Supporting information: Highly Hydrated Thin Films Obtained via Templating of the Polyelectrolyte Multilayer Internal Structure with Proteins

Aurélien vander Straeten and Christine Dupont-Gillain**

Institute of Condensed Matter and Nanosciences, Université catholique de Louvain, Place Louis Pasteur, 1 bte L4.01.10, B-1348 Louvain-la-Neuve, Belgium

aurelien.vanderstraeten@uclouvain.be
christine.dupont@uclouvain.be

1) a_w variation as a function of time using the method of Graf and Kocherbitov[1]

a_w as a function of time can be found by computing the LiCl molality as a function of time in the solution that flows through the QCM-D humidity module. The initial parameters that are known are initial water and LiCl masses, solution density and flow rate. The LiCl mass (m_s in g) and the water mass (m_w in g) variations are expressed in the following mass transfer equations:

$$\frac{dm_s}{dt} = c_{s,in}y_{in} - c_s(t)y_{out} = -\frac{m_s(t)}{V_{sol}(t)}y_{out} \tag{1}$$

$$\frac{dm_w}{dt} = c_{w,in}y_{in} - c_w(t)y_{out} = c_{w,in}y_{in} - \frac{m_w(t)}{V_{sol}(t)}y_{out} \tag{2}$$

where t is the time, y_{in} is the water influx diluting the LiCl solution (in cm^3 s^{-1}), y_{out} is the solution flux pumped out of the LiCl solution (in cm^3 s^{-1}), $c_{s,in}$ is the LiCl concentration in the water influx diluting the LiCl solution ($c_{s,in} = 0$ g cm^{-3} in the setup presented in **Figure 2**), $c_{w,in}$ the water concentration in the solution flux pumped out of the LiCl solution and V_{sol} is the volume of the LiCl solution (in cm^3). Contrarily to Graf and Kocherbitov, the volumetric pump was assumed accurate and thus $y_{in} = y_{out} = 0.1$ cm^3 s^{-1}. V_{sol} is found according to:

$$V_{sol}(t) = \frac{m_{sol}(t)}{\rho_{sol}(t)} = \frac{m_s(t) + m_w(t)}{\rho_{sol}(t)} \tag{3}$$

where m_{sol} is the mass of the LiCl solution and ρ_{sol} is the volumetric mass of the LiCl solution, found using the 5-order interpolation for density as a function of LiCl molality used by Graf and Kocherbitov.[1]

$$\rho_{sol} = Am_s^5 + Bm_s^4 + Cm_s^3 + Dm_s^2 + Em_s^1 + F \tag{4}$$

A = 0.00000013235202 B = -0.00000725374481 C = 0.00015040919573

D = -0.00170256053530 E = 0.02578077468346 F = 0.99708472909226

By solving the differential equations, the LiCl and water masses can be calculated as a function of time. Once LiCl concentration as a function of time is obtained, the resulting a_w variation as a function of time can be found. To do so, the 6-order interpolation of data reported in literature by Graf and Kocherbitov was used.[1,2]

$$a_w = Am_w^6 + Bm_w^5 + Cm_w^4 + Dm_w^3 + Em_w^2 + Fm_w^1 + G \quad (5)$$

A = 0.00000007889768 B = -0.00000498276201 C = 0.00010654893491
D = -0.00069038454736 E = -0.00214042272567 F = -0.03311780893859
G = 0.99990879748652

The obtained results are presented in **Figure S1**. The water and LiCl gravimetric masses were also experimentally determined before and after the experiment to verify the validity of the computed values. Results are also presented in **Figure S1** and it appears that the LiCl solution is well saturated and initially reaches the values used for computation. The absence of LiCl (under quantification limit of the balance) in y_{out} after 22h of experiment is also confirmed. Finally, RH, that was measured in the atmosphere of the LiCl solution, is presented in **Figure S1** and shows a good correspondence between calculated values and experimental values. Taken together, these results suggest that a_w could be accurately varied for the duration of the experiment. The computed value of a_w as a function of time will thus be used in further experiments to measure the mass of a PEM as a function of RH.

