

## Supplementary information for

# Single nanoparticle chiroptics in a liquid: optical activity in hyper-Rayleigh scattering from Au helicoids

Lukas Ohnoutek<sup>1,2</sup>, Nam Heon Cho<sup>3</sup>, Alexander William Allen Murphy<sup>1,2</sup>, Hyeohn Kim<sup>3</sup>, Dora Maria Răsădean<sup>4</sup>, Gheorghe Dan Pantoş<sup>4</sup>, Ki Tae Nam<sup>3</sup>, Ventsislav Kolev Valev<sup>1,2</sup>

<sup>1</sup> Centre for Photonics and Photonic Materials, University of Bath, Bath, BA2 7AY, UK

<sup>2</sup> Centre for Nanoscience and Nanotechnology, University of Bath, Bath, BA2 7AY, UK

<sup>3</sup> Material Science and Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea

<sup>4</sup> Department of Chemistry, University of Bath, Bath, BA2 7AY, UK

### *Chemical Information*

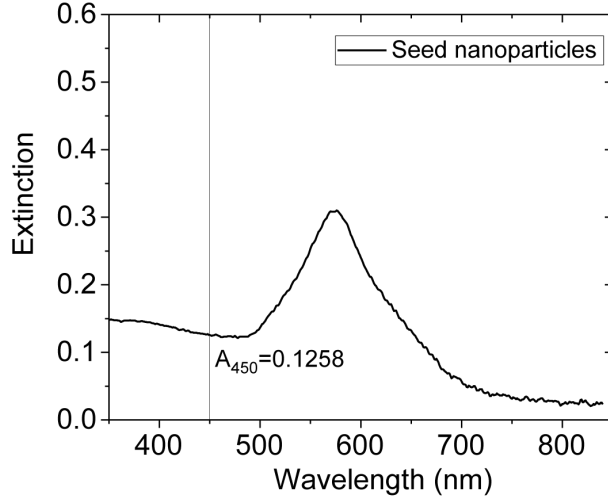
Tetrachloroauric(III) trihydrate (HAuCl<sub>4</sub>·3H<sub>2</sub>O, 99.9%), hexadecyltrimethylammonium bromide (CTAB, 99%), L-ascorbic acid (AA, 99%), and L-glutathione (γ-EC-G, 98%) were purchased from Sigma-Aldrich. D-Glutathione (γ-EC-G, 70%) was purchased from BACHEM and used without further purification. All aqueous solutions were prepared using high-purity deionized water (18.2 MΩ cm<sup>-1</sup>). Tetraethyl orthosilicate (TEOS, 99%) was purchased from Sigma-Aldrich and mPEG-SH (MW 5,000) was purchased by Biochempeg.

### *Estimating illumination volume*

In the Chirascan, light comes out of a vertical slit and it is shaped as a vertical line. The typical dimensions for the cross-section of the resulting beam are 15 mm height and 6 mm width. The length of the beam in a typical cell is 10 mm. With these dimensions, the typical volume of illumination is that of an ellipsoid:  $V = \pi \times \frac{\text{height}}{2} \times \frac{\text{width}}{2} \times \text{length}$ , i.e.  $V = 7 \times 10^{-7} \text{m}^3 \approx 10^{-6} \text{m}^3$ . This value is 11 orders of magnitude larger than the one for our HRS OA measurements. We have measured the beam cross-section at 520 nm wavelength and obtained 7 mm height and 3 mm width. With these dimensions, the calculated illumination volume in the Chirascan is  $V = 1.6 \times 10^{-7} \text{m}^3$ . According to other authors,<sup>1</sup> the Chirascan, on average has a 16 mm diameter area, which results in an illumination volume of  $V = 2 \times 10^{-6} \text{m}^3$ .

### *Determining concentration of nanoparticles*

Two independent methods were used to estimate the concentration. First, we performed an extinction-based evaluation of the nanoparticle number. The extinction spectrum of an octahedron seed solution with diameter ( $d$ )  $\approx$  50 nm shows a sharp peak (see Figure S1).



