JOURNAL OF APPLIED CRYSTALLOGRAPHY

Volume 51 (2018)

Supporting information for article:

An Optimized SEC-SAXS System Enabling High X-ray Dose for Rapid SAXS Assessment with Correlated UV Measurements for Biomolecular Structure Analysis

Timothy M. Ryan, Jill Trewhella, James M. Murphy, Jeremy R. Keown, Lachlan Casey, F. Grant Pearce, David C. Goldstone, Kelan Chen, Zhenyao Luo, Bostjan Kobe, Christopher A. McDevitt, Serena A. Watkin, Adrian M. Hawley, Stephen T. Mudie, Vesna Samardzic-Boban and Nigel Kirby

Table S1 SAXS data collection and analysis parameters

Data Collection Parameters																					
Protein sample	Glucose Isomerase	Glucose Isomerase	Thyroglobulin	Aldolase	Ovalbumin	Ovalbumin		Ribonuclease A	Aprotinin	Smchd1 WT (reported Chen et al, 2016)	Smchd1 S135C	ZnuA	ZnuA	DHDPS *E.coli*	DHDPS *T maritima*	DHDPS *A. thaliana*	TRIMCypA				
Instrument	Australian Synchrotron SAXS/WAXS beamline																				
Beam geometry	120 μm point source																				
Wavelength (Å)	1.033																				
q-range ($Å^{-1}$)	0.01 – 0.63	0.01 – 0.63	0.01 – 0.63	0.01 – 0.63	0.01 – 0.63	0.01 – 0.63		0.01 – 0.63	0.01 – 0.63	0.0076 – 0.3	0.0076 - 0.3	0.01 – 0.63	0.01 - 0.63	0.01 – 0.63	0.01 – 0.63	0.01 – 0.63	0.0058 – 0.34				
exposure time (s)	2	1	1	1	30	1		1	1	2	2	2	1	1	1	1	1				
Temperature (°C)	15	15	4	4	20	4		4	4	16	16	16	16	15	15	15	15				
Collection Mode	Conventional SEC	Coflow SEC	Coflow SEC	Coflow SEC	Conventional Static	Coflow SEC		Coflow SEC	Coflow SEC	Conventional SEC	Coflow SEC	Conventional SEC	Coflow SEC	Coflow SEC	Coflow SEC	Coflow SEC	Coflow SEC				
Column size and media	10 x 300 S200	5 x 150 S200 Increase	5 x 150 S200 Increase	5 x 150 S200 Increase	-n/a	5 x 150 S200 Increase		5 x 150 S200 Increase	5 x 150 S200 Increase	5 x 150 S200	5 x 150 S200 Increase	WTC-30S5	5 x 150 S200 Increase	5 x 150 S200 Increase	5 x 150 S200 Increase	5 x 150 S200 Increase	5 x 150 S200 Increase				
Buffer [a]	1	1	1	1	1	1		1	1	2	2	3	3	4	4	4	5				
Concentration (mg/ml)[b]	2.2	2.2	3	3	2	2 (12% dimer)			3	5	5	15	11	10	4	4	8				
Dilution from peak concentration (fold)	13.0	2.0	1.9	2.2	- N/A	Monomer: 4 Dimer: 3.4		2.4	3.8	13.0	9.3	9.4	5.0	2.0	4.0	3.6	-n.d.[c]				
Structural Parameters																					
Peaks						1	2										1	2[d]	2a	2b	3
Guiner I(0) (cm^{-1}) [std.err]	0.025 [0.0002]	0.19 [0.0001]	0.801 [0.0003]	0.16 [0.0002]	0.034 [0.0002]	0.004 [0.0007]	0.014 [0.0003]	0.013 [0.0002]	0.0036 [0.0004]	0.0164 [0.0001]	0.0225 [0.0001]	0.04 [0.0003]	0.043 [0.0002]	0.33 [0.0001]	0.074 [0.0002]	0.11 [0.0001]	0.002 [0.0008]	0.034 [0.0002]	0.036 [0.0001]	0.03 [0.0001]	0.005 [0.0004]
Guinier Rg (Å) [std.err]	32.9 [0.5]	33.1 [0.2]	60.35 [0.3]	34.9 [0.4]	25.6 [0.3]	35.5 [0.4]	23.6 [0.2]	15.2 [0.3]	10.6 [0.6]	31.6 [0.2]	31.5 [0.3]	30.2 [0.61]	22.6 [0.4]	37.8 [0.3]	36.1 [0.3]	36.9 [0.5]	67.1 [0.3]	55.2 [0.46]	56.1 [0.4]	53.9 [0.2]	41.2 [0.5]
P(r) I(0) (cm^{-1})	-n.d.	0.192 [0.0001]	0.79 [0.0001]	0.12 [0.0003]	0.035 [0.0002]	0.0039 [0.0004]	0.015 [0.0002]	0.034 [0.0002]	0.0019 [0.0001]	0.0165 [0.0001]	0.0226 [0.0001]	-n.d.	-n.d.	0.311 [0.0004]	0.101 [0.0003]	0.085 [0.0005]	0.002 [0.0005]	0.036 [0.0003]	0.037 [0.0004]	0.0305 [0.0002]	0.005 [0.0005]
P(r) Rg (Å)	-n.d.	33.2 [0.15]	61.5 [0.4]	36.2 [0.3]	26.4 [0.2]	35.1 [0.4]	23.9 [0.3]	14.9 [0.3]	11.2 [0.3]	32.3 [0.2]	32.5 [0.4]	-n.d.	-n.d.	37.4 [0.2]	36.0 [0.3]	36.5 [0.1]	68.6 [0.4]	57.3 [0.5]	58.1 [0.2]	54.2 [0.2]	43.6 [0.8]
Dmax (Å)	-n.d.	-n.d.	320.1	111.2	93.5	104.5	71.4	44.5	34	105	105	-n.d.	-n.d.	120.7	113.8	116.2	278.1	225.3	237.6	196.2	163.3
Crysol fitting results pdb ID (chi2)	-n.d	-n.d	-not available	5ald (1.103)	1ova (0.851)	1ova (0.995)	1ova[e] (1.190)	1fs3 (1.004)	5pti (1.110)	2cgn[f] (1.173)	2cgn[f] (1.191)	-n.d.	-n.d.	1yxc[g] (4.412)	1o5k[g] (2.011)	4dpp[g] (1.851)	-n.d	-n.d	-n.d	-n.d	-n.d
Normal mode chi2 of refined model	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	-n.d	1.512	1.051	1.021	-n.d	-n.d	-n.d	-n.d	-n.d

