Size-exclusion chromatography small-angle X-ray scattering of water soluble proteins on a laboratory instrument

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Size-exclusion chromatography small-angle X-ray scattering (SEC-SAXS) of water soluble proteins on a laboratory instrument Supporting Information

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Table S1: Details of proteins, SAXS experiments and analysis.

protein ^a				BS	SA				BSA dimer	RNase A	CAH	HI	OVA	OVA dimer	CA	HA	AF
Samples																	
PDB ID				4f5s	5 [1]				4f5s	9rat [2]	1v9e [3]	1ev3 [4]	1ova [5]		1aiv [6]	1ies	3 [7]
Source b				S	A				SA	GE	GE	NN C		GE	GE	S	A
M _w [kDa] d	66	66	66	66	66	66	66	66	133	14	29	35	43	86	76	476	476
ε _{280nm} [M ⁻¹ cm ⁻¹] e				438	324				n/a	9440	50420	5850	31775	n/a	88165	348	000
Buffer f	1	1	1	1	1	2	2	3	1	1	1	4	1	1	1	1	1
c [mg/ml] ^g	7.6	3.7	2.0	1.0	7.6	4.6	4.3	8.1	7.6	7.5	5.4	3.5	4.9	4.9	5.1	11.1	11.1
Volume [µl]	500	500	500	500	500	5	25	500	500	500	500	500	500	500	500	500	500
Amount of protein [mg]	3.8	1.9	1.0	0.5	3.8	0.023	0.11	4.1	3.8	3.8	2.7	1.8	2.5	2.5	2.6	5.6	5.6
Data collection																	
Instrument h	BX	BX	BX	BX	BX	BX	P12	BM29	BX	BX	BX	BX	BX	BX	BX	BX	BX
Detector 1	P300K	P300K	P300K	P300K	P300K	P300K	P2M	P1M	P300K	P300K	P300K	P300K	P300K	P300K	P300K	P300K	P300K
Wavelength [A]	1.34	1.34	1.34	1.34	1.34	1.34	1.24	0.99	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34
Flux [ph/s] J	$\sim 2.5e8$	$\sim 6 \mathrm{e7}^+$	$\sim 2.5 e8$	$\sim 2.5 e8$	$\sim 4.2 e7$	$\sim 2.5 e8$	$\sim 1e13$	$\sim 4 \mathrm{e} 11$	$\sim 2.5e8$	$\sim 2.5e8$	$\sim 2.5e8$	$\sim 2.5e8$	$\sim 2.5e8$	$\sim 2.5 e8$	$\sim 2.5 e8$	$\sim 2.5e8$	$\sim 4.2 e7$
d [mm] K	654	654	654	654	1507	654	3000	2867	654	654	654	654	654	654	654	654	1507
q_{min} [Å ⁻¹] ¹	0.011	0.011	0.011	0.011	0.0075	0.011	0.0023	0.0054	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.0075
qmax [A ⁻¹] III	0.50	0.50	0.50	0.50	0.22	0.45	0.51	0.48	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.22
Temperature [°C]	25	25	25	25	25	25	20	10	25	25	25	25	25	25	25	25	25
Collection mode	SEC	SEC	SEC	SEC	SEC	static	static	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC
Column size & media	10×300	10×300	10×300	10×300	10×300	n/a	n/a	10×300	10×300	10×300	10×300	10×300	10×300	10×300	10×300	10×300	10×300
	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc			S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc	S200 Inc
Flow rate [ml/min]	0.1	0.1	0.1	0.1	0.1	n/a	n/a	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Data analysis																	
# of frames (protein)	12	10	10	10	13	n/a	n/a	40	9	11	10	13	11	10	10	11	11
# of frames (background)	28 + 12	25 + 20	19 + 5	19 + 10	15 + 20	n/a	n/a	200 + 100	15 + 20	40 + 20	20 + 10	50 + 6	20 + 20	20 + 20	20 + 20	20 + 10	19 + 10
Data reduction																	
Estimation of a m								RAW [8, 9], Python ATSAS 2.8) [10	1							
Estimation of q_{eff}							AUTORG	RAW [Shanum (. 11] (individual	8, 9], Python ATSAS 2.8) [10 frames) BAW] (averaged da	ata)						
Estimation of q_{eff} Guinier analysis IFT							AUTORG	RAW [Shanum (. 11] (individual BAYES	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13]] (averaged da	ata)						
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina	tion						AUTORG	RAW [Shanum (. 11] (individual BAYES SAX	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14])] (averaged da	ata)						
$\begin{array}{c} \text{Estimation of } q_{eff} \\ \text{Guinier analysis} \\ \text{IFT} \\ \text{Molecular weight determina} \\ Ab \ initio \ \text{modeling} \end{array}$	tion						AUTORG DAMMII	RAW [Shanum (. 11] (individual BAYES SAX: 5 (slow mode),	8, 9], Python ATSAS 2.8) [10 I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA	9] (averaged da S 2.8) [15, 16	ata) 3]						
