# SUPPORTING MATERIAL

## Size-exclusion chromatography (SEC) measurements

Size-exclusion chromatography (SEC) was used to evaluate the fraction of oligomeric species at different BSA concentrations. Samples (100 µl) with different protein content were pre-incubated for 10 min at room temperature and then subjected to a Superdex 200 Increase 10/300 column (GE Healthcare) equilibrated with a filtrated and degassed 50 mM Tris-HCl buffer, pH 7.6, containing 100 mM NaCl, 0.1 mM EDTA, 5% glycerol and 3 mM ME. The column was operated at 25°C at 1.5 ml/min flow rate using a chromatographic ProStar325 UV/Vis HPLC/FPLC system (Varian Inc.). The elution profiles were monitored by absorbance at 280 nm.

## Zeta-potential measurements

Zeta-potential measurements were performed with a Malvern Instruments Zetasizer Nano-Z instrument (U.K.). To obtain zeta potential (ζ) the Henry equation, which includes the Smoluchowski approximation, was applied. The refractive index was fixed to 1.45.

## Guinier analysis

The averaged gyration radius for general case of polydisperse solution can be defined by the relation [44]:

$$R_{0M}^2 = \frac{\sum_k p_k n_k^2 R_{0k}^2}{\sum_k p_k n_k^2}, \qquad (1)$$

where $n_k$ – full number of electrons in particle of type-$k$ and $p_k$ – proportion of this type. Let's consider the case when we have mix solution with equal proportions of BSA monomers and dimers $p_1 = p_2 = 0.5$ (provided that both components follow Guinier law separately). If we take $R_g$ for BSA monomer and dimer from bioSAXS data base https://www.sasbdb.org/data/SASDBJ3/ and https://www.sasbdb.org/data/SASDBK3/ as 2.8 nm and 3.9 nm respectively, then averaged gyration radius (from (1)) is equal $R_{0M} = 3.7$ nm. As one can see it is quite close to $R_g$ of BSA dimer. As the Guinier analysis is mostly evaluative we can take effective averaged $R_g$ equal to $R_g$ of dimers in most cases. At larger angles the contribution from aggregates into SAXS curve is effectively zero, and the apparent gyration radius is practically equal to $R_g^{monomer}$, so there is no need to subtract the slopes for $R_g$ determination [44] (pp.149-151).

Unfortunately, it's impossible to automatically separate averaged gyration radius onto components in our case, because information about dimer proportion in solution $p_k$ and about presence of trimers and higher-order oligomers is lacking. The dimers $p_k$ could have been determined from (1), if there had been no influence on $R_g$ from oligomers of higher order. In our case we have higher order oligomers appearance (which is seen, for example, in fig.3). The only way to qualitatively evaluate gyration radii of monomers and "averaged" aggregates in solution is manually define the point of decided concavity on Guinier plot for each temperature of solution and to estimate the corresponding line slopes.

Basically, there is another way to check the reliability of proper choice of two $q$-ranges in Guinier plot: to compare $R_g$, calculated from lower $q$-range with $R_g$, calculated from $P(r)$ function. If the values are close, then Guinier approximation is still good enough. In our analysis the values of $R_g^{aggreg}$ and $R_g^{real}$ are close enough which is demonstrated in Fig.2. Of course, all that considerations could be applied only in case when it is intuitively clear where the point separating first and second $q$-range is located.

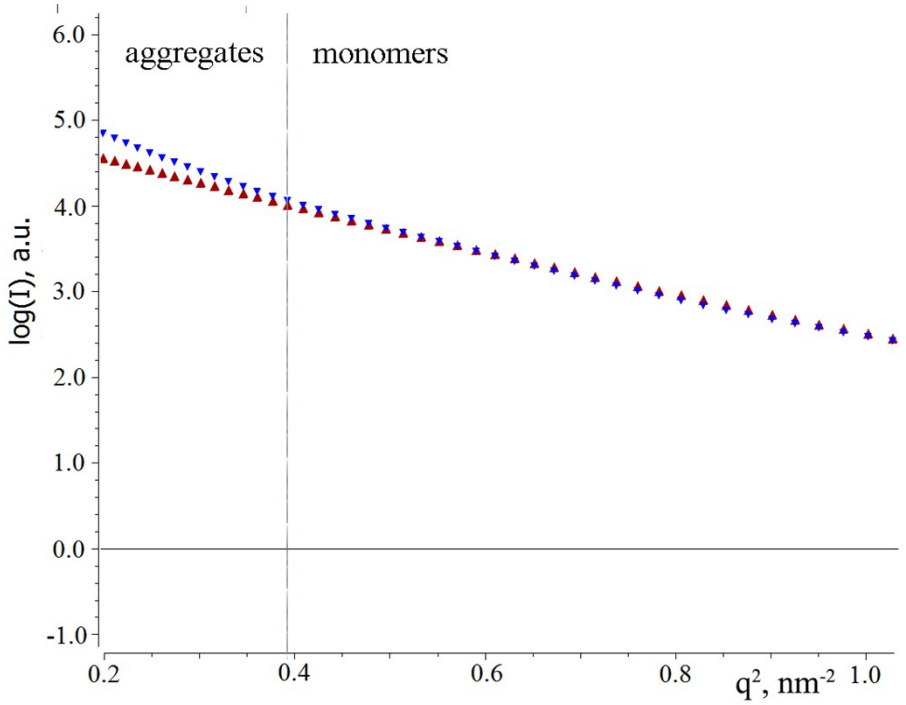



Fig.S1. The Guinier plot of solution B (c = 20 mg/ml) at room temperature and at temperature in vicinity of BSA melting point (T = 60°C).

