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Chiral Scatterometry on Chemically Synthesized Single Plasmonic Nanoparticles

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Supporting Information

ABSTRACT: Wide-spread applications of nanoparticles require large-scale fabrication techniques. Being intrinsically scalable, bottom-up nanoparticle synthesis shows an ever-growing control over particle morphology, enabling even chirally selective shapes. Significant efforts have been undertaken to refine the synthesis in order to decrease the structural spread of the particles and to purify and maximize the resulting handedness. So far, imaging technologies such as electron microscopy are mostly used to investigate the quality of the synthesis. However, for nanophotonic and plasmonic applications, the optical properties are, in fact, key. In this work, we show that single particle chiral scatterometry holds great potential



as a feedback to characterize the (chir-)optical quality of chemically synthesized nanoparticles. The spectra of single helicoid nanoparticles reveal a diverse set of chiroptical responses with hugely varying absolute chiral asymmetry in spite of the well-controlled morphology of the particles. Averaging over the single nanoparticles reproduces the solution ensemble measurement remarkably well. This demonstrates that the single particles, despite their morphological and consequently chiroptical differences, exhibit a clearly pronounced chiral spectral and structural feature. We find that the gfactor, that is, the degree of asymmetry of chiral light scattering of single nanoparticles can be up to 4 times larger than that for the ensemble. This proves that chiral scatterometry can be a highly important optical feedback for bottom-up nanoparticle synthesis as it reveals that the asymmetry of the ensemble solution can be further increased and maximized by appropriate refinement methods or by postfabrication purification.

KEYWORDS: plasmonics, chirality, single particle, chiral nanoparticles, scatterometry, chiral spectroscopy, chemical nanoparticle synthesis

hiral plasmonic structures have gathered significant interest due to the manifold potential and suspected applications, such as polarization control and manipulation,¹⁻⁶ chiral sensing,⁷⁻¹⁰ three-dimensional chiral rulers,^{11,12} switchable chiral systems,¹³⁻¹⁵ and many more.¹⁶⁻¹⁹ However, already from a very fundamental and basic viewpoint, people have been captivated by plasmonic chirality.^{12,20-30} The reason lies with their molecular counterparts: Chirality, or the structural handedness of molecules, manifests itself optically in a different absorption for left- and right-handed circularly polarized light (LCP and RCP, respectively). Consequently, the polarization state of light can be manipulated: Circular dichroism (CD) leads to differential absorption, and optical rotatory dispersion (ORD) causes the plane of linearly polarized incident light to be rotated. However, all of these effects are small for molecules due to their small light interaction cross sections. This is in stark contrast to plasmonic systems. Here, extremely large asymmetry has been found, which is linked to the

enormous dipole strength of the plasmonic resonances. Additionally, the chiral response can be tailored as there is significant control over composition, relative orientation, and position, shape, and size of individual plasmonic nanoparticles.³¹⁻³³ Moreover, it was found that the chiral response of plasmonic systems is more diverse as in the case of molecular systems as plasmons scatter and absorb light, leading to chiroptical effects in absorption as well as scattering.³⁴

There are mainly two possibilities to fabricate chiral plasmonic nanoparticles. On the one hand, top-down fabrication methods, such as electron-beam lithography, allow for a very high control of the individual nanoparticle shape and their relative position resulting in three-dimensional multilayer nanostructures.^{35–38} On the other hand, the interest in bottom-up synthesis techniques is mainly fueled by the

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scalability of these methods, allowing, in principle, to manufacture the large quantities of nanoparticles required in real-world applications. The goal of the synthesis, however, is to fabricate nanoparticles, which do not only show similar shape but also possess similar optical properties. Consequently, significant effort has been undertaken to refine the nanoparticle synthesis. Such a refinement requires feedback. In most studies, this feedback is the structural investigation of the nanoparticles *via* scanning electron microscopy (SEM) or transmission electron microscopy (TEM). However, in most cases, the optical properties are, in fact, key for future applications, which makes the optical properties appear highly appealing as a potential feedback.

