Supplementary Information

Redox Chemistry in the Pigment Eumelanin as a Function of temperature using Broad Band Dielectric Spectroscopy

K. A. Motovilov[1], V. Grinenko[2,3], M. Savinov[4], Z. V. Gagkaeva[1], L. S. Kadyrov[1], A. A. Pronin[5], Z. V. Bedran[1], E. S. Zhukova[1,5], A. B. Mostert[6], B. P. Gorshunov[1,5]

[1] *Moscow Institute of Physics and Technology, 141701, Institutsky lane 9, Dolgoprudny, Russia*
[2] *Institute for Solid State and Materials Physics, TU Dresden, 01069, Dresden, Germany*
[3] *Institute for Metallic Materials, IFW Dresden, 01069, Dresden, Germany*
[4] *Institute of Physics AS CR, Praha 8, Czech Republic*
[5]*Prokhorov General Physics Institute of the Russian Academy of Sciences, 119991, Vavilov street 38, Moscow, Russia*
[6]*Department of Chemistry, Swansea University, Singleton Park, SA2 8PP, Wales, United Kingdom*
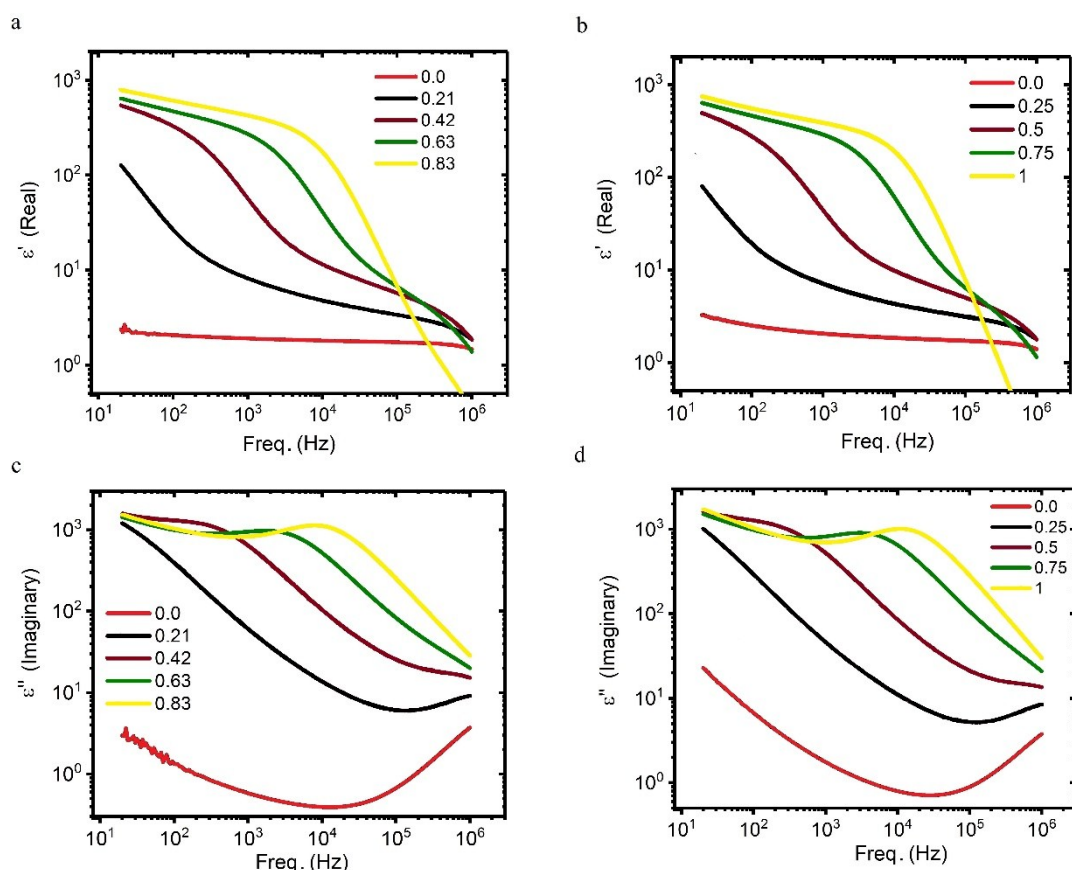
Supplementary Figures

