

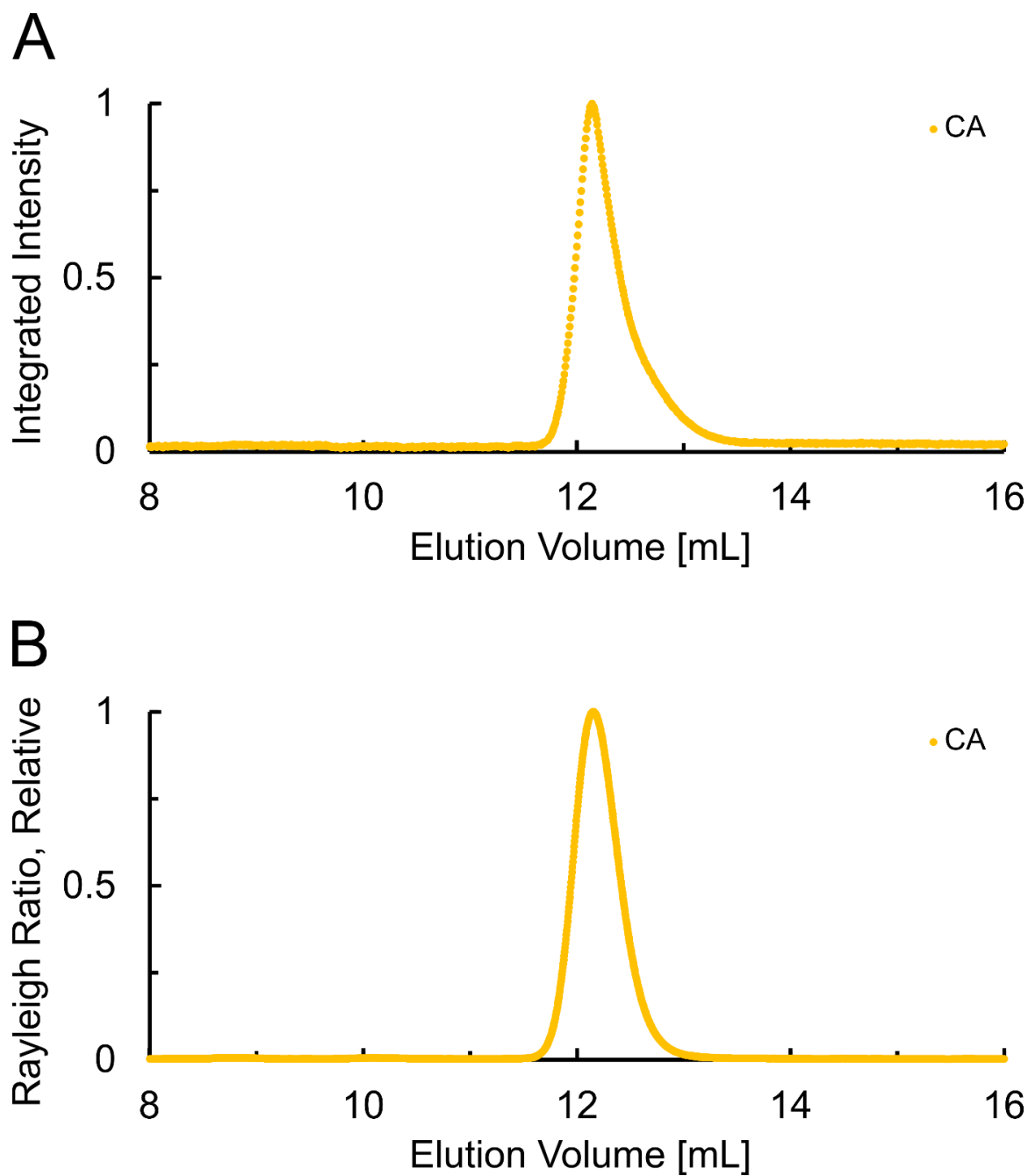
## **Adding Size Exclusion Chromatography (SEC) and Light Scattering (LS) Devices to Obtain High-Quality Small Angle X-Ray Scattering (SAXS) Data**

**Melissa A. Graewert <sup>\*</sup>, Stefano Da Vela, Tobias W. Gräwert, Dmitry S. Molodenskiy, Clément E. Blanchet, Dmitri I. Svergun <sup>\*</sup> and Cy M. Jeffries <sup>\*</sup>**

The European Molecular Biology Laboratory (EMBL) Hamburg, Notkestr. 85, 22607 Hamburg, Germany;  
sdavela@embl-hamburg.de (S.D.V.); t.graewert@embl-hamburg.de (T.W.G.);  
dmolodenskiy@embl-hamburg.de (D.S.M.); clement.blanchet@embl-hamburg.de (C.E.B.)

