Supplementary Material

Shell–Core Structural Anisotropy in Starch Granules Probed by Polarization Third-Harmonic Generation Microscopy

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1. Comparison between P-THG and P-SHG

Within the same analytical framework, quantitative metrics were extracted and compared using P-THG and P-SHG in the same corn starch granules (Figure S1). For the P-SHG analysis, the biophysical model presented in [12] was used to extract the angle φ and the SHG anisotropy ratio. The model was fitted pixel-by-pixel using an iterative least squares algorithm.

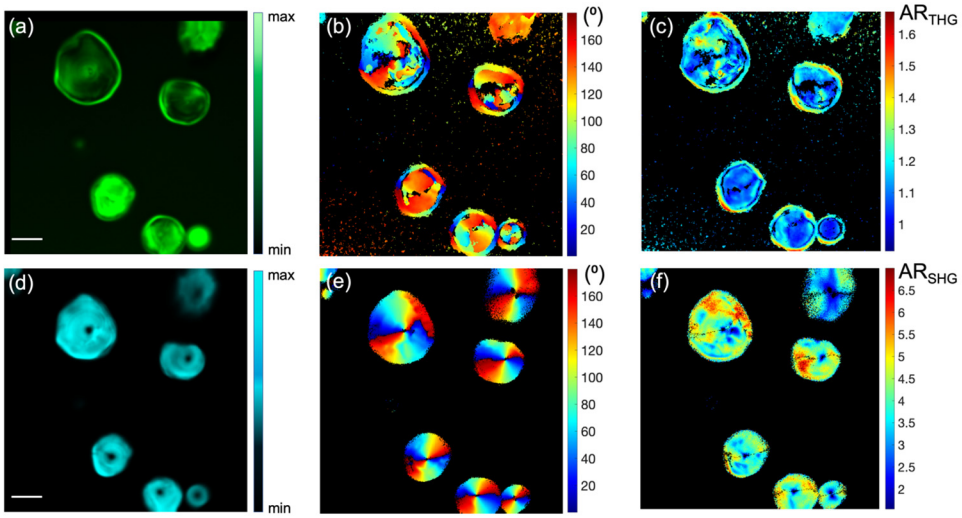


Figure S1: Comparison between P-THG and P-SHG. a) Composite THG image of corn starch granules formed using the 1542 nm beam and obtained by summing the THG images for each direction of excitation linear polarization (0^0 - 180^0 in increments of 20^0). b) Pixel-by-pixel fitting of the biophysical model to the P-THG experimental data and rendering of the angle φ. The revealed radial architecture is recognized by the evolution of the colors. c) Mapping of the AR values probes the structural anisotropy between the shell and the core. d) The flip mirrors seen in Figure 1 are put in position such that the 1030 nm beam is reaching the granules. The achromatic λ/2 retarder used for P-THG was substituted by a zero-order λ/2 retarder at 1030nm (QWPO-1030-10-2, CVI Laser). The SHG is isolated using the 514/3 bandpass filter. A composite SHG image is obtained by summing the SHG images formed after rotation of the λ/2 waveplate (0^0 - 180^0 in increments of 20^0). e) Mapping of the angle φ using the biophysical model described in [12] reveals the radial architecture of the SHG active molecule. f) Mapping of the SHG anisotropy ratio. The experimentally retrieved

mean SHG anisotropy ratio value of ~4 probes amylopectin and not amylose as the SHG source in starch [12], in both the shell and the core of the granules. Scale bars 10 μm.

By comparing intensity THG with intensity SHG imaging, (Figure S1a with S1d, respectively), it is noted that the same granules exhibit different contrast, providing different but complementary information. The radial distribution of the starch molecules is clearly probed by both P-THG and P-SHG (Figure S1b and S1e, respectively), after fitting of the P-THG and P-SHG models and pixel-by-pixel rendering of the angles φ. In Figures S1c and S1f, the AR and the SHG anisotropy ratios are presented. Mapping of the AR values probes the structural anisotropy between the shell and the core. The mean value of SHG anisotropy ratio was 4.06, σ = 0.88, which is similar to the values measured for wheat starch SHG anisotropy ratio ~3.7 [12], indicating amylopectin and not amylose as the SHG source in starch [12]. In Figure S1f, it is noted that the SHG anisotropy ratio demonstrates similar values in both the shell and the core of the granules.

