

Supplementary Information

The Supramolecular Structure of Melanin

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Image Filtering: To emphasize the periodic features of interest in the transmission electron micrograph the images were filtered in frequency space to remove frequency components outside the spatial range of interest.

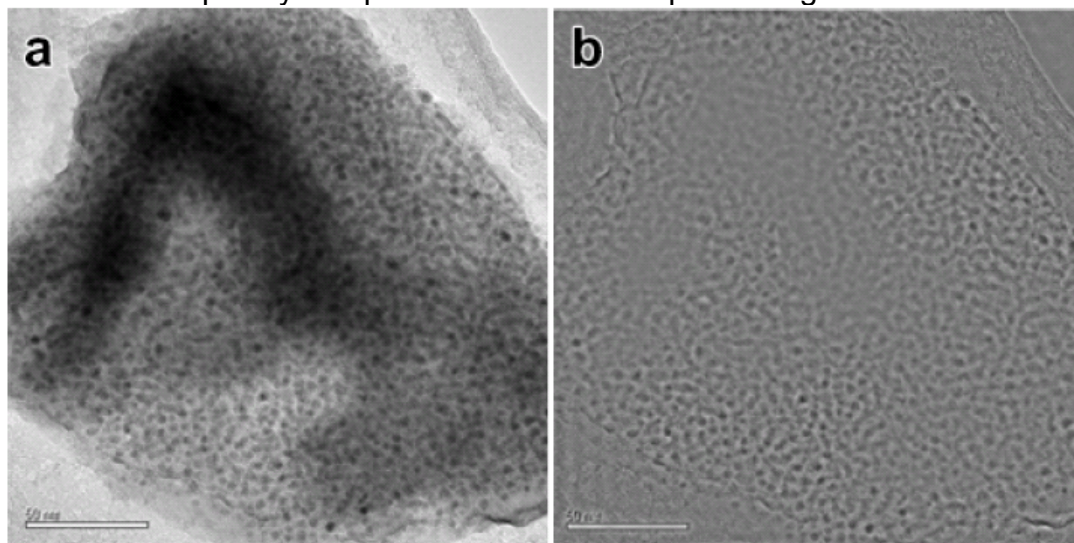


Figure S1. (a) Low resolution bright field transmission electron micrograph of synthetic eumelanin. (b) Micrograph after spatial periods outside of the range 1 – 8 nm have been removed.

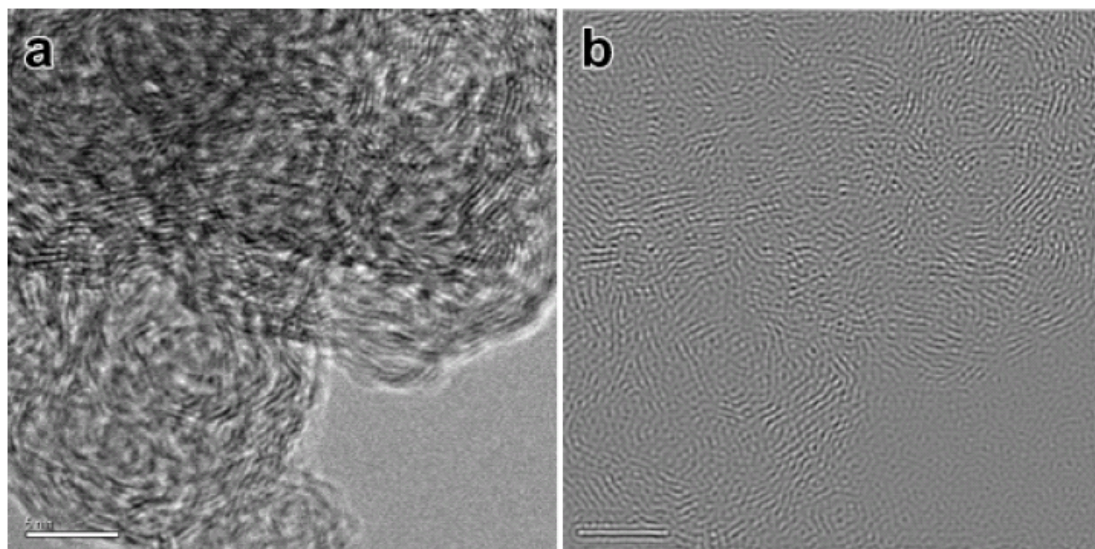


Figure S2. (a) High resolution bright field transmission electron micrograph of synthetic eumelanin. (b) Micrograph after spatial periods outside of the range 3 – 5 Å have been removed.

