Supplementary Information

Supplementary Table S1. Assignment of infrared absorption peaks in the spectra of hair medulla according to [21,23,63]

Position (cm^{-1})	Vibration	Assignment
3280	ν N-H	Protein amide A
3195	ν N-H	Protein amide B
3064	ν N-H	Protein amide B
2963	ν_{as} C-H, CH_3	CH_3 from proteins and lipids
2921	ν_{as} C-H, CH_2	CH_2 from lipids
2874	ν_s C-H, CH_3	CH_3 from proteins and lipids
2850	ν_s C-H, CH_2	CH_2 from lipids
1740	ν C=O, COOR	Carbonyl from lipid esters
1654	ν C=O, CONH	Amide I from proteins
1577	ν_{as} C=O, COO^-	Carboxylate C=O from lipids
1543	δ oop N-H	Amide II from proteins
1542	ν_{as} C=O, COO^-	In medulla minus cortex difference spectra
1515 (shoulder)	ν C-C, δ C-H	Tyrosine Tyr-OH
1470	δ_{as} C-H, CH_2	CH_2
1450	δ_{as} and δ_s C-H, CH_2 and CH_3	CH_3 and CH_2
1434, 1419	ν_s C=O, COO^-	Carboxylate
1390	δ C-H, CH_3	Lipids and proteins
1310	δ N-H and ν C-N in CONH	Amide III from proteins
1233	δ N-H and ν C-N in CONH	Amide III from proteins
1175	ν_{as} S=O, SO_3^{2-}	Cysteic acid sulfonate
1126	ν_{as} S=O, O=S=O	Cystine dioxide
1115	ν_s S=O, O=S=O	Cystine dioxide

1080	ν_s S=O, S-S=O	Cystine monoxide
1040	ν_s S=O, SO_3^{2-}	Cysteic acid sulfonate

ν_{as}: asymmetric stretching, ν_s: symmetric stretching, oop: out of plane, δ_{as}: asymmetric deformation.

Supplementary Table S2: Parameters for measuring spectral markers for hair (peak position and peak width, baseline points).

Name	**Position (cm^{-1}) & Interval (cm^{-1})**	**Baseline* (cm^{-1})**	**Note**
ν_s NH area	3280-3290 3120-3660	3120-3150 3655-3665	Amide A from proteins
ν_{as} CH_3	2955 2946-2980	2800-2920 2920-3000	Protein quantification Acyl chain length evaluation
ν_{as} CH_2 area	2915 2905-2945	2800-2920 2920-3000	Lipid quantification Lipid organization
ν_s CH_2 peak position	2850 2825-2865	2830-2865	Lipid organization
Total C-H area	2800-3005	2800-3005	Total lipid quantification
ν_s C=O ester area	1740 1725-1755	1740-1755 1475-1490	Lipid ester quantification
ν C=C area	1610 1595-1620	1620-1630 1585-1595	C=C from melanin and unsaturated lipids
COO^- νa_s C=O area	1578 1565-1590	1565-1590	Carboxylate quantification
δ_{as} C-H area	1470 1460-1485	1475-1490 1455-1460	Lipid quantification
-NH-C=O area	1240 1205-1265	1170-1235 1245-1305	Amide III from proteins

ν_{as} SO_3^{2-} area	1175 1155-1185	1120-1150 1180-1205	Cysteic acid sulfonate, marker of disulfide bridge oxidation
ν_s S-O^- area	1080 1055-1095	1040-1060 1080-1115	Cystine monoxide
ν_{as} SO_3^{2-} area	1040 1025-1055	1020-1040 1040-1080	Cysteic acid sulfonate, marker of disulfide bridge oxidation