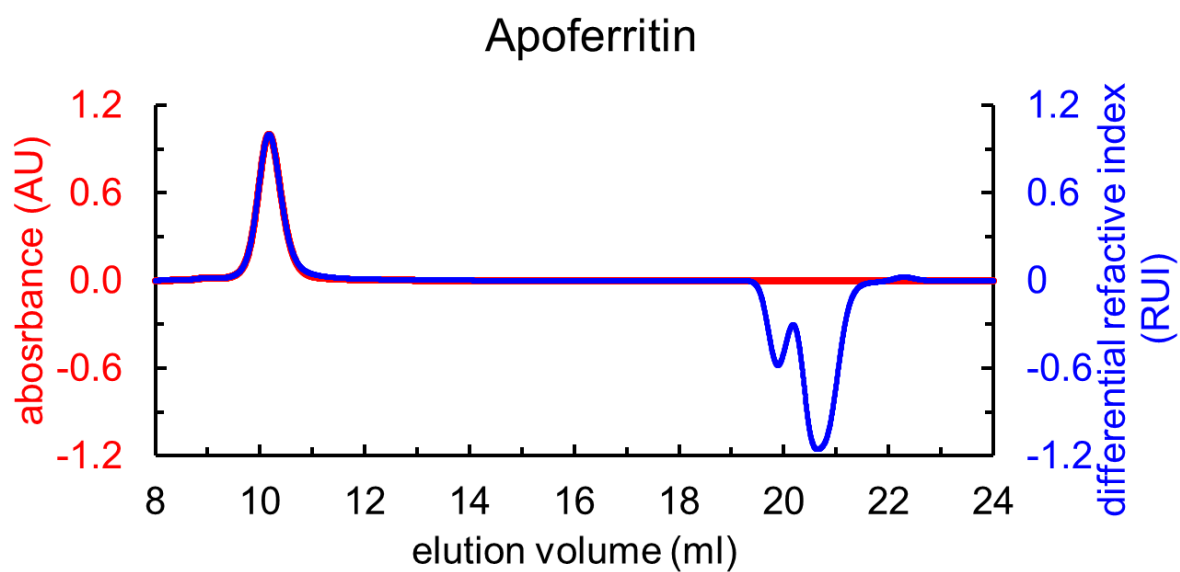
<sup>\*</sup> Correspondence: melissa.graewert@embl-hamburg.de (M.A.G.); svergun@embl-hamburg.de (D.I.S.);  
cy.jeffries@embl-hamburg.de (C.M.J.); +49-40-89902115 (M.A.G.); +49-40-89902125 (D.I.S.); +49-40-89902177  
(C.M.J.)

Supplement Figure S1



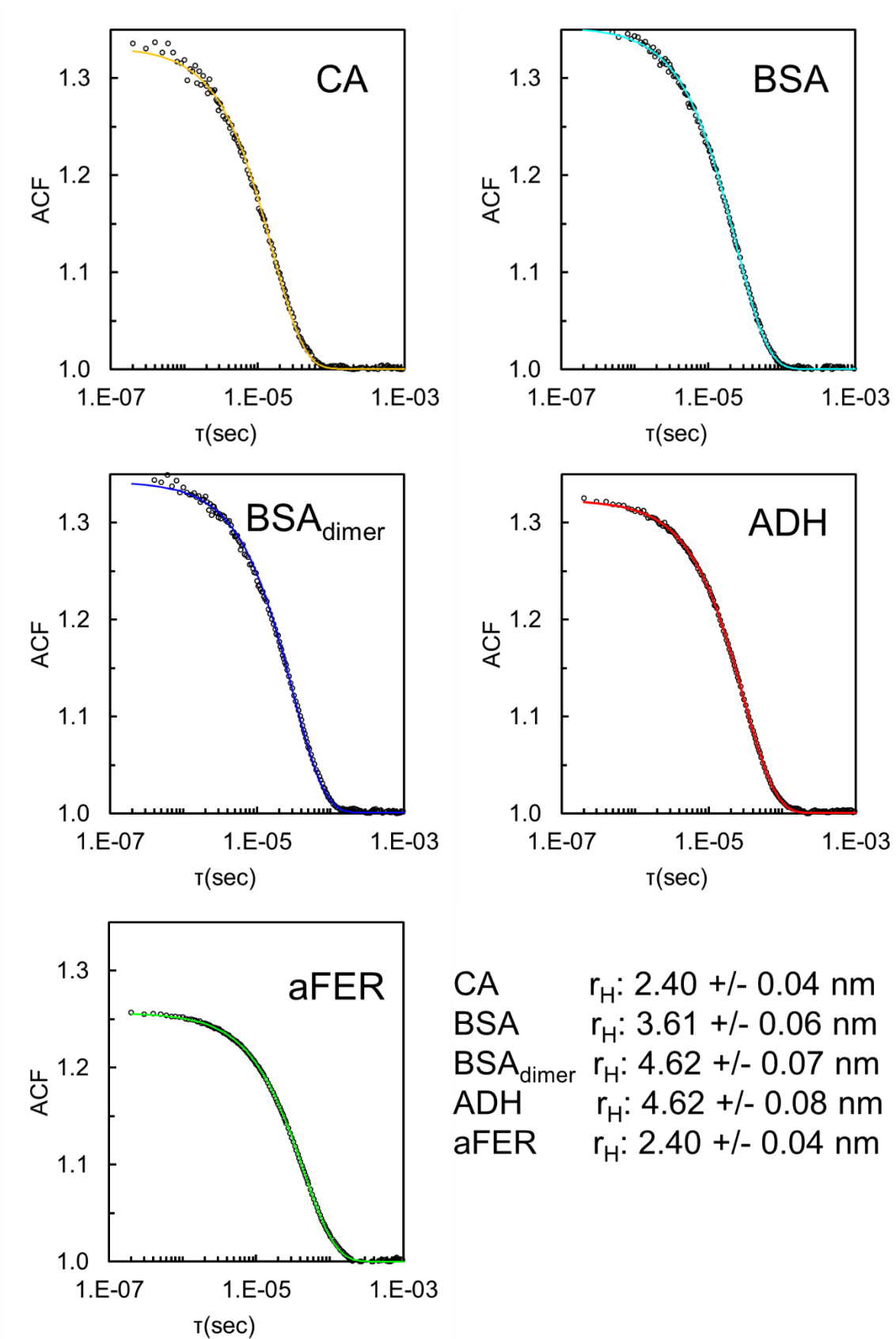
**Figure S1.** Elution profile of CA from Superdex 75 increase 10/300 column at 0.5 mL/min collected in SEC-SAXS/MALLS mode. Data is accessible as SASBDB accession code SASDFP8 (a) SAXS derived chromatogram – integrated intensities vs elution volume. (b) MALLS derived chromatogram – Rayleigh Ratio vs elution volume. Profiles are normalized for better comparison. Note, the other four samples are shown in Figure 2.