As it is seen from fig.S1 (Guinier plots for solution B at room temperature and at temperature of BSA denaturation), the boundary point can be clearly identified, and both components satisfy Guinier law separately.

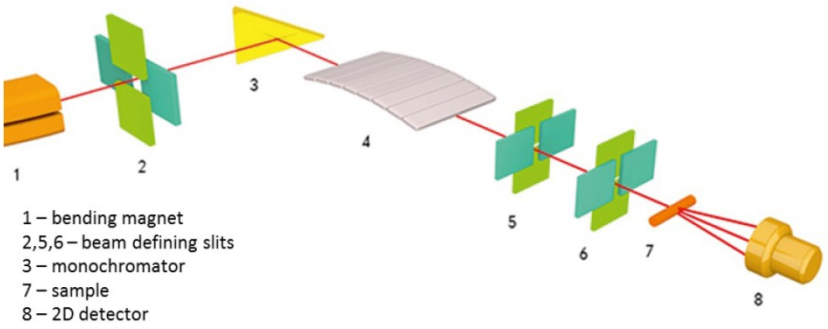



Fig.S2. Optical scheme of DICSY beamline includes 3 pair of beam defining slits, monochromator ($\lambda$ = 1.6 Å) and 2D detector Pilatus3 1M.

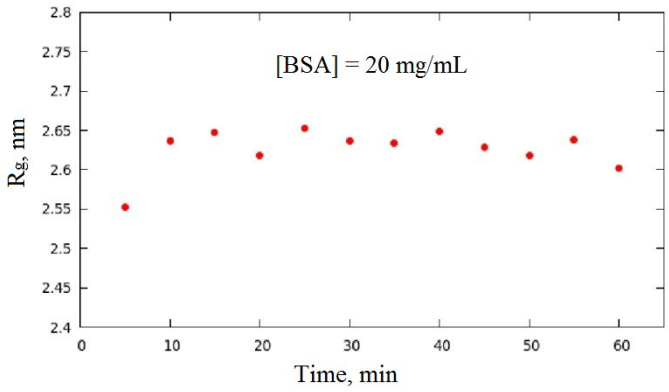
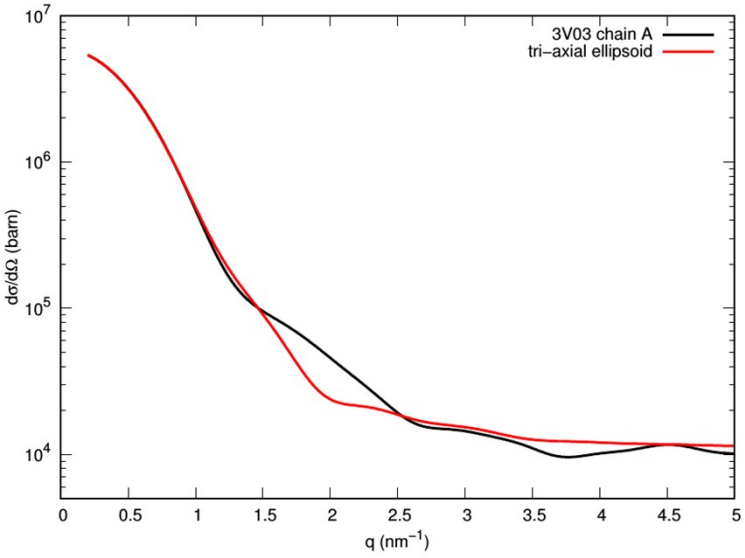



Fig.S3. First 60 min of radiation damage test at room temperature




S4. Comparison of oblate ellipsoid model and crystallographic model of BSA monomer

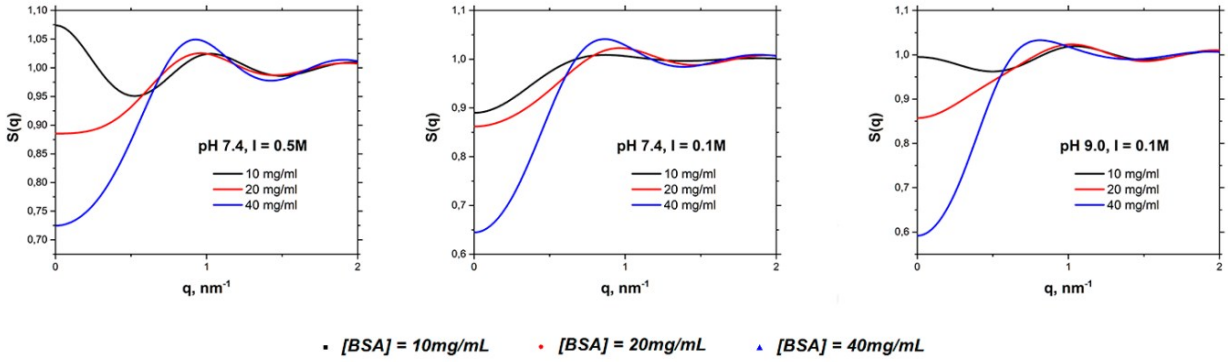



Fig.S5. Structure factors for all solutions calculated in frames of DLVO potentials on base of potentials, found from global fitting procedure.

