Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

A novel and selective silk fibroin fragmentation method

Francesca Agostinacchio [a b], *Devid Maniglio* [a b], *Emanuela Callone* [a c], *Claudio Migliaresi* [a b], *Sandra Dirè* [a c], *Antonella Motta* [a b *]

[a] Department of Industrial Engineering, University of Trento, via Sommarive 9, Trento, Italy

[b] BIOTech Research Center, European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Trento, via delle Regole 101, Trento, Italy

[c] "Klaus Müller" Magnetic Resonance Laboratory, Department of Industrial Engineering, University of Trento, Via Sommarive 9, 38123 Trento, Italy

Corresponding author: antonella.motta@unitn.it

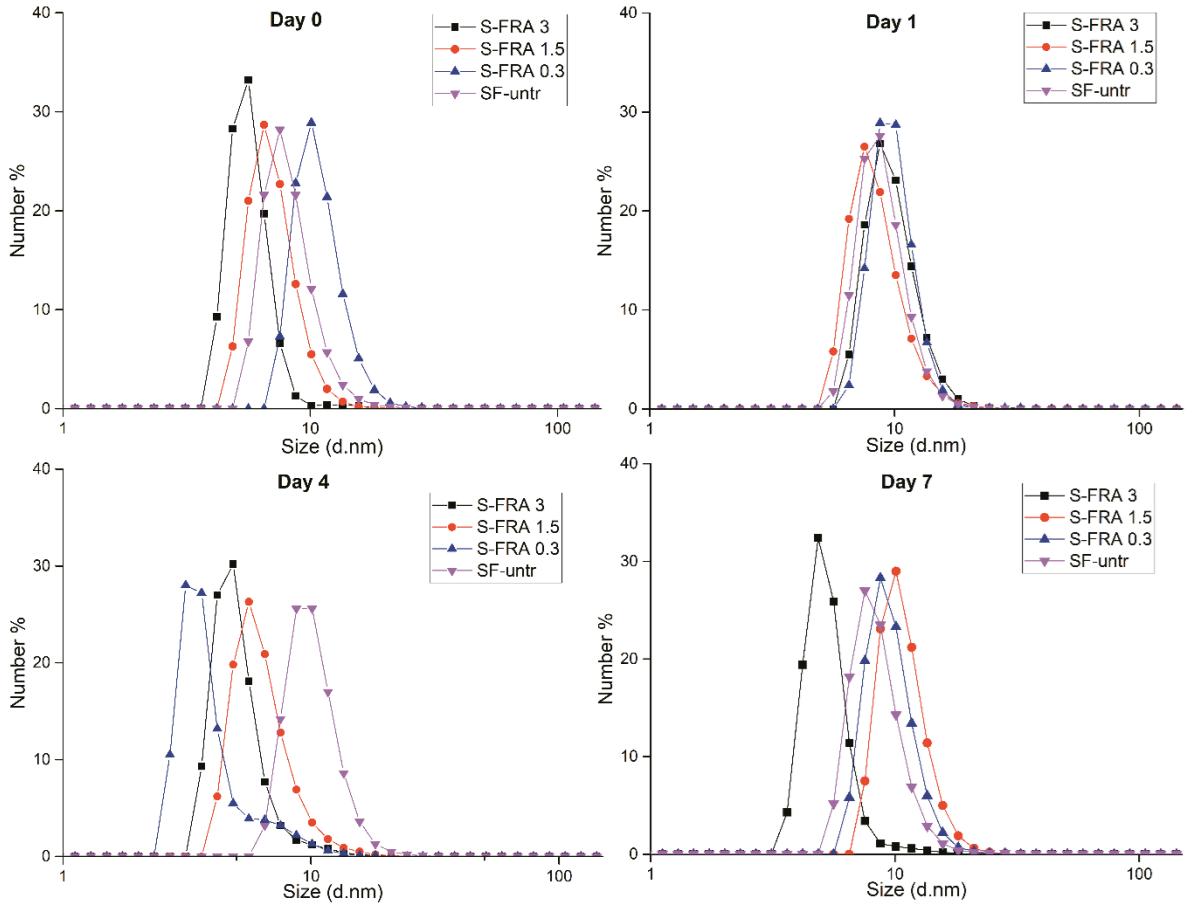

Figure S1 DLS agglomeration kinetics and particles sizes of untreated silk (SF-untr), silk fragmented with 0.3 U/mL Col G (S-FRA 0.3), 1.5 U/ml (S-FRA 1-5), 3 U/mL (S-FRA 3), measured at day 0, 1, 4, and 7 after storage at 4°C and plot according to their number percentage

