Electronic Supplementary Material (ESI) for RSC Advances.

ESI for:

Tuning the aqueous self-assembly process of insulin by a hydrophobic additive

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Polarized light microscopy images

The images in this section were obtained during the study of the spherulite size distribution of the insulin-6T system. Furthermore, in Fig. S1 is shown images from the study of insulin-only spherulite growth. Photographs of sample solutions during spherulite growth obtained at different time points clearly show that in a typical insulin-only solution, spherulites are distributed more evenly throughout the sample (Fig. S1b-d). Moreover, after 12 hours, there is no readily apparent change as a function of time in the overall size distribution of spherulites, indicating that the system has reached equilibrium.

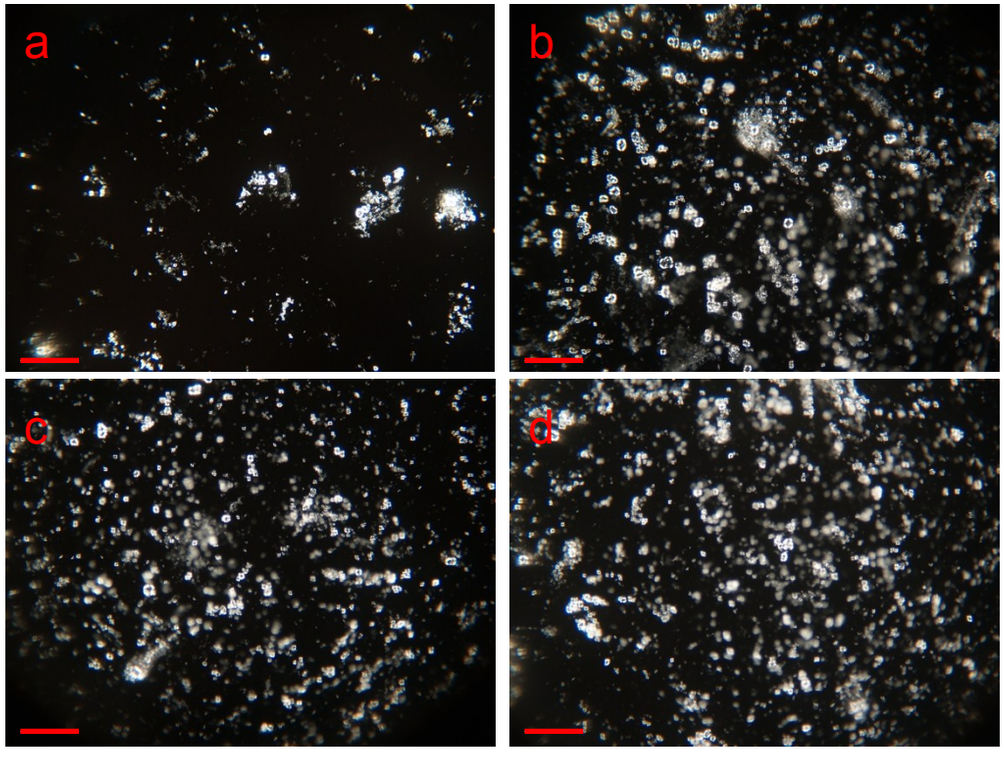

Fig. S1 Insulin-only solutions. Example images of reaction solutions. The scale bars represent 500µm (a) after 5 hours (b) after 12 hours. (c) after 22 hours (d) after 79 hours.

A typical insulin-6T solution will exhibit numerous and substantial clusters of spherulites thus resulting in a noticeable heterogenous distribution of spherulites throughout the sample (Fig. S2b).

