Supplementary Information for:

Protein detection enabled using functionalised silk-binding peptides on a silk-coated optical fibre

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Experimental

General materials

All reagents and solvents were purchased from Merck unless otherwise specified, at the highest purity level available. D-biotin (≥97.5%), 5(6)-carboxytetramethylrhodamine (TAMRA, ≥99%), Oxyma, and all Fmoc-protected building blocks including 6-(Fmoc-amino)hexanoic acid (Fmoc-Ahx-OH), Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Trp(Boc)-OH and Fmoc-Glu(tBu)-OH were purchased from ChemImpex International. Streptavidin AlexaFluor-532 conjugate was purchased from ThermoFisher (S11224). Phosphate buffer solution (100 mM) was prepared from sodium phosphate salts and adjusted to pH 7.2 with aqueous solutions of 0.1 M HCl and 0.1 M NaOH.

Aqueous silk fibroin was prepared at RMIT University according to previously reported protocols[1,2] and shipped to the University of Adelaide on ice. Alternatively, aqueous silk fibroin was purchased from Merck at 50 mg/mL and stored at -80 °C (Supelco 5154-20mL). Aliquots (1 mL) were thawed overnight at 4 °C then dialysed in water for 48 h (water changed every ~12 h) using CelluSep T2 regenerated cellulose tubular membrane dialysis tubing with a 6000-8000 Da molecular weight cut off, wall thickness 30 µm, and flat width 32 mm.[1,2] After dialysis, the silk fibroin solution was stored at 4 °C. Any solids that formed during storage were removed by centrifugation at 7800 rpm for 10 min and the supernatant retained.

Peptide synthesis

General SPPS procedure

Peptides were assembled by solid phase peptide synthesis (SPPS) using a standard Fmoc/tBu protocol, as follows:

Rink Amide PL resin (645 mg, 0.2 mmol, 0.31 mmol/g, Agilent) was swollen in 1:1 (v/v) DCM/DMF (10 mL) for 15 min, then the solution drained and the resin washed with DMF (3 × 8 mL). The resin-bound Fmoc protecting group was then removed by treatment of the resin with 20% (v/v) piperidine in DMF (8 mL) for 10 min, with intermittent stirring. The resulting solution was drained and the resin washed with DMF (3 × 8 mL), DCM (3 × 8 mL), and DMF (3 × 8 mL). A solution of the relevant *N*-terminally Fmoc-protected building block (5 equiv), HATU (5 equiv) and DIPEA (10 equiv) in DMF (10 mL) was added to the resin and allowed to react for 1 h, with intermittent stirring. The solution was drained and the resin washed successively with DMF (3 × 8 mL), DCM (3 × 8 mL), and DMF (3 × 8 mL). The *N*-terminal Fmoc group was subsequently removed by treatment of the resin with 20% (v/v) piperidine in DMF (8 mL) for 10 min with intermittent stirring. A TNBS test (see below) was used to verify that each coupling/deprotection step was successful, with retreatment as necessary. Successive couplings and Fmoc-deprotections were repeated to achieve the desired peptide sequence. After the final Fmoc-deprotection with 20% (v/v) piperidine in DMF (8 mL) for 10 min, the solution was drained and the resin washed with DMF (3 × 8 mL) and DCM (3 × 8mL) and then washed with diethyl ether (3 × 8 mL) to deswell the resin, and air-dried with suction.

TNBS Test:[3] A small spatula of swollen resin was removed and 1 drop each of TNBS solution (100 μL 5% (w/v) picrylsulfonic acid in H_2O added to 900 μL of DMF) and DIPEA solution (100 μL in 900 μL of DMF) were added, and the resin allowed to develop for 1 min. Clear/yellow beads indicated no free amine (negative, observed post successful coupling), and red/orange beads showed free amine (positive).

General resin cleavage procedure

The peptide was cleaved from the dried resin (and all amino acid side chains simultaneously deprotected) by treatment of this resin with a cleavage cocktail consisting of 92.5:2.5:2.5:2.5 (v/v) trifluoroacetic acid/triisopropylsilane/2,2'-(ethylenedioxy)diethanethiol/water (10 mL), and the suspension was placed on a table rocker for 1 h. The solution was pipetted away from the resin and concentrated to 1-1.5 mL under a stream of N_2. Diethyl ether (10 mL) was added and the mixture cooled at -20 °C overnight. The resulting precipitate was pelleted by centrifugation (7800 rpm, 15 min) and the supernatant decanted. The solid was dried under a stream of N_2, dissolved in 1:1 (v/v) acetonitrile:water, syringe filtered (20 μm PTFE syringe filter) and lyophilised to give the crude peptide as a fluffy solid.