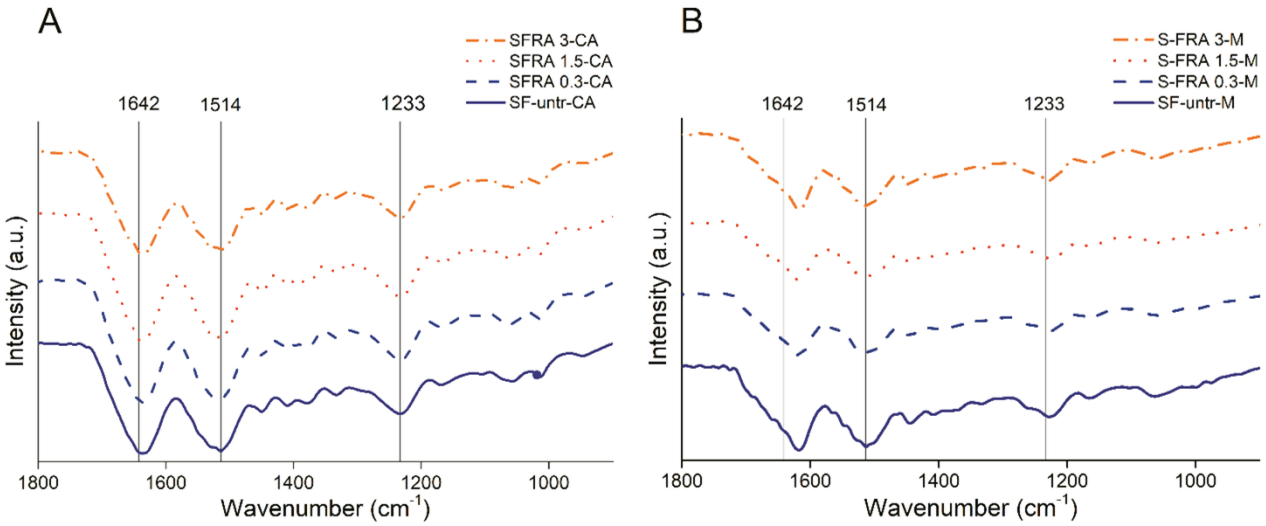

Figure S2. Comparison of FTIR spectra of untreated silk (SF-untr), silk fragmented with 0.3 U/mL Col G (S-FRA 0.3), 1.5 U/mL (S-FRA 1.5), 3 U/mL (S-FRA 3) prepared in form of films through the water-casting process (S1A) and later stabilized via methanol treatment (S1B)

