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Molecular Ordering of Layer-by-Layer Polyelectrolyte Films Studied by Sum-Frequency Vibrational Spectroscopy

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The molecular arrangement in organic thin films is crucial for their increasing technological applications. Here, we use vibrational spectroscopy by sum-frequency generation (SFG) to study the ordering of polyelectrolyte layers adsorbed on silica for all steps of layer-by-layer (LbL) self-assembly. In situ measurements during adsorption and rinsing showed that the adsorbed polymer has a disordered conformation and confirmed surface charge overcompensation upon polyelectrolyte adsorption by probing the interfacial electric field. In dry films, the polymer chains acquired a net orientational ordering, which was affected, however, by the adsorption of subsequent layers. Such a detailed characterization may allow the control of LbL film structure and functionality with unprecedented power.

Organic or hybrid organic-inorganic thin films on solids are becoming increasingly important in current technology, ranging from biomedical applications¹ to the fabrication of functional nanomaterials and devices.² Among the available techniques for producing such films, the layer-by-layer (LbL) self-assembly method pioneered by Decher and co-workers in the early 1990s presented as a simple and versatile tool for constructing multilayer assemblies on solid surfaces.^{3,4} This technique is based on alternate electrostatic adsorption of oppositely charged polyelectrolytes, although many other materials can also be incorporated into the films due to a combination of intermolecular interactions.^{2,3,5} LbL films allow precise control of the thickness and composition of each layer and are used in nanotechnology for a great variety of applications, including optoelectronic coatings,⁶ organic diodes,^{7,8} membrane models,⁹ biosensors,^{9–11} drug delivery,¹² and others. Their properties have been extensively characterized by several techniques such as atomic force microscopy,¹³⁻¹⁵ reflection-absorption infrared spectroscopy,¹³ quartz crystal microbalance,^{15,16} ellipsometry,¹⁷ and X-ray reflectivity,15 to name a few. However, the information obtained about the structure of LbL films is generally at a layer-like level, such as layer thickness, composition, or interpenetration. Therefore, a thorough structural characterization at the molecular level is often needed but is difficult to obtain with traditional techniques. This knowledge may allow us to tailor the experimental conditions to control the molecular arrangement and functionality of LbL films with unprecedented power.

Vibrational spectroscopy by sum-frequency generation (SFG) is a powerful tool to investigate molecular orientation and conformation at interfaces^{18,19} and has been recently used to investigate LbL polyelectrolyte films. We have shown with SFG spectroscopy that the drying procedure affects the film homo-

geneity.²⁰ Drying by nitrogen flow (the usual procedure) results in inhomogeneous films, while spontaneous drying leads to quite homogeneous layers. Kett et al. investigated LbL polyelectrolyte films adsorbed on a gold surface that had been coated with a self-assembled monolayer of a carboxylic-acid-terminated alky-Ithiol.²¹ They have demonstrated that adsorption of the first polyelectrolyte layer affects the conformation of the alkylthiol monolayer. Furthermore, they concluded that the subsequent layer of oppositely charged polyelectrolyte also interacts strongly with the first layer, leading to an isotropic interpenetrated bilayer. In this letter, we use SFG spectroscopy to characterize the molecular ordering of LbL polyelectrolyte films during all of the steps of LbL assembly on silica substrates (adsorption, rinsing, and drying). In situ measurements during adsorption and rinsing show that polymer chains adsorb with a disordered conformation. We also probe changes in the magnitude and sign of the electric field at the silica/film/solution interface, providing additional evidence for surface charge inversion upon adsorption of polyelectrolyte layers. Upon drying the films by spontaneous water evaporation, polymer chains acquire a net orientational ordering, which is affected, however, by the adsorption of subsequent layers. We propose a simple electrostatic model to account for the main observations.

As a model system, we have investigated LbL films formed by the well-characterized pair of polyelectrolytes,² the cationic poly(allylamine hydrochloride), PAH, and anionic poly(sodium styrene sulfonate), PSS (their molecular structures are depicted in Figure 3).PAH ($M_w = 15000$ g/mol) and PSS ($M_w = 70000$ g/mol) were obtained from Aldrich and used as received. Selfassembled LbL films were deposited on fused silica substrates (infrared grade, Esco Products Inc., 3 mm thick) cleaned by piranha solutions (H₂SO₄/H₂O₂ at 3:1 proportions by volume *-warning: piranha solutions are extremely oxidizing and should never be kept in tightly closed containers*) for 30 min, extensively rinsed with Milli-Q water (resistivity 18.3 MQ·cm), and dried by nitrogen flow right before use. Samples were

