

Supporting Information for

**Structural Investigation of a Self-Cross-Linked  
Chitosan/Alginate Dialdehyde Multilayered Film with in  
Situ QCM-D and Spectroscopic Ellipsometry**

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## S1. Preparation and Characterization of Alginate Dialdehyde

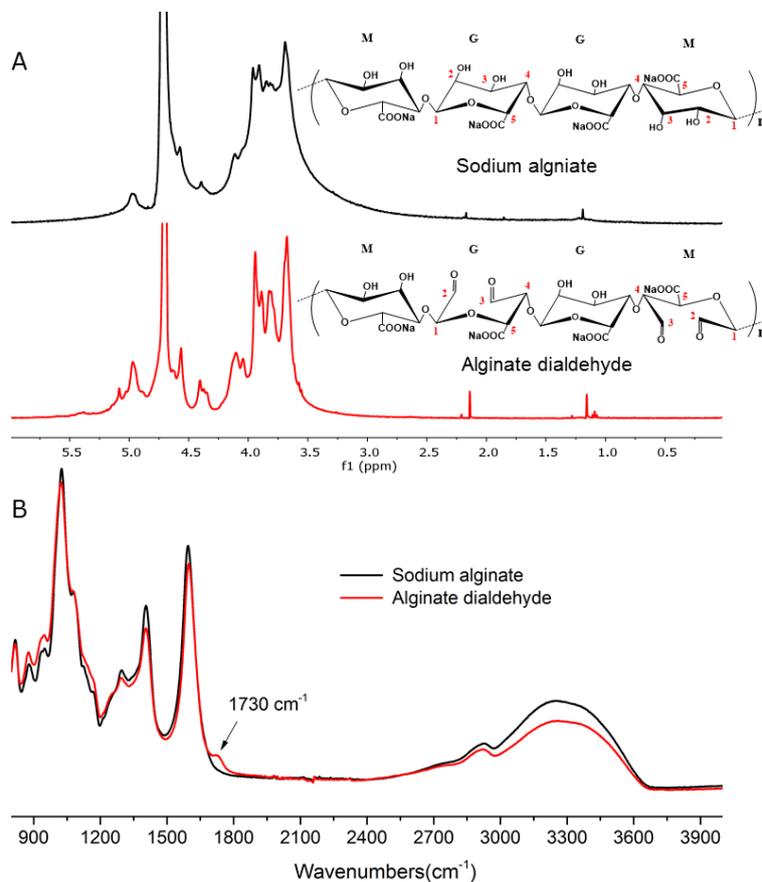
NaIO<sub>4</sub> (5.4 g) was dissolved in Milli-Q water (100 ml) to obtain a stock solution. NaAlg (5 g) was dissolved in Milli-Q water (300ml), then NaIO<sub>4</sub> (20 mol% relative to the number of repetitive units of sodium alginate) was added under stirring to reach a final volume of 500 ml with Milli-Q water. The solution was kept stirring in the dark for 24 h. The oxidation reaction was then quenched by the addition of 2 ml ethylene glycol under stirring for 0.5 h. The obtained ADA was precipitated by adding 2 g NaCl and 500 ml ethanol to the mixture. Finally, ADA was purified by dialyzing the precipitated product against Milli-Q water for 3 days followed by lyophilization.

The degree of oxidation of the synthesized ADA was determined by HONH<sub>2</sub>·HCl titration. Briefly, 17.5 g HONH<sub>2</sub>·HCl was dissolved in 150 ml Milli-Q water. Afterward, 6 ml methyl orange reagent (0.05%) was added to the solution, diluted to a final volume of 1 L, and the pH was adjusted to 4. Next, 0.1 g ADA was dissolved in 25 ml hydroxylamine hydrochloride-methyl orange solution, stirring 2 h for complete dissolution. The standardized NaOH solution was used for titration until observing the red-to-yellow endpoint. The oxidation degree of ADA was accordingly calculated to be around 18.2% following as:

$$\frac{\text{mol of CHO}}{\text{mol of uronic acid}} = \frac{V_{\text{NaOH}} \times 0.1 \text{ mol} \cdot \text{L}^{-1} \times 198 \text{ g} \cdot \text{mol}^{-1}}{m_{\text{sample}}} \quad (1)$$