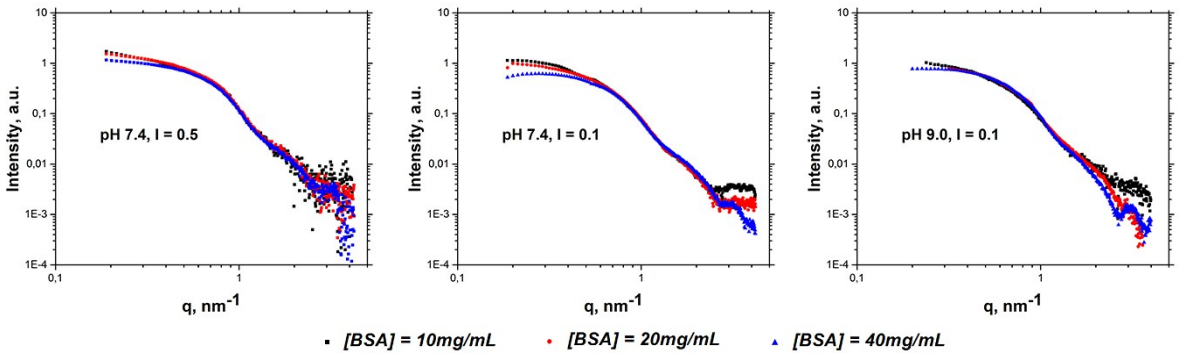



Fig.S6(a) SAXS data at 50 °C

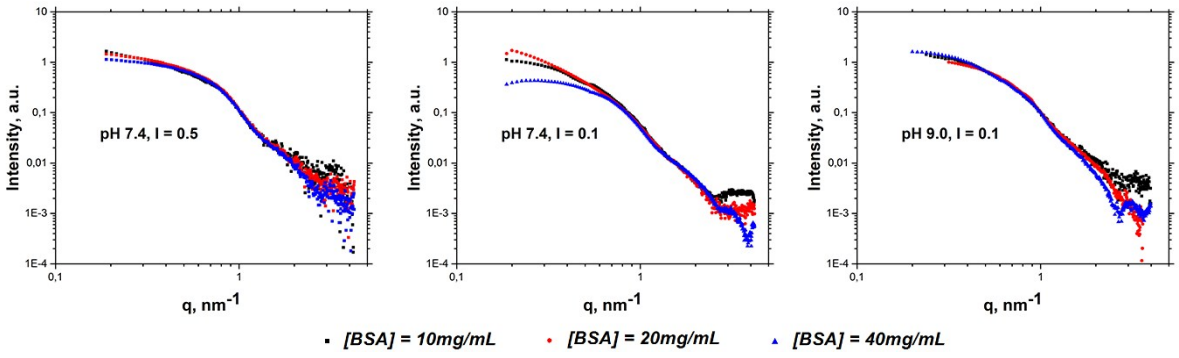



Fig.S6(b) SAXS data at 60 °C

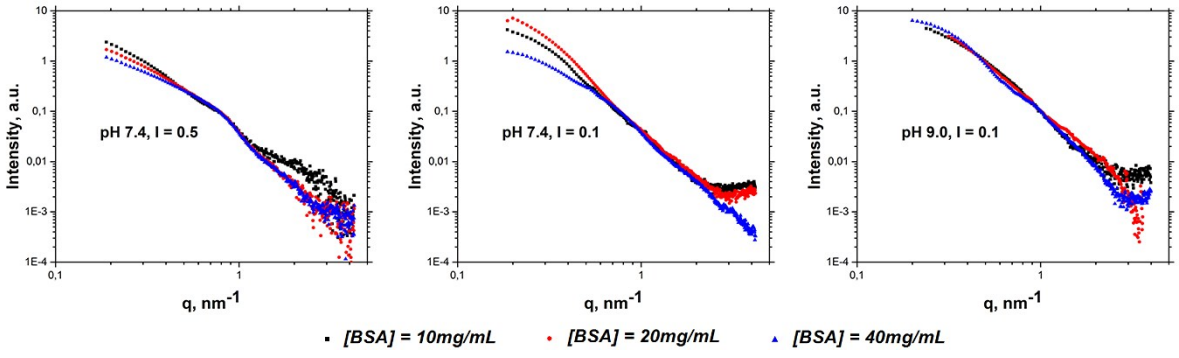



Fig.S6(c) SAXS data at 70 °C

| $T$, °C | $D_{max}$, nm | | | $R_g^{real}$, nm | | | $R_g^{monomer}$, nm | | | $R_g^{aggreg}$, nm | | | $V_{Porod}$, nm$^3$ | | | $I(0)$, a.u. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mg/ml | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** |
| **25** | 11.2 | 8.3 | 7.2 | 3.2 | 2.7 | 2.6 | 2.9 | 2.8 | 2.7 | 3.1 | 2.8 | 2.5 | 133.3 | 93.9 | 76.7 | 767.5 | 1013 | 1029 |
| **30** | 12.1 | 8.8 | 7.7 | 3.5 | 2.8 | 2.6 | 2.9 | 2.8 | 2.7 | 3.3 | 2.7 | 2.5 | 140.5 | 95 | 79 | 774.8 | 1013 | 1056 |
| **35** | 10.6 | 9.2 | 7.7 | 3.2 | 2.8 | 2.6 | 2.9 | 2.8 | 2.7 | 3.1 | 2.8 | 2.6 | 130.3 | 96.5 | 78.4 | 727.9 | 1023 | 1008 |
| **40** | 10.8 | 9.6 | 7.6 | 3.2 | 2.9 | 2.6 | 2.9 | 2.8 | 2.7 | 3.2 | 2.9 | 2.5 | 132.8 | 98 | 77.6 | 721.2 | 1054 | 953.6 |
| **45** | 12.3 | 9.9 | 7.6 | 3.5 | 3 | 2.6 | 2.9 | 2.8 | 2.7 | 3.3 | 3 | 2.5 | 142.4 | 98.9 | 78.6 | 728.4 | 1072 | 935.5 |
| **50** | 13.8 | 15.1 | 7.6 | 3.9 | 3.4 | 2.6 | 2.9 | 2.8 | 2.7 | 3.5 | 3.1 | 2.6 | 156.8 | 116.6 | 78 | 753.4 | 1153 | 919.3 |
| **55** | 14.9 | 18 | 7.8 | 4.1 | 4.5 | 2.6 | 3 | 2.9 | 2.6 | 3.5 | 3.7 | 2.6 | 154.7 | 169 | 77.2 | 751 | 1565 | 876.9 |
| **60** | 14.3 | 18.2 | 8.3 | 4.4 | 5.6 | 2.7 | 3 | 3 | 2.6 | 3.9 | 5 | 2.7 | 188.8 | 358.9 | 79 | 857.3 | 2996 | 761 |
| **65** | 15.8 | 20.5 | 14 | 5.4 | 6.6 | 4.3 | 3.2 | 3.4 | 2.7 | 5 | 6.2 | 3.5 | 374 | 1035 | 163.9 | 1307 | 6862 | 1735 |
