Low-Temperature and Water-Based Biotemplating of Nanostructured Foam-Like Titania Films Using β-Lactoglobulin

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Given the broad use of nanostructured crystalline titania films, an environmentally friendly and more sustainable synthesis route is highly desirable. Here, a water-based, low-temperature route is presented to synthesize nanostructured foam-like crystalline titania films. A pearl necklace-like nanostructure is introduced as tailored titania morphology via biotemplating with the use of the major bovine whey protein β-lactoglobulin (β-lg). It is shown that titania crystallization in a brookite-anatase mixed phase is promoted via spray deposition at a comparatively low temperature of 120 °C. The obtained crystallites have an average grain size of (4.2 ± 0.3) nm. In situ grazing incidence small-angle and wide-angle X-ray scattering (GISAXS/GIWAXS) are simultaneously performed to understand the kinetics of film formation and the templating role of β-lg during spray coating. In the β-lg/titania biohybrid composites, the crystal growth in semicrystalline titania clusters is sterically directed by the condensing β-lg biomatrix. Due to using spray coating, the green chemistry approach to titania-based functional films can be scaled up on a large scale, which can potentially be used in photocatalytic processes or systems related to energy application.

1. Introduction

Nanostructured titania has emerged to an important research topic in the field of functional materials, due to its versatile applications. For instance, the nontoxic and photoactive semiconductor is used as a photocatalyst in self-cleaning coatings,[1,2] molecule sensors,[3] photocatalytic production of hydrogen,[4,5] and as an anode material in lithium-ion batteries,[6–9] dye-sensitized solar cells,[10–13] hybrid solar cells,[14,15] and perovskite solar cells.[16,17] In several of these applications, e.g. photovoltaics, a tailored morphology in terms of porosity, surface-to-volume ratio, and size distribution is beneficial for the device performance.[18] In particular, foam-like structures are of high interest due to their mechanical advantages and robustness. Among the different approaches of templating titania nanostructures, copolymer-assisted sol–gel synthesis has been shown to provide a facile way toward a controlled morphology also for films.[19–23] Furthermore, solution processing based on copolymer-assisted sol–gel synthesis has shown high promise for economically producing nanostructured titania films via large-scale deposition techniques, such as slot-die coating[24–26] and spray deposition.[27–29] However, the synthetic copolymer templating routine comes along with comparatively toxic, harmful, or flammable organic solvents as well as a low sustainability. For an improved environmentally friendly and more sustainable scenario, water-based templating of the mesoscopic titania morphology is desirable in combination with using water-soluble biopolymers, which substitute the synthetic copolymers.[30–33]

Besides the structural properties on the mesoscopic length scale, also the crystal phase and the crystallinity affect the performance of titania-based films in applications. The polymorph titania can be found in three different phases, namely tetragonal rutile, tetragonal anatase, and orthorhombic brookite. Rutile is known to be the thermodynamic most stable crystal form, however, showing lower photocatalytic activity compared to anatase and brookite.[34] Brookite is more laborious to obtain in a pure phase, but offers also a more promising performance in photocatalytic applications, compared to anatase and rutile.[35–37] Moreover, it was observed that copolymer templating of titania...
nanostructures from an amorphous precursor such as titanium-(IV)-isopropoxide (TTIP) in a nonaqueous media showed first crystallization starting at temperatures in the range from 250 to 500 °C. To enhance low-temperature crystallization, alternative precursors such as ethylene-glycol-modified titane (EGMT) were successfully introduced to copolymer-based titania sol–gel synthesis to enable a low-temperature crystallization. However, the synthesis of tailored precursor molecules like EGMT can become comparatively laborious. Alternatively, anatase-brookite mixed crystalline titania phases were readily produced by the precipitation of titania precursors upon hydrolyzation in aqueous media. Besides this, brookite-anatase mixed systems, smaller crystallite sizes are shown to be more beneficial for photocatalytic performance, in contrast to pure anatase phase materials, in which large crystallite sizes showed improved photocatalytic performance. However, synthetic copolymers are less sustainable compared with biopolymers and biopolymers typically also match well with aqueous processing. Among the numerous biopolymers, the bovine whey protein β-lactoglobulin (β-lg) attracted interest in this direction because it forms different supramolecular structures such as amyloid fibrils upon denaturation that can act as a template material in titania-based applications. Hence, substituting synthetic copolymers with water-based biopolymers has the potential to combine low-temperature crystallization in aqueous media with a compatible templating route and to further promote the environmentally friendly synthesis of titania nanostructures.