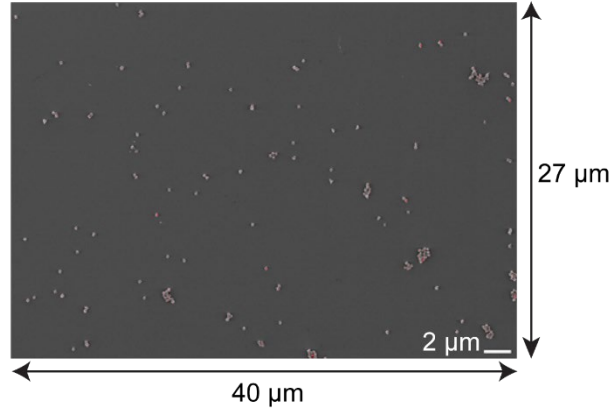
**Figure S1.** Extinction spectrum of seed nanoparticles suspension.

The absorbance at 450 nm ( $A_{450}$ ) is then substituted into the equation for approximated number of nanoparticle seeds:<sup>2</sup>

$$N = \frac{A_{450} \times 10^{14}}{d^2 \left[ -0.295 + 1.36e^{-\left(\frac{d-96.8}{78.2}\right)^2} \right]} \quad (1)$$

Assuming that each seed results in a helicoid (a conservative assumption as the alternative would result in an even smaller concentration), we can estimate the number of 432 helicoid nanoparticles in a given volume of synthesis batch. Thus, we obtain a concentration of  $\approx 7.7 \times 10^9$  NPs/mL in 5.3 mL, which is  $\approx 1.5 \times 10^9$  NPs/mL, i.e.  $\approx 1.5 \times 10^{12}$  NPs/L.

Second, we performed an SEM estimate that consisted of counting individual nanoparticles. We begin by diluting the growth solution 1:50. This dilution is required in order to separate the individual nanoparticles far enough from each other for counting to be practical. We then take a volume of 200  $\mu$ L of the diluted solution and deposit it on a 1  $\text{cm}^2$  surface. An SEM measurement is then performed and the data is shown in Figure S2.



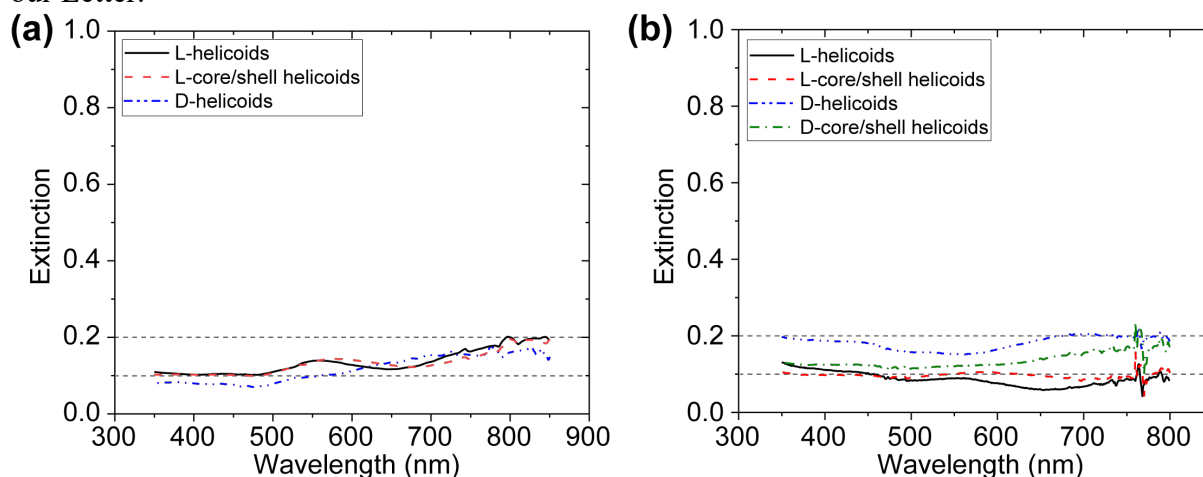
**Figure S2.** Scanning electron microscopy (SEM) of a diluted seed solution (1:50).