Fig. S1. The data is reproduced from [1]. (Top) Real component of the dielectric response *versus* frequency plots for melanin thin films exposed to H_2O (a) and D_2O (b). The imaginary component of the dielectric response can also be seen for each water type (c and d). The relative pressure/humidity

levels are indicated in the legend. The important thing to note is the qualitative similar behavior between the hydration dependence of the dielectric data for melanin (above) and the temperature dependent data shown in Figure 2. This same behavior implies the same conductivity mechanism is operative both as a function of hydration and temperatures, i.e. the comproportionation reaction.
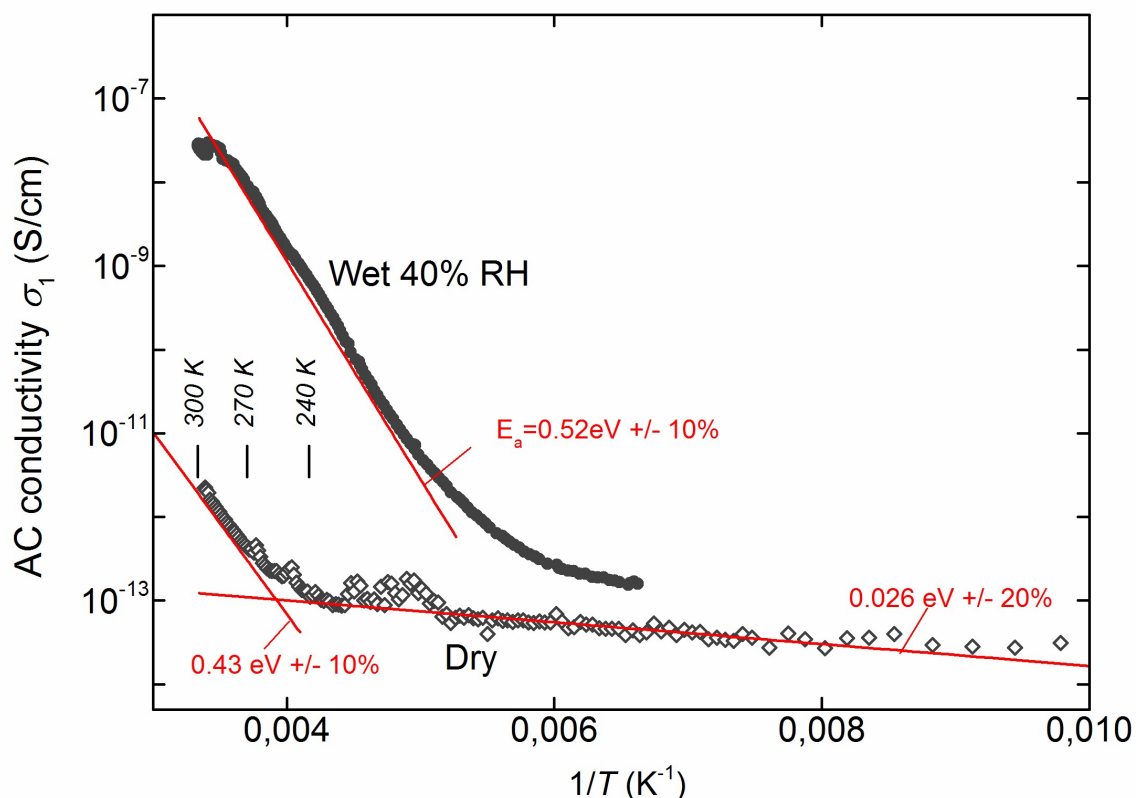

Fig. S2. Activated behavior of the real conductivity of the two melanin samples measured at a frequency 1 Hz: nearly constant loss regime (temperatures below 240 K) is characterized by an activation energy $E_a \approx 0.026$ eV±20% in dry sample (open symbols, 0% RH); hopping regime in wet sample gives E_a value of 0.52 eV±10% (closed symbols, 13.5% wt. of water) and in dry sample 0.43 eV±10% (orange curve, 0% RH). We used the real part of complex conductivity obtained at AC 1 Hz since it is the closest to DC values of conductivity which could not be obtained directly by means of our facilities at the lowest temperatures.