In this work, we present a facile and water-based sol–gel route to achieve crystalline titania films with a mixed anatase-brookite phase and a foam-like nanostructure at low temperatures. Our approach is based on the well-established titania precursor TTIP. TTIP is mixed with β-lg in acidic water to form a fibrillar biohybrid sol–gel, which is spray deposited on a heated silicon substrate. Spray deposition allows kinetic rearrangements of the biohybrid film morphology during drying on short time scales. Furthermore, spray deposition matches the sustainable synthesis route presented in this work, due to comparatively low material wastage. The deposited biohybrid films consist of a pearl necklace-like titania nanostructure that is embedded in a fibrillar β-lg biomatrix and revealed upon biopolymer extraction by ultraviolet (UV) irradiation. To reveal the kinetics of morphology formation and crystal growth during the spray deposition process, simultaneous in situ grazing incidence small-angle and wide-angle X-ray scattering (GISAXS/GIWAXS) is applied as an advanced investigation method. The morphology information of the biohybrid film is complemented by ex situ scanning electron microscopy (SEM) and ex situ GISAXS measurements. Based on this fundamental understanding of the film formation process of the biohybrid film, we gain the knowledge to control the spray deposition and achieve low-temperature and water-based biotemplating of nanostructured foam-like titania films. Such environmentally friendly and more sustainable synthesis route will be highly desirable for multiple applications in the fields of sensors, energy conversion and energy storage.

2. Result and Discussion

2.1. Surface Morphology

The sample surfaces of pristine titania, biotemplated titania after UV irradiation, and the biohybrid β-lg:titania composite films are probed with SEM (Figure 2). The surface of the pristine titania film shows a typical irregular foam-like structure with widely distributed pore sizes (Figure 2a). The surface of the biohybrid film obtained by SEM before UV irradiation (Figure 2b) shows a homogenous coverage on the macroscale but provides only limited contrast for the nonconductive biopolymer matrix for an analysis on the nanoscale. The remaining biotemplated titania film shows improved contrast in SEM after UV irradiation so that the biotemplated titania nanostructure becomes apparent. The biotemplated titania film shows a foam-like nanostructure with a pearl necklace-like arrangement of titania structures (highlighted with yellow rectangular boxes in Figure 2c), separated by pores with a fibril shape (highlighted with red rectangular boxes). This finding suggests that the titania pearl necklace-like nanostructure is present in the biohybrid sample and is sterically aligned within the voids by the surrounding β-lg fibril network, as sketched in Figure 1d. The variation of surface morphology for the biohybrid sample with different UV exposure times is shown in Figure S1 (Supporting Information).

2.2. Inner Morphology

To understand the role of the β-lg matrix in templating of the titania nanostructure and extend the accessible information
from surface to bulk morphology, static GISAXS measurements are performed. These ex situ GISAXS measurements are performed on the biohybrid film after spray deposition and the UV illuminated film. In addition to this, pristine titania and a pure ß-lg film are measured as references. The horizontal line cuts are shown in Figure 3a. Three characteristic structures are extracted from the applied model of the biohybrid and titania samples. The related radii and distance values are presented in Figure 3b,c, respectively. The biohybrid film exhibits three characteristic structures, related to a small-, medium-, and large-domain radii of (2.1 ± 0.2), (3.5 ± 0.3), and (9.6 ± 0.4) nm, respectively. These domains have a characteristic center-to-center distance of (9.6 ± 0.4), (19.1 ± 0.5), and (32 ± 1) nm, respectively. An interpretation of the morphology associated with the domain sizes is presented later in the context of the in situ GISAXS/GIWAXS discussion. After UV illumination,
the characteristic domain radii of the biotemplated titania morphology remain unchanged compared to the biohybrid sample before UV irradiation. This indicates that mainly the titania domain form factors rather than the ß-lg biomatrix contribute to the scattering intensity of the biohybrid sample, and the nanoparticle sizes of titania do not undergo obvious changes with UV irradiation. The distances, however, slightly increase after UV irradiation, to (11.4 ± 0.4) nm (small structures), (22.8 ± 0.5) nm (medium structures), and (40 ± 1) nm (large structures), respectively. The deformation of the remaining titania scaffold that is reflected in slightly increased distances after biopolymer extraction can be seen as an inverse process to the humidity uptake in porous titania scaffolds studied in our earlier work.\cite{59}

