Supporting Information

Recombinant Silk Fibroin Crystalline Regions as Biomaterial Alternatives to the Full-Length Protein

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Figure S1. Western blot analysis of the purified M300 and M150 substructures by using an anti-His antibody. For the M300 recombinant fragment the blot seems to show the presence of a truncated, lower molecular weight (~20 kDa) form; however, the SDS-PAGE (Figure 1) shows no corresponding band, suggesting that the full-length recombinant protein is the predominant form.

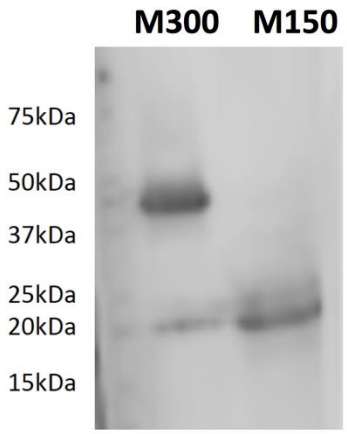
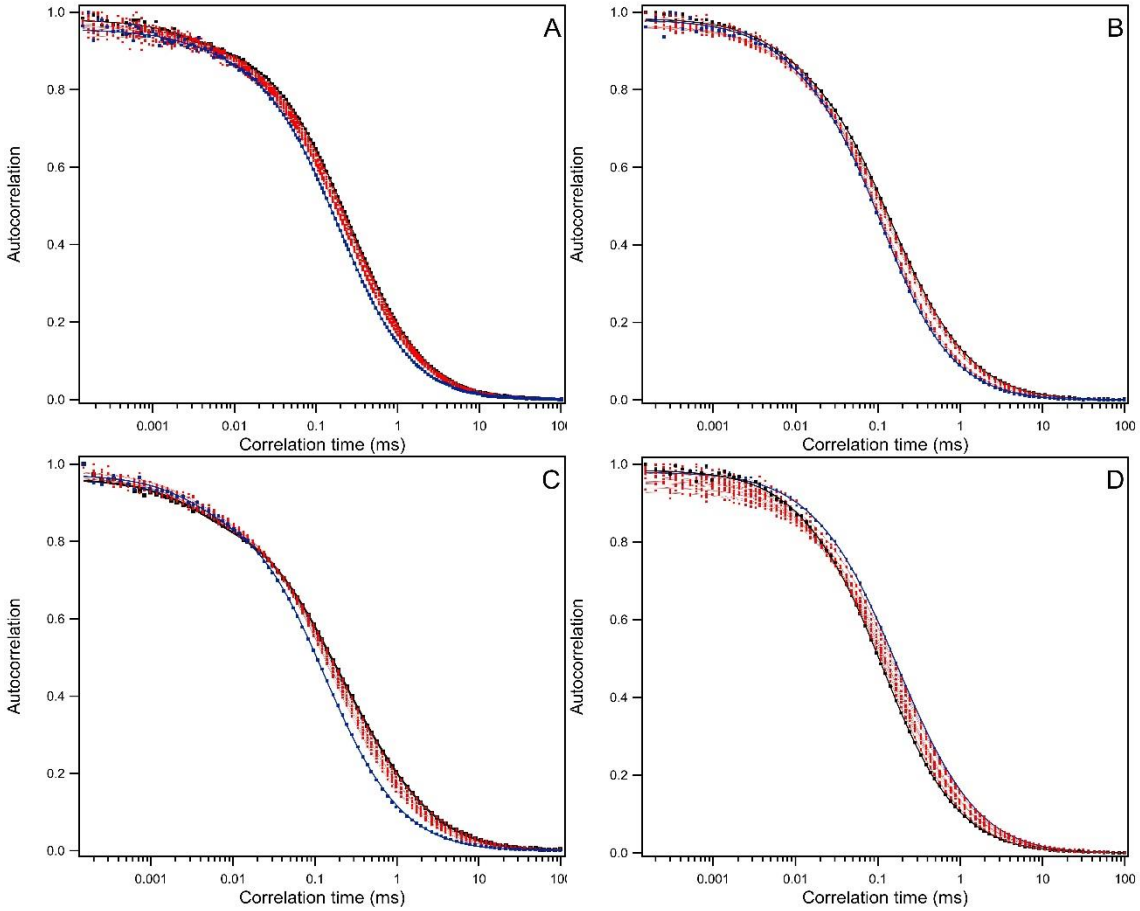


Figure S2. Autocorrelation decays for silk crystalline regions with various binding interaction partners at different concentrations (dots are experimental data; the solid line represents the fit to equation S1). Plot A shows autocorrelation decays for M300 to various concentration of fibronectin. Plot B shows autocorrelation decays for M300 to various concentration of collagen. Plot C shows autocorrelation decays for M300 to various concentration of tropoelastin. Plot D shows autocorrelation decays for M150 to various concentration of collagen.



