# Supporting Information

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### SI Materials and Methods

***piggybac* Vector Constructions.** A key element of the *piggyBac* vectors was the 2.4 kbp $A2S8^{14}$ silk-like sequence, which combines flagelliform silk-like elastic $(GPGGA)_8$ and dragline silk-like strength (linker-$alanine_8$) motifs. This sequence links the A2 flagelliform-like motif, which corresponds to a doubled A1 motif (11), to the S8 strength motif (11) to produce the [A2S8] basic repeat. The final $A2S8^{14}$ spider silk sequence, which contains 14 iterations of the [A2S8] basic repeat, was produced using the doubling strategy described by Teulé et al. (11) and cloned into pBluescript SKII+. Several other key elements needed for the *piggyBac* vector constructions were isolated by polymerase chain reactions with genomic DNA isolated from the silk glands of *Bombyx mori* strain P50/Daizo and the gene-specific primers shown in Table S1. The resulting DNA fragments included the fibroin heavy chain (fhc) major promoter and upstream enhancer element (MP-UEE), two versions of the fhc basal promoter and N-terminal domain (NTD) (exon 1/intron 1/exon 2) with different 5′- and 3′-flanking restriction sites, the fhc C-terminal domain (CTD) [3′ coding sequence and poly(A) signal], and enhanced green fluorescent protein (EGFP). In each case, the amplification products were gel-purified, and DNA fragments of the expected sizes were excised and recovered. Subsequently, the fhc MP-UEE, fhc CTD, and EGFP fragments were cloned into pSLfa1180fa (pSL); the two different NTD fragments were cloned into pCR4-TOPO (Invitrogen); and *Escherichia coli* transformants containing the correct amplification products were identified by restriction mapping and verified by sequencing. These fragments were then used to assemble the *piggyBac* vectors used in this study as follows. The synthetic $A2S8_{14}$ spider silk sequence was excised from the pBluescript SKII+ plasmid precursor with BamHI and BspEI, gel-purified, recovered, and subcloned into the corresponding sites upstream of the CTD in the pSL intermediate plasmid described above. This step yielded a plasmid designated pSL-spider 6-CTD. A NotI/BamHI fragment was then excised from one of the pCR4-TOPO-NTD intermediate plasmids described above, gel-purified, recovered, and subcloned into the corresponding sites upstream of the spider 6-CTD sequence in pSL-spider 6-CTD to produce pSL-NTD-spider 6-CTD. In parallel, a NotI/XbaI fragment was excised from the other pCR4-TOPO-NTD intermediate plasmid described above, gel-purified, recovered, and subcloned into the corresponding sites upstream of the EGFP amplimer in the pSL-EGFP intermediate plasmid described above. This produced a plasmid containing an NTD-EGFP fragment, which was excised with NotI and BamHI and subcloned into the corresponding sites upstream of the spider 6-CTD sequences in pSL-spider 6-CTD. The MP-UEE fragment was then excised with SfiI and NotI from the pSL intermediate plasmid described above, gel-purified, recovered, and subcloned into the corresponding sites upstream of the NTD-spider 6-CTD and NTD-EGFP-spider 6-CTD sequences in the two different intermediate pSL plasmids described above. Finally, the completely assembled MP-UEE-NTD-$A2S8_{14}$-CTD or MP-UEE-NTD-EGFP-$A2S8_{14}$-CTD cassettes were excised with AscI and FseI from the respective final pSL plasmids and subcloned into the corresponding sites of pBAC[3XP3-DsRedaf] (2). This final subcloning step yielded two separate *piggyBac* vectors that were designated spider 6 and spider 6-EGFP to denote the absence or presence of the EGFP marker.

**Mechnical Testing Data Analysis.** The stress/strain curves from the data set gathered for each fiber were plotted using MATLAB (Version 7.1) to determine toughness (or energy to break), Young's modulus (initial stiffness), maximum stress (ultimate or peak stress), breaking stress (stress at failure), and maximum extension (maximum percentage of strain). The statistical significance of the mechanical testing data was assessed in a one-way ANOVA analysis ($\alpha = 0.05\%$). The original hypothesis ($H_0$) was that the mean values obtained with all samples were equal. $H_0$ was rejected, and the mean values were considered to be significantly different when $F$ was larger than the $F$ critical ($F > F_{crit}$) and $P$ was <0.05. A Scheffé's test (Scheffé's critical value: $F_{SC} = 10.2$) was then used to determine the origin of the differences observed between the mean values.


