1. Introduction

The ongoing research efforts in the field of nanoparticle (NP) supercrystals lead to ever improving synthesis protocols.\textsuperscript{1–3} Constituent nanoparticles with low dispersity and high uniformity allow for well-defined supercrystals with long-range periodic order, small number of defects and large crystallite size. In the case of plasmonic nanoparticles (e.g., Ag, Au) the crystalline structure within the assemblies is directly correlated with the emergence of coupled plasmon modes.\textsuperscript{4} For instance, dark interlayer plasmons can form in supercrystals with a defined layered structure.\textsuperscript{5} Higher order modes, however, can only be observed in highly defined crystals because dispersity and the lack of periodic order result in a broadening of the according resonances. While the dispersity and uniformity of the constituent NP can be stated in well-defined quantities, the periodic order within the supercrystal lattices is usually described more qualitatively with terms such as “well-ordered”, “highly-ordered”, and so on. Quantitative statements are mostly restricted to the overall dimensions of the crystals or the crystallite size. Since the current high standards of NP supercrystals allow for more detailed studies of light matter coupling, a more detailed structural characterization is also desirable.

The most important characterization techniques electron microscopy and small-angle x-ray scattering (SAXS) provide complementary information in this respect. Electron microscopy experiments like transmission electron microscopy (TEM) allow intuitive assessments of particle uniformity and dispersity and their periodic arrangement as well as the density and nature of lattice defects. Analysis of TEM measurements yield values for NP dispersity, uniformity, nearest neighbor distances und crystallite/domain sizes. The statistics of TEM-measurements, however, are limited. The maximum domain size that can be depicted in a single measurement is restricted by the minimum magnification that allows discerning individual NP (e.g., < 200 μm² for NP diameters of 25 nm). Larger domains can be assessed by serial measurements but typically, a sampling approach is employed in contrast to a systematic mapping. Another limitation of TEM is its restriction to monolayer superlattices up to a few layers only. The number of layers can be identified by stepwise counting up to 5–6 layers depending on the specific sample, but further information like particle diameter and spacing, as well as crystal structure, are hard to obtain for multilayers. For thick multilayer crystals, the absorption of the material will prevent any meaningful analysis. As a complementary approach, SAXS at partially coherent photon sources is routinely used to determine the crystalline order of volume materials.\textsuperscript{6,7} Scanning SAXS experiments allow mapping of comparably large areas and detailed structural information can be obtained by X-ray cross-correlation analysis.
(XCCA).\textsuperscript{[8–12]} Recently, the use of XCCA in structural studies of self-assembled nanoparticles has increased,\textsuperscript{[8,13–15]} including in situ experiments of phase transitions.\textsuperscript{[16]} Form factor measurements in solution provide statistically robust data for particle diameter, shape and dispersity.\textsuperscript{[7,17]} However, slight deviations from ideal shapes or low uniformity/multiple shape variations challenge confident analysis of SAXS data. Furthermore, SAXS is not sensitive to the structure of specific single particles and thus certain types of defects like point defects will be left unnoticed. As a simplified statement EM provides a confident microscopic picture down to the single particle level, whereas SAXS provides statistically more robust structural data. In this study we use a correlated SAXS-TEM approach to study plasmonic supercrystals of gold nanoparticles (AuNP). We synthesize AuNP supercrystals with a defined layered structure, ranging from mono- to multilayer. By performing microfocus scanning SAXS experiments on AuNP supercrystal samples on TEM grids we are able to identify the same regions of interest with both methods and directly compare TEM and SAXS measurements. We analyze SAXS measurements on mono-, bi-, tri-, tetra-, and multilayers with XCCA and discuss quantitative parameters such as domain size, intensity, lattice parameters and orientational order and how they depend on the number of layers. We observe a slight increase of the narrowly distributed lattice parameters with the number of layers, large single crystalline domains up to thousands of square micrometers and a high degree of orientational order independent of the number of layers. The observed structural features can help to understand the formation mechanism of the superlattices.