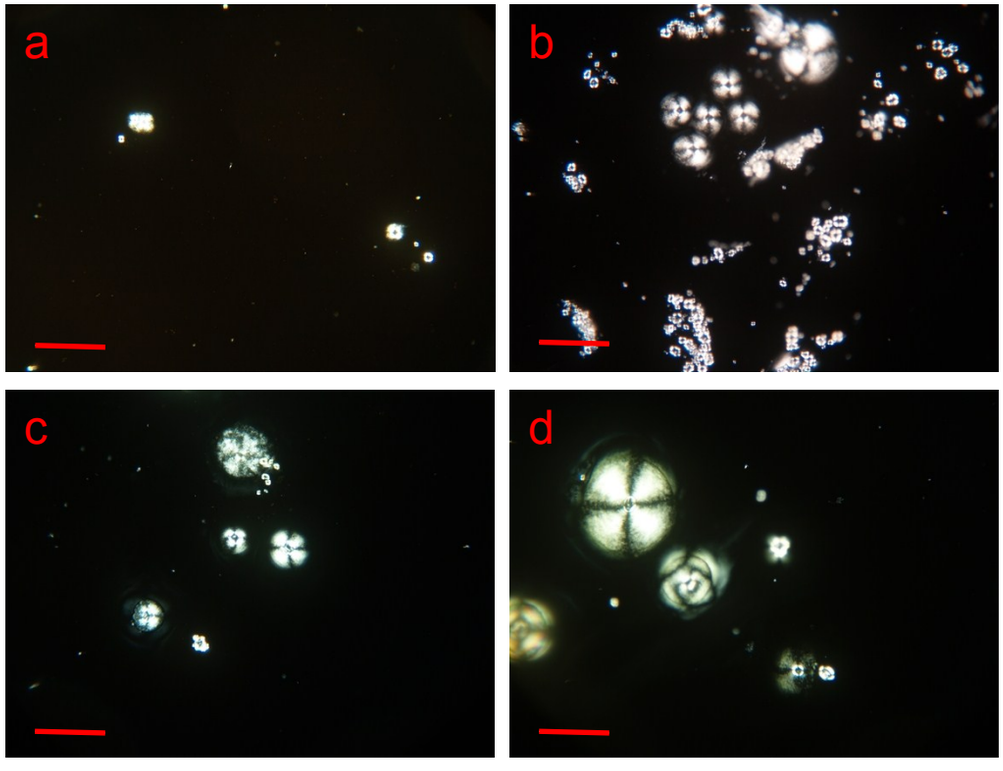

Fig. S2 Insulin-6T solutions. Example images of reaction solutions. The scale bars represent 500µm (a) after 5 hours (b) after 12 hours. (c) after 22 hours (d) after 79 hours.

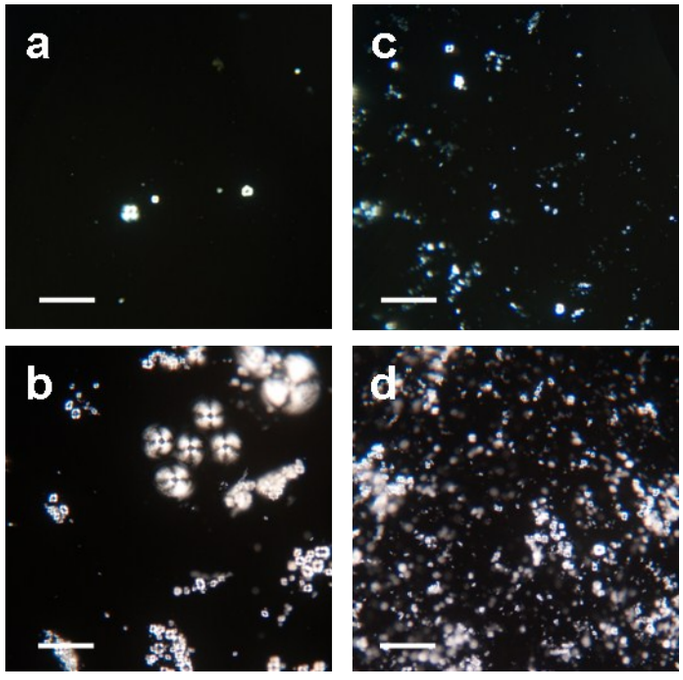

Fig. S3 Comparative images of insulin and 6T-containing solutions. The scale bars represent 500 μm. (a,b) Polarized light microscopy images of 6T-containing insulin spherulites after 5 hours (a) and 12 hours (b). (c,d) Polarized light microscopy images of insulin only insulin spherulites after 5 hours (c) and 12 hours (d).