NaAlg and ADA were dissolved in heavy water (D<sub>2</sub>O) with a concentration of 6 mg/mL for proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectrometry (Bruker Ascend™ 400) measurements (Figure S1A). The signals of the protons of G-2, G-3, G-4, M-2, M-3, M-4, and M-5 appeared in the range of 3.6–4.1 ppm and the signals in the 4.2–4.9 ppm correspond to the anomeric protons of G-1, M-1, and G-5. The new signal appeared at 4.2 ppm was assigned to the protons of G-5 of the oxidized G units. Simultaneously, two new signals corresponding to the protons of hemiacetals formed from aldehyde and hydroxyl groups appeared at 5.1 and 5.4

ppm.<sup>1</sup> NaAlg and ADA powders were used for the Fourier transform infrared (FTIR) spectroscopy (Nicolet iS50 spectrometer) measurements. As shown in Figure S1B, the corresponding peak at around 1730 cm<sup>-1</sup> represents the symmetric vibrational band of aldehyde groups.<sup>1-2</sup>



**Figure S1.** (A) <sup>1</sup>H-NMR spectra and (B) FTIR spectra of sodium alginate and alginate dialdehyde

## S2. Effective Hydrodynamic Density of the Film

The adsorbed dry mass can be calculated according to the de Feijter's formula<sup>3</sup>:

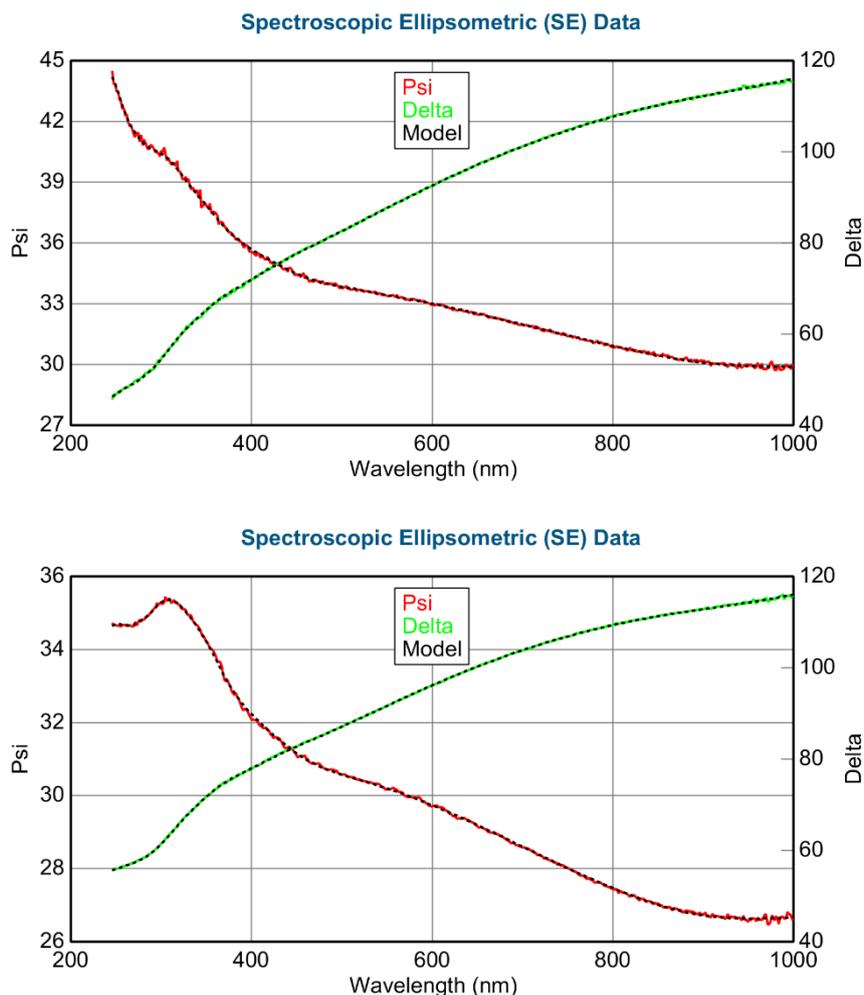
$$m_{opt} = d_f \frac{n_f - n_{sol}}{dn/dc} \quad (2)$$