Estimation of q _{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry	tion P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	AUTORG	RAW [Shanum (. 11] (individual BAYES SAX 7 (slow mode), P1/n.a.	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate) (averaged da (S 2.8) [15, 16 P1/n.a.	ata) 3] P1/n.a.	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.
Estimation of q _{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal stru Analysis of avertal structure	tion P1/n.a. cture intensi	P1/n.a. ities	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a. CR	AUTORG DAMMII P1/n.a. YSOL (ATS	RAW [Shanum (. 11] (individual BAYES SAX F (slow mode), P1/n.a. SAS 2.8) [17] w	8, 9], Python ATSAS 2.8) [10 Irames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate vith background o souting [19]) (averaged da S 2.8) [15, 16 P1/n.a. d correction e	ata) B] P1/n.a. nabled	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.
Estimation of q _{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal structure 3D representation	tion P1/n.a. cture intensi e intensities	P1/n.a. ities	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a. CR	AUTORG DAMMII P1/n.a. YSOL (ATS	RAW [Shanum (. 11] (individual BAYES SAX F (slow mode), P1/n.a. SAS 2.8) [17] w in-hous	8, 9], Python ATSAS 2.8) [10 Irames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate vith background e routine [18] PvMol] (averaged da S 2.8) [15, 16 P1/n.a. d correction e	ata) 5] P1/n.a. nabled	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.
Estimation of q _{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal structure 3D representation Results	tion P1/n.a. cture intensi e intensities	P1/n.a. ities	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a. CR	AUTORG DAMMIE P1/n.a. YSOL (ATS	RAW [Shanum (. 11] (individual BAYE: SAX 5 (slow mode), P1/n.a. 5AS 2.8) [17] w in-hous	8, 9], Python ATSAS 2.8) [1(frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate <i>i</i> th background e routine [18] PyMol	b] (averaged da .S 2.8) [15, 16 P1/n.a. correction e	ata) [] P1/n.a. nabled	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.
Estimation of q _{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak c [mg/ml] ⁿ	tion P1/n.a. cture intensi intensities 3.8	P1/n.a. ities	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a. CR	AUTORG DAMMII P1/n.a. YSOL (ATS	RAW [Shanum (. 11] (individual BAYES SAX: 5 (slow mode), P1/n.a. SAS 2.8) [17] w in-hous	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate vith background e routine [18] PyMol] (averaged da S 2.8) [15, 16 P1/n.a. d correction e	ata) B] P1/n.a. nabled 2.5	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak $c [mg/ml]^n$ Shanum $q, \sigma [A^{-1}]$	tion P1/n.a. cture intensi intensities 3.8 0.50	P1/n.a. ities 1.8 0.46	P1/n.a. 0.9 0.46	0.4 0.40	P1/n.a. 3.8 0.19	P1/n.a. CR n/a 0.33	AUTORG DAMMII P1/n.a. YSOL (ATS n/a 0.50	RAW [Shanum (. 11] (individual BAYE: SAX 7 (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate e routine [18] PyMol 	a) (averaged data (S 2.8) [15, 16 P1/n.a. 1 correction e 4.2 0.46	ata) 3] P1/n.a. nabled 2.5 0.46	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a. 2.6 0.50	P1/n.a.	P1/n.a. 3.7 0.19
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak $c \text{ [mg/m]}^n$ Shanum q_{eff} [Å ⁻¹] CRYSOL χ^2	tion P1/n.a. cture intensis i intensities 3.8 0.50 1.08	P1/n.a. ities 1.8 0.46 0.78	P1/n.a.	P1/n.a.	P1/n.a. 3.8 0.19 1.02	P1/n.a. CR n/a 0.33 1.03	AUTORG DAMMII P1/n.a. YSOL (ATS n/a 0.50 13.34	RAW [Shanum (. 11] (individual BAYE: SAX: ⁶ (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48 23.93	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate rith background e routine [18] PyMol n/a 0.50 1.19] (averaged da S 2.8) [15, 16 P1/n.a.] d correction e 4.2 0.46 1.17	ata) B] P1/n.a. nabled 2.5 0.46 0.72	P3/n.a.	P1/n.a. 2.0 0.43 0.75	P1/oblate 	P1/n.a. 2.6 0.50 1.20	P1/n.a. 3.7 0.49 6.50	P1/n.a. 3.7 0.19 1.61
