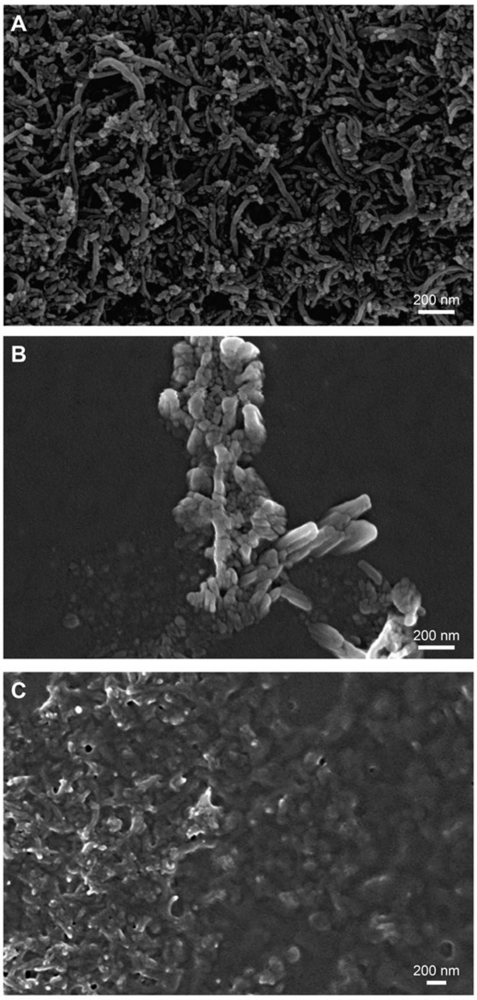
Electronic Supplementary Material (ESI) for Journal of Materials Chemistry A.

Electronic Supplementary Information

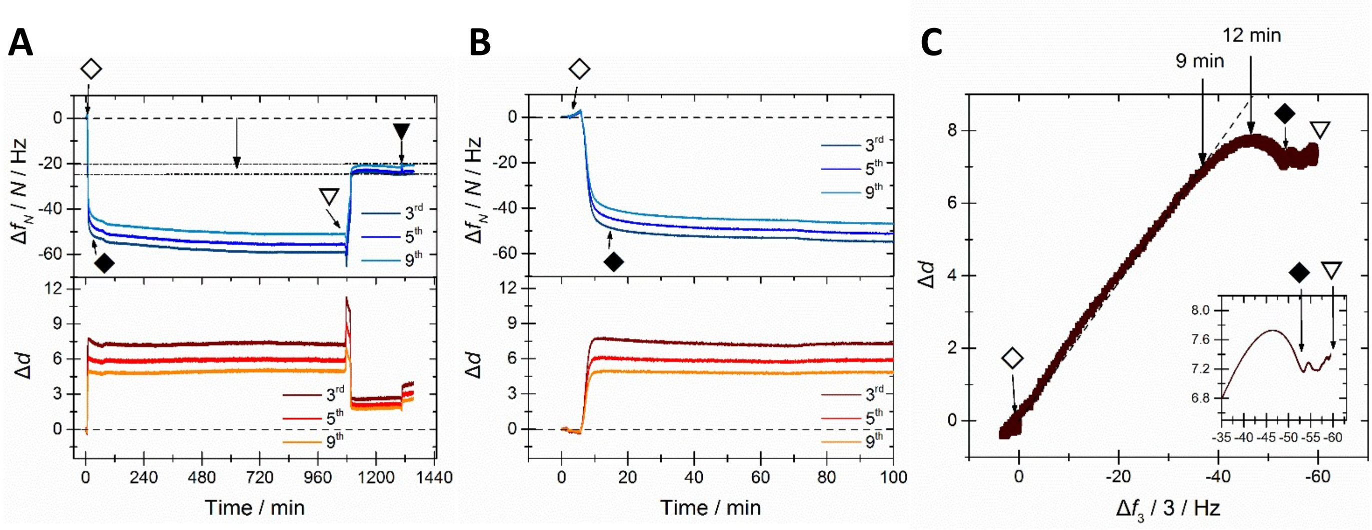


Supplementary Fig. 1 High resolution SEM images of (A) MWCNT film, (B) Film of hemin (heme *b*) and (C) MWCNT film covered by a heme-silk film.

