Electronic Supplementary Material (ESI) for Materials Advances.

Tuning Protein Delivery from Different Architectures of Layer-by-Layer Assembled onto Polymer Films

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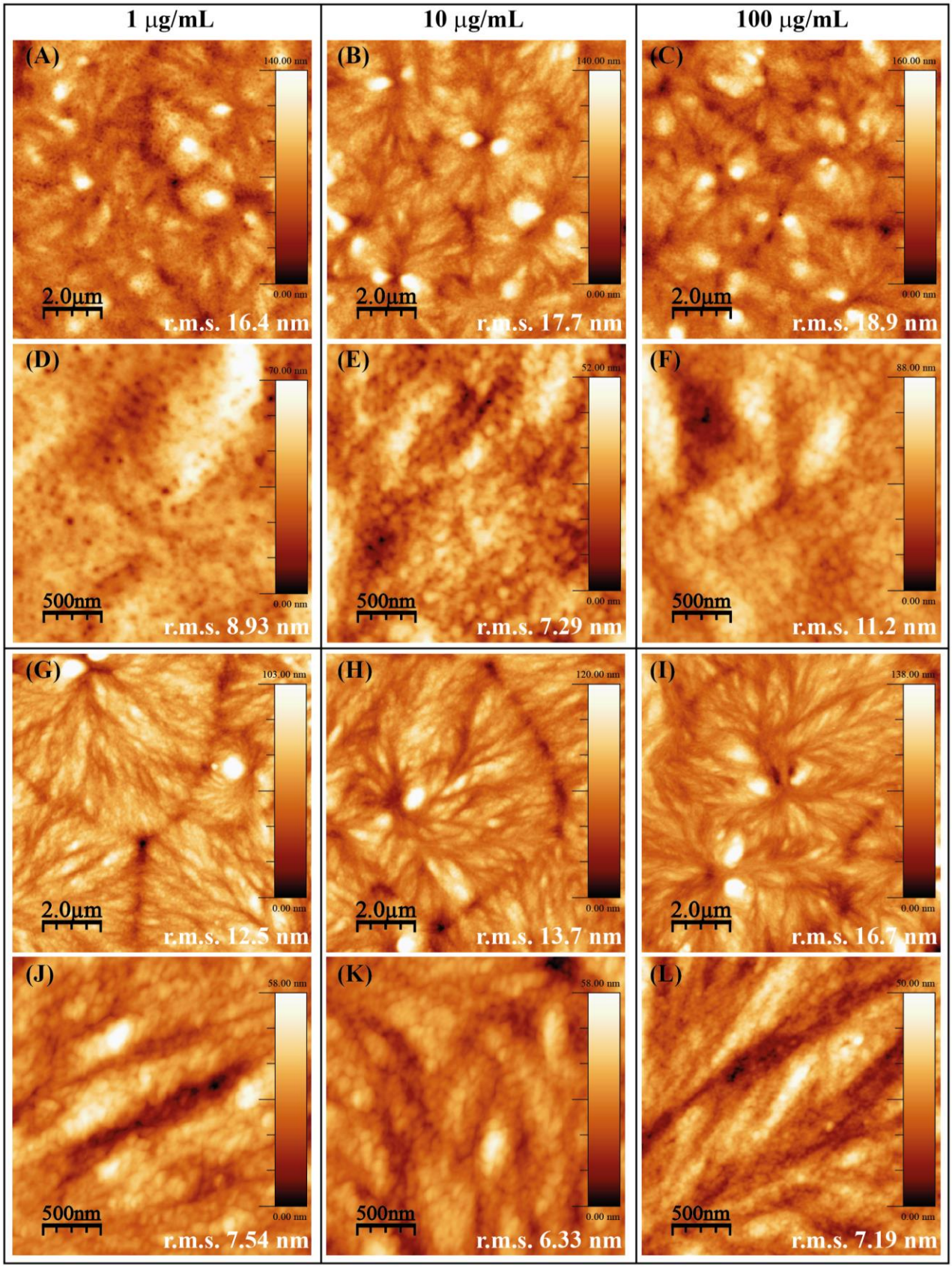

Figure S1. AFM images for LbL reservoirs (PCL/PEI)(Hep-BSA/Chi)$_{10}$ prepared by using BSA concentrations at 1 (A, D), 10 (B, E), or 100 μg/mL (C, F) in the co-solutions; LbL reservoirs (PCL/PEI)(Hep/Chi-LYS)$_{10}$ prepared by using LYS concentrations at 1 (G, J), 10 (H, K), and 100 μg/mL (I, L) in the co-solutions. Scanning areas of (10 μm x 10 μm) and (2.5 μm x 2.5 μm).