| **70** | 21.5 | 28 | 18.7 | 6.4 | 8.3 | 5.9 | 3.5 | 3.8 | 3.1 | 6.1 | 6.5 | 5.2 | 1085 | 1845 | 478 | 2637 | 6030 | 2915 |

Table.S1. Parameters table for solution B (BSA pH 7.4, I = 0.1M).

| $T$, °C | $D_{max}$, nm | | | $R_g^{real}$, nm | | | $R_g^{monomer}$, nm | | | $R_g^{aggreg}$, nm | | | $V_{Porod}$, nm$^3$ | | | $I(0)$, a.u. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mg/ml | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** |
| **25** | 14.5 | 13.7 | 7.3 | 4.1 | 3.2 | 2.5 | 2.5 | 2.7 | 2.6 | 3.6 | 2.9 | 2.5 | 140.6 | 107.3 | 79 | 758 | 3512 | 4870 |
| **30** | 16.5 | 13.4 | 7.3 | 4.4 | 3.3 | 2.5 | 2.6 | 2.7 | 2.6 | 3.6 | 3 | 2.5 | 138.6 | 111.7 | 79 | 769 | 3482 | 4837 |
| **35** | 15 | 13.8 | 7.4 | 4 | 3.3 | 2.5 | 2.5 | 2.7 | 2.6 | 3.6 | 3 | 2.6 | 140 | 114 | 80 | 766 | 3518 | 4851 |
| **40** | 15.3 | 13.7 | 7.4 | 3.9 | 3.3 | 2.5 | 2.5 | 2.7 | 2.6 | 3.2 | 3 | 2.6 | 123 | 113 | 80 | 643 | 3413 | 4822 |
| **45** | 15.5 | 13.4 | 7.4 | 4 | 3.3 | 2.5 | 2.6 | 2.6 | 2.6 | 3.3 | 3 | 2.6 | 128 | 104 | 81 | 647 | 3425 | 4808 |
| **50** | 16.3 | 13.4 | 7.5 | 4 | 3.4 | 2.5 | 2.5 | 2.6 | 2.6 | 3.2 | 3 | 2.6 | 115 | 108 | 80 | 620 | 3384 | 4831 |
| **55** | 17.4 | 13.4 | 7.6 | 4.1 | 3.3 | 2.5 | 2.5 | 2.6 | 2.6 | 3.3 | 3 | 2.6 | 120.3 | 104 | 81 | 635 | 3319 | 4770 |
| **60** | 22 | 13.5 | 8 | 5.4 | 3.5 | 2.6 | 2.5 | 2.6 | 2.6 | 3.5 | 3.1 | 2.67 | 157 | 111 | 82 | 790 | 3438 | 4802 |
| **65** | 25 | 20.6 | 14.5 | 7.5 | 5.1 | 3.6 | 2.5 | 2.7 | 2.6 | 4 | 3.7 | 3.1 | 298 | 167.4 | 109 | 1328 | 4951 | 5703 |
| **70** | 27 | 23.7 | 18.4 | 7.8 | 7 | 5.3 | 2.7 | 2.9 | 2.8 | 5.5 | 5 | 4.6 | 1023 | 699 | 267 | 3245 | 13960 | 12970 |

Table.S2. Parameters table for solution A (BSA pH 7.4, I = 0.5M).