Here, the ingress of water vapor into the pores leads to a contraction in terms of reduced center-to-center distances. For the present case of biopolymer extraction, the transition from solid to gas phase of the biomatrix during UV combustion causes isotropic expansion of the titania scaffold with slightly increasing center-to-center distances. In contrast to the biotemplated titania film, the pristine titania reference sample consists of bigger medium (radius of around (6.1 ± 0.3) nm) and large domains (radius of around (13.1 ± 0.4) nm). The small structures show the same radius ((2.1 ± 0.2) nm) as the biotemplated titania. The distances related to the small and medium domains of the pristine titania are (14.1 ± 0.4) nm and (28.2 ± 0.5) nm, respectively. For the large domains, we see no significant structural correlation in terms of center-to-center distance.

The pure ß-lg film consists of two characteristic structure sizes with a radius of (6.1 ± 0.3) nm for the small-sized structures and a radius of (12.2 ± 0.4) nm for the large-sized structures and the corresponding center-to-center distances of (19.1 ± 0.5) and (44 ± 1) nm, respectively. The obtained values of the small and large ß-lg domains are in good agreement with expected values for bundles of about 2 (small structures) and 4 (large structures) ß-lg amyloid fibrils with 6 nm in diameter.\cite{50,51}

With the obtained radii and distances, an estimation of pore sizes in the respective sample can be given by the following expression:\cite{58}

\[ R_{\text{pores}} = \frac{1}{2} D_{\text{domains}} - R_{\text{domains}} \]  

Here, \( R_{\text{pores}} \) and \( R_{\text{domains}} \) refer to the radii of the pores and the respective domains with the related distances \( D_{\text{domains}} \). By comparing the pores' radii (Figure 3d), it is found that the biotemplated titania scaffold consists of a slightly higher hierarchy in pore radii ((3.6 ± 0.7), (7.9 ± 0.8), and (10.4 ± 1.4) nm) compared to the pristine sample ((5.0 ± 0.7) and (8.0 ± 0.8) nm), respectively. It is further noticeable, that the pore radii obtained for the pure ß-lg sample (Figure 3d, (3.5 ± 0.7) and (9.8 ± 0.8) nm) coincide with the radii of the medium and large titania domains in the bioblock hybrid sample (Figure 3b, (3.5 ± 0.3) and (9.8 ± 0.4). Vice versa, the pores related to the distances of the medium and large titania domains inside the bioblock hybrid sample (Figure 3d, (6.1 ± 0.8) and (6.4 ± 1.4) nm) coincide with the small ß-lg domain radius (Figure 3b, (6.1 ± 0.3) nm). These observations are summarized as

\[ P_{\text{lg,small}} = R_{\text{hybrid,medium}} \]  

\[ P_{\text{hybrid,medium}} = P_{\text{hybrid,large}} = R_{\text{lg,small}} \]  

Here, \( R \) is the domain radius of the small, medium, or large structures of the pure ß-lg and the biohybrid sample, respectively. The corresponding pore radius is denoted with \( P \). This claims that the structure factor of the biohybrid sample representing the titania domain distances can be attributed to the surrounding ß-lg biomatrix, which is further supported by the increased titania domain distances after removal of the biopolymer by UV illumination. In conclusion the biohybrid morphology can be interpreted as voids inside the ß-lg biomatrix, which are filled with titania and as consequence, that the ß-lg domains separate the titania domains from each other.