## Supplement Figure S2



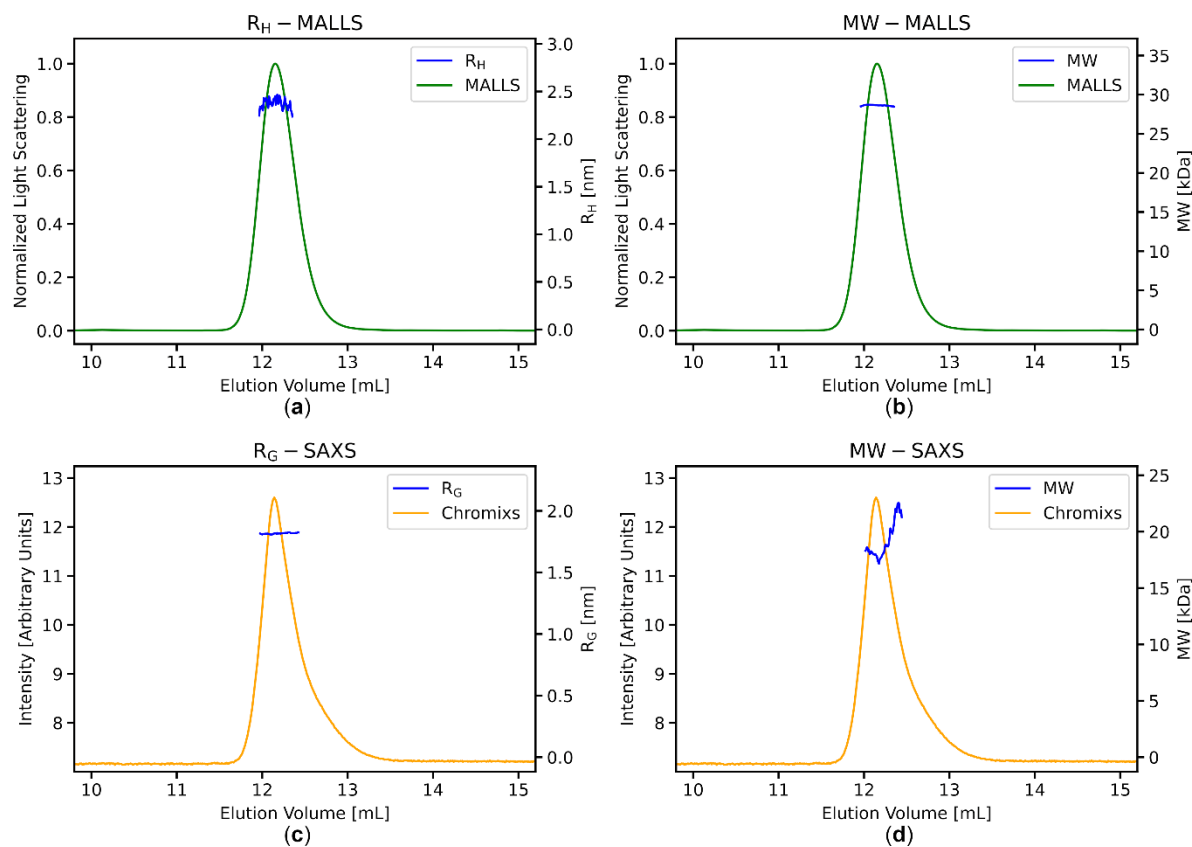
**Figure S2.** Elution profile of aFER from Superdex 200 increase 10/300 column at 0.5 mL/min collected in SEC-SAXS/MALLS mode. UV absorbance is shown in red, differential refractive index in blue.

Supplement Figure S3



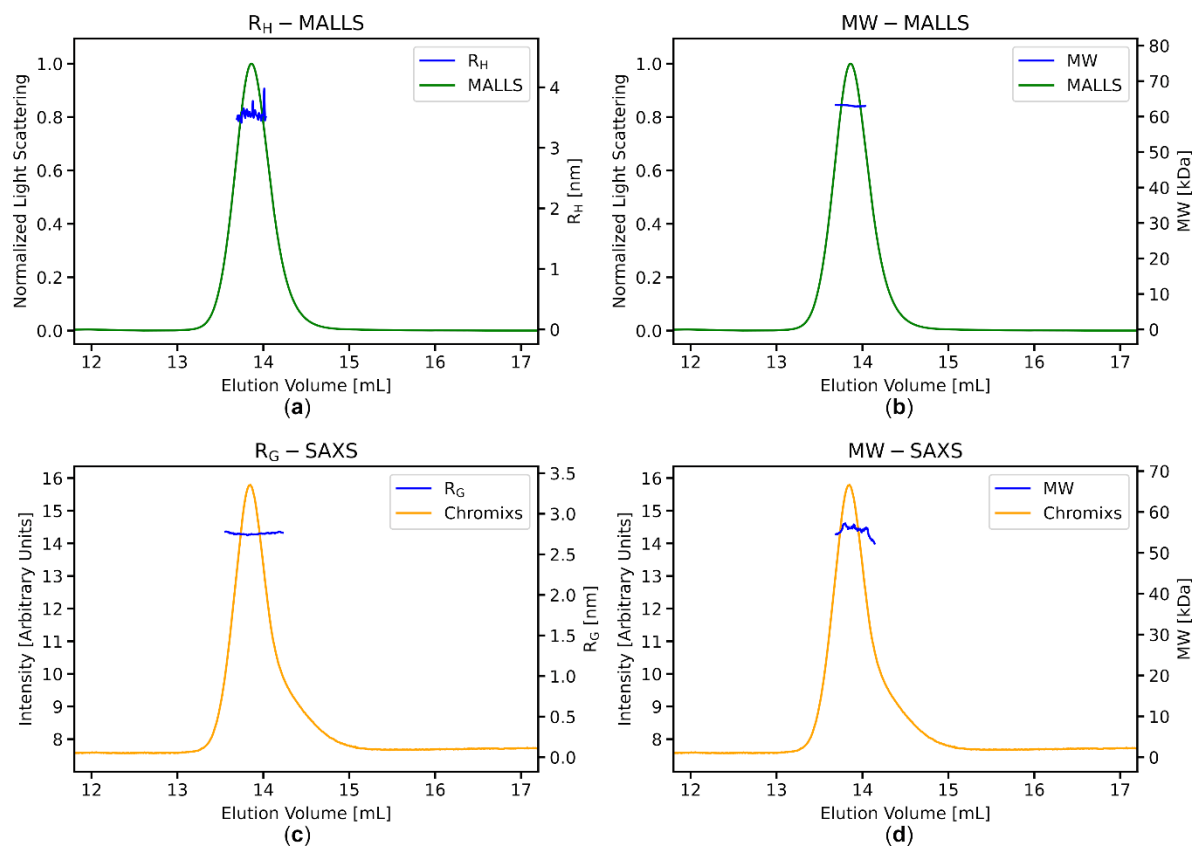
**Figure S3.** Autocorrelation functions (ACFs). Measured ACF in black, fitted nonlinear least squares algorithm. Fits shown in same colors as in Figure 5. Derived values for  $r_H$  are indicated.

