Supplementary Information

Biomimetic spinning of artificial spider silk from a chimeric minispidroin

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Supplementary results

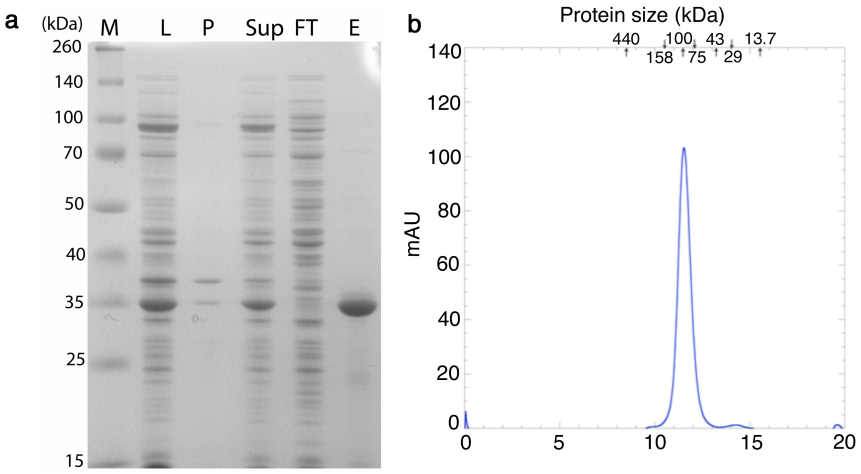
Supplementary Table 1. Estimated fraction of the different secondary structures (mean value ± standard error)

Secondary structure	NT2RepCT in solution	NT2RepCT fibers	*N. inaurata* fibers
Number of analyzed spectra	4	4	1
β-sheet (1624 cm-1 component)	25 ± 2 %	60 ± 2 %	47 %
Random coil and α-helix (1644 and 1663 cm-1)	74 ± 6 %	40 ± 2 %	38 %
β-turn (1682 cm-1)	1 ± 0 %	0 ± 0 %	16 %

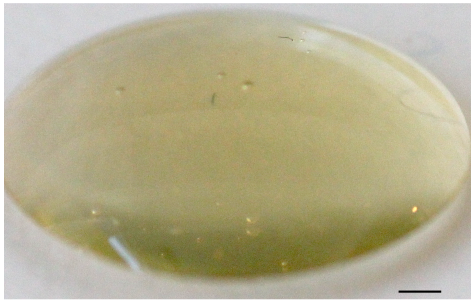
Supplementary Table 2. Comparison of diameter and mechanical properties of native dragline silk from *A. trifasciata* and different as-spun synthetic spider silk fibers. Only as-spun fibers that have been obtained by wet spinning and tested for tensile properties are included in the table. Mean value ± standard error. Mechanical properties of NT2RepCT were calculated from stress-strain curves of eight individual NT2RepCT fibers (**Supplementary Figure 7)**

	Native dragline silk *A. trifasciata* Ref[33]	NT2RepCT	eADF3 (AQ)12NR3 Ref[13]	Synthetic MaSp1 and MaSp2 Ref[4]	Flag/ MaSp2 A1S820 Ref[10]	MaSp2 1E Ref[8]	Flag GF Ref[6]
Diameter (μm)	≈3	12 ± 2	39 ± 6	61 ± 2	32 ±16	106 ± 5	37 ± 1
Extensibility (%)	17 ± 0.04	37 ± 5	7 ± 2	1.1±0.3	3.7 ±1	0.8 ± 0.3	1.1 ± 0.9
Strength (MPa)	890 ±130	162 ±8	54 ±16	33 ±7	28 ±17	14 ± 4	19 ± 5
Toughness (MJ m-3)	100 ± 40	45 ± 7	2 ± 0.8	0.2 ± 0.1	0.5 ± 0.3	0.06 ±0.03	0.12 ±0.11
Young's modulus (GPa)	11.6 ±0.7	6 ±0.8	2 ± 0.9	≈3	0.8 ±0.5	1.7 ± 0.4	n.s.

