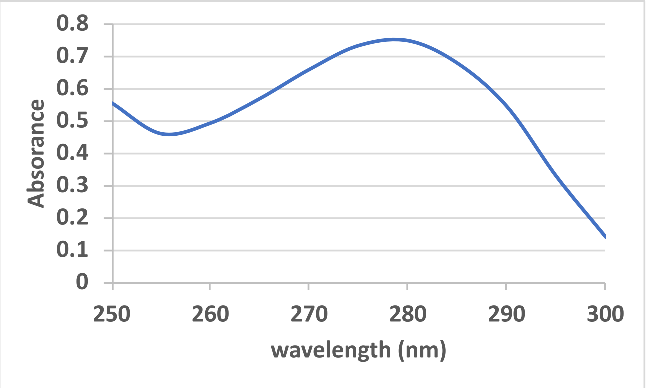
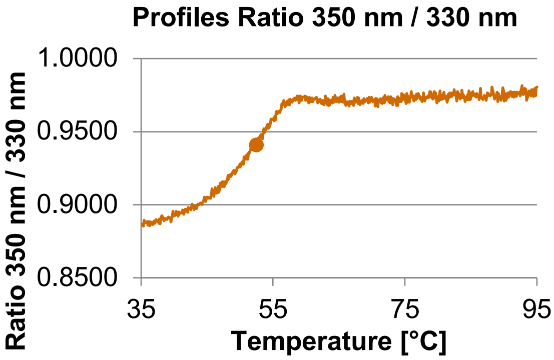
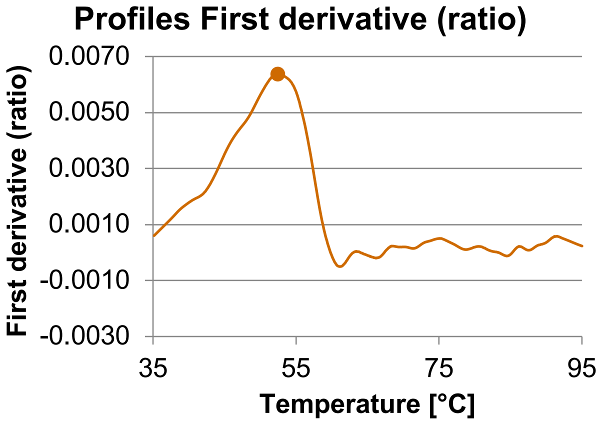
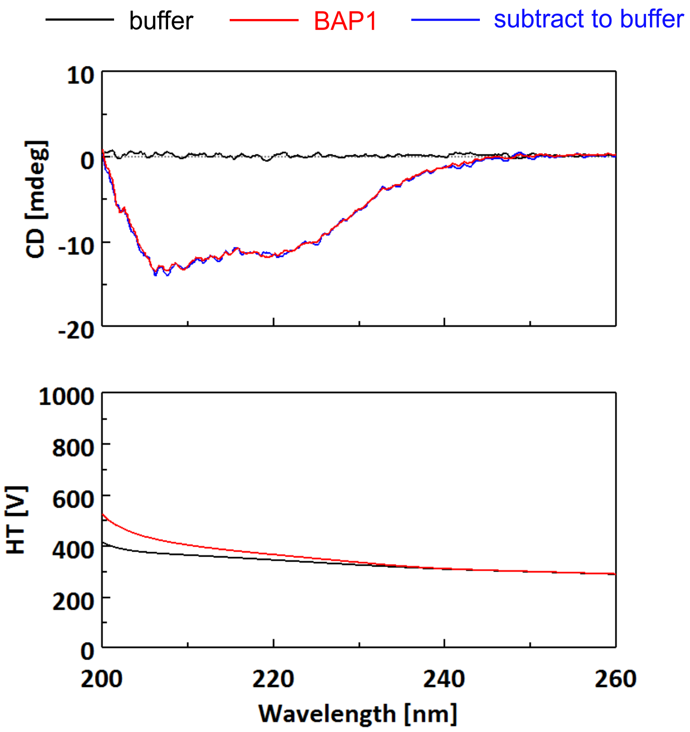
CF-01 SDS-PAGE		**CF-02 UV-Vis scan and 260/280 ratio**	
SDS-PAGE	15% gradient	Instrument	Beckman DU® 730
Protein marker	Thermo Scientific PageRuler™ Prestained Protein Ladder, 10 to 180 kDa	Sample concentration (μM)	24.14
Protein sample Protein:4x loading dye=3.1	5 μL	OD_{260}/OD_{280} *Ration < 0.7: no nucleic acid contamination	0.66
			
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.			
			