General peptide purification and characterisation

Crude peptides were purified by semi-preparative RP-HPLC on a Gilson GX-Prep RP-HPLC system equipped with a Phenomenex Aeris™ 5 μm Peptide XB-C18 100 Å 10 × 250 mm column over a linear 25-50% gradient of ACN (B) in water (A) with 0.1% trifluoroacetic acid, over 25 min, at 4 mL/min and visualised at 220 and 254 nm. Pure fractions were combined and lyophilised to give the final product. Product purity was confirmed to be >85% by analytical RP-HPLC on an Agilent Infinity 1260 analytical HPLC equipped with a Phenomenex Luna™ C18(2) 100 Å 4.6 × 250 mm column over a linear gradient of 5-50% B over 15 min, at 1.5 mL/min and visualised at 220 nm. Product identity was confirmed by high resolution mass spectrometry on an Agilent 6230 ESI MS.

SBP-Biotin: D-biotin-SYTFHWHQSWSS-NH_2

The peptide with sequence SYTFHWHQSWSS was assembled on resin as per the *General SPPS procedure*. Following *N*-terminal deprotection, a portion of the peptide-loaded resin (0.05 mmol) was swollen in 1:1 (v/v) DCM/DMF (5 mL) and washed with DMF (3 × 5 mL). A solution of D-biotin (61.1 mg, 0.25 mmol, 5 equiv), HATU (95.0 mg, 0.25 mmol, 5 equiv) and DIPEA (174 μL, 0.5 mmol, 10 equiv) in DMF (5 mL) was added to the resin and stirred intermittently for 4 h at rt. The solution was drained and the resin washed with DMF (3 × 5 mL), DCM (3 × 5 mL), and DMF (3 × 5 mL). Coupling was verified by TNBS test, then the resin was deswelled with diethyl ether (3 × 5 mL) and air-dried with suction. The crude peptide was simultaneously cleaved from the resin and globally deprotected as per the *General resin cleavage procedure*, then purified as per the *General peptide purification and characterisation procedure*, to provide SBP-Biotin as a white solid. Analytical RP-HPLC (220 nm) 92%. HRMS (ESI+) expected $[M+3H]^{3+}$ for $C_{89}H_{115}N_{23}O_{22}S$ (1889.8308): 630.9516, found $[M+3H]^{3+}$ 630.9510.

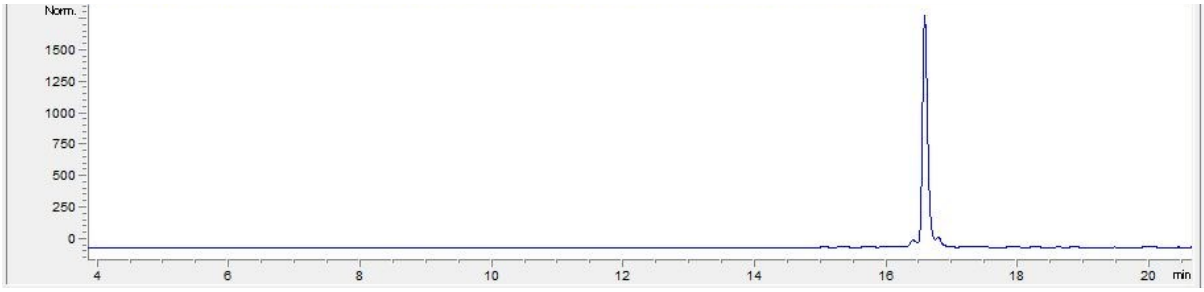


Figure S1: Analytical HPLC trace for SBP-Biotin run over a linear gradient of 5-50% ACN in H_2O with 0.1% TFA, from 5 min to 20 min, visualized at 220 nm.

SBP-TAMRA: TAMRA-Ahx-SYTFHWHQSWSS-NH_2

The peptide with sequence Ahx-SYTFHWHQSWSS was assembled on resin as per the *General SPPS procedure*. Following *N*-terminal deprotection, a portion of the resin-bound peptide (0.05 mmol) was transferred to a microwave vial (7 mL capacity) using DMF (1-2 mL). A solution of 5(6)-carboxyTAMRA (64.6 mg, 0.15 mmol, 3 equiv), 1,3-diisopropylcarbodiimide (23.2 μL, 0.15 mmol, 3 equiv) and Oxyma (21.3 mg, 0.15 mmol, 3 equiv) in DMF (2 mL) was added, then DMF was used to rinse the fluorophore into the reaction vessel to obtain a final reaction volume of 5 mL. The reaction vessel was heated by microwave irradiation to 60 °C for 30 min then cooled to rt. The resultant contents of the microwave vessel were transferred to a fritted syringe and the solution drained, then the resin washed extensively with DMF (5 × 10 mL) and DCM (5 × 5 mL) until no colour remained in the washings. The resin was then washed with diethyl ether (3 × 5 mL) and air-dried with suction. The crude peptide was simultaneously cleaved from the resin and globally deprotected according to the *General resin cleavage procedure*. The crude peptide was purified as per the *General peptide purification and characterisation procedure*, to provide SBP-TAMRA as a purple solid.[4] Analytical RP-HPLC (220 nm) 87% . HRMS (ESI+) expected $[M+3H]^{3+}$ for $C_{104}H_{121}N_{23}O_{24}$ (2075.8955): 692.9732, found $[M+3H]^{3+}$ 692.9732.