2. Results

Plasmonic supercrystals were synthesized by self-assembly of polystyrene-coated AuNP (AuNP@PSSH) at a toluene-diethylene glycol (DEG) liquid-liquid interface as described recently.\textsuperscript{[18]} The synthesis protocol (see Section 5.) yields well-defined supercrystals with crystallite sizes $> 1000 \ \mu$m$^2$, few defects and a layered structure (Figure 1). Two reference samples consisting of either mostly monolayers or mostly bi- and multilayers were prepared. Table 1 lists the basic geometrical parameters obtained by TEM and SAXS: particle diameter ($d_{\text{core}}$) and dispersity (rSD, relative standard deviation of the mean diameter for TEM and the dispersity $\Delta d$ given by the width of the Schulz–Zimm distribution in SAXS\textsuperscript{[7]}) and the lattice constants $a$. The values obtained by SAXS and TEM are in

Figure 1. Exemplary TEM measurements of plasmonic supercrystals. a) Monolayer, b) bilayer with AB stacking, c) monolayer at lower magnification d) bilayer, e) trilayer, and f) tetralayer. (scalebar in (a) 100 nm, (b) 200 nm, (c–f) 1 µm).
good agreement. The lattice constants \( a \) of the two individually prepared samples deviate just slightly (in fact, as we will show later, \( a \) was the same \( \pm 0.1 \) nm for monolayers in both samples), confirming the reproducibility of the protocol that utilizes the molar mass of the PSSH ligand to control the interparticle distances in the supercrystals.

The standard deviations of the lattice parameters \( a \) averaged over all SAXS measurements (hits) are well below 1%, underlining the crystalline long-range order of the samples. The domain sizes obtained by TEM exceed several \( \mu m \) (see Figure 1) so that microfocus SAXS measurements on defined numbers of layers are possible. We mapped selected regions of interest (ROI) with characteristic microstructures on TEM grids. In subsequent TEM-experiments, these regions could be identified and the according microstructure analyzed (Figure 2). This way, we can confidently assign the number of layers to the SAXS measurements. An overview scan is shown in the Supporting Information (Figure S1, Supporting Information). We focus on three ROI which include distinct mono- to multilayer areas and are therefore suitable for our discussion of the local structure as a function of film thickness. For thin samples as probed in this study, the scattering intensity at large \( q \) \( (q > 0.56 \text{ nm}^{-1}) \) is directly proportional to the thickness of the sample. Histograms of \( I_h \equiv I(q > 0.56 \text{ nm}^{-1}) \) then allow to determine the number of layers (Figure 3).

The TEM measurements and scanning SAXS intensity maps in Figure 2 clearly show the underlying TEM grid. The grid edges produce strong streaks in the scattering patterns raising the measured intensity, and consequently clearly highlight the grid position in the SAXS maps. Furthermore, the intensity appears to be reduced on the copper grid because of absorption of X-rays by copper. Therefore, we only used the areas inside

|                | TEM          | SAXS         |
|----------------|--------------|--------------|
|                | \( d_{ave} \) [nm] | rsd | \( a \) [nm] | \( d_{ave} \) [nm] | \( \Delta d \) | \( a \) [nm] |
| Monolayer sample | 23.4 | 4.3% | 31.4 | 24.2 | 5.8% | 32.4 |
| Multilayer sample | 23.4 | 4.3% | 30.8 | 24.2 | 5.8% | 32.8a)

*depends on the number of layers as discussed below.

Figure 2. Direct comparison of TEM (top) and scanning SAXS intensity maps (bottom) of plasmonic supercrystals for all three runs. The SAXS intensity has been averaged for \( q > 0.56 \text{ nm}^{-1} \). The according ROI were identified in subsequent TEM measurements to confirm the number of layers as indicated (top). Up to four layers can be confidently assigned with TEM as shown in Figure 1, thicker regions more than four layers (compare dark regions in run B and C indicated by 4+) can often not be confidently assigned because of the strong absorption. Note that even subtle features like small cracks can be discerned in the SAXS intensity maps. However, due to sample mounting and transfer slight deviations in the microstructure can occur.
the mesh (nominal mesh dimensions: \((38.5 \, \mu \text{m})^2\) mesh, 25 \(\mu \text{m}\) bridge width) for the determination of the number of layers by the histogram in Figure 3 (top). The corresponding maps of number of layers are shown in Figure 3 (bottom), where the gray regions reflect the masked copper grid. Apart from the large tetralayer in run A, the assignment based on the SAXS intensity is in excellent agreement with the one based on TEM analysis. An accurate and statistically robust determination of structural parameters like the lattice constant \(a\) can hardly be obtained by TEM analysis, especially for more than one layer. With the confident assignment of the number of layers by our combined approach we can now take a closer look at the SAXS results as a function of number of AuNP layers.