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assembled from aqueous solutions of PAH and PSS with 0.5 mg/mL and at pH 7, which was adjusted by addition of HCl (from Qhemis, 37%, analytical grade) or NaOH (from Aldrich, purity 99.99%). No salt was added for adjusting the ionic strength. In order to obtain homogeneous films, we used drying by slow water evaporation;²⁰ the films were dried by spontaneous evaporation only after all adsorption and rinsing steps of the LbL assembly were complete, with the substrates loosely covered by a Petri dish to avoid contamination and stored for a period of 48 h at room temperature (~23 °C) and with air humidity around 40%. Only after this period were the SFG spectra recorded.

The PAH/PSS films were studied by SFG vibrational spectroscopy, which is surface-specific and also sensitive to molecular conformation and orientation. Here, we describe only its basic features. The detailed theory and several reviews can be found elsewhere.²²⁻²⁵ Briefly, two input laser beams at frequencies of $\omega_{\rm VIS}$ and $\omega_{\rm IR}$, one visible and another in the infrared (IR) range, overlap in time and space at an interface and generate a reflected output at frequency $\omega_{\rm S} = \omega_{\rm VIS} + \omega_{\rm IR}$. This SFG signal is proportional to the square of the effective nonlinear susceptibility $\chi_{\text{eff}}^{(2)}(\omega_{\text{S}} = \omega_{\text{VIS}} + \omega_{\text{IR}})$ of the interface. SFG spectroscopy is surface-specific since $\chi^{(2)}_{eff}$ vanishes in media with inversion symmetry but may be nonvanishing at interfaces where the inversion symmetry is broken. However, if molecules adsorb with random orientations, the net SFG signal is canceled out. Conversely, if there is a substantial SFG signal, we can conclude that molecules have a net average orientation at the interface. When ω_{IR} is near the frequency of molecular vibrations, the SFG output is resonantly enhanced, yielding a vibrational spectrum of the interface. Thus, we can obtain information about the conformation and average orientational ordering of selected groups of the adsorbed polyelectrolytes in LbL films. We used a commercial SFG spectrometer (Ekspla, Lithuania) based on a pulsed Nd³⁺:YAG laser (25 ps pulse duration, 20 Hz repetition rate). Further details of the optical setup can be found in ref 20. For in situ measurements, both beams were overlapped at a silica/solution interface, formed by a silica window in contact with the polyelectrolyte solution in a Teflon cell. For each spectrum in the 2800-3800 cm⁻¹ range (OH stretches), data were collected with 100 shots/data point in 10 cm⁻¹ increments; in the 2800–3100 cm⁻¹ range (CH stretches), we showed an average of three measurements (100 shots/data point each, in 3 cm⁻¹ increments) recorded on different spots of the sample, all with excellent reproducibility.²⁰ In this work, we used the SSP (S-sum, S-visible, and P-infrared) polarization combination, which mainly probes infrared transition dipoles oriented, on average, along the surface normal. We explored CH and OH stretches in order to probe both the polymer chain and water at the film/solution interface. All spectra were divided by that of a Z-cut quartz crystal to normalize out the instrumental response. We also performed phase measurements of $\chi^{(2)}_{eff}$ from interference patterns generated by superimposing the sample SFG signal with that from a quartz crystal reference, as described in ref 26.

Figure 1a–d shows SFG spectra for LbL films of different layers recorded in situ at the silica/solution interface. It is possible to note in all spectra two broad bands due to OH stretches. The bands at 3200 and 3400 cm⁻¹ are usually attributed to water molecules in an ordered and disordered H-bonded network, respectively.^{27,28} Although this assignment has been questioned recently,^{29,30} it is not essential to the interpretation of our results, as described below. It should be emphasized that this water detected by SFG must have orien-



Figure 1. (a–d) SFG spectra for in situ measurements of LbL films in contact with the polyelectrolyte solution (squares) and with the rinsing solution (circles). The SFG spectrum of the silica/water interface at pH 7 (crosses) is also shown for comparison in (a). (e) Interference patterns for in situ phase measurements at 3200 cm⁻¹.