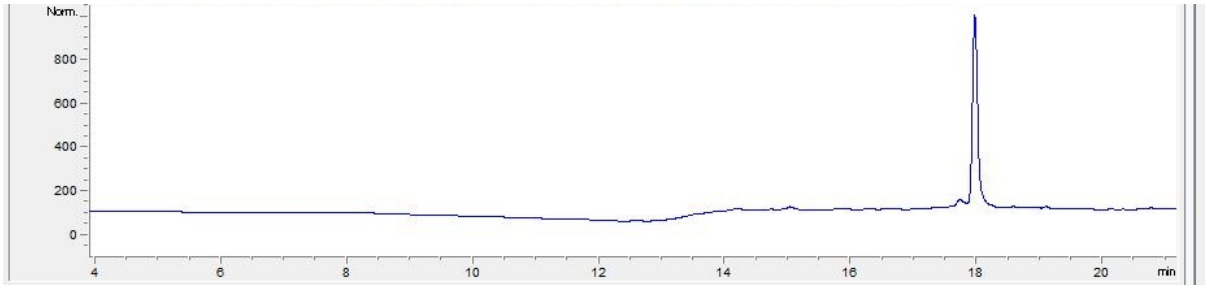


Figure S2: Analytical HPLC trace for SBP-TAMRA run over a linear gradient of 5-50% ACN in H_2O with 0.1% TFA, from 5 min to 20 min, visualized at 220 nm.

Fibre coating

Method A

A mixture of aqueous silk fibroin (65 mg/mL, 100 μL), distilled water (80 μL), and functionalised peptide (SBP-TAMRA or SBP-Biotin, 100 μM, 20 μL) was gently pipetted until homogeneous. Double-clad fibre (DCF) was cleaved with a fibre cleaver (LDC401A, Thorlabs, USA) to expose a fresh section of DCF tip, and 1-2 mm of the

fibre tip was dipped into the silk mixture for 30 s. The silk fibroin coated DCF was then dipped into 90% aq. HPLC grade methanol for 10 s to convert the silk fibroin from the silk I α-helix to the silk II β-sheet,[5,6] then removed and dried in air for at least 10 s before use.

The control fibres coated with silk fibroin only, for use in the streptavidin detection experiments, were also prepared according to Method A with the functionalised SBP replaced with water (20 μL).

Method B

Aqueous silk fibroin (65 mg/mL, 100 μL) was diluted with water (100 μL) and the mixture was gently pipetted until homogenous. DCF was cleaved with a fibre cleaver (LDC401A, Thorlabs, USA) to expose a fresh section of DCF tip and 1-2 mm of the fibre tip was dipped into the mixture and held for 30 s. The coated fibre was dipped into 90% aq. HPLC grade methanol for 10 s to convert the silk fibroin from the silk I α-helix to the silk II β-sheet,[5,6] then the silk fibroin coated fibre was removed and dried in air for 10 s. The fibre was then dipped into functionalised SBP (SBP-TAMRA or SBP-Biotin, 10 μM, 200 μL) for 30 s and dried in air for at least 10 s before use.

The functionalised SBP was replaced with free TAMRA (10 μM, 200 μL) to prepare the control fibres used in the silk coating retention experiments.

Silk fibroin only

Control fibres for the protein detection model system were prepared by coating with silk fibroin only. Aqueous silk fibroin (65 mg/mL, 100 μL) was diluted with water (100 μL) and the mixture was gently pipetted until homogenous. DCF was cleaved with a fibre cleaver (LDC401A, Thorlabs, USA) to expose a fresh section of DCF tip and 1-2 mm of the fibre tip was dipped into the mixture and held for 30 s. The coated fibre was dipped into 90% aq. HPLC grade methanol for 10 s to convert the silk fibroin from silk I to silk II,[5] then the silk fibroin coated fibre was dried in air for 10 s.

