Supporting Information for:

Encapsidation of RNA-Polyelectrolyte Complexes with Amphiphilic Block Copolymers: Toward a New Self-Assembly Route

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1. Characterization of hyaluronan-*block*-PBLG copolymer vesicles

Block copolymer vesicles were characterized by static and dynamic light scattering using an ALV/CGS-3 laser goniometer, with a 22 mW HeNe linearly polarized laser (632.8 nm) and an ALV/LSE-5004 multiple tau digital correlator. The accessible scattering angles range from 30 to 150°. All the measurements were performed at a constant temperature of 25°C. The relaxation time distributions were obtained using the Contin analysis of the scattering autocorrelation function. The relaxation frequency (Γ) is q^2-dependent in the case of a diffusive particle. The apparent diffusion coefficient (D_{app}) at a given copolymer concentration is calculated from

$$\left.\frac{\Gamma}{q^2}\right|_{q\to 0} = D_{app}$$

where q is the scattering vector defined as follows

$$q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right)$$

λ is the wavelength of the incident laser beam, n is the refractive index of the solvent and θ is the scattering angle. The hydrodynamic radius is derived from the Stokes-Einstein solution.

$$R_H = \frac{k_B T}{6\pi\eta D_{app}}$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent. The radius of gyration (R_G) was derived from the Guinier equation defined as follows:

$$\ln I = \ln I_0 - \frac{q^2 R_G^2}{3}$$

where I is the scattering intensity and I_0 is the scattering intensity at q = 0. The Guinier approximation is valid for any particle shape as long as $qR_G << 1$.

For TEM analysis, 2 µL of the dialyzed suspension of vesicles ($C_{copolymer}$ = 0.05 g/L) was deposited on a copper grid (200 mesh) coated with carbon film. After air-drying at room temperature for 30 min, the sample was stained for 3 min with 5 µL of a 1% solution of uranyl acetate. Then, the excess of staining solution was blotted away with a strip of filter paper and the grid was allowed to dry for 24 hours at room temperature. The sample was observed with a Hitachi H7650 microscope operating at an acceleration voltage of 80 kV. Images were recorded with a GATAN Orius 10.5 megapixel camera.

2. Formation of siRNA-PEI polyelectrolyte complexes

2.1 Influence of the pH

Preliminary experiments have pointed out the importance of the pH of the complexation medium on the size-polydispersity of colloidal complexes. The polydispersity index derived from the cumulant method is a dimensionless parameter defined by μ_2/Γ^2 where μ_2 is the second cumulant of the intensity autocorrelation function and Γ the average decay rate. Figure S1 shows that the polydispersity index (PDI) is around 0.15 at pH 7.6, which is characteristic of a relatively narrow distribution while the PDI lies between 0.25 and 0.35 at pH 4.8 and pH 7.2, which is typical of a rather large particle-size distribution. It is assumed that a lesser degree of protonation of the PEI favours a better ion-pairing between oppositely charged polymers and thus the formation of complexes with a higher level of molecular organization.

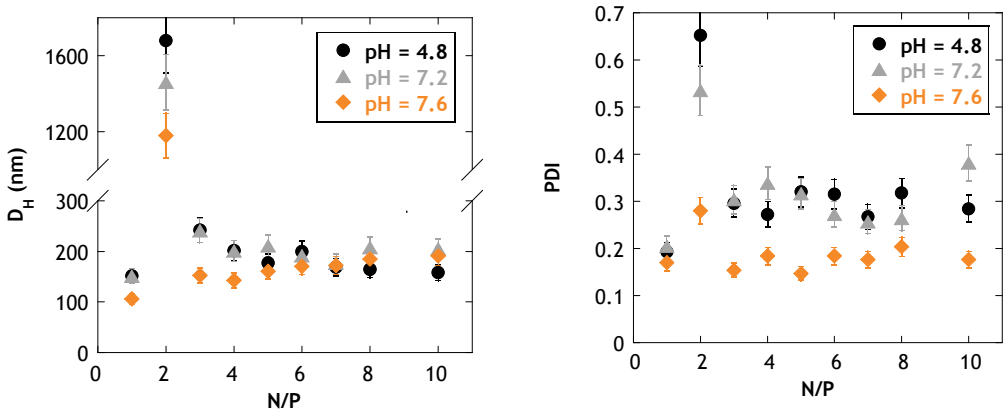

Figure S1. Hydrodynamic diameters and polydispersity indexes of siRNA-PEI polyelectrolyte complexes at different N/P ratios and various pHs (10 mM acetate buffer at pH 4.8, 10 mH Hepes buffer at pH 7.2 and 10 mM Hepes buffer at pH 7.6).