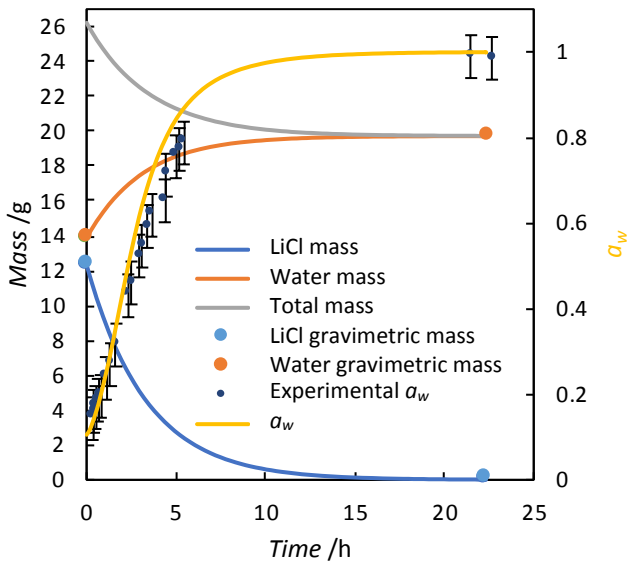

Figure S1 – Modelling of the LiCl and water masses transfer as a function of time, and the resulting water activity (a_w). In order to confirm the computed values, LiCl and water gravimetric masses are measured in initial and final solutions, and RH is measured in the air of the vessel as illustrated in **Figure 2** (error bars show the error of the instrument).

2) Water uptake on an uncoated QCM-D sensor

An experiment was carried out to verify that no water condensation occurred at the uncoated sensor surface. Indeed, the whole humidity module is thermostated and no temperature gradient should be present, especially by slowly increasing a_w which leaves time for the water sorption/desorption equilibrium to be reached. Results of mass detected on the sensor as a function of time are presented together with the calculated a_w value in **Figure S2**. It appears that no significant mass uptake could be measured. This confirms that by slowly varying a_w, the system can be used to measure mass variations in hydrated films without measurement artifacts.

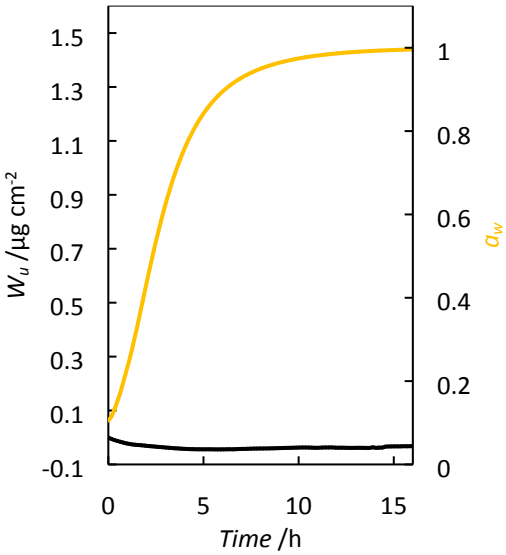

Figure S2 – Mass shift due to water uptake (W_u) with increasing a_w measured by QCM-D on a naked gold sensor.