In that sense, one question stood out from the beginning: What is the ultimate limit in the chiroptical response? How does one maximize the asymmetry of these plasmonic systems? More specifically, what is the ideal structure leading to the largest chiroptical response? In particular, the relation between the nanoscale morphology and the optical properties is of utmost interest, however, difficult or even impossible to determine. This holds, in particular, true for chiral geometries in which minute morphological differences can manifest themselves in pronounced chiroptical differences.³⁹

Recently, a water-based chemical synthesis route to fabricate three-dimensional chiral metal nanoparticles utilizing cysteine and cysteine-containing peptide additives as chiral shape modifiers has been presented.⁴⁰ This strategy has provided a possibility to synthesize various chiral helicoidal nanomaterial series^{41,42} and allowed a controlled bottom-up fabrication of *solid* symmetric left-handed, right-handed, *as well as* achiral nanoparticles. The nanoparticles of these series exhibit an exceptional chiroptical response with an ensemble asymmetry factor of up to g = 0.2, while offering full tunability of the material properties *via* the synthesis.

Nevertheless, further improvement of the chiroptical properties requires a deeper understanding of the working principles in these chemically synthesized helicoid nanoparticles. In this work, we use single particle chiral scatterometry and introduce it as an in-depth investigation method for the correlation between nanoscale morphology and far-field optical properties. We show a quantitative chiroptical comparison (forward and backward measurement) of the solid bottom-up fabricated helicoid nanoparticles, which is in contrast to previous single particle studies on aggregates.^{43–45}

We find that the single particle scattering spectra as well as the chiroptical responses of individual particles vary significantly from one another as well as from the ensemble solution response. Interestingly, individual nanoparticles were found to show a 4-fold increase of the asymmetry factor g compared to the ensemble. Despite a large spread in the single-NP asymmetry factor spectra, we show that the averaged single particle response closely resembles the ensemble solution response. Our results underpin the optical response of single NPs as a highly relevant additional feedback which can, together with structural investigations, lead to a more systematic and deeper understanding of chiral plasmonic nanostructures.

RESULTS AND DISCUSSION

Schematic representations of the ideal shape of our single Lhanded and D-handed helicoid NPs as well as the achiral hexoctahedrons are shown in Figure 1a. Their chemical synthesis is explained in detail in the Methods section. For Article



Figure 1. (a) Schematic illustration of single chiral plasmonic NPs showing the left-handed (L-handed), achiral, and right-handed (Dhanded) structure. The achiral hexoctahedrons are the seeds for the growth of the two enantiomers. (b) Schema of the optical transmission setup to measure single nanoparticle (NP) chiroptical responses. The illumination unit consists of a high intensity white light source, a polarizer (Pol.), and a quarter wave plate (QWP) to generate circularly polarized light, as well as a dark-field condenser (DF Cond.) to focus the light on the sample. The detection path contains an objective (Obj.), which collects the scattered light from the NPs, and a spectrometer.

helicoid NPs with the highest g-factor, uniform sized {111} facet enclosed gold octahedron NPs are used as presynthesized low-index seeds. Under normal growth conditions, achiral hexoctahedron nanoparticles composed of {321} facets on 48 identical triangular faces with $4/m\overline{3}2/m$ point group symmetry were synthesized. Upon addition of the chiral glutathione molecule as a chirality encoder, a preferred interaction between L-glutathione with the generated $\{321\}R$ facet in the hexoctahedron leads to a slower development of the R region. This shifts the R-S boundary toward the S region, whereas the counter-enantiomer shifts the R-S boundary toward the R region. This asymmetric growth breaks the mirror and inversion symmetry for the 432 point group symmetry generation. Viewed from the [100] direction, convex inner edges, highlighted in the achiral hexoctahedron in Figure 1a, rotate in clockwise and counterclockwise directions, resulting in pinwheel-like structures with four highly curved arms on each of the six faces of the cubic geometry. These chiral arms evolve away from the center point as its thickness increases, therefore, generating high-curvature gaps (chiral cavities).

After synthesis, the NPs are dispersed on a glass substrate *via* spin-coating at high speed, which separates them spatially. While also preventing near-field interactions, it more importantly allows for single particle measurements as the scattered light from a single particle can be recorded only for a certain minimal interparticle distance due to the size of the far-field focus, typically on the order of one resonance wavelength. The glass—air interface can introduce measurement artifacts due to the lowered symmetry. In order to measure the chiroptical properties of the nanoparticles immobilized on the surface, it is important to remove the local structural asymmetry and any potential contribution caused by this

asymmetry. We thus spin-coat a thin polymer layer (Futurrex IC1-200 spin-on glass) onto our samples. The particles are afterward embedded into a homogeneous material consisting of the glass substrate and the spin-on glass polymer layer with the same refractive index, which is crucial for avoiding symmetry breaking at the substrate-air interfaces.