Where  $n_f$  and  $n_{sol}$  are the refractive indices of hydrated film and water at  $\lambda=632.3\text{nm}$ , respectively. A refractive index increment of  $dn/dc=0.18 \text{ cm}^3/\text{g}$  was used herein.<sup>4-5</sup> The effective hydrodynamic density of the film ( $\rho_f$ ) can be calculated according to the following expression<sup>6-8</sup>:

$$\rho_f = \rho_{polymer} \frac{m_{opt}}{m_{Sau}} + \rho_0 \left(1 - \frac{m_{opt}}{m_{Sau}}\right) \quad (3)$$

where  $m_{Sau}$  is  $46.92 \text{ mg}\cdot\text{m}^{-2}$ ,  $m_{opt}$  is  $16.9 \text{ mg}\cdot\text{m}^{-2}$  (calculated from equation 2),  $\rho_{polymer}$  is  $1410 \text{ kg}\cdot\text{m}^{-3}$  (the bulk density of the polymer<sup>7</sup>), and  $\rho_0$  is  $999 \text{ kg}\cdot\text{m}^{-3}$  (density of 15 mM PBS solution at 25 °C). The effective hydrodynamic density of the film is then estimated to be about  $1100 \text{ kg}\cdot\text{m}^{-3}$ .

### S3. Ellipsometric Characterization of Bare QCM-D Sensor



**Figure S2.** Spectroscopic  $\psi$  and  $\Delta$  obtained for QCM-D sensor together with the fitted model in (top) air and (bottom) in 15 mM PBS with pH 6

The sensor was modeled as a titanium substrate coated with a silica layer. To avoid overparameterization, data fitting was only conducted for the optical constants of titanium (B-Spline function, resolution of 0.2 eV, starting material Ti from software library), while keeping the optical constants (SiO<sub>2</sub>\_JAW from software library) and thickness (25 nm) of silica fixed. By doing so, the optical behavior of the sensor substrate is modeled as a “pseudosubstrate”. The thickness of the silica coating was estimated based on the location of the oscillatory peak observed in  $\psi$  and  $\Delta$  spectra from the sensor data in the air.

#### S4. Optimization of the Optical Models

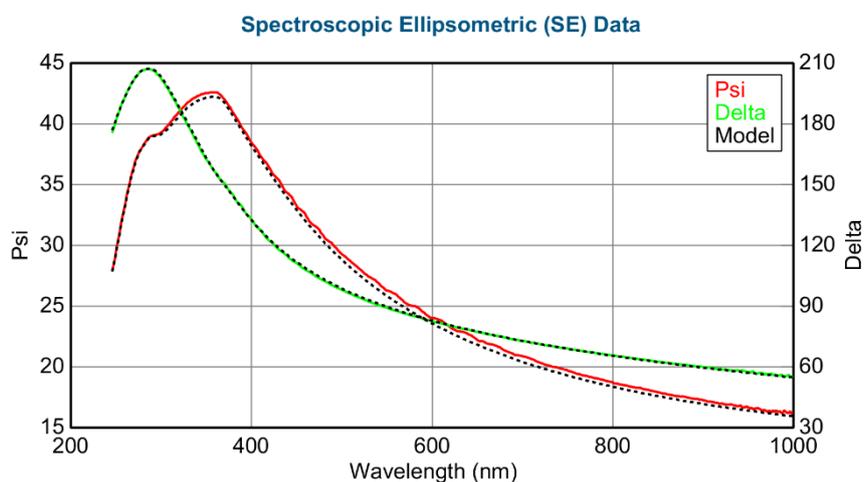
For both one-component Cauchy and two-component BEMA modeling approaches, we have checked if additional nonideality options can improve the data quality. It can be seen from Table S1 that the additional fitting parameters not only have a minor effect on the fitting quality (MSE value) but also produce a correlation between the fitting parameters and provide unphysical optical constants.