3) Use of the Sauerbrey equation

In order to convert Δf/n into a mass of water absorbed by the PEM, several mathematical models exist. The Sauerbrey equation is the simplest. It assumes laterally homogeneous thin film that are as rigid as the quartz crystal. Atomic force microscopy images of $[PAH\text{-}PPCs]_5$ and $[PAH\text{-}PSS]_5$ constructed in the very same conditions were reported by our group elsewhere and show very smooth and homogeneous surfaces (root-mean-square roughness ranging from 1 to 2 nm).[3] Considering the mass of the multilayer after construction, *i.e.* 8 and 1 µg cm^{-2} for respectively $[PAH\text{-}PPCs]_5$ and $[PAH\text{-}PSS]_5$, and a density of 1.2 g cm^{-3}, it can be computed that $[PAH\text{-}PPCs]_5$ is 67 nm thick and $[PAH\text{-}PSS]_5$ is 8 nm thick. The PEMs are thus thin films. Finally, the rigidity of the film can be estimated using the ΔD_n to -Δf/n ratio which should be lower than 4 10^{-7} Hz for a 5 MHz crystal to stay in conditions that are considered compatible with the Sauerbrey model.[4] For all measured overtones, this ratio reaches a maximum value of 8 10^{-8} Hz. A last more stringent parameter for the use of Sauerbrey equation is the Δf/n dispersions. The Δf/n measured for the different overtones (presented in **Figure 4**b and c) show a small overtone splicing for $[PAH\text{-}PPCs]_5$ and no overtone splicing for $[PAH\text{-}PSS]_5$. In order to further confirm that the Sauerbrey model can be used for $[PAH\text{-}PPCs]_5$, the mass uptake when a_w=0.999 was also computed via the Voinova model which takes into account the viscoelastic properties (using a lab-made software developed by Mc Evoy *et al.*).[5] The obtained mass was exactly the same as the one obtained using the Sauerbrey equation (data not shown) which suggests that viscoelasticity does not have to be taken into account.

4) Replicates of the sorption/desorption isotherms

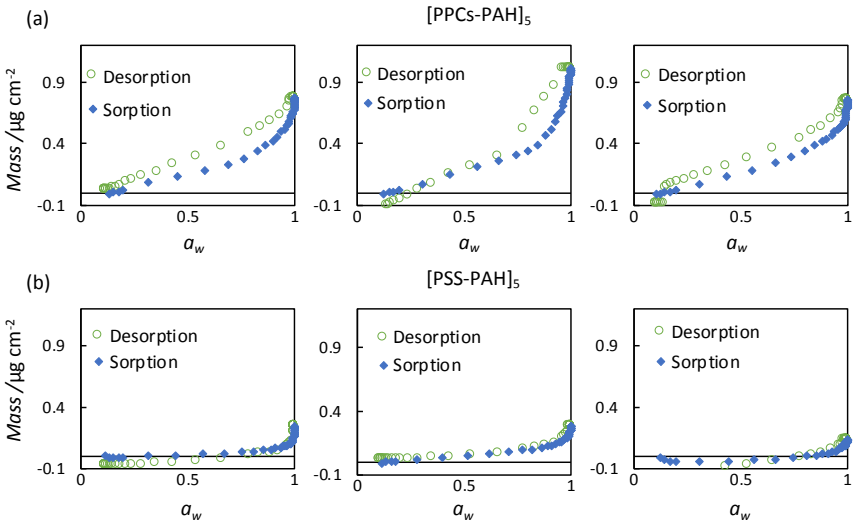

Figure S3 – Water sorption/desorption isotherms. Δm_w as a function of a_w for (a) $[PAH\text{-}PPCs]_5$ and (b) $[PAH\text{-}PSS]_5$.

5) Fitting of the Flory-Huggins equation

The Flory-Huggins equation provides a direct relationship between a_w and the polymer swelling according to[6,7]

$$a_w = (1 - S^{-1})e^{(S^{-1} + \chi S^{-2})} \tag{6}$$

where χ is the Flory-Huggins interaction parameter, and S is the swelling degree, which is defined as d_s/d_0 with d_s being the thickness at a given a_w and d_0 the thickness of the multilayer at a_w=0.11. χ is a dimensionless quantity characterizing the difference in interaction energy of a solvent molecule immersed in pure polymer compared with one in pure solvent. Its evaluation is topical since it provides insight into the polymer-solvent interaction.

