

**Electronic supplementary information (ESI)**

**Complexation of stimuli-responsive star-like amphiphilic block  
polyelectrolyte micelles with lysozyme**

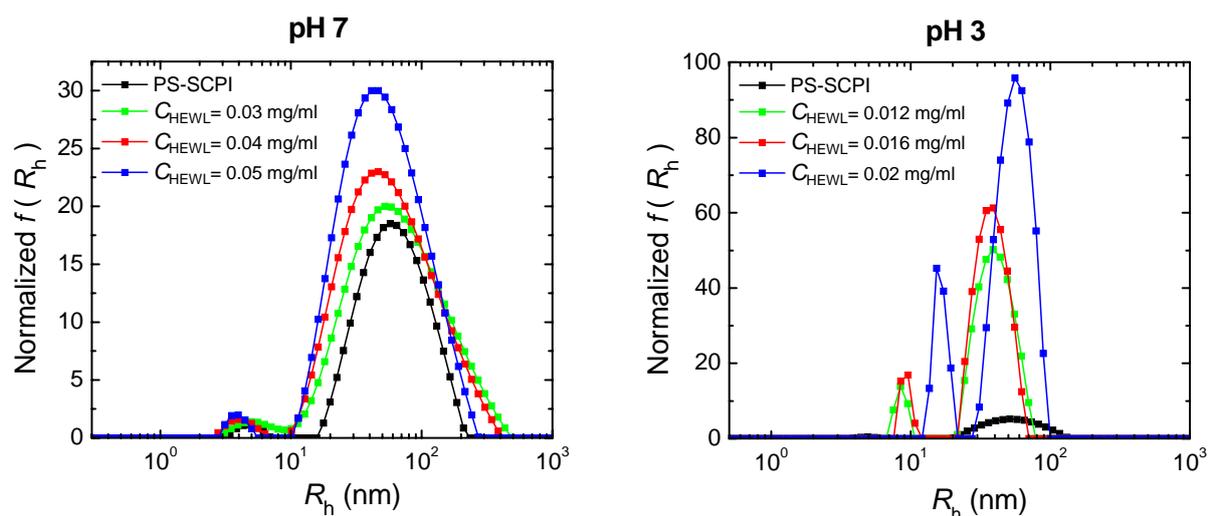
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## Scattering intensity normalized hydrodynamic radius distributions

As mentioned in the manuscript, although the hydrodynamic radius distributions of the complexes of the PS-SCPI/HEWL system at pH 7 and 3 resemble in some cases (especially at pH 7 and low protein concentration) the corresponding distribution of the neat PS-SCPI solutions, it is quite certain that they correspond to complexes rather than unimer copolymer chains and micelles, because the scattering intensity of the solutions of the complexes is higher compared to that of the diblock polyelectrolyte solution. In order to better illustrate this difference we present in Fig. S1 the scattering intensity normalized hydrodynamic radius distributions at low protein concentration values for the two different pH conditions. Specifically, each distribution has been normalized so as the maximum corresponds to the  $I/I_{\text{tol}}$  ratio, where  $I$  is the concentration independent excess scattering intensity (solvent scattering subtracted) of the solution of the complexes and  $I_{\text{tol}}$  is the scattering intensity of a toluene standard (see M. Sedláč, *J. Chem. Phys.*, 1996, **105**, 10123-10133).

