Supplementary Information for

Blending Recombinant Amyloid Silk Proteins Generate Composite Fibers with Tunable Mechanical Properties

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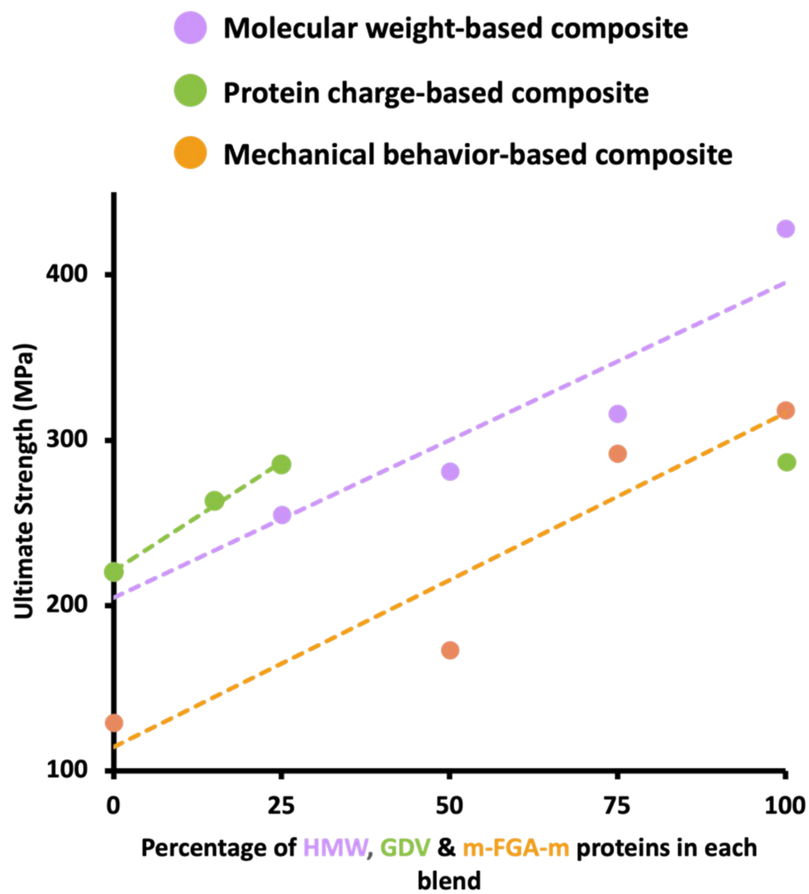
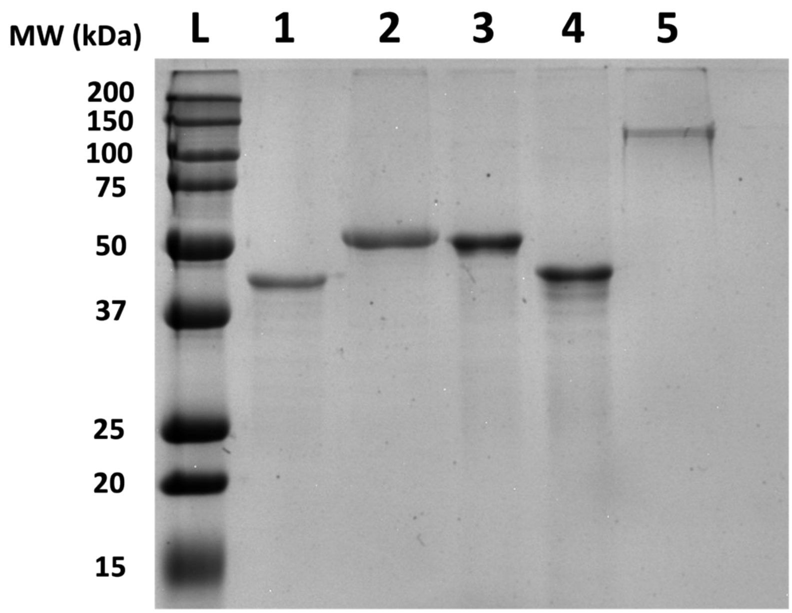
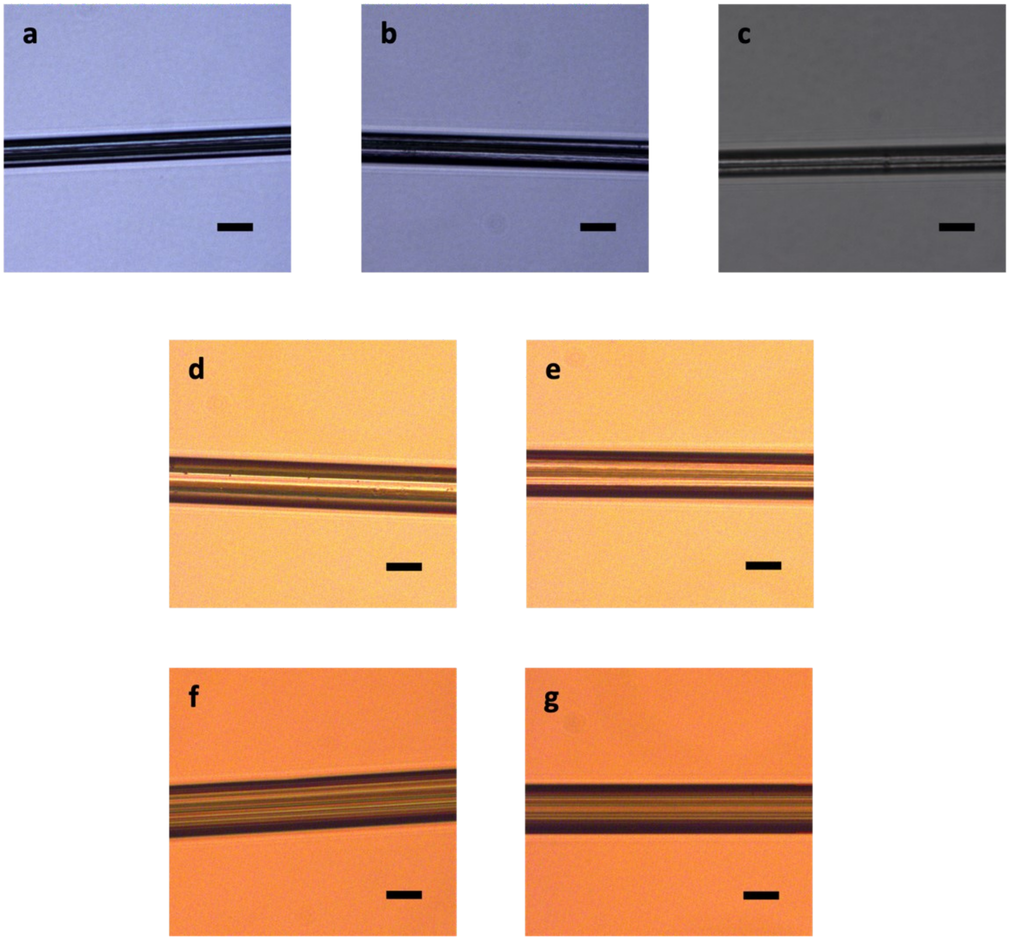