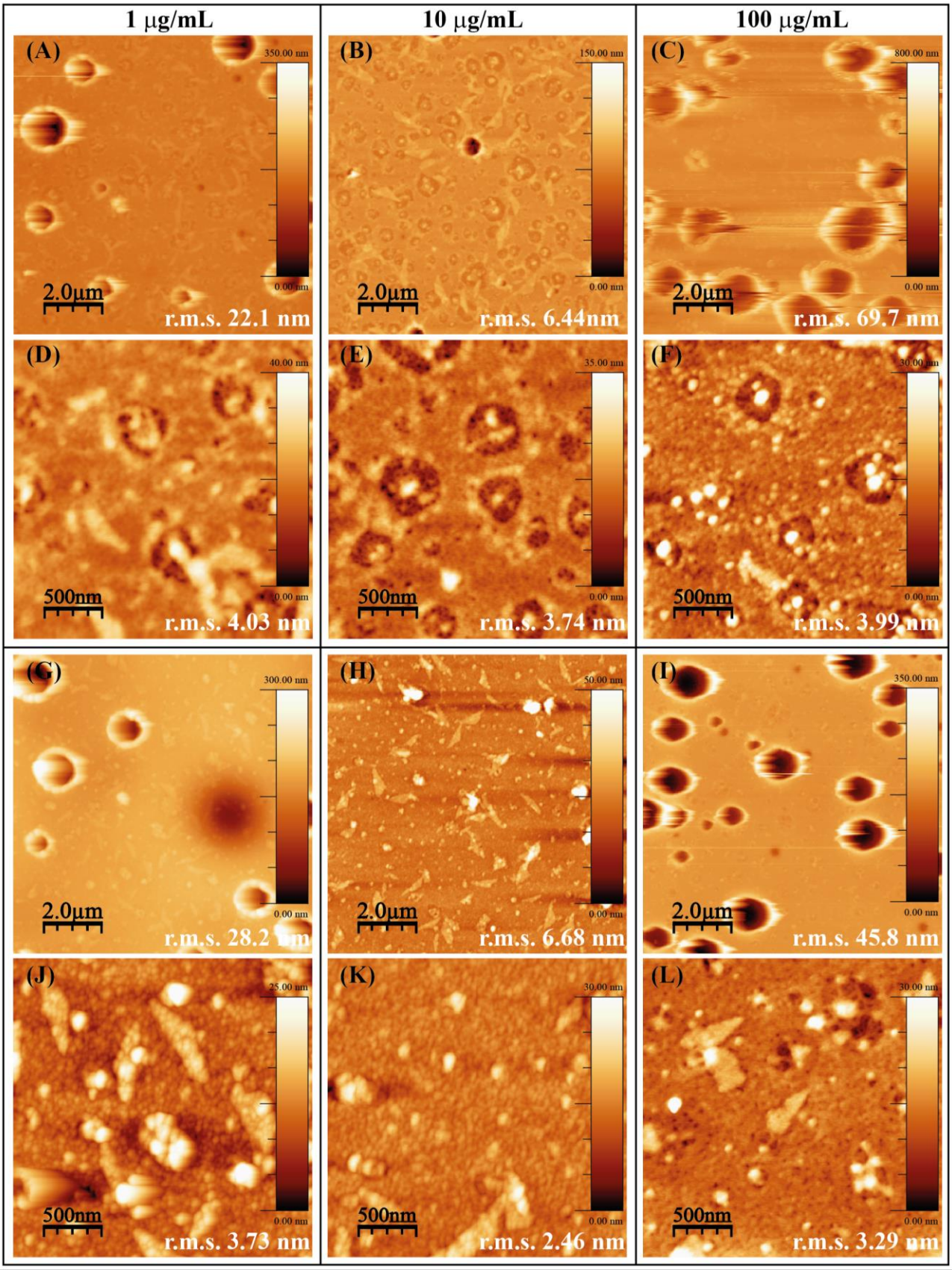

Figure S2. AFM images for LbL reservoirs (PLLA/PEI)(Hep-BSA/Chi)$_{10}$ prepared by using BSA concentrations at 1 (A, D), 10 (B, E), and 100 μg/mL (C, F) in the co-solutions; LbL reservoirs (PLLA/PEI)(Hep/Chi-LYS)$_{10}$ prepared by using LYS concentrations at 1 (G, J), 10 (H, K), and 100 μg/mL (I, L) in the co-solutions. Scanning areas of (10 μm x 10 μm) and (2.5 μm x 2.5 μm).

