Supplementary Information (SI) for Journal of Materials Chemistry B.

Recombinant silk protein condensates show widely different properties depending on sample background

Supplementary information

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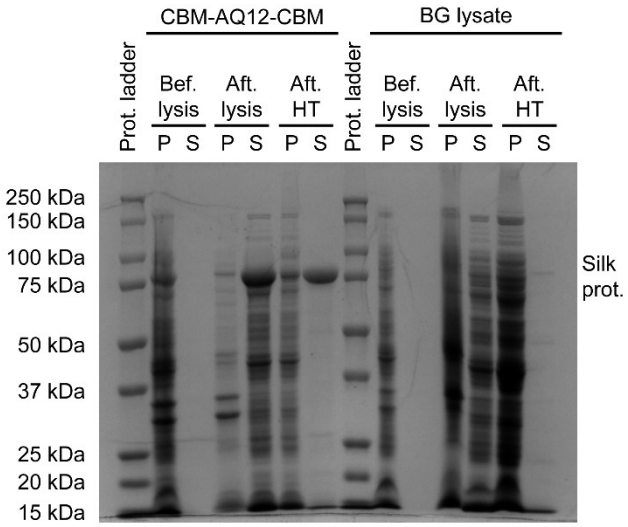
Data availability: Datasets for micropipette aspiration of IMAC silk and coalescence studies of HT silk, IMAC silk+BG lysate and IMAC silk are available on zenodo.org: DOI: 10.5281/zenodo.12529262. A representative video for the micropipette aspiration of IMAC silk is available as Supplementary Video S1. Representative videos for the coalescence of each, HT silk, IMAC silk+BG lysate, and IMAC silk condensates are available as Supplementary Videos S2–S4.

CBM-AQ12-CBM, 85.2 kDa

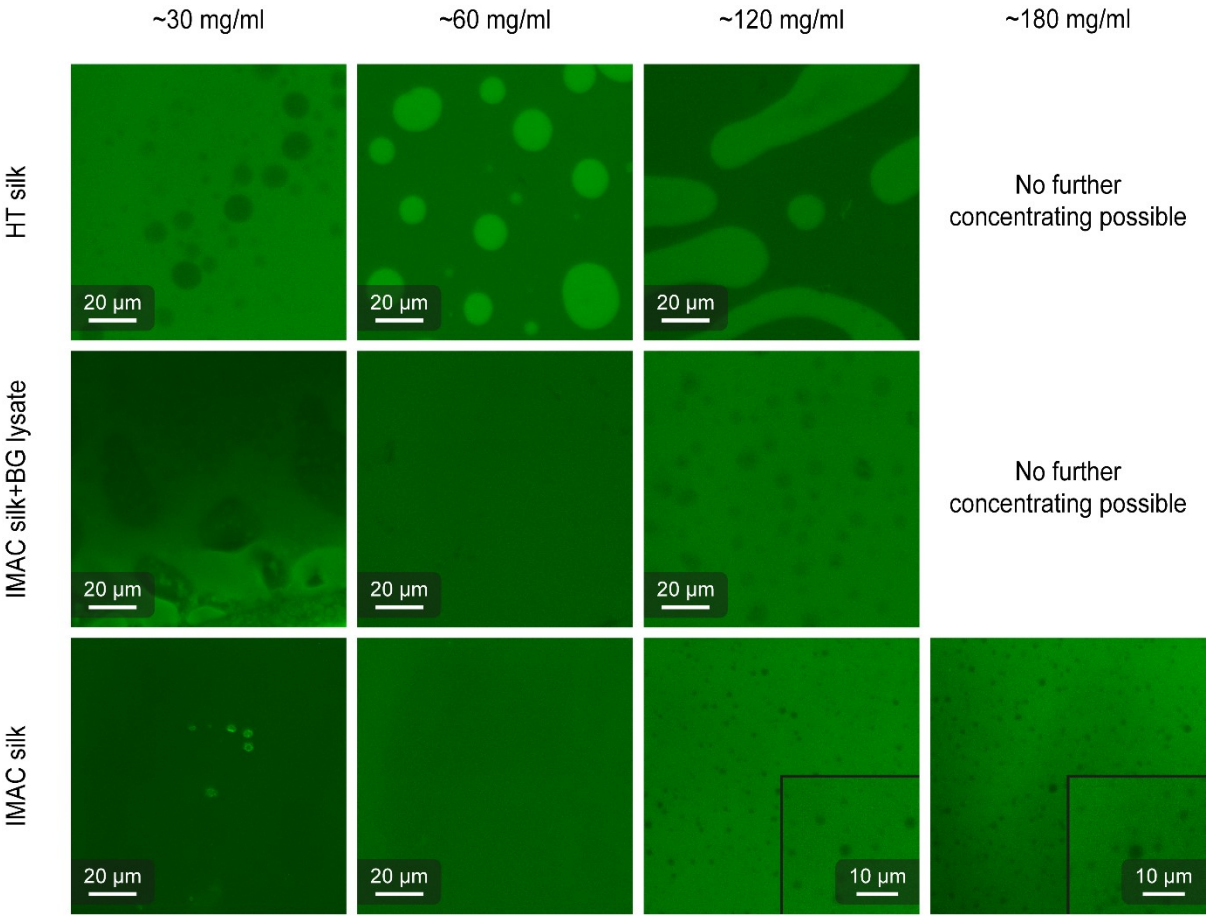
Terminal block (CBM)-*Linker*-Midblock (AQ12)-*Linker*-Terminal block (CBM)-*His-tag*

