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Melanin pigments extracted from horsehair as antibacterial agents

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Supporting Information

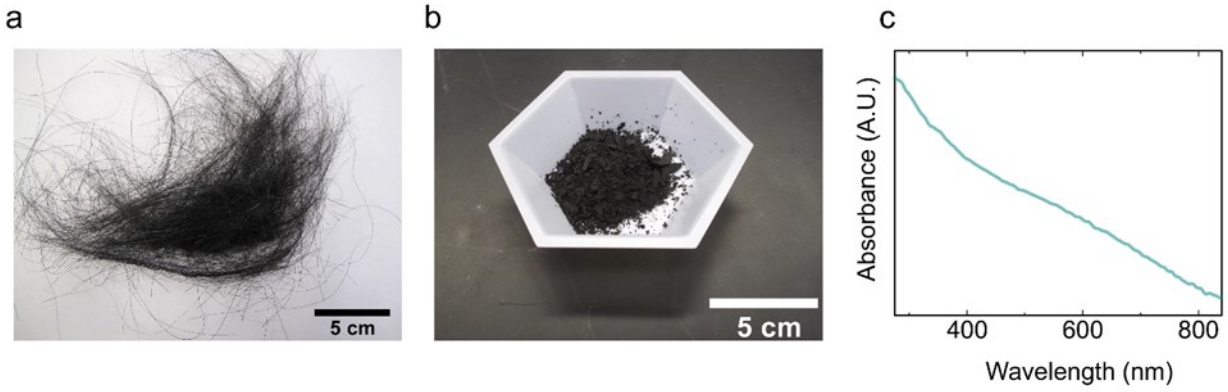

Fig. S1: (a) *Equus ferrus* hair fibers and (b) *Equus*Mel powder after extraction. (c) UV-Vis absorbance spectrum is shown for the *Equus*Mel solution (200 µg/ml). This exhibits the broad band monotonic absorbance without the distinct peaks.

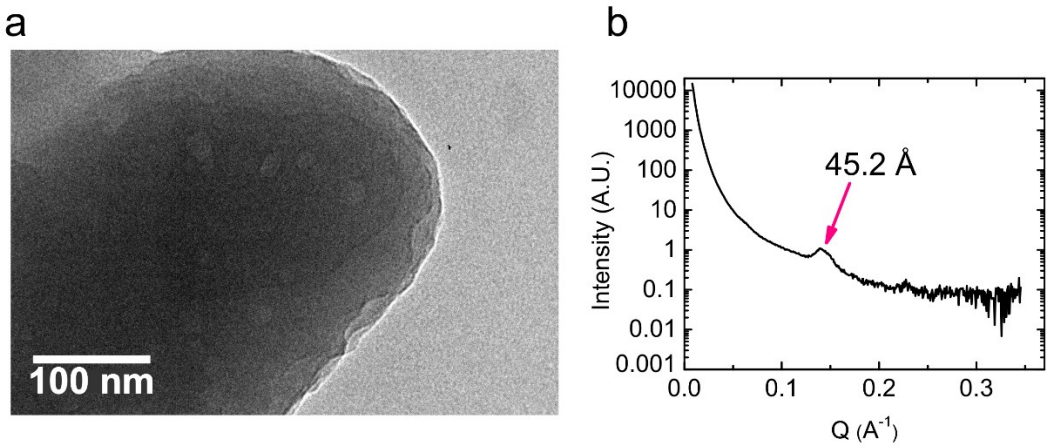

Fig. S2. Transmission electron microscopy (TEM) (a) and Small- angle X-ray scattering (SAXS) suggest that *Equus*Mel has multilayer surface with semi-crystalline structure.

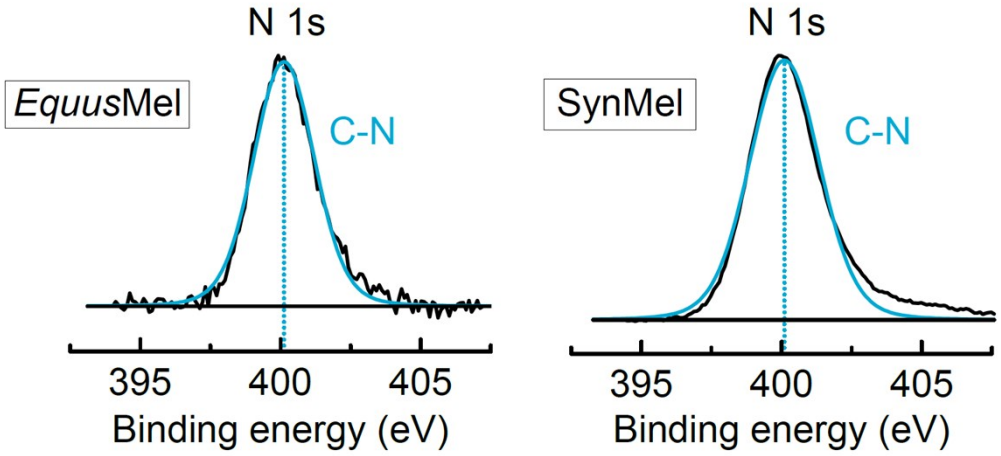

Fig. S3. High-resolution N 1s XPS spectra from *Equus*Mel and SynMel are shown. Peak fitting (blue line) is performed by CasaXPS. The peak at binding energy of 400.11 ± 0.014 represents C-N stretching from the secondary amine functional group in indole.

