Supporting Information

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Engineered Spider Silk Proteins for Biomimetic Spinning of Fibers with Toughness Equal to Dragline Silks

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1 Department of Biosciences and Nutrition, Karolinska Institutet, Neo, 14183 Huddinge, Sweden
2 Laboratory for Bioinspired, Bionic, Nano, Meta, Materials & Mechanics, Department of Civil, Environmental and Mechanical Engineering, University of Trento, Via Mesiano 77, 38123 Trento, Italy.
3 Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden
4 Present address: Spiber Technologies AB, AlbaNova University Center, SE-10691 Stockholm, Sweden
5 School of Engineering and Materials Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK.
6 Department of Physical Organic Chemistry, Latvian Institute of Organic Synthesis, Riga, LV-1006, Latvia.
7 Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, 10691 Stockholm, Sweden.
Rosetta energy (kcal/mol)

-24.6 kcal/mol

-24.9 kcal/mol

-25.1 kcal/mol

-28.3 kcal/mol

-28.3 kcal/mol
-29.4 kcal/mol

-26.5 kcal/mol

-26.5 kcal/mol

-29.1 kcal/mol

-29.1 kcal/mol
Figure S 1: Rosetta energy profiles of all engineered mini-spidroins. Bars show Rosetta energies for moving hexapeptides (indicated at the first residue of each hexapeptide), red bars indicate Rosetta energies equal or below -23 kcal/mol (dashed line). Green bars indicate Rosetta energies above the threshold and are unlikely to form steric zippers (https://services.mbi.ucla.edu/zipperdb/). Lowest Rosetta energies are indicated.
Figure S 2: Expression levels of constructs. Constructs with Thr, Val, and Ile substitutions. M- marker, BI- before induction, AE- after expression, box indicates target protein.

(AI)7-(AI)7

(AI)15-(AI)17

(AAI)3-(AAI)3

(AII)14-A14

IA6IA6I-A14

BI AE BI AE
Figure S 3: Solubility and purification of constructs evaluated by SDS-PAGE. M- marker, S- soluble fraction, P- pellet (resuspended), FT- flow-through the IMAC, C-target construct.
Figure S 4: Pearson correlation matrix (r values) of expression levels, solubility, yield, spinnability, hydrophobicity (only the repeat region), number of substitutions and Rosetta energies. Solubility, expression levels and spinnability were rated from very high (3) to none (0), (according to Table 2). * indicates statistical significance (p < 0.05).

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|               | Expression levels | Solubility after cell lysis | Average protein yield | Spinnability into fibers | Hydrophobicity | No of substitutions | Rosetta energy |
|---------------|-------------------|-----------------------------|-----------------------|--------------------------|----------------|---------------------|---------------|
| Expression levels | 1.00              | 0.30                        | 0.32                  | -0.29                    | 0.22           | -0.47               | 0.16          |
| Solubility after cell lysis | 0.30              | 1.00                        | 0.63*                 | -0.75*                   | -0.78*         | 0.77*               |               |
| Average protein yield | 0.32              | 0.23*                       | 1.00                  | -0.34                    | -0.85*         | 0.39                |               |
| Spinnability into fibers | -0.29             | -0.75*                      | 0.47                  | 1.00                     | -0.41          | -0.41               | 0.23          |
| Hydrophobicity   | 0.22              | -0.75*                      | -0.34                 | -0.41                    | 1.00           | 0.29                | -0.84*        |
| No of substitutions | -0.47             | -0.78*                      | -0.85*                | -0.41                    | 0.29           | 1.00                | -0.51*        |
| Rosetta energy   | 0.16              | 0.77*                       | 0.39                  | 0.23                     | -0.84*         | -0.51*              | 1.00          |
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Figure S 5: Mechanical properties of spinnable constructs continued. A-G) Representative stress-strain curves of all engineered proteins that were spun into fibers. Each panel shows 8 stress strain curves. H) diameter of the fibers.
Figure S6: Pearson correlation matrix (r values) of mechanical properties and secondary structure content. * indicates statistical significance (p < 0.05).

