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Chemical and Structural Diversity in Eumelanins – Unexplored Bio-Optoelectronic Materials**

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Abstract

Eumelanins, the characteristic black insoluble and heterogeneous bio-polymers of human skin, hair and eyes, have intrigued and challenged generations of chemists, physicists and biologists because of their unique structural and optoelectronic properties. Recently, an organic chemistry approach has been combined with advanced spectroscopic and imaging techniques, theoretical calculations and methods of condensed matter physics to gradually force these materials to reveal their secrets. Here we review the latest advances in the field with a view to showing how the emerging knowledge is not only helping us explain eumelanin functionality, but may also be translated into effective strategies for exploiting their properties to create a new class of biologically inspired high tech materials.

Keywords

melanins; biopolymers; 5,6-dihydroxyindoles; materials science; photochemistry

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1. Introduction

Among the broad variety of biopolymers found in Nature, few have such profound and fascinating interdisciplinary implications at the crossroads of physics, chemistry, biology and medicine as do the melanins. The reasons for this are rooted in the role of these pigments as the key components of the human pigmentary system [1–3] and their important socio-economic and clinical relevance, in relation to pigmentary disorders such as malignant melanoma, the most aggressive of skin cancers. Melanins are produced in epidermal melanocytes by tyrosinase-catalyzed oxidation of tyrosine [4] (Figure 1) via 5,6-dihydroxyindole (**1**) and 5,6-dihydroxyindole-2-carboxylic acid (**2**), the final monomer precursors. Oxidative polymerization of **1** and **2** [5] then gives rise to the black-brown variety of melanin known as eumelanin.

Working on eumelanins has usually been regarded as an intriguing, though sometimes frustrating, experience.[6] This is due to several challenging features of the system, including almost complete insolubility in all solvents, an amorphous particulate character, and extreme molecular heterogeneity. Eumelanin does however possess a number of physico-chemical properties [7] that can be used to identify and quantify the system, such as a persistent Electron Paramagnetic Resonance (EPR) signal, broadband monotonic optical absorption, peculiar excitation and emission properties.[8,9] and time dependent photo-dynamics [10–12]. Standard vibrational methods such as infra-red absorption and Raman spectroscopy [13,14] and more recently inelastic neutron scattering spectroscopy [15] have also been applied with varying degrees of success to study the vibrational finger-print of eumelanin precursors. Controlled chemical degradation giving traces of pyrrolic acids has been exploited mainly for pigment analysis in tissues [16,17] yielding only limited information as to the basic aspects of eumelanin primary-level structure. [1] Yet to-date eumelanin's fundamental structure (if indeed the term “structure” can rightly be applied to such a highly heterogeneous material), is still under intense scrutiny. [6,18]

In the 1970s, McGinness and his associates showed that natural and synthetic eumelanin behaved like an amorphous semiconductor [19,20] suggesting that it consisted of a very high molecular weight polymer made up of different units at various oxidative levels and linked randomly[21] so to fit the band gap semiconductor model. Starting from the mid 1990s a different basic supramolecular architecture for eumelanin particles has been proposed.[22–25] suggesting protomolecular structures approximately 15 Å in size made up of 4–5 planar sheets of four-to-eight 5,6-dihydroxyindole units each stacked in the z-plane with a graphite-like stacking spacing of 3.4 Å. In sepia ink eumelanin, a sequence of aggregation steps has been suggested to account for the apparent three levels of structural organization (Figure 2). [26–29]

Numerous studies using, e.g., atomic force microscopy (AFM),[26,27,30] X-ray diffraction, [31] mass-spectrometry,[32] NMR[33] and advanced quantum chemical calculations [34–36] have addressed eumelanin structure, and though most of them appear to support the stacked aggregate picture, this model has yet to see definitive proof.

With the structural debate still alive, research on eumelanins has seen a significant revival of interest in the past few years. This stems from recognition, on the part of the condensed matter physics community especially, of the broad range of technological opportunities offered by the physicochemical properties of eumelanins [37] including a re-appreciation of their semiconducting properties.