## Supplement Figure S4



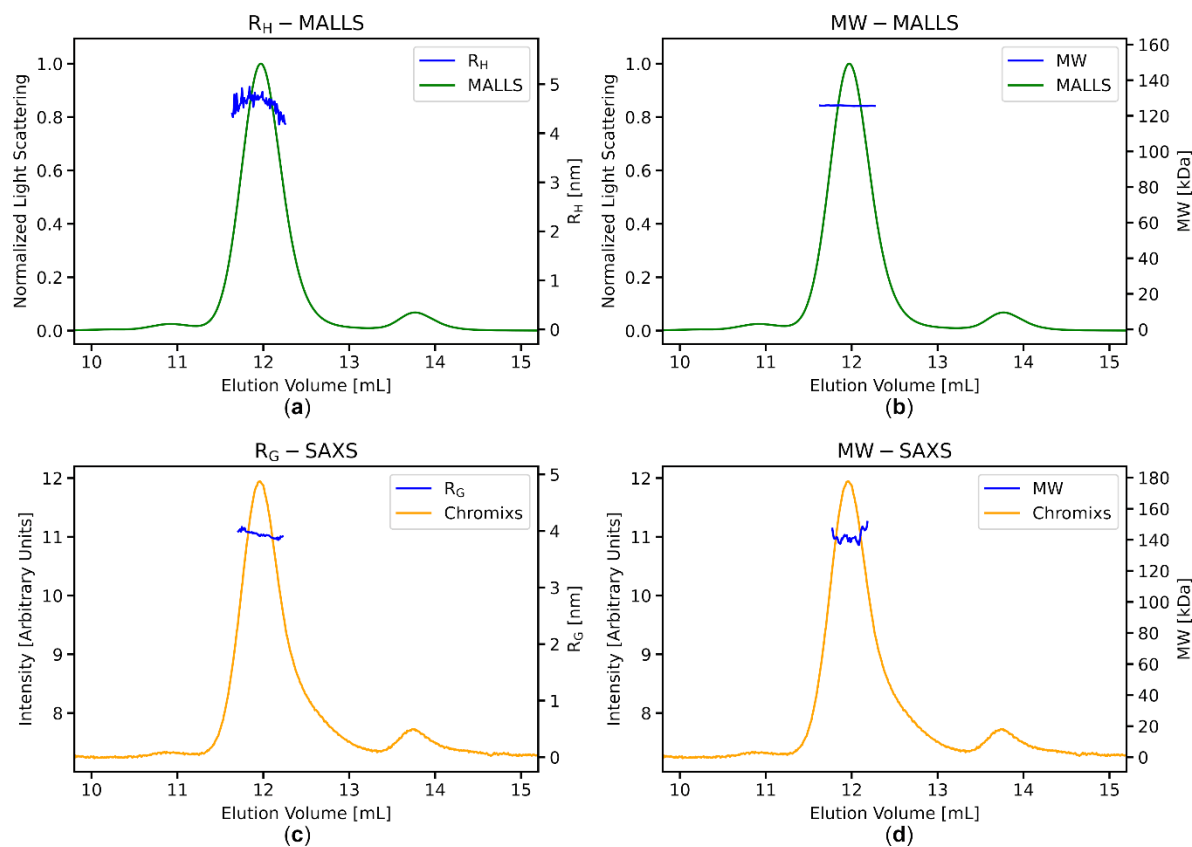
**Figure S4.** In detail characterization of CA. (a,b) MALLS derived chromatogram– Rayleigh Ratio vs elution volume in green with stable  $R_H$  (a) and  $MW_{MALLS}$  (b) across the elution peak. (c,d) SAXS derived chromatogram - integrated intensities vs elution volume produced by CHROMIXS (in orange) with stable  $R_G$  values across the elution peak (c) and Porod volume derived MW estimate (d).