|            | Diameter | Strength | Strain at break | Toughness modulus | β-sheets | α-helix/ random coil | Other secondary structures |
|------------|----------|----------|-----------------|-------------------|----------|----------------------|--------------------------|
| Diameter   | 1.00     | -0.60    | -0.76*          | -0.84*            | -0.34    | 0.53                 | -0.15                    |
| Strength   | -0.60    | 1.00     | 0.51            | 0.75*             | 0.49     | -0.65                | 0.08                     |
| Strain at break | -0.76*    | 0.51     | 1.00            | 0.95*             | 0.47     | -0.29                | -0.27                    |
| Toughness modulus | -0.84*       | 0.75*    | 0.95*           | 1.00              | 0.52     | -0.46                | -0.16                    |
| β-sheets   | -0.34    | 0.49     | 0.47            | 0.52              | 1.00     | -0.64                | -0.56                    |
| α-helix/ random coil | 0.53       | -0.65    | -0.29           | -0.46             | -0.64    | 1.00                 | -0.28                    |
| Other secondary structures | -0.15       | 0.08     | -0.27           | -0.16             | -0.56    | -0.28                | 1.00                     |
Figure S 7: Fits of absorbance spectra and second derivative of fibers spun from engineered mini-spidroins. Band 1, 5, 6, 7: β-sheets; Band 2, 3: others; Band 4: α-helix/ random-coil; Band 8: side chains.
Figure S 8: Expression and purification of (A$_3$I)$_3$-A$_{14}$ produced in a bioreactor-based E. coli fed batch culture. B) Cultivation parameters for the expression of (A$_3$I)$_3$-A$_{14}$. The stirring speed (black), pO2 (red), pH (grey), temperature (orange), feeding rate (blue), and the optical cell density (green) are shown. The induction point (22 h after inoculation) is indicated by a black dashed line. B) SDS PAGE of lane 1: total cell content 18.5 h after induction (20-fold dilution); lane 2: total cell content 21.5 h after induction (20-fold dilution); lane 3: total cell content after cell lysis; lane 4: pellet after centrifugation; lane 5: cell lysate; lane 6: flow-through; lane 7: wash using 5 mM imidazole; lane 8: eluate using 200 mM imidazole (5-fold dilution). S1-S5 reference samples of A$_{15}$-A$_{14}$. S1: 0.112 mg/mL; S2: 0.225 mg/mL; S3: 0.45 mg/mL; S4: 0.9 mg/mL; S5: 1.8 mg/mL.
Figure S 9: Mechanical properties of fibers produced from mini-spidroins expressed using a shake-flask or a bioreactor. A) Strength, B) strain at break, C) toughness modulus and D) diameter. Whiskers show standard deviation. **** p < 0.0001
Table S1: Amino acid sequences of A15-A14 and engineered constructs thereof:

| Name | Amino acid sequence |
|------|---------------------|
| A15-A14 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| (AT)7-(AT)7 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| (A3T)3-(A3T)3 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| (AV)7-(AV)7 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| (AV)7-A14 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| V15-A14 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
| (A3V)3-(A3V)3 | MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST IAQSMVQSIQLAQQRTSNKLQLANMAFASSMAIAASEEGLGSSTKTSS ISAAMSNAFLQTITGGVNVQPINEITQLVSMFAAQGMNDVSAGNSGRGQGG YGGGSGGNGAAAAAIAAAAAAGGGGQGQGGYGRQGSAAGSAAAAAAA AAAAASGQGQGGYGGCGGQGQGGYQGNGSNTSGGYGGYGTSSAAGAGVAGGS YAGAVNRLSSAEASRVSSNIAIAISGGAASAPSVISIYSGVAVSGVSSNEALIQ QALESSALYHVLSASIGNVSSVGVDSTLNVQDVSQYYV* |
15-(A3I)3

MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST
IAQSMVQSIQLAAQGRTPNKLQALNMAFASSMAEIASEEGGSLSKTSS
IASAMSNAFLQTGTVNQPFINEITQLVSMAFAQAGMNDVSAIGNSGRGG
YGQGSNGNIAAAAAIAAIAGQGGGGYGRQSGQAGSAAAIAMIAIAIA
AIAAAGSGGGGGYGGQQGGYQQGGYGSQGNTSGGGYGTSAAGAGVAGSYA
GAVNRLSSAEASRVSSNIAAIASGGASALPSVISNIYSVVAVSVGSNEALIQA
LELLSALVHVLSSAIGNVSSVGVSTLNVQDSVQGQYVG*

(A2I)4-A14

MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST
IAQSMVQSIQLAAQGRTPNKLQALNMAFASSMAEIASEEGGSLSKTSS
IASAMSNAFLQTGTVNQPFINEITQLVSMAFAQAGMNDVSAIGNSGRGG
YGQGSNGNIAAAAAIAAIAGQGGGGYGRQSGQAGSAAAIAMIAIAIA
AIAAAGSGGGGGYGGQQGGYQQGGYGSQGNTSGGGYGTSAAGAGVAGSYA
GAVNRLSSAEASRVSSNIAAIASGGASALPSVISNIYSVVAVSVGSNEALIQA
LELLSALVHVLSSAIGNVSSVGVSTLNVQDSVQGQYVG*

IA6-A6-A14

MGHHHHHHMSHTTPWTNPGLAENFMNSFMQGLSSMPGFTASQLDDMST
IAQSMVQSIQLAAQGRTPNKLQALNMAFASSMAEIASEEGGSLSKTSS
IASAMSNAFLQTGTVNQPFINEITQLVSMAFAQAGMNDVSAIGNSGRGG
YGQGSNGNIAAAAAIAAIAGQGGGGYGRQSGQAGSAAAIAMIAIAIA
AIAAAGSGGGGGYGGQQGGYQQGGYGSQGNTSGGGYGTSAAGAGVAGSYA
GAVNRLSSAEASRVSSNIAAIASGGASALPSVISNIYSVVAVSVGSNEALIQA
LELLSALVHVLSSAIGNVSSVGVSTLNVQDSVQGQYVG*
Table S 2: Hexapeptides with lowest Rosetta energies and hydropathy of the engineered mini-spidroins.

