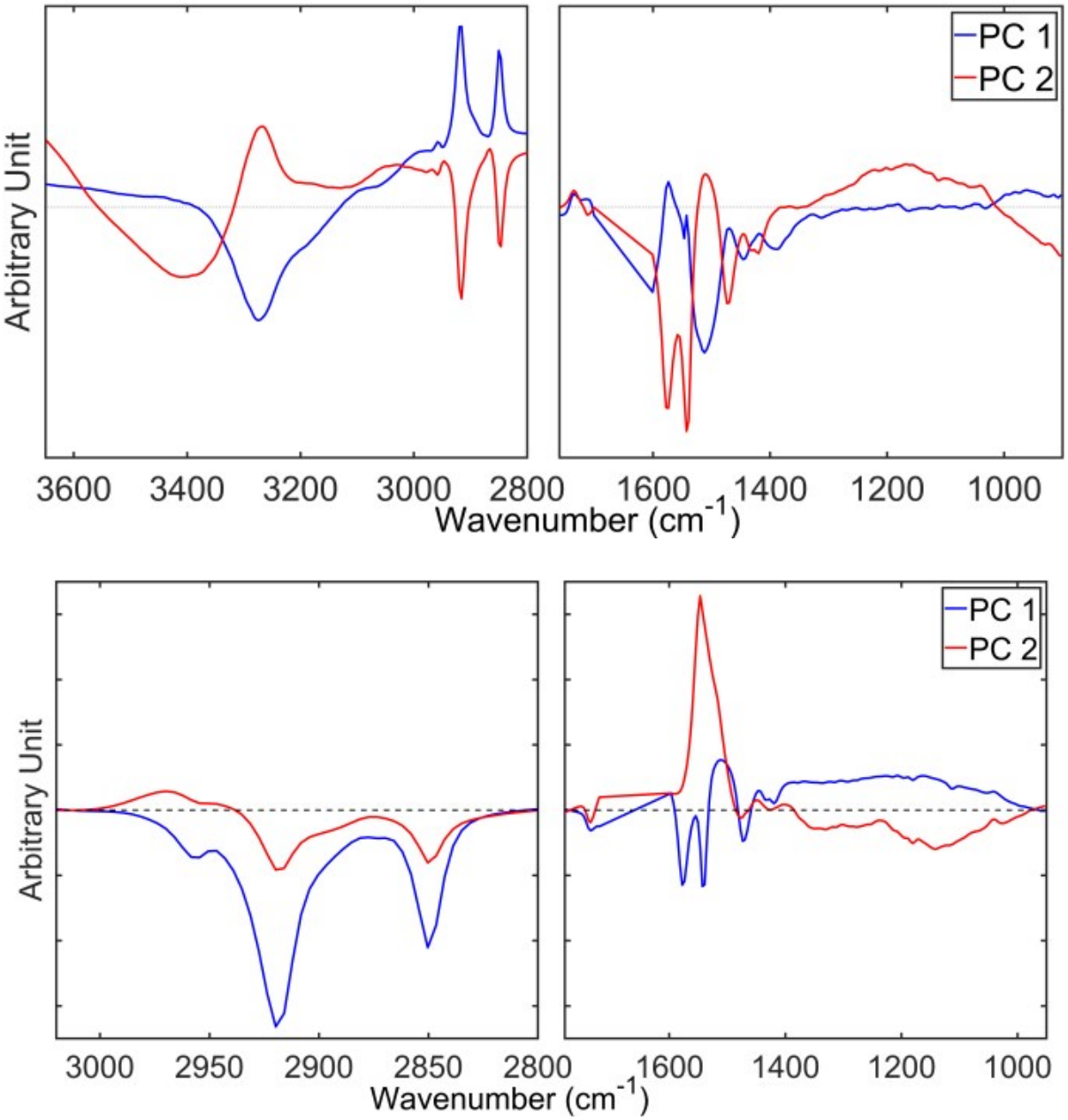
Supplementary information

Search for melanin signal in the hair medullas

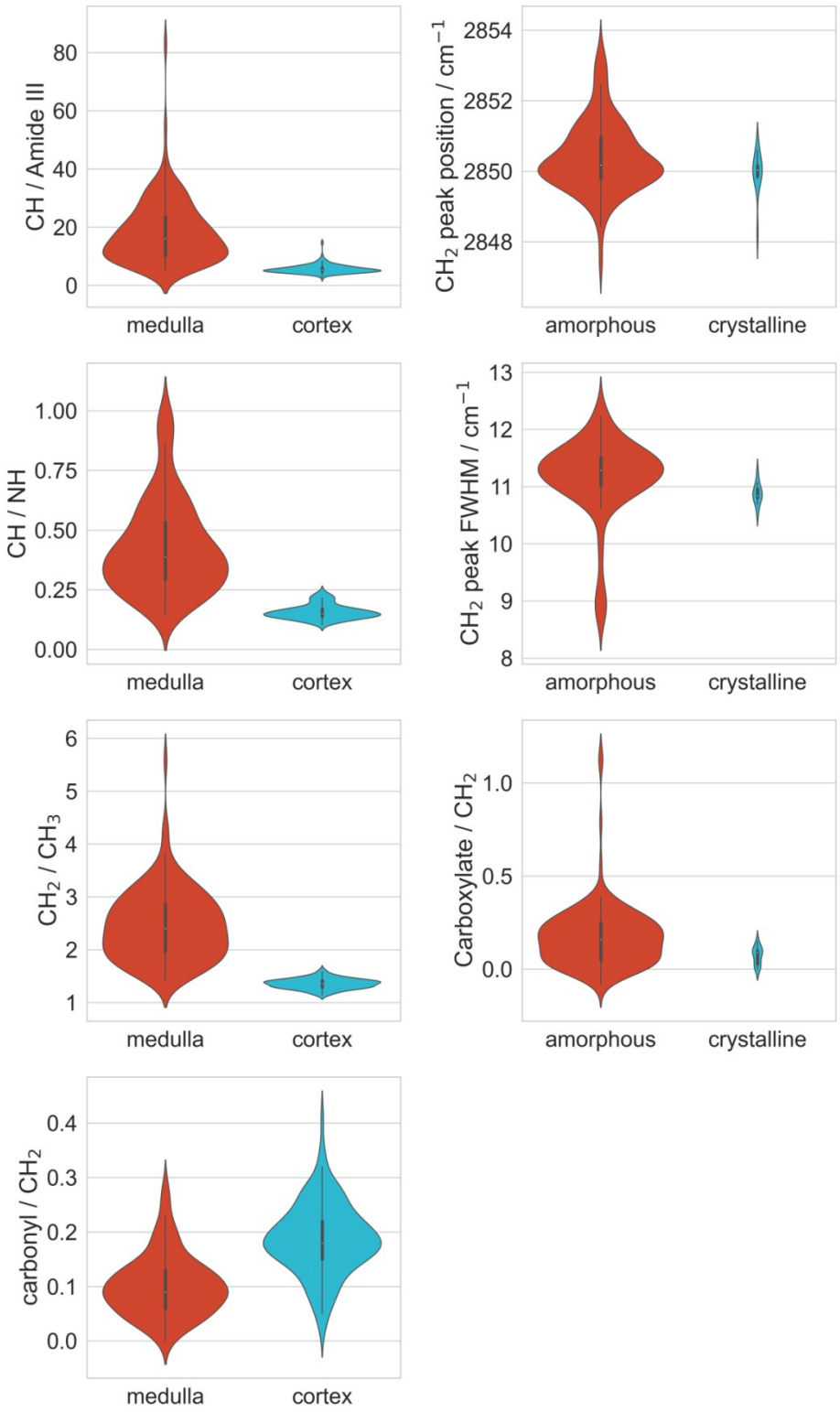
Medullas were detected visually by their dark pigmentation due to the presence of melanin. Melanin IR spectrum presents mainly broad -almost featureless- bands except for a sharp peak around 1700 cm^{-1} ascribed to carboxylic acid moieties. The dominant features are the very large bands between 1000-1400 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.