Measuring the optical properties of nanoscale biogenic spherulites: supplement

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1. EXTRACTION OF SPHERULITES

Specimens of *M. rosenbergii* were grown and maintained at the Ben-Gurion University of the Negev aqua facility in the laboratory of Prof. Amir Sagi under the following conditions: Water temperature was maintained at 27 ± 1 °C, photoperiod of 12 h light:12 h dark. Food comprised shrimp pellets (30% protein; Rangen) ad libitum three times a week. Water quality was assured by circulating the entire water volume through a biofilter maintaining all of the required water physicochemical parameters: pH 8.3 ± 0.5, nitrite 5 mg·L⁻¹. Animals were maintained under complete dark conditions for 24 h (dark-adaptation) before eyestalk collection. Animals were anesthetized in ice-cold water before dissection. The eyestalks were removed from the animal under red illumination to maintain the eyes in the dark-adapted state. The eyestalks were then fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 1× PBS buffer in the dark. Eye sections were obtained from post-larval and juvenile animals by embedding eyes in 10% agar (using 1× PBS as the medium) and cut into 100 µm sections using a vibratome. To extract the isoxanthopterin nanoparticles from the eye sections, the sections were punctured with a microneedle in the vicinity of the tapetum using a polarizing stereomicroscope. This liberated the particles from the tapetum cells. The sections were then transferred to a ca. 3 µl droplet of DDW in an Eppendorf and agitated by mild sonication for 3 minutes to liberate additional particles from the tapetum cells. A 2 µl droplet of the nanoparticle suspension was then placed on a carbon-coated, copper finder TEM grid and blotted after 1 minute. Samples were observed with a Tecnai T12 transmission electron microscope operated at 120 kV.

2. MEASURED PARTICLES

The TEM images show that the homogeneity of the constituent material varies considerably from particle to particle. To effectively compare scattering spectra to results from Mie theory, it is important for the particles to possess full rotational symmetry, which requires the particles to be sufficiently intact. For this reason, we include in our analysis only those particles whose shell appears sufficiently homogeneous and are free from visible structural defects. To illustrate this point, in Figure S1 we show examples of particles with a variety of defects. A majority of particles observed are not sufficiently intact, and out of ∼60 particles, only a few were suitable for our measurements. The values of refractive index obtained for these particles are tabulated in Section 5 of the Supporting information.

3. EXPERIMENTAL SETUP

A custom setup was built around a commercial optical microscope (Zeiss Axio Observer 5 [3]). The sample is illuminated with a ‘white’ LED (Zeiss illuminator VIS-LED). The dark field optical setup is shown in Figure S2. The incident light (Θ_i = 25°) partially enters the objective (N-Achroplan 20x, NA=0.45) and is filtered at the Fourier plane, where the collection angle is also determined (Θ_s = 17°). Two cameras are available for the Fourier plane (Blackfly S USB3, FLIR) and the image plane (PCO edge 5.5), and switching between them is achieved using flip mirrors. A spectrometer is placed after a 2f (50mm and 300mm lens respectively) correlator with a 6x magnification: collection of the scattered spectra thus is done confocally with a fiber with a diameter of 105 µm guiding light to the spectrometer (Shamrock 303i, Andor) as pinhole.

4. DETERMINATION OF DIMENSIONS

To obtain the theoretical scattering spectra for the different particles, a measurement of their radii and thicknesses was necessary. Determination of the radius and thickness was performed
**Fig. S1.** Example for different quality of isoxanthopterin particles as seen in the TEM. (a) A particle with an inhomogeneous shell (b) A particle that is non homogenous, broken and smeared. (c) Broken particle. (d) An intact particle.

**Fig. S2.** The experimental darkfield setup.

using the ImageJ software [1]. The radius was determined by measuring the area $A$ of a single particle as seen in a TEM image, and extracting the radius using the formula $A = \pi r^2$. To find the thickness of the particles, a custom plugin named “Radial Profile” was used[2]. This plugin allows us to view the radial profile of the grayscale value across the image of the spherulite. The shell thickness can be determined by locating the boundary of the shell as shown in the figure below.

**5. TANGENTIAL REFRACTIVE INDEX RESULTS**

The tangential refractive index was measured by matching the positions of the prominent peaks of the scattered spectrum to that of the theoretical spectrum resonant modes with varying tangential refractive indices as was shown in the main paper. This procedure was done for the measured particles from the two samples and its final results can be viewed in Table S1. It is apparent that the refractive index values are systematically higher in the 2nd sample. The 1st and 2nd samples were matured through 4 and 40 weeks respectively, giving a more steady state system for the 2nd
Fig. S3. Left: Particle with a perfect circle drawn around its inner radius. Right: A profile plot of normalized integrated intensities around the concentric circle drawn in the left image as a function of the distance from the center. In red is the inner radius of the particle, marked both in the image and as determined from the profile plot

sample for the crystals to form in a dense spherulite matter.

|      | $n_t(MQ)$ | $n_t(MO)$ | Average $n_t$ |
|------|-----------|-----------|---------------|
| Sample 1 |          |           |               |
| 1.66 | 1.84      | 1.84      | 1.75          |
| 1.90 | 1.89      | 1.89      | 1.90          |
| 1.86 | 1.75      | 1.75      | 1.81          |
| Sample 2 |          |           |               |
| 1.90 | 2.01      | 2.01      | 1.96          |
| 1.88 | 1.98      | 1.98      | 1.93          |
| 1.83 | 1.89      | 1.89      | 1.86          |
| 1.87 | 1.98      | 1.98      | 1.93          |

Table S1. Tangential refractive indices matched from the measured peaks location for all the measured spherulites

6. EFFECT OF THE TEM GRID ON SCATTERING RESONANCES

The finder grid used to deposit the spherulites consists of a marked copper grid, with a continuous film of formvar (thickness 5 - 10 nm) and carbon (2 - 4 nm). The presence of this film in close proximity of the spherulite affects its scattering resonances, and can shift the spectral position. In order to quantitatively estimate this effect, we performed FDTD simulations of the scattering cross section of the spherulite when placed on a TEM grid. We calculated the resonance wavelength shift for spherulites of radius $r = 200$ nm, $t = 100$ nm, $n_r = 1.4$, $n_t = 1.96$ and $n_m = n_c = 1.0$ placed on a grid with formvar thickness 10 nm and carbon film thickness 4 nm.

From the FDTD simulations we observe that the shifts only result in a slight change ($0.01$) in the estimated value of $n_t$. Therefore the error due to the substrate is much smaller than the statistical variation in measurements.
**Fig. S4.** FDTD simulations of scattering cross section in the presence (blue) and absence (red) of the Formvar and Carbon film on the TEM grid.

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