**Table S1. Fitting results for different fitting conditions**

	Modelling options	Thickness (nm)	A	B	MSE	Water content (v/v %)	Roughness (nm)	Inhomogeneity (%)
Cauchy model	basic fitting	16.0	1.510	0.004	6.915	-	-	-
	with roughness	58.9	1.407	0.003	4.382	-	11.3	-
	with grading	15.9	1.510	0.004	6.618	-	-	14.5
	with thickness nonuniformity	15.9	1.510	0.004	6.764	-	-	89.2
BEMA model	basic fitting	16.1	-	-	7.074	3.5	-	-
	with roughness	19.6	-	-	6.952	8.7	2.7	-
	with grading	15.5	-	-	7.022	0.6	-	6.9
	with thickness nonuniformity	15.5	-	-	6.832	0	-	39.4

As mentioned in the manuscript, the estimated water content for the multilayered film before crosslinking (around 3.5 v/v %) seems rather low for a hydrated polymeric film. For thin polymeric films (typically in the range of 20 nm and below) studied with ellipsometry, one could always expect a relatively strong correlation between the thickness and optical constants. This means that the small water content herein could be a result of slightly overestimated refractive index and underestimated thickness. To address this problem, we have fabricated a CHI/ADA multilayered film using the same protocol on a silicon wafer and the data was modeled using two-component BEMA (Figure S3). Accordingly, a better match between the model and experimental data is found and the estimated water content seems more physical.

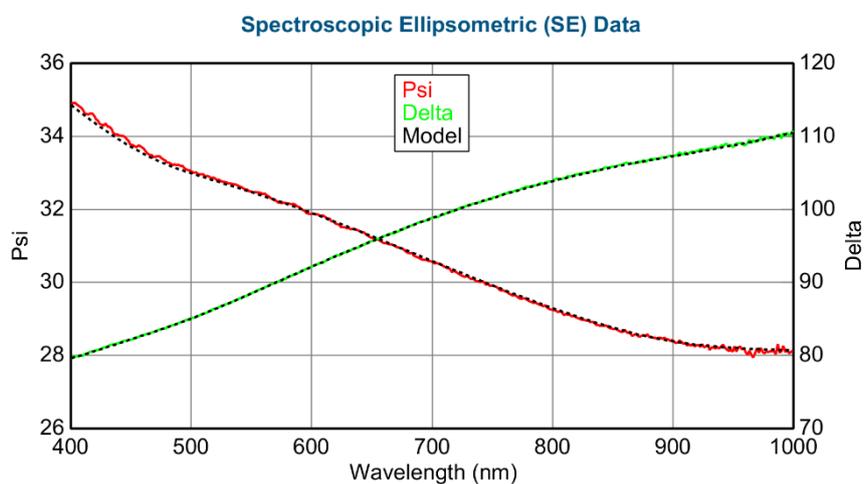
Fit Results	Optical Model
MSE = 8.863	<ul style="list-style-type: none"> <li>- Layer # 3 = <a href="#">EMA</a> Thickness # 3 = <a href="#">19.03 nm</a> (fit)</li> <li># of Constituents = <a href="#">2</a></li> <li>- Material 1 = <a href="#">Cauchy</a></li> <li style="padding-left: 20px;">A = <a href="#">1.510</a> B = <a href="#">0.00500</a> C = <a href="#">0.0000</a></li> <li>+ <b>Urbach Absorption Parameters</b></li> <li>Material 2 = <a href="#">H2O_Pribil_25</a></li> <li>EMA % (Mat 2) = <a href="#">33.0</a> (fit)</li> <li>depolarization = <a href="#">0.333</a> Analysis Mode = <a href="#">Bruggeman</a></li> </ul>
Thickness # 3 = $19.03 \pm 0.242$ nm	
EMA % (Mat 2) = $33.0 \pm 0.83$	
Total Thickness = $117.91 \pm 0.242$ nm	
	Layer # 2 = <a href="#">SIO2_JAW</a> Thickness # 2 = <a href="#">97.87 nm</a>
	Layer # 1 = <a href="#">INTR_JAW</a> Thickness # 1 = <a href="#">1.00 nm</a>
	Substrate = <a href="#">SI_JAW</a>



**Figure S3.** Spectroscopic ellipsometry data for the multilayered film on silicon wafer (in 15 mM PBS buffer at pH 6) and modeling using BEMA.

