

## Supporting Information

Engineered Recombinant Hagfish Intermediate Filament Proteins: Unraveling Domain Roles in Synthetic Fiber Formation and Mechanics

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## AA Sequences

The amino acid sequences used in making the various protein constructs presented in this study appear in Table S1.

**Table S1.** Amino acid (AA) sequence of the various recombinant hagfish intermediate filaments (rHIF). The rHIF $\alpha$  and the rHIF $\gamma$ (C387S) correspond to  $\alpha$  and  $\gamma$ , respectively.

Construct	AA sequence
<b>N-<math>\alpha</math></b>	MGHHHHHHHHHSSGHIDDDDKHMLPRMSISQTVSKSYTKSVSRGGQGVSYSQSSSHKVGGSV RYGTTYSSGGISRVLGFQGGAGGAASAGFGGSVGGSLSRVLGGSMVSGYRSGMGVGGLSLSGTAG LPVSLRGGVAGKALHAITSAFRTRVGGPGTSSVGGYGVNYSFLPSTAGPSFGGPFGGPFGGPLGP GYIDPATLSPDVTQHTRIREKQDLQTLNFKFANLVDQVRTLEQHNAILKAQISMITSPSDTPEGVNT AVVASTVTATYNAQIEDLRTTNTALHSEIDHLTTIINDITTKYEEQVEVTRTLETDWNTNKNIDNTYTLI VDLQTKVQGLDEQINTTKQIYNARVREVQAAVTGGPTAAYSIRVDNTHQAIDLTTSLQEMKTHYEVLA TKSREEAFTQVQPRIQEMAVTVQAGPQAIQAKEQIHVFKLQIDSVHREIDRLHRKNTDVEREITVIET NIHTQSDEWTNNINSLKVDLEVIKKQITQYARDYQDLLATKMSLDVEIAAYKKLLDSEETRITS
<b>CRD-<math>\alpha</math></b>	MGHHHHHHHHHSSGHIDDDDKHMLPRKQDLQTLNFKFANLVDQVRTLEQHNAILKAQISMITSP SDTPEGVNTAVVASTVTATYNAQIEDLRTTNTALHSEIDHLTTIINDITTKYEEQVEVTRTLETDWNTN KNIDNTYTLTIVDLQTKVQGLDEQINTTKQIYNARVREVQAAVTGGPTAAYSIRVDNTHQAIDLTTSLQ EMKTHYEVLATKSREEAFTQVQPRIQEMAVTVQAGPQAIQAKEQIHVFKLQIDSVHREIDRLHRKNT DVEREITVIETNIHTQSDEWTNNINSLKVDLEVIKKQITQYARDYQDLLATKMSLDVEIAAYKKLLDSEET RITS
<b><math>\alpha</math>-C</b>	MGHHHHHHHHHSSGHIDDDDKHMLPRKQDLQTLNFKFANLVDQVRTLEQHNAILKAQISMITSP SDTPEGVNTAVVASTVTATYNAQIEDLRTTNTALHSEIDHLTTIINDITTKYEEQVEVTRTLETDWNTN KNIDNTYTLTIVDLQTKVQGLDEQINTTKQIYNARVREVQAAVTGGPTAAYSIRVDNTHQAIDLTTSLQ EMKTHYEVLATKSREEAFTQVQPRIQEMAVTVQAGPQAIQAKEQIHVFKLQIDSVHREIDRLHRKNT DVEREITVIETNIHTQSDEWTNNINSLKVDLEVIKKQITQYARDYQDLLATKMSLDVEIAAYKKLLDSEET RISHGGGITITNAGTFPGGLSAAPGGGASYAMVPAGVGGVGLAGVGGYGFRRSMGGGGGGVGYGA GGGGVGYGVGGGFGGGMGMSMSRMSMGAAVGGGSYGSYSGYSGGFLSSSRAGYSARSKSYSS ARSSRIYTS
<b>N-<math>\gamma</math></b>	MGHHHHHHHHHSSGHIDDDDKHMHNLNRFEMASHSSVSYRSVRTGGTSAMIGSSGYGGSSSR AMGLGMGAAGLSMGGGFRVGSAGIGGMGISSGIGGMGISSRAGGMSAYGGAASGGAGGFVSG GVPMLGYGGGAGGFIGGVSPGIMASPAFTAGRAITSAGMSGVVGTLGPAGGMVPSLVSRLVSRDEVKNIL GTLNQLRASYVDKVRQLTIENTMEEELKNLTGGVPMSPDSTVNLENVETQVTEMLTEVSNLTLERVR LEIDVDHLRATADEIKSKYEFELGVRMQLETDIANMKRDLEAANDMRVDLDSKFNFLTEELTFQRKTQ MEELNTLKQQFGRGLPVQTSVIELDNVKSVDALNVMREEYQQVVTKNVQEAETYSKMQIDQIQ GISTQTTEQISILDKEINTLEKELQPLNVEYQRLTTYQTLGDRITDLQNRRESIDLQVFQNTYTRYEQEIEG NQVDLQRQLVTYQQLLDVKLTALDAEIATYKKLLEGQELMVT
<b>CRD-<math>\gamma</math></b>	MGHHHHHHHHHSSGHIDDDDKHMHNLNRFKNI LGTLNQLRASYVDKVRQLTIENTMEEELKN LTGGVPMSPDSTVNLENVETQVTEMLTEVSNLTLERVRLEIDVDHLRATADEIKSKYEFELGVRMQLET DIANMKRDLEAANDMRVDLDSKFNFLTEELTFQRKTQMEELNTLKQQFGRGLPVQTSVIELDNVKS VNLTDALNVMREEYQQVVTKNVQEAETYSKMQIDQIQGISTQTTEQISILDKEINTLEKELQPLNVEYQR