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak c [mg/ml] ⁿ Shanum q_{eff} [Å ⁻¹] CRYSOL χ^2 Guinier 1(0)	tion P1/n.a. cture intensi i intensities 3.8 0.50 1.08 4.6e-1	P1/n.a. ities 1.8 0.46 0.78 6.8e-2	P1/n.a. 0.9 0.46 0.82 1.5e-1	P1/n.a. 0.4 0.40 0.80 6.9e-2	P1/n.a. 3.8 0.19 1.02 3.6e-3	P1/n.a. CR n/a 0.33 1.03 4.6e-2	AUTORG DAMMII P1/n.a. YSOL (ATS 	RAW [Shanum (. 111] (individual BAYE: SAX: 5 (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48 23.93 4.9	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate 7th background e routine [18] PyMol <u>n/a</u> 0.50 1.19 1.9e-1] (averaged da S 2.8) [15, 10 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1	ata) P1/n.a. nabled 2.5 0.46 0.72 1.6e-1	P3/n.a. n/a 0.49 1.21 5.3e-2	2.0 0.43 0.75 1.9e-1	P1/oblate n/a 0.43 n/a 7.0e-2	P1/n.a. 2.6 0.50 1.20 4.9e-1	P1/n.a. 3.7 0.49 6.50 2.3e0	P1/n.a. 3.7 0.19 1.61 1.7e-2
$\begin{array}{c} \label{eq:constraint} \\ \hline {\rm Estimation of } q_{eff} \\ \hline {\rm Guinier \ analysis} \\ \hline {\rm IFT} \\ \hline {\rm Molecular \ weight \ determina} \\ \hline {\rm Molecular \ weight \ determina} \\ \hline {\rm Molecular \ weight \ observed \\ {\rm symmetry/anisometry} \\ \hline {\rm Computation \ of \ crystal \ structure} \\ \hline {\rm Computation \ of \ crystal \ structure} \\ \hline {\rm Symmetry/anisometry} \\ \hline {\rm Computation \ of \ crystal \ structure} \\ \hline {\rm Symmetry/anisometry} \\ \hline {\rm Computation \ of \ crystal \ structure} \\ \hline {\rm Results} \\ \hline \hline {\rm Peak \ c \ [mg/ml] \ ^n} \\ \hline {\rm Shanum \ } q_{eff} \ [A^{-1}] \\ \hline {\rm CRYSOL \ } \chi^2 \\ \hline {\rm Guinier \ I(0)} \\ (\Delta I(0)) \ [Arb. \ Units] \\ \hline \end{array}$	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3)	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3)	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3)	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3)	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5)	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4)	AUTORG DAMMII P1/n.a. YSOL (ATS n/a 0.50 13.34 4.5e-2 (6.1e-3)	RAW [Shanum (. 11] (individual BAYES SAX. 5 (slow mode), P1/n.a. 5AS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3)	8, 9], Python ATSAS 2.8) [1(frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate ith background e routine [18] PyMol <u>n/a</u> 0.50 1.19 1.9e-1 (5.0e-3)] (averaged da S 2.8) [15, 16 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3)	2.5 0.46 0.72 1.6e-1 (1.6e-3)	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4)	2.0 0.43 0.75 1.9e-1 (2.0e-3)	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3)	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3)	91/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2)	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4)
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak $c \ [mg/ml]^n$ Shanum $q_{eff} \ [A^{-1}]$ CRYSOL χ^2 Guinier $I(0)$ $(\Delta I(0)) \ [Arb. Units]$ Guinier R_g	tion P1/n.a. cture intensities a intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 28.1	0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4) 28.4	AUTORG DAMMII P1/n.a. YSOL (ATS 0.50 13.34 4.5e-2 (6.1e-3) 27.7	RAW [Shanum (, 111] (individual BAYE: SAX 5 (slow mode), P1/n.a. 5AS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3) 27.0	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1] (averaged da S 2.8) [15, 16 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9	2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1	3.7 0.49 6.50 (1.9e-2) 51.6	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak $c \ [mg/ml]^n$ Shanum $q_{eff} \ [A^{-1}]$ CRYSOL χ^2 Guinier $I(0)$ $(\Delta I(0)) \ [Arb. Units]$ Guinier R_g $(\Delta R_g) \ [Å]^0$ BAYES and R_s	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3 (0.3) 27.5	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3	0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.7	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 97 8	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4	AUTORG DAMMII P1/n.a. YSOL (ATS 0.50 13.34 4.5e-2 (6.1e-3) 27.7 (0.1) 28.0	RAW [Shanum (. 