Supporting Information

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Antibiotic Spider Silk: Site-Specific Functionalization of Recombinant Spider Silk Using “Click” Chemistry

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Methods

Reagents were purchased from Sigma Aldrich unless stated otherwise.

Transformation of methionine auxotroph DL41

_E. coli_ DL41 (Yale Coli Genetic Stock Center) were transformed with pJExpress401 plasmid encoding Hexa-His tag/sol tag/ Thioredoxin/ Enterokinase site/ Lys-C site/ Thrombin site/4RepCT (synthesized by Life Technologies). Transformed bacteria were streaked onto LB agar plates containing kanamycin and incubated at 37°C.

Expression and purification of 4RepCT and 4RepCT\(^{3\text{Aha}}\)

For 4RepCT containing only canonical amino acids, selected DL41 colonies were grown at 37°C in Luria- Bertani media containing kanamycin (LB\(^{\text{KAN}}\)) to an OD\(_{600}\) of 1.0 – 1.4, induced with 1 mM IPTG and incubated for a further 4 hours at 37°C. For 4RepCT\(^{3\text{Aha}}\), DL41 cells were grown at 37°C in M9 minimal media containing kanamycin and all amino acids including methionine to an OD\(_{600}\) of 1.0 – 1.4. Cells were transferred to M9 minimal media with 50 mg/L L-azidohomoalanine (L-Aha) (synthesised in-house following references 27 and 28 in main text) in place of methionine, induced with 0.5 mM IPTG and incubated overnight at 25°C. Cells were harvested by centrifugation for 12 minutes at 3500 x g, resuspended in Buffer A (20 mM Tris pH 7.5, 400 mM NaCl, 15 mM imidazole) and lysed by sonication. The lysate was centrifuged for 25 minutes at 40 000 x g and loaded onto a Ni-IMAC column (GE Life Sciences). Loosely bound proteins were eluted with Buffer A. Tightly bound 4RepCT/\(^{3\text{Aha}}\) was eluted from the column with ~250 mM imidazole in buffer containing 20 mM Tris pH 7.5 and 400 mM NaCl. Purified 4RepCT/\(^{3\text{Aha}}\) was dialysed against 20 mM Tris pH 8.0 overnight.
**Fibre formation**

Fibres were made by incubation with thrombin (5 units), Lys-C (Promega) (2 µg) or Enterokinase (5 units). Cleavage was achieved at a fusion protein concentration of 1-2 mg/ml, 1 mM potassium phosphate pH 7.5 and a total volume of 1-3 ml. The reaction was contained in sealed tubes, incubated at 30°C with gentle rocking for 4 hours.

**Conjugation of 4RepCT\(^{3\text{Aha}}\) with fluorophores**

Soluble 4RepCT\(^{3\text{Aha}}\) was labelled with 0.1 mM FAM alkyne (3′-hydroxy-6′-(2-propyn-1-yloxy)-3H-spiro[2-benzofuran-1,9′-xanthen]-3-one) (synthesised by Inderpal Kaur Sehmi) or 0.1 mM Alexa Fluor 594\(^{®}\) alkyne (Life Technologies). THPTA (3 [tris(3-hydroxypropyltriazolylmethyl)amine], sodium ascorbate and CuSO\(_4\) to final concentrations of 5.0 mM, 5.0 mM and 0.5 mM respectively. Preformed 4RepCT\(^{3\text{Aha}}\) fibres of ~1cm in length were labelled with FAM alkyne or Alexa Fluor 594\(^{®}\) as above, however 5.0 mM of Cu[I] was used in order to avoid inconsistency of catalyst concentration along the fibre structure. Reactions were protected from light and incubated at room temperature with gentle rocking for 4 hours. After the incubation period, fibres were transferred to 15 ml Falcon tubes and washed three times with 5 ml 20 mM Tris pH 7.5 and incubated overnight in 10% v/v DMSO milli-Q water at room temperature with gentle rocking.