Supplementary Figure 1. **Strength vs Protein ratio plot of various composite fibers.** The above plot shows linear change in ultimate strength with increasing percentages of a protein component across all three composites.

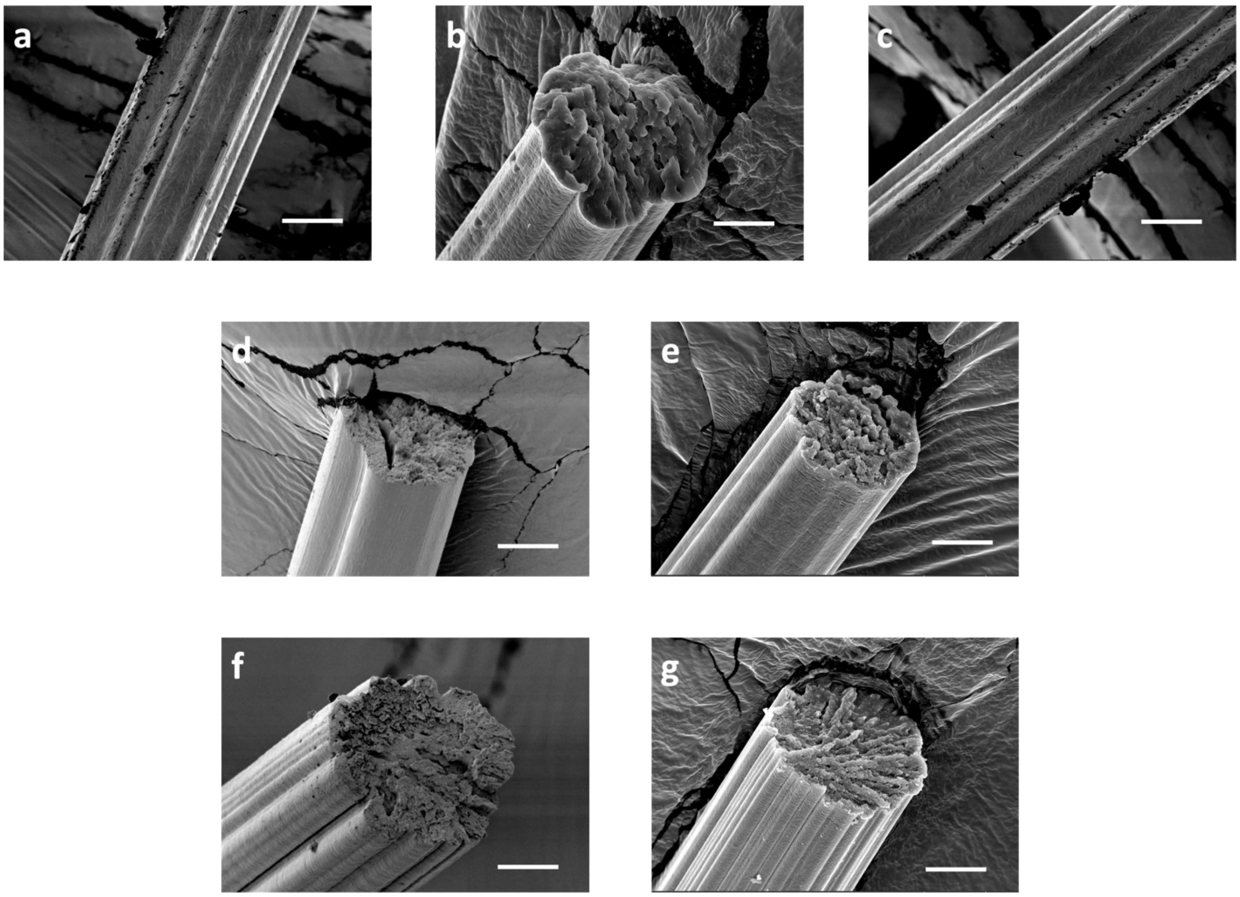


Supplementary Figure 2. **Coomassie blue-stained SDS-PAGE gel of purified proteins.** Lane L, molecular weight marker with their sizes labeled on the left. Lane 1, 16xFGAILSS protein. Lane 2, 16xGDVIEV protein. Lane 3, ^{N}M-16xFGA-^{C}M protein. Lane 4, ^{N}M-16xAAA-^{C}M protein. Lane 5, 48xFGAILSS protein.