### 2.3. Lateral Film Formation on the Crystalline Length Scale

To understand the temporal evolution of the overall morphology in the bioblock film and its crystal structure during the spray deposition process, in situ GIWAXS measurements are performed simultaneously to in situ GISAXS during the spray deposition. In Figure 4a four selected 1D intensity curves are shown against the scattering vector \( q \), taken from the 2D GIWAXS data (Figure S2, Supporting Information) by azimuthal integration at different times. It shows the rise of three distinct peaks from 1 to 53 s during spray deposition. At these given four time stages a position on the film close to the inner part of the spray cone is probed (marked as position C in Figure 1d). With fits of the intensity curves using Gaussian functions, the temporal evolution of the peak intensities, the \( q \)-values, and the distribution full width at half maximum (FWHM) is determined during film formation. The three peaks are assigned to the (121) brookite phase at (2.143 ± 0.002) Å\(^{-1}\), to the (004) anatase phase at (2.650 ± 0.001) Å\(^{-1}\), and to the (200) anatase phase at (3.318 ± 0.001) Å\(^{-1}\) of TiO\(_2\), respectively. These peaks are in good agreement with the reported brookite phase (PCPDF #29-1360) and anatase phase (PCPDF #21-1272). An estimation for the lower limit of the corresponding crystal sizes is calculated from the FWHM according to the Scherrer equation.\cite{60} The results from the Gaussian fits are presented in Figure 4b–e and show the film formation on the crystalline length scale between the turning points A and B (Figure S3, Supporting Information) of the scanning area during the entire spray experiment. In more detail, the data shows the inner, central, and outer regions of the spray cone. The temporal evolution of the Gaussian fits suggests that only the signal intensity (Figure 4b) depends on the position with respect to the spray cone, whereas \( q \)-value, FWHM, and hence crystallite sizes evolve independently of the position. Thus, the inner region of the spray cone provides a larger material deposition and hence a higher signal as compared to the central and outer regions with respect to the scanned area. The local maxima at 27 s and the local minima at 43 s correspond to the turning points A and B of the sweeping motor, respectively, and hence to the positions of highest (27 s) and lowest (11, 43 s) deposited material amount within the measured region of the spray cone. For the fixed position C, however, the intensity shows a linear increase as a consequence of the linear growth of the film.
thickness and hence the linear growth of the number of crystallites, which is characteristic of pulsed spray deposited layer-by-layer growth.[61–63] The $q$-values defining the inverse lattice spacing and their respective FWHM are given in Figure 4c,d. The temporal evolution differs for the anatase and brookite phases. The anatase peaks indexed with (004) at $(2.650 \pm 0.001)$ Å$^{-1}$ and (200) at $(3.318 \pm 0.001)$ Å$^{-1}$ remain constant during the entire spray deposition. The brookite (121) peak shifts from $(2.09 \pm 0.06)$ to $(2.143 \pm 0.002)$ Å$^{-1}$ within the first 9 s and remains constant afterward. Also, the FWHM of the brookite (121) peak shows a pronounced decay compared to the anatase crystallites from $(0.4 \pm 0.1)$ to $(0.133 \pm 0.006)$ Å$^{-1}$ and remains constant within the error bars after approximately 30 s. At the same time, anatase (200) FWHM slightly decreases from $(0.17 \pm 0.01)$ to $(0.125 \pm 0.002)$ Å$^{-1}$, whereas the FWHM of the anatase (004) peak remains constant at $(0.149 \pm 0.002)$ Å$^{-1}$. Estimating the brookite crystal size by the Scherrer equation shows nucleation centers of around $(1.3 \pm 0.3)$ nm in the early stage at 3 s, growing to crystallites of $(4.3 \pm 0.2)$ nm in diameter (Figure 4e). We suggest, that these brookite nucleation centers are formed from the amorphous phase by the rapid solvent evaporation and temperature jump from room temperature of around 25 to 120 $^\circ$C, introduced by the spray deposition on the heated substrate.[64]