In this paper, the water uptake (Δm_w) as a function of a_w was measured for $[PAH\text{-}PPCs]_5$ and $[PAH\text{-}PSS]_5$ multilayers. Using QCM-D, the dry mass of these multilayers (m_0) was measured as well. Assuming that the dry multilayer density (ρ_{PEM}) is of 1.2 g cm^{-2} and knowing that the solvent (here water) density (ρ_w) is of 1 g cm^{-2}, the swelling degree can be found according to

$$S(a_w) = \frac{\Delta m_w\, \rho_w^{-1} + m_0\, \rho_{PEM}^{-1}}{m_0\, \rho_{PEM}^{-1}} = \frac{d_s}{d_0} \tag{7}$$

Then, by fitting equation (4) to S as a function of a_w, one can estimate χ. Results of the fitting are presented in **Figure S4** for both multilayers and for both water sorption and desorption. We found that $\chi > 0$ for both multilayers (see the values for desorption and sorption, for each multilayer, on **Figure S4**). The χ value of 1.1 we found for the [PAH-PSS] system is consistent with previous report that computed a value of 0.91 $\pm$ 0.05.[8]

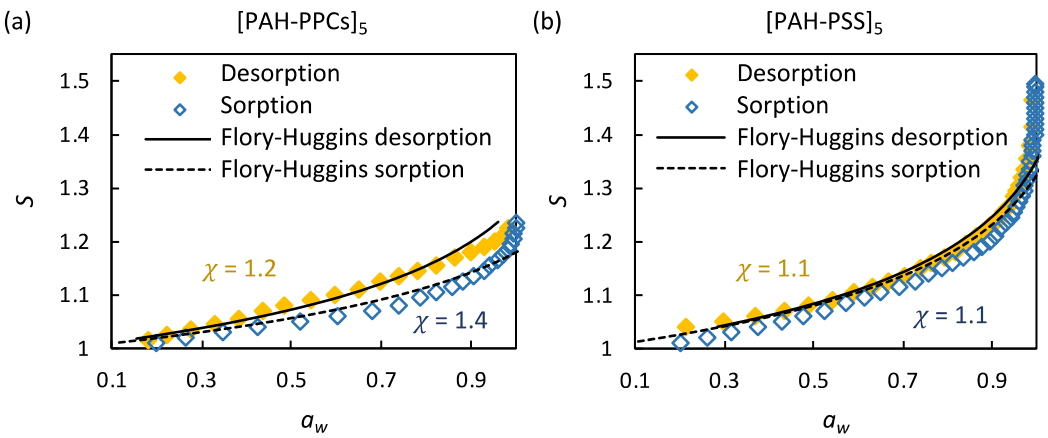

Figure S4 – S as a function of a_w for (a) $[PAH\text{-}PPCs]_5$ and (b) $[PAH\text{-}PSS]_5$ – Fitting of the Flory-Huggins equation to the data (black curves).

While the original Flory-Huggins theory assumes that χ stays constant throughout hydration and dehydration, it has been more recently proposed that it may vary with a_w. In this way, Peppas *et al.* suggested that a quadratic variation of χ as a function of $1/S$ could be used to allow for χ to vary. χ was thus defined as[9]

$$\chi(S) = \chi_1 + \chi_2 S^{-1} + \chi_2 S^{-2} \tag{8}$$

where χ_1, χ_2 and χ_3 are the fitting parameters. The data were fitted with this new condition and results are presented in **Figure 5**c and d, and in **Figure S5**. While the fitting is much better, this method did not evidence any drastic change of the χ value, therefore suggesting that the interaction between the PEs and water is relatively constant during the swelling. This result is not consistent with the positive-to-negative transition of the χ value observed at RH=60%-70% for the [PAH-PSS] system by Secrit *et al.*[10] The difference can be explained by the lower pH that was used to construct the PEMs in this paper. The consequence is that the ion-pairing between PAH and PSS is maximal.