tational order, that is, there must be a preferred average orientation for water molecules either pointing toward or away from the substrate. Hydrogen bonding interactions or the electric field at the silica/water interface can induce a net orientation of the water molecules.^{27,28} When the surface charge density is high enough, the electrostatic interaction dominates, and the shape and intensity of the large band from OH stretches in the SFG spectra from the silica/water interface can be regarded as a probe of the interfacial electric field.^{27,31} From Figure 1a, we note that the SFG spectra of the silica/water and silica/(PAH_{ads} + PAH solution) interfaces are similar, except for a slight change in the line shape (see Supporting Information for modeling parameters obtained from our best fit). However, upon replacing the PAH solution by water at pH 7 (the rinsing solution), the spectral intensity increases significantly, and the lower-frequency band (\sim 3200 cm⁻¹) now dominates the spectrum. This indicates that the interfacial electric field increased, which at first seems unexpected since rinsing is supposed to remove the loosely bound polyelectrolyte, therefore reducing the charge density. However, under the conditions of our experiment, the PAH solution contains a much larger concentration of counterions than water at pH 7. Thus, this increase in surface electric field upon rinsing is due to a reduction in screening by the counterions. Indeed, the Debye screening length for the PAH solution is about 6 nm, and it is larger than 100 nm for the rinsing solution. Upon adsorption of PSS over the first PAH layer (Figure 1b, silica/PAH/(PSS_{ads} + PSS solution) interface), we note that the SFG intensity is much reduced, indicating that the net surface charge density is now considerably smaller. Indeed, replacing the PSS solution by water at pH 7 (silica/ PAH/(PSS_{ads} + rinsing solution) interface) reduces the screening from counterions (screening length varies from about 8 to larger

than 100 nm) but does not have a significant effect on the spectrum. This happens because the net surface charge density for the silica/PAH/PSS interface is so low that the effect of water orientation by the electric field is negligible in comparison to other interactions such as hydrogen bonding,^{27,31} so that any change in screening does not affect significantly the water structure. The SFG spectra for the adsorption of the next bilayer (Figure 1c,d) are quite similar to those of the first bilayer (Figure 1a,b), displaying an alternation of spectra as the last layer is either PAH or PSS. Therefore, from the changes of both intensity and shape in the spectra of Figure 1, we can follow the changes of electric field at the silica/film/solution interface. However, measuring the intensity of the SFG spectra gives only information about the magnitude of the electric field but not its direction. The latter can be deduced by measuring the phase (sign) of $\chi_{\rm eff}^{(2)}$, which depends on the absolute orientation of the water molecules (pointing, on average, toward or away from the substrate). The relative phase between the nonresonant background and the OH resonances may be obtained by fitting the spectra, although this is difficult and less reliable for broad and overlapping OH resonances, generating controversial conclusions.^{29,30}

Alternatively, we have performed direct phase measurements²⁶ of $\chi_{eff}^{(2)}$ in situ at 3200 cm⁻¹ to determine the relative average orientation of water molecules near each LbL film. Figure 1e displays the interference patterns as a function of the phase difference between signals from the sample and the reference crystal. Since the water/silica interface (squares) is negatively charged at pH 7, that pattern should correspond to water molecules ordered by an electric field pointing toward the substrate. For the silica/(PAH_{ads} + PAH solution) interface (circles), we note an inversion from the previous interference pattern, indicating that now the net surface charge is positive. The interference patterns alternate as additional polyelectrolyte layers are assembled, showing that the net surface charge is positive when the last layer is PAH and negative when it is PSS. Therefore, SFG spectroscopy also provides confirmation of charge overcompensation upon adsorption of each polyelectrolyte layer, in agreement with zeta potential³² and surface potential³³ measurements.

It is remarkable that all spectra in Figure 1a–d are dominated by water OH stretches, with no detectable signal from CH stretches ($2800-3100 \text{ cm}^{-1}$) of the polymer chains, as confirmed by measurements with higher resolution (3 cm⁻¹, data not shown). Since both the alternation of the SFG spectra in Figure 1a–d and the UV–vis absorption spectra (data not shown) demonstrate that LbL films are assembling under our experimental conditions, this vanishing SFG signal from the polyelectrolyte chains implies that adsorbed polymer molecules have a disordered conformation when in contact with aqueous solutions. A similar interpretation has been reported for surfactant monolayers adsorbed on glass.³⁴