| $T$, °C | $D_{max}$, nm | | | $R_g^{real}$, nm | | | $R_g^{monomer}$, nm | | | $R_g^{aggreg}$, nm | | | $V_{Porod}$, nm$^3$ | | | $I(0)$, a.u. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mg/ml | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** | **10** | **20** | **40** |
| **25** | 10 | 8.7 | 7.8 | 3 | 2.8 | 2.7 | 2.8 | 2.9 | 2.8 | 2.9 | 2.5 | 2.2 | 99.5 | 89.5 | 89 | 172 | 1271 | 909 |
| **30** | 10.9 | 8.8 | 7.9 | 3 | 2.7 | 2.7 | 2.9 | 2.8 | 2.8 | 2.9 | 2.6 | 2.4 | 108 | 89 | 91 | 171 | 1246 | 907 |
| **35** | 9.9 | 8.9 | 7.9 | 3 | 2.8 | 2.7 | 2.9 | 2.8 | 2.8 | 2.9 | 2.6 | 2.6 | 107 | 89 | 88 | 171 | 1246 | 837 |
| **40** | 10.9 | 8.2 | 7.9 | 3.1 | 2.7 | 2.7 | 2.9 | 2.8 | 2.7 | 3 | 2.6 | 2.6 | 115 | 86 | 87 | 181 | 1257 | 798 |
| **45** | 11.1 | 8 | 7.9 | 3.2 | 2.6 | 2.7 | 2.9 | 2.8 | 2.7 | 3 | 2.6 | 2.6 | 120 | 85.6 | 88 | 191 | 1216 | 787 |
| **50** | 11.3 | 8.1 | 7.8 | 3.3 | 2.7 | 2.7 | 2.9 | 2.8 | 2.7 | 3.1 | 2.6 | 2.6 | 124 | 84 | 87 | 187 | 1200 | 780 |
| **55** | 11 | 8.1 | 8 | 3.2 | 2.7 | 2.7 | 2.9 | 2.8 | 2.7 | 3 | 2.6 | 2.6 | 118 | 84 | 89 | 183 | 1200 | 790 |
| **60** | 11.4 | 9.7 | 12.6 | 3.4 | 2.9 | 3.8 | 2.9 | 2.9 | 2.6 | 3.2 | 3 | 3.5 | 132 | 95 | 159 | 197 | 1285 | 1220 |
| **65** | 23.3 | 23.3 | 14.5 | 6.5 | 5.1 | 4.7 | 3.1 | 3 | 2.5 | 3.9 | 4 | 4.6 | 389 | 244 | 333 | 572 | 2629 | 2173 |
| **70** | 23.3 | 19 | 16.4 | 6.9 | 5.7 | 5.4 | 3.1 | 3.1 | 2.3 | 4.6 | 5.2 | 5.5 | 675 | 607 | 687 | 852 | 5279 | 3823 |

Table.S3. Parameters table for solution C (BSA pH 9.0, I = 0.1M).

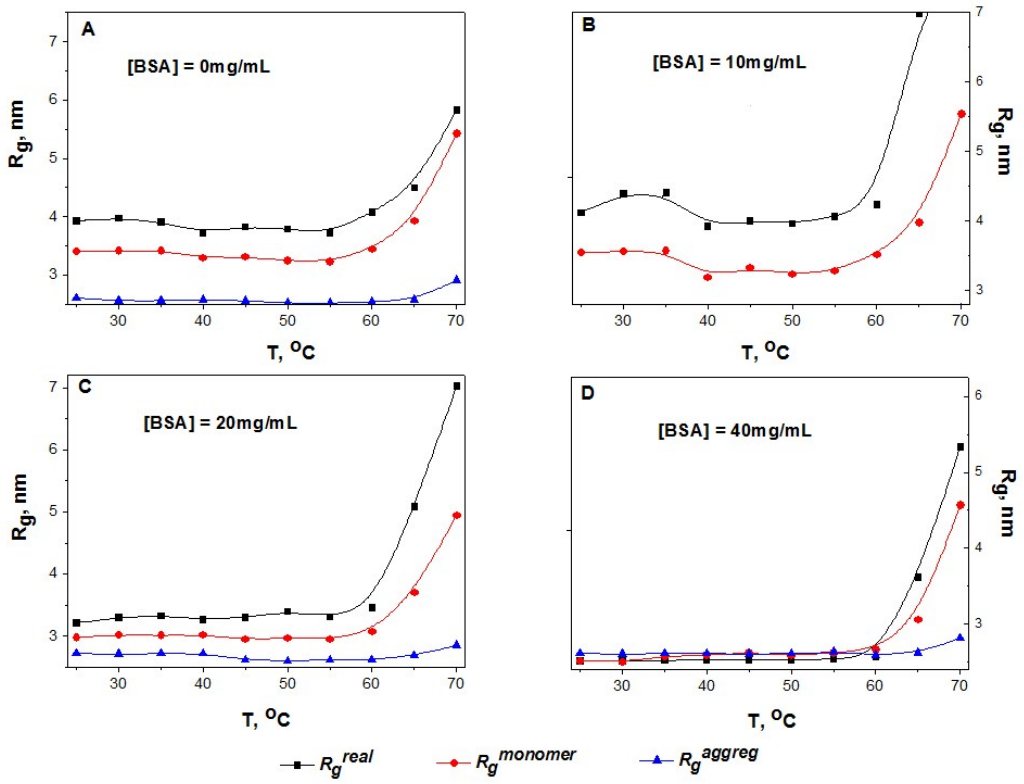



Fig.S7. $R_g(T)$ dependencies calculated by analyzing the two lines slope at Guinier plot in first ($R_g^{monomer}$) and second ($R_g^{aggreg}$) Guinier region and by $P(r)$ function ($R_g^{real}$) for pH 7.4, I = 0.5M. Plots A, B, C and D corresponding to protein concentrations c = 0; 10; 20 and 40 mg/ml, respectively.