		Inflection point (ºC)	**52.4**

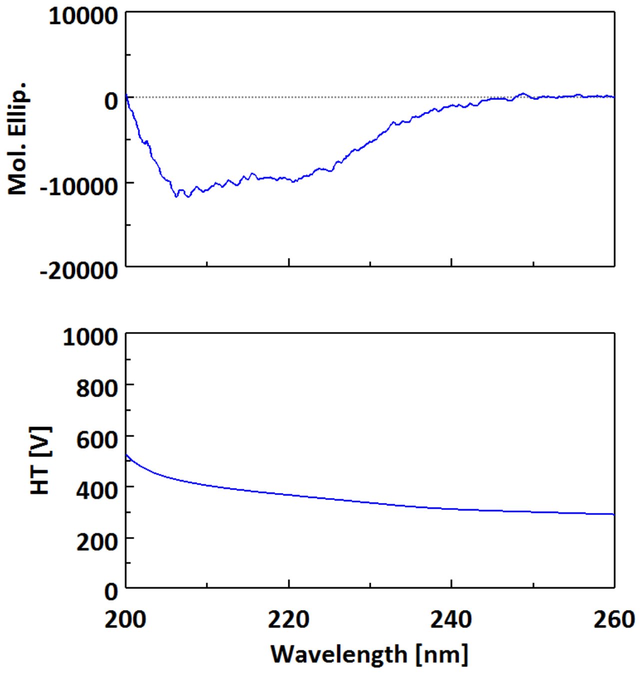
CF-04 Far-UV CD spectrum

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)



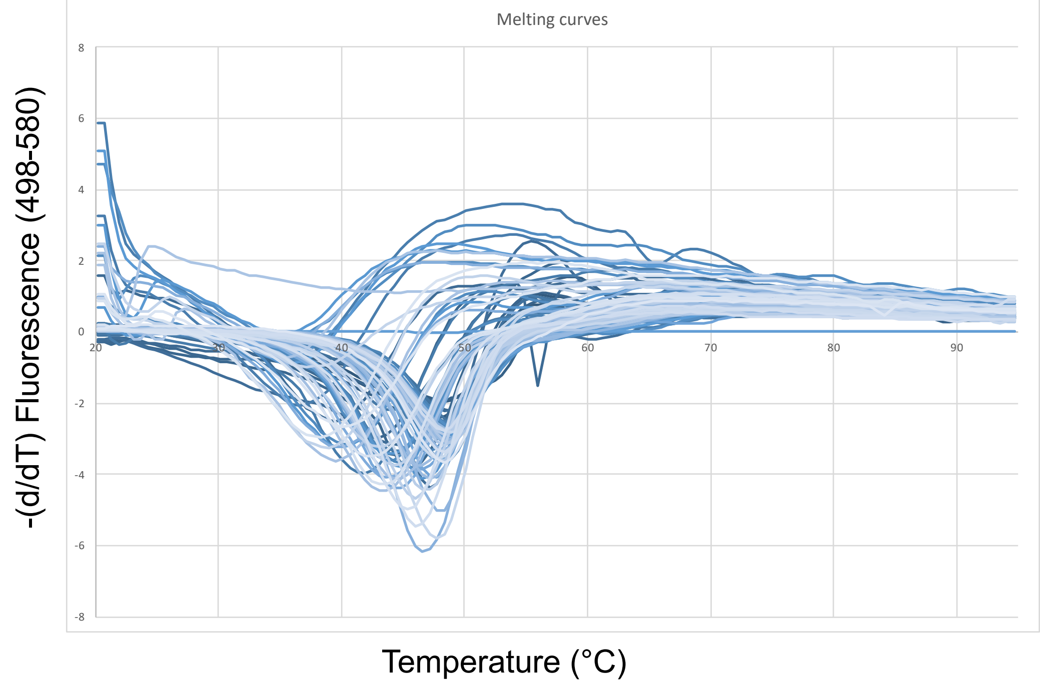
Note:

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	

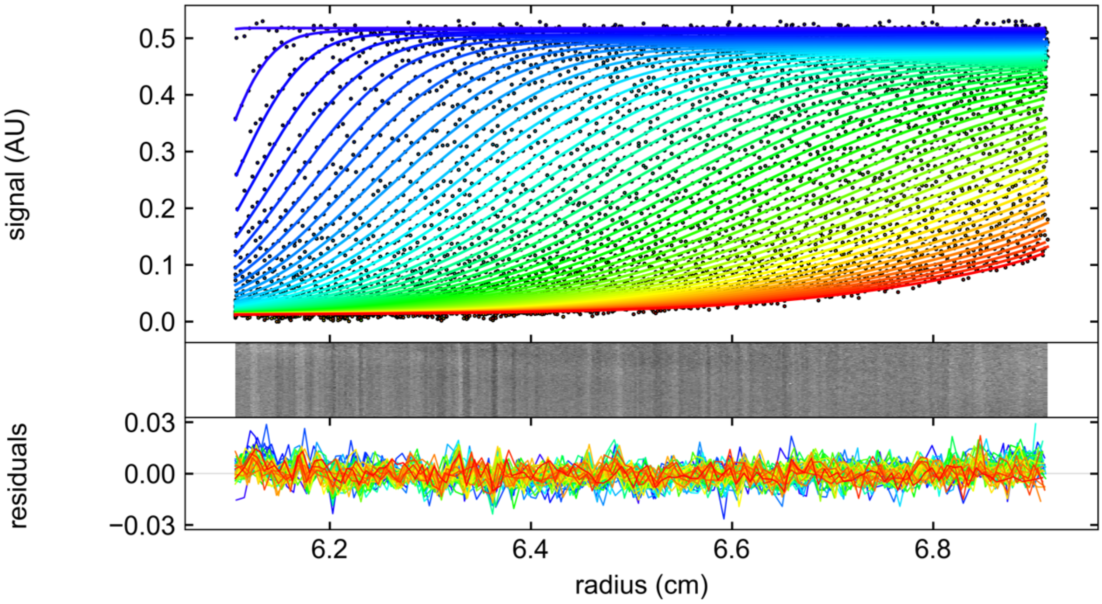
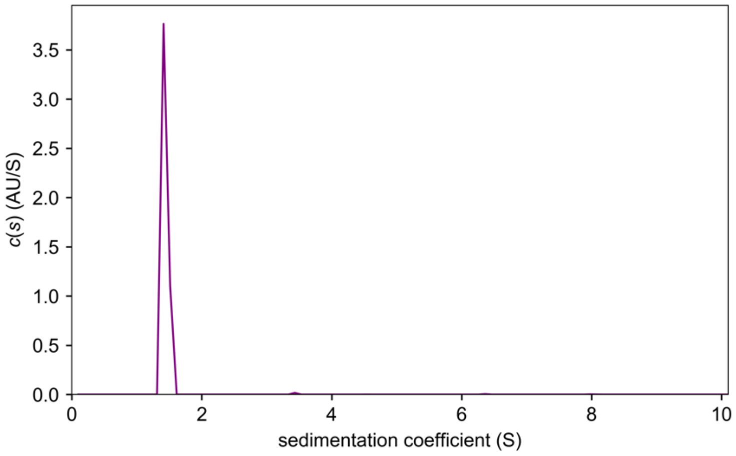
First derivative of the raw thermograms