This SEM micrograph contains 196 nanoparticles. Assuming a statistically uniform distribution of nanoparticles, i.e. similar number of individual NPs, dimers, trimers and cluster size across the surface, we can calculate the concentration. The SEM surface area is  $S = 27 \times 10^{-6} \times 40 \times 10^{-6} = 1.08 \times 10^{-9} \text{ m}^2$ , so the number of particles on 1  $\text{cm}^2 = 1 \times 10^{-4} \text{ m}^2$  can be estimated at  $1.81 \times 10^7$  NPs. The concentration of the diluted solution is therefore  $1.81 \times 10^7$  NPs in 200  $\mu$ L, which is  $\approx 9 \times 10^{10}$  NPs / L. The concentration of our nanoparticles (not diluted 50 times) is therefore  $\approx 4.5 \times 10^{12}$  NPs / L, which is in good

agreement with the  $\approx 1.5 \times 10^{12}$  NPs/L value from absorption spectra and therefore provides an indication of the range of nanoparticle concentration.

Following the silica coating process, there is usually a red-shift of the spectra, though no significant variation of extinction occurs. As an example, the extinction spectra of L-helicoids (suspended in 1 mM CTAB solution) and of silica-coated-L-helicoids (with 20 nm coating, suspended in ethanol) are shown in Figure S3(a). The slight red-shift of the peak position is caused by the refractive index change. This result indicates that there is usually a negligible amount of aggregation during the coating process. Upon preparation of the samples, there is no significant variation in concentration between the helicoids and the core/shell helicoids.

Prior to shipping the samples from Korea, their concentration was increased (10:1) in order to reduce volume. Upon arrival in the UK and prior to our HRS CD measurements, the samples were diluted (1:10). Figure S3(b) shows the extinction spectra of the samples following the concentration/dilution procedure. For ease of comparison, the data are plotted on the same scale as those in Figure S3(a). In theory, a concentration of 10:1 followed by a dilution of 1:10 should result in the same number of nanoparticles per volume. Although, upon comparing Figure S3(a) and S3(b), it is clear that the procedure has introduced some uncertainty, the concentration of the L-core/shell helicoids appears to have actually decreased. It follows that the measurement of HRS CD, in Figure 4(a) of our Letter, correspond to an even smaller concentration of nanoparticles than that which we estimated in our Letter.



**Figure S3.** Extinction spectra of the helicoids and core/shell helicoids. In (a), spectra obtained after preparation of the samples. In (b), spectra obtained prior to measuring the HRS. The samples had been concentrated (10:1) to reduce volume, prior to shipping from Korea; then they were diluted (1:10) in the UK prior to measuring.

#### *Comparison of concentration of scattering centres with the work of Verreault et al.*

In the supplementary information of Ref. 3, the authors specify the concentrations used in HRS power dependence measurements. The concentrations were between  $10^{-4}$  and  $10^{-2}$  mol/L. This corresponds to:  $\approx 6 \times 10^{22}$  molecules/ $m^3$  and  $\approx 6 \times 10^{24}$  molecules/ $m^3$ . By comparison, the concentration of nanoparticles in our measurements is between  $\approx 1.5 \times 10^{12}$  NPs/L, (i.e.  $\approx 1.5 \times 10^{15}$  NPs/ $m^3$ ) and  $\approx 4.5 \times 10^{12}$  NPs/L (i.e.  $\approx 4.5 \times 10^{15}$  NPs/ $m^3$ ), which is  $\approx 10^9$  times smaller.

*References:*

---

<sup>1</sup> Sitsanidis, E.D.; Piras, C.C.; Alexander, D.B.; Siligardi, G.; Jávorfı, T.; Hall, A.J.; Edwards, A.A. Circular dichroism studies of low molecular weight hydrogelators: The use of SRCDC and addressing practical issues. *Chirality* 2018, 30, 708-718.

<sup>2</sup> Haiss, W., Thanh, N. T. K., Aveyard, J. & Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV–Vis Spectra. *Anal. Chem.* 2007, 79, 4215–4221.

<sup>3</sup> Verreault, D.; Moreno, K.; Merlet, É.; Adamietz, F.; Kauffmann, B.; Ferrand, Y.; Olivier, C.; Rodriguez, V. Hyper-Rayleigh Scattering as a New Chiroptical Method: Uncovering the Nonlinear Optical Activity of Aromatic Oligoamide Foldamers. *J. Am. Chem. Soc.* 2020, 142 (1), 257–263