# Silk reconstitution disrupts fibroin self-assembly

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## SUPPORTING INFORMATION

**Non-sheared 1000 mg/L NSF**

Non-sheared 1000 mg/L NSF displayed a globular morphology only, with no evidence of the long, straight fibrils observed in the sheared 1000 mg/L NSF samples.
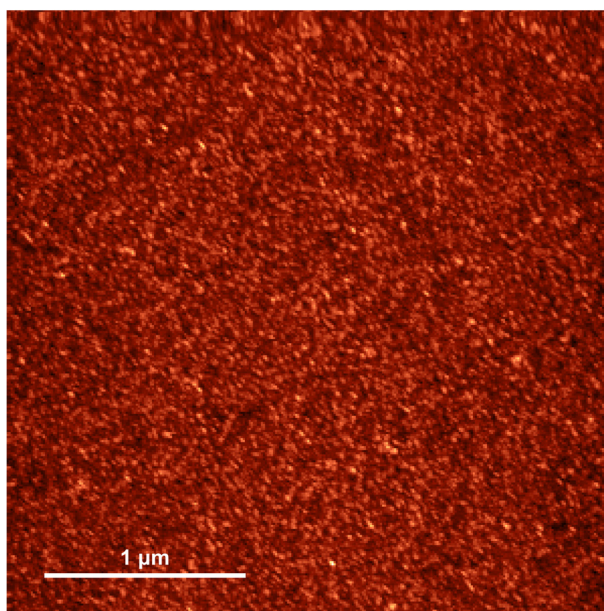


**Fig. S1.** Non-sheared 1000 mg/L NSF. Color range: 10 nm.
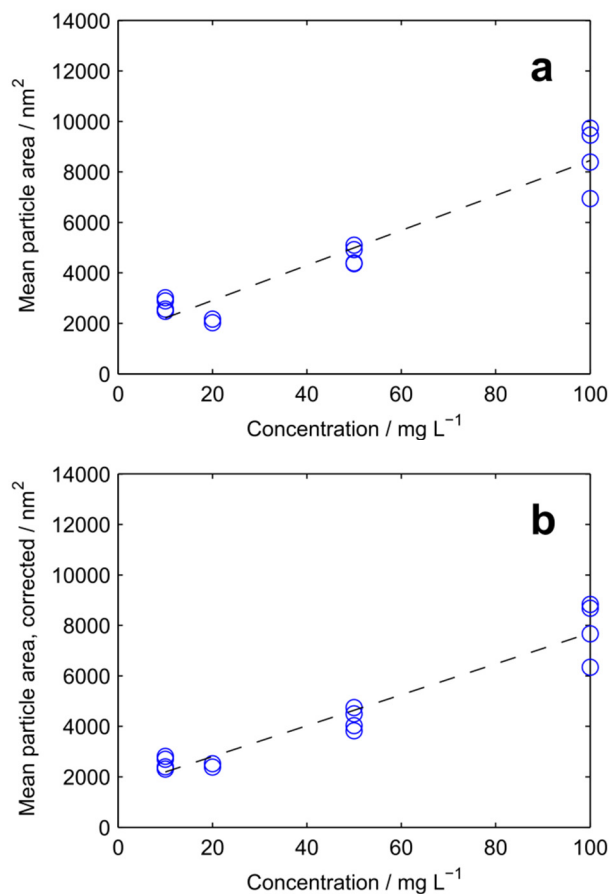
**Correction factors**



**Fig. S2.** Mean particle area of RSF assembly structures (a) before and (b) after correction according to fibril width. The black dotted line is the first-order line of best fit to the RSF data.

Due to differences in AFM probe diameter from experiment to experiment, the apparent fibril diameter changes accordingly. As a consequence of this, the measured footprint area of a particular assembly is expected to scale approximately linearly as a function of this apparent fibril diameter. To avoid that the measured footprint areas directly depend on the tip diameter, we applied a correction factor. We assumed that the fibril diameter is constant for a given fibroin concentration and consequently established a benchmark fibril diameter. For scans with larger apparent fibril diameters (allegedly due to tip size effects), we corrected the apparent aggregate areas to account for tip size-induced measurement errors (Fig. S2b). The corrected aggregate

areas (Fig. S2b) are not substantially different from the uncorrected ones (Fig. S2a). However, at low concentrations, the corrected areas provide slightly better agreement with the expected trend of increasing aggregate size as a function of fibroin concentration.