### 2.1. Lattice Constant \(a\)

Azimuthally integrated SAXS intensities \(I(q)\) have been averaged over all patterns and are shown in Figure 4a for run B. Independent from the number of layers, Bragg reflections dominate the intensity curves. All reflections can be indexed according to a 2D hexagonal lattice. With increasing number of layers, the Bragg reflections shift slightly towards smaller \(q\), suggesting an increase of the lattice constant. Thus, we calculated the lattice constant from the position of the \([10]\) reflection for all sample spots separately, and averaged it afterwards. The results are shown in Figure 4b. The lattice constant increases by 1.4\% from 32.4 nm for monolayers to 32.8 nm for multilayers (M1 and M2) where it appears to saturate.

### 2.2. Orientational Order

Similar to the SAXS analysis, the orientational order was quantified by Fourier coefficients of the intensity \(I_f\) (see Experimental Section for definitions). A perfect 2D hexagonal lattice resulting in strong Bragg reflections would yield \(I_f = 1\). Disorder as well as a different symmetry is reflected by \(0 < I_f < 1\). In experiments, setup parameters such as beam size and detection noise, as well as the width of the Bragg reflections can additionally reduce \(I_f\) values. In our case we obtain a maximum of \((I_f) = 0.96\) (Figure S2, Supporting Information). Therefore,
maxima close to 0.95 in Figure 5 reflect a very high degree of sixfold order. Because of limitations due to low intensity, in particular scattering from monolayer regions, we concentrate the analysis on the \( \{10\} \) Bragg reflection. As this reflection shows a strong six-fold symmetry, we limit the discussion to \( \ell = 6 \).

The resulting maps of the degree of sixfold order are shown in Figure 5. The top part depicts the amplitude of sixfold order \( |\hat{I}_6| \). In contrast to the intensity maps, the copper grid is less pronounced in these maps. This can be understood by the normalization of the correlation function which is calculated from relative intensity fluctuations and is thus less sensitive to absolute intensity variations.\(^{[12]}\) Cracks of the film and layer steps become visible by this approach, for example, the diagonal lines for runs A and B from top left to bottom right. Beside the amplitude of orientational order, XCCA enables access to the patch size via the orientation angle \( \omega_6 \) related to the phase of the Fourier coefficients (Figure S3, Supporting Information).\(^{[8,13]}\) Connected areas with the same \( \omega_6 \) represent a perfect lattice without measurable defects. This is shown in the bottom part of Figure 5. Although \( \omega_6 \) is calculated for the whole map including empty spots where it does not provide a meaningful measure, areas with continuous layers of AuNP seem to show large domain sizes, well beyond 20–30 \( \mu \text{m} \) in 1D. To take a closer look into the domain sizes and to neglect potential effects from the copper grid, we take a closer look into the meshes highlighted in Figure 3. Velocity plots of these meshes are shown in Figure 6.

The velocity plots highlight in particular changes of orientation at layer steps, such as in mesh ii from bi- to tri- and tetra-layers, and the gradual increase from bilayers to multilayers in mesh iii. The transition from bi- to trilayers in mesh iv results in a change of the orientation, however, this cannot strictly be observed for mono-bilayer steps. Most interestingly, mesh i consisting of tri- and tetralayers (or one large tetralayer according to TEM) shows only very weak modulations of \( \omega_6 \). This indicates that the local order and its orientations extends over the whole mesh of approximately \( (38.5 \mu \text{m})^2 \) in size. Taking a closer look on Figure 5, domains of same order appear to extend even further over different meshes, covering areas of up to 60 \( \mu \text{m} \) x 60 \( \mu \text{m} \) (run A). We note that our analysis is focused on heterogeneous regions including different numbers of layers and not on large regions with uniform thickness (i.e., number of layers). Such large uniform areas are regularly observed in TEM (cf. Figure S1, Supporting Information) and accordingly even larger single crystalline domains are conceivable. For smaller AuNP and NP of different materials large 3D supercrystals are well-established.\(^{[30]}\) For 2D crystalline superlattices, monolayer domain sizes of some square micrometers and up to 20 \( \mu \text{m} \) in 1D, as observed with EM, have been reported.\(^{[1,20–22]}\) Thus, even considering the larger particle size used herein, our XCCA analysis indicates record scale 2D single crystalline domains.