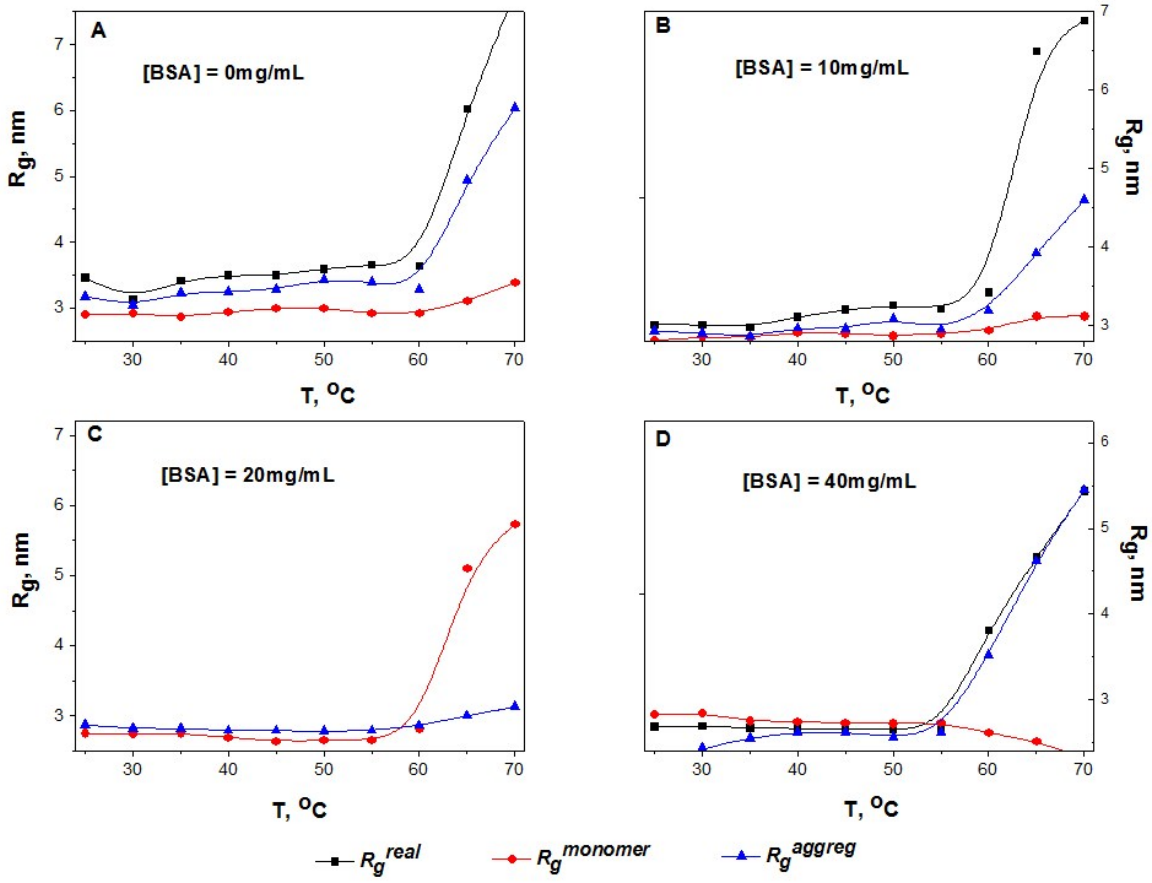



Fig.S8. $R_g(T)$ dependencies calculated by analyzing the two lines' slope at Guinier plot in the first ($R_g^{monomer}$) and the second ($R_g^{aggreg}$) Guinier region and by *P(r)* function ($R_g^{real}$) for pH 9.0, I = 0.1M. Plots A, B, C and D corresponding to protein concentrations c = 0; 10; 20 and 40 mg/ml, respectively.

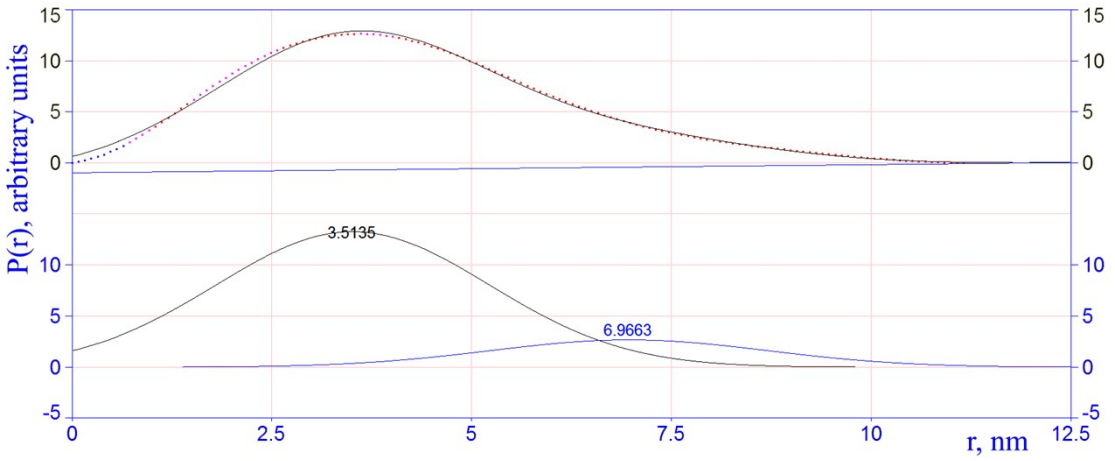



Fig.S9. Example of Gaussian decomposition of *P(r)* function for zero concentration (solution B), MDR ≈ 6.