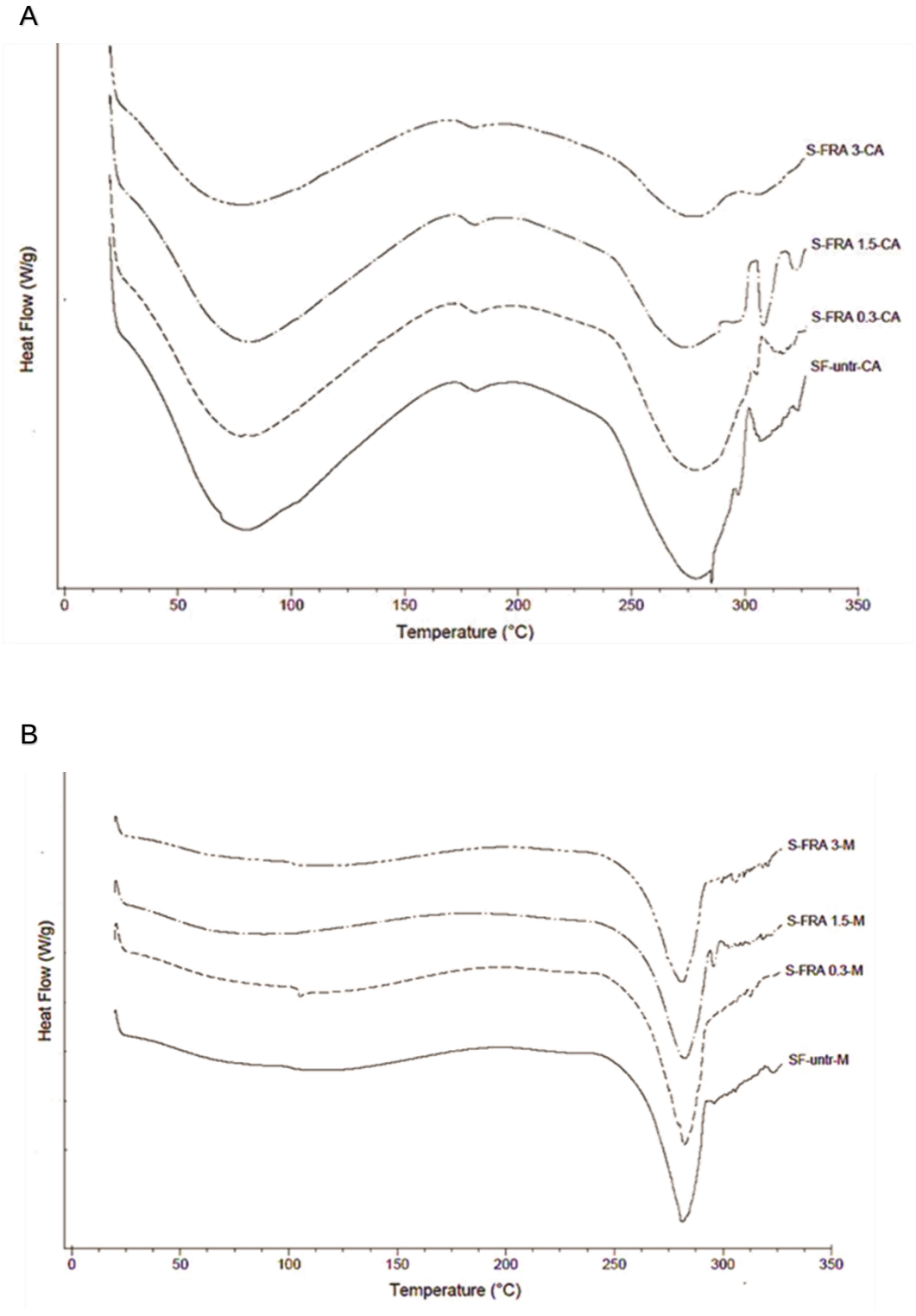

Figure S3. Comparison of DSC spectra of untreated silk (SF-untr), silk fragmented with 0.3 U/mL Col G (S-FRA 0.3), 1.5 U/mL (S-FRA 1.5), 3 U/mL (S-FRA 3) prepared in form of films via water casting (A), later stabilized via methanol treatment (B).

Table S1. ^{13}C NMR chemical shifts and assignments. according to Callone et al. [47]

δ (ppm)	Functional group	Amino acid	Secondary structure
176.8	C=O	Ala (Gln)	Silk I-like/rc*
173.8	C=O	Ala	Silk II
172.4	C=O	Ser/Tyr/Gln, Phe, Pro	-
170.7	C=O	Gly	-
155.5	Cξ	Tyr	-
131.0	Cγ/Cδ	Tyr	-
115.6	Cε	Tyr	-
64.3	Cβ	Ser	Silk II
60.6	Cβ / Cα	Ser /Pro	Silk I-like/rc
57.8	Cα	Ser	Silk I-like/rc
56.4	Cα	Ser/ Gln(Tyr)	Silk II
51.5	Cα	Ala	Silk I-like/rc
49.6	Cα /Cδ	Ala /Gln	Silk II
43.7	Cα	Gly	-
36.9	Cβ	Phe	-
30.4	Cβ/Cγ	Gln/Pro	rc
20.6	Cβ	Ala	Silk II
17.3	Cβ	Ala	Silk I