2. Recent Chemical and Physical Structure Advances

Synthetic eumelanin-like materials (on which we will focus in this mini-review) are usually produced by the enzymatic (tyrosinase, peroxidase) or chemical (ferricyanide) oxidation of tyrosine, dopa, **1** or **2**, and the final pigments may be significantly different depending on the substrate and oxidation conditions. The morphology of synthetic eumelanins as examined by scanning electron microscopy (SEM) is that of an amorphous solid on the scale of 100s of nms upwards.[38]

Insight into the basic oligomeric structural motifs of eumelanins has been pursued by investigation of the oxidative polymerization of 5,6-dihydroxyindoles.[5–39] Oxidation of **1** leads to a collection of dimers and trimers in which the indole units are linked mainly through 2,4'- and 2,7'-bondings (Figure 3).

Transition metal cations, like Ni^{2+} , Cu^{2+} or Zn^{2+} , specifically direct the oxidative coupling of **1** toward the 2,2'-dimer as the main product.[5] This effect may be used as a convenient means of exerting regio-chemical control over the coupling reaction to form more regular oligomeric scaffolds. Oxidation of dimers leads to tetramers in which different types of interring coupling modes are involved, e.g. 2,3'-, 4,4'-, and 7,7'-bonds.[40,41] Pulse radiolysis experiments coupled with DFT calculations suggested that dimers are oxidized to nearly planar extended quinone methide structures (Figure 4) absorbing strongly in the visible. [42]

Analysis of the absorption properties of **1**-derived oligomers in their reduced *o*-diphenol state indicate a gradual broadening of the chromophore with increasing molecular size but no significant, easily predictable red-shift.[43]

Polymerization of **2** is influenced by the carboxyl group at the 2-position of the indole ring which limits the range of reactive sites available for oxidative coupling, whereby a lower number of positional isomers populates the various oligomer stages with respect to **1**. Main oligomers formed by oxidative coupling of **2** include the 4,4'-biindolyl, the 4,7'-biindolyl and other minor dimers, as well as a series of trimers.[5] Oxidation of 4,4'-biindolyl yields tetramers that have been structurally characterized (Figure 5).

Notably, all of the oligomers from **2** exhibit atropisomerism[44] due to the steric constraints around the interring single bond contributing to maintain significant twist angles.

Parallel to these experimental efforts, numerous quantum chemical studies at various levels of theory have recently provided a detailed characterization of 5,6-dihydroxyindoles, [34,45–49] their quinones and oligomers. An original structural model based on tetramers consisting of four monomer units in arrangements that contain an interior porphyrin ring has been proposed mainly on theoretical grounds and time-dependent density functional theory. [35,36] Although none of the models so far proposed provides a complete and fully satisfactory explanation of eumelanin properties, the conclusions emerging from theoretical studies have provided useful guidelines for the elucidation of eumelanin properties.

3. Physico-chemical Properties and Applications

3.1 Optical and photophysical properties

The optical and photophysical properties of eumelanin are rather unique and for a comprehensive coverage readers are referred to the review by Meredith and Sarna.[7] As shown in Fig. 6, the absorbance in the ultra-violet and visible is monotonic and broad band – i.e. it is featureless and fits a single exponential in wavelength space to a high degree of accuracy.

Riesz et al. [50] have recently calculated the transition dipole strength of the eumelanin polymer across the UV and visible and shown that the system is not an unusually strong absorber relative to other organic chromophores. It was also shown that the system displays slight hyperchromism - the polymerisation process enhances the relative strength of the absorption versus the individual monomer units. The radiative quantum yield of eumelanin is tiny (<0.1%).[51] Other authors[52] have also shown that >99% of the absorbed photon energy is dissipated non-radiatively as heat within 50 ps of absorption. Thus, the eumelanin system is extremely good at dissipating UV and visible radiation. The spectral shape of the emission bears no resemblance to that of the absorbance, in a complete violation of the most basic of spectroscopic rules – the mirror image symmetry or Kasha's rule. Nighswander-Rempel et al.[8,9] have also shown that the radiative emission is dependent upon the energy of the exciting radiation – in theory, for all chromophores the emission is constant for excitation energies greater than the excitation gap of the molecule.