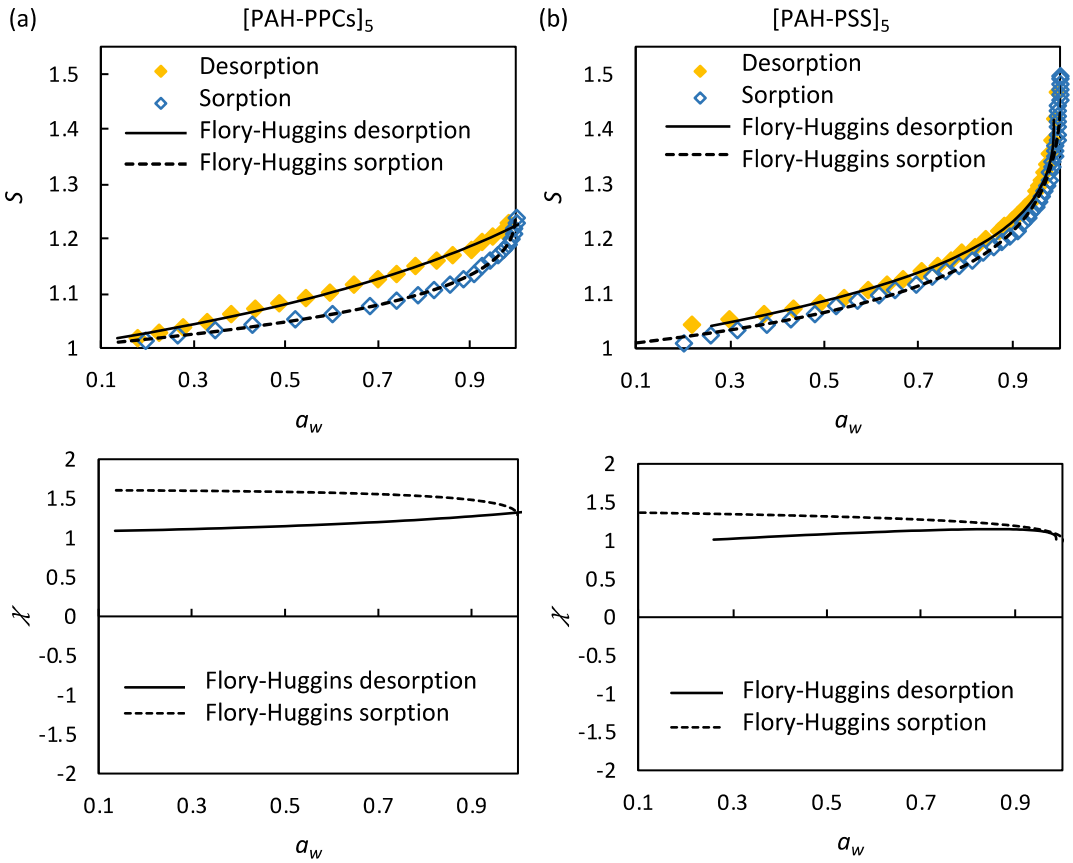

Figure S5 – S as a function of a_w for (a) $[PAH\text{-}PPCs]_5$ and (b) $[PAH\text{-}PSS]_5$ – Fitting of the Flory-Huggins equation with variable χ (black curves) – χ values computed as a function of a_w are also provided in the lower panel.

6) Δf/n and Δ*D* of the dry multilayer

The resonant frequencies of the naked sensors (before multilayer construction) were determined in the humidity chamber (a_w set at 0.11). The PEMs were then constructed in the flow module of the QCM-D. After this construction step, the coated sensors were left overnight in a desiccator before being mounted again in the humidity chamber. The a_w was set at 0.11 in the chamber and the resonant frequencies were determined again. The shift between the resonant frequency before and after multilayer construction is thus due to the multilayer mass and it was used to compute the PEM dry mass (see **Figure S6**).

