SUPPORTING INFORMATION

Revealing the dimeric crystal and solution structure of β-lactoglobulin at pH 4 and its pH and salt dependent monomer-dimer equilibrium

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Table S1. Comparisons of secondary structure elements of BLGA at different pH.

PDB-ID	pH	Oligomerization state	β-strand I	Hydrogen bonds between β-strand I in dimer interface	α-1	α- 2	α-3	α-4	EF-loop conformation (distance)[c]
1CJ5	2.0	Monomer	HIRLS		IQKV	Absent	Absent	TQLE	Closed (7.7Å)
1DV9	2.6	Monomer	IRLS		IQKVA	Absent	Absent	PTQLEE	Closed (5.8Å)
6FXB (the present structure)	4.0	Dimer	HIRLSF	5	IQKV	ISLLD	PEQS	PTQLE	Closed (6.6Å)
2AKQ	5.2	Dimer	LSF[a] & HIRLSF	3	IQKV	ISLLD	Absent	PTQLE	Closed (6.6Å)
3BLG	6.2	Dimer	IRLS	4	Absent	ISLLD	Absent	PTQLE	Closed (8.3Å)
1BEB	6.5	Dimer	IRLS	4	IQKV	ISL	PEQ	PTQL	Closed (7.1Å)
1BSQ	7.1	Dimer	IRLS	4	IQKV	ISLL	Absent	PTQL	Closed (7.0Å)
1BSO	7.3	Dimer	IRLS	4	Absent	ISLL	Absent	PTQL	Opened (14.8Å)
2Q2M	7.4	Dimer	IRLS	4	IQKVA	DISLLD	Absent	missing[b]	Opened (14.3Å)
2BLG	8.2	Dimer	IRLS	4	Absent	ISLLD	Absent	PTQLE	Opened (15.8Å)
5HTE	8.5	Dimer	IRLS	2	DIQKVA	DISLL	Absent	Absent	Opened (14.5Å)

[a]one monomer subunit of the dimer consists of LSF and the other one consists of HIRLSF. [b]residues are missing, [c]distance between C_α-P38 (AB loop) and C_α-L87 (EF loop).