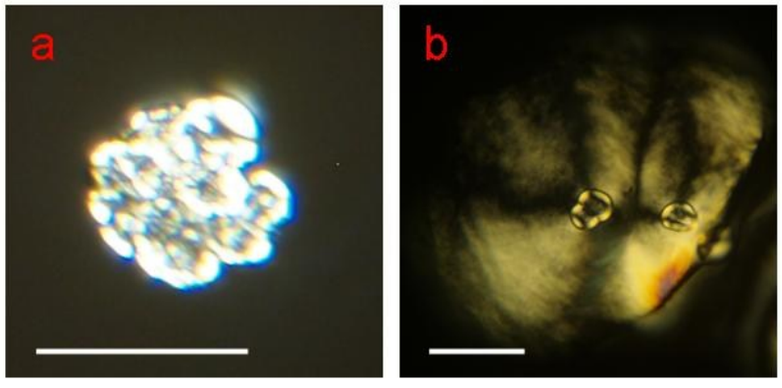

Fig. S4 Examples of merging spherulites. Polarized light microscopy images of sections of insulin-6T solutions after 46 hours. The white scalebars represent 300 μm (a) A typical merging cluster of smaller spherulite structures. (b) Merging in progress for large spherulites.

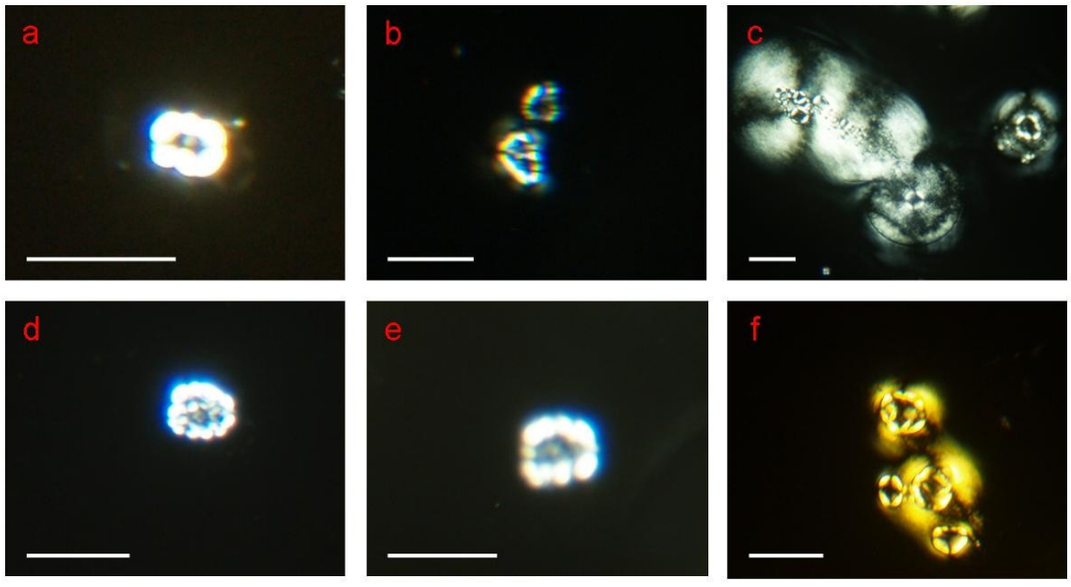

Fig. S5 Additional examples of merging spherulites. Polarized light microscopy images from sections of insulin-6T solutions. The white scalebars represent 200 μm. The images are from insulin-a6t solutions after at 12 (a,b), 22 (c-e) and 79 hours (f). Color differences are due to changes in the amount of light from the microscope lamp and exposure time adjustments made necessary for obtaining clear photographs.