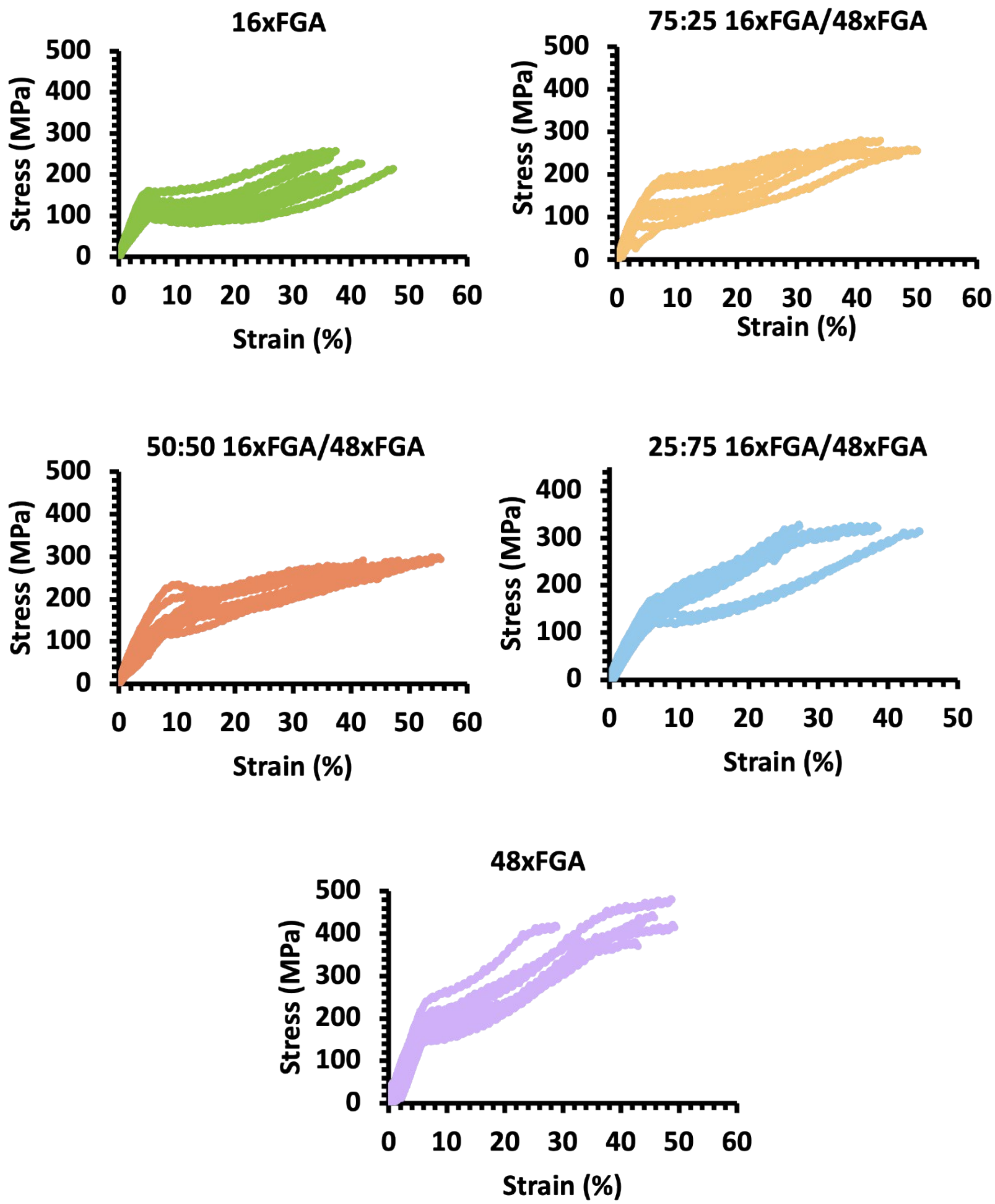


Supplementary Figure 3. **Representative optical images of composite fibers.** Scale bars (black lines) represent 20 μm. a) 75:25 16xFGA/48xFGA b) 50:50 16xFGA/48xFGA c) 25:75 16xFGA/48xFGA d) 85:15 16xFGA/16xGDV e) 75:25 16xFGA/16xGDV