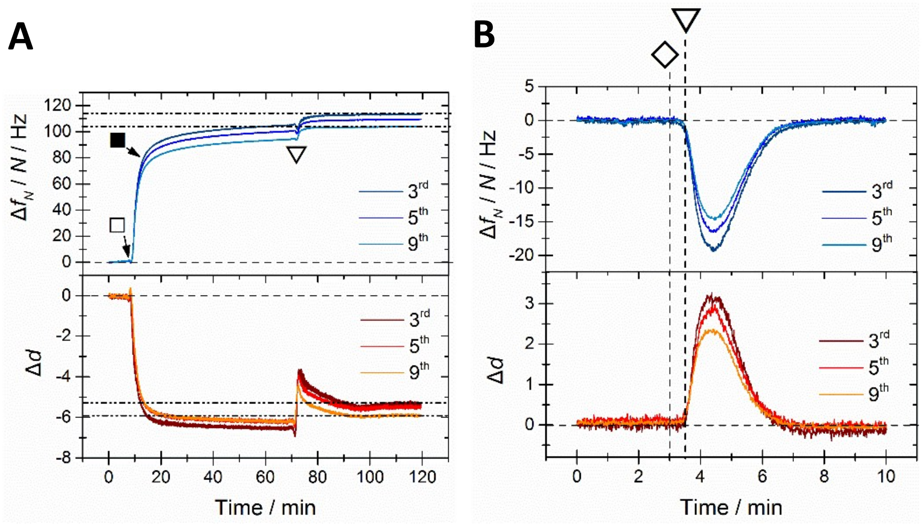
Quartz crystal microbalance measurements

New sensors were cleaned in UV-generated ozone for 10 min. A solution of honeybee silk (AmelF3) was dissolved in water at 5 mg ml^{-1} and then spin coated (40 µl solution, 4000 rpm, 30 s) on the clean sensor surface (AT-cut, gold coated quartz, 4.95 ± 0.05 MHz fundamental). The ellipsometric thickness of the silk film before and after hemin incorporation was measured using a M2000U ellipsometer (Woollam, USA) and found to be (13.98 ± 0.02) nm and (15.05 ± 0.03) nm, respectively, where the error arises from fit quality. The spin-coated sensor was placed in a Q-Sense QFM401 flow-cell module with 40 µL chamber volume. The temperature was maintained at (25 ± 0.01) °C using an integrated Peltier cooler backed by an external aluminium plate kept at 25° C by an internal water flow. A peristaltic pump was used to flow 70 vol% aqueous methanol over the sensor at 0.05 mL min^{-1} then halted as temperature equilibrated. After equilibration, a solution of 50 µg mL^{-1} hemin, 5 mM NaOH and 70 vol% methanol in water, kept at 25 °C by a heated plate was flowed at the same rate for ca. 5 min until the chamber was full of solution, and the flow was stopped. After 18 hours a 70 vol% methanol flow was reintroduced to wash the surface of uncoordinated hemin.

To a first approximation, the decrease in frequency is directly proportional to an increase in mass on the surface (i.e., the Sauerbrey equation $\Delta m = -C\Delta f$, where C = 17.8 ng cm^{-2} Hz^{-1}), and dissipation is an indicator of the stiffness of the layer, with greater dissipation a sign of a softer layer. Multiple harmonics of the 5 MHz fundamental frequency were recorded. These measurements allow for the viscoelastic properties of the film to be modelled and for deviations from the Sauerbrey mass to be captured.[1,2]



Supplementary Fig. 2 QCM-D responses of hemin on AmelF3 spin-coated gold sensor. (A) Δ*f* and Δ*d* plots showing the injection of hemin over the silk-coated sensor. Relevant time points are indicated by ◇: start of flowing hemin solution, ◆: end of flowing hemin solution and start of static equilibration ,▽: start of flowing 70 vol% methanol wash,▼: end of flowing 70 vol% methanol and start of static equilibration. In the corresponding dissipation plot a spike is seen at ▽ which is due to change in the viscosity and density bulk solution. (B) Δ*f* and Δ*d* plots showing the first 100 minutes of panel A. (C) Δ*d* vs. Δ*f* plot for the third harmonic, showing an initial increase as hemin is injected. Inset: a LOESS smoothed enlargement of the upper-right section of the plot. Points beyond ▽ are omitted due to the erratic response as solvent is changed, and do not provide further insight. The 7th harmonic has been omitted due to artefacts in the signal, though its regular inclusion is due to convention and the 3rd provides similar insight.