Software	
Primary data reduction:	ScatterBrain Version 2.82
Data processing:	ATSAS2.7 (Primus, DATGNOM, DATPOROD, DATCMP), Sigmaplot 13
Ab initio modelling:	ATSAS2.7 (DAMMIF, SREFLEX)
Computation of model intensities:	ATSAS2.7 (CRYSOL1)
3D representation:	Pymol
HPLC deconvolution:	USC SOMO HPLC
Protein parameter calculations:	MuLcH, ProtParam

[a] buffers: 1 - 46 mM sodium phosphate, 5 mM potassium phosphate, 128 mM NaCl, 22 mM KCl, 5% (v/v) glycerol, pH 7.5 (PBS).

2 - 200 mM NaCl, 20 mM HEPES, 5 % (v/v) glycerol, pH 7.5.

3 - 150 mM NaCl, 10 mM HEPES, 1.5% (v/v) glycerol 1% (wt./v) sodium azide, pH 7.5.

4 - 20 mM Tris.HCl, 150 mM NaCl, 5% (v/v) glycerol, pH 8.0.

5 - 20 mM Tris-HCl, 300 mM NaCl, 0.5 mM TCEP, 20 mM imidazole, and 10 % (v/v) glycerol, pH 7.8.

[b] Concentration calculated from 280 nm absorbance and extinction coefficeint calculated from sequence using ProtParam.

[c] – n./d. & -N/A indicate not determined and not applicable, respectively.

[d] 2 refers to the main peak, undeconvoluted.

[e] ovalbumin dimer generated using pymol.

[f] HSP90 monomer generated from this pdb structure using pymol

[g] DHDPS tetramers were generated from these dimeric structures using symmetry

Figure S1 Guinier plots for thyroglobulin (A), aldolase (B) , ribonuclease A (C), and aprotinin (D)