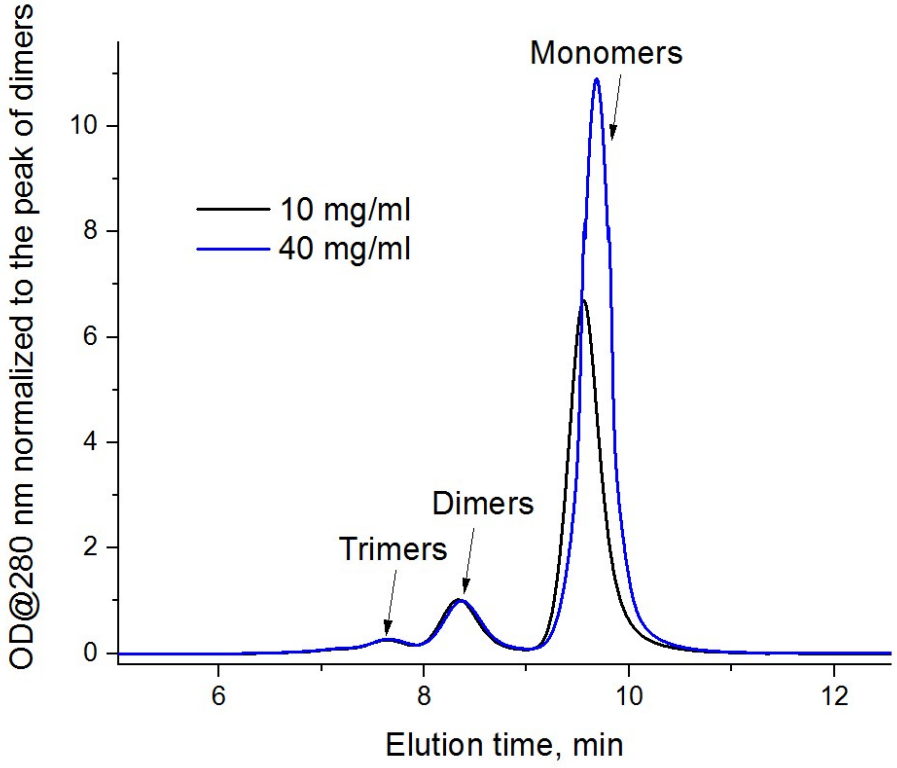



Fig.S10. Elution profiles for 10 mg/mL and 40 mg/mL BSA solutions obtained using SEC. MDR decreased by a factor of 1.5 with concentration growth.

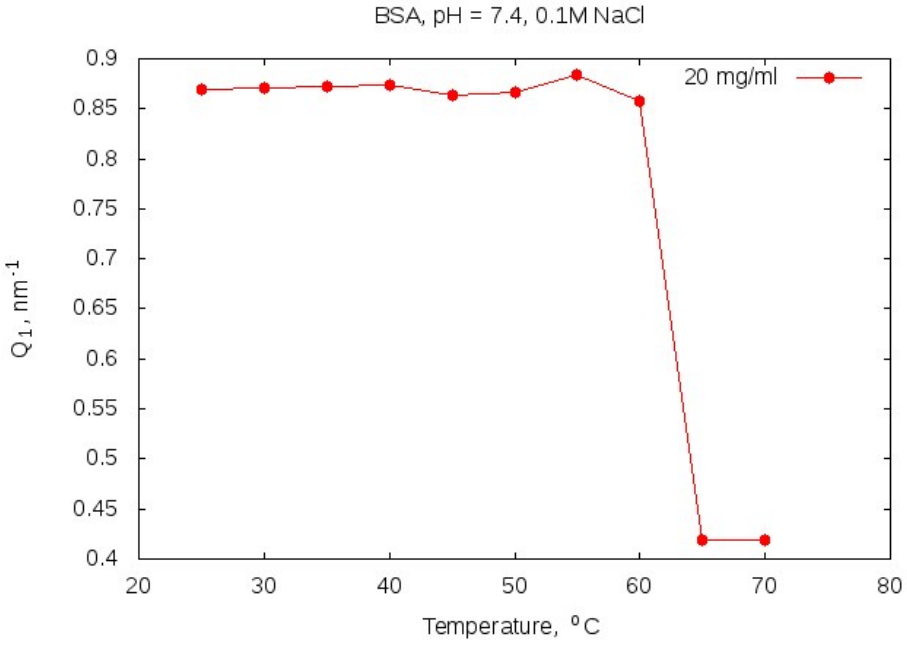


Fig.S11. Dependence of the $Q_1$ parameter versus temperature (eq 3).

| $T$,°C | $J$ ($k_BT$) | | | ellipsoid axis, Å | | |
|---|---|---|---|---|---|---|
| | c=10mg/mL | c=20mg/mL | c=40mg/mL | c=10mg/mL | c=20mg/mL | c=40mg/mL |
| 25 | 5.2 | 1.6 | 1.0 | 20x42x50 | 20x41x52 | 20x42x50 |
| 50 | 10.3 | 3.5 | 1.3 | 17x38x40 | 20x43x43 | 19x41x48 |
| 55 | 11.0 | 3.2 | 1.5 | 18x40x45 | 20x43x43 | 20x42x46 |
| 60 | 13.9 | 3.9 | 1.8 | 19x41x42 | 21x43x44 | 16x39x44 |
| 65 | 19.5 | 7.2 | 3.0 | 18x43x43 | 16x42x41 | 20x42x48 |
| 70 | 23.3 | 12.8 | 4.7 | 10x73x75 | 20x43x48 | 19x41x140 |

Table.S3. Parameters of protein-protein interaction potentials and dimensions of ellipsoidal protein model for solution A. $T$ – solvent temperature, $J$ – attractive potential depth.