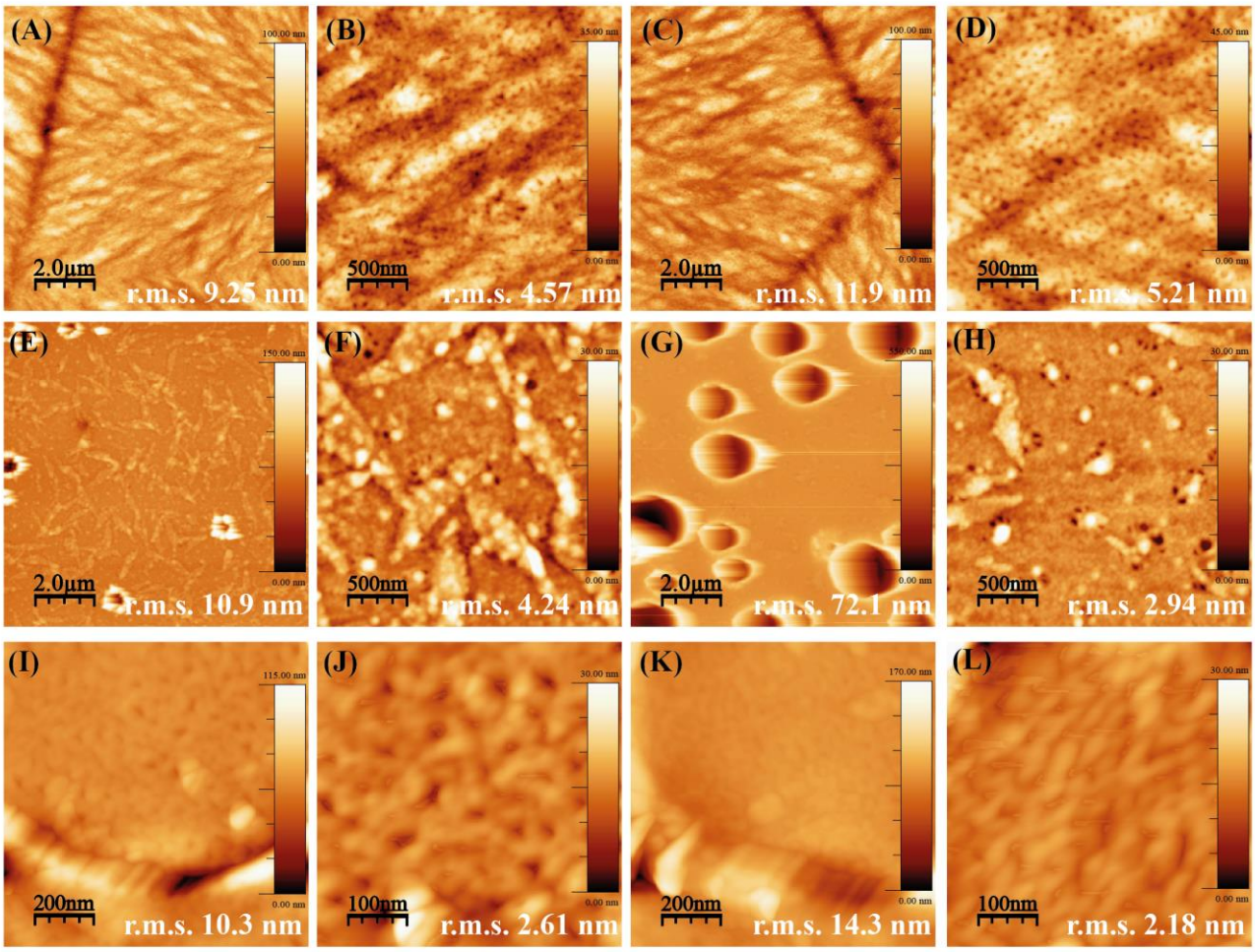

Figure S3. AFM images for LbL multilayers $(PCL/PEI)(Hep/Chi)_{10}$ (A,B), $(PCL/PEI)(Hep/Chi)_{10}(Hep)_1$ (C,D), $(PLLA/PEI)(Hep/Chi)_{10}$ (E,F), $(PLLA/PEI)(Hep/Chi)_{10}(Hep)_1$ (G,H). Scanning areas of (10 µm x 10 µm) and (2.5 µm x 2.5 µm). Images inside the holes for reservoirs $(PLLA/PEI)(Hep/Chi\text{-}LYS)_{10}$ (I,J) and $(PLLA/PEI)(Hep\text{-}BSA/Chi)_{10}$ (K,L) prepared by using protein concentrations at 100 µg/mL. Scanning areas of (1 µm x 1 µm) and (0.5 µm x 0.5 µm).