This bizarre collection of optical properties, coupled with the aforementioned quantum chemical calculations by several authors has led to a reappraisal of the high molecular heterogeneity of eumelanins[21] in terms of the chemical disorder proposition. [53] In this simple model, the broad monotonic absorption of eumelanin is in fact an ensemble average of all the individual chemically distinct species within the system. It has been calculated [37] that as few as eleven species are sufficient to create the smooth exponential profile of eumelanin across the UV and visible. The emissive behaviour of eumelanin is also naturally explained in this context by a selective pumping of sub-sets of the ensemble.

The molecular mechanism by which eumelanin dissipates absorbed radiation is still a mystery. The “multiple overlapping chromophore picture” would allow for “funneling of energy” via emission and re-absorption, but the fundamental dissipation appears too rapid for such a process. Olsen et al.[54] have recently shown that **2** can undergo relaxation by excited state proton transfer - a quantum mechanical mechanism emerging as a key player in several efficient biological processes. However, it is unclear whether this mechanism is possible in the eumelanin macromolecule or in the solid state.

3.2 Electrical properties

The electrical switching work of McGinness and co-workers [19] cemented the paradigm that eumelanins were organic semiconductors. Since those early days, many groups have observed electrical behavior apparently indicative of semiconductivity. AC and DC conductivity, photoconductivity and photothermal analysis have been used to calculate activation energies, deduce apparent band structures and carrier densities.[56,57] Several studies have shown that the electrical properties of solid state eumelanin samples are also very dependent upon the hydration state of the material.[58] This fact does not preclude the system being a semiconductor since a hygroscopic material such as eumelanin may well be expected to have an activation energy dependent upon hydration state, but due caution needs to be exercised in measurements and interpretation.

3.3 Redox, free radical and ion-binding properties

One of the most remarkable features of eumelanin is its ability to undergo electron-transfer reactions. Although the quinone/hydroquinone nature of the eumelanin subunits is a reasonable basis for explaining the observed redox properties of this material, chemical stability of the quinone groups is an issue that is not fully understood. It is believed that the quinone groups of eumelanin are mostly *o*-quinones related to 5,6-indolequinone.[59] However, free *o*-quinones, unlike *p*-quinones, are extremely unstable. It can be speculated that covalent linking of *o*-quinone subunits in eumelanin oligomers and their subsequent aggregation is a mechanism for efficient stabilization of the quinones. Such stabilization

may result from modified redox properties of the bound monomers and their reduced accessibility due to an increased steric hindrance. A pulse radiolysis investigation of synthetic dopa-melanin, using bipyridinium quaternary salts as a redox probe, revealed that the one-electron reduction potential of this eumelanin model was between -450 and -550 mV. [60] A dispersion of the redox properties of the eumelanin functional groups was also detected by potentiometric measurements. [61]

An intriguing question is whether redox properties of eumelanin change with time. This is particularly relevant for eumelanin in pigmented tissues of the human eye, such as retinal pigment epithelium, where melanin is formed early during fetal development and undergoes very little or no metabolic turnover.[62] Although no data directly answering this question have yet been obtained, physicochemical analysis of retinal pigment epithelium (RPE) melanosomes from donors of different age suggest that their age-dependent changes in photoreactivity, [63] free radical properties [64] and antioxidant capacity [65] may be determined by modifications of the eumelanin oxidation state. Interestingly, Hong and Simon [66] using X-ray photoelectron spectrometry (XPS) have shown that in bovine choroidal melanosomes the content of C=O groups, compared to C-O, increases with the age of the animal. This observation may suggest that the choroidal melanosomes become more prooxidant with age. A distinct decrease in antioxidant efficiency of bovine and porcine RPE melanosomes was observed with experimental *in vitro* photobleaching.[67,68] Such a model photoaging of bovine RPE melanosomes even stimulated their prooxidant.[68]