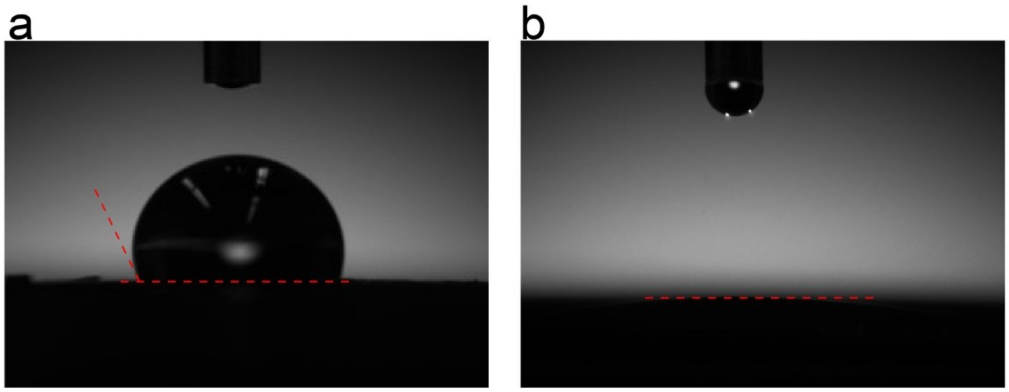

Fig. S4. Static water contact angle measurement exhibits (a) hydrophobic *Equus*Mel (contact angle= 104.7 ± 2.2°) and (b) superhydrophilic SynMel (contact angle= 0°). Melanin pellets were prepared to exhibit the flat top surface before applying 10 µl of ddH_2O.

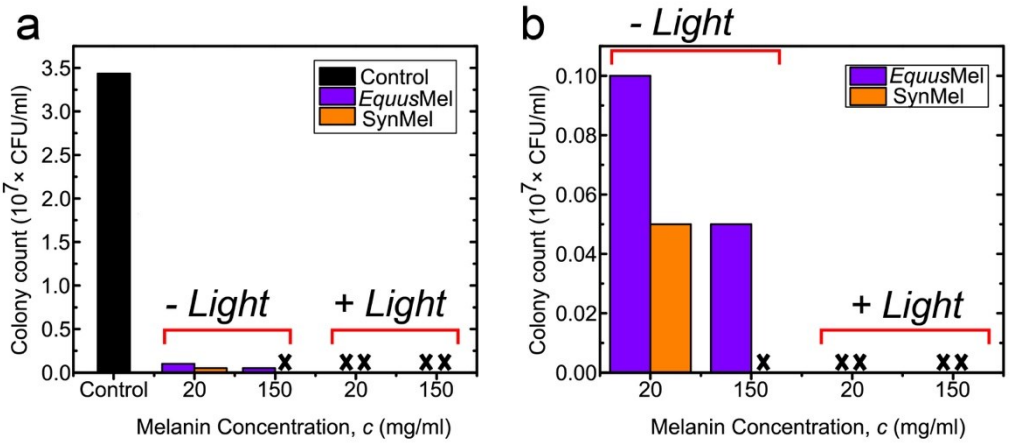

Fig. S5. (a) Antibacterial activity of *Equus*Mel and SynMel against *S. aureus* are shown after 4 h of incubation. + Light signifies that bacterial incubation with melanins occurred under ambient condition while – Light indicates light exposure was minimized as much as possible throughout the experiment from 7 days prior to incubation through colony counting. Detailed values of colony count can be found from the (b) enlarged view.

Table S1. Peak assignments of FTIR spectra for *Equus*Mel and SynMel.

Wavenumber (cm^{-1})	**Vibration mode and main functional groups**
3400 – 3200	Stretching vibration of O–H and N–H groups (carboxylic acid and phenolic OH, anime in indole, pyrrole, and amino acids)
2950 – 2850	Stretching vibration of C–H
1720 – 1706	Stretching vibration of aromatic C=O in carboxylic acid
1650 – 1600	Stretching vibration of Conjugated C=C
1342 – 1266	Stretching vibration of C−N in indole
680 – 860	Bending vibration of aromatic C−H

Table S2. Peak positions of Raman spectra of *Equus*Mel and SynMel are shown after the deconvolution using voigt function.

Peak position, cm^{-1}		Vibration mode and main functional groups
***Equus*Mel**	**SynMel**	
1224.404	1237.68445	**(α)** C–O stretching in carboxylic acid and C–OH
1348.694	1348.01362	**(β)** stretching vibration of aromatic C–N in indole
1431.441	1437.68445	**(γ)** pyrrole ring stretching
1507.377	1509.1941	**(δ)** stretching vibration of C=N in semiquinone and bending vibration of N–H
1588.081	1575.48241	**(ε)** stretching vibration of aromatic C=C in indole

Table S3. Summary of high-resolution XPS analysis of *Equus*Mel and SynMel.

	Binding energy, eV		
	O 1s	N 1s	C 1s
***Equus*Mel**	532.17 [C–OH] 533.82 [COOH]	400.12 [C–N]	284.8 [C–C]/[C=C] 287.19 [C–N]/[C–O]
SynMel	531.12 [C–O] 532.62 [C–OH]	400.10 [C–N]	284.8 [C–C]/[C=C] 287.26 [C–N]/[C–O]

Table S4. Bactericidal activity of *Equus*Mel and SynMel against *E. coli* and *S. aureus* after incubating for 4 h under ambient light.

Bacterial species	*E. coli*		*S. aureus*	
Melanin Type / Concentration (mg/ml)	***Equus*Mel**	**SynMel**	***Equus*Mel**	**SynMel**
150	100 %	100 %	100 %	100 %
20	98.12 %	100 %	100 %	100 %
5	69.06 %	98.12 %	76.67 %	98.54 %
2.5	6.87 %	61.87 %	30.1 %	93.2 %