Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information (ESI)

Pearl-necklace assembly of Human serum albumin with poly (acrylic acid) polyelectrolyte explored by small angle X-ray scattering (SAXS)

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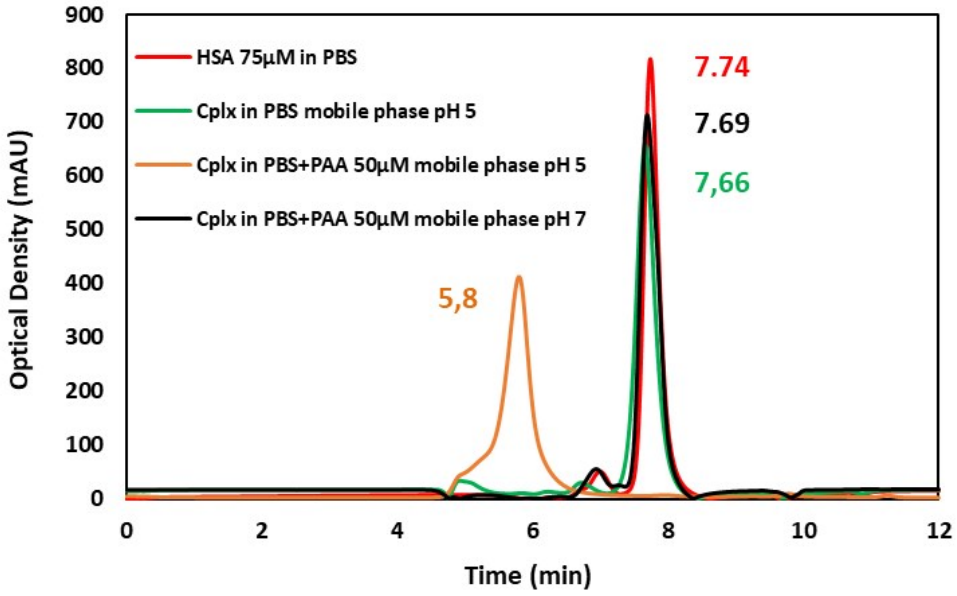
S1-Study of HSA-PAA binding reversibility

The complex stability has been examined by chromatography. For that, the single HSA at 75µM was first investigated in PBS 1X mobile phase and was detected at 7.74 min. After that, in the same mobile phase PBS 1X but at pH 5 the complex (HSA 75µM+PAA 50µM) was injected but a peak with almost the same time of retention of HSA was detected at 7.66 min. Almost the same peak was observed then at 7.69 min for a complex sample but tested this time in PBS+50µM PAA mobile phase at pH 7. Whereas in the same mobile phase but at pH 5 a different peak was obtained at 5.8 min for the complex.



S1-Figure 1. Comparison of chromatographic patterns for HSA 75µM in PBS 1X mobile phase (red peak) and for (75µM-50µM) HSA-PAA mixture in PBS 1X (black line), in 50 µM PAA solution mobile phase at pH 5 (orange peak) and at pH 7 (green peak).

These chromatographic patterns (S1-Figure 1) highlight the fact that even the samples tested are prepared under conditions that allow complexation to happen (pH 5; I=0.137M), the HSA-PAA complex tends to dissociate once put in a medium diluted in PAA or pH greater than 5. In fact, when complex was put in PBS mobile phase even at pH 5 or in PBS+PAA 50µM but at pH 7, the HSA peak was detected because this last is free in solution and not linked to PAA chains.

This outcome not only uphold the previous results regarding pH, but also prove that HSA-PAA complex is not stable in diluted medium. Therefore, the protein molecules initially bounded to the polyelectrolyte inside the samples could be then detached changing the medium, which means that this binding phenomenon that has found to take place is reversible.

S2- SAXS data analysis

The SAXS data (*I(q)* being the scattering intensity obtained from SAXS measurement) have been analysed to obtain the main properties of the HSA and the HSA-PAA complex. The home code is found in the following link:

https://github.com/CharafMerzougui/Supporting-information/blob/master/Porod%20volume%20estimation%20-%20modif%20.ipynb

Determination of the radius of gyration and *I(0)*

The radius of gyration is obtained by applying the following equation at small q:

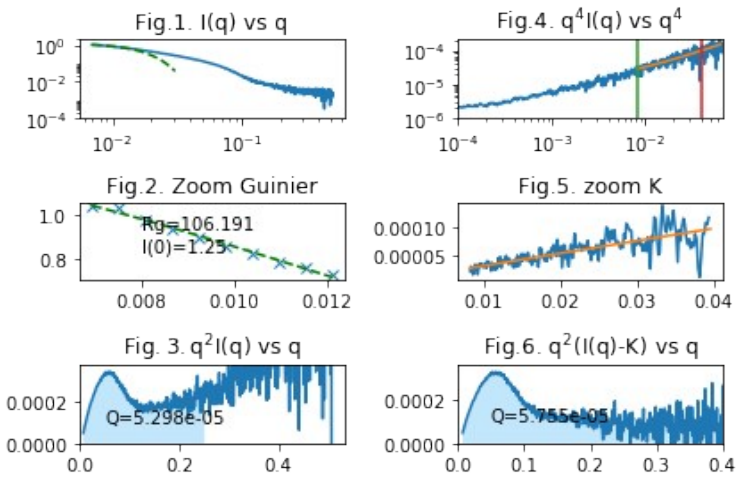
$$\lim_{q\to 0} I = I_0 exp\left(-\frac{1}{3}q^2 R_g^2\right)$$

Close to q=0, the scattering intensity of a particle is described by a Gaussian curve. The Guinier law is equivalent of a linear variation of *Ln I(q) vs* q^2 (Guinier plot), providing R_g and I_0. The code performs the curve fitting to obtain the values of R_g and I_0 for the 10 first values of q. Here q is then less than 0.012 and should then met the criteria $q.R_g < 1.5$ if R_g is less than 125 nm.

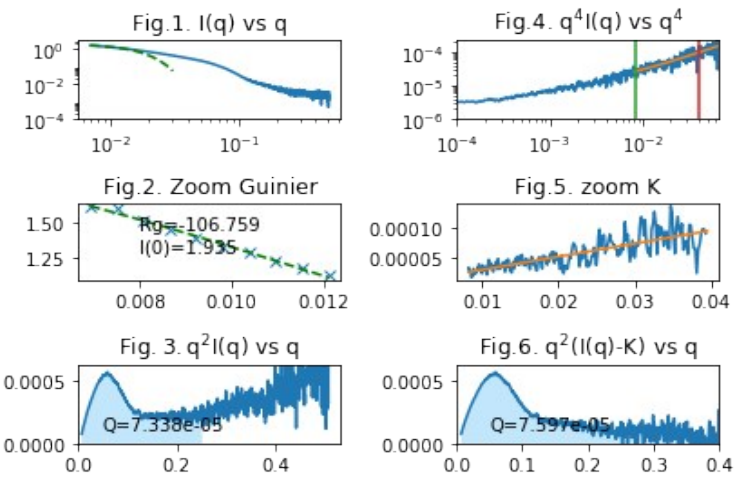
It can be checked from Fig.1 and Fig.2 that the data fitting is correct: there is no deviation from the straight line indicating no intermolecular interaction or aggregation in this concentration range.

Determination of the Porod volume

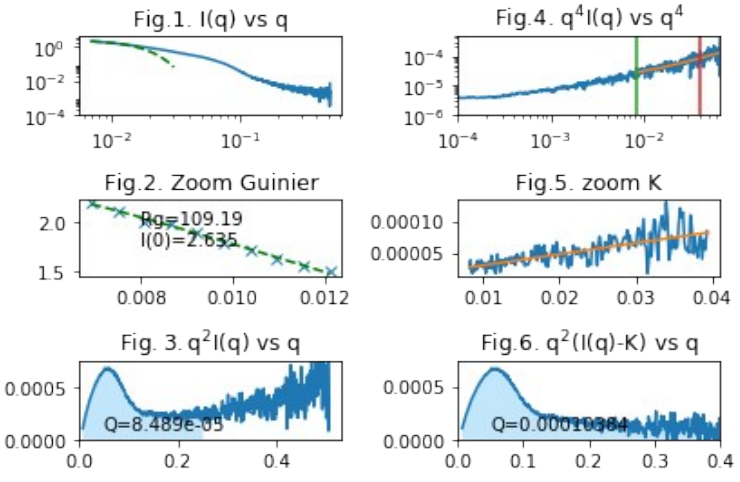
The calculations of the Porod volume (see Experimental section in the main manuscript) were done for all the studied HSA/PAA molar ratios (1.5 to 12) and were performed using the Python code mentioned above. The table below summarize the figures that show the estimation of radius of gyration R_g, the constant K as well as the invariant Q, which allow then the evaluation of the Porod volume for the studied range of HSA/PAA molar ratio.