One of the important consequences of the simultaneous presence in eumelanin of fully oxidized and fully reduced subunits, is a phenomenon known as “the comproportionation equilibrium”, in which the *o*-quinone and *o*-hydroquinone eumelanin monomers exist in equilibrium with their semi-reduced (semi-oxidized) form (for a detailed discussion and early studies see Meredith and Sarna[7]). It may seem surprising that this relationship, derived from simple solution chemistry considerations, works quite well for eumelanin, which, intuitively, should rather be described using the formalism of solid state chemistry. However, high mobility of eumelanin paramagnetic centers is inferred from the decay kinetics of the radicals that were induced by light. Thus upon termination of the *in situ* (in the resonant cavity) irradiation of eumelanin with UV or visible light, the inducible radicals decay via second-order kinetics, consistent with a random encounter of the radicals leading to their recombination. The role of highly diffusive radicals in photoprotection and phototoxicity of RPE cells was recently discussed by Seagle et al..[69–71] In these studies, the authors analyzed time-resolved EPR (TR EPR) signals with distinct spin polarization features that were induced in some eumelanin samples by nanosecond laser pulses. It must be stressed that steady-state concentration of melanin free radicals under typical physiological conditions is very low, of the order of 10^{18} spin/g, which, on average, corresponds to one free radical center per 10^3 monomer units.

A growing body of experimental evidence suggests that more than one type of free radicals exists in eumelanins. [72,73] In particular, the EPR spectrum appear to result from at least two different types of radicals – an *o*-benzosemiquinone anion radical that was strongly pH-dependent, quite labile and associated with the well-hydrated portion of eumelanin, and another radical that was pH-independent but depended upon aggregation and, therefore, probably associated with defects in the polymer backbone

Eumelanin is known to be a good chelator of multivalent metal ions such as Fe^{3+} , Mn^{3+} , Zn^{2+} and Cu^{2+} . [7,61,74,75] The binding of metal ions may involve carboxylic, amine, imine, phenolic and *o*-diphenolic groups of eumelanin exhibiting different association constants. Notably, the different binding sites of eumelanin can be activated at different pHs, which also determines the observable stability of the metal ion-eumelanin complexes.

3.4 Film preparation

An absolute pre-requisite to the full realization of eumelanin-based materials within the organic electronic or optoelectronic arena is the production of device quality thin films. Most solid state optical and electrical measurements have been performed on compressed powders which are wholly unsuitable because of their morphological variability. Control over nanoscale morphology is at the heart of modern organic electronics research and technology development. Very recently, several groups have produced synthetic eumelanin thin films[76–78] and organically soluble eumelanin derivatives.[79,80] Notably, Bothma et al. [81] have reported the first device quality synthetic eumelanin films showing enhanced optoelectronic functionality. The films exhibited solid state absorption coefficients between 10^7 and 10^6 m⁻¹ (UV-to-IR) and showed Ohmic behavior with a conductivity of $\sigma = 2.5 \times 10^{-5}$ S cm⁻¹ (relative humidity 100%, 24° C). These films can be spun-cast from organic solvents in the same way as engineered synthetic conducting polymer systems, and represent the first real opportunity to utilize the polyindolequinone system as a functional electronic or optoelectronic material. Key to the production of such films is an understanding of, and control over, the aggregation state of the system. It appears as though the insolubility of eumelanin is related to its supramolecular aggregation state. Breaking this aggregation without affecting the primary unit structure or properties is the secret which unlocks the potential of these materials. It looks as if this possibility is now a reality.