| $T$,°C | $J$ ($k_BT$) | | | ellipsoid axis, Å | | |
|---|---|---|---|---|---|---|
| | c=10mg/mL | c=20mg/mL | c=40mg/mL | c=10mg/mL | c=20mg/mL | c=40mg/mL |
| 25 | 8.5 | 6.3 | 3.8 | 17x42x42 | 15x42x40 | 17x48x41 |
| 50 | 9.0 | 5.8 | 5.3 | 19x39x57 | 19x42x49 | 17x44x41 |
| 55 | 9.2 | 5.8 | 5.5 | 19x39x59 | 18x41x48 | 17x45x40 |
| 60 | 8.0 | 7.3 | 8.3 | 16x38x62 | 18x43x49 | 17x65x41 |
| 65 | 24.0 | 10.4 | 8.7 | 19x42x58 | 14x44x70 | 17x70x70 |
| 70 | 28.9 | 20.9 | 8.5 | 23x54x65 | 17x67x62 | 17x70x75 |

Table.S4. Parameters of protein-protein interaction potentials and dimensions of ellipsoidal protein model for solution C. $T$ – solvent temperature, $J$ – attractive potential depth.

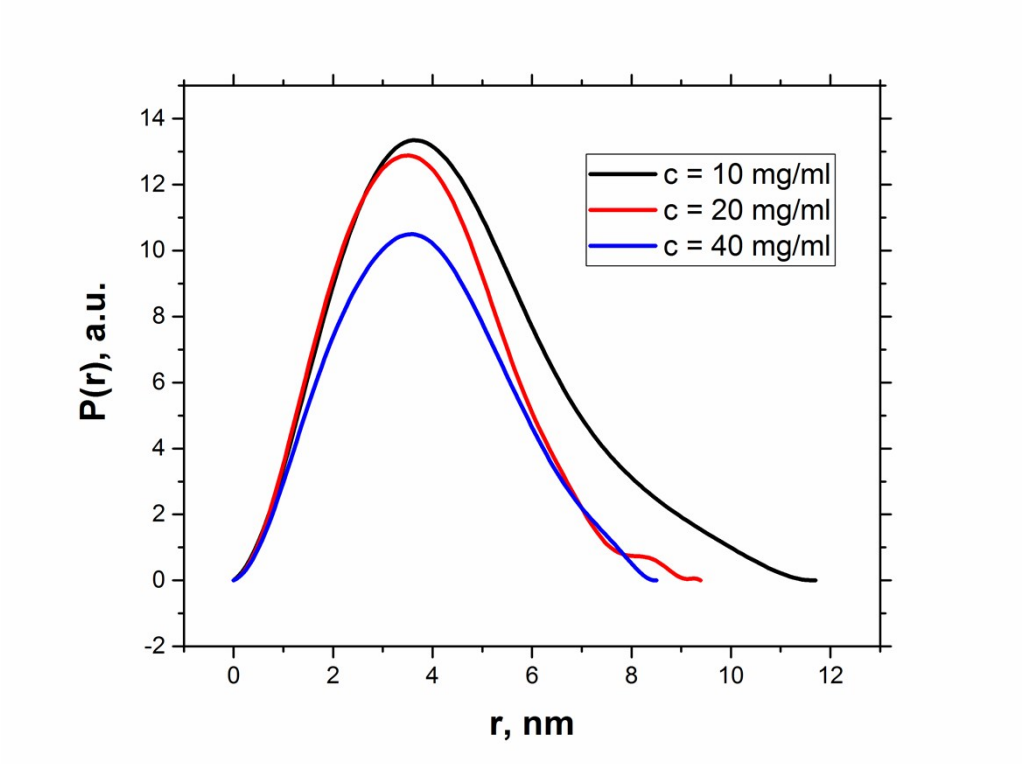



Fig.S12. *P(r)* functions for solution B at BSA concentrations 10/20/40 mg/ml, calculated on base of *I(q) /S(q)* curves.

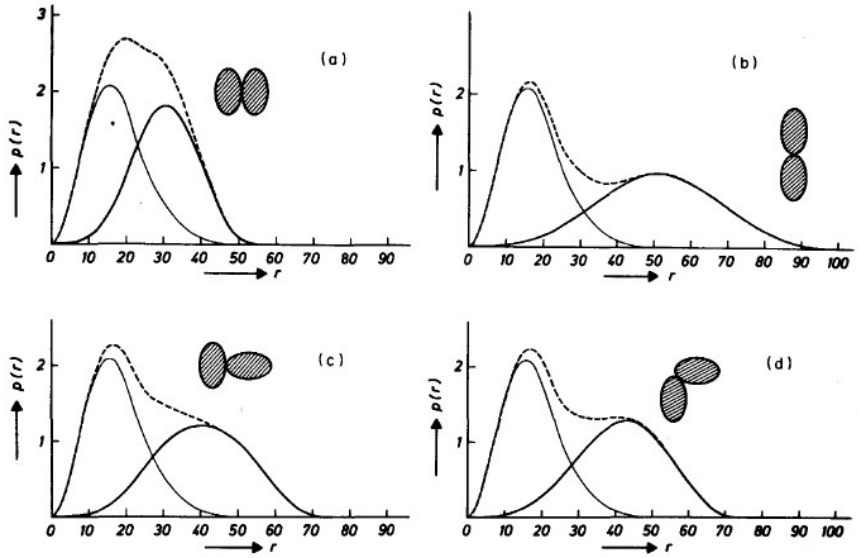



Fig.S13. Assessment of dimer formation for oblate ellipsoids using *P(r)* function analysis. (a) parallel formation, (b) linear formation, (c) T-type, (d) L-type (adapted from book of Glatter, Kratky [45]).