To measure the chiroptical response of the single NPs, we use a modified dark-field microscope (Nikon Eclipse TE2000-U).³⁹ A schema of the setup is shown in Figure 1b. We place the NPs onto the glass substrate at the sample position. With a high-intensity laser-driven plasma white light source (Energetiq EQ-99) and a subsequent combination of linear polarizer (Thorlabs LPVIS100) and broadband quarter wave plate (B. Halle RAC 5.4.20), we generate circularly polarized light. The left circularly polarized (LCP) and right circularly polarized light (RCP) are focused via a dark-field condenser onto the individual structures. Due to the high numerical aperture (NA = 0.8 - 0.95) of the condenser, only the scattered light in the forward direction (indicated by red lines in Figure 1b) is collected by the objective (magnification 60×). The primary light (blue lines) passes the objective. This allows us to resolve a single NP and detect its chiral scattering spectrum with a grating spectrometer (Princeton Instruments SP2500i) in combination with a Peltier-cooled front-illuminated CCD camera (Acton PIXIS 256E).

SEM images of four representative L-handed, achiral, and Dhanded single NPs without the polymer coating are depicted in Figure 2. When comparing the shapes of the synthesized



Figure 2. Representative SEM images showing four L-handed, achiral, and D-handed single NPs. We find clear structural differences between the single L-handed and D-handed helicoid NPs, respectively. In contrast, the achiral hexoctahedrons show very little visible structural differences. The scale bar is 200 nm.

particles with the schematic illustrations in Figure 1a, we find that both L-handed and D-handed helicoids possess the pinwheel-like morphology. The first two enantiomeric NPs clearly show the clockwise and counterclockwise rotation of the chiral arms with respect to the center symmetry point. Despite a consistent generation of a chiral motif, artifacts can exist within a batch and between batches as slight deviations from the optimum synthesis conditions, which can influence the chiral nanoparticle morphology significantly. In that sense, a "chiral motif" in our terminology means a common and persistent structural or also spectral feature (see below) which is present on average in most single nanoparticles. Representative artifacts of nanoparticle morphologies are shown in the SEM images of the two enantiomers in Figure 2. Some of the NPs have chiral features which possess underdeveloped curved arms (third L-handed particle) and some with overdeveloped protruded and extended arms (fourth L-handed particle). Additionally, we see a slight size difference between the L-handed and D-handed NPs. The achiral hexoctahedrons show significantly less structural spread, having similar shape and dimensions. For a comprehensive understanding of the synthesis and a quantification of the synthetic potential, it is now important to correlate the morphological differences with the spectral response. A priori, it is not clear which of the particle morphologies visible in Figure 2 will exhibit the strongest chiroptical response. Moreover, it is not clear which spectral signatures will survive ensemble averaging.

As mentioned, we immobilized spin-coated left-handed (L-handed), achiral, and right-handed (D-handed) nanoparticles on different substrates and embedded them into a spin-on glass. In our measurement setup, the particles will appear as bright red-colored spots due to their efficient plasmonic scattering. For each of the three cases, we have randomly picked 26 red-colored spots as locations of helicoid particles. As the particles are embedded into the spin-on glass, their position and orientation are fixed and we can locate specific particles again, allowing to re-measure the spectra as well as to measure the same spots for different illumination, for example, from the back or front side. In order to obtain reliable spectral information and a decent overview of the spectral spread, it is indispensable to have large enough sampling as well as unbiased particle selection.

Our quantitative study compares the chiroptical properties of 26 L-handed, 26 achiral, and 26 D-handed single NPs. Their single NP scattering spectra in the visible (VIS) and nearinfrared (NIR) spectral range ($\lambda = 500-1025$ nm) are shown in Figure 3 for illumination with LCP (solid) and RCP (dashed) light. The spectra in each waterfall plot are shifted vertically by 1 (the color code we used to plot the spectra for the individual particles remains the same throughout the article). This presentation of *all* 3 × 26 single nanoparticles in such a waterfall plot is essential as it allows for a quantitative and clear comparison of their scattering spectra and helps to find spectral differences.