Optical fibre sensing system

An optical fibre sensing system was built in order to stimulate fluorescence emission and collect fluorescence spectra from the SBP and silk fibroin coated optical fibres (Figure 2G and Figure S3). Double clad fibre (DCF13, Thorlabs, USA, 9μm core diameter, 105 μm inner cladding, 125 μm outer cladding) was used to fabricate the fibre sensors. A 532 nm laser (CrystaLaser, USA, 25 mW, model CL532-025) was coupled into the core of the DCF to excite the relevant fluorophore (TAMRA or AlexaFluor™ 532) located on the fibre tip. The fluorescence signal was then collected through the inner cladding of the DCF, passed through a long pass filter to remove the excitation light (532 nm, Semrock 532 LP EdgeBasic, USA), and measured with a portable spectrometer (Ocean Optics QE65000, USA). The laser power at the fibre tip was measured to be 2.7 mW. A shutter (LS6 & VCM-D1, Uniblitz, USA) was installed to minimise excess exposure of the fluorophore to excitation light, with custom LabVIEW software (National Instruments Corp, USA) used to synchronise the Ocean Optics spectrometer acquisition with a 100 ms shutter opening for each collection. A dark spectrum was also acquired immediately after each measurement with the laser shutter closed to remove the effects of ambient light from the

experiment. Before collecting fluorescence spectra, uncoated fibres were connected to the system and a background spectrum collected. This background spectrum was subtracted from all subsequent spectra. The total intensity was integrated between 570 and 600 nm for TAMRA spectra, and between 565 and 600 nm for AlexaFluor-532 spectra. The resultant integrated intensities were plotted and compared as described in the main text.

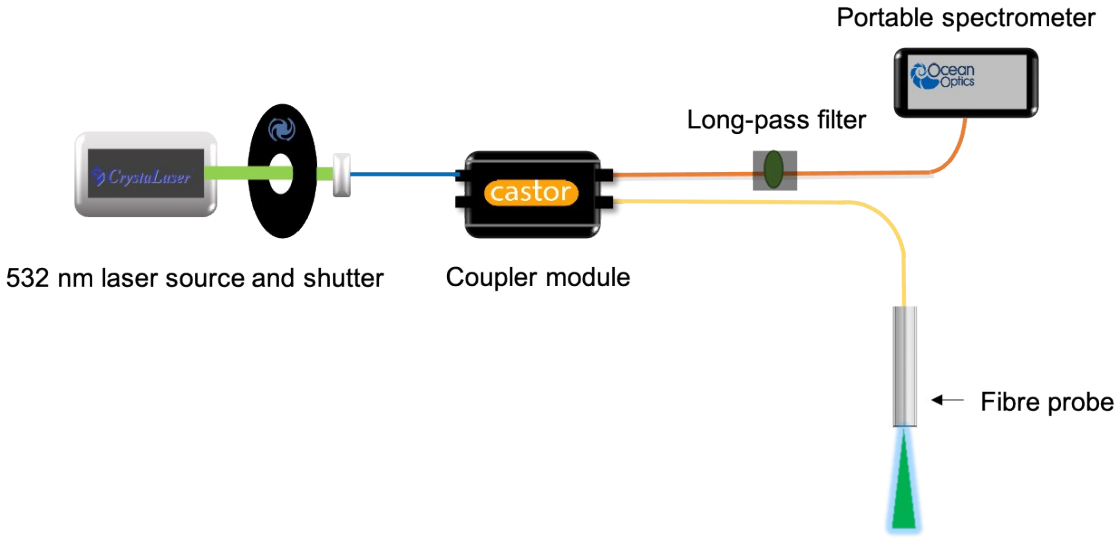


Figure S3: Schematic of the optical fibre coupled laser and spectrometer used for fluorescence spectra collection. Blue: single-mode fibre, orange: multimode fibre, yellow: double-clad fibre. Note this is an exact replica of Figure 2G in the main text.

Scanning electron microscopy

SEM (see Figure 2) of the silk coated fibres was performed on a Quanta 450 SEM in SE and BSE modes and energy dispersive X-ray (EDX) spectroscopy were also collected. Samples were dry loaded onto adhesive carbon tabs on aluminium stubs and coated with a 5 nm platinum coating. The electron beam voltage was held at 5.00 keV for SE and EDX measurements, with a working distance between 9.5 and 10.5 mm, spot size of 4, and images taken at 1500× (end on). The electron beam voltage was increased to 10.00 kV for BSE imaging. Note that images of the bare fibre and method A coated fibres have been previously published in our earlier work.[4]

Atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) was used to determine the lithium concentration in aqueous silk fibroin solutions. A standard curve for Li between 1 and 100 ppm was prepared from stock solutions of $LiOH_{(s)}$ in distilled water. Three samples of aqueous silk fibroin solutions were prepared at 50 mg/mL: the in-house silk fibroin, the Merck silk fibroin as received, and a dialysed sample of the Merck silk fibroin. The Merck silk fibroin was dialysed against 1 L of water for 48 h with the water changed every ~12 h, using CelluSep T2 regenerated cellulose tubular membrane dialysis tubing with a 6000-8000 Da molecular weight cut off, wall thickness 30 µm, and flat width 32 mm. The three silk fibroin samples were diluted 10-fold, then run on a Varian Spectra AA 250+ Atomic

Absorption Spectrophotometer. The lithium concentration (ppm) was calculated for each sample with reference to the standard curve (see Table 1 in the main text).

Silk fibroin concentration

As described in the main text, a calibration curve for silk fibroin concentration was constructed based on absorbance at 205 nm, which predominantly originates from amide bonds (see Figure 2H).[5] Absorbance at 205 nm was chosen over than the traditional 280 nm wavelength used for proteins,[6] as silk fibroin predominantly consists of the amino acids glycine (46 mol %) alanine (30 mol %), and serine (12 mol %), which do not have a strong absorbance at 280 nm.[7,8] There is also minimal tryptophan (0.2 mol %) or tyrosine (5.3 mol %) present to contribute to absorption at 280 nm.[9] Freshly prepared aqueous silk fibroin at 65 mg/mL (calculated by the dry weight of silk fibroin) was diluted to 60, 55, 50, 45, 40, and 35 mg/mL, then each stock solution was further diluted in water 200-fold and the absorbance at 205 nm measured three times per sample on a Thermofisher Nanodrop 2000 UV/Vis Spectrophotometer. This absorbance was plotted against the stock concentration, and a calibration curve calculated using GraphPad Prism 9 with a forced y-intercept of 0 (see Figure 2H).

The concentration of all silk fibroin solutions received during the course of this work were calculated according to Equation S1, with reference to this calibration curve to ensure fibre coating results were comparable. Note that these concentrations are relative to the original 65 mg/mL sample, as the extinction coefficient of the silk fibroin was not calculated. All silk fibroin solutions used in this work for fibre coating were at a final concentration of 32.5 mg/mL to ensure consistency.

$$A_{205\,nm} = 0.01306 \times Silk\ concentration\ in \frac{mg}{mL} \#S1$$

Supplemental Data

Detection of fluorescently tagged streptavidin

Optical fibres were coated with silk fibroin and SBP-Biotin by method A or B, then used to detect AlexaFluor-532 tagged streptavidin as detailed in the main text. Figure 3A shows the integrated intensity between 565 and 600 nm for one fibre of each coating type: one coated with silk fibroin only, one coated with silk fibroin and SBP-Biotin by method A, and one coated with silk fibroin and SBP-Biotin by method B. Additional plots for all three fibres prepared by each coating type are provided here, along with sample full spectra.

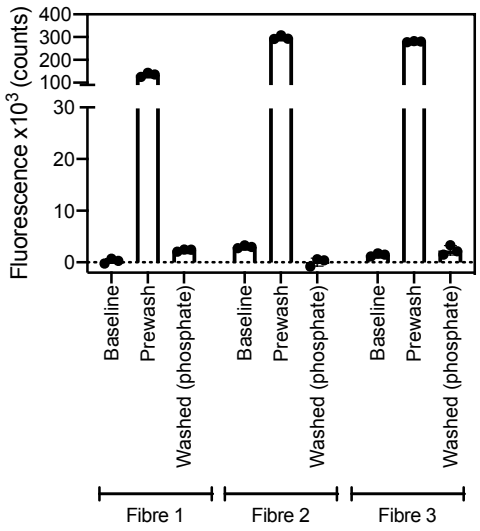


Figure S4: SBP-Biotin sensing of AlexaFluor-532 tagged streptavidin. Each point is the integrated intensity between 565 and 600 nm for a single spectrum. All fibres are control fibres coated with silk fibroin only, where all prewash fluorescence was lost upon washing with phosphate buffer (100 mM, pH 7.2). Excitation for all fibres was at 532 nm, data is plotted as mean ± standard deviation of three reads.