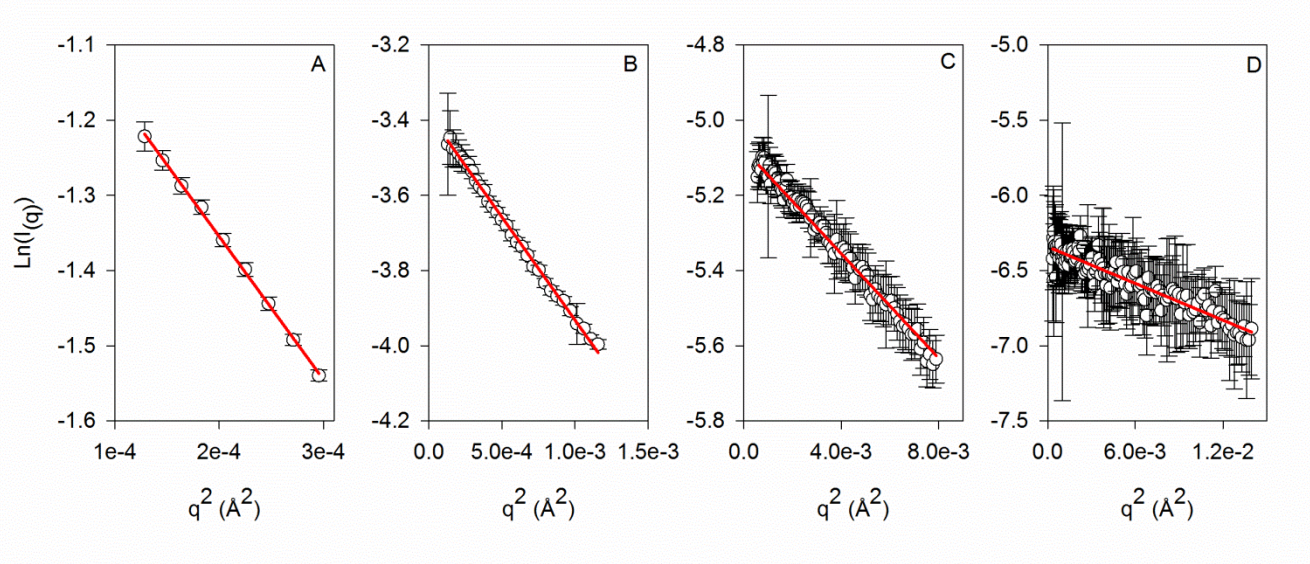

Figure S2 P(r)'s for thyroglobulin (A), aldolase (B) , ovalbumin monomer (C), ribonuclease A (D), and aprotinin (E)