First, we investigate the response of the achiral structures, depicted in the middle column. The strongest scattering for the particles, similarly to the chiral ones, appears between 700 and 900 nm, explaining the reddish appearance of the particles in the dark-field microscope. We see that the scattering spectra for RCP and LCP illumination can hardly be distinguished for all of the particles. This finding is important for two reasons: First, it demonstrates that the nanoparticle synthesis indeed delivers achiral particles of very high quality. Second, it confirms the validity of our measurement procedure as the achiral particles indeed should not show any chiroptical response. In previous works, it has been shown that minute deviations from the ideal achiral shape indeed already cause significant chiroptical activity, which makes it challenging if not impossible to perform this control experiment with, e.g., topdown fabricated structures.³⁹ Therefore, the excellent optical achirality is testimony to the synthesis strategy used.

The left column (Figure 3a) depicts the scattering spectra of the 26 L-handed single NPs. Already at first sight, the scattering for RCP and LCP illumination can be clearly distinguished for all particles, pointing to a strong chiroptical response. The



Figure 3. Scattering spectra of 3×26 randomly picked single NPs (26 L-handed, 26 achiral, 26 D-handed) for LCP (solid) and RCP light illumination (dashed). The L-handed NPs are shown in (a), achiral NPs in (b), and D-handed NPs in (c). The offset between the spectra is 1. The L-handed and D-handed NPs show a large spread in the resonance position as well as in their spectral features, especially when compared to the achiral NPs. The cause of this observation lies within the structural differences in between the L-handed and D-handed Single NPs.

exact spectral shapes and signature, however, show a surprisingly large spread. Some of the NPs show only a single peak; some possess two distinct features, whereas others show significantly reduced scattering. A similar behavior is observed for the D-handed single NP scattering spectra in Figure 3c; however, the spread in optical features seems to be less in comparison to the L-handed enantiomers. Almost all D-handed NPs show a narrow peak around $\lambda = 700-750$ nm. Overall, their scattering spectra are shifted slightly toward smaller wavelengths. The origin lies most probably within the slightly smaller dimensions of the D-handed NPs as we have observed in the SEM images shown in Figure 2. Comparing the scattering response of the L-handed and D-handed NPs, the chiroptical response of the L-handed ones seems to be larger on average, with also larger spectral spread. The D-handed NPs appear more uniform in their response.

Next, we want to study the chiroptical properties of the NPs in detail. In molecular systems, this chiroptical response is generally given as differential absorbance for RCP and LCP incident light, called circular dichroism. This does, however, not imply that the chiroptical response of any system is defined *via* absorbances. The reason for the definition lies with the properties of molecular systems which predominantly *absorb* light and show very little to no *scattering* of light (as absorbance scales with V, while scattering scales with V^2 and the molecular volume V is very small). This is in stark contrast to plasmonic systems. Here, due to its larger size compared to the size of molecules, the light scattering cross sections can even significantly surpass the absorption cross sections. Consequently, it has been demonstrated that the structural chirality of a plasmonic system manifests itself in the absorption *and* scattering response.³⁴ These two contributions can, in fact, hold different information, as spectral far-field scattering maxima do not necessarily coincide with the maximum in absorption, in particular, in systems with pronounced interaction *via* resonant light scattering. In Figure 4, we thus plot the differential scattering defined as $\Delta S = S_{\rm RCP}$ – $S_{\rm LCP}$.

The *differential* scattering spectra obtained from the scattering spectra shown in Figure 3 are depicted as solid lines in Figure 4. Please note that our structures are intrinsically highly C_4 symmetric, and thus no correction for linear artifacts, such as linear birefringence, is necessary. The differential scattering reveals that the chiroptical response is significantly more pronounced than the spectra of Figure 3. Thus, it becomes even more important for the differential scattering to be able to quantitatively compare the individual responses and the spectral chiral signature of all 3×26 single



Figure 4. Differential scattering spectra $\Delta S = S_{RCP} - S_{LCP}$ of 3 × 26 single NPs (26 L-handed, 26 achiral, 26 D-handed) in forward (solid) and backward illumination directions (dashed), respectively. The L-handed (a) and D-handed (c) NPs show a large spread in the differential scattering spectra caused by structural differences. In contrast, almost all achiral NPs show barely any chiroptical response. (d–f) Average of the differential scattering spectra of the single NPs shown (a–c), respectively. The average achiral chiroptical response is, as expected, clearly distinguishable from the chiroptical response of the enantiomers.