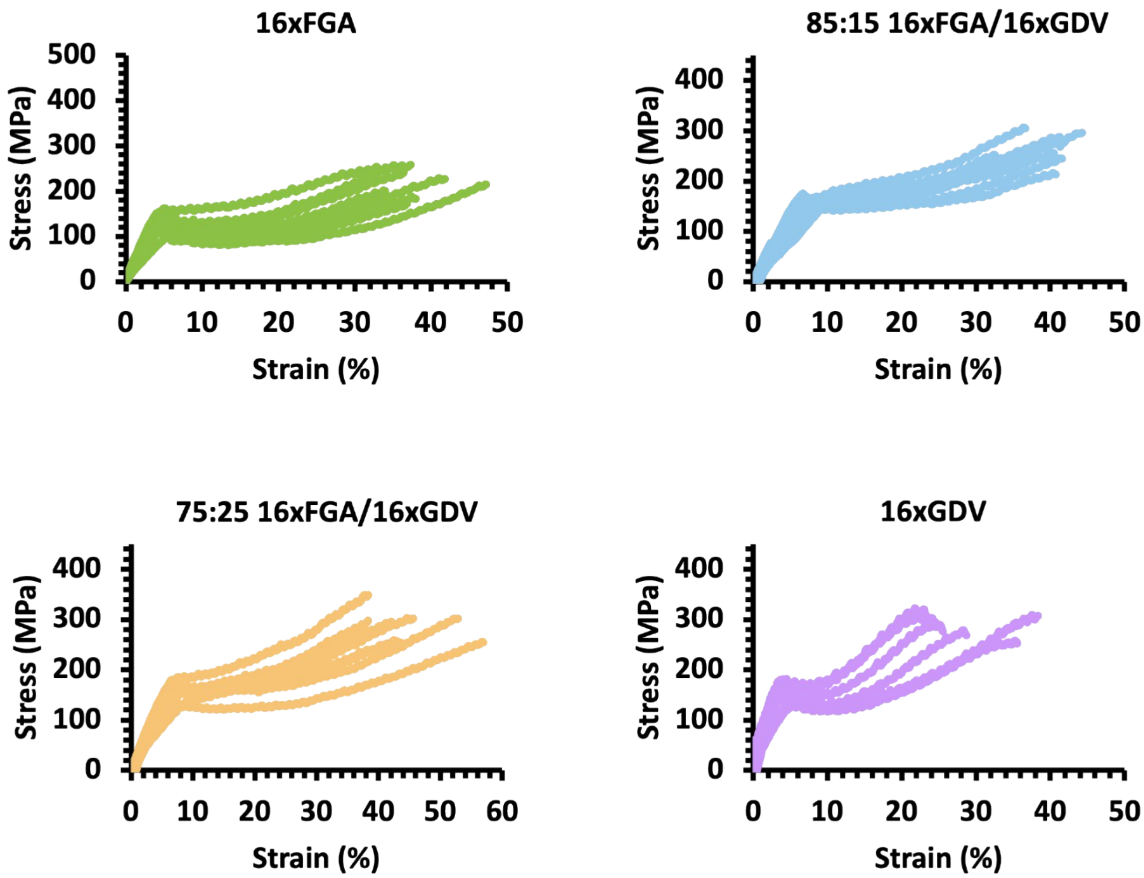
f) 75:25 ^{N}M-16xFGA-^{C}M/ ^{N}M-16xAAA-^{C}M g) 50:50 ^{N}M-16xFGA-^{C}M/ ^{N}M-16xAAA-^{C}M



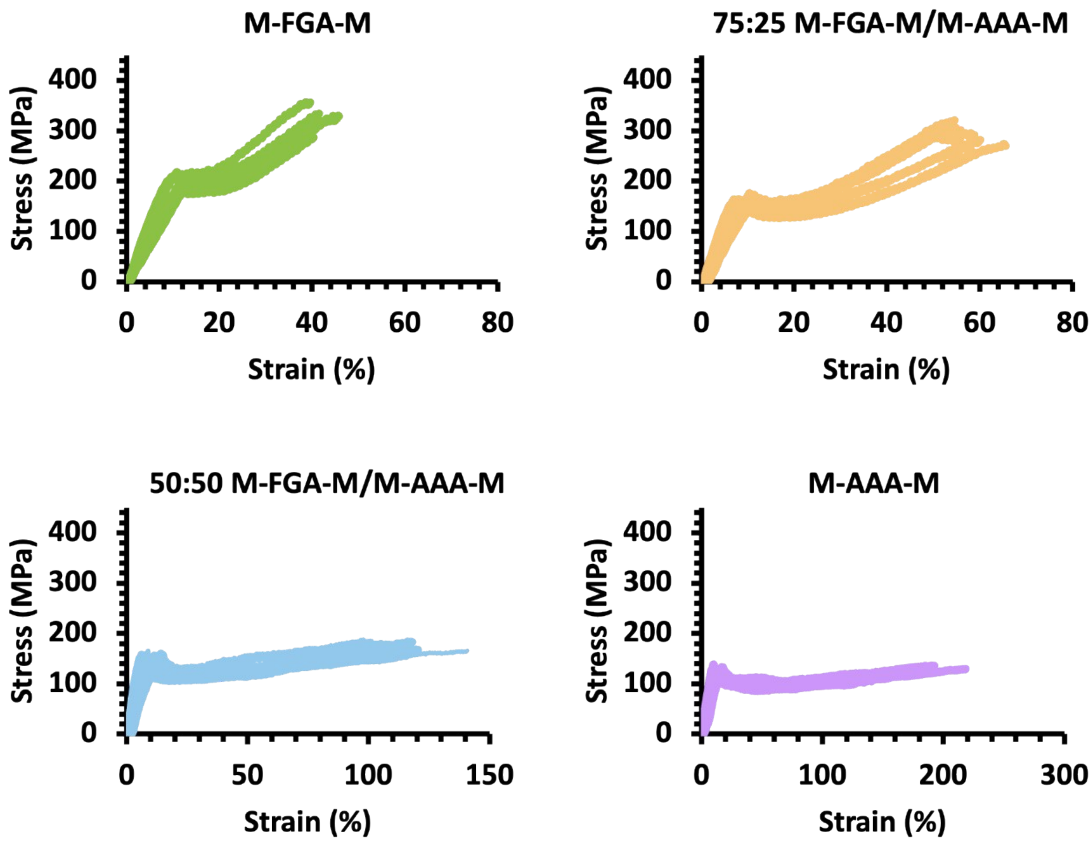
Supplementary Figure 4. **Representative SEM images of composite fibers.** Scale bars (black lines) represent 10 μm. a) 75:25 16xFGA/48xFGA b) 50:50 16xFGA/48xFGA c) 25:75 16xFGA/48xFGA d) 85:15 16xFGA/16xGDV e) 75:25 16xFGA/16xGDV f) 75:25 ^{N}M-16xFGA-^{C}M/ ^{N}M-16xAAA-^{C}M g) 50:50 ^{N}M-16xFGA-^{C}M/ ^{N}M-16xAAA-^{C}M