Simultaneous in situ GISAXS is performed to link the lateral film formation on the mesoscopic length scale to the crystal growth evolution described before. With this, complementary information to the crystallite formation in the film on a hierarchical scale can be deduced. The chosen spray parameters cause the atomization process to be in the diluted regime when arriving at the substrate. Here, the solvent has not evaporated completely and the ß-lg:TiO$_2$ phase is still dispersed in tiny droplets.[66] This enables drying kinetics on the substrate and hence morphological rearrangements that can be revealed by GISAXS.[67] Figure 5a shows the horizontal line cuts taken from the individual 2D GISAXS data (Figure S4, Supporting Information) at the selected position C close to the center of the spray cone. The obtained radii for the small, medium, and large structures are presented in Figure 5b. The small structures (green triangles) grow in radius from $(1.6 \pm 0.2)$ to $(2.1 \pm 0.2)$ nm within the first 30 s of spray deposition and remain constant bars from 0 to 15 s (Figure 4e). The crystal size of anatase (200) shows a slight increase from $(3.4 \pm 0.2)$ to $(4.5 \pm 0.1)$ nm and remains constant after that (Figure 4e). These results indicate that the anatase crystallites are already formed during the hydrothermal sol–gel process and slightly grow preferentially toward the (200) direction, whereas the brookite crystallites form and grow as a consequence of spray coating on the heated substrate.

**2.4. Lateral Film Formation on the Mesoscopic Length Scale**

Simultaneous in situ GISAXS is performed to link the lateral film formation on the mesoscopic length scale to the crystal growth evolution described before. With this, complementary information to the crystallite formation in the film on a hierarchical scale can be deduced. The chosen spray parameters cause the atomization process to be in the diluted regime when arriving at the substrate. Here, the solvent has not evaporated completely and the ß-lg:TiO$_2$ phase is still dispersed in tiny droplets.[66] This enables drying kinetics on the substrate and hence morphological rearrangements that can be revealed by GISAXS.[67] Figure 5a shows the horizontal line cuts taken from the individual 2D GISAXS data (Figure S4, Supporting Information) at the selected position C close to the center of the spray cone. The obtained radii for the small, medium, and large structures are presented in Figure 5b. The small structures (green triangles) grow in radius from $(1.6 \pm 0.2)$ to $(2.1 \pm 0.2)$ nm within the first 30 s of spray deposition and remain constant
afterward. The medium structures (red circles) decrease from 
\((4.4 \pm 0.3)\) nm to 
\((3.4 \pm 0.3)\) nm during the first 30 s of film formation and remain constant afterward. A symmetric correlation between the shrinking medium and the growing small structure sizes is noticeable. The small structures are associated with the crystalline phase as they are in good agreement with the values for the brookite and anatase crystallite sizes obtained from GIWAXS. Hence, we conclude that the medium structures can be associated with the amorphous phase that is consumed during crystallite growth. The particle size distributions of small and medium structures both narrow with time (Figure 5d). This effect is more pronounced for the crystalline phase than for the amorphous phase and reflects the growing brookite crystallite size, which is approaching the constant size of anatase crystallites. Furthermore, the intensity related to the crystalline phase is 80% higher than the amorphous phase intensity.

Besides the structure sizes, also the center-to-center distances vary for the crystalline and the amorphous domains during film formation (Figure 5c). The distance related to the TiO₂ crystallites (green circles) decreases from 
\((10.2 \pm 0.4)\) to 
\((9.4 \pm 0.4)\) nm. The distance between the amorphous domains (red squares) decreases from 
\((20.5 \pm 0.5)\) to 
\((18.9 \pm 0.5)\) nm. The distributions of the shrinking crystalline and amorphous center-to-center distances show also narrowing in the FWHM (Figure 5e). As discussed earlier, we relate the distances of titania domains to separating β-lg domains. Hence, we conclude the driving force for the observed changes in the titania domain distances and their distribution widths to be the condensation of the surrounding biomatrix upon drying on the heated substrate.

By comparing the temporal evolution of crystalline domain distance with the radius of the crystalline and amorphous phase, a common relation turns out, that can be described by

\[
R_{\text{amorphous}} = \frac{D_{\text{crystalline}}}{\sqrt{3}} - R_{\text{crystalline}}
\]

Here, \(R_{\text{amorphous}}\) is the radius of the amorphous domain. \(D_{\text{crystalline}}\) and \(R_{\text{crystalline}}\) are the distance and the radius of the crystallites, respectively. The term \(\frac{D_{\text{crystalline}}}{\sqrt{3}}\) describes the radius of an isosceles triangle. This link between amorphous and crystalline phases suggests that the titania morphology consists of semicrystalline clusters, built by an amorphous domain being centered within surrounding growing crystallites that are arranged in an isosceles triangle.