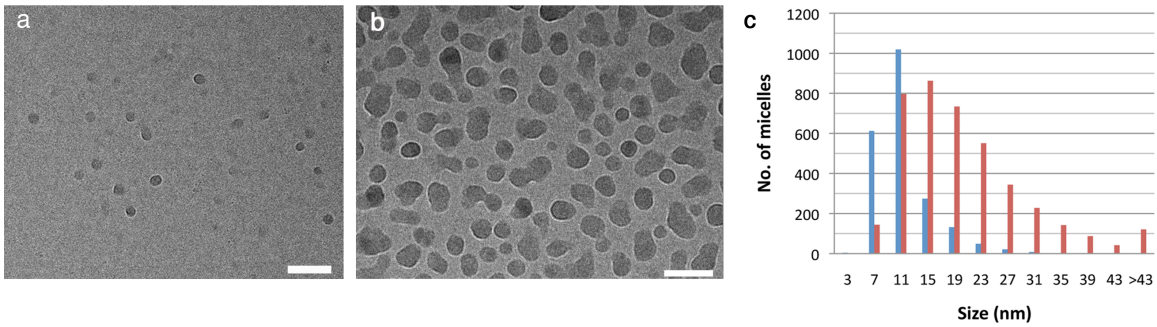
n.s.: not specified



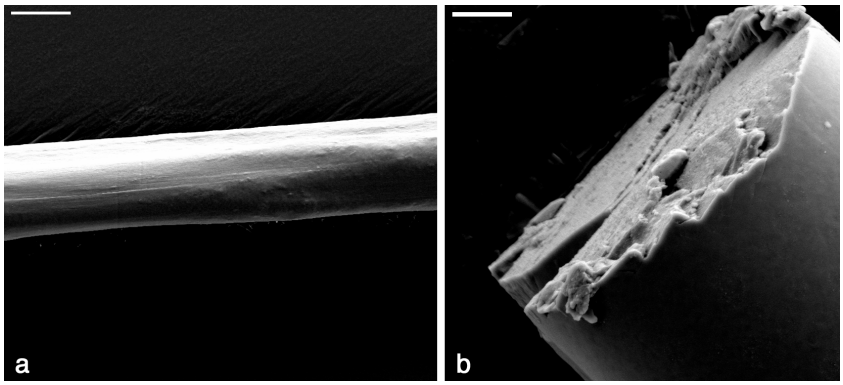
Supplementary Figure 1. (a) SDS-PAGE of different steps during NT2RepCT purification. M = Spectra Broadrange protein marker (sizes in kilodalton is shown to the left), L = total cell lysate, P = pellet, Sup = supernatant after centrifugation of whole cell lysate, FT = flow through Ni-NTA column, E = NT2RepCT eluted from the Ni-NTA column. (b) Size exclusion chromatography of NT2RepCT. The elution positions and molecular masses of calibrant proteins are indicted above the chromatogram.