nanoparticles. This is again achieved (similar to Figure 3) by making use of the shown waterfall plot. First, we turn our attention to the reference achiral NPs: We observe a close to zero chiroptical response for all 26 particles. As already mentioned, this is important as a control experiment to make sure the optical detection path performs flawlessly while also revealing the excellent NP quality. As we used the same scale on all panels, it is obvious that the small observed spectral features for the achiral structures are most likely measurement artifacts but, in any case, fully negligible when compared to the response of the chiral NPs. The results of another important control experiment are depicted as the dashed lines: Due to the encapsulation and immobilization of the nanoparticles, we can flip the sample and thus revert the illumination direction. The handedness of a chiral particle is a geometric property and thus independent of the illumination direction. However, in general, a sufficiently large chiral nanoparticle will not have exactly the same spectral response for all possible illumination directions due to retardation effects. This behavior is most obvious for an

elongated spiral illuminated along the long axis or perpendicular to it. However, for exactly counterpropagating light fields, the response needs to be close to identical. The reason for this control experiment is simple: Only in case of a true chiral response, the differential scattering spectra will show reasonable agreement. Contributions of, for instance, polarization conversion or elliptical birefringence in contrast will flip sign. When inspecting the differential scattering spectra for forward and backward illumination for the achiral NPs, we see that also these spectra show little to no signal, again underlining the achirality of the NPs. The optical response of one particularly interesting particle is depicted in dark green (13th particle from the bottom): We observe a comparably strong differential scattering signal; however, it nearly perfectly flips sign for opposite illumination direction. This indicates that the observed signal, in fact, most probably originates from linear birefringence. For reasons unclear, locally, the symmetry seems to be reduced, causing linear birefringence, which can be



Figure 5. (a-c) g-factor spectra $g = 2\frac{S_{RCP} - S_{LCP}}{S_{RCP} + S_{LCP}}$ for all 3 × 26 single NPs (26 L-handed, 26 achiral, 26 D-handed). The calculation of the g-factor allows for a comparison of the optical activity to solution (ensemble) measurements. In panel (d), we plot the averaged g-factor of the 26 L-handed (red), 26 achiral (black), and 26 D-handed NPs. Additionally, the g-factor of the respective NP solution is plotted in lighter colors in (e). The agreement between averaged single NPs and NP solution in terms of resonance position and absolute values of the g-factor is very good. We obtain similar maximum g-factor values of approximately ±0.2.

mistaken for a chiroptical signal without proper control experiments.

We now turn to the chiroptical response of the L-handed NPs depicted in the left panel of Figure 4. Again, the differential scattering for forward and backward illumination is plotted as solid and dashed lines, respectively. Most of the chiroptical features are present in the wavelength range from λ = 750 nm to λ = 950 nm. In addition, some of the L-handed NPs show features at smaller wavelengths around $\lambda = 600-650$ nm. Compared to the scattering spectra, the differential scattering very prominently indicates a surprisingly large spectral spread of the particles. We observe spectral signatures of varying number of modes and at different spectral positions. The relative strength of these modes as well as their absolute values vary significantly. Some signatures, such as the one around 900 nm, are extremely pronounced in some spectra while being nearly absent in others. A subset of particles exhibits spectral signatures below 700 nm, which are not at all detectable for others. In general, the spectral spread is much larger than we would have naively expected from the SEM images as well as from the maturity of the synthesis strategy. This, in fact, points at two important implications: First, also in these particles, seemingly minor structural deviations lead to significant spectral changes. Moreover, it underpins that there is significant potential for improvement of the overall chiroptical response of the ensemble. The spectra measured for opposite illumination directions show excellent agreement with their counterparts. There are minor differences, which we ascribe to inaccuracies in alignment (single NP measurements are highly sensitive to possible small positioning differences) and smaller retardation effects. However, the overall spectral features and their relative strength are in excellent agreement, underlying that we are indeed measuring the true chiroptical response.

The right panel of Figure 4 finally depicts the differential scattering for the D-handed NPs for forward and backward illumination. The overall observations are very similar as in

case of the L-handed NPs. The spectra for forward and backward illumination show reasonable agreement, validating our measurements. In general, the chiroptical response is smaller as well as blue-shifted in comparison to the L-handed NPs. Also, we observe smaller overall spread of the spectral response in terms of number of modal signatures and overall amplitudes. We ascribe this observation as well as the blue shift to the slightly smaller size of the D-handed NPs, which causes an overall blue-shift as well as a less pronounced mode splitting.