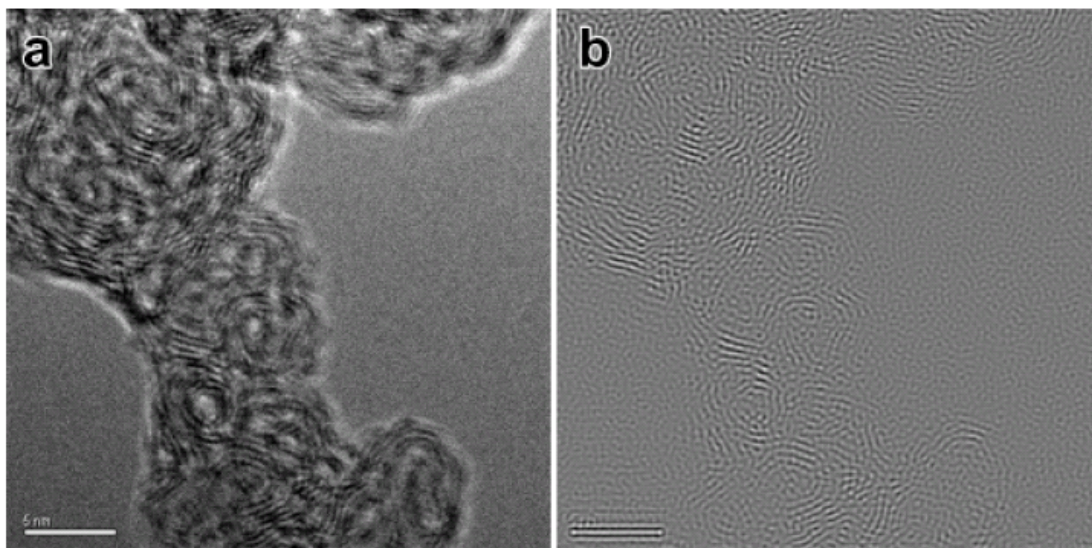


Figure S3. (a) High resolution bright field transmission electron micrograph of synthetic eumelanin. (b) Micrograph after spatial periods outside of the range 3 – 5 Å have been removed.

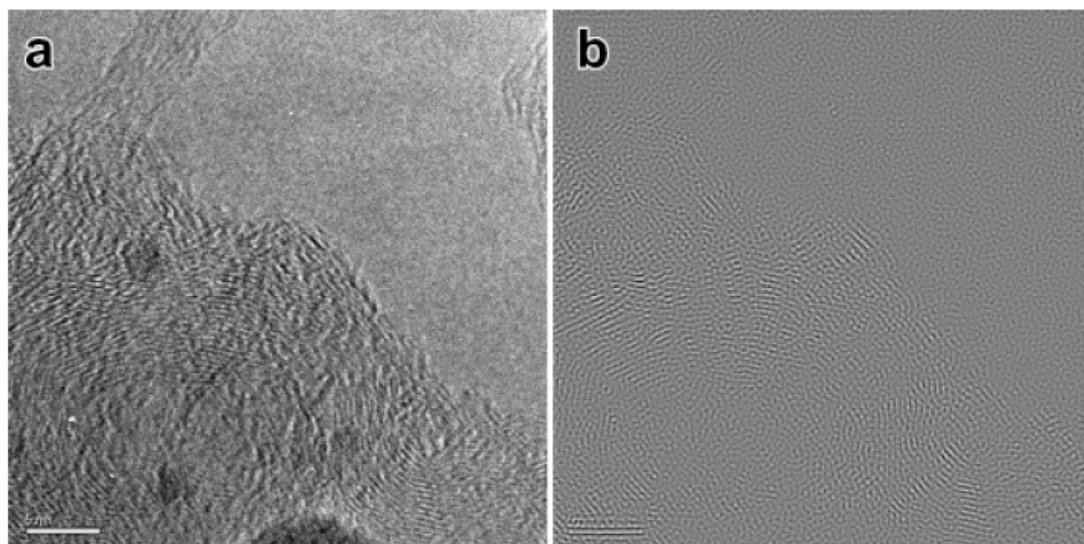


Figure S4. (a) High resolution bright field transmission electron micrograph of bovine eumelanin. (b) Micrograph after spatial periods outside of the range 3–5 Å have been removed.

Photoluminescence: The photoluminescence of synthetic eumelanin (corrected for reabsorption and inner filter effects¹) in water and the de-stacked eumelanin in DMF were examined (See Fig. S5 and Fig. S6). In both cases the fluorescence was found to be very weak (radiative quantum yield $\ll 1\%$) and the profile was that of an inhomogeneously broadened Gaussian whose peak shifted with excitation wavelength. Both of these features are characteristic of synthetic eumelanin and have been extensively reported in the literature¹⁻³.