LLTTYQTLGDRLTDLQNRRESIDLQVFQNTYTRYEQEIEGNQVDLQRQLVITYQQLLDVKTALDAEIATYKK  
LLEGQELMVT

**γ-C** MGHHHHHHHHHSSGHIDDDDKHMHNLNRFKNI<sub>L</sub>GLNQLASVVDKVRQLTIENETMEEELKN  
LTGGVPMSPDSTVNLENVETQVTEMLTEVSNLTLERVLEIDVDHLRATADEIKSKYEFELGVRMQLET  
DIANMKRDLEAANDMRVDLDSKFNFLTEELTFQRKTQMEELN<sub>L</sub>KQQFGRLGPVQTSVIELDNVKS  
NLTDALNVMREEYQQVVTKNVQEAETYSKM<sub>Q</sub>IDQIQGISTQTTEQISILDKEINTLEKELQPLNVEYQR  
LLTTYQTLGDRLTDLQNRRESIDLQVFQNTYTRYEQEIEGNQVDLQRQLVITYQQLLDVKTALDAEIATYKK  
LLEGQELMVRTAMADDFAHATVVRSGTLGGASSSSVGYGASSTLGAISGGYSTGGGASYSAGAGGA  
SYSAGAGGASYGVGGGYSGSSAMMEGSSSGHSMYSSSSMKRSSSKSASASAGGYGTSGHDSTIIL  
QQTS

### Bioreactor results

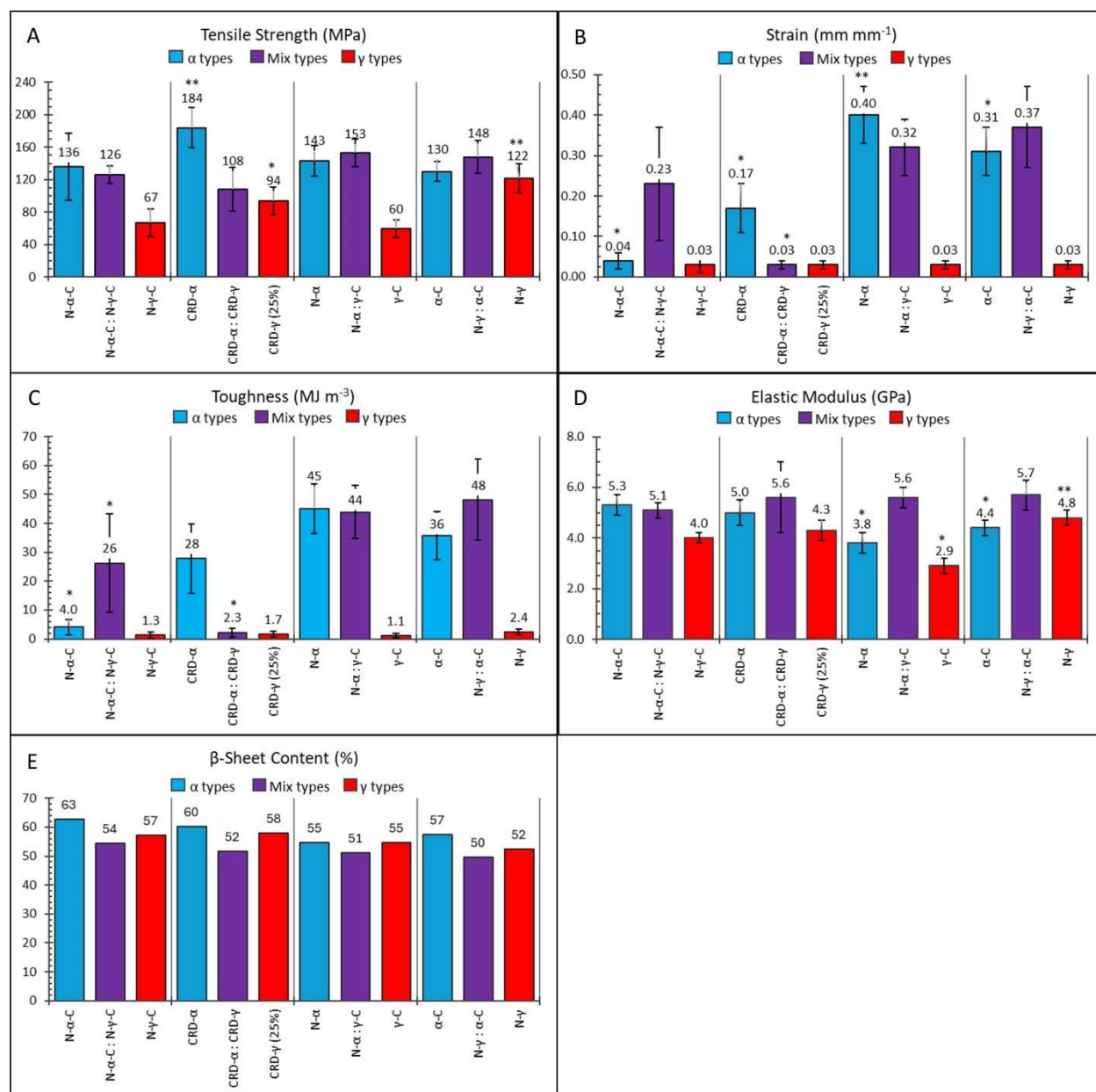
Results for each of the bioreactor runs (Table S2) performed for this study (N-α, CRD-α, α-C, N-γ, CRD-γ, and γ-C).

