SUPPORTING INFORMATION FOR:

Shear-induced self-assembly of native silk proteins into fibrils studied by atomic force microscopy

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1. Analysis of the Heights of Single Fibroin Molecules and Fibroin Clusters

The height of single proteins and protein clusters was determined by taking the maximum of AFM topography cross-sections. This procedure was carried out for the same 91 single molecules and 85 clusters as the other analyses presented in the main manuscript (protein volumes etc.); the results are shown in Figure 1. Single molecules feature a relatively broad distribution around 0.6 nm, whereas clusters consisting of two or three subunits exhibit a narrower distribution centered around 1.3 nm. The reason for this is two-fold. First, not all of the single-molecule objects will contain a heavy chain protein. Some of them may just represent light-chain or P25 proteins. These two smaller proteins are expected to exhibit a smaller height. Second, the lateral extent of single molecules may be smaller than the effective tip–sample interaction area. Consequently, the apparent height is an effective average of areas containing the protein and areas without protein, thus representing the substrate with a topography level of zero (see Santos et al.,[a] Ref. 36 in the main manuscript).

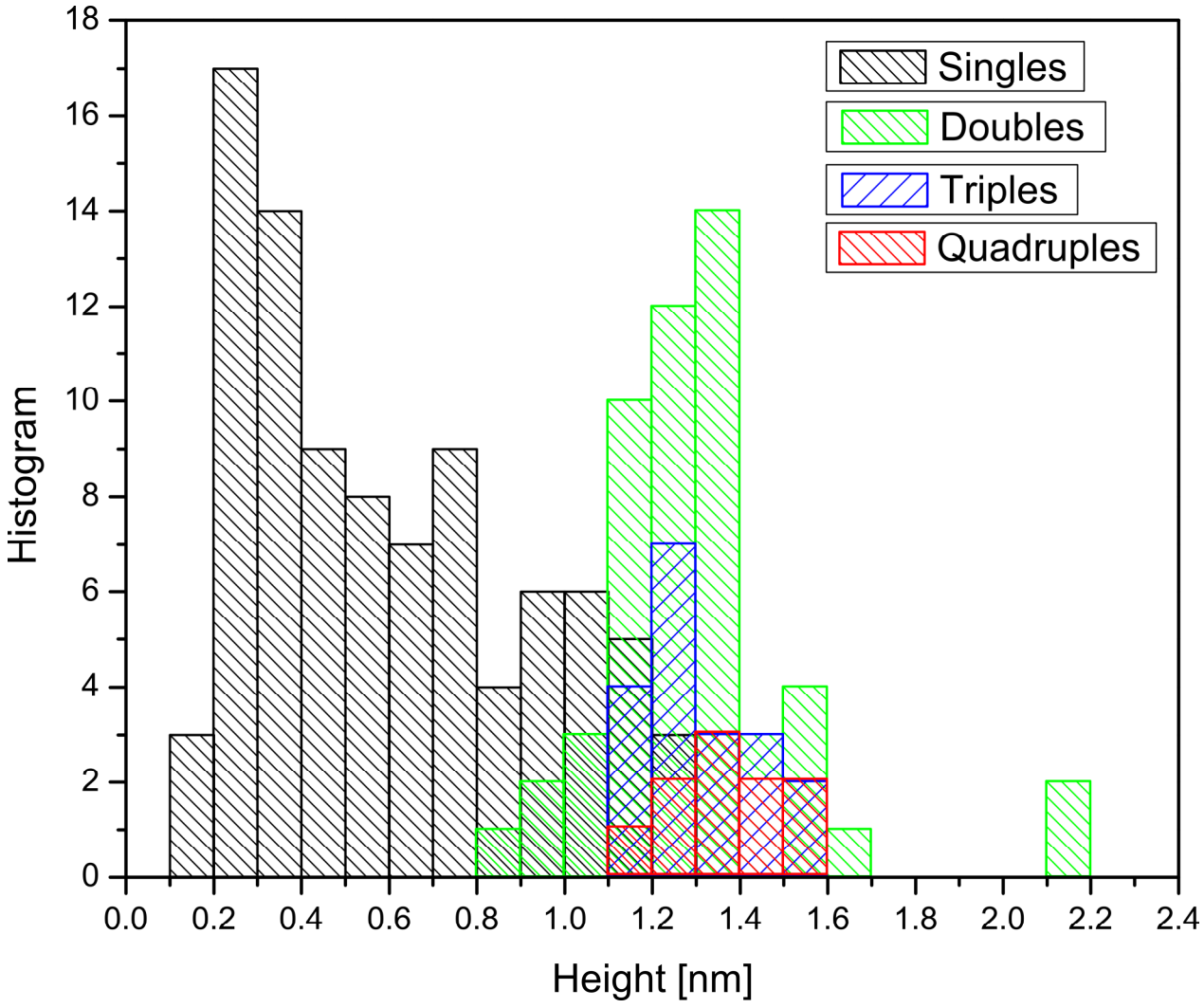

Figure 1. Histogram showing the heights of single protein molecules and protein clusters consisting of 2–4 subunits.