11] (individual BAYE: SAX: ⁵ (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3) 27.0 (0.1) 27.1	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate rith background e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8] (averaged da S 2.8) [15, 10 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7	atta) 3] P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6 (0.3) 17.9	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 10.1	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7 (2.9) 26.4	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 20.4	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52 2
$\begin{array}{c} \hline \text{Estimation of } q_{eff} \\ \hline \text{Guinier analysis} \\ \hline \text{IFT} \\ \hline \text{Molecular weight determina} \\ \hline Ab initio modeling \\ \hline \text{symmetry/anisometry} \\ \hline \text{Computation of crystal structure} \\ \hline \text{3D representation} \\ \hline \hline \textbf{Results} \\ \hline \hline \text{Peak } c \ [mg/ml]^{n} \\ \hline \text{Shanum } q_{eff} \ [A^{-1}] \\ \hline \text{CRYSOL } \chi^{2} \\ \hline \text{Guinier } I(0) \\ (\Delta I_{q}) \ [Ab] \\ \hline \text{Guiner } R_{g} \\ (\Delta R_{g}) \ [A] \\ \hline \text{BAYESapp } R_{g} \\ (\Delta R_{g}) \ [A] \\ \hline \end{array}$	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3 (0.3) 27.5 (0.1)	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3 (0.1)	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.1 (0.1)	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5 (0.2)	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 27.8 (0.2)	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4 (0.1)	AUTORG DAMMII P1/n.a. YSOL (ATS 0.50 13.34 4.5e-2 (6.1e-3) 27.7 (0.1) 28.0 (0.1)	RAW [Shanum (. 111] (individual BAYE: SAX: 5 (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3) 27.0 (0.1) 27.1 (0.1)	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate rith background e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8 (0.2)] (averaged da S 2.8) [15, 10 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7 (0.1)	atta) P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6 (0.3) 17.9 (0.1)	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 19.1 (0.1)	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7 (0.1)	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7 (2.9) 36.4 (0.2)	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 30.4 (0.1)	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0 (0.1)	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52.3 (0.1)
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina <i>Ab initio</i> modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak c [mg/ml] ⁿ Shanum q_{eff} [Å ⁻¹] CRYSOL χ^2 Guinier 1(0) ($\Delta I(0)$) [Årb. Units] Guinier R_g (ΔR_g) [Å] EAYESAPP R_g (ΔR_g) [Å] Crystal R_g [Å]	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3 (0.3) 27.5 (0.1) 27.1	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3 (0.1) 27.1	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.1 (0.1) 27.1	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5 (0.2) 27.1	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 27.8 (0.2) 27.1	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4 (0.1) 27.1	AUTORG DAMMII P1/n.a. YSOL (ATS n/a 0.50 13.34 4.5e-2 (6.1e-3) 27.7 (0.1) 28.0 (0.1) (0.1) 27.1	RAW [Shanum (. 