2. Arithmetic simulations of C and D parameters

In Figure S2, the arithmetic simulations of C parameter of Equation (6), ranging from 0.1 to 0.5 in steps of 0.1 and the arithmetic simulations of D parameter ranging from 0.1 to 0.5 in steps of 0.1, are presented. We note that the P-THG curves that better match the experimental data correspond to C = 0.3 and D = 0.5.

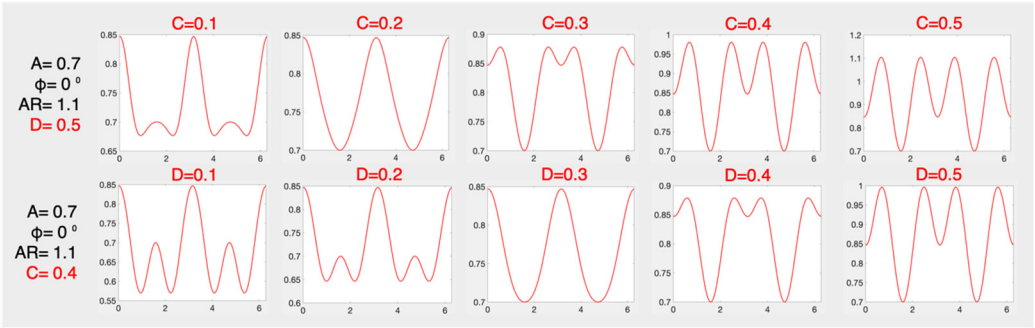


Figure S2: Arithmetic simulations of C and D parameters. The P-THG curves that better match the experimental data correspond to C = 0.3 and D=0.5.

3. Effect of the tilted-off plane molecules

In order to examine the effect of the tilted-off plane molecules in P-THG measurements, we performed P-THG at different z-planes of a granule. Because of the 3D radial molecular architecture, the z-planes outside the equator contain tilted-off plane molecules, with angles that depend on their distance from the equator, and the longer the distances from the equator, the bigger the tilted-off plane angles.

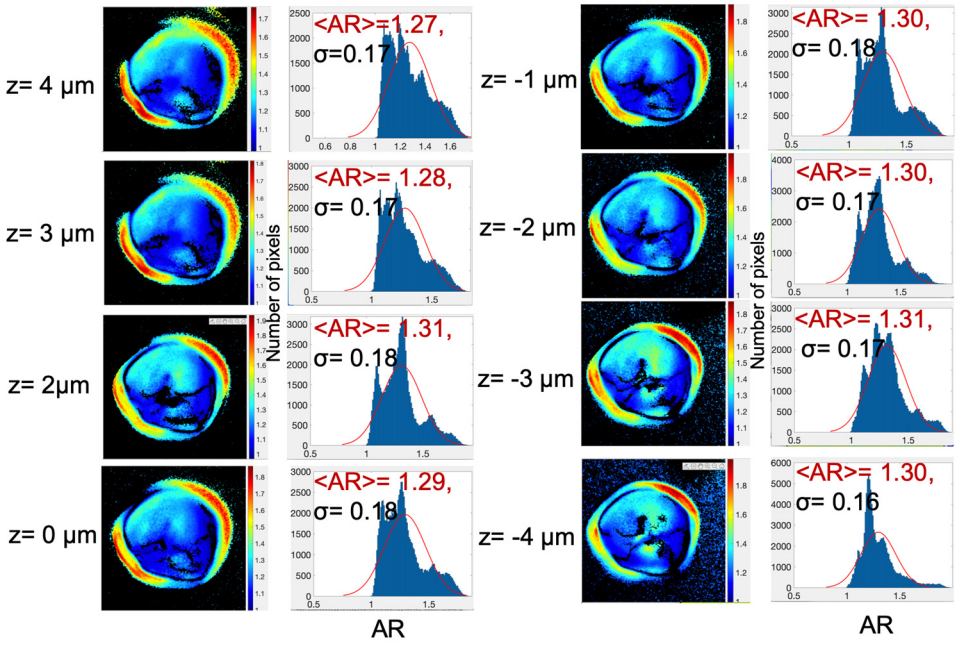


Figure S3: Pixel-wise maps of the AR between different z-planes.

From the experimental data of Figure S3, it is observed that the mean AR values slightly change between different z-planes. Nevertheless, the shell–core anisotropy contrast is present between different z-planes, because the tilted-off plane molecules are affecting the AR values of both the shell and the core of the granule.