Equation S1. Standard autocorrelation analysis equation.

$$G(\tau) = \frac{< \delta F(t) \delta F(t+\tau) >}{< F(t) >^2}$$

Where: G is the exponential decay of the autocorrelation function
t is time
F(t) is the function of fluorescence intensity at time t
δF(t) is the function of fluctuations of the fluorescence signal which is defined as s the deviations from the temporal average of the signal δF(t)=F(t)-<F(t)>
τ is delay time

Equation S2. Function used to fit the autocorrelation decays.

$$G(\tau) = \left(1 + \frac{T}{1-T} e^{-\frac{\tau}{\tau_{triplett}}}\right) \bullet \frac{\rho_1}{\left(1 + \frac{\tau}{\tau_D}\right)} \bullet \frac{1}{\left(1 + \left(\frac{r_0}{z_0}\right)^2 \left(\frac{\tau}{\tau_D}\right)\right)^{1/2}} + \left(1 + \frac{T}{1-T} e^{-\frac{\tau}{\tau_{triplett}}}\right) \bullet \frac{\rho_2}{\left(1 + \frac{\tau}{\tau'_D}\right)} \bullet \frac{1}{\left(1 + \left(\frac{r_0}{z_0}\right)^2 \left(\frac{\tau}{\tau'_D}\right)\right)^{1/2}}$$

Where: G is the exponential decay of the autocorrelation function
T is the fraction of fluorophores in triplet dark state
$\tau_{triplet}$ is the fluorophores' triplet relaxation time
ρ_1 and ρ_2 are contribution of the silk proteins and their binding complex
τ_D and τ'_D are the diffusion times for silk proteins and binding complex
r_0 and z_0 are the radial and axial axes of the three-dimensional observation volume

Equation S3. Equation used for the calculation of the faction of M300 or M150, respectively, bound to collagen, tropoelastin or fibronectin. The diffusion time, triplet relaxation time and the fraction of fluorophores in triplet dark state were determined from autocorrelation decays obtained in the absence of binding partners (collagen, tropoelastin, and fibronectin). Separate experiments were conducted in which the radial and axial axes were determined independently using the autocorrelation decay of Atto488, FluoSpheres Carboxylate-Modified Microspheres with diameter 0.1 and 0.02 µm. Fluctuations from intersystem crossing were utilized with fluctuations from diffusion of free and bounded fluorophore labeled silk protein in and out of the observation volume to evaluate the amount of silk proteins that are bound to the ligands.

$$f = \frac{\rho_2}{\rho_1 + \rho_2}$$

Figure S3. Molar mass determination for the M300 crystalline region by SEC-MALS.