## Supplement Figure S5



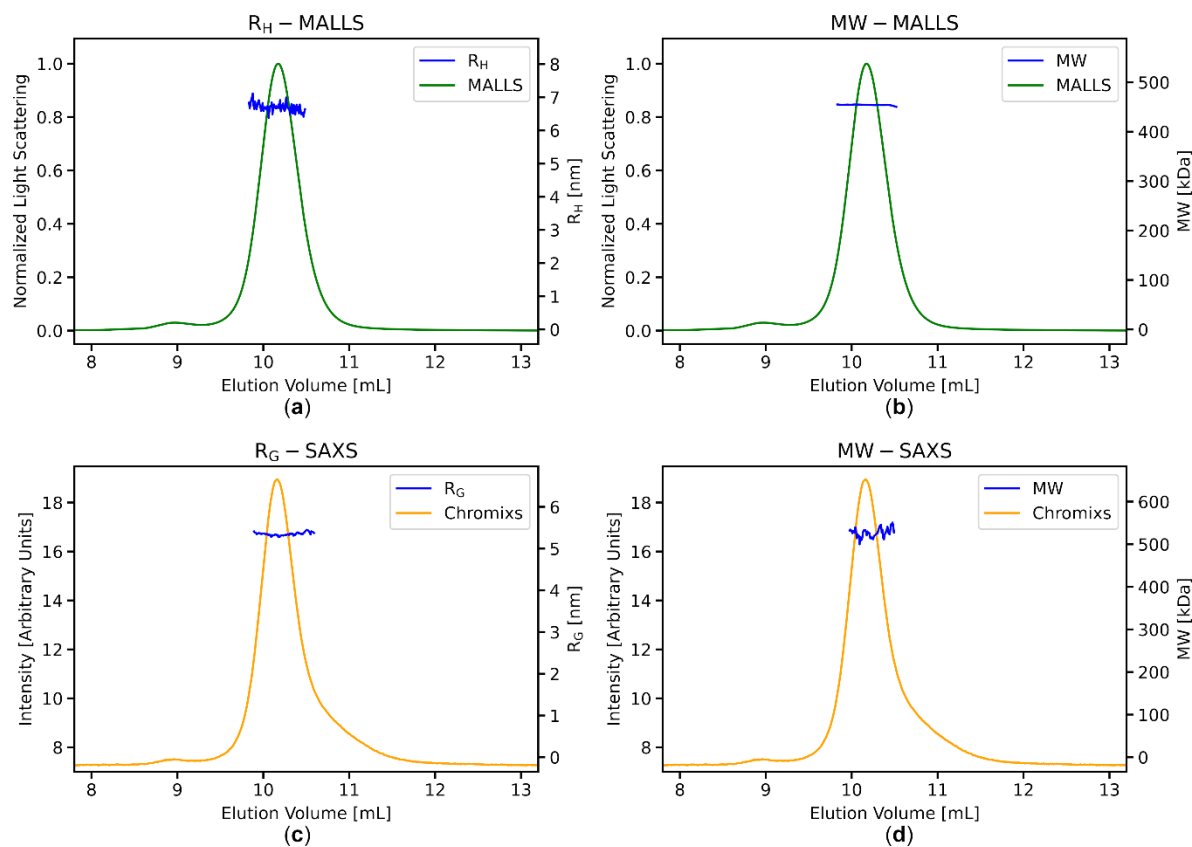
**Figure S5.** In detail characterization of monomeric BSA. (a,b) MALLS derived chromatogram– Rayleigh Ratio vs elution volume in green with stable  $R_H$  (a) and  $MW_{MALLS}$  (b) across the elution peak. (c,d) SAXS derived chromatogram - integrated intensities vs elution volume produced by CHROMIXS (in orange) with stable  $R_G$  values across the elution peak (c) and Porod volume derived MW estimate (d).

## Supplement Figure S6



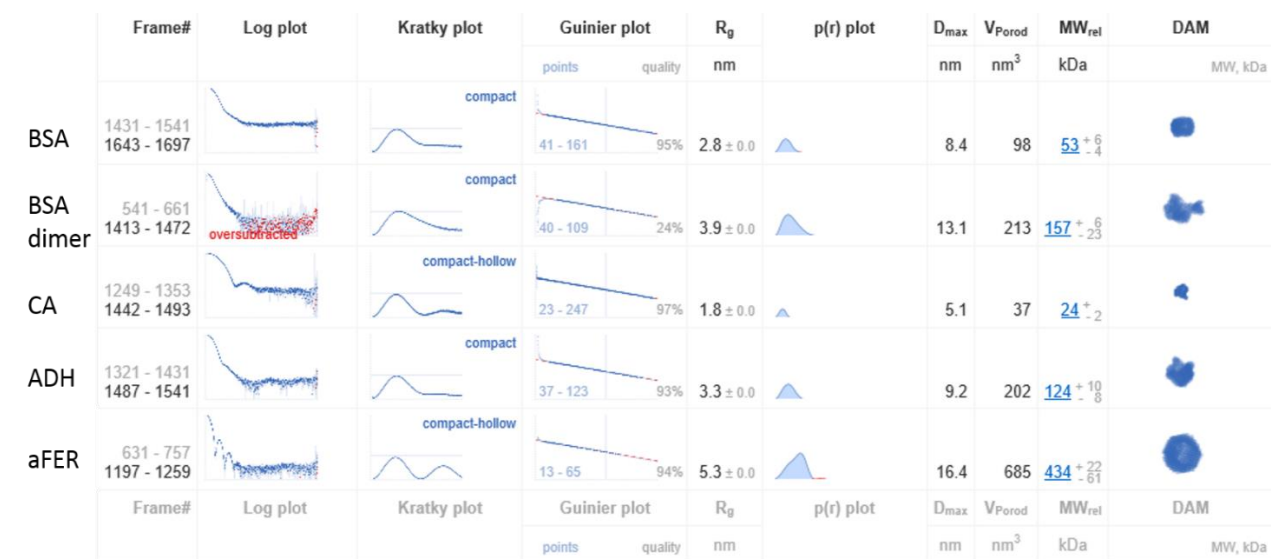
**Figure S6.** In detail characterization of dimeric BSA. **(a,b)** MALLS derived chromatogram– Rayleigh Ratio vs elution volume in green with stable  $R_H$  **(a)** and  $MW_{MALLS}$  **(b)** across the elution peak. **(c,d)** SAXS derived chromatogram - integrated intensities vs elution volume produced by CHROMIXS (in orange) with stable  $R_G$  values across the elution peak **(c)** and Porod volume derived MW estimate **(d)**.