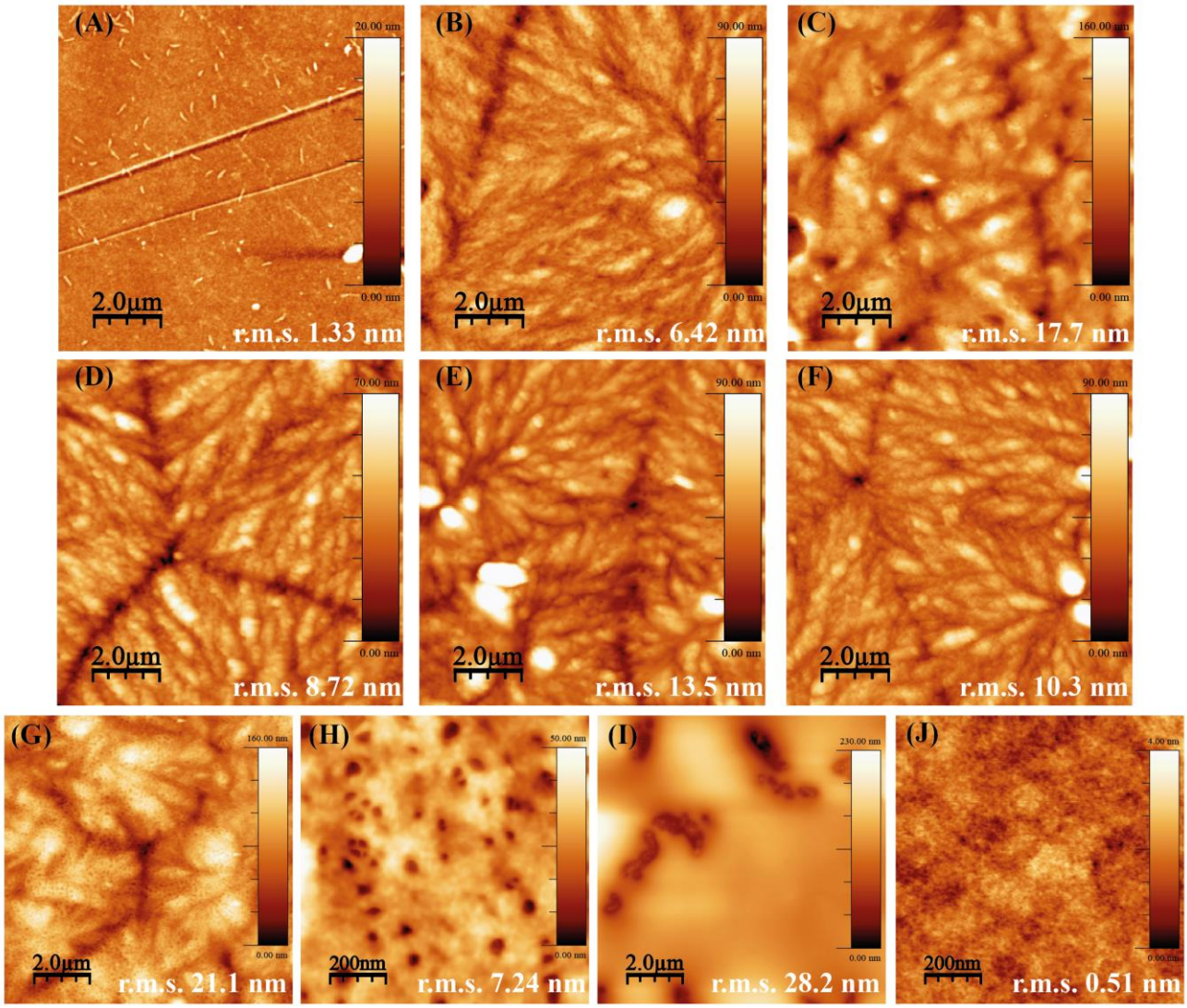

Figure S4. AFM images for LbL reservoirs (A) $(PCL/PEI)(Hep/Chi)_1(Hep\text{-}BSA/Chi)_3(Hep/Chi)_6$, (B) $(PCL/PEI)(Hep/Chi)_4(Hep\text{-}BSA/Chi)_3(Hep/Chi)_3$, (C) $(PCL/PEI)(Hep/Chi)_7(Hep\text{-}BSA/Chi)_3$; (D) $(PCL/PEI)(Hep/Chi)_1(Hep/Chi\text{-}LYS)_3(Hep/Chi)_6$, (E) $(PCL/PEI)(Hep/Chi)_4(Hep/Chi\text{-}LYS)_3(Hep/Chi)_3$, and (F) $(PCL/PEI)(Hep/Chi)_7(Hep/Chi\text{-}LYS)_3$; scanning areas of (10 µm x 10 µm). AFM images for controls $(PCL/PEI)(Hep\text{-}BSA/Chi)_{10}$ (G,H) and $(PCL/PEI)(Hep/Chi\text{-}LYS)_{10}$ (I,J); scanning areas of (10 µm x 10 µm) and (1 µm x 1 µm).