1. Teulé F, Furin WA, Cooper AR, Duncan JR, Lewis RV (2007) Modifications of spider silk sequences in an attempt to control the mechanical properties of the synthetic fibers. *J Mater Sci* 42:8974–8985.
2. Horn C, Schmid BG, Pogoda FS, Wimmer EA (2002) Fluorescent transformation markers for insect transgenesis. *Insect Biochem Mol Biol* 32:1221–1235.


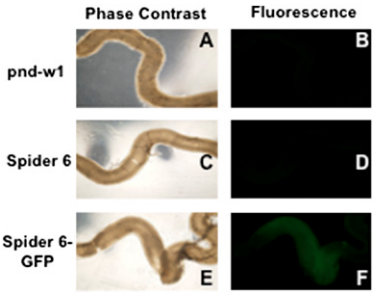


**Fig. S1.** Expression and localization of a chimeric silkworm/spider silk protein in pnd-w1 (*A* and *B*), Spider 6 (*C* and *D*), and Spider 6-GFP (*E* and *F*) silkworm silk glands. Silk glands were excised, bombarded with the spider 6 or spider 6-GFP *piggyBac* vectors, and examined by phase contrast (*A*, *C*, and *E*) or fluorescence microscopy (*B*, *D*, and *F*), as described in *Materials and Methods*.

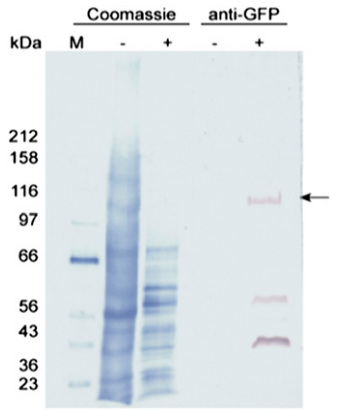


**Fig. S2.** Expression of a chimeric silkworm/spider silk protein in silkworm silk glands. Silk glands were excised, bombarded with the empty *piggyBac* (–) or spider 6-GFP *piggyBac* (+) vectors, and incubated in Grace's medium for 48–72 h, as described in *Materials and Methods*. The glands were then homogenized in 0.03 M sodium phosphate buffer (pH 7.4), mixed with Laemmli buffer, and samples were analyzed by SDS/PAGE with Coomassie staining (lanes 1–3) or immunoblotting (lanes 4 and 5) with an anti-GFP antibody. The numbers on the left side of the image indicate the sizes of protein standards in kilodaltons, and the arrow on the right side marks a major immunoreactive band with an apparent molecular weight of ~116 kDa, which was detected only in extracts of silk glands bombarded with the spider 6-GFP construct.

**Table S1. Synthetic spider silk protein content in composite silkworm/spider silk fibers**

| | | | | | | Spider 6 | | | Spider 6 GFP, line 1 | | | Spider 6-GFP, line 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A2S8[14] standards | | | | | Test 1 | Test 2 | Average | Test 1 | Test 2 | Average | Test 1 | Test 2 | Average |
| Intensity | 2,922 | 3,030 | 3,139 | 3,224 | 3,120 | 3,150 | | 3,213 | 3,051 | | 2,953 | 2,795 | | |
| Micrograms in well | 3.00 | 4.00 | 5.00 | 6.00 | 4.81 | 5.10 | | 5.72 | 4.13 | | 3.17 | 1.63 | | |
| Milligrams in undiluted solution | | | | | 1.28 | 1.36 | | 1.53 | 1.10 | | 0.85 | 0.43 | | |
| Percentage of total protein | | | | | 4.33 | 4.60 | 4.47 | 5.15 | 3.72 | 4.44 | 2.82 | 1.45 | 2.13 | |

A total of 30 mg of degummed silk from two individual transgenic animals in each line was sequentially extracted as described in *Materials and Methods*, and samples of the final extract were used for immunoblotting assays to estimate the proportions of synthetic spider silk to total protein in each fiber type. Known amounts of the synthetic spider silk protein from *E. coli* were used as the standards, and band intensities were estimated by laser-scanning densitometry using a Bio-Rad GelDoc system, as described in *Materials and Methods*.