11] (individual BAYES SAX. 5 (slow mode), P1/n.a. 5(AS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3) 27.0 (0.1) 27.1 (0.1) 27.1	8, 9], Python ATSAS 2.8) [10 I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate vith background e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8 (0.2) 38.7] (averaged da S 2.8) [15, 16 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7 (0.1) 14.4	atta) P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6 (0.3) 17.9 (0.1) 18.3	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 19.1 (0.1) 18.8	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7 (0.1) 22.8	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7 (2.9) 36.4 (0.2) n/a	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 30.4 (0.1) 30.3	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0 (0.1) 53.0	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52.3 (0.1) 53.0
$\begin{array}{c} \hline \text{Estimation of } q_{eff} \\ \hline \text{Guinier analysis} \\ \hline \text{IFT} \\ \hline \text{Molecular weight determina} \\ \hline Ab initio modeling \\ \text{symmetry/anisometry} \\ \hline \text{Computation of crystal structured} \\ \hline \text{3D representation} \\ \hline \textbf{Results} \\ \hline \hline \textbf{Results} \\ \hline \hline \textbf{Peak } c \ [mg/ml]^{\text{In}} \\ \hline \textbf{Shanum } q_{eff} \ [\text{A}^{-1}] \\ \hline \textbf{CRYSOL } \chi^2 \\ \hline \textbf{Guinier } I(0) \\ (\Delta I(0)) \ [\text{Arb. Units}] \\ \hline \textbf{Guinier } R_g \\ (\Delta R_g) \ [\text{Å}] \\ \hline \textbf{BAYESapp } R_g \\ (\Delta R_g) \ [\text{\AA}] \\ \hline \textbf{Crystal } R_g \ [\text{\AA}] \\ \hline \textbf{BAYESapp D max} \end{array}$	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3 (0.3) 27.5 (0.1) 27.1 79.6	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3 (0.1) 27.1 81.0	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.1 (0.1) 27.1 (0.1) 27.1	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5 (0.2) 27.1 73.0	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 27.8 (0.2) 27.1 78.9	P1/n.a. CR 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4 (0.1) 27.1 78.9	AUTORG DAMMII P1/n.a. YSOL (ATS 0.50 13.34 4.5e-2 (6.1e-3) 27.7 (0.1) 28.0 (0.1) 27.1 79.1	RAW [Shanum (. 11] (individual BAYES SAX: ⁵ (slow mode), P1/n.a. SAS 2.8) [17] w in-hous 0.48 23.93 4.9 (1.8s-3) 27.0 (0.1) 27.1 (0.1) 27.1 72.6	8, 9], Python ATSAS 2.8) [10 frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate ith backgroun e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8 (0.2) 38.7 115.5] (averaged da S 2.8) [15, 16 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7 (0.1) 14.4 41.1	atta) B] P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 18.6 (0.3) 17.9 (0.1) 18.3 44.2	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 19.1 (0.1) 18.8 49.2	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7 (0.1) 22.8 65.2	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7 (2.9) 36.4 (0.2) n/a 106.1	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 30.4 (0.1) 30.3 84.0	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0 (0.1) 53.0 119.6	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52.3 (0.1) 53.0 120.4
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak $c \ [mg/ml]^n$ Shanum $q_{eff} \ [Å^{-1}]$ CRYSOL χ^2 Guinier $I(0)$ $(\Delta I(0)) \ [Årb. Units]$ Guinier R_g $(\Delta R_g) \ [Å]$ BAYESapp B_g $(\Delta R_g) \ [Å]$ EAYESAPP D_{max} $(\Delta D_{max}) \ [Å] P$ Comman ($M = M = M = M = M = M = M = M = M = M =$	tion P1/n.a. cture intensities intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) (0.3) 27.5 (0.1) 27.1 27.1 (12.2) (12.2)	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3 (0.1) 27.1 27.1 81.0 (9.1) (9.1)	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.7 (0.1) 27.1 (0.1) 27.1 (0.1) 27.4 (4.7) (4.7)	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5 (0.2) 27.1 73.0 (7.4)	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 27.8 (0.2) 27.1 27.1 27.1 27.9 (7.9) (7.9) (7.9)	P1/n.a. CR n/a 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4 (0.1) 27.1 27.1 27.1 27.1 27.1 27.1 27.1 27.1	AUTORG DAMMII P1/n.a. YSOL (ATS 2YSOL (ATS 2YSOL (ATS 27.7 (0.1) 28.0 (0.1) 27.1 (0.5) (0.5) (0.5)	RAW [Shanum (. 