Based on this, we have tried to improve the fitting quality for the multilayered film on the QCM-D sensor by excluding the UV range from the modeling (Figure S4). Accordingly, a thickness of around 23 nm and a more physical water content value of around 34 v/v % was obtained. For the rest of the data (pH cycle and multivalent ions), excluding the UV range from modeling was shown to have a minor effect on the fitting parameters.

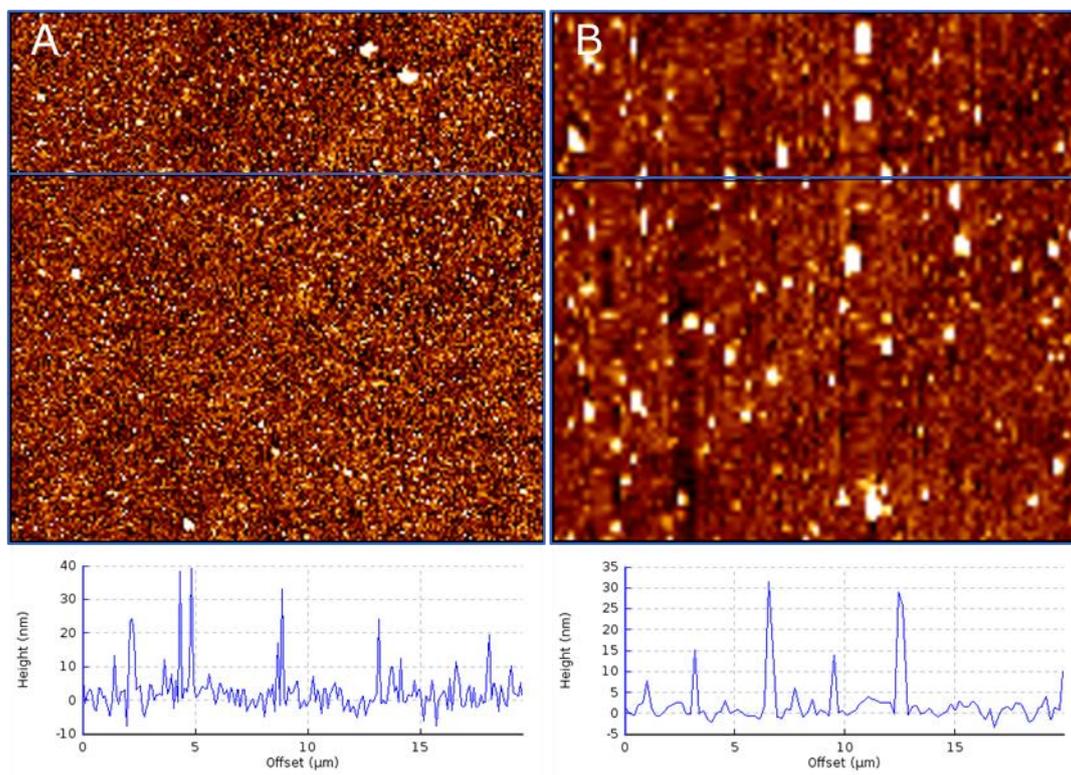
Fit Results	Optical Model
<p>MSE = 1.584</p> <p>Thickness # 2 = <math>23.07 \pm 0.150</math> nm</p> <p>EMA % (Mat 2) = <math>34.1 \pm 0.44</math></p>	<ul style="list-style-type: none"> <li>- Layer # 2 = <a href="#">EMA</a> Thickness # 2 = <a href="#">23.07 nm</a> (fit)</li> <li style="padding-left: 20px;"># of Constituents = <a href="#">2</a></li> <li>- Material 1 = <a href="#">Cauchy</a></li> <li style="padding-left: 20px;">A = <a href="#">1.510</a> B = <a href="#">0.00500</a> C = <a href="#">0.0000</a></li> <li>+ <b>Urbach Absorption Parameters</b></li> <li style="padding-left: 20px;">Material 2 = <a href="#">H2O_Pribil_25</a></li> <li style="padding-left: 20px;">EMA % (Mat 2) = <a href="#">34.1</a> (fit)</li> <li style="padding-left: 20px;">depolarization = <a href="#">0.333</a> Analysis Mode = <a href="#">Bruggeman</a></li> </ul> <hr/> <p>Layer # 1 = <a href="#">SiO2_JAW</a> Thickness # 1 = <a href="#">25.00 nm</a></p> <hr/> <p>+ Substrate = <a href="#">B-Spline</a></p>



**Figure S4.** Spectroscopic ellipsometry data for the multilayered film (in 15 mM PBS buffer at pH 6) on QCM-D sensor and modeling using BEMA in the wavelength range of 400 – 1000 nm.