2.2 AFM analysis

AFM analysis of siRNA-PEI complexes evidenced the presence of a dense core resulting from the segregation of polyelectrolyte complexed segments surrounded by a thin shell of uncomplexed PEI ensuring the colloidal stabilization. AFM also reveals the softness of the polyelectrolyte particles as evidenced by a length to height ratio higher than 20 (Figure S2).

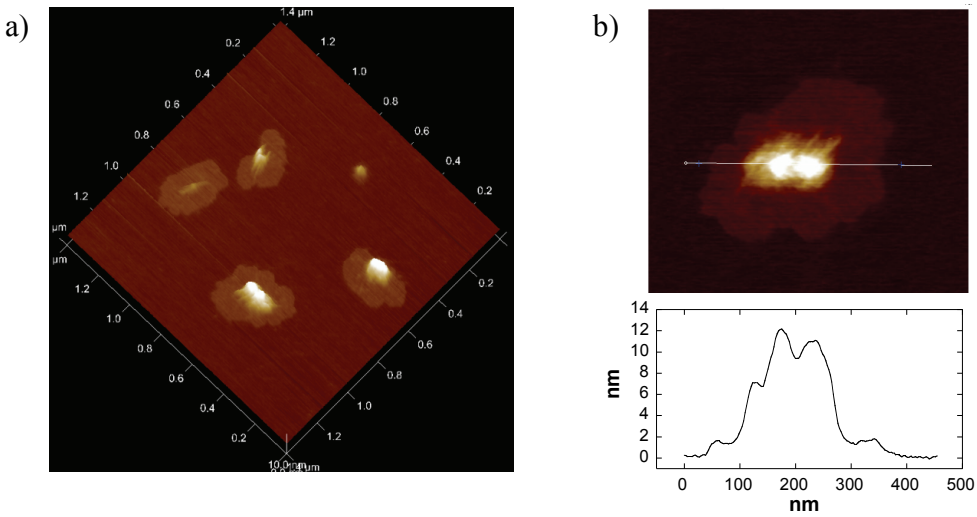

Figure S2. AFM pictures and height profiles of naked siRNA-PEI polyelectrolyte complexes at two size-scales: a) 1400 nm x 1400 nm, b) 600 x 600 nm.

3. Hyaluronan- and copolymer-modified siRNA-PEI complexes

3.1 Light scattering analysis

The whole process of interaction between hyaluronan or copolymer and siRNA-PEI complexes was followed by dynamic light scattering. Figure S3 is the complement of the data set in the main paper (*see* Figure 2). It shows the variation of the light scattering intensity and the polydispersity index as a function of the concentration of hyaluronan or HYA-*b*-PBLG copolymer. It is worth