Finally, we discuss the effect of film thickness on the degree of orientational order. While the lattice constant was found to depend on the number of layers, the question arises if this can also be observed for \( |\hat{I}_6| \). Increase of the degree of local order as well its variation has been reported for heterogeneously ordered films of AuNP.\(^{[8]}\) The amplitudes of the Fourier coefficients \( |\hat{I}_6| \) averaged over the particular number of layers are plotted in Figure 7.

Besides \( \ell = 6 \), we show results for \( \ell = 2 \) as an example for even coefficients that do not reflect the dominating symmetry. Scattering patterns from supercrystals are typically centrosymmetric, that is, \( I(q) = I(-q) \) which is known as Friedel’s law. Therefore we also show \( \ell = 5 \) which is thus a measure of background and noise contribution. Obviously, the sixfold order dominates. Most importantly, there is no dependence on the number of layers. Only for monolayer steps we observe an increase of \( |\hat{I}_6| \) which is connected to the increase of intensity with the number of layers and thus the visibility of Friedel’s law. We find similar results for other even symmetries that are not multiples of six. The constant sixfold local symmetry as a function of the number of layers suggests that the self-assembled films show a well-developed local order already for monolayers that does not develop significantly with a growing number of layers.

3. Discussion

Self-assembly of small nanoparticles with short ligands has been studied for many years now. However, experimental
control of self-assembly is still challenging because of complex processes involving various parameters and forces over many length and time scales.\footnote{1,2,23} The same holds true for larger AuNP, coated with polymer ligands.\footnote{22} To understand the self-assembly of such systems, two perspectives are helpful: the ensemble and the pairwise particle-particle interaction.\footnote{23} In a simplified view on the ensemble level, the assembly can be understood as an optimization of a hard-sphere (considering the whole AuNP-core/polymer-shell system as one hard sphere) packing, that reduces the overall free energy by increasing the free volume entropy (maximized local degrees of freedom for each constituent outweighs the reduced configurational entropy of the ensemble).\footnote{1} This approach is established, for example, for predicting the structural variety of binary nanoparticle superlattices.\footnote{24–26} Following this picture, the crystallinity of the AuNP supercrystals indicates that a thermodynamic minimum is reached. The closest packing of the “hard-spheres” (AuNP + polymer shell) results in a non-closed packing of the AuNP-cores, that can be tuned via the thickness of the polymer shell (i.e., molecular mass of the ligand), a major advantage of polymers compared to small molecular ligands.\footnote{18,21,22,26,27} The particle-particle interactions in such systems are dominated by the polymer shell. Because the system is nonpolar, electrostatic contributions can be neglected, leaving Van-der-Waals attractive forces between the AuNP-cores and repulsive forces upon compression of the polymer shells at decreasing interparticle distances.\footnote{12,23} However, the interparticle ligand-ligand interactions itself change during the self-assembly process. Being dispersed in the solvent toluene, a good solvent for polystyrene, the interaction of polymer segments with solvent molecules is favored over polymer–polymer interactions.\footnote{28} Accordingly, the AuNP@PSSH are well dispersed in toluene and do not aggregate. In the final formed superlattices, no toluene is present, and ligand-ligand interactions are favored over interactions with air or the polar liquid subphase, resulting in coherent films (thin films would also form from bare polystyrene-ligand solutions without particles).\footnote{38} Because the attractive ligand-ligand interactions are rather weak (no hydrogen-bridges, chemical cross-linking etc.), the resulting superlattice films are also fragile and brittle. Thus, the interparticle ligand-ligand interactions gradually change, during slow evaporation of the solvent, from repulsive to weakly attractive. The weak interactions are the key for reaching a thermodynamic minimum during assembly, because they allow for structural rearrangements in the forming lattices.\footnote{2,22} The other factor in this respect is the liquid subphase providing mobility for the arranging particles.\footnote{24} The viscosity of the liquid subphase affects the 2D-Brownian motion of the floating particles and forming superlattice segments at the liquid-liquid and the liquid-air interface and therefore the kinetics of the assembly. The kinetics are related to the formation of defects, which can be understood as local minima of the free energy landscape often termed kinetic trap states.\footnote{9} From these considerations we can conclude that the synthesis protocol provides a thermodynamically and kinetically well-balanced system, that allows the drying AuNP@PSSH to reach a thermodynamic minimum in the form of crystalline superlattices. The layered crystalline structure is thermodynamically favored and the particle-particle