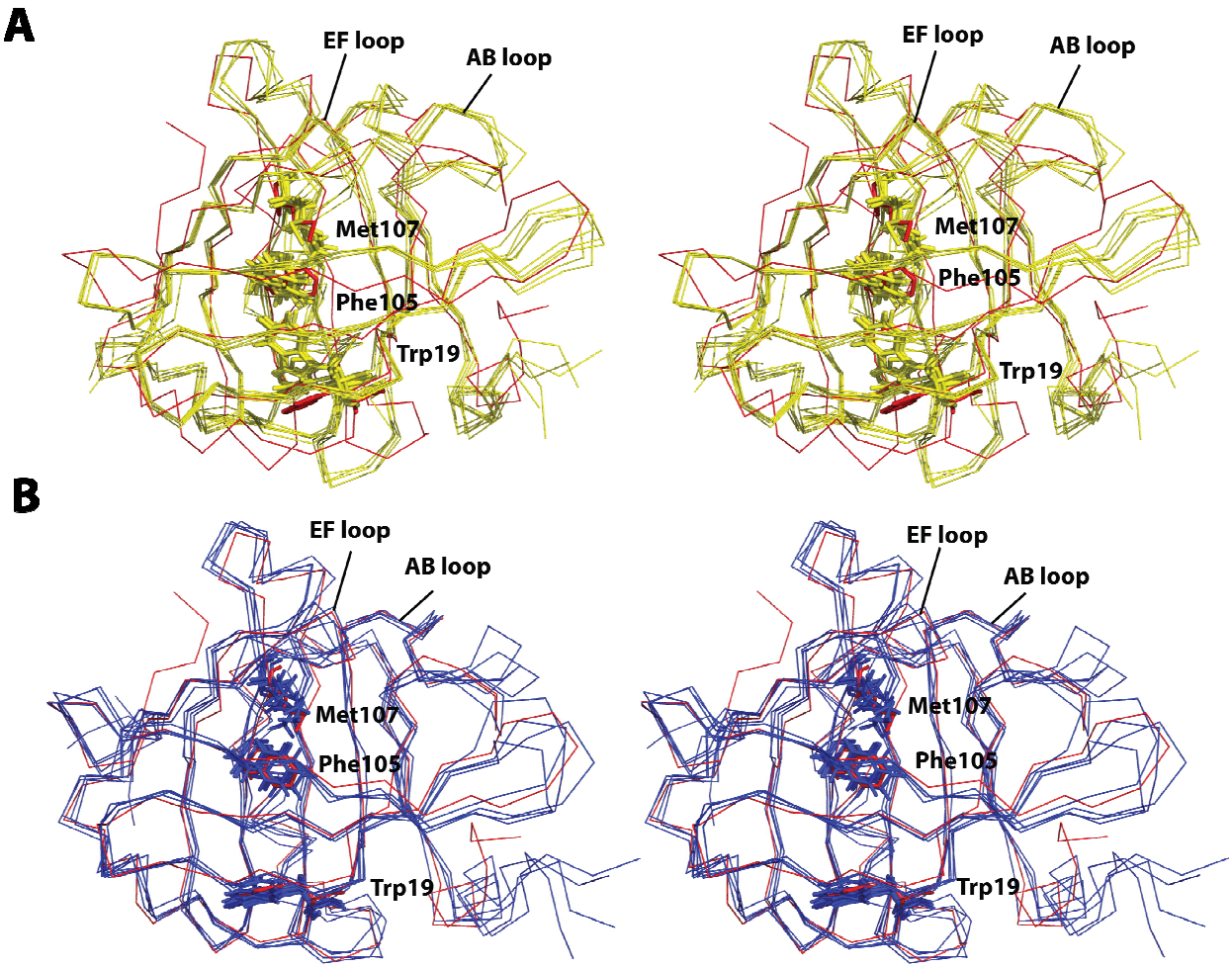

Figure S1. Stereo views of BLGA showing structural comparisons. (A) Superimposed single subunit structure (chain B) of BGLA at pH 4.0 (red, 6FXB) and the monomer NMR structures of BLGA at pH 2.0 (yellow, 1CJ5, only first five models are shown for clarity). (B) Superimposed single subunit structure (chain B) of BGLA at pH 4.0 (red, 6FXB) and the monomer NMR structures of BLGA at pH 2.6 (blue, 1DV9, only first five models are shown for clarity).