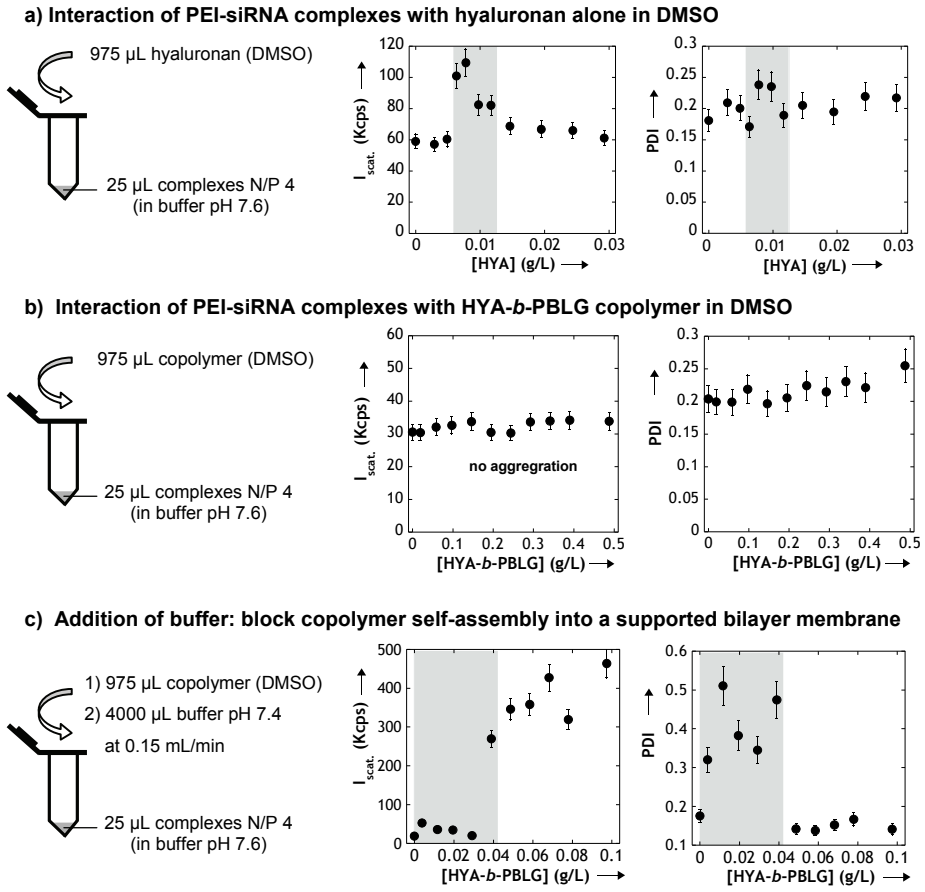

Figure S3. a) Interaction of hyaluronan (HYA) with siRNA-PEI complexes (N/P = 4, C_{siRNA} = 30 µM) in DMSO/Hepes buffer (97.5/2.5) (v/v). b) and c) Two-step assembly of HYA-*b*-PBLG copolymer around siRNA-PEI polyelectrolyte complexes. First step (b): interaction of the copolymer with complexes in DMSO/Hepes buffer (97.5/2.5) (v/v). Second step (c): addition of Hepes buffer in the medium up to a final DMSO/buffer composition of 20/80 (v/v). The light scattering intensity ($I_{scat.}$) and the polydispersity index (PDI) derived through a cumulant analysis of resulting particles are plotted as a function of the concentration of hyaluronan (a) or copolymer (b and c). The grey area represents the domain of colloidal instability where particles sediment.

noticing that the values of light scattering intensity follow the same trend as those of hydrodynamic diameters plotted in Figure 2 except when an excess of buffer was added in the medium. Namely, we observe a strong increase of the scattering intensity above $C_{copolymer}$ = 0.03 g/L (Figure S3.c) while the hydrodynamic diameter strongly decreases in the same range of concentration in relation with the dissociation of the aggregates (Figure 2.c in the paper). This apparently contradictory observation can be explained by considering that the copolymer-coated complexes siRNA-PEI have a higher intrinsic scattering power related to the refractive increment index (dn/dc) than the naked particles of complexes. Therefore, this is another evidence of the formation of a copolymer membrane onto the complex particles. The relatively low variation of the polydispersity index (PDI ~ 0.2-0.25) at various stages of preparation reflects the stability of the colloidal assemblies in different solvent conditions.

3.2 Stability of copolymer-coated siRNA-PEI complexes

The stability of the glycopolypeptide-modified siRNA-PEI complexes at various stages of preparation was assessed by gel retardation assay in presence of SYBR green using the same experimental procedure as for naked siRNA-PEI complexes (*see* the Materials and methods section). The final concentration of siRNA in complexes is 2.2 10^{-3} g/L, which is relatively low with respect to the sensitivity of the SYBR green. Therefore, both the concentration of siRNA and copolymer were increased by a factor 2 for gel electrophoresis experiments with SYBR

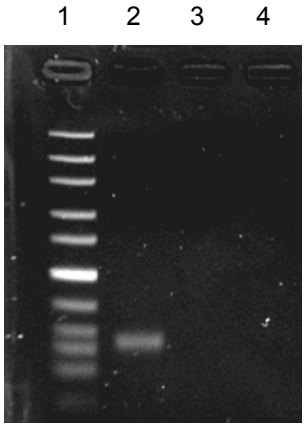

Figure S4. Gel retardation assay of siRNA in various conditions:

lane 1: 10-300 bp DNA ladder

lane 2: free siRNA (23 bp, C_{siRNA} = 4.4 10^{-3} g/L)

lane 3: siRNA-PEI complexes (N/P = 4), modified with copolymer in DMSO (C_{siRNA} = 2.2 10^{-2} g/L, $C_{copolymer}$ = 0.5 g/L)

lane 4: siRNA-PEI complexes (N/P = 4) modified with copolymer in DMSO and after addition of 10 mM Hepes buffer at pH 7.4 (C_{siRNA} = 4.4 10^{-3} g/L, $C_{copolymer}$ = 0.1 g/L)

green. No significant difference was observed by DLS in terms of hydrodynamic sizes and size dispersities at such concentrations (results not shown). Figure S4 evidences the absence of siRNA release from complexes after addition of the copolymer solution in DMSO and after addition of 10 mM Hepes buffer pH 7.4.