**Table S2.** The bioreactor runs OD<sub>600</sub> values, cell mass, and reactor size.

Protein Construct	OD <sub>600</sub> at induction	Final OD <sub>600</sub>	Wet cell mass (g)	Bioreactor size (L)
N-α	86	138	338	2
CRD-α	84	128	276	2
α-C	69	96	224	2
N-γ	38	81	132	10
CRD-γ	46	74	172	2
γ-C	58	113	221	2

### Mechanical results

The primary mechanical and structural results presented in the main paper were converted to graphical form in Figure S1. These figures help visualize the differences in properties for the different constructs.



**Figure S1.** Mechanical and structural properties of the different constructs spun from 20% w/v dopes (CRD-γ at 25%) into SW with a 2X2X stretch factor application. A. Tensile strength, B. Strain, C. Toughness, D. Elastic modulus, E. β-sheet content. Panels A-D are averages with standard deviation error bars. \* Indicates a significantly worse property for that protein group, while \*\* indicates a significantly better property for that protein group.

The mechanical properties of the different protein constructs at various protein concentrations, stretch factors, and bath contents appear in separate tables corresponding to the protein type

for each table. These tables only include constructs, concentrations, stretch factors, and bath contents for successfully formed fibers. Table S3 focuses on the rHIF $\alpha$  constructs that need the C-terminus for fiber formation in dH<sub>2</sub>O. In the table, rHIF $\alpha$  is reduced to N- $\alpha$ -C to indicate the full-length construct that includes both termini.

**Table S3.** Mechanical properties from various concentrations of the rHIF $\alpha$  constructs spun at multiple stretch factors. Here, dH<sub>2</sub>O is deionized water, and SW is salt water from Instant Ocean.