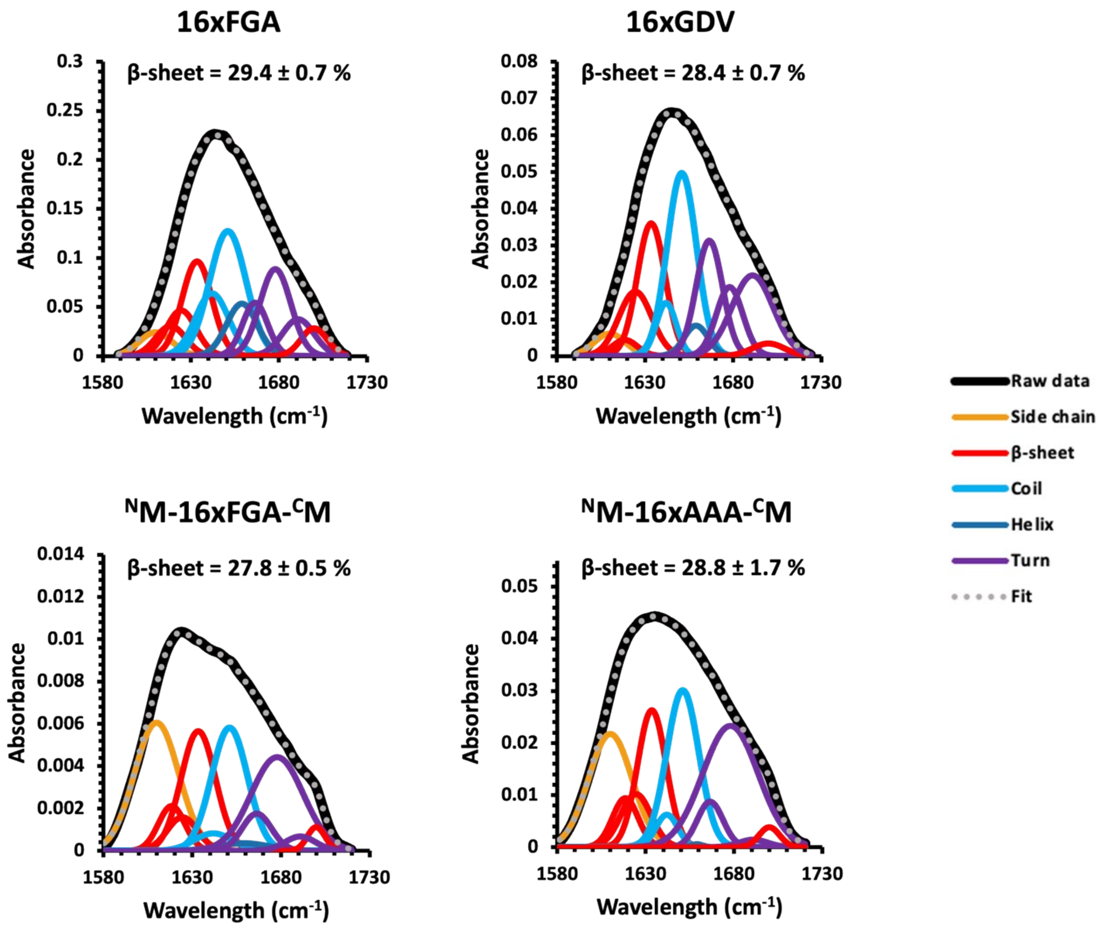
Supplementary Figure 5. Stress-strain curves of individual HMW/LMW composite fibers.



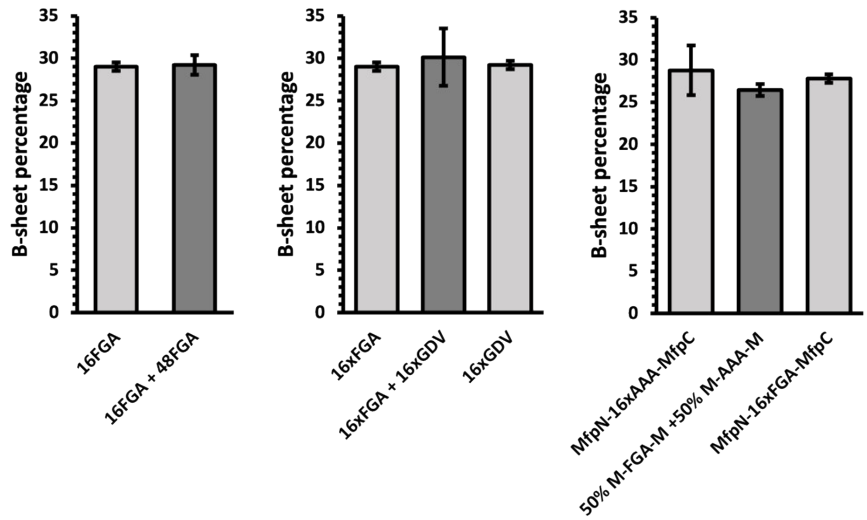
Supplementary Figure 6. Stress-strain curves of individual protein charge-based composite fibers.