**Figure 5.** Orientational order. Top: Map of sixfold order $|\hat{I}_6|$ for the three runs. Bottom: Orientation angle $\omega$.
interactions are sufficiently weak to allow rearrangements. In particular, single particles, or even small islands, on a monolayer (same for bi- and multilayers) are practically never observed. The number of layers cannot be perfectly controlled today, but it is related to the number of particles in the drying solution in a given geometry. This number can be controlled via the particle concentration or via the volume.

Previous experiments indicate that the evaporation time affects the lattice constant \(a\). For a fast evaporation we observed slightly larger \(a\) compared to a slow evaporation (Figure S4, Supporting Information), suggesting a contraction of the AuNP@PSSH lattice in the late stage of assembly to maximize the increasingly attractive ligand-ligand interactions. After complete evaporation of the solvent, further contraction is not possible. No change of the lattice constant was observed for aging dry samples. Because the observed increase of \(a\) is a small subnanometer effect, it has to be interpreted with care. One could speculate that the contraction of the lattice at the late stage of assembly is possible for monolayers with all particles being mobile on the liquid subphase as illustrated in Figure 8. In contrast, once a second layer has formed, further contraction is limited. Assuming that thicker crystals with more layers start growing at an earlier point of the assembly process, the lattice contraction would be stopped earlier. In consequence the

![Figure 6. Velocity plots of the four meshes labeled in Figure 3. The length of the arrows is given by \(|\hat{I}_6|\), its direction by \(\omega_6\).](image)

![Figure 7. Mean Fourier coefficients \(|\hat{I}_\ell|\) for \(\ell = 2, 5, 6\), as a function of number of layers for the three runs. The error bars reflect the rms error.](image)
lattice constant would gradually increase with the number of layers as indicated by our results shown in Figure 4b. No peak broadening was observed for an increasing number of layers (Figure 4a) indicating that the lattice constants within domains of a given number of layers are constant.

4. Conclusion

The combination of TEM, SAXS, and XCCA enables a detailed and confident structural characterization of plasmonic supercrystals, combining statistically robust structural data from SAXS with a detailed microscopic picture obtained by TEM analysis and XCCA. Supported by direct comparison of TEM measurements and SAXS-maps, we show that the correlation of SAXS intensity and sample thickness can be used to categorize intensity maps and analyze the SAXS-results as a function of layer number. We find a subnanometer but significant increase of the lattice constant with increasing number of layers, record-scale single-crystalline domains up to thousands of square µm and a high degree of orientational order irrespective of the number of layers. The structural details obtained in this study will help to understand and further optimize the self-assembly process of AuNP@PSSH, yielding well-defined plasmonic supercrystals.

5. Experimental Section

Plasmonic Supercrystals Synthesis: Supercrystals were synthesized with Polystyrene-thiol (PSSH) functionalized AuNP ($d_{core} = 23.4$ nm, $M_w$(PSSH) = 11 400 g mol$^{-1}$) by self-assembly at a toluene-diethylene glycol (DEG) liquid-liquid interface as described recently.$^{[16]}$ By adjusting the volume of the AuNP@PSSH solution (7.1 nm) in toluene that was evaporated on the liquid subphase DEG, one sample with mostly monolayers (300 µL) and one sample with mostly bi- and multilayers (500 µL) was prepared.