Construct		Water Type	n	Toughness (MJ/m <sup>3</sup> )	Tensile Stress (MPa)	Strain (mm/mm)	Diameter (micron)	Elastic Modulus (GPa)
15% w/v								
1X1X	N- $\alpha$ -C	dH <sub>2</sub> O	6	1.08 ± 0.9	44.8 ± 8.1	0.03 ± 0.02	50.2 ± 1.0	2.1 ± 0.3
	$\alpha$ -C	dH <sub>2</sub> O	6	11.3 ± 9.7	66.5 ± 15	0.23 ± 0.20	36.4 ± 4.4	3.1 ± 0.9
	N- $\alpha$ -C	SW	10	0.51 ± 0.3	50.5 ± 14	0.02 ± 0.01	66.3 ± 1.2	3.0 ± 0.2
	N- $\alpha$	SW	8	3.70 ± 2.9	50.4 ± 14	0.11 ± 0.09	56.4 ± 6.2	2.9 ± 0.8
	CRD- $\alpha$	SW	10	1.22 ± 0.7	93.0 ± 28	0.02 ± 0.01	59.7 ± 3.1	4.2 ± 0.3
	$\alpha$ -C	SW	9	0.87 ± 0.8	64.3 ± 32	0.02 ± 0.01	69.7 ± 9.6	3.1 ± 0.9
1.5X1.5X	N- $\alpha$ -C	dH <sub>2</sub> O	9	24.0 ± 7.0	66.7 ± 7.6	0.42 ± 0.14	35.0 ± 2.6	2.5 ± 0.8
	N- $\alpha$ -C	SW	9	0.49 ± 0.2	53.1 ± 12	0.02 ± 0.00	56.5 ± 4.5	3.4 ± 0.3
	N- $\alpha$	SW	5	0.85 ± 0.7	46.5 ± 18	0.02 ± 0.01	47.4 ± 2.5	2.9 ± 0.4
	CRD- $\alpha$	SW	7	3.20 ± 1.1	144 ± 16	0.04 ± 0.01	42.5 ± 2.7	5.1 ± 0.4
	$\alpha$ -C	SW	10	1.13 ± 0.5	87.6 ± 12	0.02 ± 0.00	42.5 ± 1.5	4.6 ± 0.4
2X2X	N- $\alpha$ -C	dH <sub>2</sub> O	9	19.3 ± 9.6	60.8 ± 9.5	0.38 ± 0.20	35.5 ± 3.5	2.5 ± 0.8
	N- $\alpha$ -C	SW	10	27.7 ± 9.2	98.6 ± 14	0.33 ± 0.14	34.2 ± 1.9	3.6 ± 0.8
	N- $\alpha$	SW	7	15.8 ± 12	79.6 ± 33	0.21 ± 0.14	34.5 ± 1.9	3.5 ± 0.7
	CRD- $\alpha$	SW	10	13.4 ± 4.6	207 ± 26	0.08 ± 0.02	33.2 ± 2.5	6.6 ± 0.8
	$\alpha$ -C	SW	10	10.3 ± 4.2	128 ± 15	0.10 ± 0.03	34.2 ± 2.1	4.5 ± 0.4
2.5X2.5X	N- $\alpha$ -C	SW	10	21.0 ± 9.9	137 ± 23	0.18 ± 0.08	30.5 ± 1.9	4.5 ± 0.9
	$\alpha$ -C	SW	8	2.55 ± 2.0	111 ± 26	0.04 ± 0.02	31.6 ± 5.1	4.5 ± 1.1
20% w/v								
1X1X	N- $\alpha$ -C	dH <sub>2</sub> O	10	2.50 ± 1.4	62.3 ± 13	0.05 ± 0.02	58.3 ± 5.7	2.5 ± 0.3
	$\alpha$ -C	dH <sub>2</sub> O	9	2.17 ± 1.9	54.9 ± 28	0.06 ± 0.04	54.5 ± 4.1	2.3 ± 0.2
	N- $\alpha$ -C	SW	10	0.94 ± 0.3	74.7 ± 12	0.03 ± 0.01	73.3 ± 3.7	3.3 ± 0.5
	N- $\alpha$	SW	10	1.27 ± 0.9	58.5 ± 15	0.04 ± 0.03	73.9 ± 3.8	2.5 ± 0.4
1.5X1.5X	N- $\alpha$ -C	dH <sub>2</sub> O	13	18.6 ± 16	74.8 ± 18	0.30 ± 0.27	45.2 ± 4.7	2.7 ± 0.5
	$\alpha$ -C	dH <sub>2</sub> O	10	17.7 ± 11	69.6 ± 14	0.32 ± 0.17	26.2 ± 2.8	2.6 ± 0.8
	N- $\alpha$ -C	SW	8	1.29 ± 0.6	90.9 ± 20	0.03 ± 0.01	60.8 ± 9.7	3.9 ± 0.5
	N- $\alpha$	SW	8	35.5 ± 17	76.7 ± 9.8	0.58 ± 0.25	41.1 ± 4.2	3.4 ± 0.6
	CRD- $\alpha$	SW	7	3.78 ± 1.9	96.6 ± 8.1	0.06 ± 0.02	60.8 ± 2.1	3.4 ± 0.3
	$\alpha$ -C	SW	10	1.88 ± 0.6	96.5 ± 19	0.03 ± 0.01	58.0 ± 3.4	3.4 ± 0.4
2X2X	N- $\alpha$ -C	dH <sub>2</sub> O	18	16.5 ± 12	91.5 ± 25	0.19 ± 0.11	38.5 ± 4.1	3.1 ± 0.4
2.5X2.5X	N- $\alpha$ -C	SW	9	2.31 ± 1.2	147 ± 40	0.03 ± 0.01	38.5 ± 4.7	5.9 ± 0.6
	N- $\alpha$	SW	10	25.6 ± 6.5	200 ± 26	0.17 ± 0.03	23.1 ± 1.3	5.3 ± 0.7
	CRD- $\alpha$	SW	8	40.8 ± 9.4	178 ± 27	0.30 ± 0.07	37.2 ± 2.2	4.5 ± 0.5
	$\alpha$ -C	SW	10	19.4 ± 8.1	188 ± 38	0.13 ± 0.04	34.5 ± 1.4	5.2 ± 0.6
25% w/v								
1X1X	N- $\alpha$ -C	dH <sub>2</sub> O	8	3.68 ± 2.8	60.5 ± 12	0.08 ± 0.06	64.7 ± 5.2	2.5 ± 0.7
	N- $\alpha$	dH <sub>2</sub> O	8	7.05 ± 3.4	67.6 ± 22	0.13 ± 0.06	46.1 ± 11	2.8 ± 0.8
	$\alpha$ -C	dH <sub>2</sub> O	4	0.57 ± 0.6	34.4 ± 18	0.03 ± 0.02	39.5 ± 6.6	2.4 ± 0.8
	N- $\alpha$ -C	SW	10	0.71 ± 0.2	55.6 ± 7.6	0.03 ± 0.01	80.9 ± 1.9	2.4 ± 0.2
	N- $\alpha$	SW	8	1.35 ± 0.2	66.6 ± 8.3	0.04 ± 0.00	81.4 ± 4.4	2.3 ± 0.2