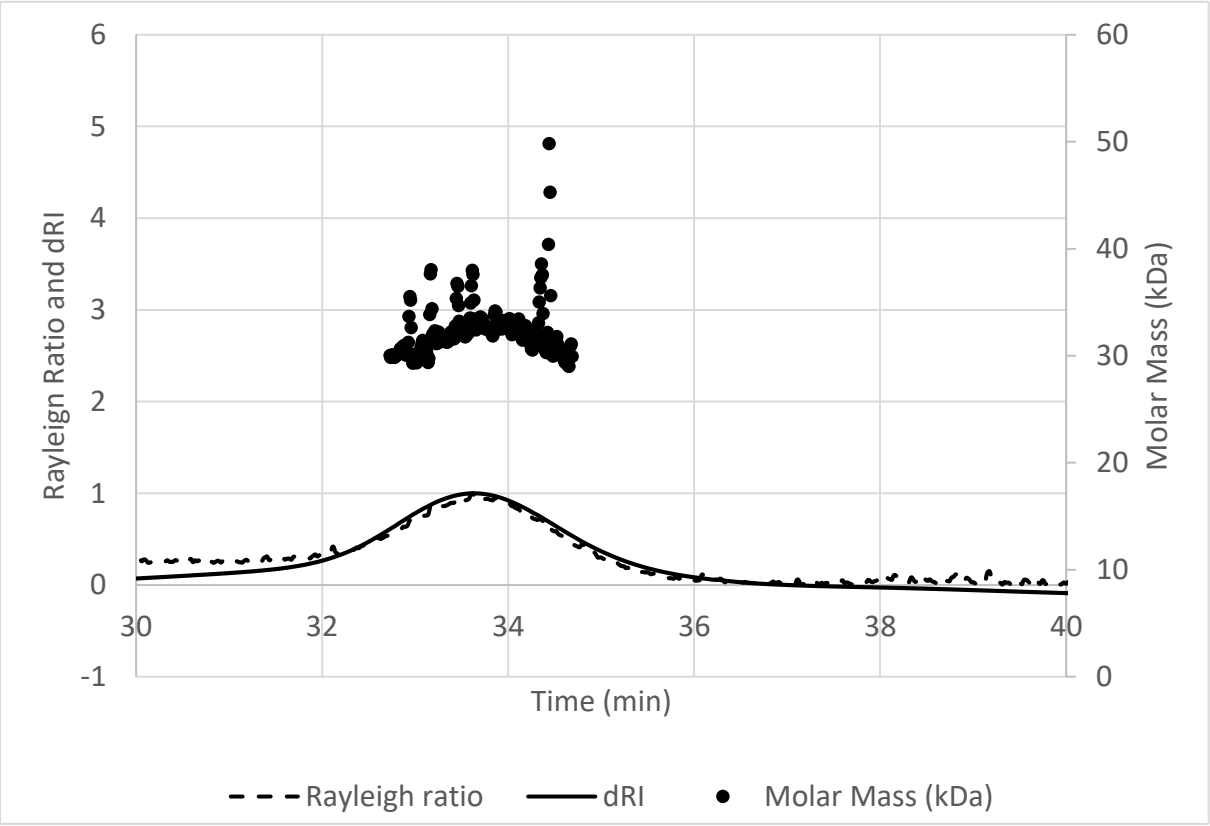


Figure S4. CD spectra of M150 crystalline region at different temperatures, indicating no significant secondary structure changes.

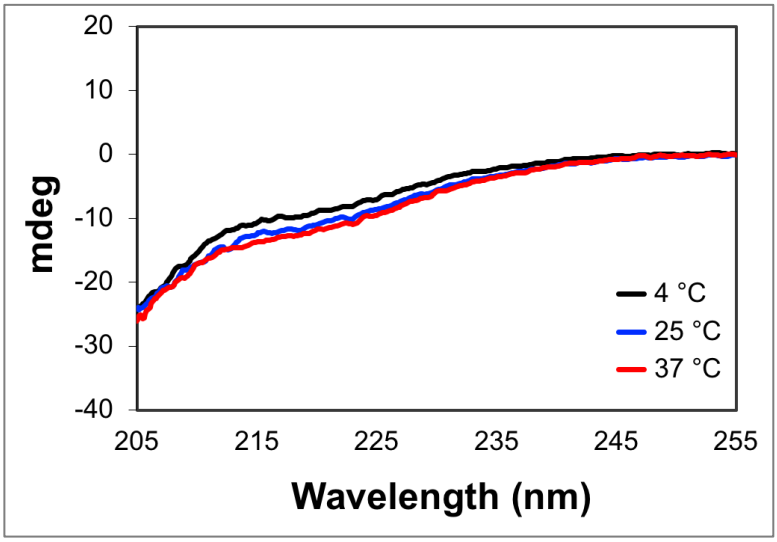


Figure S5. FTIR analyses of full-length SF with or without methanol treatment.