Figure 2 presents SFG spectra (ex situ) for LbL films assembled from pH 7 solutions and dried as described above. In contrast to in situ measurements, there are now five vibrational resonances from CH stretches at around 2845, 2878, 2920, 2950, and 3050 cm⁻¹, which are assigned to the CH₂ symmetric stretch, CH stretch, CH₂ asymmetric stretch, Fermi resonance between the CH₂ symmetric stretch and the CH₂ bending mode overtone, and aromatic CH stretch modes, respectively.^{35–37} These strong CH peaks imply that for the dry films, the polymer chains do have a net orientational order. However, a quantitative discussion of these spectra is difficult because the polyelectrolyte molecules probably adsorb with a



Figure 2. SFG spectra (ex situ measurements) of LbL films assembled with pH 7 solutions and dried by slow evaporation. The inset shows in detail the CH stretch of the aromatic ring of PSS.

conformation that leads to broad angular distributions for CH groups. Moreover, the SFG spectra contain contributions from both PAH and PSS. Thus, we have focused on a qualitative interpretation.

The top spectrum of Figure 2 shows that the first layer of PAH is adsorbed with a net backbone orientation, probably due to the electrostatic interaction with the negatively charged substrate. With an additional PSS layer, the spectrum changes both in intensity and shape since it may now have contributions from both PAH and PSS. However, the spectral intensities do not increase with the deposition of an additional bilayer, and the spectral shape alternates according to the last layer being either PAH or PSS. This indicates that polyelectrolyte layers are not oriented on average in the same direction as the film grows and suggests that the conformations of the previously adsorbed layers are affected by the subsequent layers in such a way that only the topmost layer has a net orientational order and contributes to the SF spectra while the inner layers adopt an approximately random conformation that is inactive for SFG. Specifically, when the last layer is PAH, we do not observe the weak peak at around 3050 cm⁻¹ but do only when the last layer is PSS. This is reasonable because this resonance is attributed to the aromatic ring present only in PSS. However, this peak vanishes for the (PAH/PSS)/PAH film, indicating that this PSS layer reoriented and assumed a centrosymmetric molecular orientation since it is now located between two layers of equal charge density that result in no preferential orientation for its charged groups. A similar effect has been recently reported by Kett et al.²¹ for LbL films of different polyelectrolytes. However, they attributed the effect only to interpenetration and complexation of oppositely charged polyelectrolyte layers, which resulted in an isotropic arrangement of the polymer chains. Even though interpenetration is significant for LbL films, it is certainly not complete as individual layers can still be identified in the films.³⁸ Therefore, this mechanism cannot completely explain our results since the PSS layer in the (PAH/PSS)/PAH film is quite disordered, even with a noninterpenetrated region of PSS in the film. Therefore, we propose that this rearrangement results mainly from the electrostatic interactions among the layers, in addition to the disordering effect of layer interpenetration. Indeed, other experiments with different pH values, ionic strengths, and substrates indicate that this is a general phenomenon (manuscript in preparation). On the basis of these results, we propose the model sketched in Figure 3 to illustrate the structure of the in situ and ex situ LbL films. Layer interpenetration was not included for clarity of presentation. Note the



Figure 3. Model for in situ (top) and ex situ (bottom) adsorbed layers of PAH/PSS on silica from pH 7 solutions. PSS chains are represented by a red line and PAH by a blue line in the model for the in situ structure. In the bottom panel, the cationic groups of PAH are displayed in blue, and anionic groups of PSS are in red and yellow. Water molecules within the film are represented in light blue near the charged groups.

presence of water molecules in the dry films as we have previously shown that they continue to be hydrated,²⁰ in agreement with previous reports³⁸ and with important implications for bioactive LbL films.¹⁰

In summary, sum-frequency vibrational spectroscopy allowed us to probe the structure of polyelectrolyte layer-by-layer films during all steps of the self-assembly procedure. We showed that the adsorbed layers, while in an aqueous environment, are in a disordered conformation. It was also possible to follow the adsorption of polyelectrolyte layers by probing changes in the magnitude and sign of the electric field at the silica/solution interface via its effect on the water vibrational spectrum. This provided direct evidence for surface charge overcompensation upon polyelectrolyte adsorption. Drying the films by spontaneous water evaporation led to a net orientational ordering of the polymer chains, which was affected, however, by the adsorption of subsequent layers via electrostatic interactions. This detailed structural characterization at the molecular level is very important for many applications of layer-by-layer films, such as, for example, in biosensors or optoelectronic devices. Such knowledge may allow a rational design of the experimental conditions to fabricate organic films with the desired molecular arrangement.

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Supporting Information Available: Description of the curve fitting for the SFG spectra of Figure 1a-d. This material is available free of charge via the Internet at http://pubs.acs.org.

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