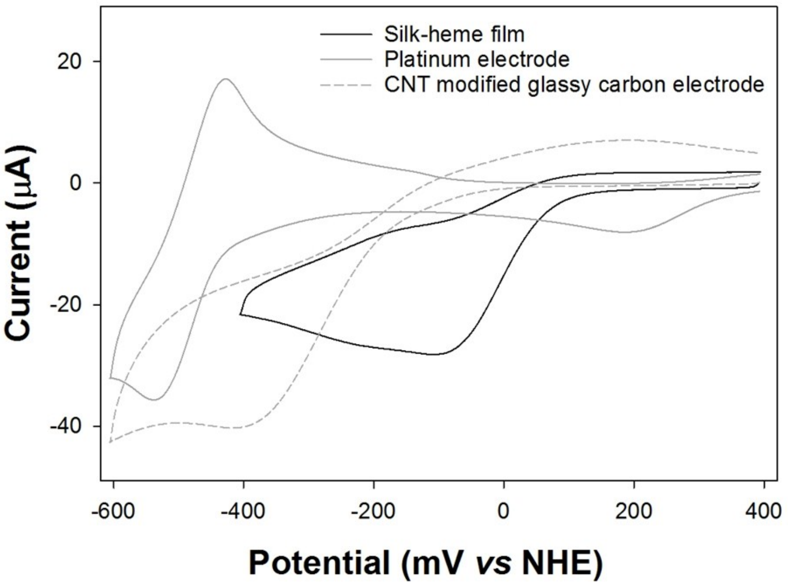


Supplementary Fig. 3 (A) Δ*f* and Δ*d* plots showing the removal of a spin-coated silk film via trypsin digest. □: 0.05% w/v trypsin flowed over the sensor ■: flow halted and sensor remains immersed in trypsin ▽: water flowed to wash sensor and remove trypsin (B) Δf and Δd plots demonstrating the lack of coordinated hemin for short exposure to 70% methanol. ◇: hemin solution flowed over sensor ▽: after 30 seconds 70 vol% methanol flow resumed.

Supplementary Fig. 22 shows the representative QCM-D traces for hemin incorporation into a spin-coated silk film. The symbols in the Δ*f* vs. time trace (Supplementary Fig. 2S2A&B) correspond to the equivalent points in Δ*d* vs Δ*f* (Supplementary Fig. 2S2C) plots. The sensor was first equilibrated with methanol. The open diamond indicates when the methanolic hemin solution was injected and allowed to flow over the surface of the sensor for roughly 4 min, after which point the flow was switched off (black diamond). The vertical arrow in panel A indicates a net frequency decrease after washing of the sensor and equilibration of between 20–24 Hz (dash-dot lines). This corresponds to a Sauerbrey mass of 0.35–0.42 µg cm^{-2} of coordinated hemin. Taken with the increase in thickness, this change corresponds to a density increase of approximately 0.3 g cm^{-3}. Figure S3 shows the removal of spin-coated silk from a sensor by trypsin digestion, allowing us to calculate the mass of silk in the film. The frequency change is 104–114 Hz, which corresponds to a Sauerbrey film mass of 1.84–2.02 µg cm^{-2}, meaning the ratio of hemin to silk in the film after washing is around 20%. Panel B of Figure S3 also shows the inability of hemin to coordinate in the film before the silk has formed its coiled-coil structure.

The ‘*d* vs *f*’ plot in panel C of Supplementary Figure 2 ‘normalises’ the energy loss based on the adsorbed mass.[3] Steeper slopes typically correspond to layers that are softer. The majority of Supplementary Fig. 22C (between the white and black diamonds) corresponds to the initial injection from 0 to 4 min, and the 6 min after the flow was stopped. Effectively this covers the initial interaction of hemin with the film. The more interesting aspect of this is that after 10 min the gradient becomes negative as mentioned previously as the adlayer stiffens, which is potentially an indication of beta-sheet formation and hydrogen bonding that occurs with an aqueous methanol stabilisation. The mass gradually increased over the next 16 h while the dissipation remained practically constant. When the hemin-free methanol was again flowed over the sensor surface (open triangle), the frequency traces increased and the dissipation dropped. These effects are attributed to difference in the density and viscosity of the hemin-free and hemin-containing methanol solutions.

A control was run in which the hemin solution flowed continually over the surface for ca. 30 s (Figure S3B). This did not provide enough contact time for a measurable increase in film mass to occur; all hemin is washed from the surface as the silk protein must restructure before coordination, and the timescale was too short for this to occur.