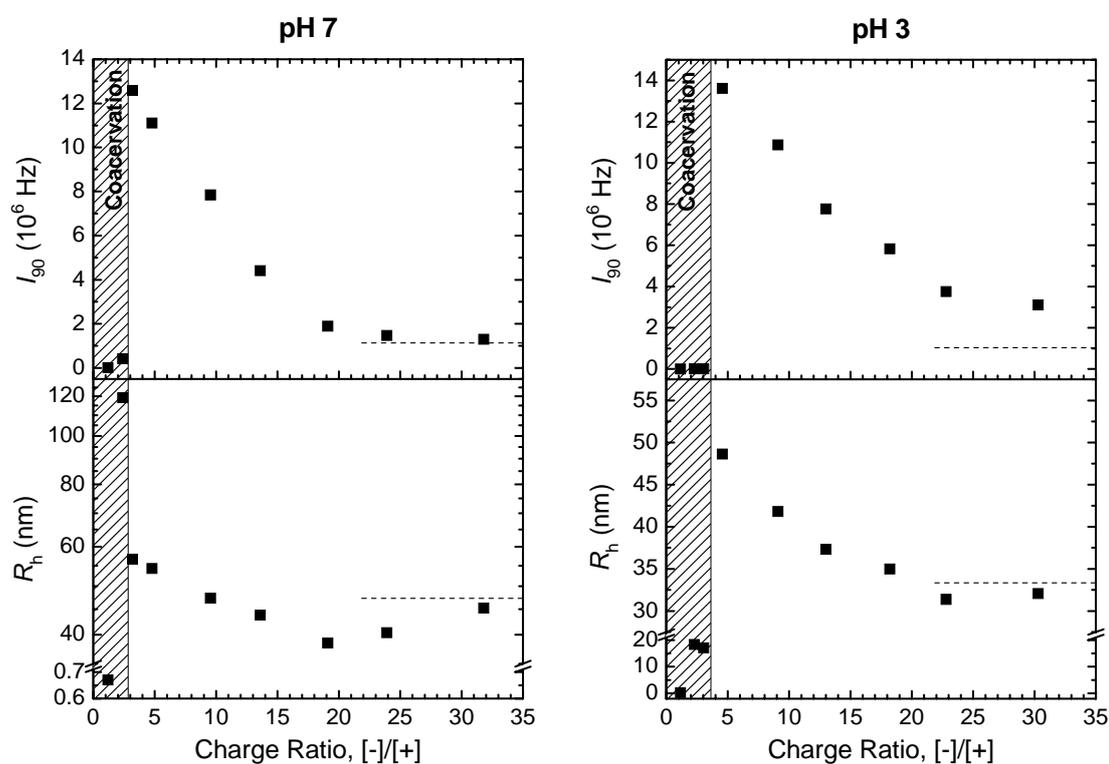
In this way it is evident that even at the lowest  $C_{\text{HEWL}}$  values the amplitude of the distributions of the complexes is higher than that of the PS-SCPI sample, thus proving that the mass of the scattering species in the solutions of the complexes is larger than that of the pure diblock polyelectrolyte solutions. Furthermore, the immense difference of amplitude observed at pH 3 provides further confirmation of the proposed aggregation of the block polyelectrolyte micelles complexed with protein.



**Fig. S1** Scattering intensity normalized hydrodynamic radius distributions obtained from CONTIN analysis of the DLS autocorrelation functions at  $90^\circ$  for the solutions of the complexes at low protein concentration values for the PS-SCPI/HEWL system at pH 7 and 3. The corresponding distributions for neat PS-SCPI solutions are included for comparison.

## Charge ratio dependence

A more direct comparison of the mass, size and solution behavior of the complexes formed at pH 7 and 3 is possible through the representation of the DLS results as a function of the  $[-]/[+]$  charge ratio, which is defined as the molar ratio of the total number of negative to positive charges in the bulk solution. Fig. S2 shows the charge ratio dependence of the light scattering intensity at  $90^\circ$ ,  $I_{90}$ , and hydrodynamic radius,  $R_h$ , for the solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl. The horizontal dashed lines indicate the corresponding values for the neat PS-SCPI solution.



**Fig. S2** Light scattering intensity at  $90^\circ$ ,  $I_{90}$ , and hydrodynamic radius,  $R_h$ , as a function of the  $[-]/[+]$  charge ratio for the solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl. The horizontal dashed lines indicate the corresponding values for the neat PS-SCPI solution.