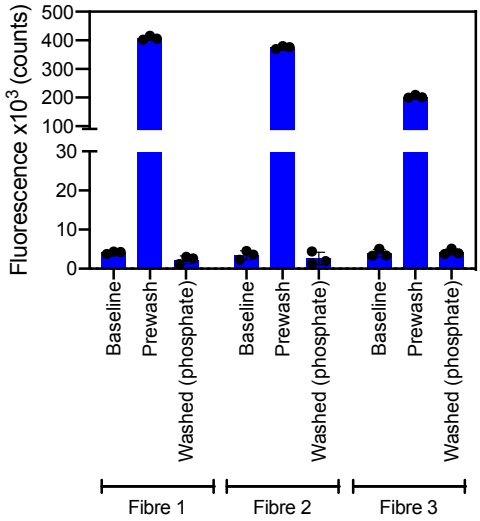


Figure S5: SBP-Biotin sensing of AlexaFluor-532 tagged streptavidin. Each point is the integrated intensity between 565 and 600 nm for a single spectrum. All fibres were coated with silk fibroin and SBP-Biotin by method A, where all prewash fluorescence was lost upon washing with phosphate buffer (100 mM, pH 7.2). Excitation for all fibres was at 532 nm, data is plotted as mean ± standard deviation of three reads.

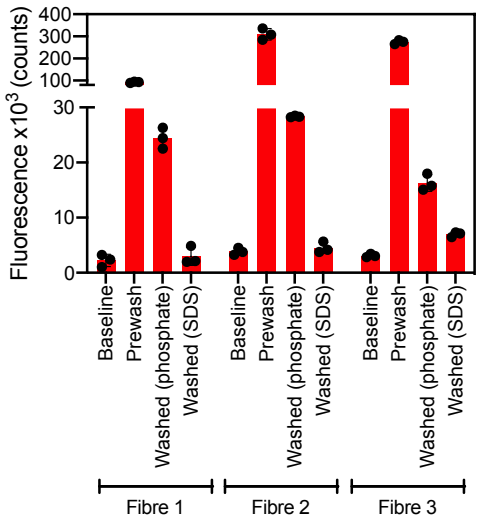
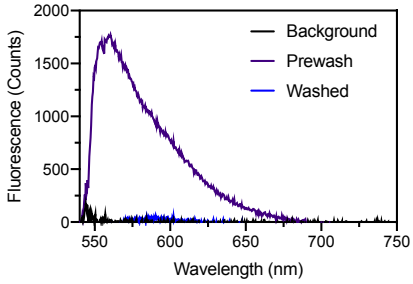
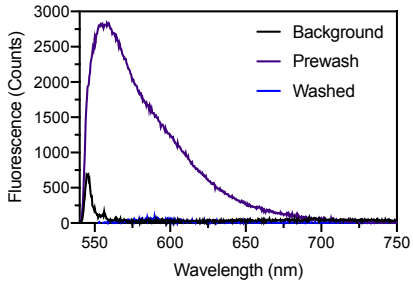


Figure S6: SBP-Biotin sensing of AlexaFluor-532 tagged streptavidin. Each point is the integrated intensity between 565 and 600 nm for a single spectrum. All fibres were coated with silk fibroin and SBP-Biotin by method B. Washing with phosphate buffer(100 mM, pH 7.2) did not remove all prewash fluorescence. Washing with 2% aqueous sodium dodecyl sulfate (SDS) removed all fluorescence. Excitation for all fibres was at 532 nm, data is plotted as mean ± standard deviation of three reads.

A)



B)



C)

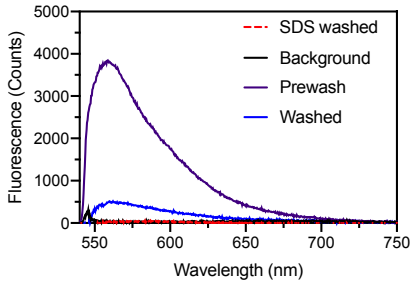


Figure S7: Sample full spectra for an optical fibre coated with A) silk fibroin only, B) silk fibroin and SBP-Biotin by method A, and C) silk fibroin and SBP-Biotin by method B. Black) background fluorescence before exposure to AlexaFluor-532 tagged streptavidin. Purple) Fluorescence spectrum after dipping in streptavidin solution. Blue) Fluorescence spectrum after

washing 3 x 30 s in phosphate buffer (100 mM, pH 7.2). Red, dashed) Fluorescence spectrum after washing 3 x 30 s in sodium dodecyl sulfate (SDS, 2% aqueous). Excitation wavelength for all spectra was 532 nm.