11] (individual BAYES SAX: ⁵ (slow mode), P1/n.a. SAS 2.8) [17] w in-hous <u>n/a</u> 0.48 23.93 4.9 (1.8e-3) 27.0 (0.1) 27.1 (0.1) 27.1 (0.1) 27.1 72.6 (0.3) (0.3)	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate rith background e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8 (0.2) 38.7 115.5 (3.2) (3.2)] (averaged da S 2.8) [15, 10 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7 (0.1) 14.4 14.4 (6,4) (6,4)	atta) 3] P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6 (0.3) 17.9 (0.1) 18.3 44.2 (2.6)	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 19.1 (0.1) 18.8 49.2 (1.4) (1.4)	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7 (0.1) 22.8 (0.1) 22.8 (1.3) (1.3)	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.7 (2.9) 36.4 (0.2) n/a 106.1 (0.6)	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 30.4 (0.1) 30.3 84.0 (1.2) (1.2)	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0 (0.1) 53.0 119.6 (0.5) (0.5)	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52.3 (0.1) 53.0 120.4 (1.7) 120.4
Estimation of q_{eff} Guinier analysis IFT Molecular weight determina Ab initio modeling symmetry/anisometry Computation of crystal structure 3D representation Results Peak c [mg/ml] ⁿ Shanum q_{eff} [A ⁻¹] CRYSOL χ^2 Guinier $I(0)$ (ΔI_q) [A ¹ σ BAYESapp R_g (ΔR_g) [Å] Crystal R_g [Å] BAYESapp D_{max} (ΔD_{max}) [Å] P Crystal D_{max} [Å] BAYESapn M	tion P1/n.a. cture intensities a intensities 3.8 0.50 1.08 4.6e-1 (3.2e-3) 28.3 (0.3) 27.5 (0.1) 27.1 79.6 (12.2) 92.5 4.2	P1/n.a. ities 1.8 0.46 0.78 6.8e-2 (1.1e-3) 28.1 (0.6) 28.3 (0.1) 27.1 81.0 (9.1) 92.5 2.4	P1/n.a. 0.9 0.46 0.82 1.5e-1 (3.3e-3) 27.7 (1.0) 27.1 (0.1) 27.1 69.4 (4.7) 92.5 2.7	P1/n.a. 0.4 0.40 0.80 6.9e-2 (2.8e-3) 29.0 (1.7) 27.5 (0.2) 27.1 73.0 (7.4) 92.5 2.4	P1/n.a. 3.8 0.19 1.02 3.6e-3 (5.1e-5) 27.9 (0.6) 27.8 (0.2) 27.1 78.9 (7.9) 92.5 2.4	P1/n.a. CR n/a 0.33 1.03 4.6e-2 (4.5e-4) 28.4 (0.4) 28.4 (0.4) 28.4 (0.1) 27.1 78.9 (4.5) 92.5 92.5	AUTORG DAMMII P1/n.a. P1/n.a. YSOL (ATS 0.50 13.34 4.5e-2 (6.1e-3) 27.7 (0.1) 28.0 (0.1) 27.1 79.1 (0.5) 92.5 7 0	RAW [Shanum (. 11] (individual BAYE: SAX: 5 (slow mode), P1/n.a. SAS 2.8) [17] w in-hous n/a 0.48 23.93 4.9 (1.8e-3) 27.0 (0.1) 27.1 (0.1) 27.1 72.6 (0.3) 92.5 \$ e \$	8, 9], Python ATSAS 2.8) [1(I frames), RAW Sapp [12, 13] SMoW [14] damfilt (ATSA P2/prolate rith background e routine [18] PyMol n/a 0.50 1.19 1.9e-1 (5.0e-3) 41.1 (1.4) 40.8 (0.2) 38.7 115.5 (3.2) 147.5 2.2] (averaged da S 2.8) [15, 10 P1/n.a. d correction e 4.2 0.46 1.17 1.7e-1 (1.1e-3) 14.9 (0.2) 14.7 (0.1) 14.4 41.1 (6.4) 46.8 2,7	atta) P1/n.a. nabled 2.5 0.46 0.72 1.6e-1 (1.6e-3) 18.6 (0.1) 18.3 44.2 (2.6) 58.0	P3/n.a. n/a 0.49 1.21 5.3e-2 (4.9e-4) 20.0 (0.3) 19.1 (0.1) 18.8 49.2 (1.4) 54.9 2.7	2.0 0.43 0.75 1.9e-1 (2.0e-3) 23.5 (0.4) 23.7 (0.1) 22.8 65.2 (1.3) 74.2 2 5	P1/oblate n/a 0.43 n/a 7.0e-2 (4.0e-3) 36.4 (0.2) n/a 106.1 106.1 (0.6) n/a 2.8	P1/n.a. 2.6 0.50 1.20 4.9e-1 (3.9e-3) 30.1 (0.4) 30.4 (0.1) 30.3 84.0 (1.2) 106.1 5.0	P1/n.a. 3.7 0.49 6.50 2.3e0 (1.9e-2) 51.6 (0.6) 52.0 (0.1) 53.0 119.6 (0.5) 150.3 6.0	P1/n.a. 3.7 0.19 1.61 1.7e-2 (2.4e-4) 53.2 (1.1) 52.3 (0.1) 53.0 120.4 (1.7) 150.3 4.4
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a Proteins: BSA: bovine serum albumin, RNase A: ribonuclease A, CAH: carbonic anhydrase, HI: human insulin, OVA: ovalbumin, CA: conalbumin, HAF: horse apoferritin