2. Alternate Protein Volume Analysis

As an alternate route to estimate protein volumes from AFM topography data we followed the technique proposed by Piesantra et al.[b] (Ref. 40 in the main manuscript). In this technique, a threshold is first placed at half the height of the objects. The volume above the threshold is directly taken from the AFM data and multiplied by two to estimate the volume of the entire object. This method provides a better model for the suspected spherical or cylindrical morphology of many biomolecules, which is not fully accessible via AFM. Moreover, it alleviates the problem of systematic broadening of structures due to the size of the tip, as the most significant part of this broadening occurs in the "bottom half" of the topography. We obtained protein volumes that were consistently at least a factor of two smaller (average: 315 nm^3) than the volumes obtained by our main method described in the manuscript (750 nm^3 on average).

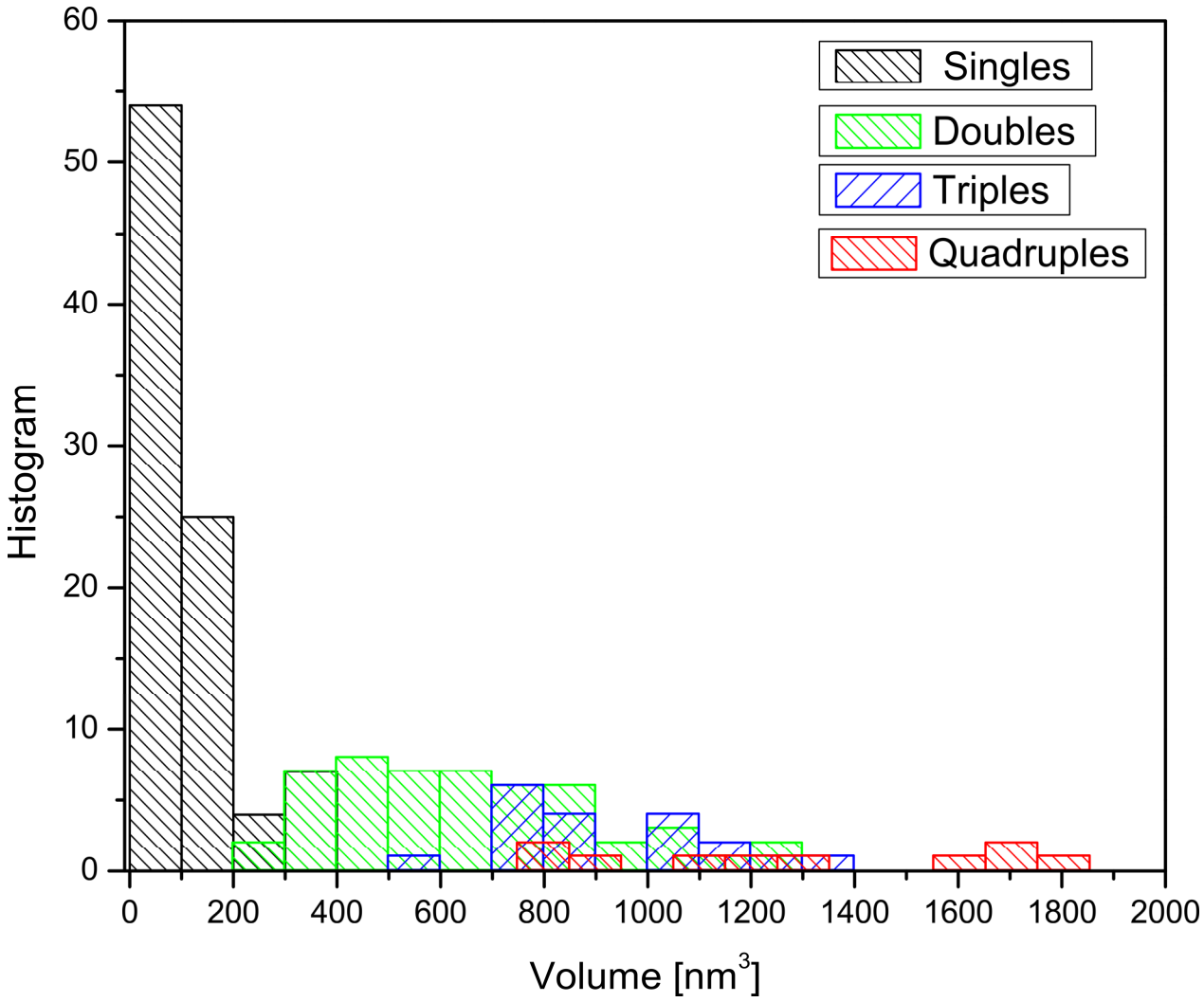

Figure 2. Volume analysis based on the model of Pietrasanta et al.