Additionally, we calculate the average of the single NP differential scattering spectra and plot them in Figure 4d-f. As expected from the individual spectra, the average of the achiral NPs is close to zero over the entire spectral range and is clearly distinguishable from the L- and D-handed response. They both show in parts, especially for $\lambda < 700$ nm, mirror symmetry in the differential scattering. Again, we find a good agreement between the averages for the NPs being measured in forward (solid) and backward direction (dashed).

As mentioned above, the full chiral answer of a plasmonic structure constitutes scattering and absorption contributions. As we are analyzing the scattering part in this work, a direct comparison of the chiroptical response measured here to the absorptive CD measurements of the NPs in solution presented before⁴⁰ requires proper normalization. The asymmetry gfactor represents a convenient normalized quantity facilitating this comparison, defined as $g = 2 \frac{S_{\rm RCP} - S_{\rm LCP}}{S_{\rm RCP} + S_{\rm LCP}}$. In Figure 5, we plot the asymmetry factor for all 3×26 single NPs in panels a-c. We deliberately plotted each set of 26 measurements in one panel to once more demonstrate the diverse nature of the spectra (for better comparability of the spectral shape, we plot all 3 \times 26 single NP g-factor spectra in a waterfall plot in Figure S1). When the spectra for the L-handed and D-handed NPs are examined, there seems to be no common pattern but more a random distribution of modes at different spectral positions. The asymmetry factors for the achiral NPs show very



Figure 6. Single NP g-factor as well as the corresponding SEM images of six selected (a) L-handed and (b) D-handed NPs (labeled with I– VI). Note that the NPs are completely embedded in a spin-on glass layer, hence the SEM images are blurrier than the images in Figure 2. We see that the distinct differences in the g-factor spectra are caused by huge differences in the shape as well as in the dimensions of the NPs. Interestingly, some chiroptical responses correspond to dumbbell-shaped pairs of L-handed NPs, which was not observable in the initial dark-field scattering measurements. The scale bar is 200 nm.

little response when compared to the handed particles, in accordance with the previous discussion. Strikingly, when performing an averaging³⁹ by individually averaging the sets of 3×26 measurements of Figure 5a-c, we observe a clear and pronounced chiral feature in the spectra, as shown in panel d of Figure 5. The averages of the L-handed and D-handed NPs show a remarkably strong dispersive chiral feature with good mirror symmetry (apart from the previously discussed slight spectral blue shift of the D-handed NPs). The achiral average exhibits barely any features at all, as expected. Even more remarkable, the spectra show excellent agreement with the solution measurements of the same particles obtained with a Jasco CD spectrometer in transmission, as plotted in Figure 5e (note that this measurement can only be performed up to 775 nm). Despite a small spectral red shift of the average due to a refractive index increase (from water n = 1.33 to spin-on-glass n = 1.41), we obtain almost identical spectral shapes as well as absolute asymmetry values (note the identical y scale). Fundamentally speaking, it is remarkable that the g-factor spectra obtained from these two different types of measurements (scattering vs absorption) as well as from these completely different nanoparticle arrangements (average of single particles vs solution ensemble) are almost identical.⁴⁶

This finding is important for a number of reasons: The individual measurements contain clearly significantly more details and information when compared to the average (see also Figure S2 for a comparison of pairs of single NPs with mirror-symmetric chiroptical responses and similar scattering spectra).^{39,43–45,47} In fact, it is stunning how many modal

signatures vanish in the average while only a single dispersive feature remains. This immediately leads to an important question: Is this feature a consequence of a common chiral motif among all particles or is this the signature of a certain subset of particles which survives the averaging while the other signatures and particles cancel each other out? This question cannot be answered at this point, but is highly relevant for future applications. If the signature is indeed related to a certain subset of particles, these particles could be identified and purification strategies developed which would immediately increase the overall asymmetry. This point is underlined by another important finding of the single-particle measurements: The asymmetry of a few single NPs significantly outperforms the maximum g-factor values of the averaged NPs as well as of the solution, which is on the order of $g \approx \pm 0.2$. We find Lhanded single particles with values of up to g = -0.8, which is 4 times larger than the average.