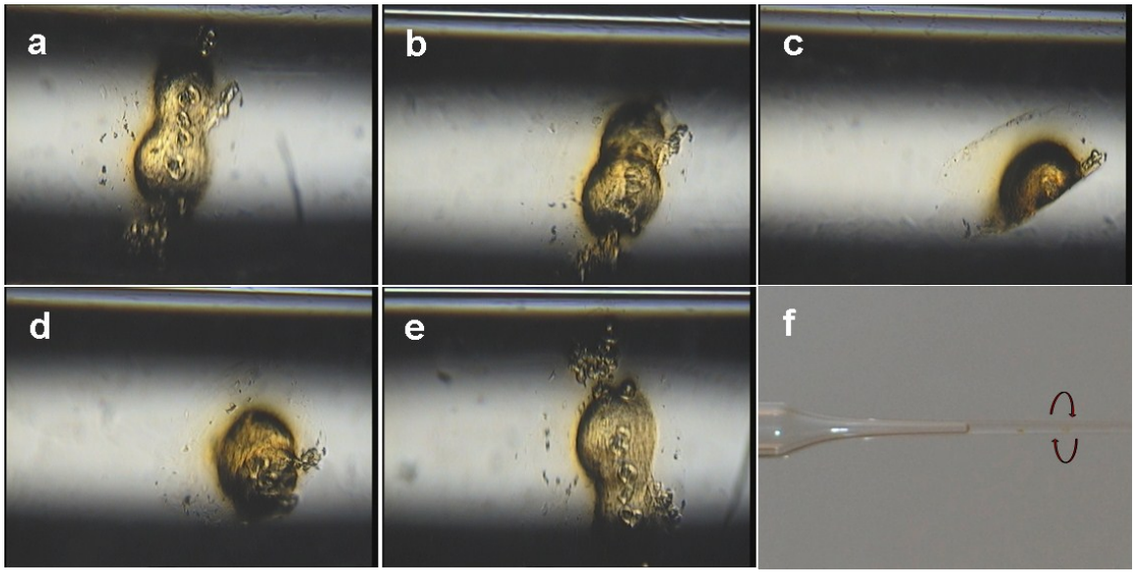

Fig. S6 The isolated structure of figure 2e-f viewed from different angles. The structure was sucked into a glass pipette that was subsequently rotated and photographed using a light microscope. (a) 0° (b) 45° (c) 90° (d) 135° (e) 180° (f) Photograph of the isolated structure in the glass pipette used to obtain figures S7 (a) – (e).

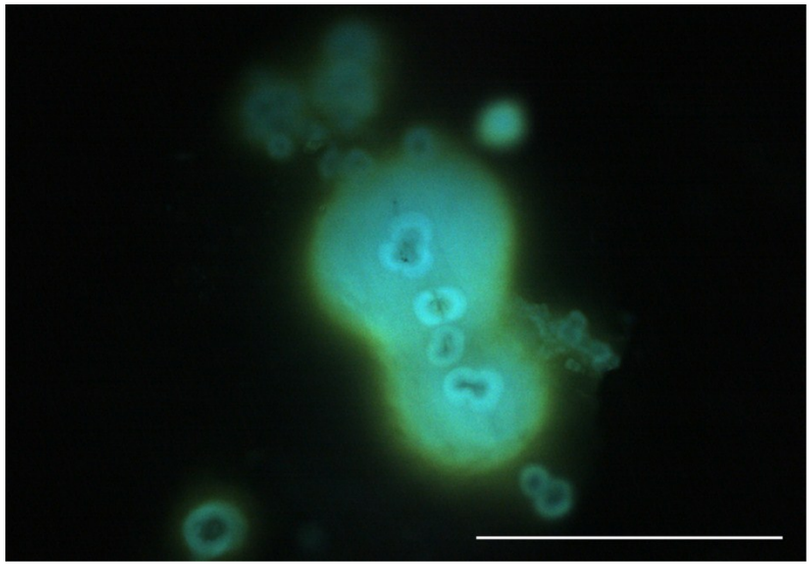

Fig. S7 The structure seen in Fig. S6 viewed with a fluorescence microscope using a 405nm excitation wavelength. The scale bar represents 500 μm.