 $^{\rm b}$ GE: GE Healthcare, SA: Sigma-Aldrich, NN: Novo Nordisk A/S

^c Formulation:

Dissolve the protein in water. Add Zn(OAc)₂, phenol, NaCl and sodium phosphate buffer. Adjust concentration to 600 μ M HI in 7 mM sodium phosphate (pH 7.4), 60 mM phenol, 200 μ M Zn(OAc)₂ and 23 mM NaCl. Check pH of the sample and gently adjust to pH 7.4 using small amounts of HCl or NaOH [19].

^d M_w : molecular weight

 $e \varepsilon_{280 nm}$: extinction coefficient at 280 nm

f Buffers: 1 - PBS, pH 7.4

2 - $50~\mathrm{mM}$ HEPES, pH 7.5

3 - PBS, 1 mM DTT, pH 7.4

4 - 7 mM sodium phosphate, 60 mM phenol, 200 $\mu \rm M~Zn(OAc)_2,$ 23 mM NaCl, pH 7.4

 $\stackrel{\mathrm{g}}{\cdot} c$: concentration

h BX: Xences BioXolver L (commercial laboratory instrument), P12: BioSAXS beamline P12, EMBL-Hamburg (synchrotron), BM29: BioSAXS beamline ESRF, Grenoble (synchrotron)

i P300K: windowless DECTRIS Pilatus 300K without beamstop, P2M: DECTRIS Pilatus 2M, P1M: DECTRIS Pilatus 1M

 $^{\rm j}$ flux at the sample position

^k d: sample-detector distance

 $\stackrel{1}{\overset{m}{=}} \begin{array}{c} q_{min} \text{: smallest measured } q \\ \overset{m}{\overset{m}{=}} q_{max} \text{: largest measured } q \end{array}$

ⁿ concentration at the maximum of the elution peak

^o R_g : radius of gyration

 ${}^{\mathrm{p}} D_{max}$: longest extension of the protein ${}^{\mathrm{q}} N_p$: number of good parameters

r ${\scriptstyle \overset{r}{N_{S}:}}$ number of Shannon channels

* NB for the measurement on the 4 mg/ml BSA sample, the intensity of the direct beam was lower than for the other measurements.

Contrast calculation

The scattering of a protein sample in the forward direction I(0) is proportional to $(\Delta \rho)^2$, where $\Delta \rho = \rho_{\text{protein}} - \rho_{\text{buffer}}$ is the scattering contrast and ρ_{protein} and ρ_{buffer} are the scattering length densities of the protein and the buffer, respectively. They are given by

$$\rho_{\text{protein}} = \rho_{\text{e,protein}} \cdot r_0 = \rho_{\text{M,protein}} \cdot r_0 / \bar{v} \quad \text{and} \quad \rho_{\text{buffer}} = \rho_{\text{e,buffer}} \cdot r_0 \tag{1}$$

where $r_0 = 2.82e-13$ cm is the classical electron radius, $\rho_{M,protein} = 3.22e23$ e/g [20] is the protein electron density per mass, $\bar{v} = 0.74$ cm³/g is the voluminosity of the protein and $\rho_{e,protein} = 4.34e23$ e/cm³ and $\rho_{e,buffer}$ are the protein and buffer electron densities, respectively. Using the electron densities for PBS buffer, $\rho_{e,PBS} = 3.37e23$ e/cm³, and glycerol, $\rho_{e,gly} = 4.12e23$ e/cm³, the relative scattering contrast and forward scattering intensity in PBS with glycerol and DTT, respectively, are summarized in the following tables.