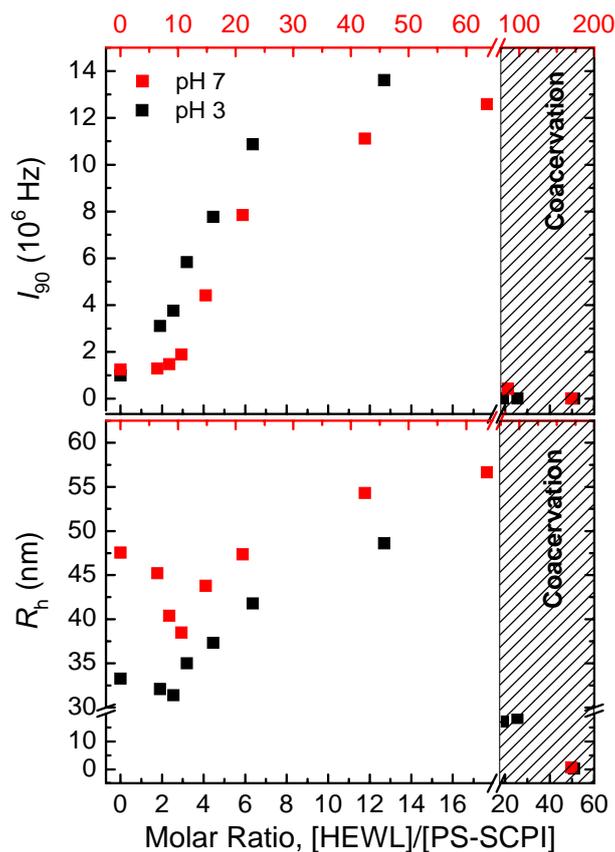
As it can be seen, at both pH conditions the complexes exhibit similar behavior. Specifically, at low charge ratio values, *i.e.* high degree of charge neutralization, coacervation of the complexes takes place, due to enhanced aggregation of the PS-SCPI micelles complexed with protein. The maximum mass and size of the complexes is observed at charge ratio values of about 3 at pH 7 and about 5 at pH 3, while further increase of charge ratio results in the decrease of the mass and the size of the complexes, since the degree of charge

neutralization decreases, the solubility of the complexes increases and their aggregation is hindered. At the highest charge ratio values the size of the complexes increases denoting their more extended conformation, which stems from the decreasing number of interacting protein molecules per SCPI polyelectrolyte chain (or equivalently the smaller degree of charge neutralization). In general, the mass of the complexes formed at pH 3 is larger than that at pH 7, while the opposite applies for their size, providing evidence of the more extended aggregation of the complexes and their more compact structure.

### **Molar ratio dependence**

An alternative representation of the DLS results of the present study is shown in Fig. S3, where the  $I_{90}$  and  $R_h$  values for the solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl are presented as a function of the molar ratio of the two components. That is, the ratio of the total moles of the protein to that of the diblock polyelectrolyte in the bulk solution,  $[\text{HEWL}]/[\text{PS-SCPI}]$ , which basically denotes the number of protein molecules corresponding to each polyelectrolyte chain of the micellar corona. Note that the molar ratio values of the solutions of the complexes at pH 7 correspond to the top  $x$  axis, while the range of the  $x$  axes has been chosen appropriately so as the vertical points indicate solutions with similar charge ratio values.

Through this representation is once again evident that the mass of the complexes formed at pH 3 is larger than that of the complexes at pH 7, while their size is smaller, because of their more aggregated state and less extended conformation of potentially solvated chains. The additional interest here lies on the comparison of the solution behavior of the complexes at the two pH conditions as a function of the number of protein molecules that interact with each polyelectrolyte chain. As it can be seen, although the total number of interacting HEWL molecules per SCPI chain at pH 3 is significantly smaller than the corresponding at pH 7, the combination of the reduced charge density of the SCPI polyelectrolyte block and the increased net positive charge of the protein molecule at pH 3 causes a larger degree of charge neutralization, thus leading to enhanced decline of solubility and increased aggregation of the polyelectrolyte micelles complexed with protein, even at low molar ratio values. Also noteworthy is the difference in the maximum interaction ratio that can be achieved maintaining at the same time the solubility of the aggregates of the complexes, which at pH 7 is calculated to be about 64 protein molecules per polyelectrolyte chain, while at pH 3 is about 13.



**Fig. S3** Light scattering intensity at  $90^\circ$ ,  $I_{90}$ , and hydrodynamic radius,  $R_h$ , as a function of the  $[\text{HEWL}]/[\text{PS-SCPI}]$  molar ratio for the solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl.