5. Summary and Outlook – Towards New Functional Materials

Though unavoidably incomplete, the foregoing account should give some taste of the mixed feelings of achievement, expectations and new challenges that characterize the current age of eumelanin research. Although a unified perspective of eumelanin structure is not yet available, knowledge is currently increasing and the information known about eumelanin from a variety of physical techniques is being gradually placed into a better defined conceptual framework. The emphasis on “blackness” of electroactive materials features prominently in ongoing research, but a caveat is raised that not all that is black is a eumelanin, and not all eumelanins share the same features in terms of robustness and functional activity. A fundamental difference exists between natural and synthetic eumelanins, whereby extrapolation of data from one type of pigment to the other is not justified. Synthetic eumelanin-inspired materials may be produced by different methodologies which may be optimized as knowledge of the chemistry of 5,6-dihydroxyindole polymerization increases. Novel structural variants and derivatives of eumelanin building blocks are currently being designed [59,82] and experimentally evaluated for preparing new rationally designed materials. The basic structural organization depends on monomer composition and synthetic conditions which may have a significant impact on the overall organization of the aggregates. These synthetic efforts, coupled with the advances in the creation of thin film structures are what is needed to transition the field from “biochemical and biophysical oddity” to genuine functional material. A summarizing view of the possible range of application of functional eumelanin films is provided in Figure 7.

It is also instructive to look further afield than possible applications in electronics or optoelectronics for eumelanin materials. For example, Lee et al.[83] have recently shown that a polydopamine derived eumelanin-like material inspired by the adhesive proteins secreted by mussels can be made into a functional coating that sticks to an unprecedented array of organic and inorganic substrates. There have also been a few recent studies on the magnetic properties of melanins – a virtually unexplored facet of the property map.[84]

Within this framework, the oligomer model emphasizes the importance of molecular diversity (chemical disorder) as a possible key feature underpinning structure-property

relationships.[37] Much work has to be done to unequivocally confirm that this is the correct structure-property model. Answers to these questions are now coming from a number of ongoing studies, which will pave the way to the design of melanin-inspired functional materials armed with the new structure-property toolkit.

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Biographies



Marco d'Ischia obtained his degree in Chemistry from the University of Naples Federico II, Italy, where he is Professor of Organic Chemistry since 2001. His main research interests focus on the chemistry of natural bioactive products and heterocyclic compounds, including melanins and melanogenesis, the oxidation chemistry of biomolecules in relation to

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Alessandra Napolitano graduated in Chemistry in 1984 at the University of Naples Federico II. In 2001 she was made Associate Professor of Organic Chemistry. Her main research interests lie in the field of heterocyclic compounds, with special reference to hydroxyindoles and benzothiazines, oxidative chemistry of phenolic natural products, food chemistry, lipid peroxidation, and analytical chemistry. Currently she is involved in several research projects dealing with the chemistry of natural pigments, including pheomelanins, and the chemical bases of diseases.



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Paul Meredith is an Associate Professor of Physics in the School of Physical Sciences and Centre for Organic Photonics and Electronics at the University of Queensland in Australia. He is also a Smart State Senior Fellow and currently serves as Vice President of Materials Development for XeroCoat Pty Ltd. Associate Professor Meredith obtained his PhD in Materials Physics from Heriot-Watt University in Edinburgh and was also a DTI Research Fellow in Soft Matter Physics at the Cavendish Laboratory, Cambridge University. His main research interests include the physics and chemistry of melanins and also the development and understanding of new organic optoelectronic materials for applications such as solar cells and chemi-sensors.