MGNLKVEFYNSNPSDTTNSINPQFKVTNTGSSAIDLSKLTLRYYYTVDGQKDQTFWCDHAA
IIGSNGSYNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNY
TQSNDYSFKSASQFVEWDQVTAYLNGVLVWGKEP*SASASASAGASAAASAGAGAGA*GPY
GPGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAG
GYGPGSGQQGPGQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGGYGPGSGQQGP
GQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPG
QQGPGQQGPYGPGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQQGPYG
PGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGG
YGPGSGQQGPGQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGGYGPGSGQQGPG
QQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQ
QGPGQQGPYGPGASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQQGPYGP
GASAAAAAAGGYGPGSGQQGPGQQGPGQQGPGQQGPGQQGPYGPGASAAAAAAGGY
GPGSGQQGPGQQGPGQQGPGQQGPGQQ*ASASASAAASAASTVANSSS*NLKVEFYNSNP
SDTTNSINPQFKVTNTGSSAIDLSKLTLRYYYTVDGQKDQTFWCDHAAIIGSNGSYNGITSN
VKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDYSFKSAS
QFVEWDQVTAYLNGVLVWGKE*LEHHHHHH*

Supplementary Figure S1: Protein Sequence for CBM–AQ12–CBM.



Supplementary Figure S2: SDS-PAGE of CBM–AQ12–CBM and BG lysate for Pellet (P) and Supernatant (S) before cell lysis, after cell lysis and after heat treatment (HT). Before lysis the silk protein (85 kDa) is present in the pellet. After lysis and after HT the silk protein is present in the supernatant. After HT some faint bands indicating other heat stable proteins are visible. No band indicating the presence of the silk protein is visible in the BG lysate.



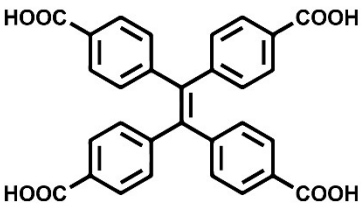
Supplementary Figure S3: Fluorescence microscopy images with free eGFP of concentrated samples of HT silk, IMAC silk and IMAC silk+BG lysate. Free eGFP partitions into the dilute phase. Samples with ~30, 60, 120 and 180 mg/ml were imaged. HT silk forms condensates at 30 mg/ml and

inverted phase can be observed at 60 and 120 mg/ml. IMAC silk+BG lysate forms condensates at 30 mg/ml and based on the partitioning of protein aggregates (into the dense phase) inverted phase can be observed at 60 and 120 mg/ml. However, condensate formation seems to be influenced by the addition of free eGFP. IMAC silk forms condensates at 120 and 180 mg/ml. Inverted phase was not observed.