* rc : random coil

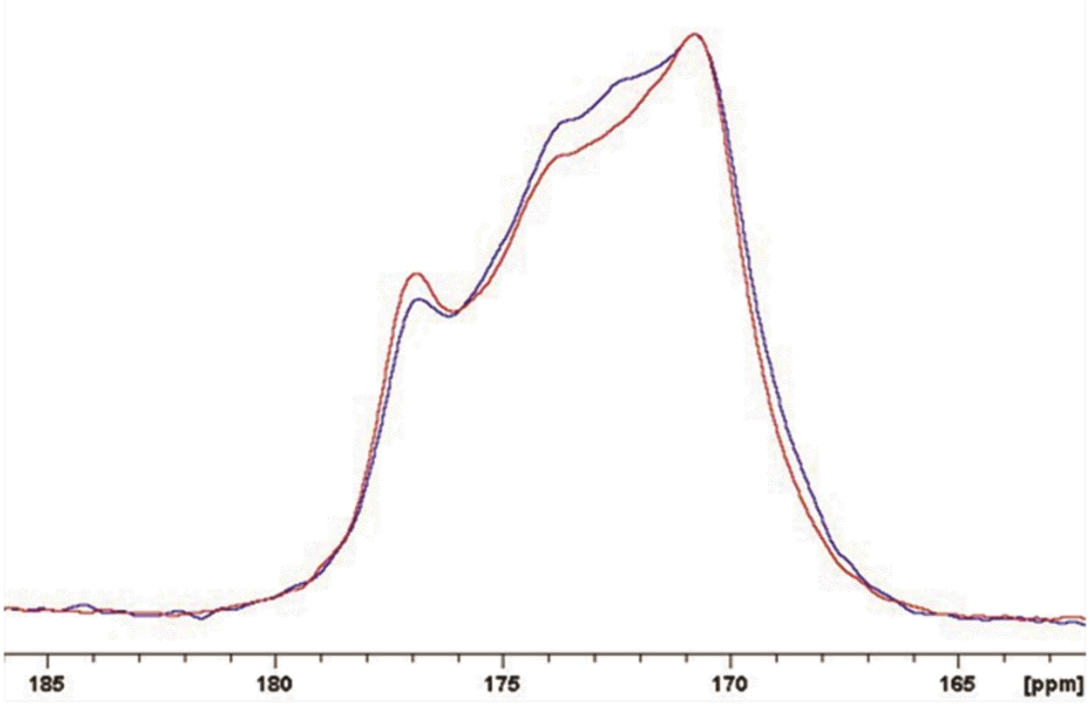

Figure S4. Superposition of the C=O resonances of the SF-untr-WV (blue line) and S-FRA3-WV (red line) samples.

It is interesting to note that the C=O peak, usually described with three components (Ala Silk I and II and Gly), shows at least four/five components, probably due to the characteristic peak sharpness induced by water-vapor treatment, which highlights also the contribution of Ser and Tyr to the C=O resonance.

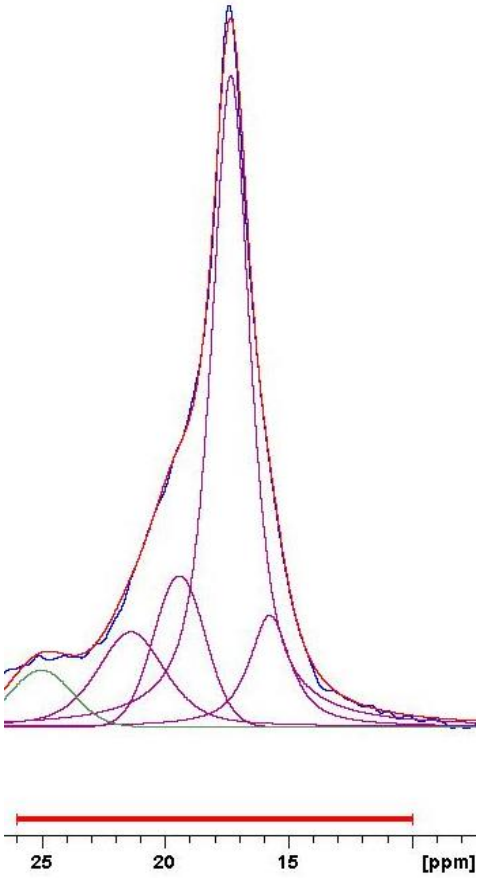

Figure S5. Profile fitting of Ala Cβ peak of S-FRA 3 sample