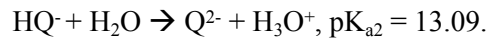**Estimation of the activation energy for the comproportionation reaction.**

The overall reaction for comproportionation reaction is:

$$H_2Q + Q + 2H_2O \rightarrow 2SQ^- + 2H_3O^+,$$
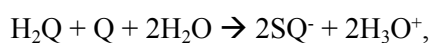
where H_2Q is hydroquinone, Q is quinone, SQ^- is semiquinone and H_3O^+ is hydronium. The pK_a's for DHI has been determined to be 9.54 and 13.09 [2] and is similar to DHICA. Thus:

$$H_2Q + H_2O \rightarrow HQ^- + H_3O^+, \; pK_{a1} = 9.54$$

and

$$HQ^- + H_2O \rightarrow Q^{2-} + H_3O^+, \; pK_{a2} = 13.09.$$
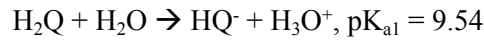
Assuming ideal behavior we can write:

$$K_{a1} = \frac{[HQ^-][H_3O^+]}{[H_2Q][H_2O]} = e^{-\frac{\Delta G_{r,a1}}{RT}},$$

$$K_{a2} = \frac{[Q^{2-}][H_3O^+]}{[HQ^-][H_2O]} = e^{-\frac{\Delta G_{r,a2}}{RT}}.$$

The Gibbs free energy of reaction for A1 can then be determined for each as follows:

$$K_{a1} = 10^{-9.54} = \frac{[HQ^-][H_3O^+]}{[H_2Q][H_2O]} = e^{-\frac{\Delta G_{r,a1}}{RT}},$$

$$10^{-9.54} = e^{-\frac{\Delta G_{r,a1}}{RT}},$$

$$\ln 10^{-9.54} = -\frac{\Delta G_{r,a1}}{RT},$$

$$-9.54 \ln 10 = -\frac{\Delta G_{r,a1}}{RT},$$

$$9.54 \ln 10 = \frac{\Delta G_{r,a1}}{RT},$$

$$9.54 RT \ln 10 = \Delta G_{r,a1}.$$

Assuming room temperature of 298 K:

$$9.54 \times 8.314 \, JK^{-1}mol^{-1} \times 298 \, K \times \ln 10 = \Delta G_{r,a1},$$

$$23636 \, Jmol^{-1} \times \ln 10 = \Delta G_{r,a1},$$

$$23636 \, Jmol^{-1} \times 2.303 = \Delta G_{r,a1},$$

$$\Delta G_{r,a1} = 54433 \, Jmol^{-1},$$

$$\Delta G_{r,a1} = 9.039 \times 10^{-20} J,$$

$$\Delta G_{r,a1} = 0.562 \, eV.$$

Repeating the calculation for A2 one gets:

$$K_{a2} = 10^{-13.09} = \frac{[Q^{2-}][H_3O^+]}{[HQ^-][H_2O]} = e^{-\frac{\Delta G_{r,a2}}{RT}},$$

$$10^{-13.09} = e^{-\frac{\Delta G_{r,a1}}{RT}},$$

$$\ln 10^{-13.09} = -\frac{\Delta G_{r,a1}}{RT},$$

$$-13.09\ln 10 = -\frac{\Delta G_{r,a1}}{RT},$$

$$13.09\ln 10 = \frac{\Delta G_{r,a1}}{RT},$$

$$13.09 RT \ln 10 = \Delta G_{r,a1}.$$

Assuming room temperature of 298 K:

$$13.09 \times 8.314 \, JK^{-1}mol^{-1} \times 298 \, K \times \ln 10 = \Delta G_{r,a1},$$

$$32431 \, Jmol^{-1} \times \ln 10 = \Delta G_{r,a1},$$

$$32431 \, Jmol^{-1} \times 2.303 = \Delta G_{r,a1},$$

$$\Delta G_{r,a1} = 74690 \, Jmol^{-1},$$

$$\Delta G_{r,a1} = 1.24 \times 10^{-19} J,$$

$$\Delta G_{r,a1} = 0.771 \, eV.$$

Now, turning to the redox chemistry reactions, we focus on the one electron half reactions. Currently, there is no information available for indole quinones, but there is information available for 1,2, benzoquinone units [3], [4]. This entity is a reasonable proxy since it is know that in melanin during synthesis break up of the indole quinone occur frequently [5], i.e. you will have 1,2-benzoquinone units. Thus

$$Q + e^- \rightarrow SQ^- \quad , E_0 = -0.21 \text{ V}$$

and

$$SQ^- + e^- \rightarrow Q^{2-} \quad , E_0 = +0.043 \text{ V}.$$
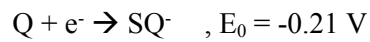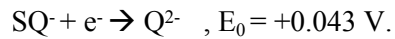
Working with the first reaction one gets:

$$E_0 = -\frac{RT}{zF} \ln K_{red1},$$

$$E_0 = -\frac{RT}{zF} \ln K_{red1},$$

$$zFE_0 = -RT \ln K_{red1},$$

$$\Delta G_{r,red1} = -RT \ln K_{red1}.$$

Hence, since we know E_0, we have the Gibbs free energy

$$\Delta G_{r,red1} = zFE_0 = 1 \times 9.6485 \times 10^4 \, Cmol^{-1} \times -0.21 \, V,$$

$$\Delta G_{r,red1} = -20261 \, Jmol^{-1},$$

$$\Delta G_{r,red1} = -3.365 \times 10^{-20} \, J,$$

$$\Delta G_{r,red1} = -0.21 \, eV.$$

Repeating the above calculation for the second half reaction:

$$E_0 = -\frac{RT}{zF} \ln K_{red2},$$

$$E_0 = -\frac{RT}{zF} \ln K_{red2},$$

$$zFE_0 = -RT \ln K_{red2},$$

$$\Delta G_{r,red1} = -RT \ln K_{red2}.$$

Hence, since we know E_0, we have the Gibbs free energy

$$\Delta G_{r,red1} = zFE_0 = 1 \times 9.6485 \times 10^4 \, Cmol^{-1} \times 0.043 \, V,$$

$$\Delta G_{r,red1} = 4149 \, Jmol^{-1},$$

$$\Delta G_{r,red1} = 6.89 \times 10^{-21} \, J,$$

$$\Delta G_{r,red1} = 0.043 \, eV.$$

With all the above, we are able to estimate the Gibbs free energy of the overall comproportionation reaction. Recalling, the equilibrium constant for the overall reaction will have to have the form of

$$K_{comp} = \frac{[SQ^-]^2 [H_3O^+]^2}{[Q][H_2Q][H_2O]^2},$$

which can be constructed as follows:

$$\frac{[HQ^-][H_3O^+]}{[H_2Q][H_2O]}\frac{[Q^{2-}][H_3O^+]}{[HQ^-][H_2O]}\frac{[SQ^-][SQ^-]}{[Q^{2-}]}\frac{1}{[Q]} = K_{a1}K_{a2}\frac{K_{red1}}{K_{red2}} = K_{comp}$$