Transmission Electron Microscopy: TEM measurements were performed using a Jeol JEM-1011 instrument operating at 100 kV. Samples of self-assembled AuNP@PSSH films were carefully skinned off with a carbon coated copper grid held by a tweezer. The grid was then dried in vacuum for at least 1 h. TEM measurements were analyzed with ImageJ (version 1.50i) to determine the parameters summarized in Table 1. The indicated mean values were obtained from analyses of at least three images from different spots on the samples. To identify the regions of interest mapped in the SAXS experiments, overview maps (Figure S1, Supporting Information) were used to identify characteristic structural features.

Coherent SAXS: The coherent SAXS experiment were performed at beamline P10 of PETRA III (DESY, Hamburg, Germany) at an X-ray energy of 8.2 keV. The samples were placed in the in house sample chamber that has been evacuated afterwards. The scattering patterns were measured using an Eiger X4M detector with 2167 x 2070 pixels with a pixel size of (75 µm)$^2$. This detector was placed 5 m downstream the sample using an evacuated flight path. In order to obtain micrometer scanning resolution, the beam was focused by a stack of compound refractive lenses to a size of 1.2 µm x 1.0 µm (horizontal x vertical) at the sample position.$^{[29]}$ To scan the sample, we used piezo stages and step sizes of 1 µm in horizontal and vertical direction. Runs were performed covering regions of interest of 140 µm x 150 µm (run A) and 100 µm x 100 µm (runs B and C), respectively. The exposure time was 1 s per sample spot. The mean particle diameter and dispersity was determined from a form factor sample (Figure S5, Supporting Information), that is, AuNP@PSSH in toluene, because for the supercrystals the I(q) are dominated by the Bragg reflections preventing form factor fitting.

X-ray Cross Correlation Analysis: The XCCA method allows to access orientational order information via angular correlation functions from 2D scattering patterns. In standard SAXS experiments this information is typically lost due to the averaging over the azimuthal angle:

$$I(q) = \frac{|\langle I(q, \varphi) \rangle|}{|\langle I(q, \varphi) \rangle|}$$

where

$$q = \frac{4 \pi}{\lambda} \sin \left(\frac{\theta}{2}\right)$$

denotes the modulus of the wave vector transfer, $\lambda$ the wavelength of the X-rays and $\theta$ the scattering angle. In XCCA, the correlation function probing orientational order is given by$^{[9]}

$$C(q, \Delta) = \frac{\langle I(q, \varphi) I(q, \varphi + \Delta) \rangle_\varphi - \langle I(q, \varphi) \rangle^2_\varphi}{\langle I(q, \varphi) \rangle^2_\varphi}$$

Orientational order can be quantified by the Fourier coefficients of C of order l with respect to the angle difference $\Delta$,$^{[11,12]}$ which is connected by the Wiener–Khinchin theorem to the Fourier coefficients of the intensity

$$\hat{C}_l(q) \sim \frac{1}{N} \left| \int_{-\pi}^{\pi} I(q, \varphi) e^{-iql} d\varphi \right|^2$$

**Figure 8.** Illustration of the final stage of self-assembly: mono- versus bilayer (not to scale).
For thin films in particular, XCCA enables quantification of the extent of sample orientation by the phase \( \Omega \) of the Fourier coefficients

\[
\hat{I}_\ell(q) = \int \hat{I}(q) \exp(\Omega \ell \cdot q) \, dq
\]

In this way, domain size of few \( \mu \text{m} \) are reported for self-assembled films of gold nanoparticles (10) and PbS nanocrystals (14). The orientation of the sixfold symmetry in the scattering pattern and thus the probed sample area is given by

\[
\omega_k = \frac{\Omega}{\ell}
\]

\[
(6)
\]

### Supporting Information

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

### Acknowledgements

This work is supported by the Cluster of Excellence ‘Advanced Imaging of Matter’ of the Deutsche Forschungsgemeinschaft (DFG)—EXC 2056-project ID 390715994. The authors acknowledge DESY (Hamburg, Germany), a member of the Helmholtz Association HGF, for the provision of experimental facilities. Parts of this research were carried out at beamline P10 of PETRA III. Open access funding enabled and organized by Projekt DEAL.

### Conflict of Interest

The authors declare no conflict of interest.

### Keywords

nanomaterials, small-angle X-ray scattering, self-assembly, supercrystals, X-ray cross-correlation analysis

Received: May 25, 2020

Revised: July 8, 2020

Published online: August 13, 2020