Retention of SBP-TAMRA on optical fibres

Optical fibres were coated with silk fibroin and SBP-TAMRA or free TAMRA by method B, then washed by submerging the fibre tips in water on a short term (3 x 30 s) or a long term (18 h) basis as detailed in the main text. First, three control fibres were prepared with free TAMRA used in place of SBP-TAMRA, to demonstrate the necessity of the SBP component for retention of TAMRA on the silk coated fibre (see Experimental section). These control fibres were coated by method B such that the free TAMRA was isolated to the silk surface. Fluorescence spectra were collected, then the fibres were washed by submerging the fibre tips three times in water for 30 seconds. Fluorescence spectra were collected again and the percentage of fluorescence retained was calculated (Figure S8, see Figures S10 and S11 for full fluorescence spectra). This showed that only 5% of the fluorescence signal was retained after washing (Figure S8, striped blue bar). Next, the fluorescence retention of three fibres bearing surface bound SBP-TAMRA (method B) was calculated through the same washing and fluorescence spectra collection procedure. In this case, 90% of the fluorescence signal obtained before washing was retained after washing in water (Figure S8, solid blue bar). The optical fibres functionalised by method B are thus stable to repeated submersion in water, with minimal SBP-TAMRA removed. Therefore, the SBP is required to retain TAMRA on optical fibres coated by method B.

Next, the long-term adherence of the silk coatings on optical fibres was investigated. A set of three control fibres (coated with free TAMRA only) and three SBP-TAMRA coated fibres were prepared by method B and fluorescence spectra acquired, and the fibre tips were soaked in water overnight. Fluorescence spectra were collected again after this soak and the fluorescence retention calculated (Figure S8, see Supplementary Information Figure S10 and S11 for full spectra). Only 3% of the original fluorescence signal was retained after soaking the control fibres (Figure S8, striped red bar), while 68% was retained for the SBP-TAMRA coated fibres (Figure S8, solid red bar). This demonstrates that the method B coating of silk fibroin and SBP-TAMRA is stable on the fibre for extended periods of time, while free TAMRA is not. Overall, these washing experiments demonstrate that silk fibroin and SBP-TAMRA coatings prepared by method B remain adhered to the optical fibre under repeated or prolonged exposure to water. These results are in agreement with the observed stability of fibres coated with SBP-TAMRA by method A (Figures S9-11).[4]

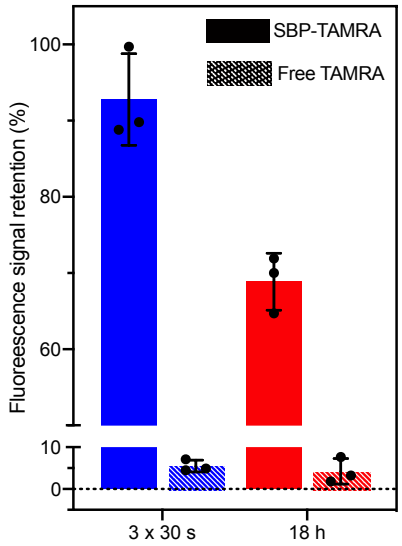


Figure S8: Percentage fluorescence retention on method B coated fibres, calculated from comparison of the integrated intensity (570 to 600 nm) of fluorescence spectra obtained before and after washing each fibre. Each point shown is the average of three spectra for one fibre, with a total of three fibres plotted per bar as mean ± standard deviation. The solid bars represent fibres coated with silk fibroin and SBP-TAMRA, while the striped bars represent fibres coated with silk fibroin and free TAMRA. Left, fibres washed in water for 3 x 30 s (blue) or soaked overnight (red). All spectra were collected with an excitation wavelength of 532 nm.

The calculated fluorescence retention shown in Figure S8 for the method B coated fibres is replotted here with comparison to the method A coatings.[4] Sample full spectra are also provided for each coating type.

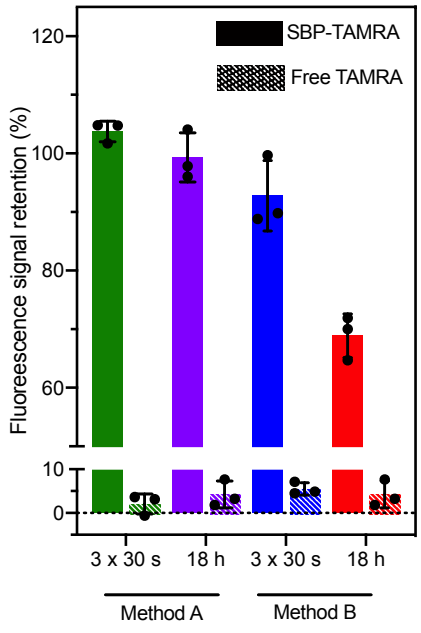


Figure S9: Percentage fluorescence retention on silk fibroin coated fibres, calculated from comparison of the integrated intensity (570 to 600 nm) of fluorescence spectra obtained before and after washing each fibre. Each point shown is the average of three spectra for one fibre, with a total of three fibres plotted per bar as mean ± standard deviation. The solid bars represent fibres coated with silk fibroin and SBP-TAMRA, while the striped bars represent fibres coated with silk fibroin and free TAMRA. Left, fibres coated by method A and washed in water for 3 x 30 s (green) or soaked overnight (purple).