The measurements and results above can only be explained by deviations in shape, size, dimensions, relative features, *etc.* between the individual particles. In particular, random uncontrolled deviations would most likely rather cancel out in an ensemble than being detectable. It is therefore highly compelling to try and analyze the morphology of the individual particles we measured optically and determine which particle is the "best", hence resulting in the largest asymmetry factor. Such an analysis is, in fact, not straightforward. First, even if the particles would be freely accessible, a determination of their three-dimensional morphology would only be possible by advanced TEM techniques. Additionally, the investigation is hindered by the fact that the particles are fully incorporated into the spin-on glass layer. In order to at least partially overcome this issue, we used a focused ion beam tool equipped with a laser interferometer stage (Raith ionLine Plus) to determine the exact coordinates of the particles and to selectively thin/mill the polymer overcoating (for further details, please see the Methods section). Selected SEM images of these particles are shown in Figure 6 for six single L-handed in panel a and for six D-handed single NPs in panel b (micrographs of additional particles can be found in Figures S3 and S4). The color code for each particle matches the color used in all previous figures. Please note that we milled as much of the spin-on glass as possible without destroying or damaging the particles. However, there are still parts of the layer left on top of as well as around the particles which causes the shown SEM images to exhibit a certain "blurriness" due to additional electron scattering in this layer.

The most prominent finding is that we measured a number of L-handed pairs of particles instead of single ones. Spincoating of particles can lead to certain unavoidable clustering. This finding is also testimony to our unbiased selection of measurement spots. The spectral response of the particle pairs is, in fact, highly interesting. Pair III depicted in pink shows barely any chiroptical response when compared to the other particles. This is a first hint that certain spectral signatures of different particles might indeed cancel out (particle pair V in orange shows similar behavior). Particles I and II possess the highest asymmetry factor of g = 0.5 and 0.8. Interestingly, the main chiroptical features are in the same spectral region (λ = 600-750 nm) as the monodispersive average response in Figure 5d (red curve). Previously reported simulation data of the helicoid nanoparticles showed a strong correlation between the chiroptical response and the helicoid NP geometrical parameters such as the gap depth, width, particle size, and tilt angle, resulting in a maximum asymmetry factor of around $g \approx$ 0.8 for the given pinwheel-like chiral nanostructures.⁴⁰ Due to the spin-on glass layer as well as the inability to observe all six facets of the particles, it is difficult to access the precise shape of the particle. However, the best visible top facet ([100] view) closely resembles the ideal pinwheel-like structure, as shown in Figure 1a. Further detailed investigation with appropriate statistics is needed, but this finding provides two valuable insights. First, it might again point to a potential subset of particles which is responsible for the persistent chiral spectral motif visible in Figure 5d,e. Second, the observed significantly larger single NP asymmetry factor compared to the ensemble value suggests that there is potential to further improve the already impressive chiroptical properties of the NPs grown via the amino acid and peptide directed synthesis approach. In contrast, particles IV and VI are clearly deformed. Despite limited visibility, particle IV shows an underdevelopment of chiral arms and cavities, causing it to be smaller in size. Particle VI is an example for a particle with overdeveloped chiral arms, similar to the last L-handed particle in Figure 2. Significant extrusion and overgrowth of one of the chiral arms coincides also with a smaller core body. For both particles IV and VI, it is not straightforward to identify the chiral shape, still it is clearly presented as indicated by their chiroptical response, which is surprisingly large. Their chiral response, however, clearly differs from the average response of the L-handed NPs in Figure 5d.

In panel b of Figure 5, we show similar results for six Dhanded single NPs. The SEM images underline the point made above: From the images, it is very difficult to access the true and full morphology. The six particles appear significantly more uniform than the six L-handed NPs in panel a. Yet, there is still a significant spread in the spectral features which we cannot correlate with any particular structural differences. Particles II—VI appear to be more similar to the ideal D-handed shape with counterclockwise rotation of chiral arms, as shown in Figure 1a, whereas the shape of particle I seems ill-formed. However, clear assignments require significantly more statistics and merit further in-depth study.