3. Orientation of Fibrils with Respect to Spinning Direction

The orientation of the "beads-on-a-string" type fibers observed after spin-coating the 1:10 fibroin solutions (shown in Figures 3 and 4 in the main manuscript and discussed in detail) is highly correlated with the radial direction of the sample. As shown in Figure 3, the direction of the fibers is perpendicular to the spinning axis of the sample during spin-coating. This further demonstrates that the observed protein self-assembly is shear-induced through spin-coating.

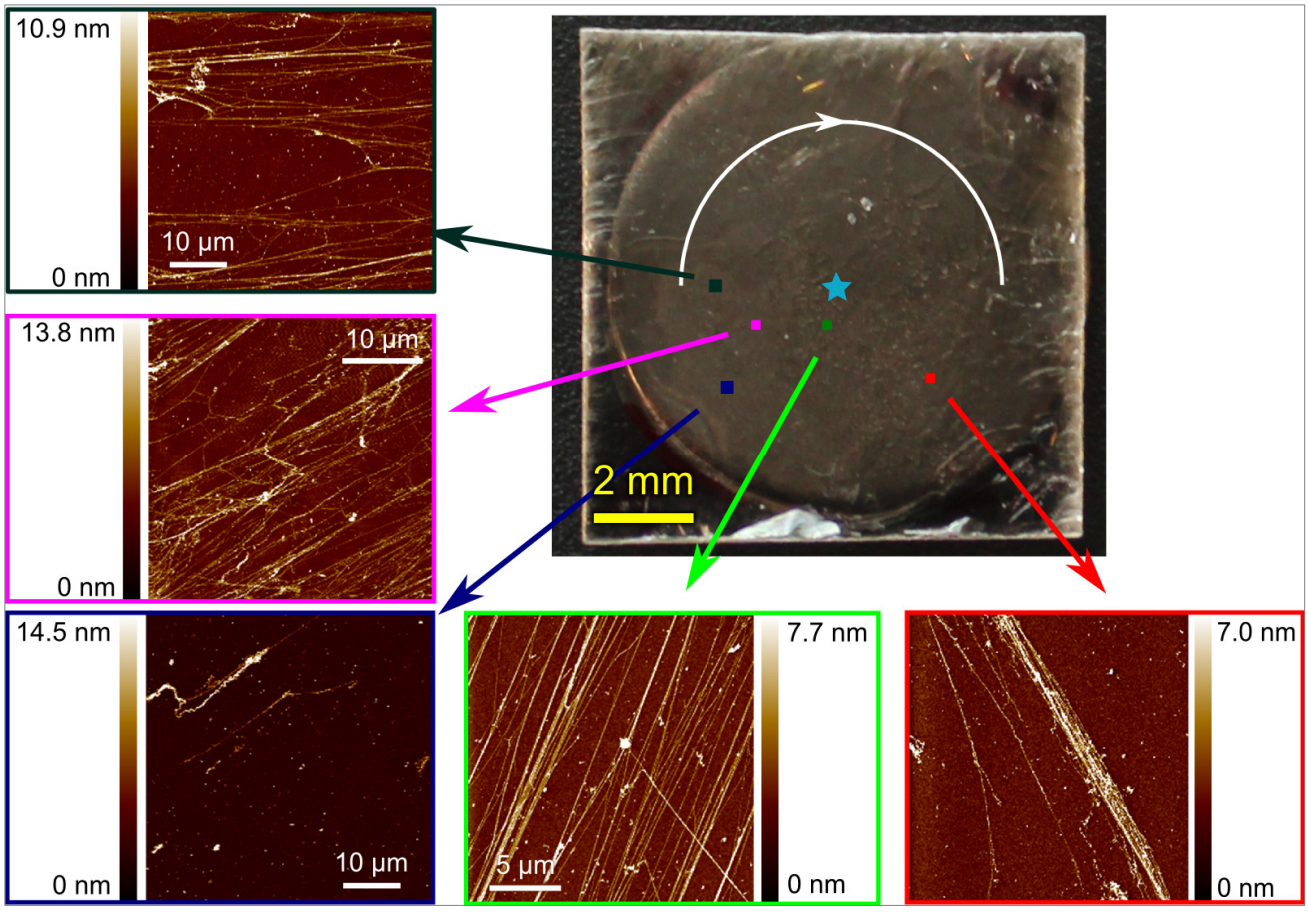

Figure 3. The large image in the top right corner shows a photograph of the sample from top. A square mica slide (about 12 × 12 mm) is mounted on a metallic puck (about 11 mm in diameter). The rotation axis of the spin coater is indicated by the light blue star. The five pictures grouped around the photograph are AFM topography scans of the sample taken at the indicated positions. All the fibrils visible in the AFM scans are perpendicular to the spinning axis, in radial direction.

a) Santos, S.; Barcons, V.; Christenson, H. K.; Font, J.; Thomson, N. H. PLoS ONE 2011, 6: e23821.

b) Pietrasanta, L. I.; Thrower, D.; Hsieh, W.; Rao, S.; Stemmann, O.; Lechner, J.; Carbon, J.; Hansma, H. Proc. Natl. Acad. Sci. USA 1999, 96, 3757–3762.