	CRD- $\alpha$	SW	9	2.35 $\pm$ 1.1	98.4 $\pm$ 23	0.04 $\pm$ 0.01	78.1 $\pm$ 4.5	3.0 $\pm$ 0.4
	$\alpha$ -C	SW	10	1.42 $\pm$ 0.5	78.5 $\pm$ 14	0.03 $\pm$ 0.01	78.8 $\pm$ 5.0	2.9 $\pm$ 0.4
1.5X1.5X	N- $\alpha$ -C	dH <sub>2</sub> O	10	27.2 $\pm$ 14	61.6 $\pm$ 7.2	0.53 $\pm$ 0.27	51.9 $\pm$ 5.4	2.1 $\pm$ 0.8
	N- $\alpha$	dH <sub>2</sub> O	7	3.39 $\pm$ 2.8	64.7 $\pm$ 3.1	0.07 $\pm$ 0.05	56.0 $\pm$ 3.2	2.5 $\pm$ 0.1
	$\alpha$ -C	dH <sub>2</sub> O	9	21.4 $\pm$ 11	68.2 $\pm$ 7.6	0.37 $\pm$ 0.18	34.3 $\pm$ 0.9	2.3 $\pm$ 0.2
	N- $\alpha$ -C	SW	10	21.5 $\pm$ 23	85.4 $\pm$ 11	0.29 $\pm$ 0.29	57.0 $\pm$ 5.8	3.0 $\pm$ 0.4
	N- $\alpha$	SW	6	2.32 $\pm$ 1.1	69.9 $\pm$ 2.3	0.05 $\pm$ 0.02	69.2 $\pm$ 4.0	2.5 $\pm$ 0.2
	CRD- $\alpha$	SW	8	3.46 $\pm$ 1.4	113 $\pm$ 19	0.05 $\pm$ 0.01	66.4 $\pm$ 4.9	3.5 $\pm$ 0.3
	$\alpha$ -C	SW	9	34.6 $\pm$ 16	94.4 $\pm$ 5.7	0.42 $\pm$ 0.18	54.9 $\pm$ 2.0	2.8 $\pm$ 0.7
2X2X	N- $\alpha$ -C	SW	10	30.0 $\pm$ 8.2	152 $\pm$ 10	0.23 $\pm$ 0.05	40.6 $\pm$ 0.5	4.2 $\pm$ 0.2
	N- $\alpha$	SW	9	44.0 $\pm$ 5.2	110 $\pm$ 10	0.48 $\pm$ 0.09	47.5 $\pm$ 2.5	3.1 $\pm$ 0.2
	CRD- $\alpha$	SW	8	17.0 $\pm$ 11	212 $\pm$ 37	0.11 $\pm$ 0.05	40.8 $\pm$ 3.2	5.3 $\pm$ 0.8
	$\alpha$ -C	SW	9	27.3 $\pm$ 5.2	117 $\pm$ 11	0.26 $\pm$ 0.05	47.6 $\pm$ 1.1	3.5 $\pm$ 0.6
2.5X2.5X	N- $\alpha$ -C	SW	9	19.6 $\pm$ 5.3	139 $\pm$ 10	0.17 $\pm$ 0.04	40.7 $\pm$ 0.6	4.7 $\pm$ 1.1
	N- $\alpha$	SW	10	28.0 $\pm$ 5.1	198 $\pm$ 11	0.18 $\pm$ 0.03	38.4 $\pm$ 0.7	4.5 $\pm$ 0.2
	CRD- $\alpha$	SW	8	28.9 $\pm$ 20	189 $\pm$ 29	0.18 $\pm$ 0.10	41.5 $\pm$ 2.0	4.6 $\pm$ 0.4
	$\alpha$ -C	SW	10	32.2 $\pm$ 11	118 $\pm$ 45	0.35 $\pm$ 0.11	44.4 $\pm$ 7.5	3.1 $\pm$ 1.5

Mechanical data of the fibers from combining the rHIF $\alpha$  and rHIF $\gamma$ (C387S) constructs in a 1:1 ratio (Table S4). In this table, the rHIF $\alpha$  and rHIF $\gamma$ (C387S) full-length proteins are indicated by  $\alpha$  and  $\gamma$ , respectively.

**Table S4.** Mechanical properties from various concentrations of the 1:1 rHIF $\alpha$ : rHIF $\gamma$ (C387S) constructs spun at multiple stretch factors. Here, dH<sub>2</sub>O is deionized water, and SW is salt water from Instant Ocean.