CONCLUSION

In conclusion, we have carried out a quantitative and consistent comparison of the optical and chiroptical properties of chemically synthesized single plasmonic NPs. We used single particle chiral scatterometry in combination with scanning electron microscopy to investigate the influence of structural differences on the single particle chiroptical response. The differential scattering spectra of 26 left-handed and 26 right-handed helicoid NPs showed a huge spread in spectral positions of chiroptical features as well as in absolute values of the optical activity. Consistency was proven with an illumination of the NPs from front- and backside, where the chiroptical responses showed very good agreement. The chiroptical response of additionally measured 26 achiral octahedral NPs was close to zero and clearly distinguishable from the chiroptical response of the two enantiomers. Focused ion beam milling allowed for a structural investigation of selected helicoid NPs and showed structural differences in terms of an over- and underdevelopment of chiral arms and cavities, which strongly influenced the single particle chiroptical response. A comparison of the averaged single NP scattering asymmetry factor to the asymmetry factor of the NPs ensembled in solution resulted in almost identical g-factor spectra. Among measured individual nanoparticles, the highest asymmetry factor was found to be g = 0.8, which is a 4-fold increase compared to that of the solution ensemble. Our findings clearly show that there is significant potential to further increase the asymmetry of the helicoid NP ensemble solution by an appropriate refinement during synthesis or by postfabrication purification. Consequently, the presented results have immediate implications in nanoparticle synthesis by using single particle chiral scatterometry as a feedback for the development and fabrication of high-quality chemical synthesis methods. In addition, our findings can help to develop refinement methods which ensure particles which do not only show little inhomogeneities in their structural properties but, in particular, in their optical response.

METHODS

Nanoparticle Synthesis. Tetrachloroauric(III) trihydrate (HAuCl₄·3H₂O, 99.9%), hexadecyltrimethylammonium bromide (CTAB, 99%), L-ascorbic acid (AA, 99%), and L-glutathione (γ -E-C-G, 98%) were purchased from Sigma-Aldrich. D-Glutathione (γ -E-C-G, 70%) was purchased from BACHEM and used without further purification. All aqueous solutions were prepared using high-purity deionized water (18.2 M Ω cm⁻¹).

Octahedral seeds were synthesized as reported previously,⁴⁸ centrifuged (6.708g, 150 s) twice, and dispersed in aqueous CTAB (1 mM) solution. In a typical synthesis, 0.8 mL of 100 mM CTAB and 0.2 mL of 10 mM gold chloride trihydrate was added into 3.95 mL of deionized water to form a [AuBr₄]⁻ complex. Au³⁺ was then reduced to Au⁺ by the rapid injection of 0.475 mL of 100 mM AA solution. The chiral growth of nanoparticles was initiated by adding 5 μ L of 5

mM L,D-glutathione solution and 50 μ L of seed solution into the growth solution. The growth solution temperature was maintained at 30 °C in a water bath for 2 h, where the pink solution gradually became blue with a large scattering. In the case of achiral hexoctahedral nanoparticles, all synthesis conditions were maintained the same except the addition of the glutathione molecule. The particle solution was centrifuged twice (1.677*g*, 60 s) to be dispersed in a 1 mM CTAB solution for further characterization.

Dilution and Spin-Coating. The prepared nanoparticle solution was diluted to 10 vol % with 1 mM CTAB solution. Then, residual CTAB was removed from the diluted particle solution through centrifugation using high-purity deionized water while keeping the final volume constant. We dispensed 200 μ L of the diluted nanoparticle solution and dropped it on a marked 1 × 1 cm² clean glass substrate. Afterward, we spin-coated the solution at 5000 rpm for 30 s to obtain evenly spread nanoparticles with a spacing to prevent optical coupling. See Figure S5 for an optical image showing particles dispersed on the substrate.

Definition of *g***-Factor.** We define the transmission *g*-factor as $g_{\rm T} = 2\frac{T_{\rm LCP} - T_{\rm RCP}}{T_{\rm RCP} + T_{\rm LCP}}$ and the scattering *g*-factor as $g_{\rm S} = 2\frac{S_{\rm RCP} - S_{\rm LCP}}{S_{\rm RCP} + S_{\rm LCP}}$, meaning that we interchange the RCP and LCP contributions. This is simply an inversion of sign, and we link this definition to the contribution of absorption and scattering to the extinction cross section $\sigma_{\rm ext} = \sigma_{\rm abs} + \sigma_{\rm scat}$.

Focused Ion Beam Milling of Overcoating. Although we measured the chiroptical response of the 3×26 single NPs, we saved the x-y coordinates of each particle relative to a chosen fix point. Afterward, this allowed us to mark each particle in a separately taken overview dark-field image of the sample. Subsequently, this image was used to locate all particles with a Raith ionLine Plus system equipped with a laser interferometer stage and mill down the spin-on glass layer over an area of $1 \times 1 \mu$ m at each particle position.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b04046.

Further detailed single NP g-factor spectra (waterfall plots), pairs of L- and D-handed NPs with similar scattering spectra and mirror-symmetric chiroptical responses, as well as further SEM investigations of the measured single NPs and the correlation to their chiroptical responses (PDF)

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Notes

The authors declare no competing financial interest.

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