In addition to the small structures (crystallites) and medium structures (amorphous phase), the large structures extracted from the horizontal line cuts decrease in radius from 
\((10.2 \pm 0.4)\) to 
\((9.4 \pm 0.4)\) nm during the time of spray deposition (Figure 5b) and in distance from 
\((290 \pm 30)\) to 
\((32 \pm 1)\) nm (Figure 5c). The evolution of the large structures equals the center-to-center distances of amorphous phase and crystalline phase in the following way, suggesting the large structures to be agglomerates of adjacent semicrystalline clusters:

\[
R_{\text{agglomerates}} = \frac{D_{\text{crystalline}}}{2} - \frac{1}{2} R_{\text{amorphous}}
\]
Here, \( R_{\text{agglomerates}} \) is the domain radius of the large structures built up by the semicrystalline clusters. \( D_{\text{amorphous}} \) and \( D_{\text{crystalline}} \) are the distances of the amorphous and the crystalline domain, respectively.

Hence, we explain the resulting film morphology as semicrystalline titania clusters, formed by amorphous domains (medium structures) inside an isosceles triangular way arranged crystalline domains (small structures), that form agglomerates (large structures) with the adjacent semicrystalline clusters, inside the pores of the surrounding \( \beta \)-lg biomatrix. Besides the lateral film formation, the biohybrid film shows a uniform distribution along the vertical direction without the presence of any enrichment layers, which can be seen in the vertical line cuts taken at \( q_y = 0 \, \text{nm}^{-1} \) in Figure S5 (Supporting Information). This indicates also the absence of any roughness correlation between the interfaces.

2.5. Steric Templating of Titania by Biopolymer

To further investigate the dependence of the film formation on the condensing \( \beta \)-lg biomatrix and to link it with the crystal growth obtained from GIWAXS, a Kratky-like analysis is performed (Figure 6a). The rise of an intensity maximum is noticeable during the film formation. These bell-shaped intensities are fitted with Gaussian functions to extract the respective center values for \( q_y,c \) with the corresponding FWHM and hence to obtain information about the overall morphology formation, including the contributions of the crystalline, amorphous, and agglomerated domains. The evolution of the obtained \( q_y,c \) shows an inverse correlation with the center-to-center distance associated with the crystalline phase and according to Equation (6) with the radius of the agglomerates

\[
D_{\text{crystalline}} = R_{\text{agglomerates}} \sim \frac{1}{q_y,c} 
\]

This result further confirms that the morphology inside the biomatrix mainly consists of the semicrystalline clusters and their agglomerates. The corresponding FWHM of the Kratky-like intensity bell narrows during film formation, indicating a rearrangement into a more ordered assembly of the overall morphology. Interestingly, the Kratky-like FWHM shows the same trend in temporal evolution when compared to the brookite (121) FWHM evolution in Figure 6b. The ratio of the Kratky-like FWHM and brookite (121) FWHM converges to 1 and follows the evolution of the ratio obtained by dividing the crystalline phase distance’s FWHM by the amorphous phase distance’s FWHM (Figure 6c). The convergence to 1 indicates that the film formation has finished since no changes appear anymore in the size distribution on the crystalline length scale (brookite (121) FWHM) and the overall morphology (Kratky-like intensity bell FWHM). Moreover, Figure 6c reflects, that the brookite crystallization and the overall morphological formation toward a more ordered spatial assembly are directly linked to the same mechanism.

The temporal evolution of the morphology can be interpreted as the coalescence of the structures that is driven by the shrinking of the center-to-center distances as seen from the interdependence of radii and distances in Equations (5)–(7). As the semicrystalline clusters and their agglomerates are embedded in and separated by the biomatrix made up of the \( \beta \)-lg fibrillar network (Equations 1–4), the shrinking center-to-center distances can be related to the condensation of the \( \beta \)-lg biomatrix. Thus, we explain the common mechanism for film formation in the fibrillar \( \beta \)-lg network, which acts as a steric template for the semicrystalline clusters and their agglomerates, building up the pearl necklace-like titania morphology. Since not all the water solvent evaporates during spray deposition, the biohybrid film is deposited in a swollen state of the biomatrix. The condensation mechanism takes place as the biomatrix gradually shrinks upon drying on the heated substrate, allowing for kinetic rearrangements toward higher spatial order, and eventually stops after the film has fully dried. The mechanism of steric \( \beta \)-lg biotemplating is illustrated in Figure 7. It is further notable that the correlation of distribution widths obtained by Kratky-like analysis may serve as a tool for future evaluation of simultaneous GISAXS/GIWAXS data.