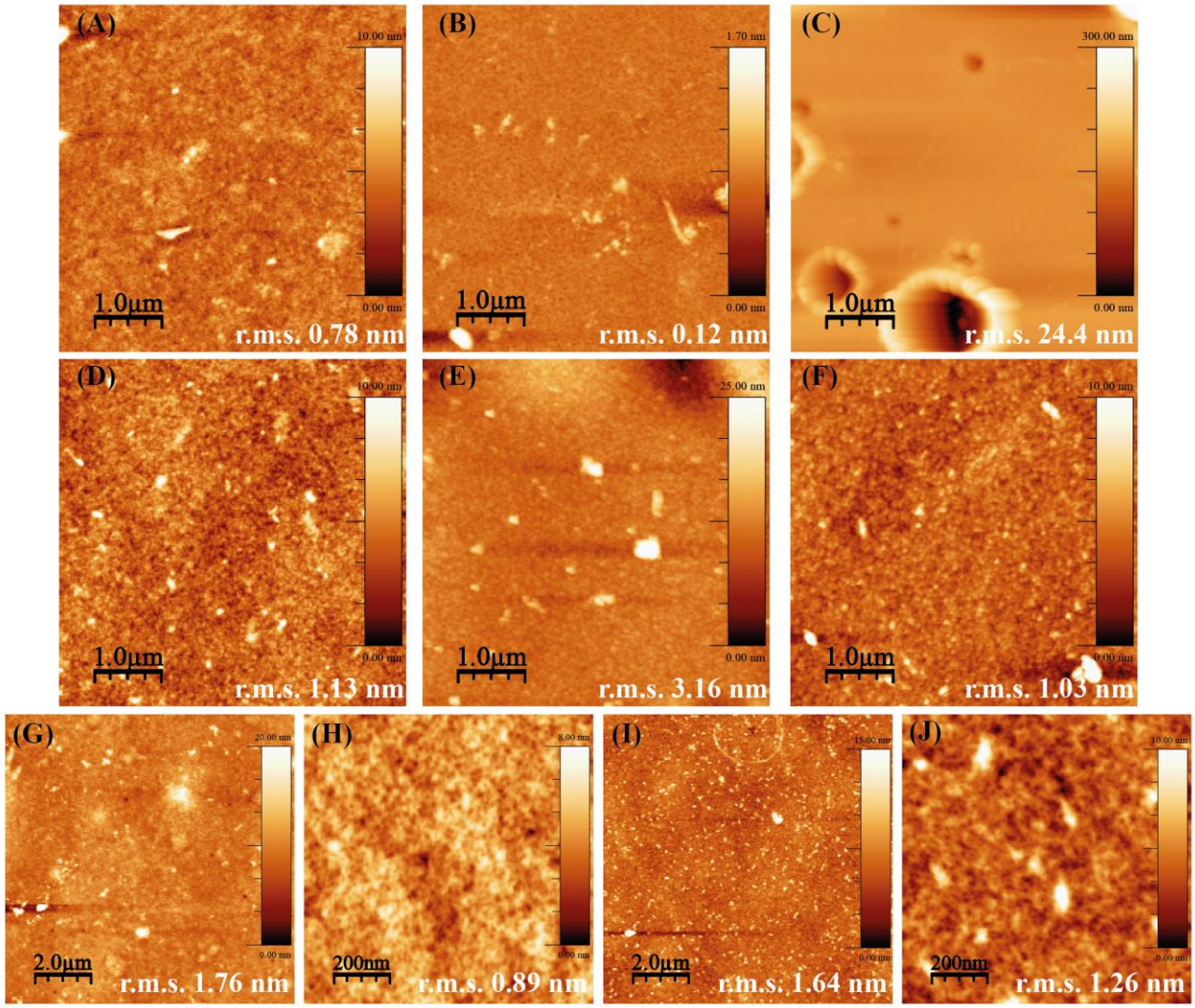

Figure S5. AFM images for LbL reservoirs (A) (PLLA/PEI)(Hep/Chi)$_1$(Hep-BSA/Chi)$_3$(Hep/Chi)$_6$, (B) (PLLA/PEI)(Hep/Chi)$_4$(Hep-BSA/Chi)$_3$(Hep/Chi)$_3$, and (C) (PLLA/PEI)(Hep/Chi)$_7$(Hep-BSA/Chi)$_3$, (D) (PLLA/PEI)(Hep/Chi)$_1$(Hep/Chi-LYS)$_3$(Hep/Chi)$_6$, (E) (PLLA/PEI)(Hep/Chi)$_4$(Hep/Chi-LYS)$_3$(Hep/Chi)$_3$, and (F) (PCL/PEI)(Hep/Chi)$_7$(Hep/Chi-LYS)$_3$; scanning areas of (5 µm x 5 µm). AFM images for controls (PLLA/PEI)(Hep-BSA/Chi)$_{10}$ (G,H) and (PLLA/PEI)(Hep/Chi-LYS)$_{10}$ (I,J); scanning areas of (10 µm x 10 µm) and (1 µm x 1 µm).