This has the temperature dependence of:

$$K_{comp} = e^{-\frac{\Delta G_{r,comp}}{RT}} = e^{-\frac{\Delta G_{r,a1}}{RT}}e^{-\frac{\Delta G_{r,a2}}{RT}}e^{-\frac{\Delta G_{r,red1}}{RT}}e^{\frac{\Delta G_{r,red2}}{RT}},$$

$$K_{comp} = e^{-\frac{\left(\Delta G_{r,a1} + \Delta G_{r,a2} + \Delta G_{r,red1} - \Delta G_{r,red2}\right)}{RT}}.$$

Therefore:

$$\Delta G_{r,comp} = \Delta G_{r,a1} + \Delta G_{r,a2} + \Delta G_{r,red1} - \Delta G_{r,red2}.$$

$$\Delta G_{r,comp} = 0.562\ eV + 0.771\ eV - 0.21\ eV - 0.043\ eV.$$

$$\Delta G_{r,comp} = 1.08\ eV.$$

Overall reaction energy of 1.08 eV. We do note that this is a calculation done only based on monomers, however, the purpose of this calculation is to yield and approximate value for comparison to the data obtained in the study.

Quinhydrone explanation

To understand the background to our proposal, we give a short discussion on the structural knowledge of melanin. It is accepted that melanin is a structurally disordered material with constituent indolequinone building blocks with various redox states (see scheme in Fig.1). These blocks are cross-linked and organized into oligomer sheets [6]–[11], which then stack to form the aforementioned grains. Reduced (hydroquinones – H_2Q) and half-reduced (semiquinones - SQ) states are Brønsted acids where the pK_a of the generic melanin semiquinone is presumably closer to 6.8, which is the pK_a for the DHI monomer unit [12]. Furthermore, in the 1960-80s, presence of quinhydrone in melanin was intensively articulated mainly in EPR/ESR studies, since melanin is a quinone/hydroquinone system [13]–[15]. But this articulation has fallen by the way side in recent

years. The presence of quinhydrone in melanin is not an unreasonable supposition from knowledge of model quinone compounds. For example, *p*-benzoquinone, unlike melanin, can be relatively easily crystallized and studied with the X-ray diffraction technique. It was demonstrated that pressures of 1,5-3 GPa induce cooperative transfer of proton and/or electron in crystals of *p*-benzoquinhydrone derivatives leading to formation of stable *p*-benzosemiquinone [16], [17]. In 2006 Molčanov *et al*. [18] demonstrated by means of variable-temperature X-ray diffraction combined with EPR and DFT analysis, that *p*-benzosemiquinone can be considered as an excited state of *p*-benzoquinhydrone, and formation of *p*-benzosemiquinone from *p*-benzoquinhydrone can be induced by high pressure or X-ray irradiation under atmospheric pressure. This conversion happens by a single-crystal-to-single reaction and radical molecules are stabilized by low-barrier hydrogen bonds; in other words, great structural transformations are not needed for the chemical transition. At the same time, it is well established for the *p*-benzoquinones that quinhydrone complex is much more stable in solid dry state [19] and is rather labile in solution owing to water intercalative H-bonding interactions.

Unfortunately, the investigations of ability of more close analogs of melanin, i.e. *o*-quinones, to form *o*-quinhydrones were not performed intensively till now. We could find only one structural study devoted to formation of *ortho*-quinhydrone from phenanthrene-9,10-dione and phenathrene-9,10-diol [20]. The authors particularly underline stability of the novel compound in dry state and instability in solution.

Therefore, taking the above discussion into account, we propose an additional chemical pathway for melanin's redox chemistry that is depicted in Figure 1, where we show the quinohydrone complex and the reaction pathways II and III. Introduction of water will move the system towards formation of anions, predominantly semiquinone anion SQ^-. According to the current most developed concept, the conductivity mechanism (at least in the dark) of melanin is due to the reactions IV and V, which is perturbed by the presence of water and yields protons and anionic semiquinones. These charge carriers provide the main contribution to the observed conductivity by their diffusion through the water matrix and SQ^--anions [21]–[24]. At the same time, it should be noted that according to the

X-ray, infrared and thermally depolarization current [25]–[27] studies of dry melanin, certain portion of water molecules always remain incorporated in the material.