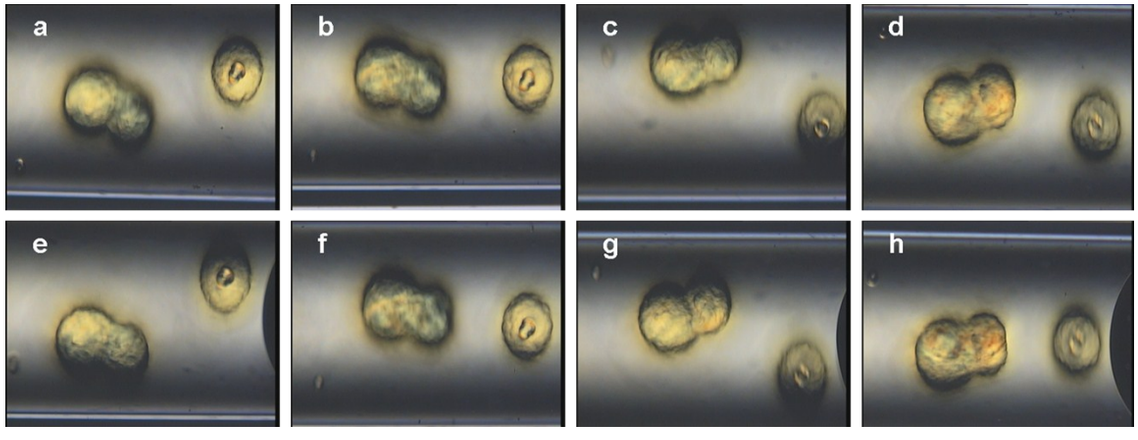

Fig. S8 An additional isolated merged structure viewed from different angles in an identical manner as that of the structure in Fig. S6. (a) 0° (b) ~45° (c) ~90° (d) ~135° (e) ~180° (f) ~225° (g) ~270° (h) ~315°

Determination of spherulite size distributions

In order to obtain further insight into the mechanism of spherulite formation we performed an experiment where we followed the size distribution of spherulites over time for the reaction of the 6T-insulin composite material. Due to difficulties in obtaining

reliable data from a single reaction-chamber (caused by solvent evaporation and spherulites obscuring one another), we opted for setting up 7 parallel reactions, and analyzing these one after the other at the appropriate time.

A stock solution was prepared, from which 100 µl samples were distributed into 7 vials, which were subsequently heated at 65 °C. The reaction was quenched at the appropriate time by transferring the entire content to a microscope slide, followed by analysis of the still wet sample by polarized light microscopy. In this way we could make an estimate of the size distribution of spherulites, as well as the number of spherulites at the time when the sample was quenched (Fig. S10a). Moreover, in Fig. S10b is also shown the size distribution of spherulites for reaction mixtures at 12, 22, and 79 hours. For practical reasons we only included spherulites with a diameter larger than 150 µm in the statistics. All of the statistics shown in Fig. S10a and S10b, are the result of 3 independent measurements. Each solution was photographed using a 4 times magnification lens and yielding, depending on the viscosity of the solution, up to 20 photos per solution to be analyzed for spherulite size and number of spherulites. Size estimation and exact counting of spherulites was at times (most commonly for spheres 150-300 µm in size and the first 12 hours) made challenging due to the presence of clusters of spherulites which could be interpreted either as a single larger unit or several smaller. In borderline cases several smaller units were favoured. Fortunately, the most difficult samples to analyze from the clustering point of view were also the samples with the highest number of spherulites, thus providing a sounder statistical basis for the mean size value estimate. However, considering ambiguousness due to clusters, intermediates and less than optimal focus and lighting for each and every spherulite, results should be considered to be a rough estimate of the size distribution of spherulites. We also performed a similar qualitative experiment with solutions containing native insulin (Fig. S1); however, these samples contained a too large number of spherulites for counting to be practical.