	Constructs	Water Type	n	Toughness (MJ/m <sup>3</sup> )	Tensile Stress (MPa)	Strain (mm/mm)	Diameter (micron)	Elastic Modulus (GPa)
15% w/v								
1X1X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	11	2.48 $\pm$ 1.5	58.6 $\pm$ 8.1	0.05 $\pm$ 0.03	52.9 $\pm$ 0.6	2.4 $\pm$ 0.1
1.5X1.5X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	15	27.7 $\pm$ 14	64.1 $\pm$ 6.2	0.52 $\pm$ 0.29	41.6 $\pm$ 3.1	2.5 $\pm$ 0.2
	$\alpha$ : $\gamma$	SW	7	0.34 $\pm$ 0.2	40.4 $\pm$ 11	0.01 $\pm$ 0.00	50.3 $\pm$ 0.4	3.0 $\pm$ 0.1
2X2X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	20	28.9 $\pm$ 9.3	91.8 $\pm$ 15	0.38 $\pm$ 0.14	30.5 $\pm$ 2.1	2.9 $\pm$ 0.5
	$\alpha$ : $\gamma$	SW	7	22.1 $\pm$ 12	56.5 $\pm$ 18	0.50 $\pm$ 0.13	36.6 $\pm$ 0.4	3.1 $\pm$ 0.5
20% w/v								
1X1X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	8	2.24 $\pm$ 1.4	49.8 $\pm$ 11	0.06 $\pm$ 0.02	70.3 $\pm$ 5.5	2.1 $\pm$ 0.5
	N- $\alpha$ : $\gamma$ -C	dH <sub>2</sub> O	9	1.97 $\pm$ 1.3	62.8 $\pm$ 18	0.04 $\pm$ 0.02	57.7 $\pm$ 3.7	3.2 $\pm$ 0.5
	N- $\gamma$ : $\alpha$ -C	dH <sub>2</sub> O	8	5.11 $\pm$ 4.4	59.6 $\pm$ 7.6	0.11 $\pm$ 0.08	52.2 $\pm$ 2.2	2.9 $\pm$ 0.2
	$\alpha$ : $\gamma$	SW	8	1.38 $\pm$ 1.1	84.0 $\pm$ 21	0.02 $\pm$ 0.01	66.9 $\pm$ 7.8	4.8 $\pm$ 0.3
	CRD- $\alpha$ : CRD- $\gamma$	SW	8	0.65 $\pm$ 0.4	70.1 $\pm$ 19	0.02 $\pm$ 0.01	66.5 $\pm$ 2.2	5.5 $\pm$ 0.9
	N- $\alpha$ : $\gamma$ -C	SW	8	0.82 $\pm$ 0.6	79.3 $\pm$ 30	0.02 $\pm$ 0.01	65.3 $\pm$ 4.9	5.4 $\pm$ 0.3
	N- $\gamma$ : $\alpha$ -C	SW	8	0.36 $\pm$ 0.2	53.0 $\pm$ 20	0.01 $\pm$ 0.00	65.1 $\pm$ 4.0	5.0 $\pm$ 0.2
1.5X1.5X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	12	37.6 $\pm$ 4.8	68.5 $\pm$ 8.2	0.63 $\pm$ 0.07	45.5 $\pm$ 2.2	2.2 $\pm$ 0.2
	N- $\alpha$ : $\gamma$ -C	dH <sub>2</sub> O	7	1.74 $\pm$ 1.5	64.6 $\pm$ 8.9	0.04 $\pm$ 0.03	50.3 $\pm$ 3.0	3.0 $\pm$ 0.4
	N- $\gamma$ : $\alpha$ -C	dH <sub>2</sub> O	10	41.7 $\pm$ 20	74.5 $\pm$ 5.5	0.60 $\pm$ 0.28	39.1 $\pm$ 3.8	3.0 $\pm$ 0.3
	$\alpha$ : $\gamma$	SW	10	1.78 $\pm$ 1.3	90.7 $\pm$ 29	0.03 $\pm$ 0.01	49.1 $\pm$ 1.6	5.1 $\pm$ 0.1
	CRD- $\alpha$ : CRD- $\gamma$	SW	8	1.03 $\pm$ 1.0	77.8 $\pm$ 40	0.02 $\pm$ 0.01	49.0 $\pm$ 1.5	5.8 $\pm$ 0.6
	N- $\alpha$ : $\gamma$ -C	SW	6	2.95 $\pm$ 2.1	117 $\pm$ 21	0.04 $\pm$ 0.02	52.2 $\pm$ 4.7	5.7 $\pm$ 0.2
	N- $\gamma$ : $\alpha$ -C	SW	7	0.50 $\pm$ 0.3	63.3 $\pm$ 19	0.02 $\pm$ 0.01	62.7 $\pm$ 6.6	5.1 $\pm$ 0.3
2.5X2.5X	$\alpha$ : $\gamma$	SW	12	31.8 $\pm$ 12	133 $\pm$ 8.6	0.27 $\pm$ 0.10	34.5 $\pm$ 1.1	4.9 $\pm$ 0.2
	N- $\alpha$ : $\gamma$ -C	SW	10	25.3 $\pm$ 6.9	239 $\pm$ 16	0.13 $\pm$ 0.03	30.5 $\pm$ 1.0	7.0 $\pm$ 0.5

	N- $\gamma$ : $\alpha$ -C	SW	9	42.0 $\pm$ 16	189 $\pm$ 25	0.26 $\pm$ 0.10	32.0 $\pm$ 1.4	6.7 $\pm$ 0.4
25% w/v								
1X1X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	10	0.74 $\pm$ 0.3	50.6 $\pm$ 7.7	0.02 $\pm$ 0.01	81.8 $\pm$ 1.7	2.6 $\pm$ 0.1
	$\alpha$ : $\gamma$	SW	9	0.65 $\pm$ 0.5	64.4 $\pm$ 22	0.02 $\pm$ 0.01	71.6 $\pm$ 7.1	4.5 $\pm$ 0.3
1.5X1.5X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	10	3.52 $\pm$ 2.3	56.8 $\pm$ 9.3	0.07 $\pm$ 0.04	64.8 $\pm$ 1.0	2.6 $\pm$ 0.1
	$\alpha$ : $\gamma$	SW	9	0.59 $\pm$ 0.4	65.2 $\pm$ 21	0.02 $\pm$ 0.00	77.3 $\pm$ 8.4	4.4 $\pm$ 0.4
2X2X	$\alpha$ : $\gamma$	dH <sub>2</sub> O	10	37.4 $\pm$ 17	156 $\pm$ 24	0.29 $\pm$ 0.12	30.6 $\pm$ 1.7	4.5 $\pm$ 0.5
	$\alpha$ : $\gamma$	SW	8	3.34 $\pm$ 3.1	99.4 $\pm$ 26	0.04 $\pm$ 0.03	51.6 $\pm$ 7.1	5.0 $\pm$ 0.3
	$\alpha$ : $\gamma$	dH <sub>2</sub> O	11	29.8 $\pm$ 12	87.4 $\pm$ 16	0.40 $\pm$ 0.13	38.4 $\pm$ 2.3	2.4 $\pm$ 0.5
2.5X2.5X	$\alpha$ : $\gamma$	SW	10	15.1 $\pm$ 14	125 $\pm$ 13	0.13 $\pm$ 0.10	45.3 $\pm$ 4.6	5.1 $\pm$ 0.2

The final table (Table S5) looks at the mechanical properties of rHIF<sub>(C387S)</sub> constructs. N- $\gamma$ -C indicates the full-length construct (of rHIF<sub>(C387S)</sub>) that includes both termini.

**Table S5.** Mechanical properties from various concentrations of the rHIF<sub>(C387S)</sub> constructs spun at multiple stretch factors. Here, dH<sub>2</sub>O is deionized water, and SW is salt water from Instant Ocean.