3. Conclusion

In the present work, we investigate the film formation of biohybrid titania:\( \beta \)-lg composites with respect to the influence of \( \beta \)-lg on the titania morphology during spray deposition on a heated
substrate. For this, in situ GISAXS and GIWAXS techniques are combined and complemented with static GISAXS and SEM imaging to understand the formation process on the crystal-line and nanometer length scale. We observe that the titania nanostructure consists of agglomerates of semicrystalline clusters with a mixed brookite-anatase crystal phase of around $(4.2 \pm 0.3)$ nm crystal grain size. In these semicrystalline clusters, the amorphous phase is consumed by the growing crystalline phase. We show that the distribution widths obtained from a Kratky-like representation serve as a good tool to link the analysis of GISAXS to GIWAXS. As a result, we see a correlation of the brookite distribution width extracted from GIWAXS with both, the amorphous and crystalline domain distance distribution widths obtained from GISAXS. The shrinking distances of the titania domains are attributed to the condensing β-lg biomatrix, in which semicrystalline titania clusters are agglomerating. This interpretation is further supported by the findings from ex situ SEM and GISAXS measurements. In SEM, the biotemplated titania exposed to UV irradiation shows a foam-like nanostructure provided by a pearl necklace-like titania scaffold, introduced by the fibrillar β-lg biomatrix. Ex situ GISAXS measurements reveal, that the pore size of a pure β-lg reference agrees with the domain radii of the biohybrid titania morphology and vice versa. These results lead to the conclusion that the β-lg biomatrix acts as a promising biotemplate for the creation of foam-like titania nanostructure with a pearl necklace-like order by sterically directing the morphology formation. Therefore, this work presents an approach to a water-based, low-temperature route for the synthesis of nanostructured foam-like brookite-anatase mixed titania films by spray deposition. It is promoting an environmentally friendly and more sustainable fabrication of titania-based functional films with the possibility to an upscaling on large scale being of interest for multiple applications from the fields of sensors, energy conversion and energy storage.

4. Experimental Section

Materials: The biopolymer β-lactoglobulin (β-lg, lyophilized powder, ≥90% PAGE) and titanium(IV)isopropoxide (97%) (TTIP) were purchased from Sigma-Aldrich, Germany, and used without any further purification. Hydrochloric acid (37%) was purchased from Carl Roth, Germany. The aqueous solutions were based on deionized water (Milli-Q, 18.2 MΩ cm$^{-1}$).

Sample Fabrication: Figure 1 schematically shows the main sample fabrication steps. 12 mL hydrochloric acid (HCl) was diluted to $10 \times 10^{-3}$ M HCl with deionized water and used as a host solvent. First, 4 mL of $10 \times 10^{-3}$ M HCl was mixed with 148 µL TTIP. Second, 20 mg of β-lg was added and kept stirring for 30 min at room temperature to form a biohybrid sol–gel (Figure 1a). After that, the β-lg:TTIP sol–gel was kept stirring in a sand bath at a temperature of 90 °C for 22 h, to promote the hydrothermal synthesis of TiO$_2$ nanoparticles (NPs) (Figure 1b). Also, the heating under acidic conditions led to denaturation and unfolding of β-lg to fibrillar aggregates.[48,49] These aggregates electrostatically bound to the TiO$_2$ NPs and acted as a templating network. The sol–gel was eventually immersed and quenched in a water bath at the temperature of 4 °C. Spray deposition of the resulting β-lg:TiO$_2$ hybrid dispersion was performed with an air atomizing nozzle of type JAUCO D555000, purchased from Spraying System Co (Figure 1c). The nozzle provided a cone-shaped spray coat with an opening angle of 10°, which results in a circular film of the biohybrid composite (Figure 1d). The radius of the covered area depended on the sample-substrate distance as well as the opening angle of the spray cone. Precleaned silicon substrates were placed at a distance of 20 cm to the nozzle and heated to 120 °C. Pulsed spray deposition based on 500 µL of β-lg:TiO$_2$ solution was performed in intervals of 0.2 s spraying and 2.8 s drying, followed by 10 s of annealing. Hence, a single spray cycle lasted 3 s in total. A nitrogen flow set to 1 bar was used as carrier gas with a flow rate of 125 mL s$^{-1}$. The pulsed
deposition was controlled by the magnetic valve MEBH-5/2-1/8-B, purchased from Festo SE & Co. KG. The as-deposited biohybrid samples were irradiated with UV-light (HTC 400-241, Osram AG) for 24 h, to remove the biomatrix by combustion and to obtain the biotemplated, nanostructured titania films. The pristine titania reference sample was fabricated in the same way without adding β-lg and UV irradiation. In addition, a pure β-lg reference sample was fabricated in the same way, without adding TTIP and UV irradiation.