## Supplement Figure S7



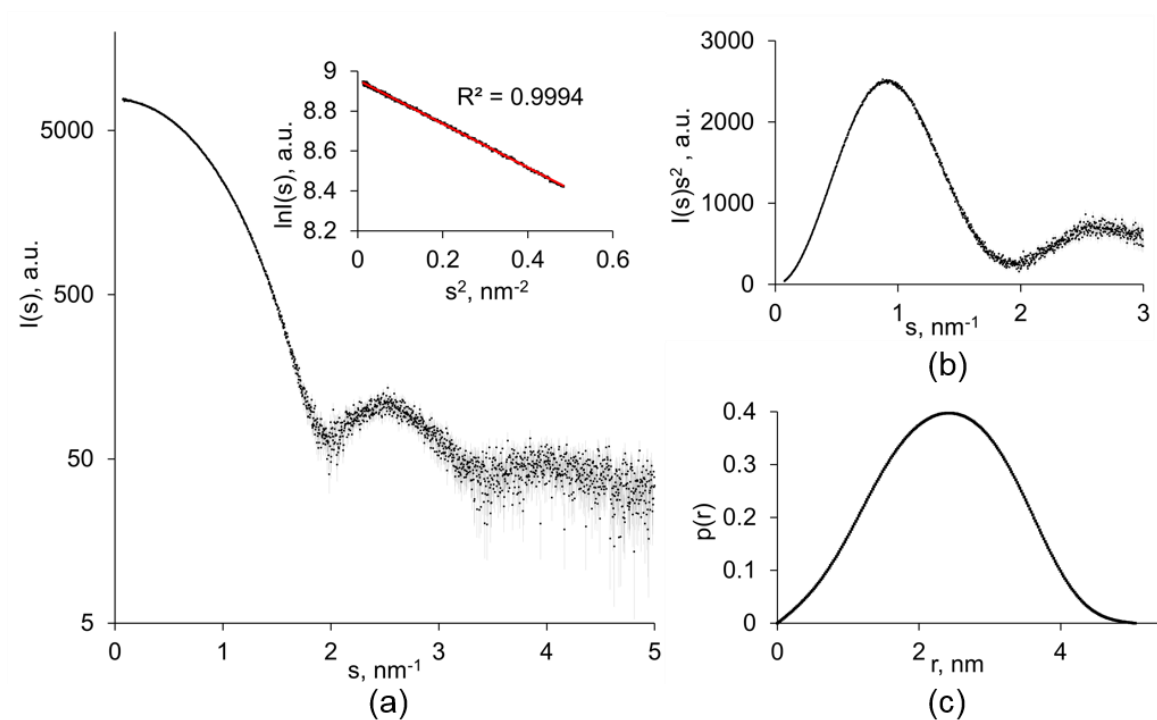
**Figure S7.** In detail characterization of aFER. **(a,b)** MALLS derived chromatogram– Rayleigh Ratio vs elution volume in green with stable  $R_H$  **(a)** and  $MW_{MALLS}$  **(b)** across the elution peak. **(c,d)** SAXS derived chromatogram - integrated intensities vs elution volume produced by CHROMIXS (in orange) with stable  $R_G$  values across the elution peak **(c)** and Porod volume derived MW estimate **(d)**.

## Supplement Figure S8



**Figure S8.** Automated output summary from SASFLOW/CHROMIXS. Key parameters and structural analysis are shown for all samples measured. Figure has been cropped for better visuality.

## Supplement Figure S9



**Figure S9.** SASBDB entry. SASDFP8- Carbonic anhydrase from bovine erythrocytes SEC-SAXS coupled to multiangle laser and quasi-elastic light scattering (MALLS and QELS);