Supplementary Table S1: Overview of the values for the micropipette aspiration of the IMAC condensates. Radius of the pipette R_p, radius of the condensate R_0, applied pressure ΔP, critical pressure ΔP_c, aspiration rate $\dot{L}_a$, retraction rate $\dot{L}_r$, surface tension γ, bulk viscosity η. n.d. = could not be determined.

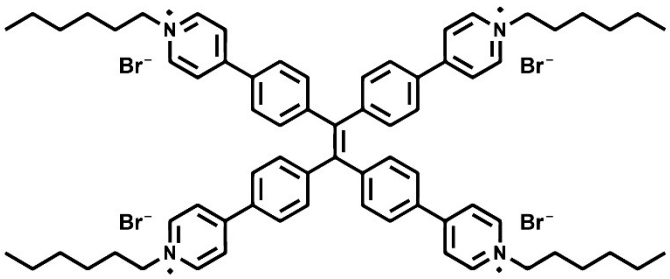
Number	R_p (µm)	R_0 (µm)	ΔP (Pa)	ΔP_c (Pa)	$\dot{L}_a$ (µm/s)	$\dot{L}_r$ (µm/s)	γ (µN/m)	η (Pa s)
1	9.3	46.6	20.0	9	1.9	n.d.	51.9	5.7
2	9.3	54.4	20.0	7	1.3	n.d.	39.0	9.8
3	9.3	45.9	20.0	10.3	1.8	1.9	59.5	5.3
4	9.3	57.1	20.0	9.7	1.8	1.8	53.8	5.5
5	9.3	130.8	20.0	10.0	4.2	4.2	50.0	2.3
6	9.3	70.7	20.0	11.0	3.3	4.0	58.4	2.7
7	9.3	42.4	20.0	7.0	2.2	n.d.	41.4	5.8
8	9.3	126.3	20.0	9.1	1.8	n.d.	45.7	6.1
9	10.80	46.5	20.0	10	2.0	2.0	70.2	5.7