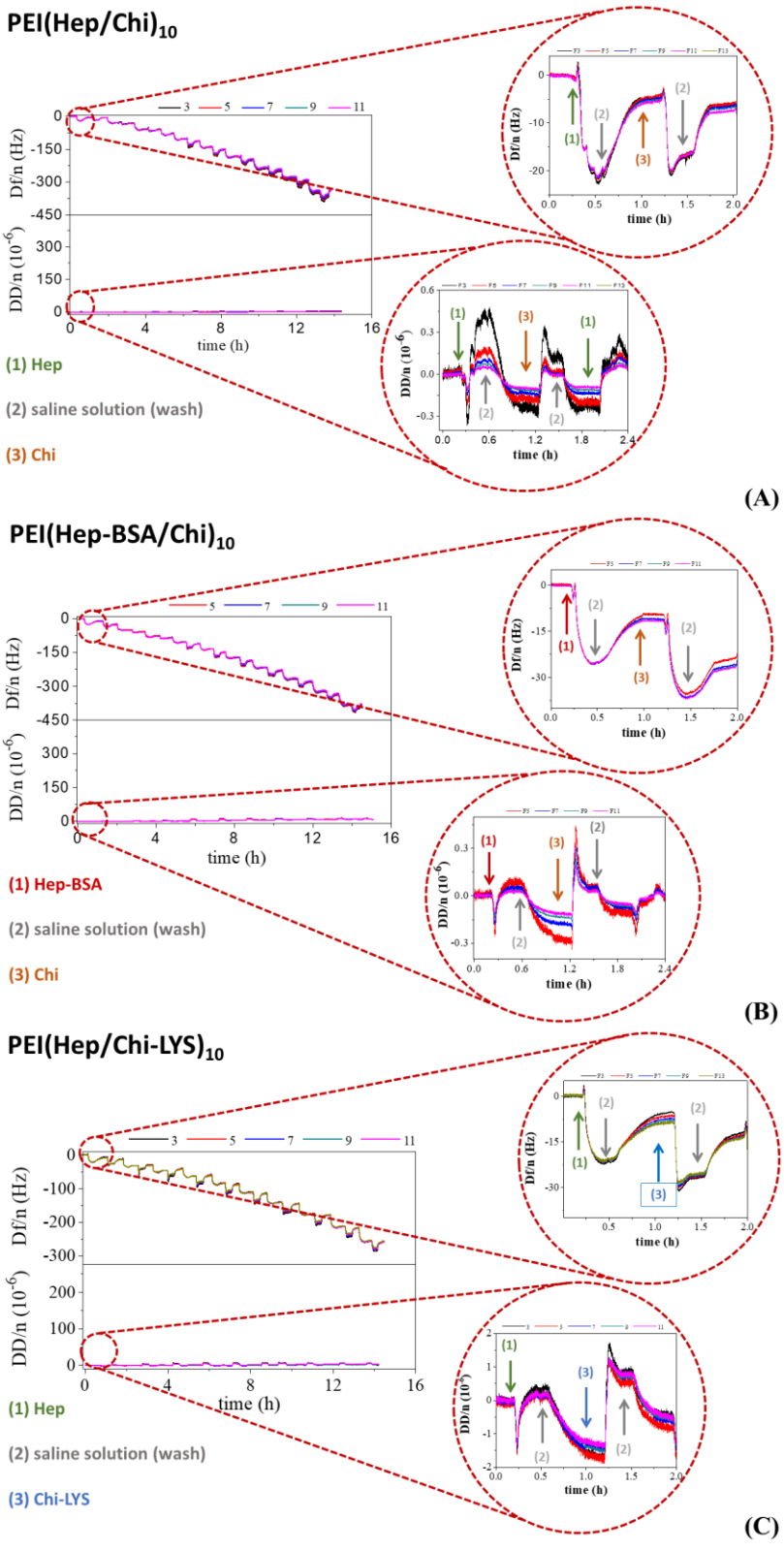

Figure S6: Resonance frequency normalized by overtone (Δf/n) and Dissipation for each overtone (ΔDn) as a function of time for ten-layer pairs for (A) Hep/Chi, (B) Hep-BSA/Chi and (C) Hep/Chi-LYS, respectively. The arrow (1) (green) indicates the addition of Hep for (A) and (C), the arrow (1) (red) indicates the addition of Hep-BSA for (B), the arrow (2) (gray) indicate the wash for (A), (B) and (C), the arrow (3) (brown) indicate the addition of Chi for (A) and (C) and the arrow (3) (blue) indicates Chi-LYS for (C).