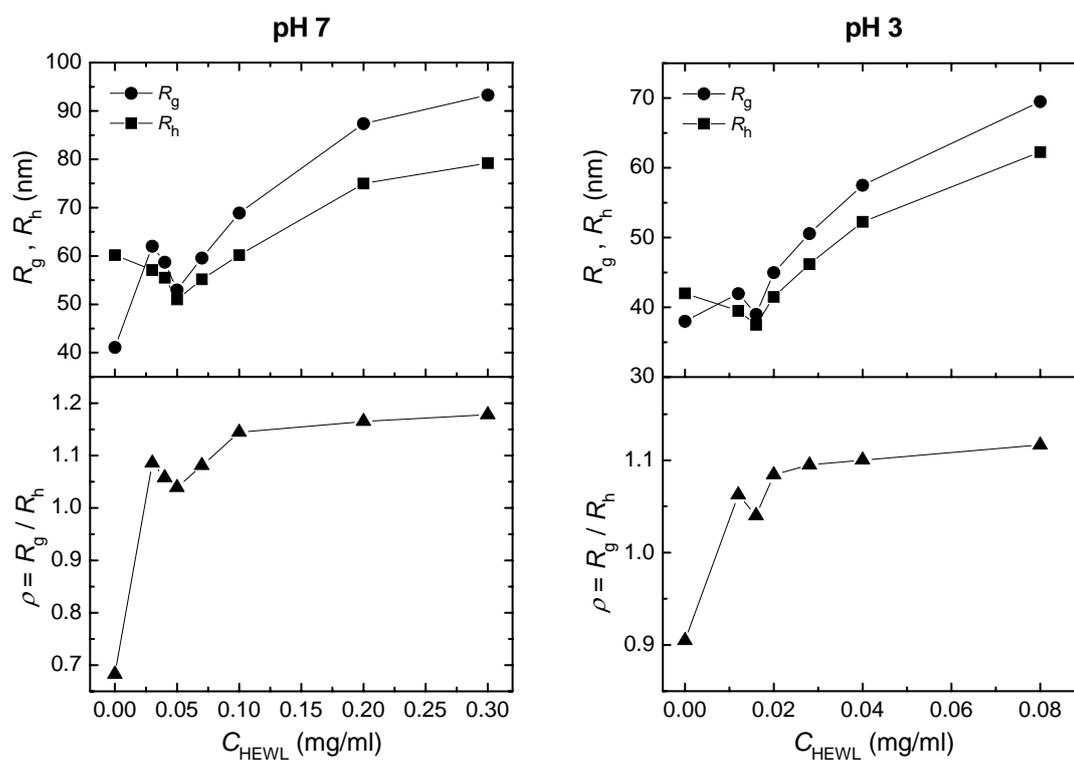
### Static light scattering results

The results of the multi-angle SLS measurements for the stable solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl, regarding the values of the radius of gyration,  $R_g$ , and hydrodynamic radius,  $R_h$ , along with their characteristic ratio  $\rho = R_g/R_h$  are presented in Fig. S4 as a function of  $C_{\text{HEWL}}$ .

As expected at both pH conditions the transition of the  $R_g$  and  $R_h$  values is similar to that observed for the  $R_h$  values derived from the DLS measurements at  $90^\circ$ , and in accordance to the changes of the size of the complexes. It is worth noting that for the solutions of the complexes the  $R_g$  values are greater than the corresponding  $R_h$  values, in contrast to the neat PS-SCPI solution. Apparently, the incorporation of the protein molecules in the corona of the polyelectrolyte micelles causes a significant increase of the  $R_g$ , due to the change in the radial distribution of mass. Moreover, SLS results correspond more closely to the size of the larger scattering species in solution, *i.e.* the complexes that the polyelectrolyte micelles form with

protein rather than the unimer copolymer chains, and for this reason the  $R_g$  and  $R_h$  values are generally greater than the  $R_h$  values estimated using cumulants analysis of the DLS measurements.

In addition, the transition of the characteristic ratio  $\rho$  denotes the conformational changes of the complexes. Specifically, the initial small decrease stems from the shrinkage of the polyelectrolyte corona of the micelles caused by complexation at pH 7, while at pH 3 indicates the compact structure of the aggregated polyelectrolyte micelles complexed with protein formed at low  $C_{\text{HEWL}}$  values. The subsequent increase is caused by the primary aggregation of the complexes at pH 7 or their secondary aggregation at pH 3, which apparently leads to more loose aggregate structures. Finally, the observed marginally smaller  $\rho$  values at pH 3 constitute evidence of the more extended aggregation and more compact structure of the formed complexes.



**Fig. S4** Radius of gyration,  $R_g$ , and hydrodynamic radius (derived from multi-angle measurements),  $R_h$ , as a function of  $C_{\text{HEWL}}$  for the solutions of the PS-SCPI/HEWL system at pH 7 and 3 and at 0.01 M NaCl.