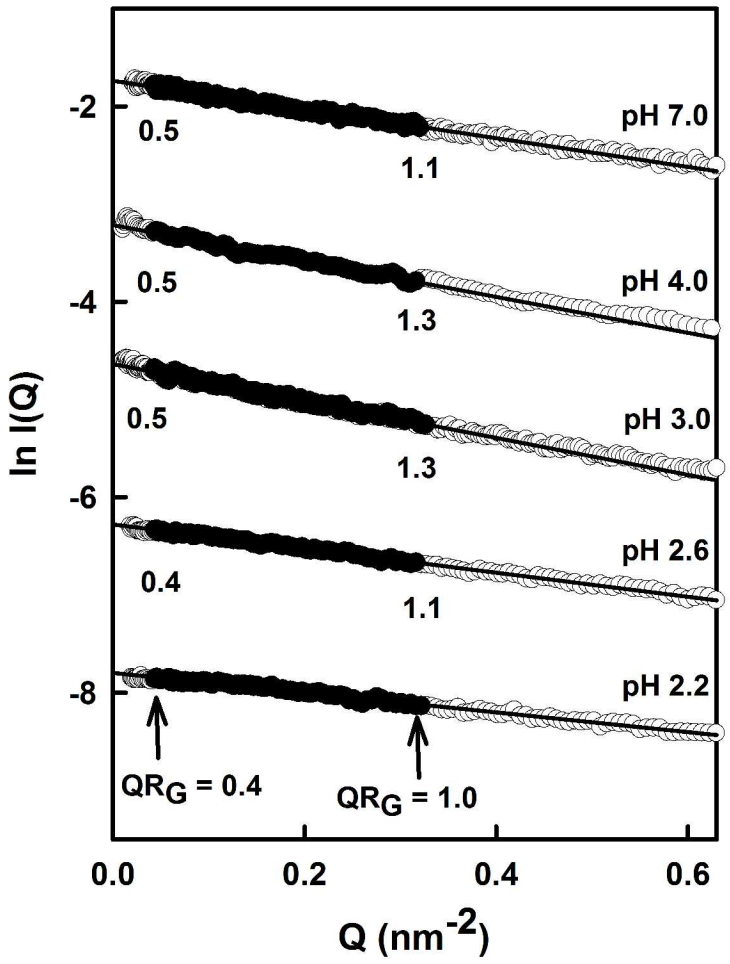

Figure S2. Guinier R_G analyses of BLGA at various pH. BLGA was studied at 1 mg/mL in 10 mM buffer: glycine (pH 2.2 and 2.6), sodium citrate/citric acid (pH 3.0 and 4.0) and Tris-HCl (pH 7.0). Open circles correspond to the experimental SAXS data, while filled circles correspond to the Q ranges used to determine R_G in the Q fit range of 0.21–0.57 nm^{-1}.

Table S2. SAXS data for BLGA (1 mg/mL) at different pH and NaCl concentration

pH	R_G (nm)[a]	Length L (nm)	K_D (μM)	R_G (nm)[a] NaCl			Length L (nm) NaCl		
				50 mM	100 mM	150 mM	50 mM	100 mM	150 mM
2.2	1.73 ± 0.02 1.78 ± 0.03	5.6	345.6	2.24 ± 0.02 2.25 ± 0.03	2.37 ± 0.01 2.37 ± 0.04	2.38 ± 0.02 2.38 ± 0.02	7.4	7.8	7.9
2.6	1.94 ± 0.03 1.95 ± 0.02	6.5	54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3.0	2.39 ± 0.03 2.38 ± 0.04	7.1		2.33 ± 0.03 2.29 ± 0.03	2.33 ± 0.01 2.34 ± 0.04	2.33 ± 0.02 2.33 ± 0.03	7.1	7.7	7.6
4.0	2.32 ± 0.04 2.32 ± 0.02	7.2		2.46 ± 0.01 2.44 ± 0.04	2.44 ± 0.02 2.42 ± 0.04	2.41 ± 0.02 2.41 ± 0.01	7.7	7.7	7.8
7.0	2.09 ± 0.02 2.14 ± 0.03	7.4	9	n.d. n.d.	2.30 ± 0.01 2.31 ± 0.03	2.33 ± 0.01 2.31 ± 0.04	n.d.	7.8	7.8

a: The upper value is obtained by Guinier analyses, and the lower is from GNOM $P(r)$ analyses; n.d.: not determined

Table S3. SAXS data for BLGA (2 mg/mL) at different pH values

pH	R_G (nm)[a]	Length L (nm)	K_D (μM)
2.2	1.80 ± 0.02 1.80 ± 0.03	5.7	352.8
2.6	2.01 ± 0.03 2.08 ± 0.02	7.1	53.7
3.0	2.42 ± 0.02 2.43 ± 0.04	7.8	
4.0	2.31 ± 0.02 2.34 ± 0.02	7.5	
7.0	2.22 ± 0.02 2.27 ± 0.04	7.8	9.6

a: The upper value is obtained by Guinier analyses, and the lower is from GNOM $P(r)$ analyses