In order to prevent single pixels and other processing artifacts to be counted, a minimum cutoff was imposed on both particle area and skeletonized fibril length distributions. After applying the threshold, single pixels and other unrealistically small features occasionally resulted due to imperfect flattening and the binary nature of the threshold. Since all fibrils measured in low concentration scans were about 15 nm in width, all fibrils less than 15 nm in length and all particles less than $\pi \times (7.5 \text{ nm})^2 \approx 176 \text{ nm}^2$ in area were discarded.

## Consistency between scans

To demonstrate that our technique—AFM scanning of spin-coated fibroin—yields quantitative results, we analyzed consistency of fibril morphology across multiple scanning areas of the same sample, as well as across multiple samples of equivalent concentration. We therefore collected multiple scans from samples of the same concentration by moving our scanning area in 5 μm or 10 μm intervals along a radial axis (relative to the spin-coating center) of each sample. Upon inspection, these equivalent concentration scans appeared to retain a similar surface coverage and morphology, and as expected, scans from a different prep appeared similar as well (Fig. S3). We then conducted Kruskal–Wallis tests on the same-concentration fibril length samples to test the null hypothesis that the samples were drawn from the same distribution. Since the returned $p$-values were all greater than 0.13 (Table S1), we failed to reject the null hypothesis—which is noteworthy for such large sample sizes. Additionally, the close agreement in area fraction between scans of the same concentration across different preparations asserts the rigor and consistency of our experimental setup (Fig. S4).
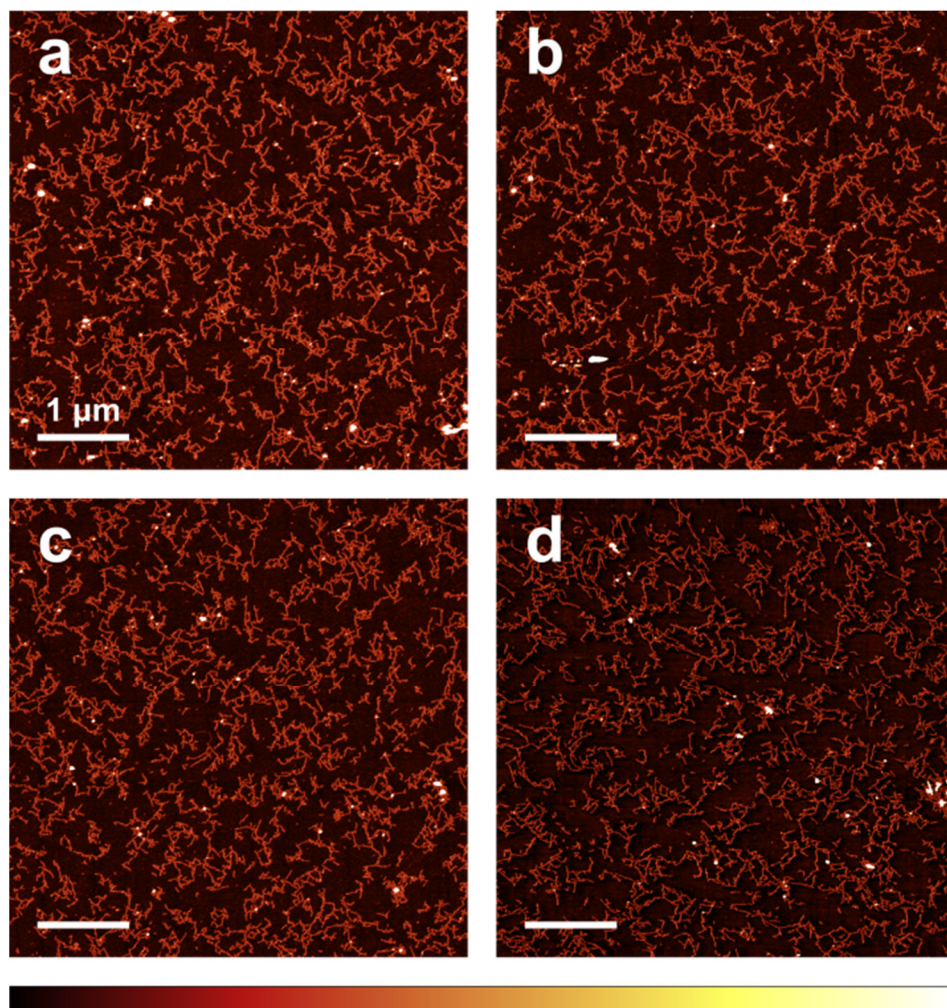
**Fig. S3.** Scans of sheared 100 mg/L RSF at three different locations on the same sample (a–c) and on a separate date, with a different sample and different tip (d). The consistency in fibril morphology between images supports our claim that our quantitative analysis results are robust and representative. Scale bars: 1 μm. Color bar: 2 nm.