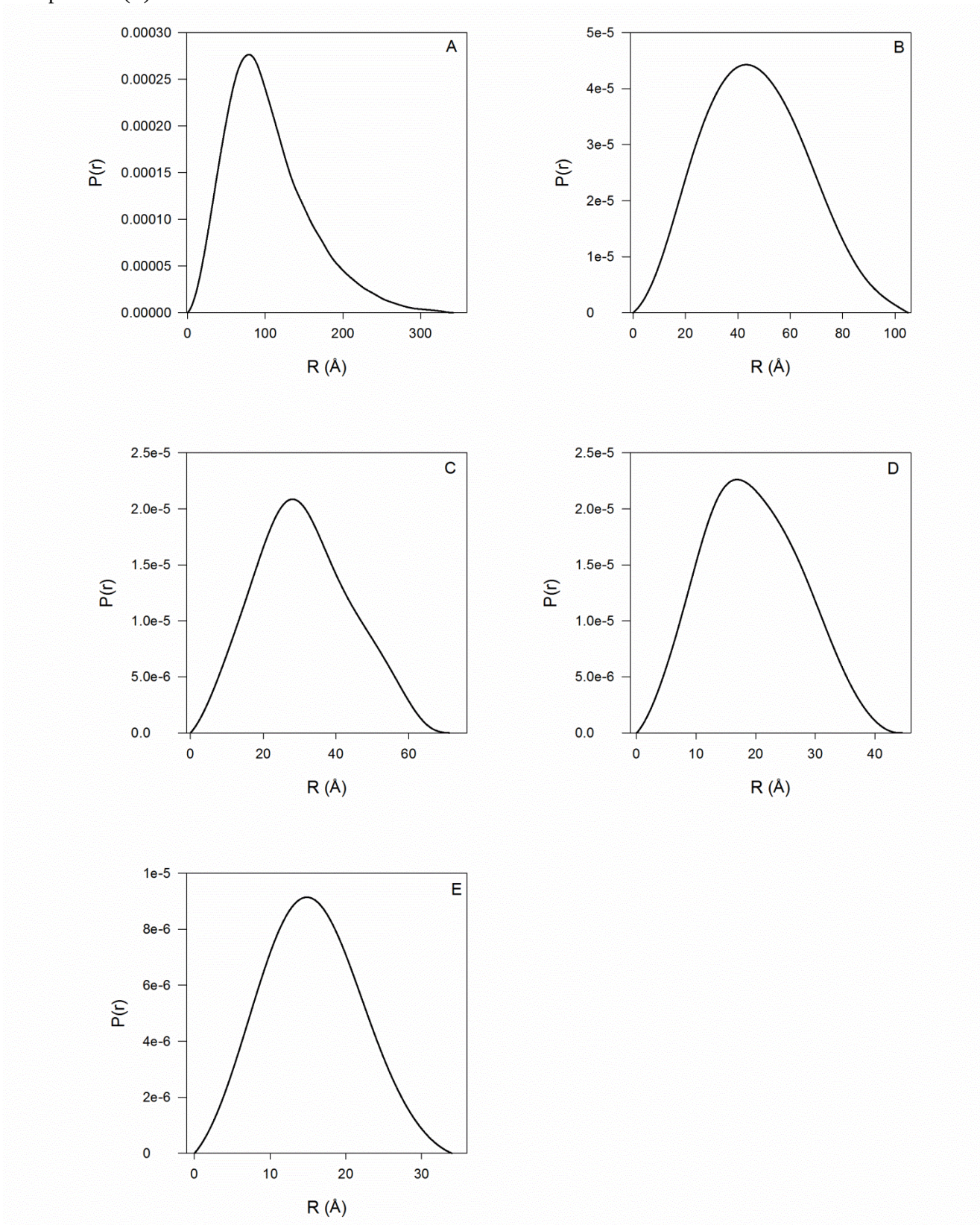

Figure S3 Kratky plots for thyroglobulin (A), aldolase (B) , ovalbumin monomer (C), ribonuclease A (D), and aprotinin (E).