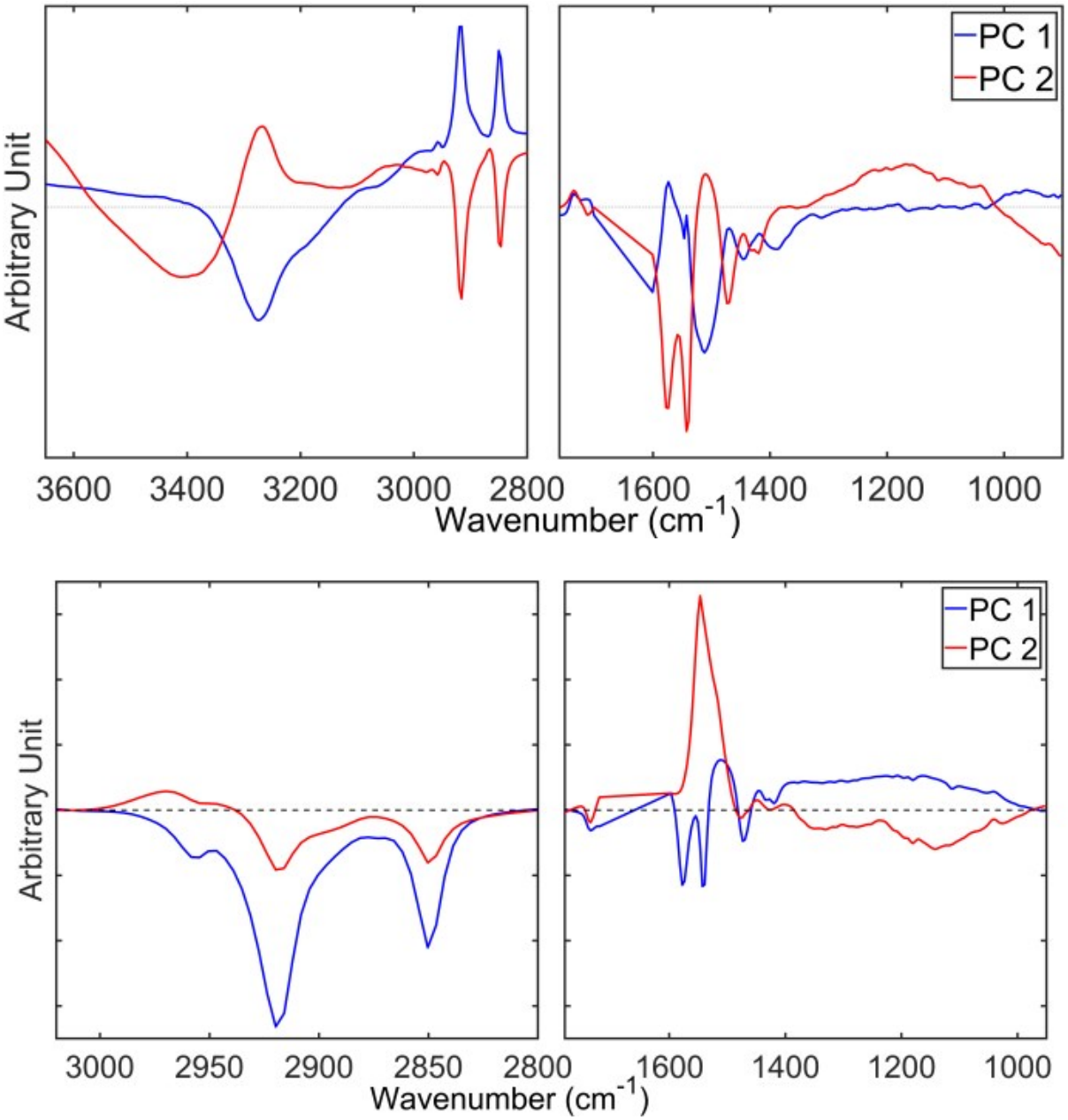
*Baseline: positions of the 2 baseline points. When two pairs of values are indicated, the algorithm was used in the 'minimum between 2 points' search mode, wherein the baseline was automatically adjusted to pass by the local minimum in each of the 2 ranges. ν_{as}: asymmetric stretching, ν_s: symmetric stretching, δ_{as}: asymmetric deformation.

