

Experimental:

X-ray absorption near-edge structure spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS) were employed to analyze the nature of the synthetic eumelanin solid sample. The X-ray absorption spectra were measured at the XAFS-1 beamline of the LNLS, Laboratorio Nacional do Luz Síncrotron, Campinas, São Paulo, Brazil. XANES and EXAFS spectra at the Fe K-edge (7112 eV) were recorded in air at room temperature in transmission mode with three ion chambers as detectors: one before the sample to measure the incident X-ray intensity (I_0), one after the sample and before the corresponding reference metal foil to measure the intensity after the sample (I_1), and one after the metal foil (I_2). The absorption spectrum of the sample and the metal foil were expressed as $\log(I_0/I_1)$ and $\log(I_1/I_2)$, respectively. The spectrum of the metal foil was used to calibrate the absolute energy scale for the corresponding sample spectrum by positioning the absorption edge at the first inflection point. Monochromator at the beamline was equipped with Si(111) crystals. The 0.3 mm vertical aperture of the beam definition slits in the hutch provided a resolution of about 2.5 eV at the Fe K-edge.

Results and discussion:

XANES:

In order to extract information about the electronic state of Fe and its site symmetry in synthetic melanin, Fe K-XANES spectrum of that sample and the corresponding ones from different Fe-oxides reference compounds were recorded. Figure 1.a shows the Fe-K XANES spectra of three different Fe-oxide reference compounds as well as the spectrum of the synthetic eumelanin (solid sample). XANES spectra were analyzed using standard procedures: a linear background was fitted to the pre-edge region and then subtracted from the entire spectrum, and the jump of the spectrum was normalized to unity with the post-edge asymptotic value.

One qualitative but relevant consideration is about the pre-edge region of the XANES spectra. In effect, the feature at the pre-edge region of the transition metal 1s edges can be directly related to the symmetry of the metal atoms. The intensity of the pre-edge peak (at 7114 eV) is much larger for compounds in which the metal site has tetrahedral symmetry than for (distorted) octahedral systems. This is clearly explained by de Groot as follows [de Groot, F.M.F., *J. Electron Spectrosc. Relat. Phenom.*, **1994**, 529]: the pre-

edge region is related to transitions to the 3d bands. Both direct $1s \rightarrow 3d$ quadrupole transitions and dipole transitions to 4p character hybridized with the 3d band are possible. For the quadrupole transitions, the matrix elements are only about 1% of the dipole transition, but on the other hand, the amount of 3d character is by far larger than the p character. In tetrahedral systems, the local mixing of p and d nature is symmetry allowed, while for a system with inversion symmetry such as octahedral symmetry, it is “forbidden”. This rule is relaxed in the solid, and if the density of states is calculated, one finds a small admixture of p states into the 3d band. This admixture is less than for tetrahedral systems, which explains the small pre-edge. If an octahedral metal site is distorted then, depending on the particular distortion, more p character will be mixed into the 3d band. The result is that a distortion of the octahedron will show up as an increased intensity of the pre-edge peaks. In summary, the rule is the higher the symmetry the lower the intensity of the pre-peak. In our case, for the melanin sample, the experimental evidence from the XANES spectrum is consistent with the presence of Fe ions in higher symmetry, i.e. octahedrally coordinated ions.

The higher the oxidation state of the metal, the more positive the overall charge of the atom, and more energy is required to excite an electron out of an orbital [Shulman, R. G., Yafet, Y., Eisenberger, P., Blumberg, W. E. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1384]. The first formally allowed electric dipole transition is the $1s \rightarrow 4p$ transition. Due to the size of the 4p orbital, it overlaps with p orbitals of the ligands, either through σ - or π -bonding. Consequently, this transition is sensitive to the oxidation state and the ligand environment of the metal. For certain symmetries around the metal, the formally electric-dipole forbidden $1s \rightarrow 3d$ transition can be observed, which occurs at a lower energy than the main edge transitions [Roe, A. L., Schneider, D. J., Mayer, R. J., Pyrz, J. W.; Widom, J., Que, L., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1676]. This transition gains intensity due to the mixing of metal 3d and 4p orbitals, and gives information about the ligand as well as about the oxidation state and symmetry of the metal complex [DuBois, J. L., Mukherjee, P., Stack, T. D. P., Hedman, B., Solomon, E. I.; Hodgson, K. O. *J. Am. Chem. Soc.* **2000**, *122*, 5775. Westre, T. E., Kennepohl, P., DeWitt, J. G., Hedman, B., Hodgson, K. O., Solomon, E. I. *J. Am. Chem. Soc.* **1997**, *119*, 6297].