a



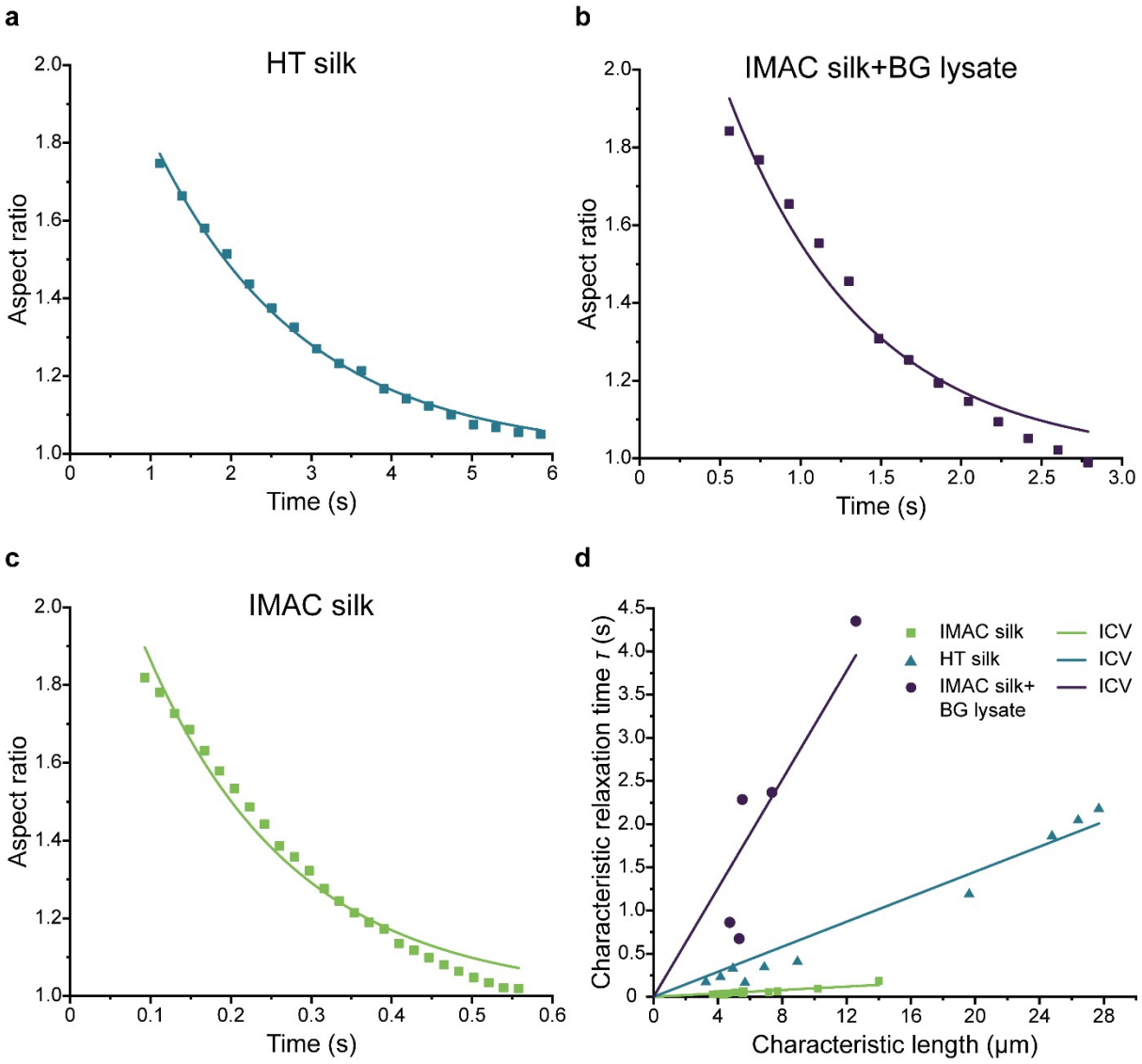
TPE4COOH

b

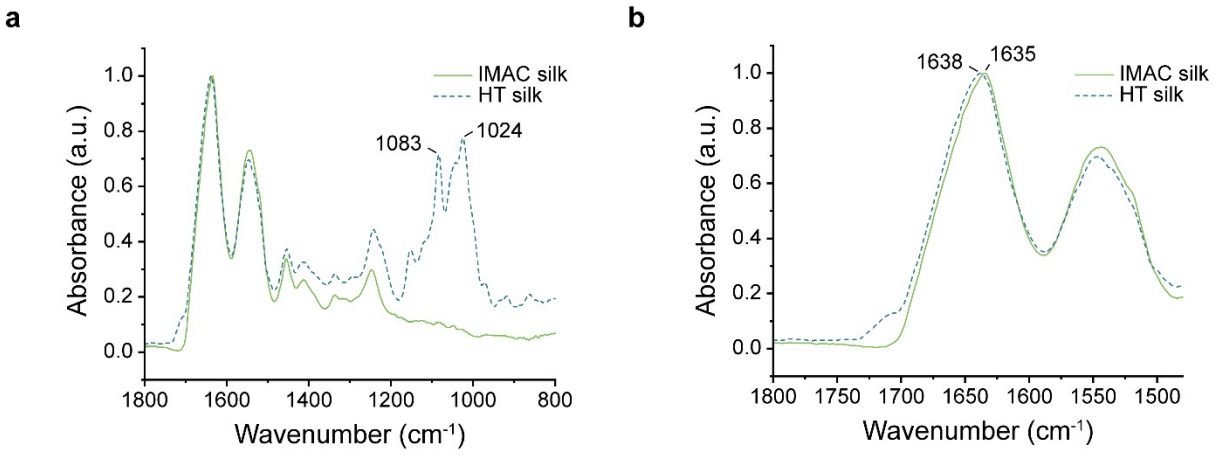


TPE4PH

Supplementary Figure S4: AIE structures a) 4,4',4'',4'''-(Ethene-1,1,2,2-tetrayl)tetrabenzoic acid (TPE4COOH). **b)** 4,4',4'',4'''-(ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(1-hexylpyridin-1-ium) bromine (TPE4PH).