**Fluorescence imaging**

Labelled fibres were imaged using a Nikon Eclipse Ti fluorescence microscope with FITC (467-498 excitation 513-556 emission) and Texas Red (542-582 excitation 604-644 emission) filter cubes. Silks were observed under 60 X (Nikon Apo TIRF NA 1.49 objective) and 4 X (Nikon Plan Fluor NA 0.13 objective) magnification.
Conjugation 4RepCT^{3Aha} with levofloxacin

4RepCT^{3Aha} fibres of ~1 cm in length were reacted with 9.27 mM of alkyne bearing linker (glycidyl propargyl ether) as described in ‘conjugation with fluorophores’ (in the case of non-functionalised and levofloxacin control fibres, linker was replaced with water). After incubation, fibres were washed with 3 ml of buffer containing 20 mM Tris pH 7.5, 1 mM EDTA (wash buffer) four times. After washing, linker-labelled and levofloxacin control fibres were refluxed at 65°C for 18 hours in methanol containing 50 mM levofloxacin (non-functionalised fibre was refluxed in methanol only). All fibres were washed with 2 ml of methanol four times, 2 ml of wash buffer twice and stored in methanol.

Tensile strength measurements

A total of 3 4RepCT^{3Aha} fibres and 3 FAM modified 4RepCT^{3Aha} fibres were removed from solution and mounted onto cardboard windows. Once straightened, the fibres were glued into place using cyanoacrylate super glue (LOCTITE, Henkel Corporation). Glue was allowed to dry and the fibre-mounted cardboard window fixed into the grips of the tensile tester (Mecmesin MultiTest 2.5 i) equipped with a 2N load cell. The sides of the cardboard window were carefully cut, and tensile test initiated. Fibres were pulled vertically at a speed of 5 mm/min until the fibre broke (failed) and the load at failure recorded.

Unmodified silk strength of 17.79 MPa with a standard deviation of 13.96 (average of 3 measurements).

FAM-modified silk strength of 17.53 MPa with a standard deviation of 14.36 (average of 3 measurements).
Scanning electron microscopy (SEM) and Energy-Dispersive X-ray spectroscopy (EDX)

Adhesive carbon pads were fixed atop aluminium SEM stubs, and were used to secure fibre samples on top of the stub. Once secure, fibre samples were coated with a fine layer of platinum and transferred into the sample carousel of the SEM (FEI Quanta 650). Images were taken at 5.00 kV. For EDX analysis uncoated fibre samples were placed into the carousel of the SEM (FEI Quanta 650) and analysed using an Oxford instruments Xmax 150 detector.

Supplementary Figure 1: EDX spectra of functionalised 4RepCT\(^{3}\text{Aha}\) fibres. (A) An unwashed FAM conjugated 4RepCT\(^{3}\text{Aha}\) fibre contains a negligible amount of copper – 0.1% of the specimen weight, shown in the map sum spectrum. The area in which copper is found (between 8 – 10 keV) is magnified in the inset picture and shows that the copper signal is noisy, indicating that copper content is low (B) A FAM conjugated fibre after one 20 mM Tris buffer pH 7.5 wash does not contain any detectable copper. (C) A FAM conjugated fibre after identical washing steps to those used on levofoxacin conjugated fibres does. The fibre does not contain any detectable copper.
Zone of inhibition assay

A culture of *E. coli* NCTC 12242 was grown with shaking (180 rpm) at 37°C in Mueller Hinton broth (Oxoid) to an OD$_{600}$ 0.1 and diluted 100 x in the same medium. The diluted culture was spread onto Mueller-Hinton agar (Oxoid) plates using a cotton swab. Non-functionalised, levofloxacin control and levofloxacin-functionalised fibres (previously soaked for 72 hours in buffer containing 50 mM MES pH 5.5) were placed in the centre of the agar plate. Plates were incubated at 37°C for 120 hours.

Inhibition of bacterial growth in liquid media

Non-functionalised, levofloxacin control and levofloxacin-functionalised fibres (purged of methanol) were equilibrated in tubes containing 2 ml LB broth and incubated at 37°C for 18 hours (triplicate of each fibre type). After incubation, 10 µl of an overnight culture of *E. coli* NCTC 12242 was added to each 2 ml of LB broth containing fibres and LB alone, again in triplicate. Tubes were incubated at 37°C with shaking (180 rpm) and OD$_{600}$ measured after 24 hours.