To obtain the mean oxidation state of the iron in the sample a linear relationship between the shift of the absorption edge and the iron (average) oxidation state was considered in a reasonable first approximation by using different Fe-oxides as reference

compounds [Ramallo-López, J.M., Lede, E.J., Requejo, F.G., Rodríguez, J., Kim, J.Y., Rosas-Salas, R., Domínguez, J.M., *J. Phys. Chem. B*, **2004**, *108*, 20005]. The energy shift of the edge absorption relative to the metal reference compound was determined by the position of the inflection point at the edge region in the absorption spectrum. Thus the Fe^{2+} to Fe^{3+} ratio in melanin was determined from the chemical shifts of Fe edge at the absorption spectra assuming the presence of only Fe in +3 and +2 oxidation state in the melanin sample as earlier [González Orive, A., Dip, P., Gimeno, Y., Díaz, P., Carro, P., Hernández Creus, A., Benítez, G., Schilardi, P.L., Andrini, L., Requejo, F.G., Salvarezza, R.C., *Chem. Eur. J.*, **2007**, *13*, 473; Ide-Ektesabi, A. A., Kawakami, T., Watt, F., *Nuclear Instruments and Methods in Physics Research B*, **2004**, *213*, 590. Ide-Ektesabi, A.S., Fujisawa, S. Yoshida, *J. App. Phys.* **2002**, *91*, 1613]. According to this procedure, the energy shift of Fe K-XANES edge in our aumelanin solid sample is 7.3 (figure 1.b), which implies a relationship - averaged on the (homogeneous) sample - for $\text{Fe}^{2+}/\text{Fe}^{3+}$ of about 1/9.

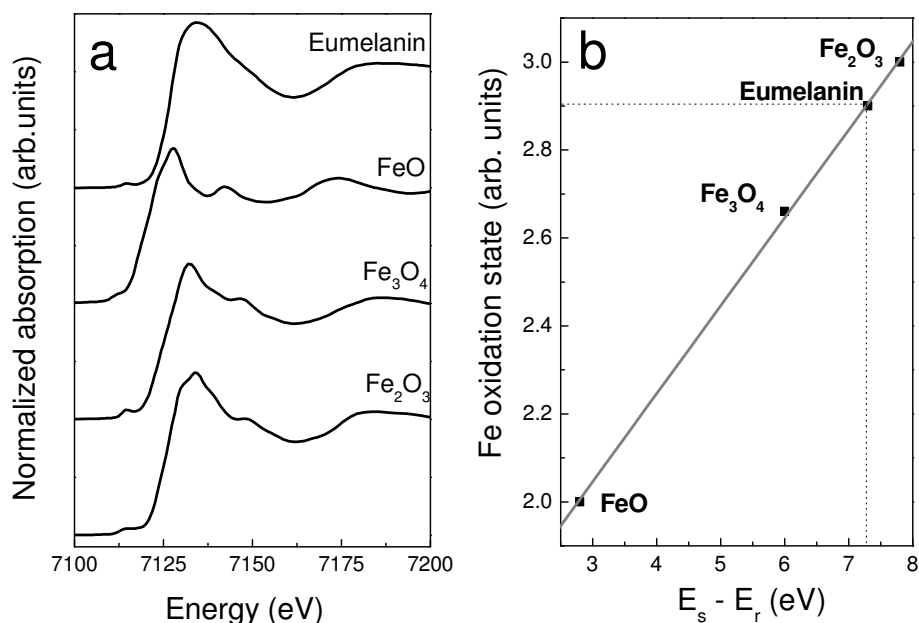


Fig.S1: (a) Fe K XANES spectra of oxide reference compounds (Fe_2O_3 , Fe_3O_4 and FeO) and pure melanin. (b) Chemical shift between the energy edge of the absorption spectrum of the samples (E_s) and metallic Fe^0 (E_r). This chemical shift in the synthetic melanin spectrum was used to determine the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio in the sample (see text).

EXAFS:

In order to establish the structure of the local environment of Fe ions in eumelanin we perform the EXAFS analysis on the Fe K-edge absorption spectra of eumelanin sample. EXAFS analysis was based on the conventional method of Fourier filtering [*X-ray Absorption: Principles, Applications, Techniques of EXAFS, SEXAFS, and XANES*, Koningsberger, D.C. and Prins, R. (Eds.) 1988, Wiley, New York. Newville, M., Ravel, B., Haskel, D., Rehr, J.J., Stern, E.A., Yacoby, Y. *Physica B*, **1995**, 208, 154]. Previously, a linear background was fitted to the pre-edge region of the raw data and then subtracted from the entire spectrum while a quadratic function was fitted and subtracted above the edge region and then the jump of the spectrum was normalized to unity with the post-edge asymptotic value. Because of the low-Z backscatterers in melanin, the Fourier transform was weighted by k^3 with a Hanning window between 2.5 \AA^{-1} and 12.5 \AA^{-1} and back-transformed between 1 and 4 \AA . As we already pointed out at the analysis of the XANES pre-edge region, we can assume a similar Fe-(octahedral) environment than Fe in Fe_2O_3 . Then it is reasonable to extract theoretical phases and amplitudes for fitting purposes (using Atoms and Feff package [Zabinski, S. I., Rehr, J. J., Ankudinov, A., Albers, R. C., Eller, M. J., *Phys. Rev. B*, **1995**, 52, 2995. Ravel, B., Newville, M., *J. Synchrotron Rad.*, **2005**, 12, 537]) from Fe_2O_3 experimental data. The pre-edge peak region of the eumelanin most closely resembles that of Fe_2O_3 (figure 1.a) suggesting that the first coordination shell of Fe can be represented by only one type of Fe atom in a structurally distorted arrangement close to Fe in Fe_2O_3 with a well defined bond length. No multiple scattering considerations were taken into account for the fitting of first neighbours and the $S_0^2 = 0.79$ value was experimentally obtained from the fitting of Fe_2O_3 reference compound.

Figure 2.a shows the Fourier transform of the experimental EXAFS oscillation corresponding to the melanin sample (hollow circles) and the corresponding one to the fitted function to the data (full line) considering the first coordination shell with six oxygen atoms and the second coordination shell of Fe atoms (see table 1). Figure 2.b shows Fourier back-transform of the experimental oscillation filtered in the region 1-4 \AA (first and second neighbour contribution) and the corresponding fitted oscillation. Table 1 shows the fitted EXAFS parameters for the first shell of coordination for each type of neighbour atom (Fe and O).

According to the fitted average coordination number N (see Table 1) the probable structure is an iron atom coordinated to three pairs of oxygen atoms. In a previous work Kropf and coworkers [Kropf, A. J., Bunker, B. A., Eisner, M., Moss, S. C., Zecca, L.,

Stroppolo, A., Crippa, P. R., *Biophys. J.*, **1998**, 75, 3135] assign two pairs of oxygen atoms as forming part of two quinone-like structures having (as in present case) a first-shell coordination number of about six. These authors suggest that the remaining pair of oxygen atoms can be attributed to the hydration of the sample, out of the plane determined by the two quinone-like structures. In present case the corresponding Fe-O bond of 2.18 Å for oxygen from hydration results about 0.2 Å larger than the four oxygen atoms bonded to Fe from the quinone structures. Another remarkable point from the EXAFS fittings is the coordination of Fe with other Fe ions. According to the average coordination number fitted in this case, even this not corresponds to the Fe coordination as in a bulk oxide structure, the Fe centers appear linked with other ones, apparently forming small melanin clusters with more than one Fe ion inside (see text).

Coordination Shell	Average Coordination Number	Distance (Å)	Debye-Waller Factor (Å ⁻²)
O ₁	4.2 ₁	2.00 ₅	0.008 ₁
O ₂	1.6 ₃	2.18 ₅	0.008 ₁
Fe ₁	0.6 ₅	2.81 ₅	0.004 ₃
Fe ₂	0.5 ₅	2.95 ₅	0.004 ₃
Fe ₃	0.3 ₅	3.24 ₅	0.004 ₃

Table 1: fitted EXAFS parameters for the first shell of coordination for each type of neighbour atom (Fe and O).

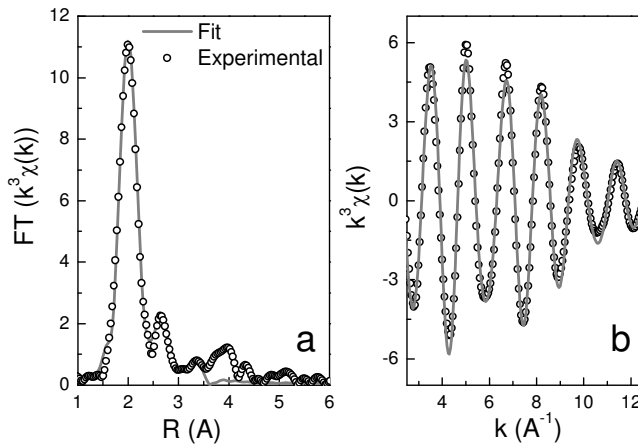


Figure S2: a) Experimental Fourier transform of the data (hollow circles) and the back-transform of the fitted oscillation. b) Experimental data corresponding of the filtered oscillation between 1 and 4 Å (hollow circles) and the corresponding fitted oscillation (full line).