Supplementary Materials

Differential Scanning Fluorimetry provides high throughput data on silk protein transitions.

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(SM1) Animals, Silk Preparation and Dye Preparation

Animals: *Bombyx mori* were reared at the Padua Silk Station in Italy feeding on white mulberry (*Morus alba*) and shipped to Oxford; *Antherea pernii* were reared in Oxford on oak (*Quercus robur*) leaves until they were ready to spin. *Nephila edulis* were all reared in-house and fed on fruitflies when small and houseflies when large.

Native Silk Sample Preparation: The worms were killed by decapitation and the glands were immediately dissected out, rinsed in distulled water for a few seconds and then submerged in type II demineralised water at 22 ± 2 °C. For the preparation of stock solutions the epithelium was very carefully removed (avoiding shearing the dope) and the gland contents freed of any gland tissue. With spiders the dope was taken from the middle part of the gland. With silkworms the silk dope was taken from two distinct gland sections, the posterior-middle section (*pmm*) and the posterior section (pm) of the gland; for detailed definitions of the divisions and their products see Akai[1]. These gland sections were chosen in order to minimise the presence of Sericin in the stock solution[2,3]; spiders do not have sericin.

Silk solutions were then prepared by carefully (to minimise shear) transferring the native silk dope material into 1.5 ml Eppendorf tubes, which were then filled with ultra-pure (Milli-Q filtered) water. Because of the smaller quantities of silk dope extractable from the spider ampulate glands, we always combined the contents of two glands (from the same spider). The Eppendorf tubes were then placed horizontally on an orbital shaker in a cold room (at 5°C) and shaken overnight for ca 9 Hours at 60 rpm in order to solubilise and mix the native silk dope. DSF runs were done the following day.

Solution concentration was determined by transferring 200 µL of the overnight solution onto pre-weighed Aluminium foil to oven-dry overnight at 60°C followed by dry-weight determination and calculation of the silk solution concentration (w/v) performed in duplicate.

Reconstituted 'Silk' Sample (RSF) Preparation: RSF was either (i) prepared in-house following the classical RSF silk reconstitution method that follows on well-

established procedures[3,4,5] or – as control - (ii) given to us by Orthox Ltd, which uses a comparable but proprietary technique to prepare its clinical grade RSF.

For the in-house RSF preparation, *Bombyx mori* silkworm cocoons were stored at ambient temperature and humidity until required when they were degummed in a 0.1% Na_2CO_3 solution at 70 ± 1 °C for 3 hours. To prepare RSF, 250 mg of silk fibres were solubilised per millilitre of 9M lithium bromide (LiBr). The solution was kept at this temperature for 20 minutes at 70 ± 1 °C using a temperature controlled bath. The solution of solubilised fibres was then transferred to a dialysis bag (VISKING® molecular weight cut off 12-14 kDa). To remove the LiBr, the silk solutions were dialysed against demineralized water type II (resistivity > 1 MΩcm) changed every two hours automatically over 48 hours at 7 °C.

Dye preparation: At all times using rubber gloves were worn to avoid surface contamination, which could interfere with fluorescence measurements. Sypro® orange dye was used as dye of choice and after experimenting with other dyes (Figure S1). Our Sypro® orange dye was supplied by Sigma Aldrich in a concentrated form in DMSO (di-methyl sulfoxide) and as it is light sensitive was stored in foil at 5°C. For our standard 2% v/v concentration we added 4 µL of the Sypro Orange concentrate to 196 µL of ultra-pure (Milli-Q) water. Aliquots (20 µL) were transferred from the stock silk solutions to 0.2 ml Eppendorf tubes supplied by Qiagen using plastic pipettes with the tips cut-off (5 mm from the original length) to minimise shearing of the sample while it was drawn-in and expelled-out of the pipette. Sypro® orange Dye was then added to the silk solution aliquots with 5, 10, 20 & 30 µL for the different concentrations. Once the dye had been added, the preparations were homogenised overnight at 5°C using an Orbital Shaker at 40 rpm before beginning the measurements in the Quiagen real-time PCR instrument.