Supplementary information

Search for melanin signal in the hair medullas

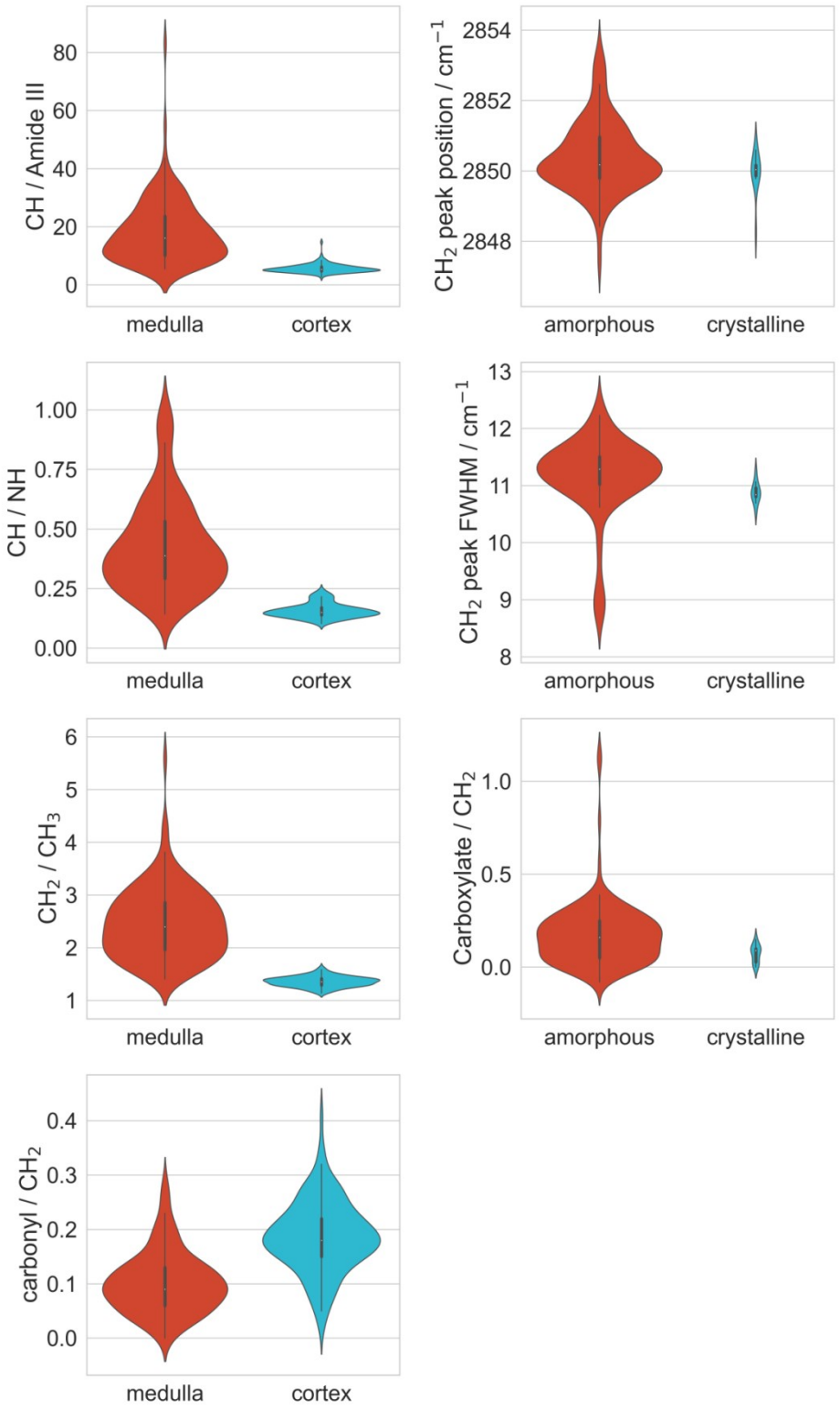
Medullas were detected visually by their dark pigmentation due to the presence of melanin. Melanin IR spectrum presents mainly broad -almost featureless- bands except for a sharp peak around 1700 cm^{-1} ascribed to carboxylic acid moieties. The dominant features are the very large bands between 1000-1400 cm^{-1} arising from hydroxyl, alcoholic, and phenolic groups, the peak around 1610 cm^{-1} assigned to the C=C vibration and the large OH peak at 3000-3400 cm^{-1} [64]. The most suitable peaks for detecting melanin are the intense 1610 cm^{-1} peak which might appear between the two amide bands of keratins and KAPs and the sharper COOH peak at 1710 cm^{-1} which might appear between the 1736 cm^{-1} ester peak and the foot of the amide I band. We searched for the presence of melanin using these peaks but we could not detect any obvious sign in any of the 134 medulla and difference spectra. Thus, we conclude that infrared microspectroscopy doesn't appear to be a suitable method to study melanin *in situ* in hair medulla.

Supplementary Figures



Supplementary Figure S1. Loadings plots from principal components analysis of medulla – cortex difference spectra (top) and from medulla spectra (bottom). PC1 and PC2 capture respectively 36 and 21% of the spectral variance of the difference spectra; 52 and 22% of the medulla spectra. For the PCA of difference spectra, the loadings of PC1 show an anti-correlation of lipid signal (C-H, 2800-3000 cm^{-1}) and protein signal (N-H at 3290 cm^{-1}) and those of PC2 show a correlation between the C-H signal and the carboxylate signal (1575, 1542, 1470, 1430, 1420 cm^{-1}). The narrow peak at 1542.7 cm^{-1} from calcium carboxylates in PC2

correlated with other carboxylate peaks and with the O-H peak and anti-correlated with the protein N-H peak. For the PCA on medulla spectra, PC1shows correlation between the lipid C-H peaks and C=O peaks from calcium carboxylate. The PC2 shows an anti-correlation between the amide II peak at 1545 cm^{-1} and the lipid C-H and esterified lipid C=O peaks.



Supplementary Figure S2. Spectral marker values for hair medulla and cortex. Violin plot of the values from Table 1. Peak area ratios, peak position and peak FWHM of various peaks were used as spectral markers to evaluate the lipid concentration (CH/Amide III, CH/NH, CH_2/CH_3),

the lipid phase (CH_2 peak position), lipid crystallinity (CH_2 FWHM), and carboxylate or carbonyl concentration in the medullas.