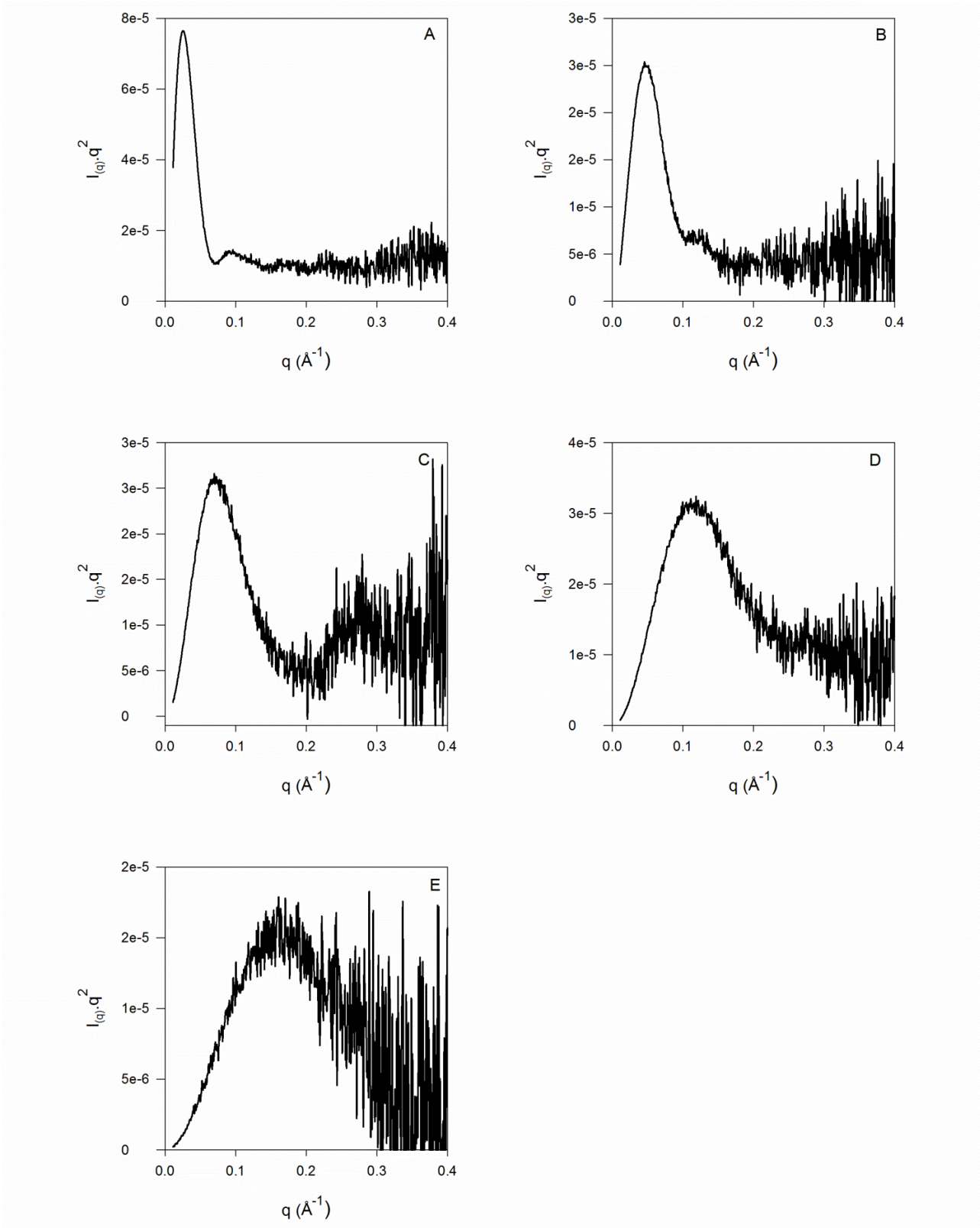

Figure S4 Elution profile of the MW stds on an Agilent Advance Biosec 300 angstrom 5 x 300 Sec column.

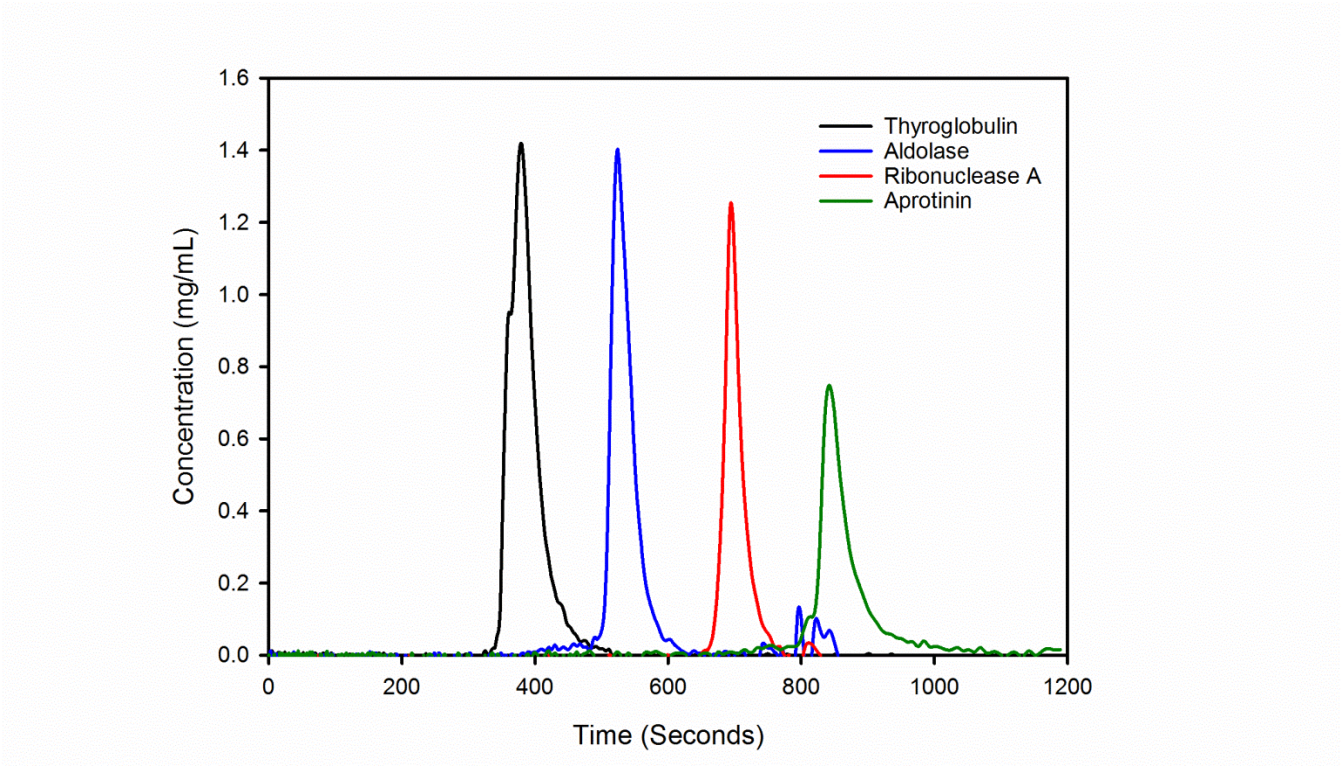

Figure S5 Guinier plots for the ovalbumin dimer (A), and monomer (B) datastets