ELECTRONIC SUPPORTING INFORMATION

Recognition-Driven Layer-by-Layer Construction of Multiprotein Assemblies on Surfaces: Biomolecular Toolkit for Building Up Chemoresponsive Bioelectrochemical Interfaces

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Complementary QCM-D Experiments

As an attempt to observe the evolution of the film properties during the multilayer growth we followed the sequential layer-by-layer bioconjugation at different overtones. In accordance to the Sauerbrey equation,

$$\Delta m = -\frac{C\Delta f}{n} \qquad [1]$$

this would indicate that Δf scales with n in case of having a rigid film (in our experimental setup C = 17.7 Hz^{-1} ng cm^{-2}). Strictly speaking this is valid only for uniform rigid films with material properties indistinguishable from those of the crystal resonator.[1] Obviously, this is not the case of soft matter-based architectures attached to the resonator in a liquid environment. However, in some cases dealing with soft matter the mechanical properties of the film resembles those of a rigid layer in the sense of the Sauerbrey equation. Recently, Notley *et al.*[2] reported that the parameter $\Delta f\,/n$ in the growth of polyelectrolyte multilayers is independent of frequency throughout the assembly indicating that the film is rigid enough to use the Sauerbrey equation as a first approximation. In a similar fashion, we propose analyzing $\Delta f\,/n$ at different overtones as a strategy to obtain information of the multilayer growth. It is worth mentioning that we do not attempt to carry out a quantitative measurement of the mass coverage using the Sauerbrey equation. Our goal is to explore the variation in rigidity/softness at different growth stages by simply studying the departure from the Sauerbrey scaling. Figure S1 shows the plots Δf_n versus time obtained at different overtones normalized by the overtone number. As a result, we observed a Sauerbreyian response ($\Delta f_n/n$ = constant) only for the first Con A monolayer. Beyond the first protein layer, the interfacial architecture becomes less rigid and more dissipative, probably due to the significant amount of solvent or electrolyte trapped into the protein film.

[1] G. Z. Sauerbrey, *Phys.* **1959**, *155*, 206.
[2] S. M. Notley, M. Eriksson, L. Wagberg, *J. Colloid. Interface Sci.* **2005**, *292*, 29.

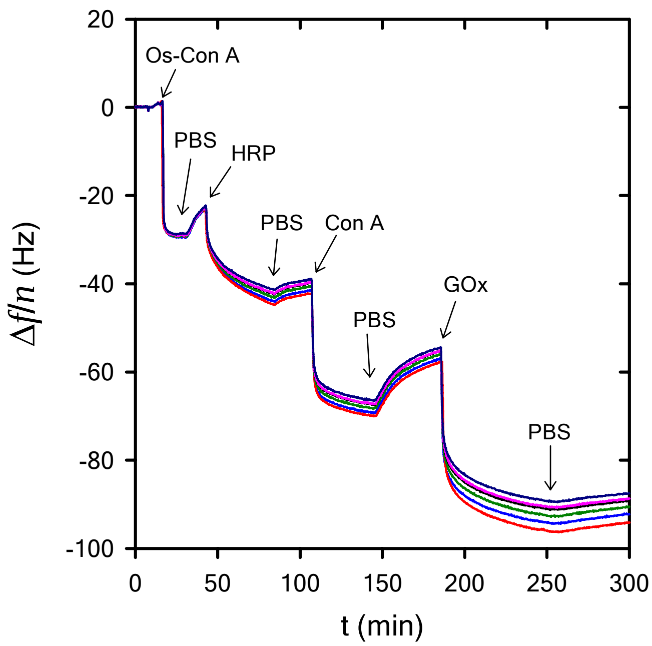

Figure S1. Representation of normalized frequency ($\Delta f/n$) at different overtones obtained during the multilayer growth. The different overtones correspond to: (red trace) n = 3; (blue trace) n = 5; (green trace) n = 7; (black trace) n = 9; (pink trace) n = 11; (dark blue trace) n = 13.