c(gly) [%v/v]	$\rho_{\rm e,PBS,gly} \ [\rm e/cm^3]$	$\Delta \rho_{\rm PBS,gly} \ [e/cm^2]$	$\frac{\Delta \rho_{\rm PBS,gly}}{\Delta \rho_{\rm PBS}} \ [\%]$	$\frac{I(0)_{\text{PBS,gly}}}{I(0)_{\text{PBS}}} = \frac{(\Delta \rho_{\text{PBS,gly}})^2}{(\Delta \rho_{\text{PBS}})^2} \ [\%]$
1	3.38e23	2.70e10	99.2	98.5
2	3.39e23	2.68e10	98.4	96.9
3	3.39e23	2.66e10	97.7	95.4
5	3.41e23	2.62 e10	96.1	92.4
7	3.42e23	2.57e10	94.6	89.4
10	3.45e23	2.51e10	92.2	85.1

c(DTT) [mM]	$\rho_{\rm e,PBS,DTT} \ [e/cm^3]$	$\Delta \rho_{\rm PBS,DTT} \ [e/cm^2]$	$\frac{\Delta \rho_{\rm PBS,DTT}}{\Delta \rho_{\rm PBS}} \ [\%]$	$\frac{I(0)_{\text{PBS,DTT}}}{I(0)_{\text{PBS}}} = \frac{(\Delta \rho_{\text{PBS,DTT}})^2}{(\Delta \rho_{\text{PBS}})^2} [\%]$
1	3.37e23	2.72e10	99.9	99.9
2	3.37e23	2.72e10	99.9	99.8
3	3.37e23	2.72e10	99.8	99.7
5	3.37e23	2.72e10	99.7	99.5
7	3.37e23	2.71e10	99.6	99.3
10	3.38e23	2.71e10	99.5	99.0

 $\Delta \rho_{\text{PBS}}$, $\Delta \rho_{\text{PBS,gly}}$ and $\Delta \rho_{\text{PBS,DTT}}$ are the scattering contrasts of a protein in pure PBS buffer, in PBS buffer with glycerol and in PBS buffer with DTT, respectively, and $I(0)_{\text{PBS, II}}$ and $I(0)_{\text{PBS,DTT}}$ are the corresponding forward scattering intensities.











Figure S1: Selection of sample and buffer regions for data analysis of SEC-SAXS data from BSA samples with stock concentrations of 8, 4, 2 and 1 mg/ml. *Top panels:* Integrated intensity as a function of frame index (i.e. time), *bottom panels:* Individual 30s frames. A-E: Xenocs BioXolver L, F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble.





Figure S2: Selection of sample and buffer regions for data analysis of Xenocs BioXolver L SEC-SAXS data. *Top panels:* Integrated intensity as a function of frame index (i.e. time), *bottom panels:* Individual 30s frames.



Figure S3: Pictures of our laboratory-based SEC-SAXS setup. Left: Overall view of the mobile HPLC unit next to the SAXS instrument, middle: connection of the HPLC tubing to the flow-through cell, right: fraction collector after the SAXS exposure cell.



Figure S4: UV traces (HPLC unit and SAXS exposure cell) together with the forward scattering intensity I(0) (left axis) and the radius of gyration R_g (right axis) of each individual frame across the monomer peak of BSA. A-E: Xenocs BioXolver L, F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble.



Figure S5: Guinier plots of BSA data. A-F: SEC-SAXS data, G and H: static SAXS data. A-E and G: laboratory instrument (Xenocs BioXolver L), F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble, H: synchrotron BioSAXS beamline P12, EMBL-Hamburg.



Figure S6: Concentration-normalized static SAXS measurements of 5 mg/ml BSA on our laboratory instrument (Xenocs BioXolver L, 60s exposure) and of 4 mg/ml BSA on a synchrotron BioSAXS beamline (P12, EMBL-Hamburg, 1s exposure) on absolute scale. The synchrotron data was scaled to overlap with the BioXolver data. The inset shows the corresponding pair-distance distribution functions p(r).



Figure S7: UV traces (HPLC unit and SAXS exposure cell) together with the forward scattering intensity I(0) (left axis) and the radius of gyration R_g (right axis) of each individual frame across the monomer peak of different proteins. *NB:* for HI, no chromatogram is available due to the presence of phenol, which strongly absorbs at 280 nm, in the running buffer.



Figure S8: Guinier plots of SEC-SAXS data of ribonuclease A (A), carbonic anhydrase (B), human insulin (C), ovalbumin monomer and dimer (D and E, respectively), conalbumin (F) and apoferritin (G and H).

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