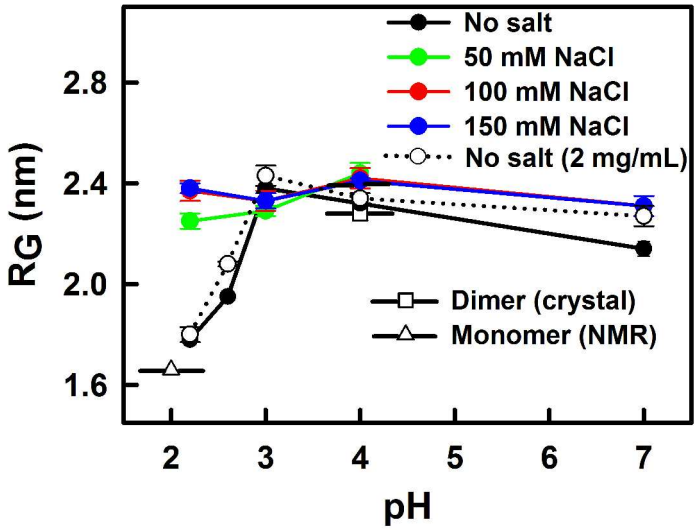

Figure S3. pH and NaCl concentration dependence of GNOM R_G parameters for BLGA. BLGA at 1 mg/mL (50–150 mM NaCl) and at 2 mg/mL (0 M NaCl) was analysed in 10 mM glycine (pH 2.2 and 2.6), sodium citrate/citric acid (pH 3.0 and 4.0) and Tris-HCl (pH 7.0). GNOM R_G values for BLGA are indicated. Comparison of experimental GNOM R_G values with the theoretical R_G values calculated for the pH 4.0 dimer crystal structure (present structure, open square) and a monomer NMR structure (1CJ5, open triangle) are shown.

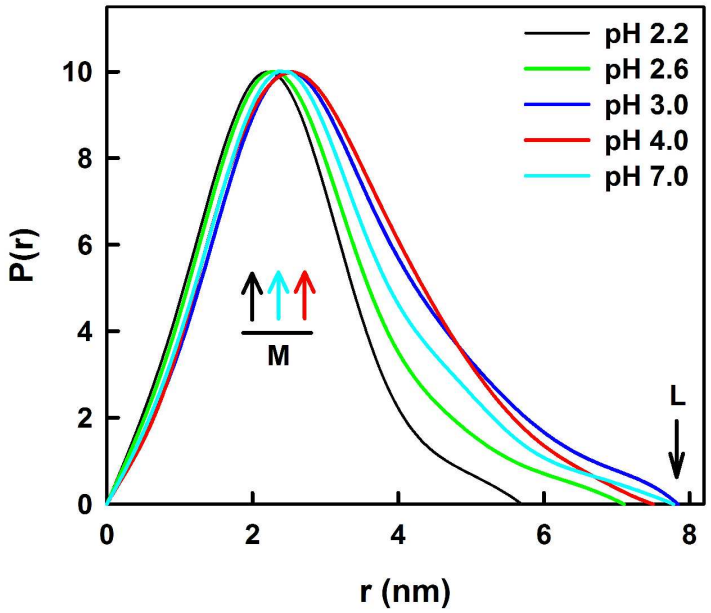

Figure S4. Experimental $P(r)$ analyses of BLGA. Distance distribution function $P(r)$ analyses of BLGA (2 mg/mL) at various pH. The most frequently occurring distance M and the maximum length L for BLGA are indicated. The L was measured when the $P(r)$ curve reached zero at a large r value.