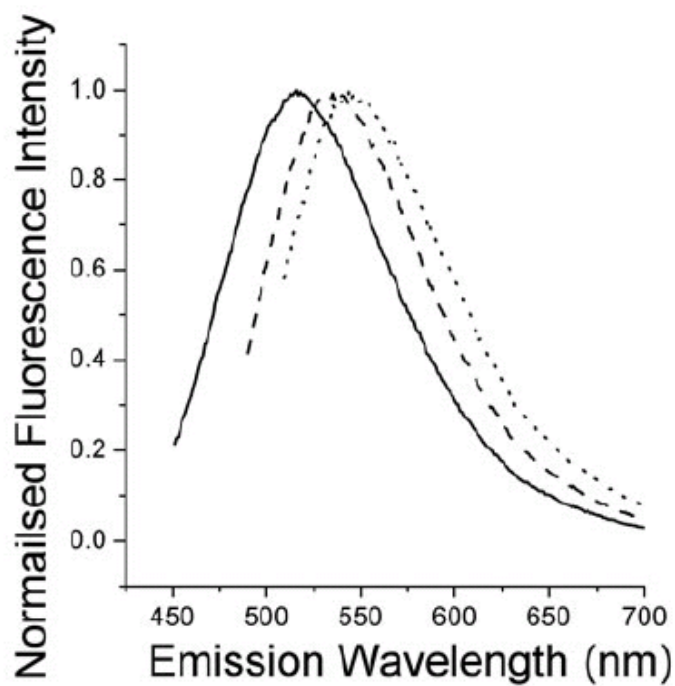


Figure S5. Photoluminescence of 0.005 % (w/w) solution of synthetic eumelanin in water with the excitation wavelengths of 440 nm (solid), 480 nm (dashed) and 500 nm (dotted).

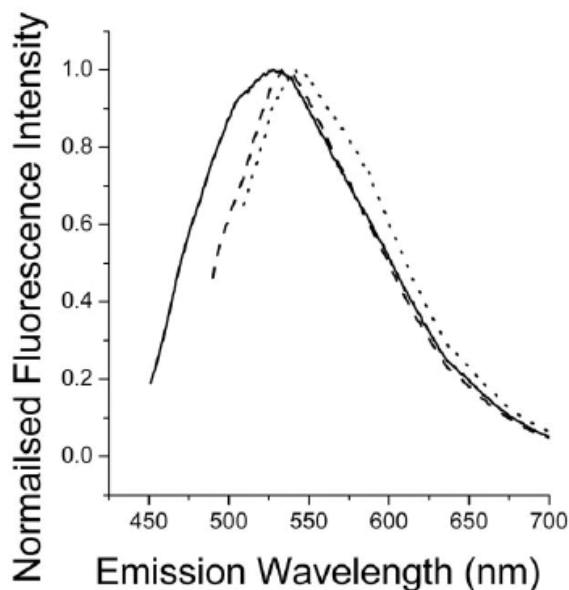


Figure S6. Photoluminescence of 0.005 % (w/w) solution of synthetic eumelanin in DMF, the excitation wavelengths of 440 nm (solid), 480 nm (dashed) and 500 nm (dotted).

Additional Fourier analysis: Fourier analysis was conducted on the LVHRTEM images obtained from both the de-stacked synthetic eumelanin and also for those obtained from the sepia eumelanin granules. Fig. S7 shows that both of the images of the DMF de-stacked synthetic eumelanin have no feature at 3.7 – 4.0 Å. There is a broad peak centred on 5 Å which is likely due to the characteristic size scale of the monomer indoles which are on the order of 5 Å⁴.

The scaled radial power spectrum of the sepia eumelanin shown in Fig. S8 shows a peak occurring at around 3.5 – 4.5 Å. The lower quality of the image and fewer stacked regions in the focal plane of the LVHRTEM is responsible for the broadened peak in the Fourier analysis.

