Optical Anisotropy of Flagellin Layers: In Situ and Label-Free Measurement of Adsorbed Protein Orientation Using OWLS

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The surface adsorption of the protein flagellin was followed in situ using optical waveguide lightmode spectroscopy (OWLS). Flagellin did not show significant adsorption on a hydrophilic waveguide, but very rapidly formed a dense monolayer on a hydrophobic (silanized) surface. The homogeneous and isotropic optical layer model, which has hitherto been generally applied in OWLS data interpretation for adsorbed protein films, failed to characterize the flagellin layer, but it could be successfully modeled as an uniaxial thin film. This anisotropic modeling revealed a significant positive birefringence in the layer, suggesting oriented protein adsorption. The adsorbed flagellin orientation was further evidenced by monitoring the surface adsorption of truncated flagellin variants, in which the terminal protein regions or the central (D3) domain were removed. Without the terminal regions the protein adsorption was much slower and the resulting films were significantly less birefringent, implying that intact flagellin adsorbs on the hydrophobic surface via its terminal regions.