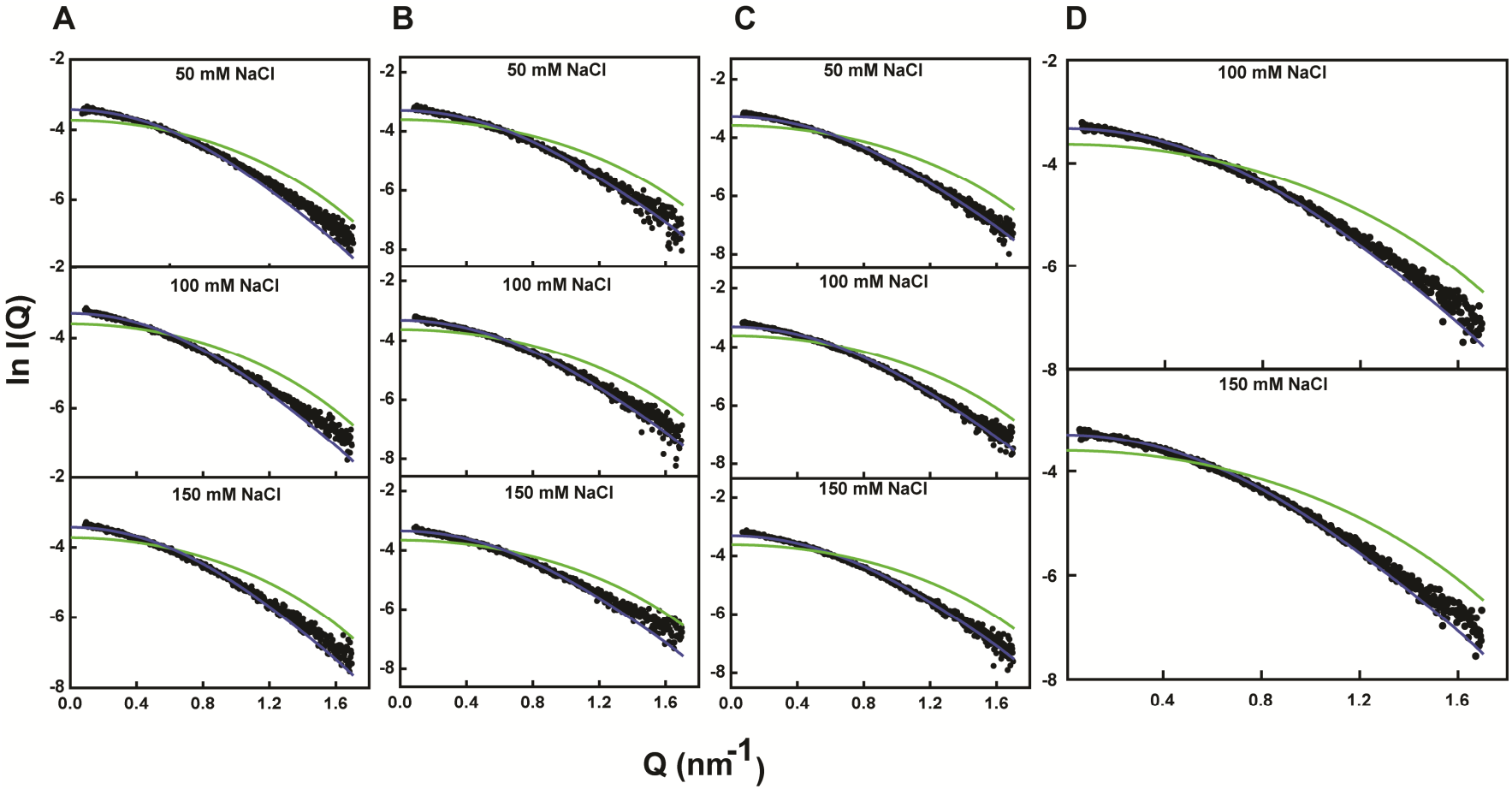

Figure S5. X-ray scattering curve fits for the monomer and dimer BLGA structures. BLGA (1 mg/mL) was studied in the presence of 50, 100 and 150 mM NaCl at pH 2.2 (A), pH 3.0 (B), pH 4.0 (C) and in the 100 and 150 mM NaCl at pH 7.0 (D). The experimental scattering data are denoted by points (●), the green lines correspond to the monomer BLGA NMR structure (1CJ5) and the blue lines correspond to the present pH 4.0 dimer crystal structure (6FXB). The *Q* range employed 0.21–0.57 nm^{-1}.