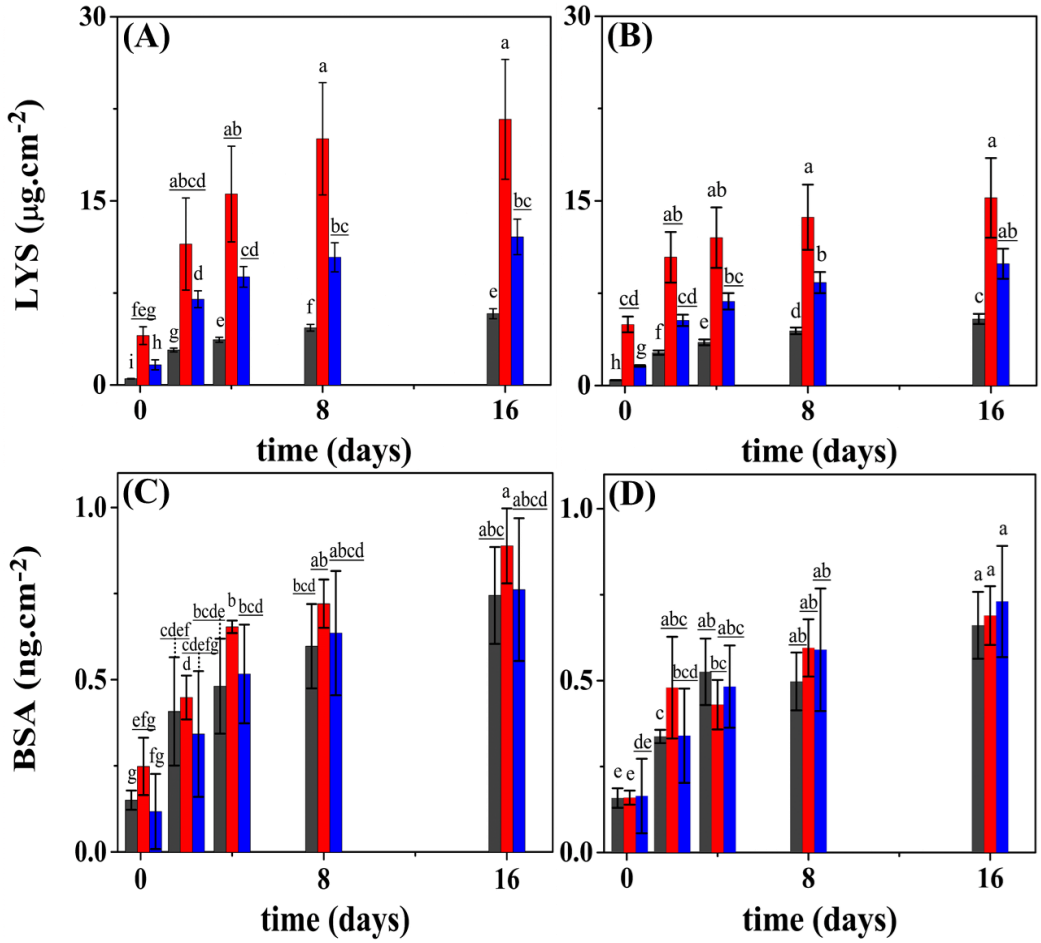

Figure S7: Release profiles for BSA or LYS from 10layer pairs of Hep/Chi LbL reservoirs built onto PCL or PLLA films. Proteins were placed within the 5 top layers (red bars), evenly distributed (blue bars), or within the 5 bottom layers (black bars) of the reservoirs. (A) LYS reservoir on PCL substrate; (B) LYS reservoir on PLLA substrate (C) BSA reservoir on PCL substrate; (D) BSA reservoirs on PLLA substrate. Similar letters indicate no statistically significant difference.

Additional discussion on the results of Figure 8

The introduction of a reservoir based on Hep-BSA monolayer causes a frequency shift, which is stabilized at -40 Hz (Fig. 8C, arrow 4). The rinsing with a saline solution (Fig. 8C, arrow 2) does not induce a considerable release of both negative species deposited onto the thin film. The introduction of Chi causes a frequency decrease in the first moment (Fig. 8C, arrow 3), but promptly turns back to values near the baseline previous obtained for Hep-BSA adsorbed. Furthermore, when the system is rinsed, it shows a frequency increase, related to the removal of part of the adsorbed protein and polyanion. However, during the assembly of the second layer-pair, the addition of Hep-BSA solution causes a frequency shift in the same magnitude of the first covered layer, $\Delta f_n/n \approx -74$ Hz, (Fig. 8C, arrow 4). A comparison between the first and second addition of coacervate indicates a little split on the overtones, observed when the first and second addition of Chi are compared. The second Chi addition causes a frequency shift until stabilization takes place, with a little split on the overtones. This situation is changed during rinsing, likely due to the removal of the extra polycation and the coordination of Hep-BSA/Chi, leading to a frequency shift at - 70 Hz (Fig. 8C, arrow 3).

