Materials and Methods

Materials

Recombinant honeybee silk proteins used to make the silk films were produced via fermentation in *Escherichia coli* and freeze dried to form water-soluble sponges as previously described, then stored at room temperature until required.[1,2] Mesoporphyrin IX dihydrochloride, Pd(II) meso-Tetra (4-carboxyphenyl)porphine, zinc phthalocyanine tetrasulfonic and zinc phthalocyanine were purchased from Frontier Scientific, while Triruthenium dodecacarbonyl and all other chemicals were purchased from Sigma-Aldrich.

Preparation of ruthenium(II) CO mesoporphyrin IX (RuMP)

RuMP was prepared following slight modifications to published methods.[3,4] 180 mg of Ru_3CO_2 and 60 mg of mesoporphyrin IX dihydrochloride were refluxed under N_2 for 48 h in 30 mL of degassed glacial acetic acid. Reaction progress was monitored using UV-Vis spectroscopy. Once completed, the reaction mixture was cooled in an ice-water bath and the reaction product precipitated through the dropwise addition of chilled reverse osmosis (RO) H_2O. The reaction product was then separated from the reaction mixture by vacuum filtration, and washed with RO H_2O, yielding a deep red-purple product. The reaction product was collected and dried under vacuum for 96 hours. UV-Vis spectroscopy and NMR were used to confirm the formation of RuMP, using previously detailed NMR assignments.[4]

Methods

Macrocycle solution preparation

Macrocycle solutions were prepared by dissolving each macrocycle in 1 mL of 0.1M NaOH and 9 mL of 70% methanol added to give a final solution of 1 mg/10 mL. Solutions were then stored at room temperature and wrapped in aluminium foil until required. Solutions were used within two days, or freshly prepared for further experiments.

Silk film preparation

Silk films were prepared using two different methods. Firstly, 20-100 µL of a 10-15 mg/mL aqueous solution of silk sponge dissolved in H_2O was pipetted onto a well plate and air-dried for 24 h. The volume of solution determined the size of the film. Thicker films were prepared through repetition of this process, with additional layers of solution added once the previous solution had dried to form a film. Once dried, the films were stabilised by overnight soaking in 70% methanol, a process used to induce β-sheet formation and make the films insoluble in water.[5] To introduce a macrocycle into the film, the methanol soak was replaced with a soak in the macrocycle solution, which both stabilised the films and introduced the macrocycle to the films for binding. After soaking overnight, the solution was removed and the silk film was washed 2-3 times with 70% methanol for 30 minutes. During the drying and macrocycle introduction processes, the well plate was stored under aluminium foil to prevent photo-degradation. An alternative method of film preparation involved peeling a thin layer of silk sponge and directly applying a few drops of macrocycle solution. The piece of sponge was left soaking in the solution for approximately 5 minutes before the excess solution was removed. The sponge layer was then washed twice in 70% methanol before use.

Spectroscopy

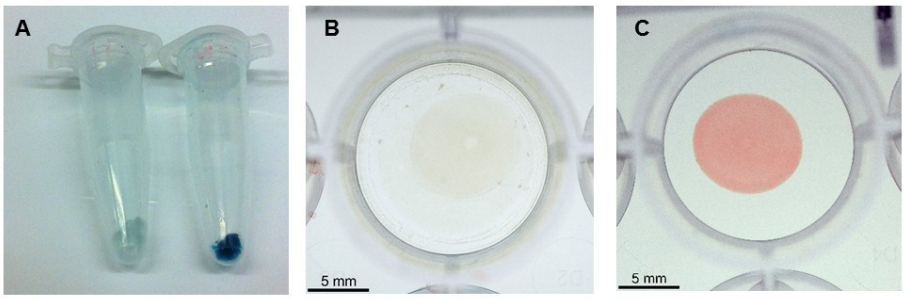
Absorption measurements were performed using either a SpectraMax M2 plate reader (Molecular Devices, USA) or an Ocean Optics Spectrometer (fibre optic with Mini D2T light source).