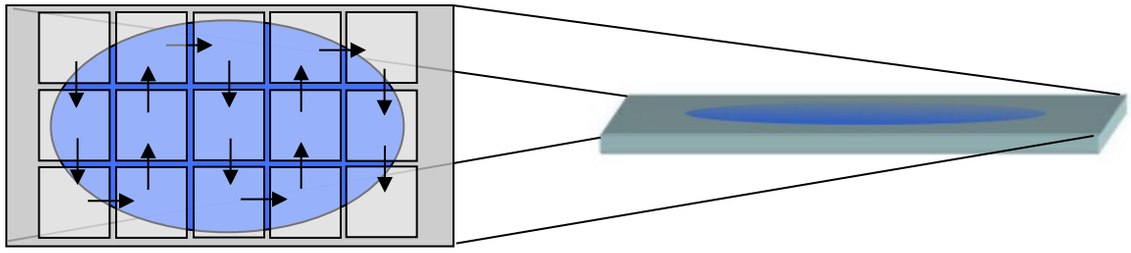

Fig. S9 Schematic drawing of the procedure for photographing sample solutions yielding the information in Fig. S10a and b. A microscope slide with sample solution on it was gradually moved and photographs taken according to the path indicated by arrows. A small gap was intentionally left between each area photographed so as to avoid overlapping images.

The data in Fig. S10a indicate that with increasing time, the number of spherulites increases up to 22 hours, after which the number of spherulites slowly decreases. In contrast, the average diameter of the spherulites continues to slowly increase throughout the reaction. However, with increasing diameter the growth slows down. The size distribution (Fig. S10b) after 12, 22, and 79 h is broad in all cases, but it is clear that it is gradually shifting towards larger diameters. Fig. S2 shows representative photographs of 6T-insulin solutions at different time points from the investigation described above . Furthermore, for comparison Fig. S1 shows representative photographs of insulin-only solutions at different time points.