The adsorption of Chi and LYS, followed by the initial addition of Hep (Fig. 8E, arrow 1) and after that the rinsing with the saline solution (Fig. 8E, arrow 2) presented similar behavior, observed with multilayers systems formed by (Au/PCL/PEI)(Hep/Chi)$_2$ and (Au/PCL/PEI)(Hep-BSA/Chi)$_2$, respectively. However, the addition of Chi and LYS presented a noteworthy behavior (Fig. 8E, arrow 5): at the first moment, it is observed a frequency shift due to an interaction between Hep and Chi/LYS. When total adsorption of Chi-LYS is achieved, expressed by the stabilization of the frequency at – 44 Hz, the rinsing takes place, and the first layer-pair is formed (Fig. 8E, arrow 2). Nevertheless, the rinsing can remove part of the polyanion previously adsorbed, a situation caused by a strong complexation between Hep and Chi.[34–36]

By comparing the adsorption of Hep-BSA or Chi-LYS, it is observed that while rinsing, the frequency shift achieves the same value as before the addition of protein co-dissolved with the

polyelectrolyte (Figs. 8C and 8E, respectively). It indicates that a very low quantity of Hep-BSA and Chi-LYS remain onto the system. The interesting observation is the activation of the surface by the absorption of polyelectrolytes of opposite charge takes place, inducing the formation of multilayers system.[34–36] The addition of Hep in the second bilayer does not cause overtone splitting, where the stabilization took place at – 62 Hz (Figure 8E, arrow 1). The addition of Chi and LYS caused a minor split on overtones, similarly to that observed with Hep and BSA, as well as a frequency shift, followed by the rinsing when the system stabilized at -73 Hz.