**Table S2. Statistical analysis of mechanical data from all silk fiber samples**

| | Break stress | Maximum stress | Maximum strain | Young's modulus | Toughness |
|---|---|---|---|---|---|
| ANOVA *P* value | <0.00001 | <0.00001 | <0.00001 | <0.00001 | <0.00001 |
| Origin of differences (Scheffé's test results) | | | | | |
| Spider 6 | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from all other groups. | Different from parental control (pnd-w1). |
| Spider 6-GFP, line 1 | Different from native spider dragline silk. | Different from native spider dragline silk. | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from all other groups. | Different from parental control (pnd-w1). |
| Spider 6-GFP, line 4 | Different from parental control (pnd-w1) and native spider dragline silk. | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from parental control (pnd-w1) and different from native spider dragline silk. | Different from all other groups. | Different from parental control (pnd-w1). |
| pnd-w1 | Different from native spider dragline silk, spider 6, and spider 6-GFP (line 4). | Different from native spider dragline silk, spider 6, and spider 6-GFP (line 4). | Different from all three transgenic silkworms. | Different from all other groups. | Different from all other groups. |
| Dragline | Different from all other groups. | Different from all other groups. | Different from all three transgenic silkworms. | Different from all other groups. | Different from parental control (pnd-w1). |

**Table S3. PCR amplification of key components of the *piggyBac* vectors used in this study**

| Primer No. | Name | Sequence (5′–3′) | Restriction site(s) added | Template DNA | Primer combination for PCRs | Amplification products and sizes |
|---|---|---|---|---|---|---|
| 1 | Major pro (SP) | TAACTCGAGGCTCAAAGCCTCATCCCAATTTGGAG | 5′ XhoI | *B. mori* genomic DNA | 1 and 2 | fhc major promoter −5,000 to −3,844 (1,157 bp) |
| 2 | Major pro (ASP) | ATACCGCGGTGCAGAAGACAAGCCATCGCAACGGTG | 3′ SacII | | | |
| 3 | UEE (SP) | ATACCGCGGAAAGATGTTTTGTACGGAAAGTTTGAA | 5′ SacII | | 3 and 4 | fhc enhancer −1,659 to −1,590 (70 bp) |
| 4 | UEE (ASP) | TTAGCGGCCGCCGAACCCTAAAACATTGTTACGTTACGTT ACTTG | 3′ NotI | | | |
| 5 | fhc pro + NTD (SP) | TAAGCGGCCGCGGGAGAAAGCATGAAGTAAGTTCTTTA AATATTACAAAAA | 5′ NotI | | 5 and 6(–) 5 and 7(+) | Spider 6 EGFP (–) or (+) expression cassettes |
| 6 | fhc Pro + NTD (ASP) | ATAGGATCCACGACTGCAGCACTAGTGCTGCTGAAATCGC | 3′ BamHI | | | fhc basal promoter and 5′ cds +62,118 to +63,816 (1,744 bp) |
| 7 | fhc Pro + NTD (ASP for EGFP) | ATATCTAGAACGACTGCAGCACTAGTGCTGCTGAAATCGC | 3′ XbaI | | | |
| 8 | EGFP (SP) | CAATCTAGACGTGAGCAAGGGCGAGGAGCTGTTCACC | 5′ XbaI | pEGFP-N1 plasmid DNA | 8 and 9 | EGFP (720 bp) |
| 9 | EGFP (ASP) | TAAGGATCCAGCTTGTACAGCTCGTCCATGCCGAGAG | 3′ BamHI | | | |
| 10 | fhc CTD (SP) | ATACCCGGGAAGCGTCAGTTACGGAGCTGGCAG | 5′ XmaI | *B. mori* genomic DNA | 10 and 11 | fhc 3′ cds and poly(A) signal +79,021 to +79,500 (480 bp) |
| 11 | fhc CTD (ASP) | CAAGCTGACTATAGTATTCTTAGTTGAGAAGGCATAC | 3′ SalI | | | |