	Construct	Water Type	n	Toughness (MJ/m <sup>3</sup> )	Tensile Stress (MPa)	Strain (mm/mm)	Diameter (micron)	Elastic Modulus (GPa)
15% w/v								
1X1X	N- $\gamma$ -C	dH <sub>2</sub> O	10	1.11 $\pm$ 0.84	52.9 $\pm$ 11	0.03 $\pm$ 0.01	54.7 $\pm$ 0.5	2.8 $\pm$ 0.2
	$\gamma$ -C	dH <sub>2</sub> O	10	10.5 $\pm$ 8.8	79.6 $\pm$ 24	0.14 $\pm$ 0.09	51.0 $\pm$ 10	2.8 $\pm$ 0.8
	N- $\gamma$ -C	SW	10	0.40 $\pm$ 0.3	49.7 $\pm$ 19	0.01 $\pm$ 0.01	47.7 $\pm$ 8.8	3.8 $\pm$ 0.4
	$\gamma$ -C	SW	9	0.04 $\pm$ 0.0	10.9 $\pm$ 5.3	0.01 $\pm$ 0.00	68.6 $\pm$ 5.8	1.7 $\pm$ 0.8
1.5X1.5X	N- $\gamma$ -C	dH <sub>2</sub> O	10	34.3 $\pm$ 12	70 $\pm$ 2.6	0.54 $\pm$ 0.19	37.3 $\pm$ 0.4	2.9 $\pm$ 0.2
	N- $\gamma$	dH <sub>2</sub> O	11	1.23 $\pm$ 0.7	58.9 $\pm$ 15	0.03 $\pm$ 0.01	42.6 $\pm$ 4.6	2.7 $\pm$ 0.6
	N- $\gamma$ -C	SW	10	0.40 $\pm$ 0.3	48.1 $\pm$ 21	0.01 $\pm$ 0.01	46.9 $\pm$ 4.3	3.8 $\pm$ 0.3
	N- $\gamma$	SW	7	1.09 $\pm$ 0.4	73.4 $\pm$ 11	0.03 $\pm$ 0.01	38.7 $\pm$ 2.0	3.3 $\pm$ 0.9
	$\gamma$ -C	SW	10	0.16 $\pm$ 0.1	24.5 $\pm$ 11	0.01 $\pm$ 0.00	53.7 $\pm$ 3.0	2.5 $\pm$ 0.2
2X2X	N- $\gamma$ -C	dH <sub>2</sub> O	7	28.8 $\pm$ 15	92.7 $\pm$ 21	0.35 $\pm$ 0.15	29.4 $\pm$ 2.8	3.4 $\pm$ 0.6
	N- $\gamma$ -C	SW	11	4.28 $\pm$ 3.1	105 $\pm$ 20	0.05 $\pm$ 0.03	31.6 $\pm$ 1.3	4.4 $\pm$ 0.5
	N- $\gamma$	SW	9	25.0 $\pm$ 8.5	83.4 $\pm$ 4.0	0.35 $\pm$ 0.11	29.2 $\pm$ 0.5	3.4 $\pm$ 0.3
	$\gamma$ -C	SW	8	0.34 $\pm$ 0.3	33.2 $\pm$ 18	0.02 $\pm$ 0.01	40.9 $\pm$ 3.0	2.5 $\pm$ 0.8
20% w/v								
1X1X	N- $\gamma$ -C	dH <sub>2</sub> O	11	1.98 $\pm$ 1.3	46.7 $\pm$ 4.7	0.05 $\pm$ 0.03	76.9 $\pm$ 2.3	2.1 $\pm$ 0.1
	N- $\gamma$	dH <sub>2</sub> O	8	17.1 $\pm$ 16	73.1 $\pm$ 18	0.27 $\pm$ 0.26	38.8 $\pm$ 3.5	3.1 $\pm$ 0.6
	$\gamma$ -C	dH <sub>2</sub> O	9	0.26 $\pm$ 0.3	28 $\pm$ 10	0.02 $\pm$ 0.01	54.2 $\pm$ 1.7	2.1 $\pm$ 0.2
	N- $\gamma$ -C	SW	10	0.41 $\pm$ 0.2	41.8 $\pm$ 12	0.01 $\pm$ 0.00	62.5 $\pm$ 7.2	3.5 $\pm$ 0.3
	N- $\gamma$	SW	9	0.92 $\pm$ 0.6	67.7 $\pm$ 28	0.02 $\pm$ 0.01	55.5 $\pm$ 3.6	3.6 $\pm$ 0.7
	CRD- $\gamma$	SW	9	1.43 $\pm$ 0.9	60.7 $\pm$ 23	0.04 $\pm$ 0.01	113 $\pm$ 29	2.0 $\pm$ 0.7
1.5X1.5X	N- $\gamma$ -C	dH <sub>2</sub> O	12	50.8 $\pm$ 6.6	70.0 $\pm$ 6.4	0.84 $\pm$ 0.09	43.5 $\pm$ 2.0	2.5 $\pm$ 0.3
	N- $\gamma$	dH <sub>2</sub> O	8	63.7 $\pm$ 43	101 $\pm$ 38	0.78 $\pm$ 0.27	30.4 $\pm$ 5.1	3.2 $\pm$ 0.8
	$\gamma$ -C	dH <sub>2</sub> O	5	0.39 $\pm$ 0.4	33.6 $\pm$ 15	0.02 $\pm$ 0.01	42.5 $\pm$ 5.8	2.1 $\pm$ 0.3
	N- $\gamma$ -C	SW	8	0.47 $\pm$ 0.2	50.9 $\pm$ 10	0.02 $\pm$ 0.00	46.7 $\pm$ 3.3	3.7 $\pm$ 0.3
	N- $\gamma$	SW	6	0.87 $\pm$ 0.4	75.1 $\pm$ 22	0.02 $\pm$ 0.01	47.4 $\pm$ 3.0	3.7 $\pm$ 0.6
	CRD- $\gamma$	SW	10	1.20 $\pm$ 0.9	74.2 $\pm$ 31	0.03 $\pm$ 0.01	59.0 $\pm$ 5.8	3.3 $\pm$ 0.5
	$\gamma$ -C	SW	10	0.54 $\pm$ 0.3	48.6 $\pm$ 11	0.02 $\pm$ 0.01	59.2 $\pm$ 8.6	2.6 $\pm$ 0.3
2X2X	N- $\gamma$ -C	dH <sub>2</sub> O	11	38.5 $\pm$ 9.9	95.9 $\pm$ 20	0.46 $\pm$ 0.03	36.5 $\pm$ 3.6	3.2 $\pm$ 0.7
	N- $\gamma$	dH <sub>2</sub> O	10	59.2 $\pm$ 23	92.8 $\pm$ 29	0.75 $\pm$ 0.14	28.2 $\pm$ 3.2	3.0 $\pm$ 0.8
	$\gamma$ -C	dH <sub>2</sub> O	8	0.35 $\pm$ 0.3	33.4 $\pm$ 14	0.02 $\pm$ 0.01	37.4 $\pm$ 1.3	2.1 $\pm$ 0.4
2.5X2.5X	N- $\gamma$ -C	SW	10	9.87 $\pm$ 7.5	87.2 $\pm$ 10	0.13 $\pm$ 0.09	35.6 $\pm$ 2.9	4.1 $\pm$ 0.3
	N- $\gamma$	SW	10	15.6 $\pm$ 8.7	178 $\pm$ 32	0.11 $\pm$ 0.07	27.4 $\pm$ 2.9	4.9 $\pm$ 1.2
	$\gamma$ -C	SW	11	4.76 $\pm$ 3.0	78.8 $\pm$ 8.8	0.08 $\pm$ 0.04	33.1 $\pm$ 1.5	3.4 $\pm$ 0.2