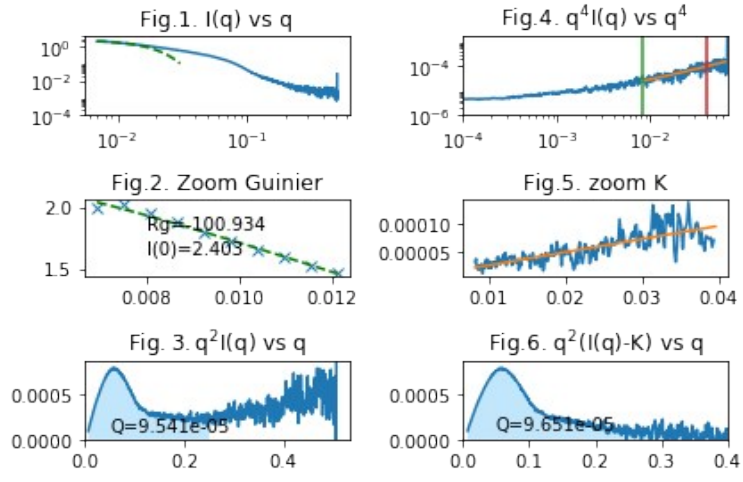
[HSA]/[PAA] =1.5



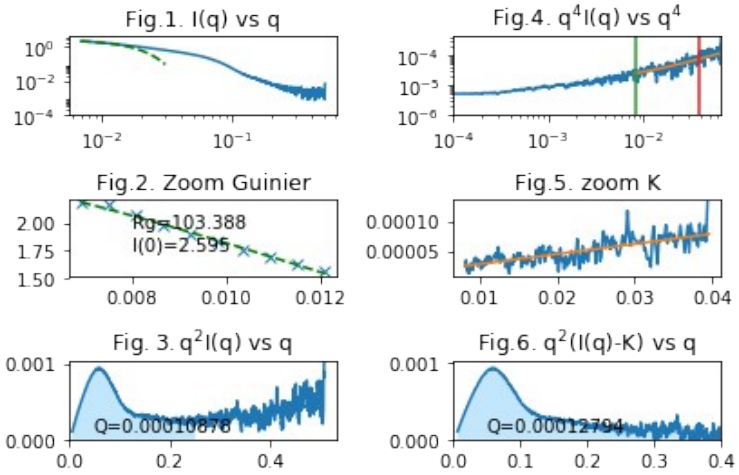
[HSA]/[PAA] =3



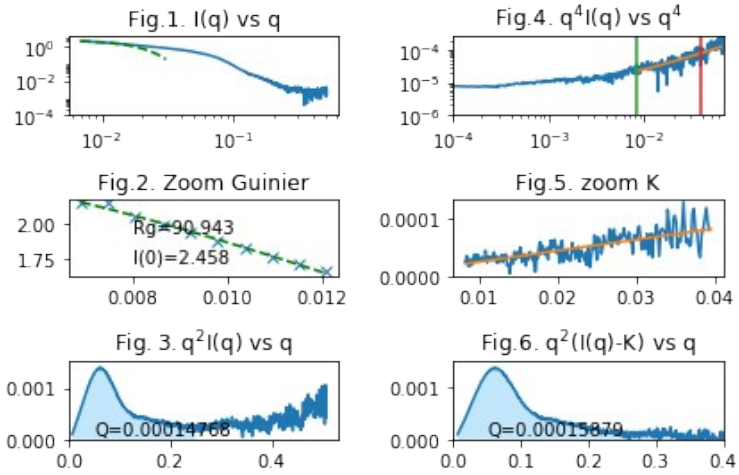
[HSA]/[PAA] =3.6



[HSA]/[PAA] =4.5

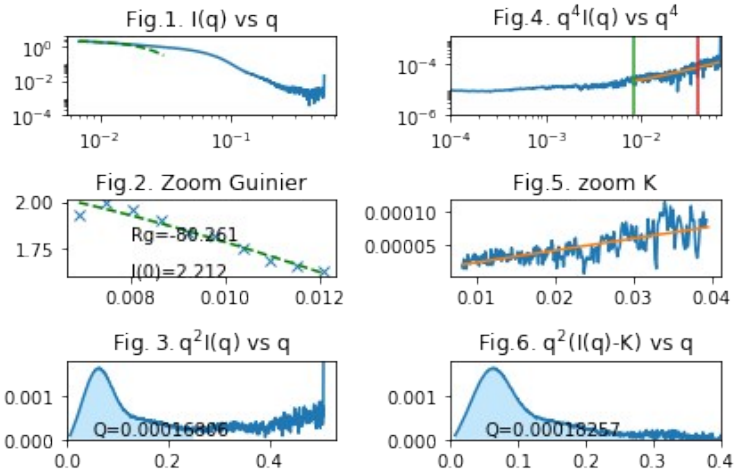


[HSA]/[PAA] =6

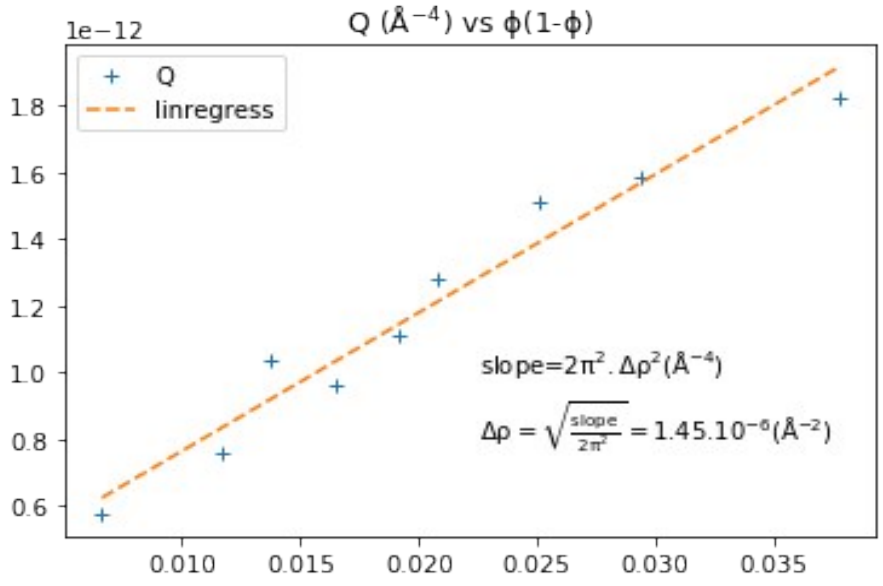


[HSA]/[PAA] =9

[HSA]/[PAA] =12



It has to be noted that the value of Q has been plotted as a function of $\phi(1-\phi)$ to estimate the value of $\Delta\rho$ of the mixture. The obtained result 1.45. 10^{-6} Å^2 (S2-Figure 1) seems to be consistent with the contrast of proteins found to be around 2. 10^{-6} Å^2 (Svergun et Koch, 2003) and also with what was found for surfactants (Bombelli et al., 2002).



S2-Figure 1. Calculation of the contrast Δρ of the mixture HSA+PAA from the slope of the linear regression of the invariant Q as function of ϕ(1-ϕ) for an HSA/PAA malar ratio ranging from 1.5 to 12.

S3- Stochiometry Model for the volume fraction calculation of the HSA, PAA, HSA-PAA complex

One considers a mixture of HSA and PAA with the molar concentration $[HSA]_0$ and $[PAA]_0$ respectively that allows the formation of a complex with the following stoichiometry:

$$n\,HSA\ +\ 1\,PAA \rightarrow 1\,complex(PAA-nHSA)$$

For a progress of the complexation corresponding to a molar concentration in complex [c]:

$$[PAA] = [PAA]_0 - [c]$$

$$[HSA] = [HSA]_0 - (n * [c])$$

If the PAA is on excess ($[HSA]_0 < n[PAA]_0$), the composition in the mixture is:

$$[c] = \frac{[HSA]_0}{n}$$

$$[PAA] = [PAA]_0 - \frac{[HSA]_0}{n}$$

If HSA is on excess ($[HSA]_0 > n[PAA]0$), the composition in the mixture is:

$$[c] = [PAA]_0$$

$$[HSA] = [HSA]_0 - (n * [PAA]_0)$$

Knowing the concentration, one can define the volume fraction of the mixture as:

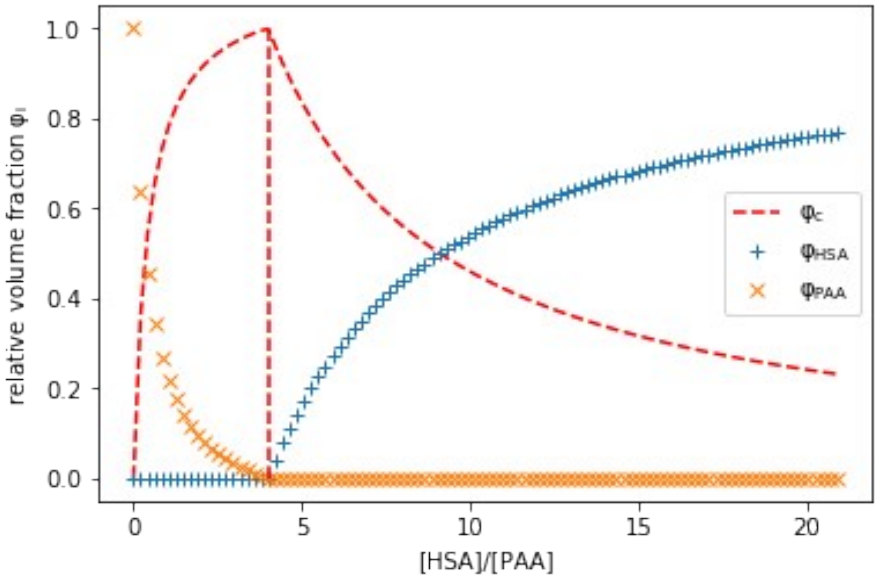
$$\phi = N_{Avo}\ ([c]V_c + [HSA]V_{HSA} + [PAA]V_{PAA})$$

Where V_c, V_{HSA}, V_{PAA} are the volume of a molecule of complex, HSA and PAA respectively that were evaluated from SAXS analysis using Primus of isolated solute and found to be around: 500.10^3, 100.10^3 and 50.10^3 Å^3 for complex, HSA and PAA and respectively.

The relative volume fraction of the complex in the mixture is then:

$$\varphi_c = \frac{N_{Avo}.[c].V_c}{\phi}$$

From this complexation model, one can then determine the evolution of the relative volume fraction of HSA, PAA and the complex (S3-Figure 1) for a given value of the stoichiometry coefficient n.



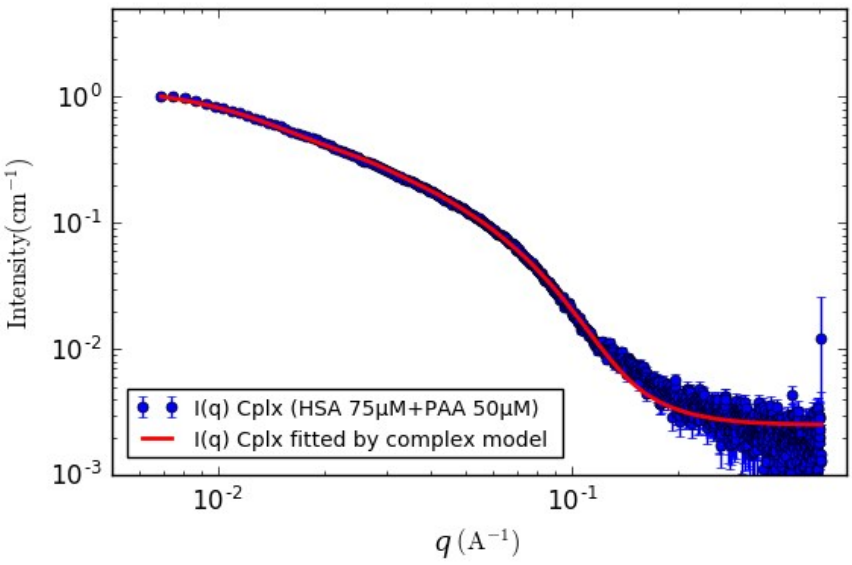
S3-Figure 1. The relative volume fraction of HSA, PAA and the complex as a function of [HSA]/[PAA] molar ratio calculated with the complexation model for a stoichiometry coefficient n=4

The computation was carried out using a Python code found in the following link:

https://github.com/CharafMerzougui/Supporting-information/blob/master/Porod%20volume%20estimation%20-%20modif%20.ipynb

S4-HSA-PAA complex structure modelling

As shown in the paper, the first analysis of autocorrelation *P(r)* of the complex revealed an elongated form such as a cylinder (Radius≈40 Å; Length≈400 Å), a shape that was found to fit well the SAXS experimental *I(q)* data of the HSA-PAA complex (red line S4-Figure 1). But, the radius obtained from the fit using Sasview yields 25 Å, which is smaller than the radius of the complex assimilated to a cylinder obtained from the *P(r)* (39 Å). That could be due to the fact that the value of complex radius estimated from the cross section gyration radius R_c using Sasview provides a mean value of the distances forming the section of the complex. The calculated value reflects probably an average of the radius of HSA and the diameter of the polymer, which is smaller than that of HSA.



S4-Figure 1. The SAXS scattering intensity for HSA-PAA complex as dotted thick blue line, fitted by cylinder model (Radius≈25 Å ; Length≈400 Å) as red line.