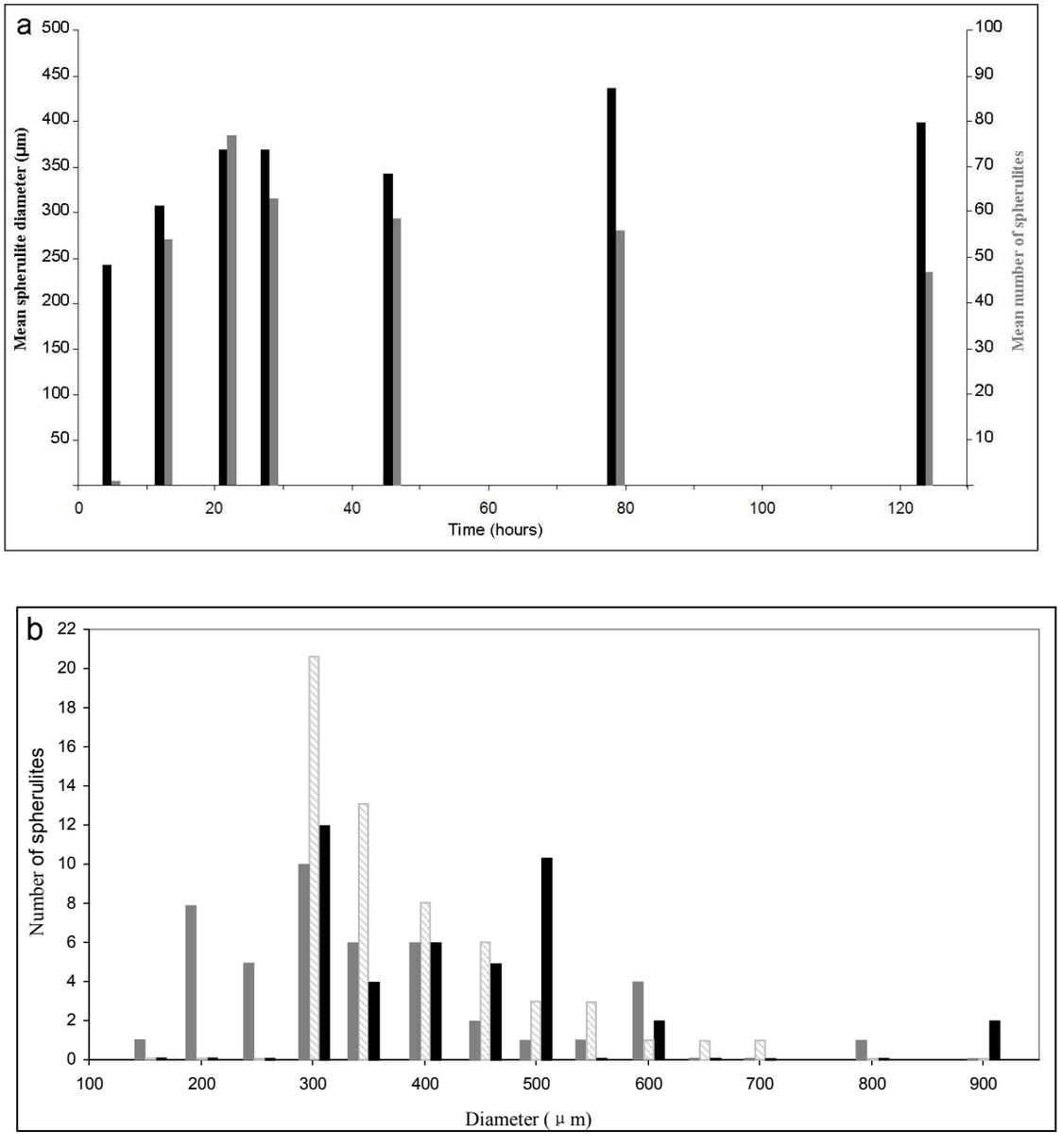

Figure S10 (a) Correlation between mean spherulite size and mean number of spherulites ≥ 150µm over time. The mean diameter of the spherulites at different time points are shown as black bars and the mean number of spherulites as grey. (b) Correlation between mean size and mean number of spherulites ≥ 150µm over time. In grey, striped light grey and black are shown the size distribution of spherulites in samples after 12, 22 and 46 hours, respectively.

Drying of spherulites and water content in spherulites

Upon drying the 6T-insulin spherulites shrink. If water is then added, the spherulite swells to roughly its former size. This process results in a loss of the Maltese cross pattern when investigated by polarized light microscopy. Drying thus seems to give rise to irreversible structural changes.

The weight change upon removing the water content from a sphere by drying was estimated to be about 50%.

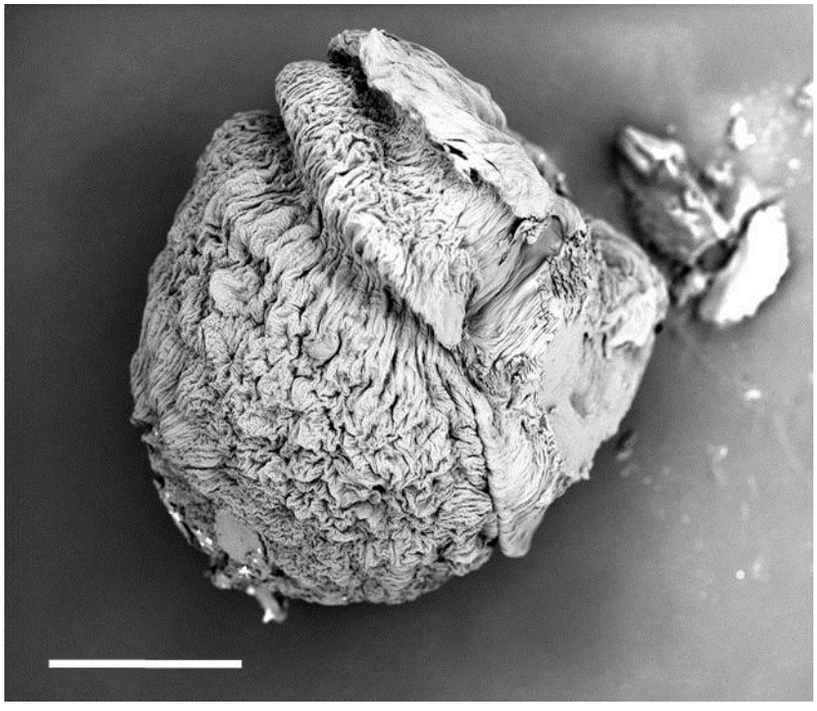

Fig. S11 SEM image of a sphere left to dry in open air. The white scale bar represents 140 μm. The smaller grooves on the surface of the dried sphere are due to the drying process. The two large grooves stems from compression of the spherulite during handling of the sample.