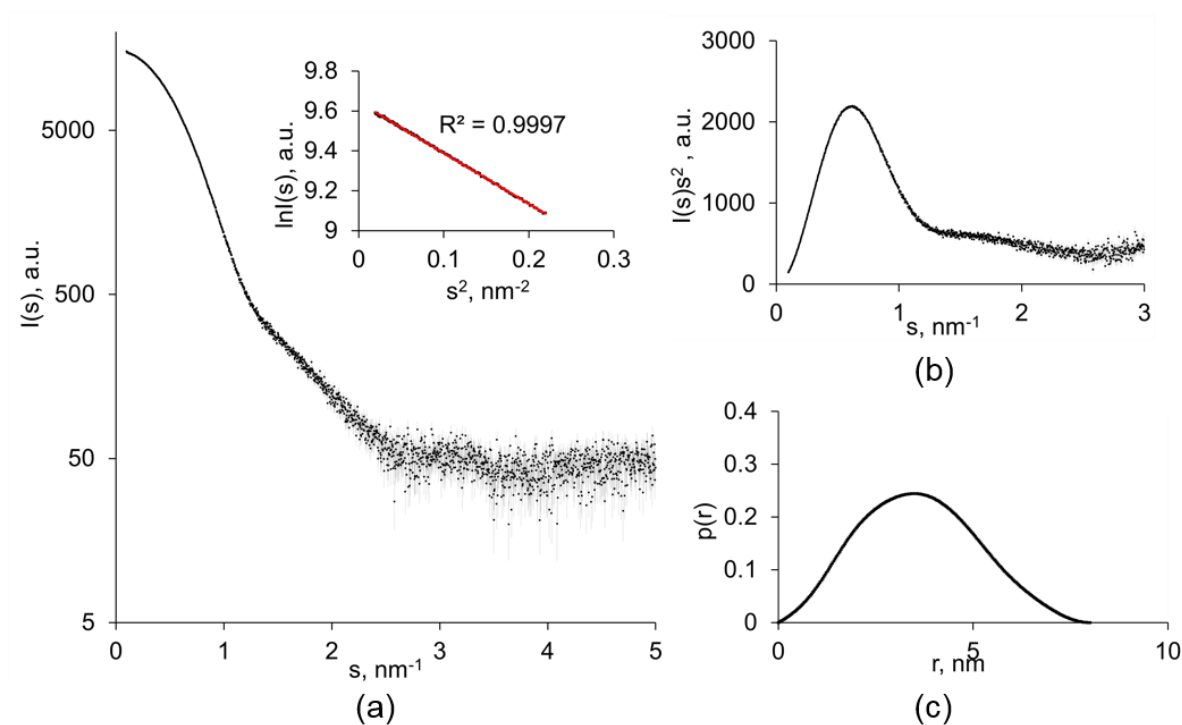
$MW_{\text{experimental}}$  28 kDa

$MW_{\text{expected}}$  29 kDa

$\text{Porod}_{\text{Volume}}$  37  $\text{nm}^3$

- (a) SAXS scattering plot in log-linear scale. Guinier analysis is shown as inlay.
- (b) Kratky plot
- (c)  $p(r)$  function

## Supplement Figure S10



**Figure S10.** SASBDB entry. SASDFQ8- Bovine serum albumin, purified monomer SEC-SAXS coupled to multiangle laser and quasi-elastic light scattering (MALLS and QELS);

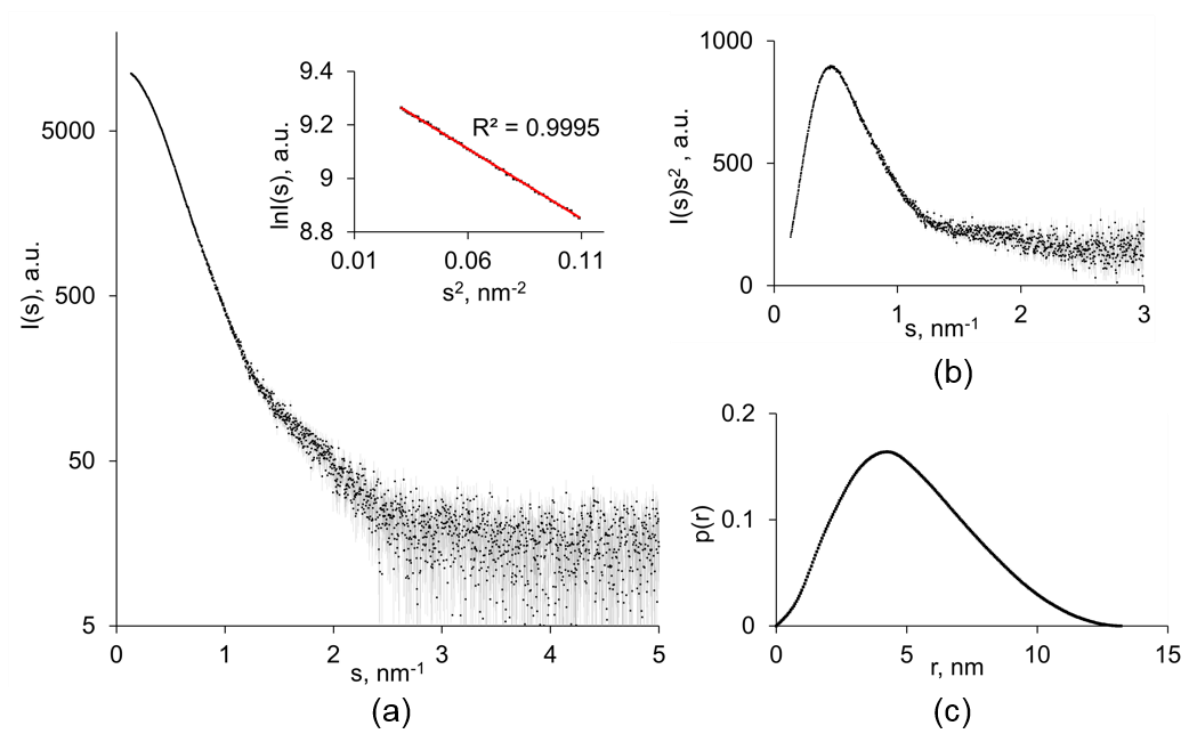
$MW_{\text{experimental}}$  63 kDa

$MW_{\text{expected}}$  66 kDa

$\text{PorodVolume}$  98  $\text{nm}^3$

- (a) SAXS scattering plot in log-linear scale. Guinier analysis is shown as inlay.
- (b) Kratky plot
- (c)  $p(r)$  function

## Supplement Figure S11



**Figure S11.** SASBDB entry. SASDFR8- Bovine serum albumin, purified dimer SEC-SAXS coupled to multiangle laser and quasi-elastic light scattering (MALLS and QELS);