## Measures of effect size

Non-parametric measures of effect size (MES) were preferred over a standard analytical null hypothesis significance test (NHST), such as a $t$-test, because of the non-normality of the distributions (Fig. 3b), the large difference in sample size (for particle area, $N_{RSF} = 655$ and $N_{NSF} = 51$), and the susceptibility of NHST to misinterpretation (which is especially pernicious when testing large samples).[1,2] We employed two MES that do not require normally distributed data sets, Cohen's $U_3$ and the area under the receiver operating curve (AUROC). Cohen's U3 gives

the proportion of data in the first set that falls below the median of the second set, while the AUROC measures how well two distributions can be separated by a single delineation value.[1] Both of these tests therefore measure the overlap between two distributions, with a result of 0.5 returned in cases of perfect overlap and a result of 1 indicating that the population underlying the second tested distribution is greater than the first underlying population. Confidence intervals were calculated using 10,000 bootstrap iterations: in each iteration, new samples were created by randomly resampling from the existing samples with replacement, and the MES was conducted on the new samples.[1]

| Distribution tested | Number of distributions tested | Mean number of fibrils per distribution | $p$-value |
|---|---|---|---|
| 10 mg/L RSF | 4 | 297 | 0.14 |
| 10 mg/L SF | 2 | 284 | 0.16 |
| 20 mg/L RSF | 2 | 653 | 0.86 |
| 50 mg/L RSF | 4 | 1098 | 0.42 |
| 100 mg/L RSF | 4 | 3357 | 0.22 |
| 200 mg/L RSF | 4 | 10,575 | 0.14 |

**Table S1.** Kruskal–Wallis tests of fibril branch lengths for each concentration, where the tested null hypothesis is that the branch length distributions from separate scanning areas were sampled from the same distribution.
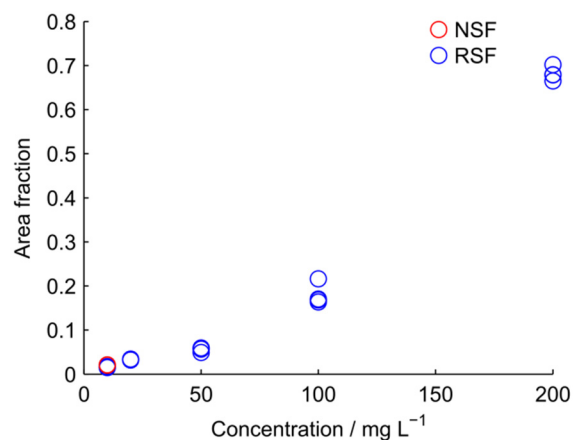
**Fig. S4.** Area fraction of fibrillar coverage in scans of RSF (blue) and SF (red) assembly. To obtain an area fraction, images were first thresholded using a consistent standard relative to each scan's histogram. Each area fraction was corrected by the scan's fibril width.

## Particle area means and medians

The mean particle area in RSF scans increased relative to concentration, while median particle area exhibited greater fluctuations. The increase in mean is attributed to the greater prevalence of outliers (i.e. larger particles) in the higher concentration scans.
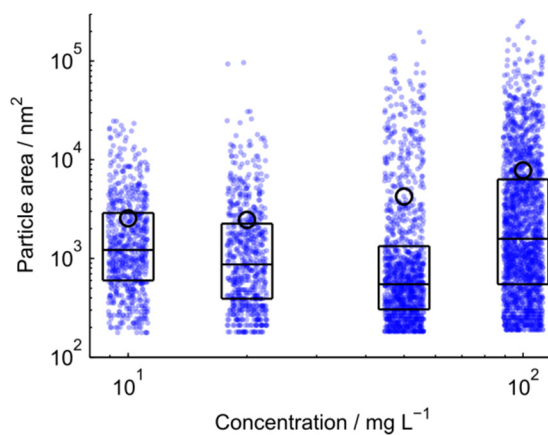


**Fig. S5.** Sizes of particles in 10 mg/L–100 mg/L RSF scans, with the *x* and *y* axes log-scaled. Each blue data point represents a single particle area (randomly *x*-displaced from its concentration for ease of viewing), each black circle is the mean of the concentration, and each box represents the first quartile, median, and third quartile of the concentration.

# References

(1)     Hentschke, H.; Stüttgen, M. C. *Eur. J. Neurosci.* **2011**, *34*, 1887–1894.

(2)     Anderson, D.; Burnham, K.; Thompson, W. *J. Wildl. Manage.* **2000**, *64*, 912–923.