Right, fibres coated by method B washed in water 3 x 30 s (blue) or soaked overnight (red). All spectra were collected with an excitation wavelength of 532 nm.

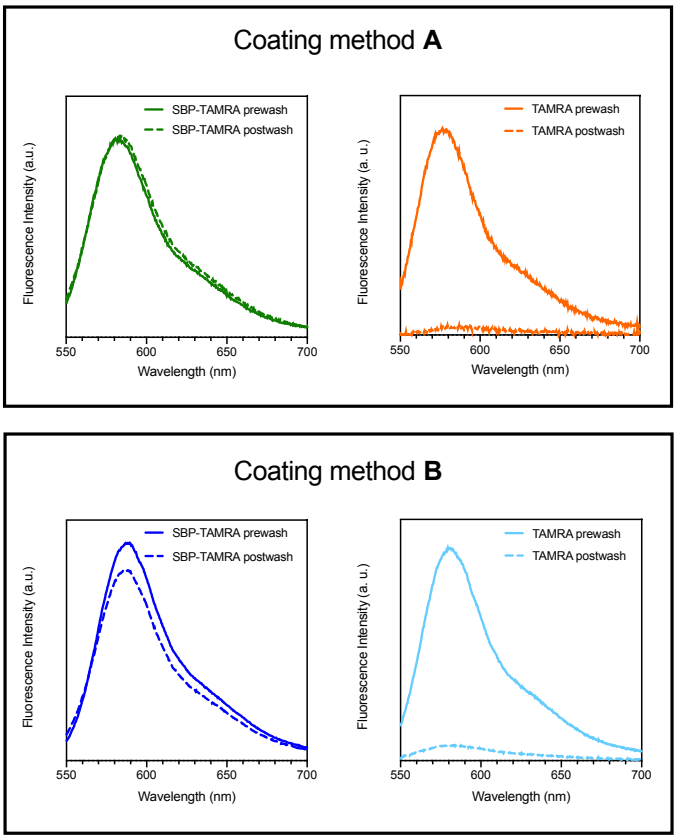


Figure S10: Full spectra obtained for sample optical fibres, coated with silk fibroin and SBP-TAMRA or free TAMRA then washed 3 x 30 s in water. Excitation at 532 nm for all spectra.

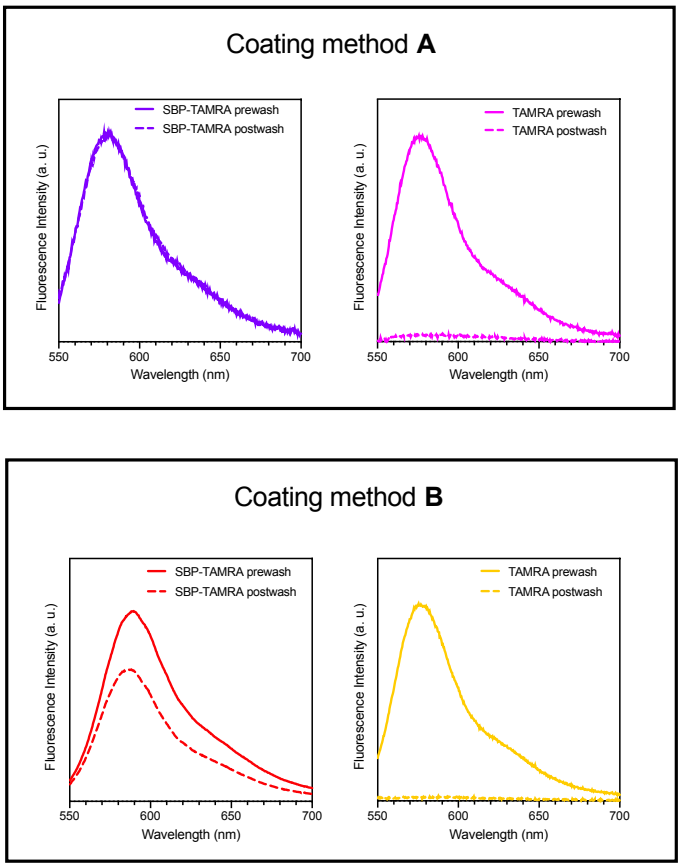


Figure S11: Full spectra obtained for sample optical fibres, coated with silk fibroin and SBP-TAMRA or free TAMRA then soaked in water for 18 h. Excitation at 532 nm for all spectra.

References

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