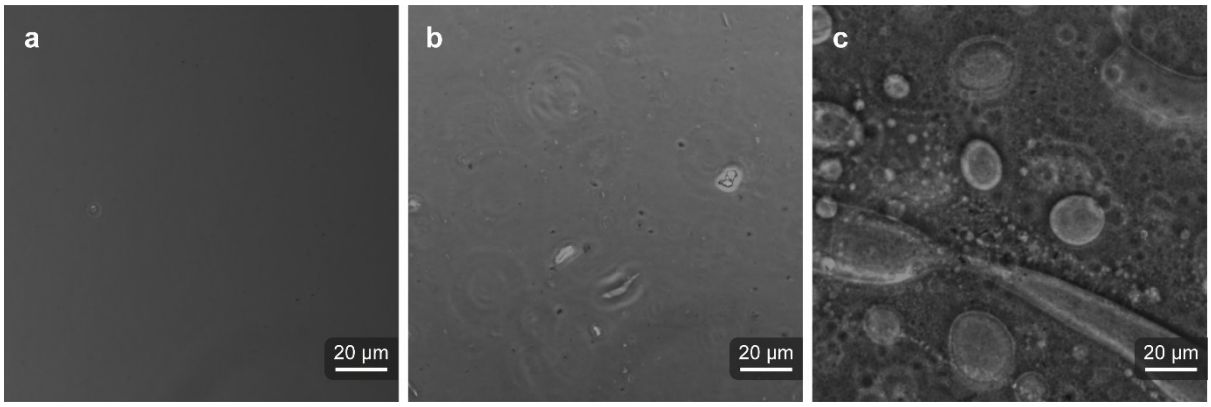


Supplementary Figure S5: Inverse capillary velocity (ICV) analysis of HT silk, IMAC silk and IMAC silk+BG lysate. a-c) Representative graphs of the aspect ratio vs. time of coalescence events (squares) from video recordings and fit with $AR(t) = 1 + (AR_0 - 1)e^{-\frac{t}{\tau}}$ (solid lines) for HT silk **(a)** (n=10), IMAC silk+BG lysate **(b)** (n=5), and IMAC silk **(c)** (n=11). **d)** Characteristic relaxation time τ, derived from fits, plotted against the characteristic length. The slope of the linear fit equals the ICV.



Supplementary Figure S6: FTIR analysis of IMAC silk (126 mg/ml) and HT silk (67 mg/ml). Spectra were acquired with PerkinElmer FTIR with ATR, 4 cm^{-1} resolution, 1 cm^{-1} interval, 32 scans in the range from 2000–750 cm^{-1}. b) Zoom-in for the range 1800–1500 cm^{-1}.

To investigate the secondary structure of proteins depending on the presence of background molecules we performed FTIR spectroscopy (PerkinElmer FTIR with ATR) of 10 µl condensated IMAC (126 mg/ml) and HT silk (67 mg/ml) solution with 4 cm^{-1} resolution, 1 cm^{-1} interval and 32 scans in the range from 2000–750 cm^{-1} (Figure R1). In both samples we see a clear peak in the Amide I region indicating a combination of α-helices, amorphous regions, and β-sheets. In the HT sample the presence of nucleic acids such das DNA and/or RNA is indicated by peaks in the region from 1200-900 cm^{-1} (Figure R1a). The Amide I peak is 1638 cm^{-1} in HT and 1635 cm^{-1} in IMAC silk (Figure R1b). This is a very small shift of 3 cm^{-1} which might indicate a conformational change in the proteins. However, also nucleic acids give a signal in the same region as the Amide I peak. Moreover, also other proteins being present in the sample background of HT silk might lead to this shift in the Amide I. Therefore, it is not possible to comment further on the secondary conformation of IMAC and HT silk. Additionally, HT silk shows a strong peak around 1000 cm^{-1}. This might correspond to the presence of sugars and nucleic acids in the sample (Z. Movasaghi et al. (2008) Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues, Applied Spectroscopy Reviews, 43:2, 134-179, DOI: 10.1080/05704920701829043).



Supplementary Figure S7: Light microscopy images of (a) IMAC silk. (b) IMAC silk with ssDNA from salmon testes (Deoxyribonucleic acid sodium salt from salmon testes, Sigma-Aldrich). (c) IMAC silk with sonicated ssDNA from salmon testes. IMAC silk with sonicated ssDNA showed condensates after 90 min of concentrating, while IMAC silk without DNA or with DNA that was not sonicated showed no condensates even after 180 min.