Understanding the mechanisms driving the formation of complexes of polyelectrolytes and proteins of opposite charges is of a fundamental importance as such complexes are often encountered in biological or industrial situations [1]. A large variety of macroscopic structures can be formed depending on the kind and strength of the interactions involved in the system. We show here how the combination of SANS with contrast matching permits the full determination of the structures formed by a model system. This system, made of lysozyme (positively charged protein) and PSSNa (negatively charged polyelectrolyte), is both model from the physico-chemical point of view and from a neutron contrast point of view because $\rho_{\text{PSSNa}} = \rho_{\text{lyso}}$. 

Firstly, we have used PSSD to watch separately the protein and the polyelectrolyte inside the complexes. Three main structures can be formed in the system when changing the ratio of negative to positive charges $[-]/[+]_{\text{intro}}$ and the length of the PSS chains [2] for a given pH and a given ionic strength (50mM): (i) For $[-]/[+]_{\text{intro}} < 5$ and for long PSS chains, the structure is a network formed by PSS chains cross-linked by lysozyme (Fig 1.b). Macroscopically, samples are gels. (ii) For $[-]/[+]_{\text{intro}} < 5$ and for short PSS chains, lysozyme and PSS chains are embedded in dense 3-D aggregates that arrange in a fractal network at a larger scale (Fig 1.c). Macroscopically, samples are liquid. (iii) For $[-]/[+]_{\text{intro}} > 5$ and whatever the chain length, the internal structure of the lysozyme changes. After an initial strong electrostatic binding, lysozyme is progressively unfolded thanks to an hydrophobic contact with PSS. The two chainlike objects are finally organized in a homogeneous costructure (not shown here). We focus then on the role of $[-]/[+]_{\text{inner}}$ structure on the dense 3-D aggregates formed by small chains [3]. The primary complexes are always formed with radii around 10 nm and organize at a higher scale in aggregates of fractal dimension 2.1. The systematic use of the contrast matching of PSS$_d$ in SANS have allowed the determination of the species composition and the water content, yielding the compactness and the inner charge ratio $[-]/[+]_{\text{inner}}$. The primary complexes have: (i) an inner charge ratio $[-]/[+]_{\text{inner}}$ close to 1 whatever $[-]/[+]_{\text{intro}}$, (ii) a high total volume fraction (0.25 to 0.4), (iii) an increases of the radius with an increase of $[-]/[+]_{\text{intro}}$ from 75 Å up to 150 Å and a (iv) shell of PSS chains when $[-]/[+]_{\text{intro}} > 1$. There are free proteins if $[-]/[+]_{\text{intro}} < 1$ and free PSS chains if $[-]/[+]_{\text{intro}} >> 1$. This inner charge stoechiometry is recovered for different charge density of components (tuned by pH for lysozyme and by sulfonation rate for PSS) and proves the dominant role of direct electrostatic interactions in complexation. The primary complexes aggregate at a higher scale with a fractal dimension of 2.1 characteristic of Reaction Limited Colloidal Aggregation often found in charged systems. The size of primary complexes is limited by the total ionic strength of solution.

We present now to experiments with specific labelling tricks that takes benefit from $\rho_{\text{PSSNa}} = \rho_{\text{lyso}}$ to go further in the understanding of the complexation process.

**Conformation of a PSS chain within complexes [4]:**
The pictures of the two types complexes that can be formed when $[-]/[+]_{\text{intro}} < 5$ (gel or dense globules) suggest that the transition between the two regimes correspond to the overlapping concentration of the chains $c^*$: when the chains are in semi-dilute regime, the network pre-formed by PSS chains is cross-linked by proteins but when they are in dilute regime, chains collapse with proteins to form globules. In order to check this assumption, the persistence length $l_p$ of the chain within the gel has to measured to get $c^*$. $c^*$ is indeed proportional to $1/N_1^{3/2} l_p^{3/2}$ where N is the number of repetitions units per chain. Experimentally, the system shifts from one regime to another for an N lying between 350 and 600 for [PSS] = 0.1 mol/L with [lyso] = 40g/l and I = 50mM. But if one calculate $c^*$ with $l_p = 50\text{Å}$ (value taken from literature for pure PSS solutions), the system must stay in semi-dilute regime for $N > 30$. The chains should thus be largely shrunk when interacting with proteins.
In order to measure $l_p$, we have made several mixtures of PSSH, PSS D and lysosyme in a solvent that matches both PSSH and lysosyme with $[\text{PSS}]+[\text{PSSD}] = 0.1 \text{ mol/L}$ and $[\text{lyso}]= 40g/l$. The only remaining terms in the scattered intensity are due to the correlations between deuterated PSS monomers $S_{2D}(q)$ that write:

$$S_{2D}(q) = \Phi_D S_{1D}(q) + \Phi_D^2 S_{2DD}(q)$$

where $\Phi_D$ is the volume fraction of PSSD chains, $S_{1D}$ the intra-chains signal and $S_{2DD}$ the structure factor between monomers from distinct chains. As shown in insert of figure 2.a and 2.c, the interpolation at $\Phi_D = 0$ enable a direct measurement of $S_{1}(q)$:

$$S_{1D}(q)/\Phi_D = S_{1D}(q) + \Phi_D S_{2DD}(q)$$

The measurement made with or without lysosyme are presented in figure 2.a and 2.c. The comparison of the form factor in Kratky plot show a large shrinking of the chains with lysozyme. A fit of the form factor by a wormlike model show that the $l_p$ value of 50Å is recovered without proteins but the $l_p$ is reduced to 20Å when interacting with proteins. Finally, a calculation of $c^*$ with $[\text{PSS}] = 0.1 \text{ mol/L}$ and $l_p = 20Å$ show that the transition form dilute to semi-dilute regime should occur for $N \sim 500$ that perfectly matches the value obtained experimentally.

**Experimental proof of the counter-ions release during complexation [5].**

The counter-ions release is commonly considered as one of the main driving process of the complexation due to the entropic gain associated with the release. Though this assess is confirmed by simulations, there is not yet an experimental direct proof of such release. We have tested this hypothesis with a 'black or white' experiment by performing SANS measurement on samples that should scatter or not when all species except counterions are matched. This is based on the hairy shell of PSS chains that surrounds the globular complexes in diluted regime as soon as the introduced charge ratio $[-]/[+]_{\text{intro}}$ is higher than 1. In case of release, counterions must be all expelled from the core of the hairy globules but not completely from the shell as there should remains Manning condensation on the free PSS chains. The only samples that should provide a scattering are the ones for which $[-]/[+]_{\text{intro}} > 1$ because the counterions decorate the shell (see figure 3). The matching is experimentally performed by replacing the usual Na+ counterions of hydrogenated PSS chains by deuterated TAM+ counterions. Such counterions have a large contrast with a 57%H$_2$O/43%D$_2$O solvent that matches both lysozyme and PSS H chains. Results are presented in figure 3 and unambiguously prove the counterions release : 'naked' samples do not scatter though hairy samples scatter. The counterions scattering is proportional to the one of the chains (obtained with PSSD chains and hydrogenated TAM+, not shown here). This confirms that the counterions decorate the shell.

**Figure 2.** Measurement of form factor within complexes : (a) and (c) experimental value of $I(q)/\Phi_D = f(\Phi_D)$ and interpolation to $\Phi_D = 0$ without proteins (a) or with proteins (c). Insert show the principle of experiment. (b) Comparison of form factor in Kratky plot and scattering modelization with model of wormlike chains.

**Figure 3.** Upper part : Contrast of globular complexes made with lysosyme and hydrogenated PSS chains with deuterated TAM+ counter-ions in either a 100%D$_2$O solvent or in a 57%H$_2$O/43%D$_2$O solvent. Lower part : low q SANS scattering in a 57%H$_2$O/43%D$_2$O solvent. From left to right : increase of the $[-]/[+]_{\text{intro}}$.}