Fluorescence emission and phosphorescence lifetime analyses were performed using an FLS980e Fluorescence Spectrometer (Edinburgh Photonics, UK). These analyses were performed on macrocycle films or solutions in 3 mL airtight cuvettes under three different conditions: air, nitrogen (N_2) gas, or argon gas. Nitrogen and argon gas were supplied from pressurised gas canisters with controlled flow rates and applied to the cuvettes through an airtight septum with a long needle. A secondary shorter needle was used to allow excess air to escape. Gas was passed through the cuvette for a period of 20 minutes or more to ensure complete replacement of the existing conditions inside the cuvette. Fluorescence emission scans were completed using excitation wavelengths determined from the major peaks of the absorption spectrum of the corresponding film or solution. Phosphorescence lifetime analyses were completed using the same excitation wavelength and the emission wavelength determined from the emission scan. Phosphorescence lifetimes were determined using curve fitting software provided by Edinburgh Photonics. Lifetimes were fit according to an exponential decay equation (Equation 1), with the quality of the fit determined using a chi-square test.

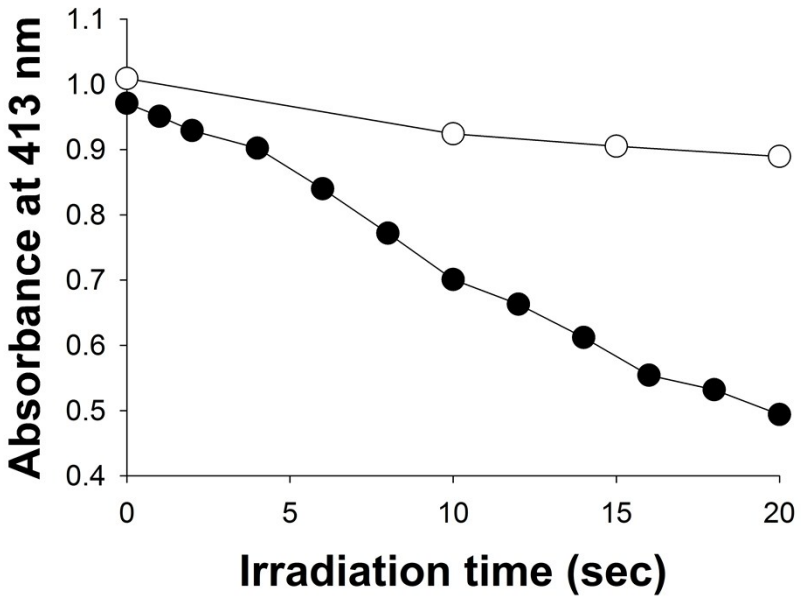
$$R(t) = B_1 e^{(-t/\tau_1)} + B_2 e^{(-t/\tau_2)} + B_3 e^{(-t/\tau_3)} + B_4 e^{(-t/\tau_4)} \quad (1)$$

Singlet oxygen production assays

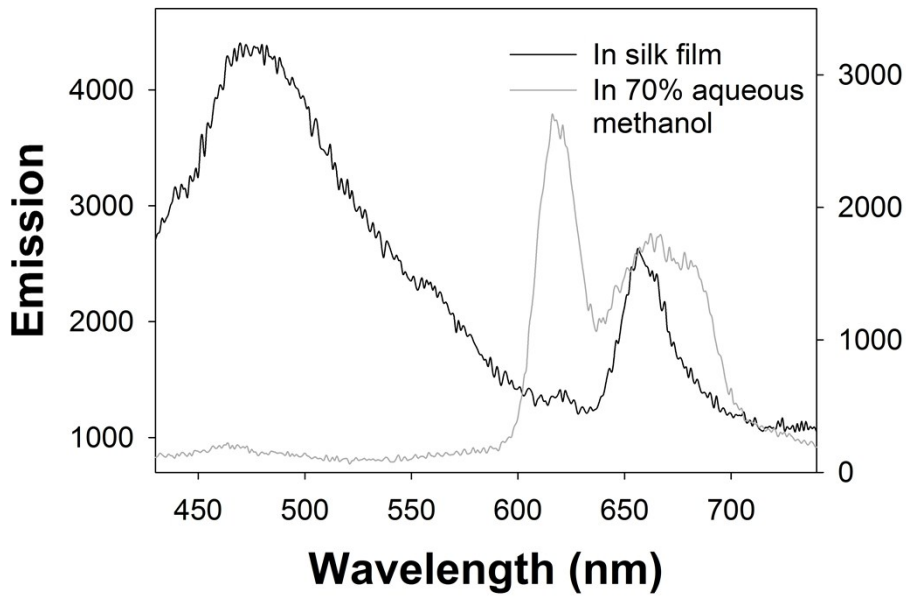
Singlet oxygen studies were carried out using a tungsten lamp (240 W, 20 V). A H_2O filter, to filter off ultraviolet and far infra-red radiation and an interference filter (Wratten Special Filter 29), were placed before the light source. Experiments were carried out in a spectrochemical cell of 1 cm pathlength using DMSO as a solvent and 1,3-diphenylisobenzofuran (DPBF) as a singlet oxygen scavenger (3×10^{-5} M).