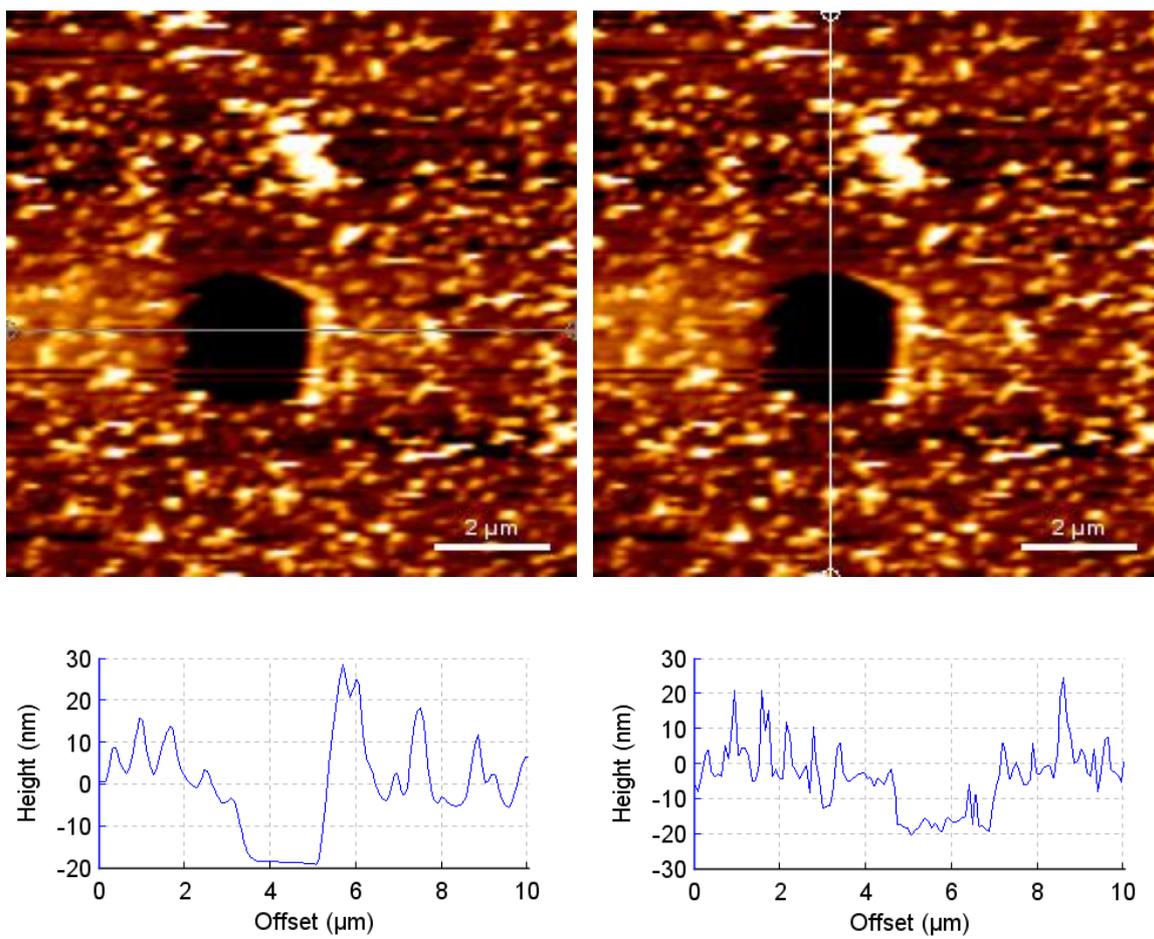
## S5. Topography of the Film

The surface topography of the fabricated multilayered film was studied using AFM tapping mode imaging. As shown in Figure S5, self-assembled structures can be found on the multilayered film, with a height in the range of 10-100 nm.