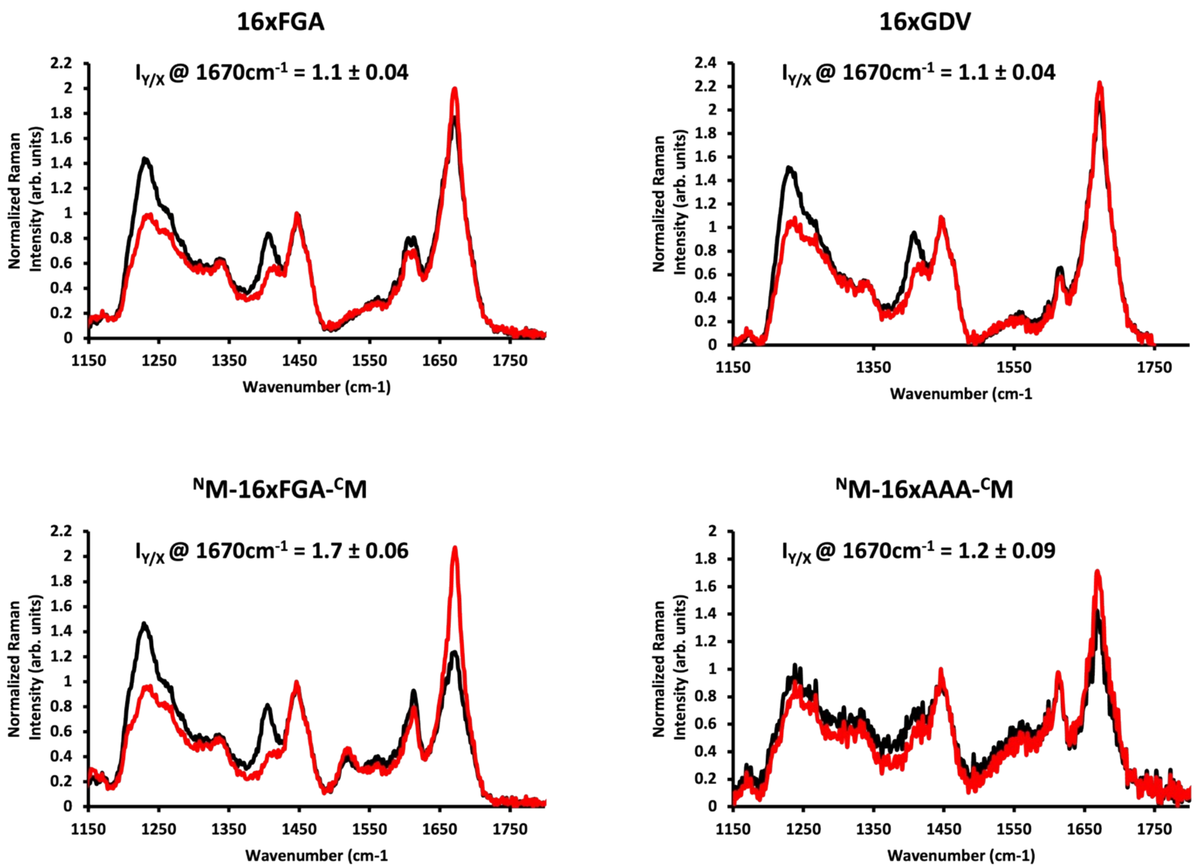
Supplementary Figure 7. Stress-strain curves of individual mechanical behavior-based composite fibers.



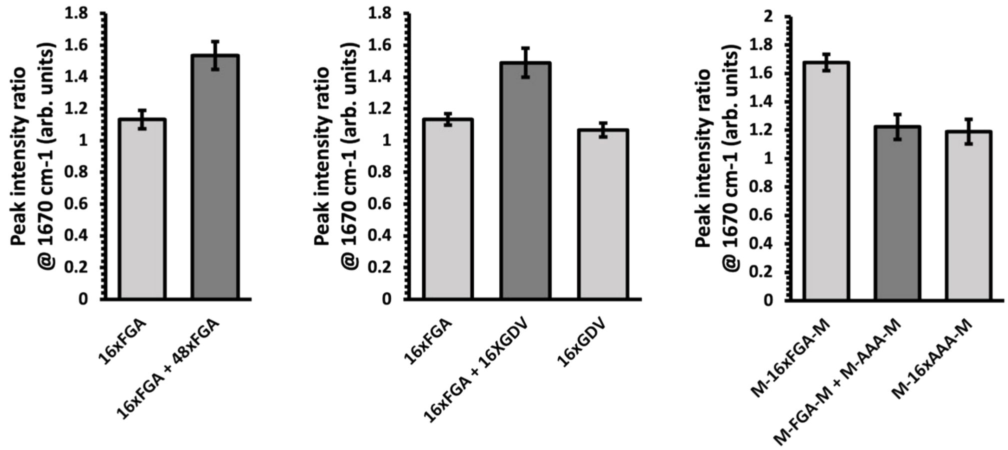
Supplementary Figure 8. FTIR analysis of pure protein fibers. The FTIR spectra from the pure protein fibers were used to deconvolute the amide I peak into a set of 11 Gaussian peaks to calculate the β-sheet content based on previously reported methods (see Methods).



Supplementary Figure 9. Deconvoluted β-sheet content of pure vs. composite protein fibers. Comparison of β-sheet content in the pure vs. composite protein fibers calculated based on amide 1 peak deconvolution from their FTIR spectra. The mean percentages were obtained by averaging the values from 3 fibers and the error bars denote the standard deviation of those values.



Supplementary Figure 10. Raman spectromicroscopy analysis of composite fibers. Raman spectra were acquired for fibers oriented perpendicular (red line) or parallel (black line) to the polarization of the incident laser. Spectra were normalized to the intensity of the peak at 1460 cm^{-1}, which corresponds to orientation insensitive CH_2 bending (see Methods). Spectra shown are the average of spectra acquired on three separate fibers for each fiber state. As a measure of orientation sensitivity, the average ratio of the amide I peak (1670 cm^{-1}) intensity at 0° to that at 90° is shown above each spectrum.



Supplementary Figure 11. Peak intensity ratio of pure vs. composite protein fibers.

Comparison of the peak intensity ratio at 1670 cm^{-1} in the pure vs. composite protein fibers. The mean values were obtained by averaging the values from 3 fibers and the error bars denote the standard deviation of those values.

Supplementary Table 1. Amino acid sequences of the proteins used in this study.