Supplementary Fig. 4 Comparison of oxygen reduction by heme-silk films, polished platinum electrode (BASi) and glassy carbon electrode modified with multiwalled carbon nanotubes in an aerated 50 mM phosphate buffer (pH 7.4). Scan rate, 100 mV s^{-1}

Calibration of N_C (collection efficiency of the RRDV)[4]

When calibrating the collection efficiency of the RRDV (N_C), N_2 saturated 0.1 M pH 7.0 phosphate buffer solution and 0.004 M $K_3Fe(CN)_6$ were used as the electrolyte. The electrodes were the same as those used in the ORR measurements. The electrode was rotated at 2500 rpm and the amperometric current–time measurements were performed. The voltage of the disk and ring were set to 0.1 V and 1.5 V vs. NHE, respectively. The reactions taking place on the disk and ring are:

$$\text{Disk: } Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$$

$$\text{Ring: } Fe(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-} + e^-$$

The measurement lasted 60 s and the disk and ring currents (I_d and I_r) over the last 10 s were averaged. The measurement was repeated once with the disk disconnected. The ring current (I_{r0}) over the last 10 s was averaged. I_{r0} includes all the anodic currents that are not from $Fe(CN)_6^{4-}$ reduced on the disk. They may be related to the oxidation of water, free $Fe(CN)_6^{4-}$ or any impurities.

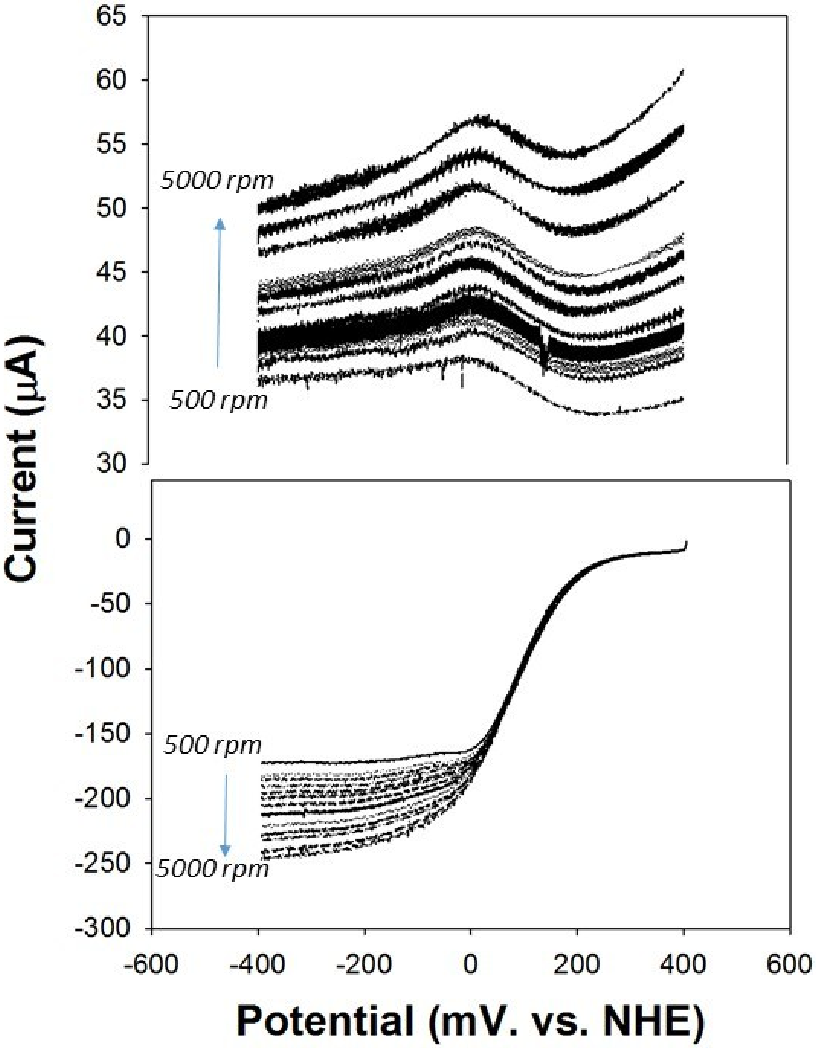
$$I_r = 4.07 \text{ mA cm}^{-2} \quad I_{r0} = 2.55 \text{ mA cm}^{-2} \quad I_d = 1.07 \text{ mA cm}^{-2}$$

(surface area of disk electrode : 0.125 cm^2, surface area of ring electrode : 0.188 cm^2)