**Figure S5.** AFM tapping mode topography images ( $20 \times 20 \mu\text{m}^2$ ) of the film determined in air (A) and in solution (B).

To further assess the thickness of the multilayered film, the sample on the QCM-D sensor was scratched ( $2 \times 2 \mu\text{m}^2$ ) using contact mode imaging in pH 6 buffer. Afterward, the film including the scratched area was scanned using tapping mode imaging and the cross-section profiles were obtained (Figure S6). Accordingly, the film thickness can be estimated to be around 15-20 nm. It should be noted that since tapping mode imaging is based on a mechanical response (deflection) measured by the cantilever, AFM thickness is closer to the ellipsometry thickness and the topmost parts of the film with high water content might not be seen.

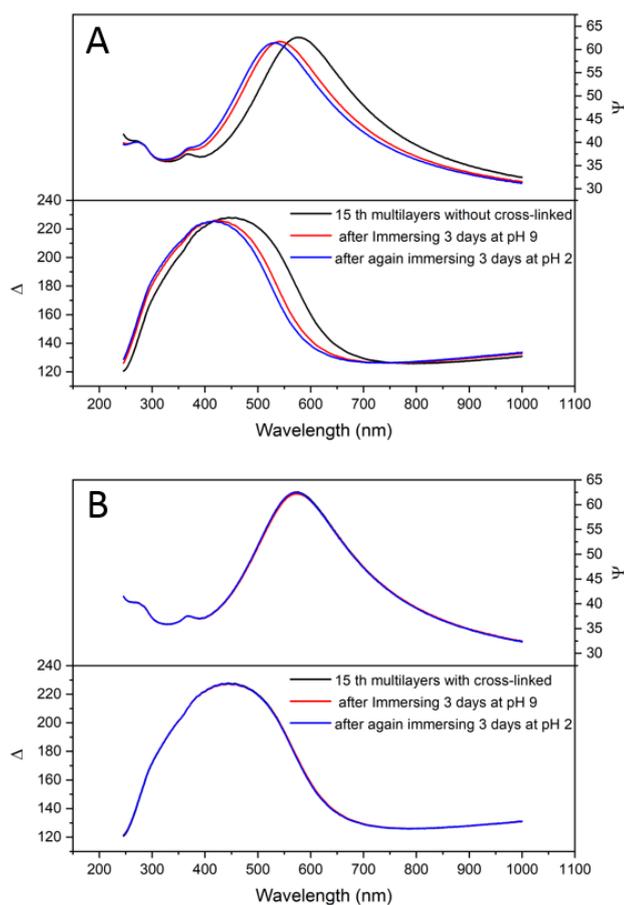


**Figure S6.** AFM tapping mode topography images ( $10 \times 10 \mu\text{m}^2$ ) and the cross-section profiles of the scratched film determined in pH 6 buffer.

The film thickness (from the base of aggregates) is estimated to be around 15-20 nm.

## S6. Stability of the Crosslinked Multilayered Film

To check the effect of post-modification using sodium cyanoborohydride, we have prepared two multilayered film samples on silicon wafer, one without post-modification and one with post-modification. Afterward, the dry thickness of the samples was measured in air. Then, the samples were immersed in pH 9 solution for 3 days followed by another 3 days immersion in pH 2 solution. As can be seen from the raw and modeled ellipsometric data, the sample without post-modification does not fully dissolve, however, a notable decrement in thickness is found (14.1 nm  $\rightarrow$  8 nm (after pH 9)  $\rightarrow$  6 nm (after pH 2)). On the other hand, the sample with post-modification showed notable stability and the thickness remained almost unchanged (13.7 nm  $\rightarrow$  13.5 nm (after pH 9)  $\rightarrow$  13.3 nm (after pH 2)).



**Figure S7.** Spectroscopic ellipsometry data for the multilayered film on silicon wafer without (A) and with (B) post-modification using sodium cyanoborohydride. The measurements were conducted in air.

## Reference

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