Protein	Amino acid sequence
16xFGAILSS	MAKTKGTASGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQG TSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGL GGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGF GAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGG AGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYG GLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGT SGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLG GQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFG AILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGA GQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGG LGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTS GRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGG QGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGA ILSSGGAGQGGYGGLGSQGTSSGHHHHHHHHHH
48xFGAILSS	MAKTKGTASGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQG TSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGL GGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGF GAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGG AGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYG GLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGT SGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLG GQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFG AILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGA GQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGG LGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTS GRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGG QGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGA ILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAG QGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGL GSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSG RGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQ GAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAIL SSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQ GGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLG SQGTSGRGGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGR GGLGGQGAGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQG AGFGAILSSGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILS SGGAGQGGYGGLGSQGTSGRGGLGGQGAGFGAILSSGGAGQG

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Supplementary Table 2. Summary of mechanical properties of 16xFGAILLS fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m^3)	Diameter (μm)
16FGA-1	202	3.9	34	43	17
16FGA-2	188	3.5	37	44	17
16FGA-3	173	4.1	36	40	17
16FGA-4	229	3.3	41	58	20
16FGA-5	252	5.0	35	55	19
16FGA-6	251	4.0	36	54	20
16FGA-7	214	2.0	47	53	16
16FGA-8	258	4.8	37	70	16
Average	221	3.8	38	52	18
Standard Deviation	32	0.9	4	10	1.5

Supplementary Table 3. Summary of mechanical properties of 75:25 16xFGA/48xFGA fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (µm)
75:25 16FGA/48FGA-1	253	4.6	29	57	19
75:25 16FGA/48FGA-2	260	4.2	48	101	19
75:25 16FGA/48FGA-3	250	3.8	33	62	19
75:25 16FGA/48FGA-4	261	3.5	40	73	17
75:25 16FGA/48FGA-5	247	1.2	46	65	18
75:25 16FGA/48FGA-6	281	3.4	41	81	19
75:25 16FGA/48FGA-7	252	3.4	40	61	18
75:25 16FGA/48FGA-8	240	4.5	36	53	18
Average	256	3.6	39	69	18
Standard Deviation	12	1.1	6	16	1

Supplementary Table 4. Summary of mechanical properties of 50:50 16xFGA/48xFGA fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m^3)	Diameter (µm)
50:50 16FGA/48FGA-1	280	3.8	36	79	20
50:50 16FGA/48FGA-2	252	2.5	44	81	18
50:50 16FGA/48FGA-3	277	2.3	35	74	18
50:50 16FGA/48FGA-4	291	4.3	53	124	18
50:50 16FGA/48FGA-5	267	2.1	43	78	18
50:50 16FGA/48FGA-6	272	2.9	37	61	18
50:50 16FGA/48FGA-7	292	2.1	42	83	19
50:50 16FGA/48FGA-8	299	1.6	53	107	20
50:50 16FGA/48FGA-9	292	2.6	48	92	19
50:50 16FGA/48FGA-10	290	2.1	53	102	20
Average	281	2.6	44	88	19
Standard Deviation	14	0.8	7	18	0.9

Supplementary Table 5. Summary of mechanical properties of 25:75 16xFGA/48xFGA fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m^3)	Diameter (µm)
25:75 16FGA/48FGA-1	319	3.5	25	49	16
25:75 16FGA/48FGA-2	327	3.7	35	88	18
25:75 16FGA/48FGA-3	305	2.7	24	42	17
25:75 16FGA/48FGA-4	320	3.3	33	74	16
25:75 16FGA/48FGA-5	293	2.4	40	63	16
25:75 16FGA/48FGA-6	310	3.9	32	71	16
25:75 16FGA/48FGA-7	329	3.6	27	57	17
25:75 16FGA/48FGA-8	323	3.3	36	84	17
25:75 16FGA/48FGA-9	315	3.9	44	82	16
Average	316	3.4	33	68	17
Standard Deviation	11	0.5	7	16	0.7

Supplementary Table 6. Summary of mechanical properties of 48xFGAILLS fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m^3)	Diameter (µm)
48FGA-1	432	3.7	41	118	13.6
48FGA-2	422	4.7	49	135	13.8
48FGA-3	380	4.0	42	102	14.2
48FGA-4	428	3.7	43	114	14.4
48FGA-5	445	3.8	43	120	13.9
48FGA-6	482	4.9	47	153	13.9
48FGA-7	417	4.1	40	103	13.8
48FGA-8	419	4.5	29	83	14.1
Average	428	4.2	42	116	13.9
Standard Deviation	29	0.5	6	21	0.2

Supplementary Table 7. Summary of mechanical properties of 16xGDVIEV fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m^3)	Diameter (µm)
16xGDV - 1	296	6.0	24	44	12
16xGDV - 2	258	3.2	35	56	12
16xGDV - 3	322	6.7	22	54	11
16xGDV - 4	311	7.3	23	50	11
16xGDV - 5	279	4.7	28	47	12
16xGDV - 6	228	5.3	45	62	10
16xGDV - 7	309	5.1	38	68	10
Average	286	5.5	31	54	11
Standard Deviation	34	1.3	9	8	0.7

Supplementary Table 8. Summary of mechanical properties of 85:15 16xFGA/16xGDV fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (μm)
85:15 FGA/GDV - 1	256	3.4	40	70	31
85:15 FGA/GDV - 2	260	3.4	36	62	27
85:15 FGA/GDV - 3	216	2.9	40	60	29
85:15 FGA/GDV - 4	287	3.5	41	74	26
85:15 FGA/GDV - 5	245	2.5	37	63	30
85:15 FGA/GDV - 6	245	3.5	41	68	24
85:15 FGA/GDV - 7	252	2.7	32	54	29
85:15 FGA/GDV - 8	296	1.8	43	82	26
85:15 FGA/GDV - 9	271	2.6	38	67	26
85:15 FGA/GDV - 10	306	3.1	36	71	26
Average	264	2.9	38	67	27
Standard Deviation	27	0.6	3	8	2.2

Supplementary Table 9. Summary of mechanical properties of 75:25 16xFGA/16xGDV fibers.