$MW_{\text{experimental}}$  126 kDa

$MW_{\text{expected}}$  133 kDa

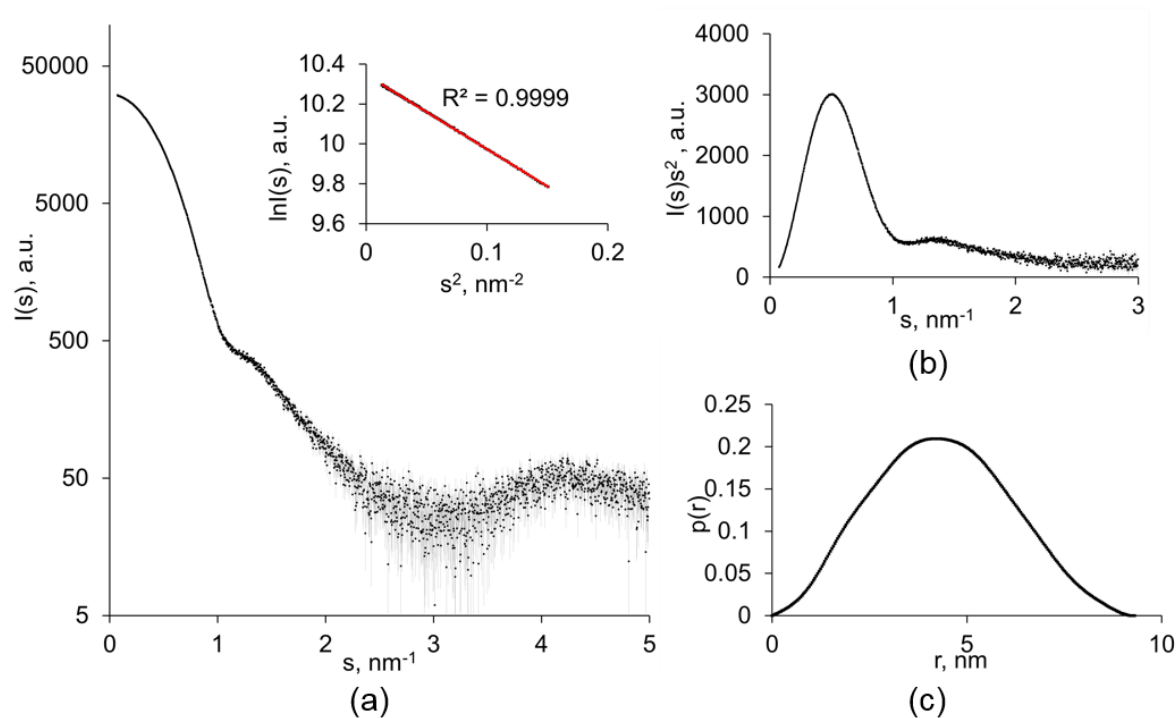
$\text{Porod}_{\text{Volume}}$  211  $\text{nm}^3$

(a) SAXS scattering plot in log-linear scale. Guinier analysis is shown as inlay.

(b) Kratky plot

(c)  $p(r)$  function

## Supplement Figure S12



**Figure S12.** SASBDB entry. SASDF58- Yeast alcohol dehydrogenase 1 SEC-SAXS coupled to multiangle laser and quasi-elastic light scattering (MALLS and QELS);

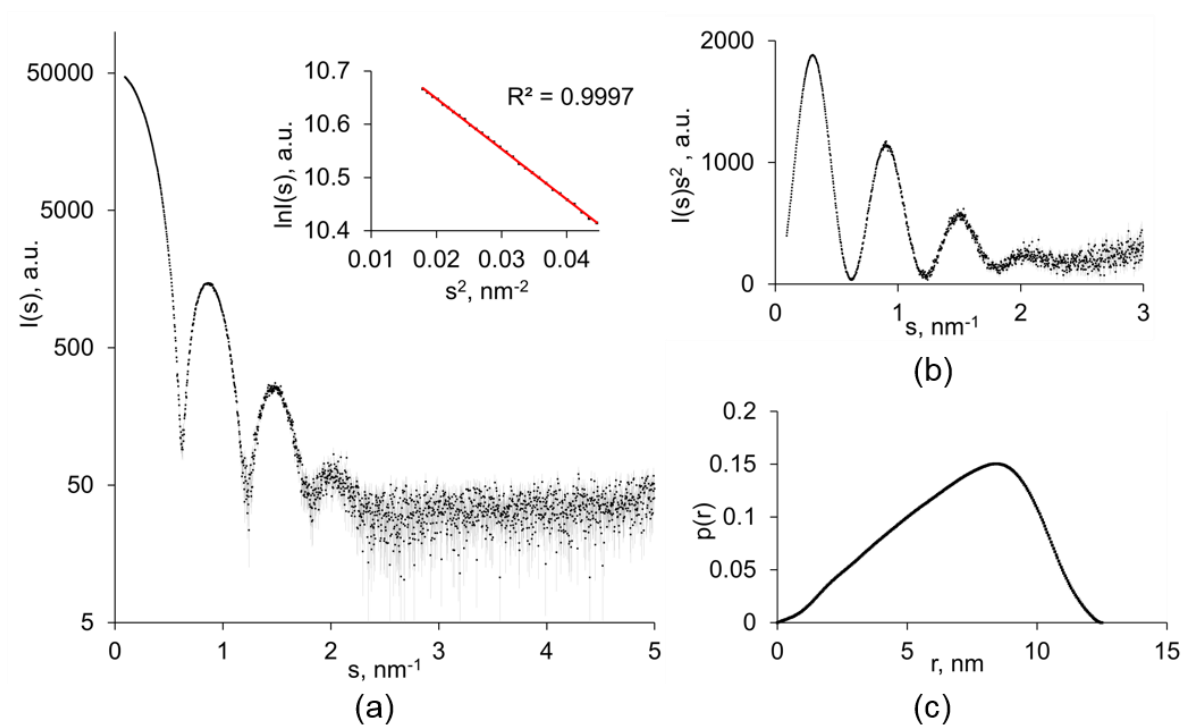
$MW_{\text{experimental}}$  142 kDa

$MW_{\text{expected}}$  147 kDa

$\text{PorodVolume}$  201  $\text{nm}^3$

- (a) SAXS scattering plot in log-linear scale. Guinier analysis is shown as inlay.
- (b) Kratky plot
- (c)  $p(r)$  function

## Supplement Figure S13



**Figure S13.** SASBDB entry. SASDFN8-Apoferritin horse spleen SEC-SAXS coupled to multiangle laser and quasi-elastic light scattering (MALLS and QELS); Apoferritin light chain.

$MW_{\text{experimental}}$  454 kDa

$MW_{\text{expected}}$  479 kDa

$\text{PorodVolume}$  679  $\text{nm}^3$

- (a) SAXS scattering plot in log-linear scale. Guinier analysis is shown as inlay.
- (b) Kratky plot
- (c)  $p(r)$  function