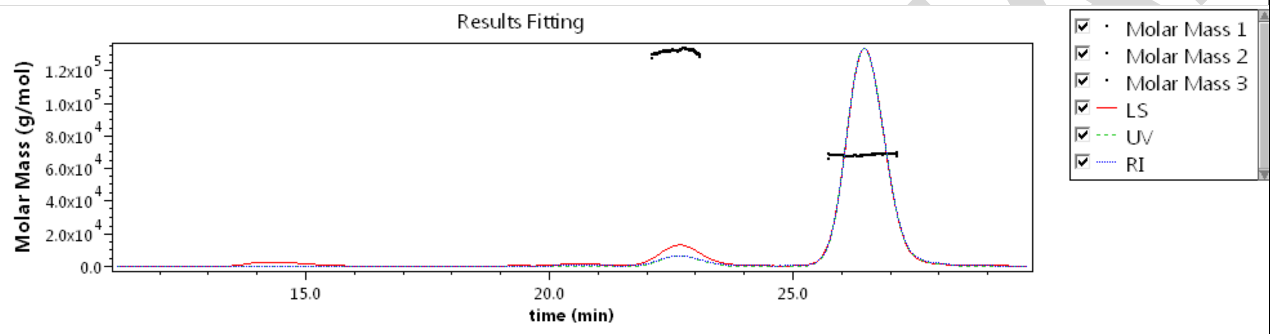
Melting temperatures of individual screening conditions

Salt	pH		4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5
		1	2	3	4	5	6	7	8	9	10	11	12
0	A	48.35	40.12	38.36	42.47	43.65	45.41	46.00	48.35	48.35	47.76	48.35	48.94
0.05M	B	45.41	37.77	42.47	44.24	41.30	46.59	46.00	47.17	46.00	46.59	46.00	46.59
0.1 M	C	*ND*	35.42	39.54	43.06	44.24	46.59	47.17	48.35	48.35	48.35	47.76	48.94
0.15 M	D	46.59	35.42	39.54	43.65	44.24	46.59	47.76	48.35	48.35	48.35	46.59	48.94
0.2 M	E	35.42	35.42	40.12	44.24	44.82	46.59	47.76	48.94	48.94	48.35	48.94	48.94
0.25 M	F	36.01	35.42	40.71	43.65	44.82	47.17	48.35	48.94	48.94	48.35	48.94	48.35
0.5 M	G	36.01	36.60	41.89	44.24	44.24	47.17	48.94	48.35	48.94	48.94	48.35	48.35
1.0 M	H	47.17	39.54	44.24	46.00	43.06	48.35	47.76	48.94	47.76	48.35	47.76	48.35

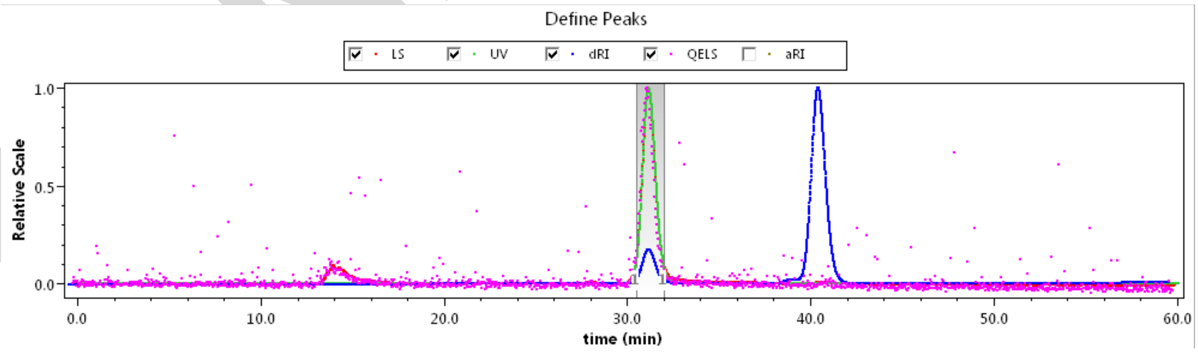
Note: pH 7.0-9.5, salt concentration 0-1M is the recommended buffer condition
ND indicates not determined