## **Comparison between the complexation of the PS-SCPI polyelectrolyte micelles with HEWL and that of the SCPI homopolyelectrolyte chains with HEWL**

At pH 7 the observed behavior is similar for both systems, in the sense that as the number of interacting protein molecules per polyelectrolyte chain increases, whether it concerns the polyelectrolyte chains of the corona of the PS-SCPI micelles or the polyelectrolyte chains of the SCPI-54K sample, the degree of charge neutralization becomes higher resulting in the aggregation of the complexes and the adaptation of more compact structures. The main difference between the two systems lies upon the maximum number of interacting protein molecules per polyelectrolyte chain that can be achieved, while at the same time maintaining the solubility of the aggregates of the complexes. For the PS-SCPI/HEWL system it was found that this ratio is about 64 protein molecules per polyelectrolyte chain (see Fig. S3), whereas the corresponding number for the HEWL/SCPI-54K system is about 26 (see M. Karayianni *et al.*, *Biomacromolecules*, 2011, **12**, 1697–1706, Supporting Information, Figure S3). Of course, the fact that the two types of macromolecules carry different total number of charged groups (due to the differences in the degrees of polymerization and functionalization) must be considered. Specifically, the SCPI block of the PS-SCPI diblock copolymer carries 1.5 times more charged groups than the SCPI-54K polyelectrolyte sample (812 to 540 charged monomeric units). Nevertheless, the increased interaction of the PS-SCPI polyelectrolyte copolymer with the protein molecules is larger than expected based on the difference in the total number of charges and thus cannot be accounted for entirely by this fact. Apparently, the more extended conformation of the SCPI polyelectrolyte chains of the micellar corona, compared to that of the SCPI-54K sample, allows for the interaction with an increased number of protein molecules. Moreover, the stereochemical constraints imposed by the micellar morphology avert to a greater extent the aggregation of the polyelectrolyte chains complexed with protein, as denoted by the smaller degree of aggregation of the complexed micelles as to the corresponding degree of aggregation of the complexed polyelectrolyte homopolymer chains.

On the other hand, at pH 3 and low protein concentration values the small degree of interaction between the polyelectrolyte chains of the PS-SCPI micelles and protein molecules most likely enables each protein molecule to interact with more than one polyelectrolyte chains thus contributing to the observed aggregation of the micelles complexed with protein, which is similar to the observed behavior in the case of the SCPI-54K polyelectrolyte sample. The difference here is that as the protein concentration increases the degree of aggregation of

the complexed micelles shows a parallel increase, while for the SCPI-54K sample the corresponding aggregation degree of the complexed polyelectrolyte chains was reduced. It seems that, in contrast to the behavior of the polyelectrolyte homopolymer, the topological constraints of the micellar morphology hinder the possible conformational rearrangements of the complexed polyelectrolyte chains that would allow for the compensation of the induced reduction of their solubility. Therefore, in the case of the PS-SCPI polyelectrolyte micelles the reduction of their solubility by complexation results into further aggregation. However, the more extended conformation of the polyelectrolyte chains of the micellar corona once again permits an increased interaction with the protein molecules. In particular, each polyelectrolyte chain of the corona of the PS-SCPI micelles interacts with about 13 protein molecules, whereas the SCPI-54K polyelectrolyte chains with about 4, meantime preserving the solubility of the aggregates of the complexes.

### **Analysis of the circular dichroism (CD) spectra**

The detailed results regarding the percentages of protein secondary structure derived from the analysis of the far-UV CD spectra using the SELCON3, CDSSTR and CONTIN algorithms of the CDPro software, along with the K2D neural network are presented in Table S1.

**Table S1** Detailed results regarding the percentages of protein secondary structure derived from the analysis of the far-UV CD spectra of three representative solutions at  $C_{\text{HEWL}} = 0.1, 0.2$  and  $0.3$  mg/ml of the PS-SCPI/HEWL system at pH 7 and 0.01 M NaCl, and of neat HEWL at 0.5 mg/ml concentration

Sample	SELCON3				CDSSTR				CONTIN				K2D		
	$\alpha$ -helix	$\beta$ -sheet	turns	random	$\alpha$ -helix	$\beta$ -sheet	turns	random	$\alpha$ -helix	$\beta$ -sheet	turns	random	$\alpha$ -helix	$\beta$ -sheet	random
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
neat HEWL	30	17	20	31	35	16	17	32	33	16	16	36	31	11	58
$C_{\text{HEWL}} = 0.1$ mg/ml	29	17	19	32	35	15	17	33	33	16	15	36	31	11	58
$C_{\text{HEWL}} = 0.2$ mg/ml	31	17	19	32	35	15	17	33	33	16	17	34	31	13	56
$C_{\text{HEWL}} = 0.3$ mg/ml	31	16	19	32	35	17	17	31	33	16	17	35	31	11	58