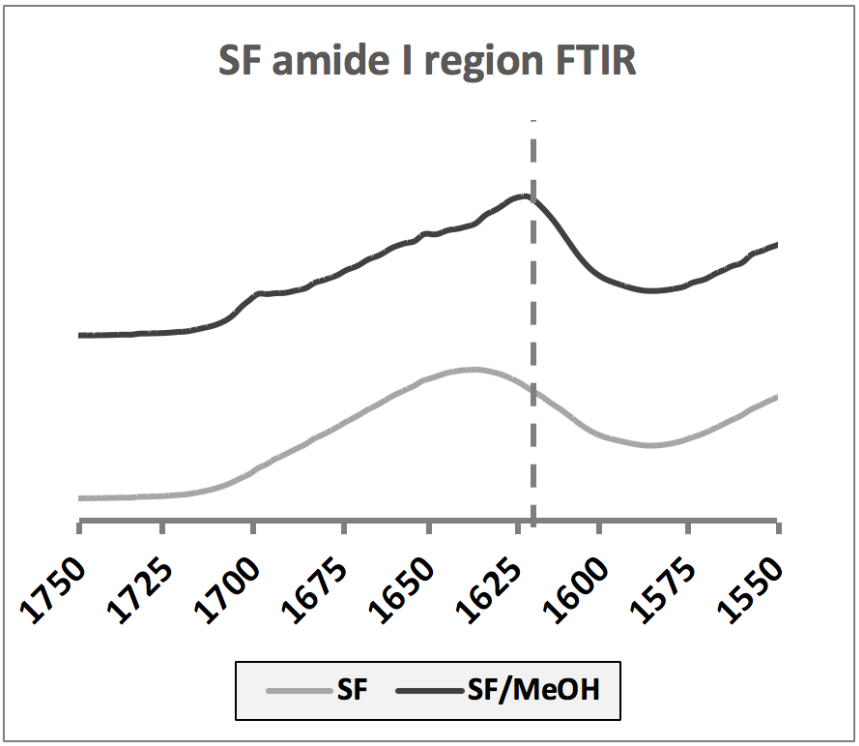
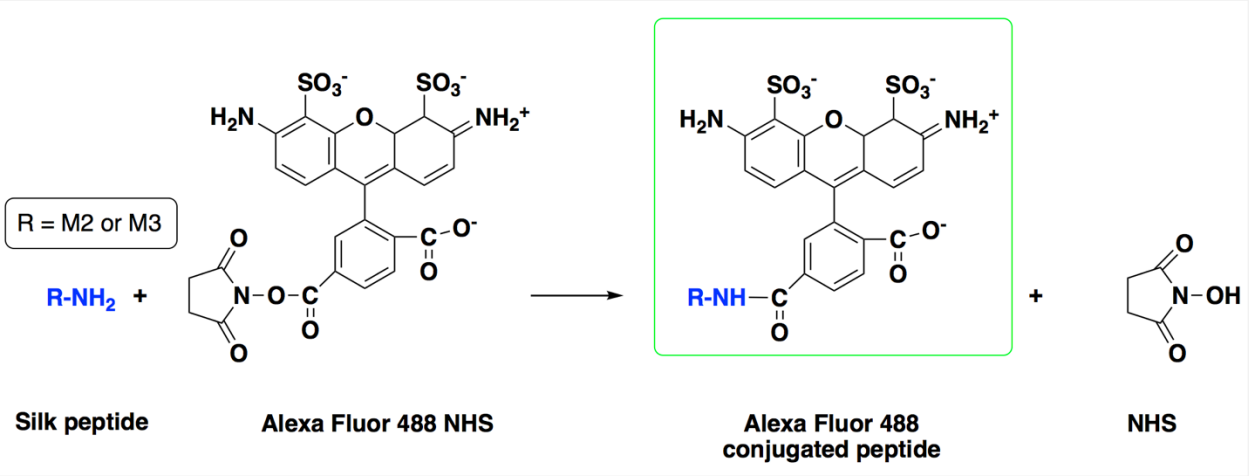


Figure S6. Conjugation reaction of the M300 and M150 substructures with Alexa Fluor 488 N-hydroxysuccinimide (Alexa Fluor NHS).



Equation S4. Equation used for the calculation of the equilibrium dissociation constant (K_d).

$$f = \frac{\text{[bound silk crystalline region]}}{\text{[free silk crystalline region]+[bound silk crystalline region]}} = \frac{\text{[Free ligand]}}{\text{Kd+[Free ligand]}}$$

Table S1. Protparam sequence analyses for human collagen I, human tropoelastin, human fibronectin and M300 and M150 crystalline regions.

	Collagen I	**Fibronectin**	**Tropoelastin**	**M300**	**M150**
UniProtKB/Swiss-Prot ID	P02452	P02751	P15502	NA	NA
Ala (A)	9.5%	4.0%	21.4%	31.9%	31.8%
Arg (R)	4.8%	5.1%	1.3%	0.0%	0.0%
Asn (N)	1.9%	4.1%	0.0%	0.0%	0.0%
Asp (D)	4.5%	5.2%	0.4%	0.0%	0.0%
Cys (C)	1.2%	2.5%	0.3%	0.0%	0.0%
Gln (Q)	3.3%	5.4%	1.2%	0.0%	0.0%
Glu (E)	5.1%	5.9%	0.7%	0.0%	0.0%
Gly (G)	26.7%	8.3%	19.3%	49.7%	49.3%
His (H)	0.6%	2.1%	0.1%	0.0%	0.0%
Ile (I)	1.6%	4.9%	2.1%	0.0%	0.0%
Leu (L)	3.3%	5.3%	5.9%	0.0%	0.0%
Lys (K)	3.9%	3.1%	4.6%	0.0%	0.0%
Met (M)	0.9%	1.1%	0.0%	0.0%	0.0%
Phe (F)	1.8%	2.2%	2.1%	0.0%	0.0%
Pro (P)	19.0%	7.8%	12.6%	0.0%	0.0%
Ser (S)	4.1%	8.1%	1.7%	11.9%	12.8%
Thr (T)	3.1%	10.9%	1.4%	0.0%	0.0%
Trp (W)	0.4%	1.6%	0.0%	0.0%	0.0%
Tyr (Y)	0.9%	4.3%	2.0%	5.0%	4.7%
Val (V)	3.2%	8.1%	12.8%	1.4%	1.4%
Total number of negatively charged residues	141	271	141	0	0
Total number of positively charged residues	128	201	128	0	0
Grand average of hydropathicity (GRAVY)	-0.788	-0.525	0.617	0.274	0.267