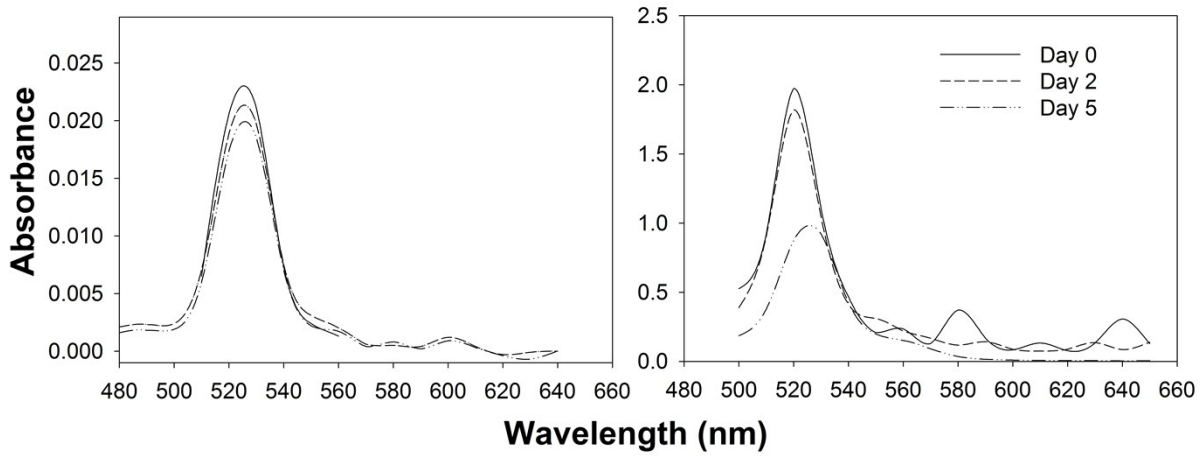
Supplementary Figure S1: A Photographs of silk sponges which have been soaked in methanol solutions containing zinc phathlocyanine (ZnPc -left) and zinc phathlocyanine tetrasulfonate (ZnPcTs - right) and washed with 70% aqueous methanol. The dark blue colour of the sponge noted with ZnPcTs indicates binding to the silk in contrast to that seen with ZnPc. **B** and **C** – Silk films to which ruthenium mesoporphryin IX **(B)** and palladium-meso-tetra-(4-carboxyphenyl)porphyrin **(C)** have been added from 70% methanol solutions.



Supplementary Figure S2: Comparison between the rate of DPRF degradation (absorbance max 413 nm) by zinc phthalocyanine tetrasulfonic acid in the presence of oxygen (solid circles) compared to anaerobic DMSO solutions (open circles).



Supplementary Figure S3: Comparison of the emission spectrum for ruthenium mesoporphyrin IX in solution and immobilised in a silk film following excitation at 400 nm.



Supplementary Figure S4: Photostability of palladium-meso-tetra-(4-carboxyphenyl)porphyrin (PdPor) in silk film (left) compared to in 70% methanol solution (right) over five days.

References

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