Name	**Peak Stress (MPa)**	**Modulus (GPa)**	**Strain at Break (%)**	**Toughness (MJ/m^3)**	**Diameter (µm)**
75:25 FGA/GDV - 1	297	3.1	38	71	28
75:25 FGA/GDV - 2	348	4.0	38	84	26
75:25 FGA/GDV - 3	304	1.9	53	95	25
75:25 FGA/GDV - 4	296	2.6	42	76	28
75:25 FGA/GDV - 5	258	2.4	42	73	29
75:25 FGA/GDV - 6	278	3.6	37	68	28
75:25 FGA/GDV - 7	261	3.4	35	61	27
75:25 FGA/GDV - 8	255	3.4	57	88	28
75:25 FGA/GDV - 9	303	3.2	44	87	27
75:25 FGA/GDV - 10	255	3.6	44	78	27
Average	285	3.1	43	78	27
Standard Deviation	30	0.6	7	10	1.1

Supplementary Table 10. Summary of mechanical properties of ^{N}M-16xFGA-^{C}M fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (µm)
M-FGA-M - 1	287	2.2	40	72	32
M-FGA-M - 2	357	2.7	38	89	29
M-FGA-M - 3	273	2.9	34	60	32
M-FGA-M - 4	330	2.6	45	93	30
M-FGA-M - 5	334	2.9	41	87	29
M-FGA-M - 6	325	3.1	45	101	31
Average	318	2.7	40	84	31
Standard Deviation	32	0.3	4	15	1.1

Supplementary Table 11. Summary of mechanical properties of 75:25 ^{N}M-16xFGA-^{C}M / ^{N}M-16xAAA-^{C}M fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (µm)
75:25 M-FGA-M/M-AAA-M - 1	258	2.0	57	90	33
75:25 M-FGA-M/M-AAA-M - 2	273	2.9	57	101	32
75:25 M-FGA-M/M-AAA-M - 3	274	2.2	65	110	31
75:25 M-FGA-M/M-AAA-M - 4	316	2.8	53	102	31
75:25 M-FGA-M/M-AAA-M - 5	307	2.3	53	101	31
75:25 M-FGA-M/M-AAA-M - 6	303	2.2	53	114	32
75:25 M-FGA-M/M-AAA-M - 7	284	1.9	60	106	32
75:25 M-FGA-M/M-AAA-M - 8	292	3.6	49	110	31
75:25 M-FGA-M/M-AAA-M - 9	321	2.3	53	104	28
Average	292	2.5	55	104	31
Standard Deviation	22	0.5	5	7	1.4

Supplementary Table 12. Summary of mechanical properties of 50:50 ^{N}M-16xFGA-^{C}M / ^{N}M-16xAAA-^{C}M fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (µm)
50:50 M-FGA-M/M-AAA-M - 1	167	2.2	140	184	33
50:50 M-FGA-M/M-AAA-M - 2	177	2.0	110	146	32
50:50 M-FGA-M/M-AAA-M - 3	190	2.3	97	151	31
50:50 M-FGA-M/M-AAA-M - 4	184	2.1	114	167	31
50:50 M-FGA-M/M-AAA-M - 5	169	1.8	120	161	33
50:50 M-FGA-M/M-AAA-M - 6	182	2.1	100	152	27
50:50 M-FGA-M/M-AAA-M - 7	158	2.5	114	142	29
50:50 M-FGA-M/M-AAA-M - 8	160	2.9	95	122	28
Average	173	2.2	111	153	30
Standard Deviation	12	0.3	15	21	2.2

Supplementary Table 13. Summary of mechanical properties of ^{N}M-16xAAA-^{C}M fibers.

Name	Peak Stress (MPa)	Modulus (GPa)	Strain at Break (%)	Toughness (MJ/m³)	Diameter (µm)
M-AAA-M - 1	134	1.3	135	144	38
M-AAA-M - 2	130	2.4	165	175	39
M-AAA-M - 3	118	1.8	125	120	39
M-AAA-M - 4	130	1.3	125	134	36
M-AAA-M - 5	132	2.4	215	225	42
M-AAA-M - 6	139	2.2	205	215	38
M-AAA-M - 7	119	1.3	140	135	39
M-AAA-M - 8	129	2.4	190	192	39
Average	129	1.9	163	167	39
Standard Deviation	7	0.5	37	40	1.5

Supplementary Note 1. Determination of overall charges for proteins used in charge-based composites.

The protein charges reported in this study were determined using an online calculator tool (www.protpi.ch). The protein charge was calculated for 16-mer amyloid-silk proteins including the amyloid peptides, linker regions and His-tag.

These charges can also be manually calculated by summing up the charges carried by the ionizable groups in the amino acids (the N-terminus, C-terminus and the side chains of acidic and basic amino acids) at a neutral pH of 7.4 by considering their respective pKa values. The calculation of charges at pH 7.4 for the proteins used in charge-based composites (16xFGA & 16xGDV) is outlined below:

16xFGAILSS

Ionizable group	Amino acid	pKa	# of occurrences	Charge
N-terminus	Methionine	9.1	1	+1
C-terminus	Histidine	1.7	1	-1
Basic side chains	Arginine	12.1	16	+16
	Lysine	10.7	2	+2
	Histidine	6	10	0, since histidine's pI of 7.6 is very close to pH 7.4.
				Total charge = +18

16xGDVIEV

Ionizable group	Amino acid	pKa	# of occurrences	Charge
N-terminus	Methionine	9.1	1	+1
C-terminus	Histidine	1.7	1	-1
Acidic side chains	Aspartic acid	3.7	16	-16
	Glutamic acid	4.1	16	-16
Basic side chains	Arginine	12.1	16	+16
	Lysine	10.7	2	+2
	Histidine	6	10	0, since histidine's pI of 7.6 is very close to pH 7.4.
				Total charge = -14

Note, the table's method only give a rough estimation as it does not consider partial charges. For example, a fraction of Histidine groups at pH 7.4 is positively charged. Online calculators consider this fraction, thus often give charge values with decimals.