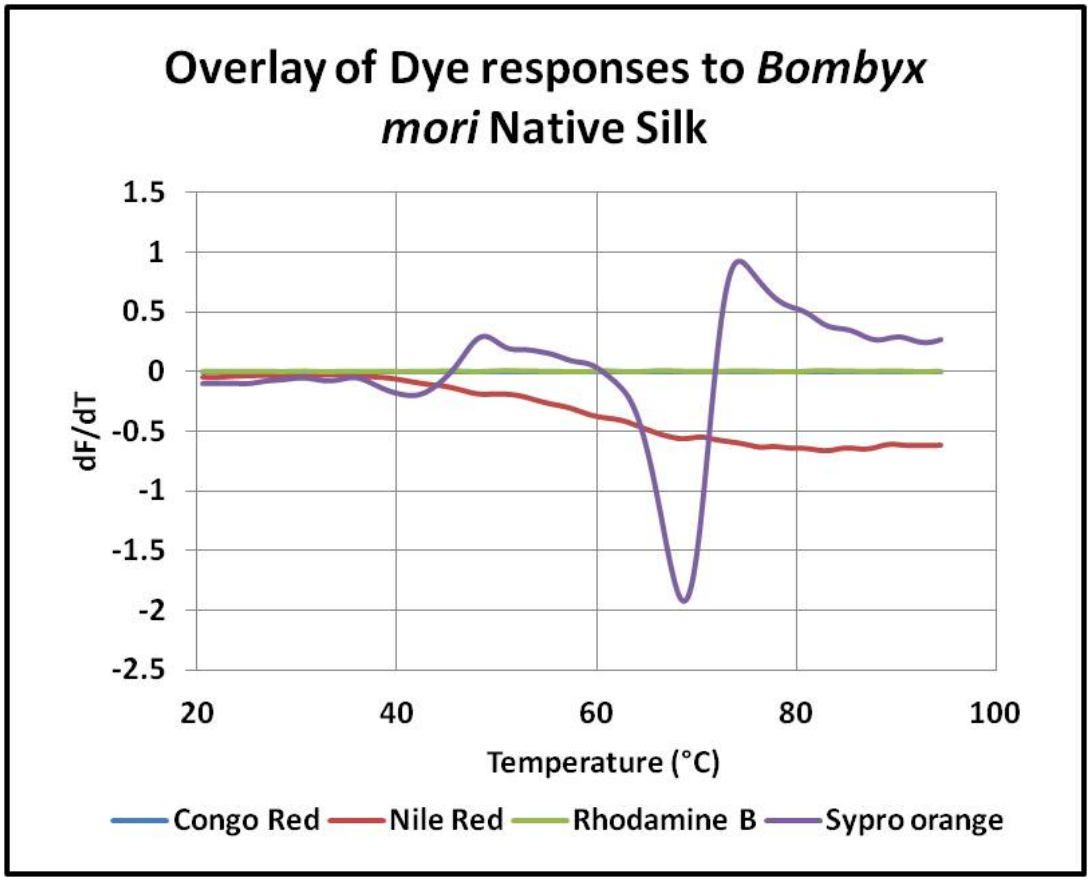

FIGURE S1 Signals of different dyes tried on samples of *Bombyx mori* native silk feedstock.

(SM2) Additional Experimental Details

Experimental Details: All samples were measured in 0.2 ml Eppendorf tubes on the Rotor-Gene Q real-time PCR by Quiagen (www.quiagen.com) using the following temperature program (i) hold at 25°C for 2 minutes; (ii) equilibrate at 20°C and hold for 5 minutes; (iii) ramp from 20°C to 95°C in 0.4°C steps (hold for 10 Seconds at each step). Each measuring step lasted 30 seconds. The Eppendorfs were always closed and positioned as soon as samples had been prepared in order to minimize evaporation. The nature of the measurement (rapid spinning generating centripetal forces) minimizes the possibility of bubble formation during a temperature ramp and the sealed Eppendorfs prevent evaporation from the sample as the temperature is raised. All samples were immediately re-run as controls using the same settings, thus testing whether complete denaturation had occurred during the first run.

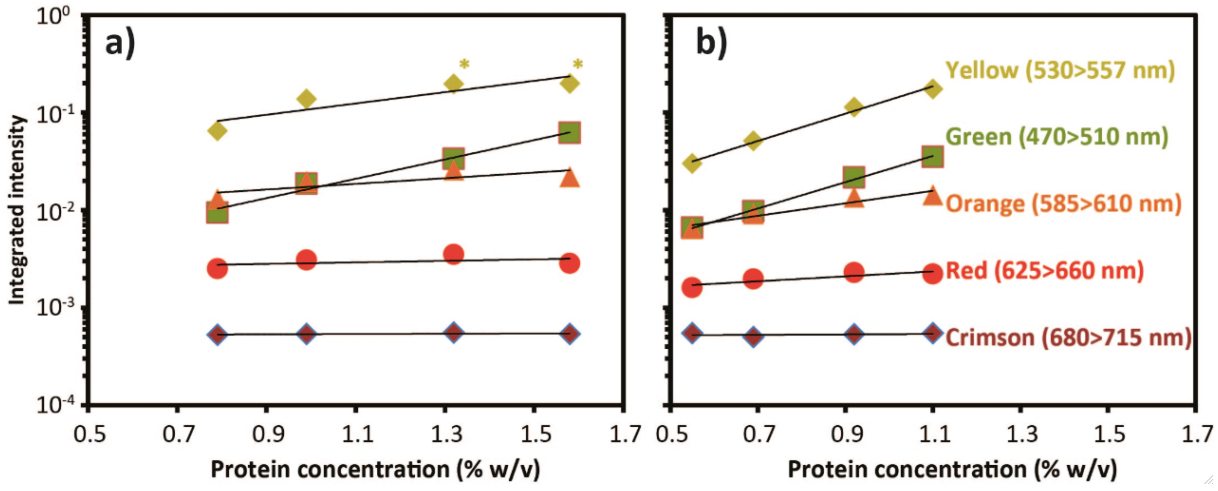

FIGURE S1 Rotor-Gene Q measures the fluorescence simultaneously at multiple wavelengths. (a) Fluorescence melting peak intensity from Sypro® orange mixed with native silk protein from the posterior section (*pm*) as a function of the dye. (b) Fluorescence melting peak intensity from Sypro® orange mixed with native silk protein from the posterior-middle section (*pmm*). The star symbols represent concentrations that saturated the detector. The five channels (green yellow, orange, red and crimson) of the instrument excited at 470, 530, 585, 625 and 680 nm and emitted at 510, 557, 610, 660 and 715 nm, respectively. The log of the integrated intensity is proportional to the concentration. The yellow channel (excitation at 530 nm and emission at 557 nm) offers the best sensitivity with the lowest detection limit because it matches closest the excitation and emission of Sypro® orange at 569 nm [50]. However, the yellow channel signal saturated at the silk-dope concentrations typically used in our experiments (around 1%). To allow us to probe the wider dynamic range we focused our analysis on the second most sensitive channel, green, which excites at 470 nm and emits at 510 nm.

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