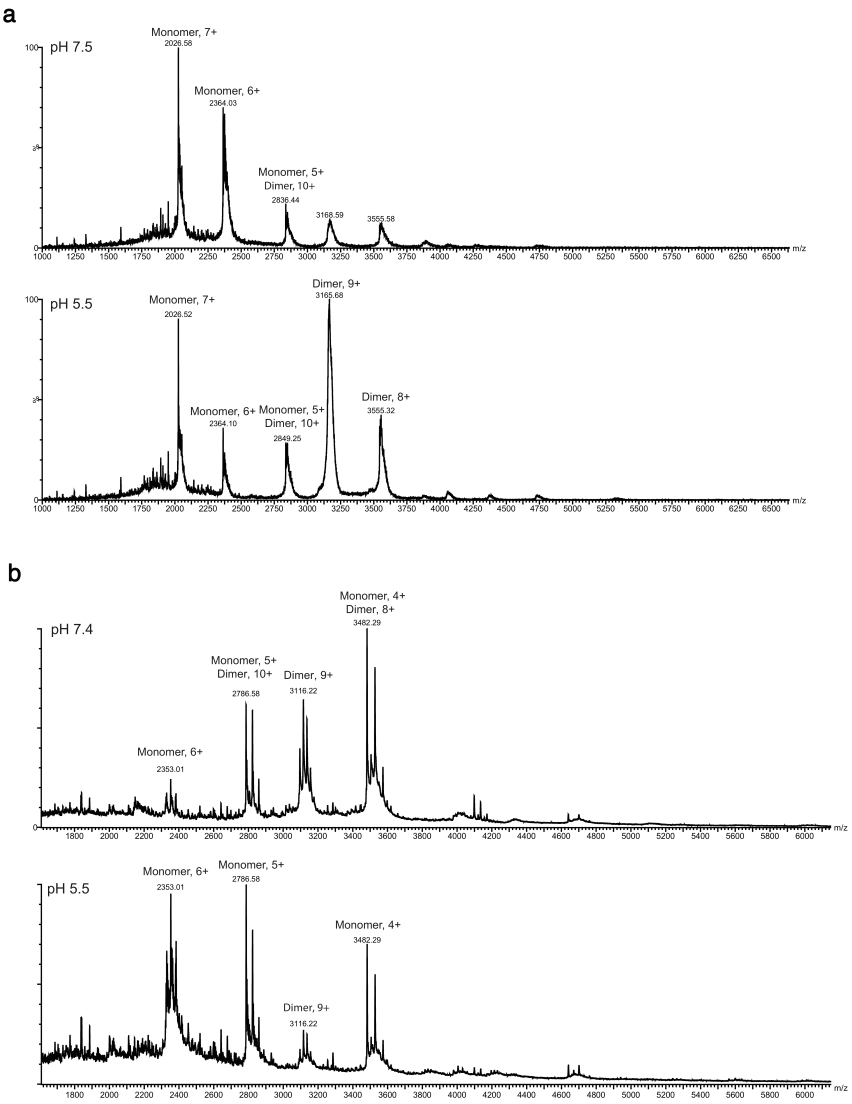
Supplementary Figure 2. Photograph of NT2RepCT at 300 mg/ml. Scale bar 0.1 cm.



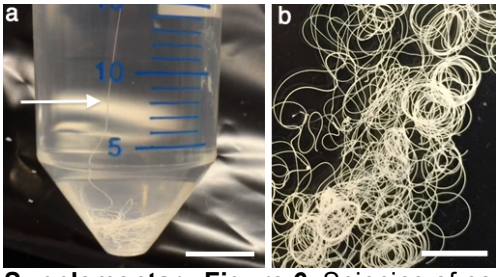
Supplementary Figure 3. (a) Cryo-electron micrograph of un-sheared NT2RepCT at pH 8.0. (b) Cryo-electron micrograph of sheared NT2RepCT at pH 7.5. (c) Size distribution of NT2RepCT micelles before (blue bars) and after (red bars) shearing. Scale bars 50 nm.



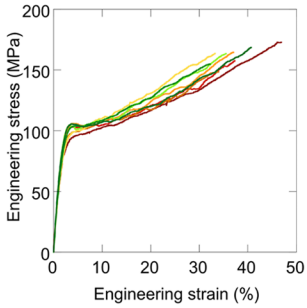
Supplementary Figure 4. Scanning electron micrographs of NT2RepCT fibers. (a) As-spun fiber. (b) Fracture surface of a fiber stretched until failure to examine the fiber interior core. Scale bars (a) 10 μm (b) 2 μm.



Supplementary Figure 5. (a) NT dimerizes at low pH. (b) Low pH induces dimer destabilization of CT and shifts the protein towards higher charge states, with similar kinetics as observed for NT2RepCT aggregation, see main Figure 2.



Supplementary Figure 6. Spinning of non-spidroin protein (bovine serum albumin, BSA) into a methanol coagulation bath results in continuous fibers that can be collected in (a) the bottom of a Falcon tube and (b) can be poured into a Petri dish without breaking. Scale bars 1 cm.



Supplementary Figure 7. Engineering stress/strain curves for eight separate NT2RepCT fibers. Mechanical properties calculated from these curves, and fiber diameters, are given in **Supplementary Table 2**.

Supplementary Movie 1. Spinning NT2RepCT in a biomimetic spinning device. Fibers form instantaneously as the highly concentrated spinning dope hits the pH 5.0 aqueous buffer.

Supplementary Movie 2. As-spun NT2RepCT fibers can be rolled up in a dry state on a frame.