Thus, the dimer complex of the quinhydrone with charge transfer has additional degrees of freedom in comparison with monomers. Appearance of water leads to disintegration of the complex and disappearance of the corresponding degrees of freedom, their contribution to the set of states and, as a consequence, to decrease of melanin (not water) heat capacity. At higher temperatures (above 220 K), certain increase of specific heat is observed in wet melanin that transforms into a decrease above $\approx$240 K. We associate its origin with the water transformation into liquid phase and its subsequent evaporation from the sample.

References

[1]   M. Sheliakina, A. B. Mostert, and P. Meredith, "Decoupling Ionic and Electronic Currents in Melanin," *Adv. Funct. Mater.*, vol. Accepted, 2018.

[2]   L. K. Charkoudian and K. J. Franz, "Fe(III)-Coordination Properties of Neuromelanin Components: 5,6-Dihydroxyindole and 5,6-Dihydroxyindole-2-carboxylic Acid," *Inorg. Chem.*, vol. 45, no. 9, pp. 3657–3664, 2006.

[3]   A. J. Swallow, "Physical Chemistry of Semiquinones," in *Quinones in Energy Conserving Systems*, B. Trumpower, Ed. Academic Press, 1982, pp. 59–72.

[4]   S. Steenken and P. Neta, "Electron transfer rates and equilibriums between substituted phenoxide ions and phenoxyl radicals," *J. Phys. Chem.*, vol. 83, no. 9, pp. 1134–1137, May 1979.

[5]   M. S. Blois, "The Melanins: Their Synthesis and Structure," in *Photochemical and Photobiological Reviews*, Boston, MA: Springer US, 1978, pp. 115–134.

[6]   P. Meredith and T. Sarna, "The physical and chemical properties of eumelanin," *Pigment Cell Res.*, vol. 19, no. 6, pp. 572–594, Dec. 2006.

[7]   A. A. R. Watt, J. P. Bothma, and P. Meredith, "The supramolecular structure of melanin," *Soft Matter*, vol. 5, no. 19, p. 3754, Sep. 2009.

[8]   M. D'Ischia, A. Napolitano, V. Ball, C. T. Chen, and M. J. Buehler, "Polydopamine and eumelanin: From structure-property relationships to a unified tailoring strategy," *Acc. Chem. Res.*, 2014.

[9]   K. B. Stark, J. M. Gallas, G. W. Zajac, M. Eisner, and J. T. Golab, "Spectroscopic Study and Simulation from Recent Structural Models for Eumelanin: II. Oligomers," *J. Phys. Chem. B*, vol. 107, no. 41, pp. 11558–11562, 2003.

[10]   J. M. Gallas, K. C. Littrell, S. Seifert, G. W. Zajac, and P. Thiyagarajan, "Solution Structure of Copper Ion-Induced Molecular Aggregates of Tyrosine Melanin," *Biophys. J.*, vol. 77, no. 2, pp. 1135–1142, Aug. 1999.

[11]   G. W. Zajac, J. M. Gallas, J. Cheng, M. Eisner, S. C. Moss, and A. E. Alvarado-Swaisgood, "The fundamental unit of synthetic melanin: a verification by tunneling microscopy of X-ray scattering results," *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1199, no. 3, pp. 271–278, Apr. 1994.

[12]   B. Szpoganicz, S. Gidanian, P. Kong, and P. Farmer, "Metal binding by melanins: studies of colloidal dihydroxyindole-melanin, and its complexation by Cu(II) and Zn(II) ions," *J. Inorg. Biochem.*, vol. 89, no. 1–2, pp. 45–53, Apr. 2002.

[13]   R. Arnaud, G. Perbet, A. Deflandre, and G. Lang, "Electron spin resonance of melanin from hair. Effects of temperature, pH and light irradiation," *Photochem. Photobiol.*, vol. 38, no. 2, pp. 161–168, Aug. 1983.