**Film Characterization:** In situ GISAXS/GIWAXS measurements were performed at the beamline P03 MiNaXs of the storage ring PETRA III (DESY, Hamburg, Germany).[16] Nozzle and substrate were mounted in a spray chamber, which was mounted into the beamline (Figure S5, Supporting Information). Spray deposition was carried out with the spray protocol described above. The X-ray wavelength was 0.984 Å, corresponding to a photon energy of 12.6 keV. Dectris Ltd. Pilatus 1M detector and Dectris Ltd. Pilatus 300K detector were placed in 4871 and 326 mm distance to the sample position, to simultaneously record the 2D GISAXS and GIWAXS signals, respectively. Both detectors had a pixel size of (172 x 172) m². 2D in situ GISAXS and GIWAXS images were taken with an exposure time chosen to be 0.1 s to ensure a high time resolution. The Si substrate was aligned to an incident angle of $\theta_i = 0.425^\circ$, which ensured a proper separation of the material characterization scattering signal called Yoneda peak and the signal from specular reflection (Figure S7, Supporting Information). The whole setup was periodically sweeping with 0.5 mm s⁻¹ in a range of 8 mm perpendicular to the beam direction, to protect the sample from radiation damages by the high brilliance X-ray illumination (Figure S8, Supporting Information). This also probed the film formation for different positions in the spray cone within the scanning area defined by the turning points A and B as sketched in Figure 1d. The 2D GISAXS images were reduced to 1D intensity distributions as a function of the scattering vector $q$, (nm⁻¹) by use of the software DPDAK.[69] These horizontal line cuts provide lateral information about the morphology and were taken at the Yoneda peak position of the β-lg:TIO₂ biohybrid material (at $q_y = 0.6$ nm⁻¹), which corresponded to a critical angle of $\alpha_c = 0.13^\circ$. To extract the lateral information, the horizontal line cuts were modeled by applying cylindrical form factors on a 1D paracrystal in the framework of the distorted wave Born approximation (DWBA), using the effective interface approximation (EIA) in combination with the local monodisperse approximation (LMA).[60] This lateral information was modeled in terms of form factors (structure sizes) and structure factors (center-to-center distances). 2D GIWAXS images were reshaped with the MATLAB-based software package GXSQUI and 1D intensity distributions as functions of $q$ (Å⁻¹) were obtained by azimuthal integration.[60] These intensity distributions contained information about the crystalline phase evolution during spray deposition. The data were fitted with Gaussian functions to extract q-spacings, distribution widths at half maximum, and intensities of the crystalline phases. Optical microscope images were taken during spray deposition by a camera mounted into the spray chamber (Figure S3, Supporting Information). SEM measurements were performed with a 3.5 mm working distance and an accelerating voltage of 5 kV (Zeiss NVision 40). SEM measurements complement the in situ GISAXS/GIWAXS data with a real-space surface characterization of the as-deposited β-lg:TIO₂ sample after spray coating, the nanostructured, UV-treated TIO₂ sample, and the pristine titania as a reference.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords**

β-lactoglobulin-biotemplating, low-temperature crystallization, nanostructured titania, spray deposition, water-based synthesis

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