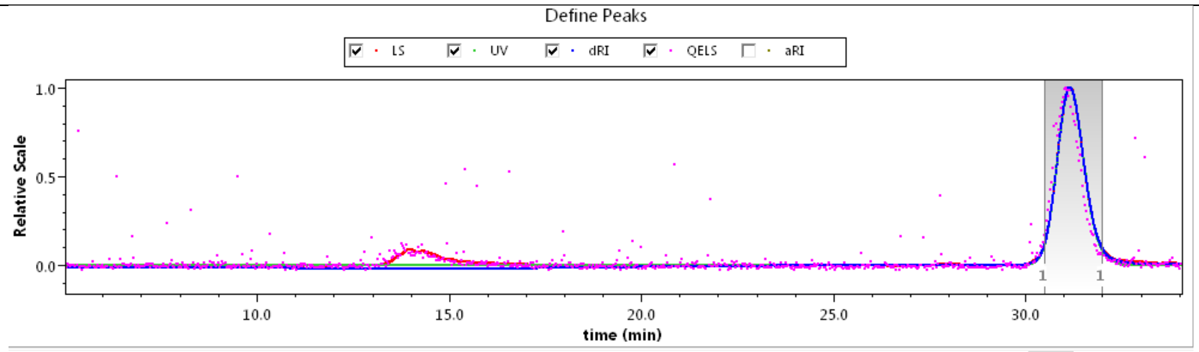
CF-06 AUC SV mode	
Data collection parameters:	
Sample buffer	PBS pH7.4, 10% glycerol, 0.02% NaN_3
Detection mode	UV absorption at 280nm
Rotation speed (rpm)	40 000
Duration (hour)	5
Sample temperature (°C)	20
Buffer density (g/ml)*	1.034
Buffer viscosity (η)*	0.0138995
* calculated by Sednterp based on the buffer composition	
Raw data fitting	
	
SV distribution	
	
Molecular weight estimate (kDa)	26.6 (monomer)

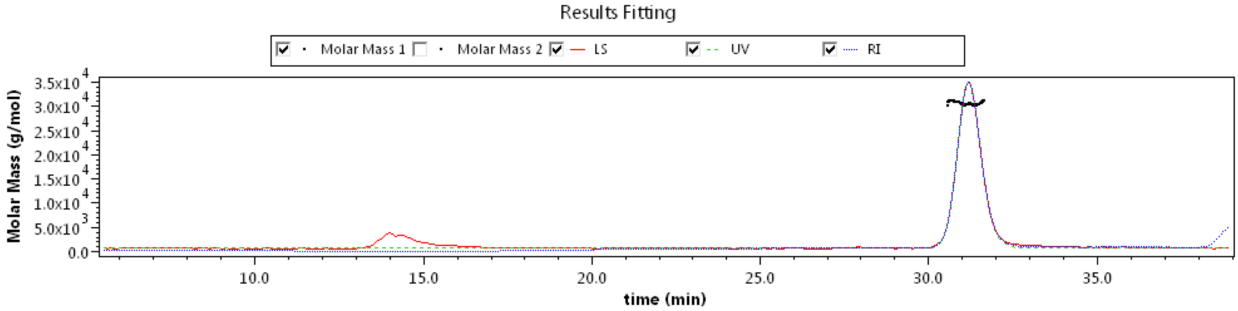
CF-07 SEC-MALS	
Data collection parameters:	
Sample buffer	PBS pH7.4, 10% glycerol, 0.02% NaN_3
SEC column	GE 10/300 Superdex 200 increase
Flow rate	0.5 ml/min
Sample temperature (°C)	ambient
Control sample (BSA)	1.0 mg/ml
Calculated MW of BSA (kDa) Theoretical value 66.5 kDa	131.9 ± 0.82 (dimer) 68.09 ± 0.32 (monomer)



Sample 1	1.0 mg/ml
Calculated MW (kDa)	30.42 ± 0.023 (monomer)
Polydispersity	Mw/Mn: 1.000 (± 1.097%), Mz/Mn: 1.000 (± 1.901%)







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