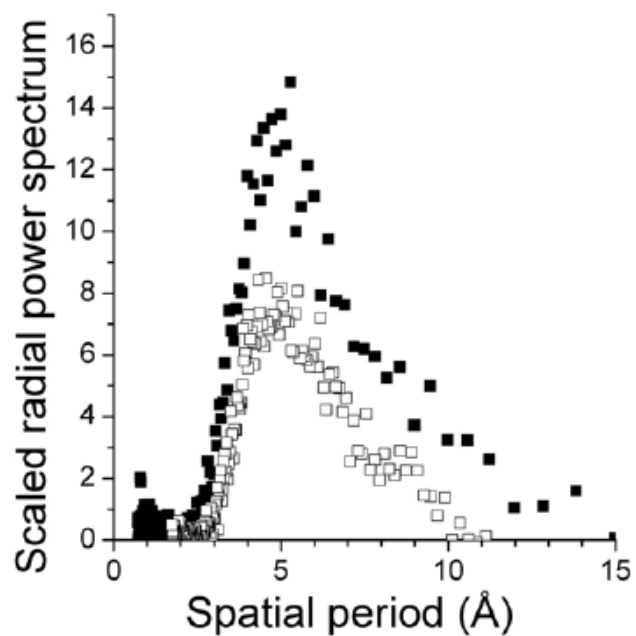


Figure S7. Scaled radial power spectrum of the LVHRTEM images of DMF destacked eumelanin shown in Fig.6b (open black squares) and Fig.6a (solid black squares).

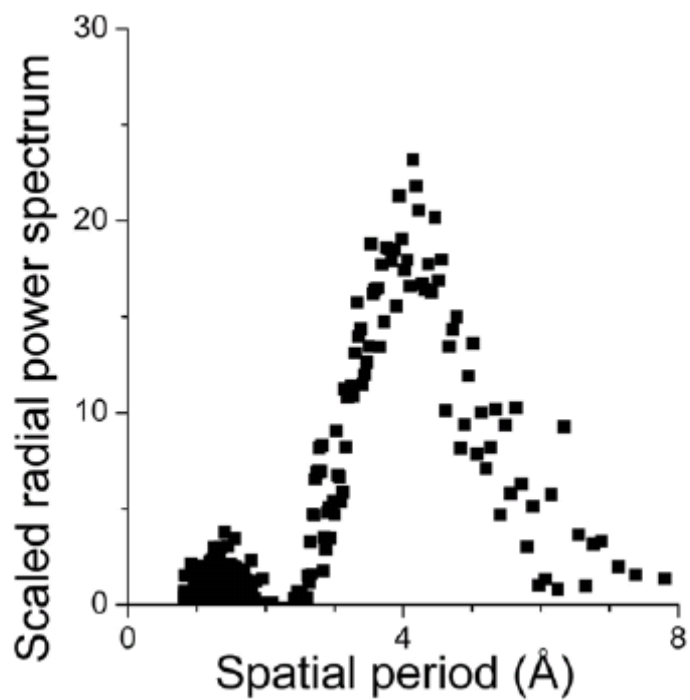


Figure S8. Scaled radial power spectrum of sepia eumelanin LVHRTEM shown in Fig.5c.

Supplementary Methods

Synthetic eumelanin synthesis: Synthetic eumelanin was synthesized and purified following the standard literature procedure⁵. Briefly, 15 g of DL-dopa, purchased from Sigma-Aldrich (Sydney, Australia), was dissolved in 3 L of high-purity 18.2 M Ω milliQ de-ionized water. The pH of the solution was adjusted to pH 8 using a concentrated ammonia solution. Air was bubbled through the solution while it stirred for 3 days. Concentrated hydrochloric acid was then added to the solution to bring the pH down to 2. The resulting black precipitate was washed several times with 0.01 M hydrochloric acid and then with de-ionized water. The precipitate was then dried overnight in an oven at 80°C.

Absorption measurements: Absorbance spectra were recorded using a Varian (Palo Alto, CA) Cary 300 UV-Vis spectrophotometer with a 300 nm/min scan speed and 2 nm bandpass. All spectra were collected using a 1 cm square quartz cuvette. Solvent scans (obtained under identical conditions) were used for background correction.

Fluorescence Measurements: Photoluminescence excitation spectra were recorded using a Jobin Yvon (Paris, France) Fluoromax 3 fluorimeter. All spectra were collected using a 1 cm square quartz cuvette. A band-pass of 3 nm and an integration time of 0.5 s were used. Again, solvent scans were acquired for background correction. The spectra were corrected for attenuation of the probe and reabsorption of the emission according to the procedure outlined by Meredith *et. al.*¹.

Supplementary References

1. Meredith, P. & Riesz, J. Radiative relaxation quantum yields for synthetic eumelanin. *Photochem. Photobiol.* **79**, 211-216 (2004).
2. Nighswander-Rempel, S. P., Riesz, J., Gilmore, J. & Meredith, P. A quantum yield map for synthetic eumelanin. *J. Chem. Phys.* **123**, 194901 (2005).
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4. Powell, B. J. 5,6-Dihydroxyindole-2-carboxylic acid: a first principles density functional study. *Chem. Phys. Lett.* **402**, 111-115 (2005).
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