25% w/v								
1X1X	N-γ-C	dH <sub>2</sub> O	7	4.32 ± 4.1	56.5 ± 5.2	0.09 ± 0.07	60.7 ± 8.9	2.1 ± 0.2
	N-γ	dH <sub>2</sub> O	5	0.61 ± 0.6	32.7 ± 35	0.05 ± 0.06	70.2 ± 26	2.0 ± 1.7
	γ-C	dH <sub>2</sub> O	9	0.06 ± 0.0	10.7 ± 4.5	0.02 ± 0.00	61.4 ± 7.5	1.0 ± 0.2
	N-γ-C	SW	11	0.99 ± 0.4	71.8 ± 13	0.03 ± 0.01	69.4 ± 3.5	3.1 ± 0.2
	N-γ	SW	9	0.39 ± 0.4	32 ± 16	0.02 ± 0.01	111 ± 7.3	2.2 ± 0.3
	CRD-γ	SW	11	0.62 ± 0.5	51.9 ± 27	0.02 ± 0.01	87.8 ± 22	3.1 ± 0.9
	γ-C	SW	14	0.38 ± 0.2	40.7 ± 7.2	0.02 ± 0.00	79.0 ± 3.8	2.4 ± 0.3
1.5X1.5X	N-γ-C	dH <sub>2</sub> O	10	21.9 ± 10.5	73.3 ± 16	0.33 ± 0.14	46.0 ± 4.4	2.5 ± 0.5
	γ-C	dH <sub>2</sub> O	12	0.77 ± 0.3	48.4 ± 10	0.03 ± 0.01	51.0 ± 8.3	2.3 ± 0.5
	N-γ-C	SW	10	1.13 ± 0.3	78.4 ± 8.8	0.03 ± 0.00	63.3 ± 1.0	3.3 ± 0.1
	N-γ	SW	8	2.12 ± 1.0	102 ± 23	0.04 ± 0.01	64.6 ± 9.1	3.3 ± 0.6
	CRD-γ	SW	11	1.54 ± 0.8	79.9 ± 11	0.03 ± 0.01	63.3 ± 3.7	3.9 ± 0.2
2X2X	N-γ-C	dH <sub>2</sub> O	11	12.1 ± 5.3	94.6 ± 28	0.15 ± 0.05	37.3 ± 4.4	3.2 ± 0.7
	N-γ-C	SW	12	22.2 ± 13	116 ± 9.7	0.21 ± 0.11	43.0 ± 1.2	4.2 ± 0.2
	N-γ	SW	6	0.66 ± 0.6	58.7 ± 28	0.02 ± 0.01	61.5 ± 2.0	3.2 ± 0.9
2.5X2.5X	N-γ-C	SW	11	13.6 ± 8.1	144 ± 13	0.11 ± 0.06	39.2 ± 3.0	4.8 ± 0.3
	N-γ	SW	12	37.9 ± 27	108 ± 6.5	0.42 ± 0.29	53.3 ± 2.3	3.5 ± 0.2