[14]   R. D. Lillie, P. T. Donaldson, L. L. Vacca, P. P. Pizzolato, and S. K. Jirge, "Reduction and azo coupling of quinones," *Histochemistry*, vol. 51, no. 2–3, pp. 141–152, 1977.

[15]   C. C. Felix, J. S. Hyde, T. Sarna, and R. C. Sealy, "Interactions of melanin with metal ions.

Electron spin resonance evidence for chelate complexes of metal ions with free radicals," *J. Am. Chem. Soc.*, vol. 100, no. 12, pp. 3922–3926, Jun. 1978.

[16]   K. Nakasuji *et al.*, "Exploration of new cooperative proton-electron transfer (PET) systems: first example of extended conjugated quinhydrones, 1,5-dihalo-2,6-naphthoquinhydrones," *J. Am. Chem. Soc.*, vol. 113, no. 5, pp. 1862–1864, Feb. 1991.

[17]   Y. Uchida *et al.*, "Temperature and Pressure Effects on Intermolecular Interactions in Charge Transfer Crystals of 2-(4-Methoxyphenyl)-1,4-benzoquinone and Quinhydrone," *Bull. Chem. Soc. Jpn.*, vol. 75, no. 4, pp. 695–703, Apr. 2002.

[18]   K. Molčanov, B. Kojić-Prodić, and M. Roboz, "Structural characterization of *p* -benzosemiquinone radical in a solid state: the radical stabilization by a low-barrier hydrogen bond," *Acta Crystallogr. Sect. B Struct. Sci.*, vol. 62, no. 6, pp. 1051–1060, Dec. 2006.

[19]   D. J. G. Ives and G. J. Janz, *Reference Electrodes*. New York: Academic Press, 1961.

[20]   F. Calderazzo, C. Forte, F. Marchetti, G. Pampaloni, and L. Pieretti, "Reaction of Phenanthrene-9,10-dione with Phenanthrene-9,10-diol: Synthesis and Characterization of the First ortho-Quinhydrone Derivative," *Helv. Chim. Acta*, vol. 87, no. 4, pp. 781–789, Apr. 2004.

[21]   A. B. Mostert *et al.*, "Role of semiconductivity and ion transport in the electrical conduction of melanin," *Proc. Natl. Acad. Sci.*, vol. 109, no. 23, pp. 8943–8947, Jun. 2012.

[22]   S. B. Rienecker, A. B. Mostert, G. Schenk, G. R. Hanson, and P. Meredith, "Heavy Water as a Probe of the Free Radical Nature and Electrical Conductivity of Melanin," *J. Phys. Chem. B*, vol. 119, no. 48, pp. 14994–15000, 2015.

[23]   J. Wünsche *et al.*, "Protonic and Electronic Transport in Hydrated Thin Films of the Pigment Eumelanin," *Chem. Mater.*, vol. 27, no. 2, pp. 436–442, Jan. 2015.

[24]   A. B. Mostert, S. B. Rienecker, C. Noble, G. R. Hanson, and P. Meridith, "The photoreactive

free radical in eumelanin," *Sci. Adv.*, vol. 4, no. 3, p. eaaq1293, Mar. 2018.

[25]   M. Bridelli, R. Capelletti, and P. R. Crippa, "Electret state and hydrated structure of melanin,"
Bioelectrochemistry Bioenerg., vol. 8, no. 5, pp. 555–567, Oct. 1981.

[26]   B. Bilińska, "Progress of infrared investigations of melanin structures," *Spectrochim. Acta
Part A Mol. Biomol. Spectrosc.*, vol. 52, no. 9, pp. 1157–1162, Aug. 1996.

[27]   M. G. Bridelli, R. Capelletti, and P. R. Crippa, "Infrared-spectroscopy of eye and synthetic
melanins at various values of pH and hydration," *Physiol. Chem. Phys.*, vol. 12, no. 3, pp.
233–240, 1980.