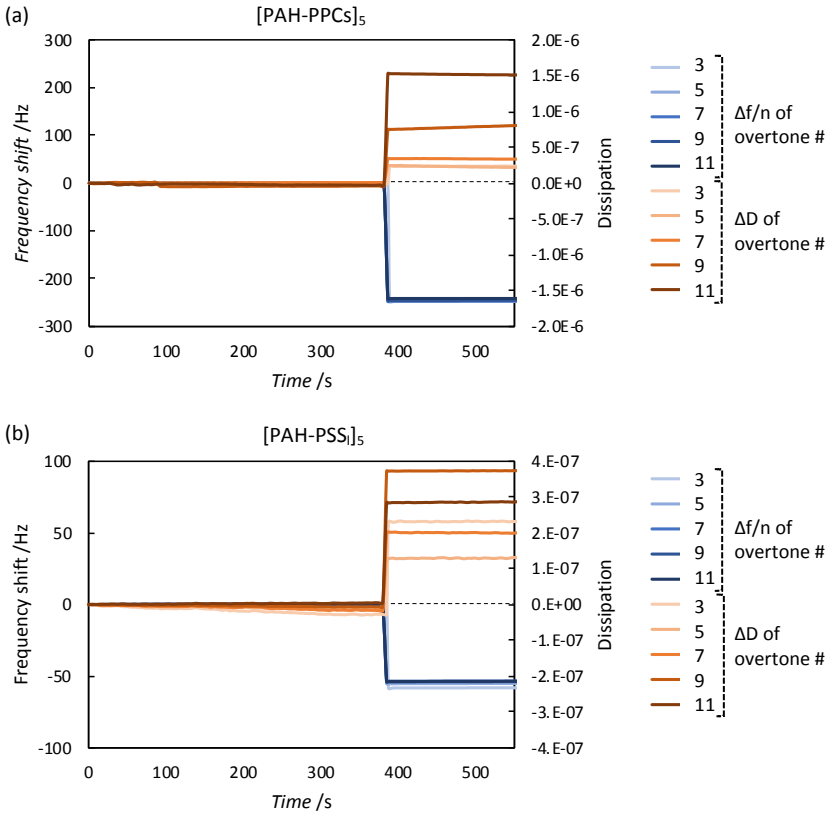

Figure S6 – Δf/n and Δ*D* due to (a) $[PAH\text{-}PPCs]_5$ or (b) $[PAH\text{-}PSS]_5$ construction at $a_w = 0.110$ (measured in the humidity chamber).

6) References

(1) Graf, G.; Kocherbitov, V. Determination of Sorption Isotherm and Rheological Properties of Lysozyme Using a High-Resolution Humidity Scanning QCM-D Technique. *J. Phys. Chem. B* **2013**, *117* (34), 10017–10026.

(2) Robinson, R. A. The Water Activities of Lithium Chloride Solutions up to High Concentrations at 25°. *Trans. Faraday Soc.* **1945**, *41* (0), 756–758.

(3) vander Straeten, A.; Bratek-Skicki, A.; Jonas, A. M.; Fustin, C. A.; Dupont-Gillain, C. Integrating Proteins in Layer-by-Layer Assemblies Independently of Their Electrical Charge. *ACS Nano* **2018**, *12* (8), 8372–8381.

(4) Reviakine, I.; Johannsmann, D.; Richter, R. P. Hearing What You Cannot See and Visualizing What You Hear: Interpreting Quartz Crystal Microbalance Data from Solvated Interfaces. *Anal. Chem.* **2011**, *83* (23), 8838–8848.

(5) Mc Evoy, K. Improving Quantification of Proteins at Interfaces through a Better and Practical Modeling of Quartz Crystal Microbalance Response. UCL 2012.

(6) Lounis, F. M.; Chamieh, J.; Leclercq, L.; Gonzalez, P.; Geneste, A.; Prelot, B.; Cottet, H. Interactions between Oppositely Charged Polyelectrolytes by Isothermal Titration Calorimetry: Effect of Ionic Strength and Charge Density. *J. Phys. Chem. B* **2017**, *121* (12), 2684–2694.

(7) Shultz, A. R.; Flory, P. J. Phase Equilibria in Polymer-Solvent Systems. II. Thermodynamic Interaction Parameters from Critical Miscibility Data. *J. Am. Chem. Soc.* **1953**, *75* (16), 3888–3892.

(8) Kügler, R.; Schmitt, J.; Knoll, W. The Swelling Behavior of Polyelectrolyte Multilayers in Air of Different Relative Humidity and in Water. *Macromol. Chem. Phys.* **2002**, *203* (2), 413–419.

(9) G. Mikos, A.; A. Peppas, N. Flory Interaction Parameter χ for Hydrophilic Copolymers with Water. *Biomaterials* **1988**, *9* (5), 419–423.

(10) Secrist, K. E.; Nolte, A. J. Humidity Swelling/Deswelling Hysteresis in a Polyelectrolyte Multilayer Film. *Macromolecules* **2011**, *44* (8), 2859–2865.