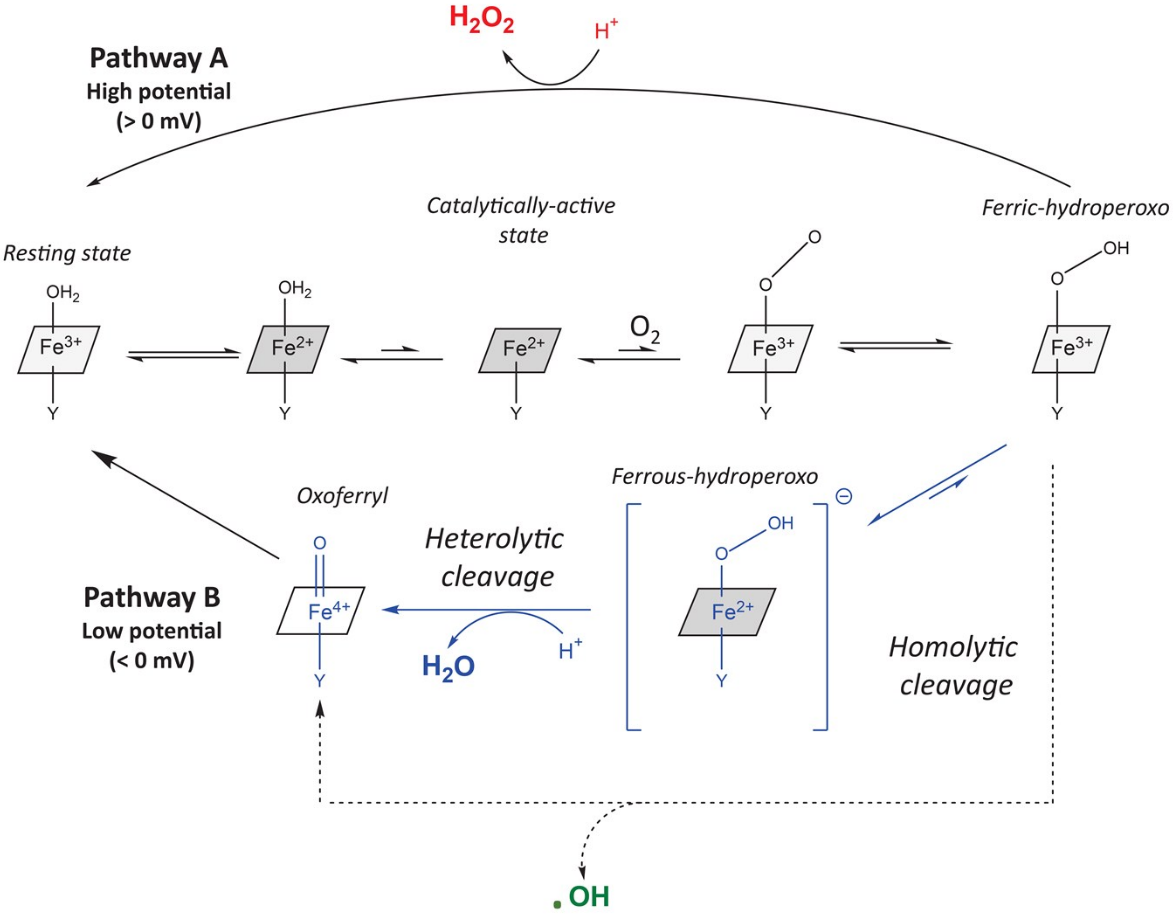
The N_C value is calculated by using the following equation:

$$N_c = \frac{I_r - I_{r0}}{I_d}$$

The N_C value of the heme-silk-CNT electrode was 1.42.



Supplementary Fig. 5 Rotating ring disk voltammetry (RRDV) 100 mM phosphate buffer, pH 7. Scan rate – 100 mV/sec. Top – Pt ring electrode at different rotation rates. Pt ring held at 1.5V *vs.* NHE. Bottom – disk electrode linear sweep voltammetry.



Supplementary Scheme 1: Plausible catalytic cycle for oxygen reduction by heme-silk films. In Pathway A, following protonation, hydrogen peroxide is released from the ferric-hydroperoxo. In Pathway B, the ferric-hydroperoxo is reduced and water is the product following heterolytic O-O cleavage. A third pathway is possible if homolytic cleavage occurs, producing hydroxyl-radicals (dotted line), which damages the catalytic heme center. This pathway leads to loss of activity within a few turnovers in simple porphyrin systems. Modified from ref 5.

References

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