| Construct       | Example of hexapeptide | Rosetta energy (kcal/mol) | Hydropathy |
|-----------------|-------------------------|---------------------------|------------|
| A₁₅₋A₁₄         | AAAAAA                  | -24.6                     | -0.168     |
| (AT)₇-(AT)₇     | ATATAT                  | -24.9                     | -0.617     |
| (A₃T)₃₋(A₃T)₃  | AAATAA                  | -25.1                     | -0.36      |
| (AV)₇-(AV)₇     | AVAVAV                  | -28.3                     | 0.263      |
| (AV)₇₋A₁₄      | AVAVAV                  | -28.3                     | 0.047      |
| V₁₅₋A₁₄         | VVVVVV                  | -29.4                     | 0.294      |
| (A₃V)₃₋(A₃V)₃  | AVAAAV                  | -26.5                     | 0.017      |
| (A₃V)₃₋A₁₄     | AVAAAV                  | -26.5                     | -0.076     |
| (AI)₇-(AI)₇     | AIAIAI                  | -29.1                     | 0.317      |
| A₁₅₋(AI)₇       | AIAIAI                  | -29.1                     | 0.074      |
| (AIA₂)₃₋(AIA₂)₃ | AIAIAI                  | -26.8                     | 0.109      |
| (A₃I)₃₋(A₃I)₃  | AIAIAI                  | -26.8                     | 0.04       |
| (A₁₄I)₃₋A₁₄    | AIAIAI                  | -26.8                     | -0.064     |
| A₁₅₋(A₃I)₃     | AIAIAI                  | -26.8                     | -0.064     |
| (A₁₄I)₄₋A₁₄    | AIAIAI                  | -27.1                     | -0.029     |
| IA₈₋A₁₄₋A₁₄    | AAAIAA                  | -26.1                     | -0.064     |
Table S3: Mechanical properties of spinnable constructs and their standard deviation:

|                      | Strength (MPa) | Strain at break (%) | Toughness modulus (MJ/m^3) | Diameter (μm) | Young's modulus (MPa) |
|----------------------|---------------|---------------------|----------------------------|--------------|-----------------------|
| **A_{15}-A_{14}**    | 44.09 ± 19.64 | 47.96 ± 55.82       | 18.19 ± 20.34              | 13.40 ± 3.70 | 1685 ± 466            |
| **(A_3T)^3-(A_3T)_3**| 67.80 ± 30.58 | 8.31 ± 15.15        | 4.70 ± 12.34               | 19.41 ± 8.51 | 2183 ± 921            |
| **(A_3V)^3-(A_3V)_3**| 70.76 ± 24.37 | 3.26 ± 1.82         | 1.31 ± 1.07                | 17.12 ± 2.95 | 2786 ± 861            |
| **(A_3V)^3-A_{14}**  | 64.51 ± 19.73 | 78.59 ± 59.89       | 49.58 ± 45.26              | 9.09 ± 2.68  | 3348 ± 1121           |
| **(A_3I)^3-A_{14}**  | 131.63 ± 31.87| 160.44 ± 37.00      | 145.63 ± 42.18             | 4.16 ± 0.78  | 3501 ± 948            |
| **A_{15}-(A_3I)_3**  | 78.78 ± 34.87 | **203.52 ± 120.39** | 125.33 87.99               | 10.88 ± 2.59 | 3045 ± 964            |
| **(A_3I)^3-A_{14}**  | 45.41 ± 9.73  | 84.51 ± 89.26       | 37.01 ± 40.61              | 12.11 ± 3.33 | 2463 ± 653            |
| **(A_3I)^3-A_{14}^a**| 95.68 ± 39.53 | 150.76 ± 57.09      | 90.96 ± 45.19              | 6.13 ± 4.00  | 2854 ± 868            |

a) protein expressed in a bioreactor

Table S4: Secondary structure content as determined by FTIR spectroscopy:

|                          | A_{15}-A_{14} | (A_3T)^3-(A_3T)_3 | (A_3V)^3-(A_3V)_3 | (A_3V)^3-A_{14} | (A_3I)^3-A_{14} | A_{15}-(A_3I)_3 | (A_2I)^4-A_{14} |
|--------------------------|--------------|-------------------|-------------------|-----------------|-----------------|----------------|----------------|
| α-helix/random coil (%)  | 40.2         | 38.5              | 32.9              | 30.3            | 31.3            | 35.0           | 39.5           |
| β-sheets (%)             | 40.1         | 40.0              | 40.7              | 44.7            | 43.2            | 47.0           | 32.3           |
| Other (%)                | 19.7         | 21.5              | 26.4              | 25.0            | 25.5            | 18.0           | 28.3           |