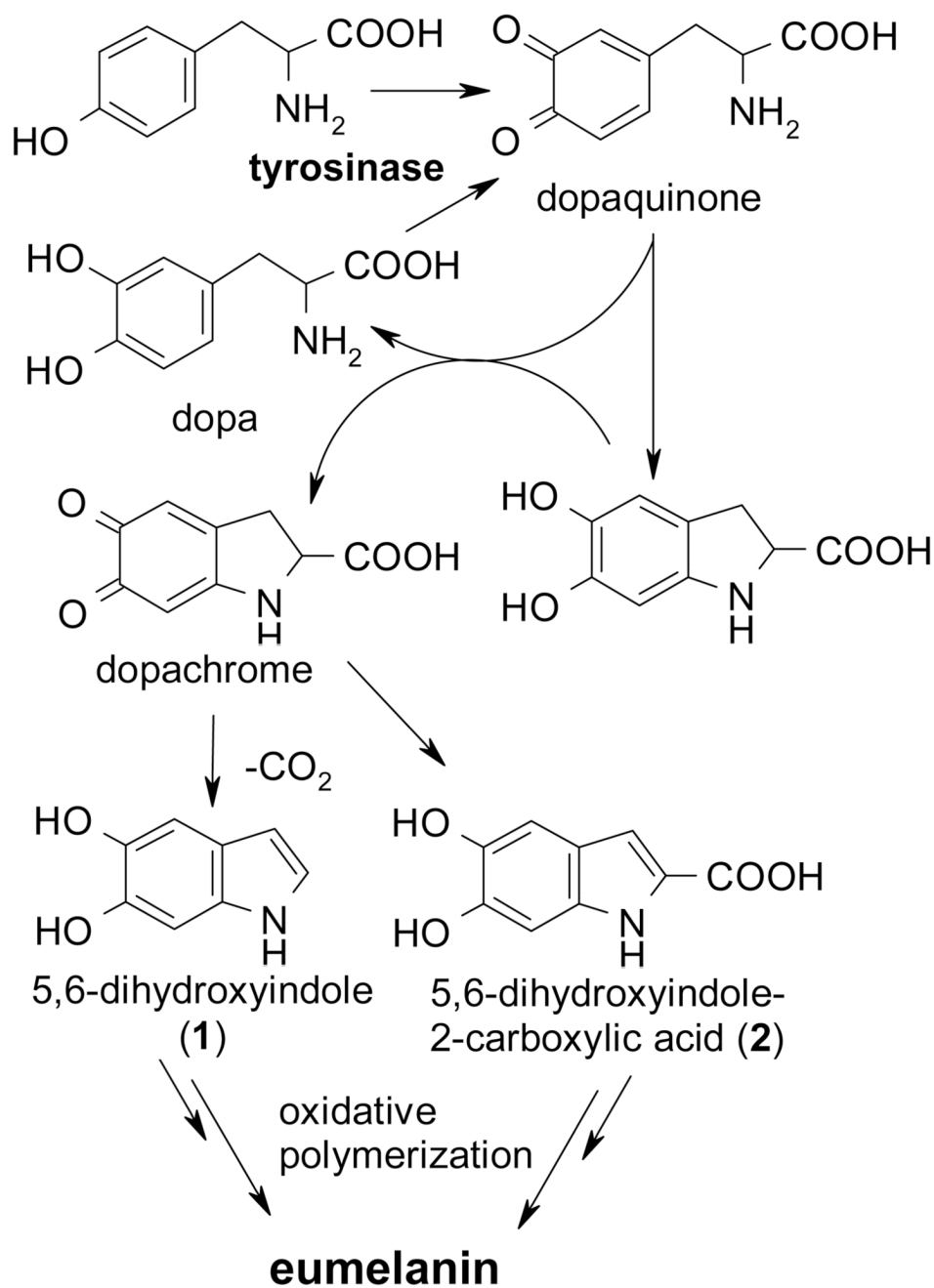


Figure 1.
The tyrosinase catalysed oxidation of tyrosine to create the brow-black pigment called eumelanin.[1]

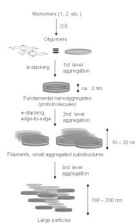


Figure 2.

A schematic view of the hierarchical aggregate structure proposed for sepia eumelanin.[26–28]

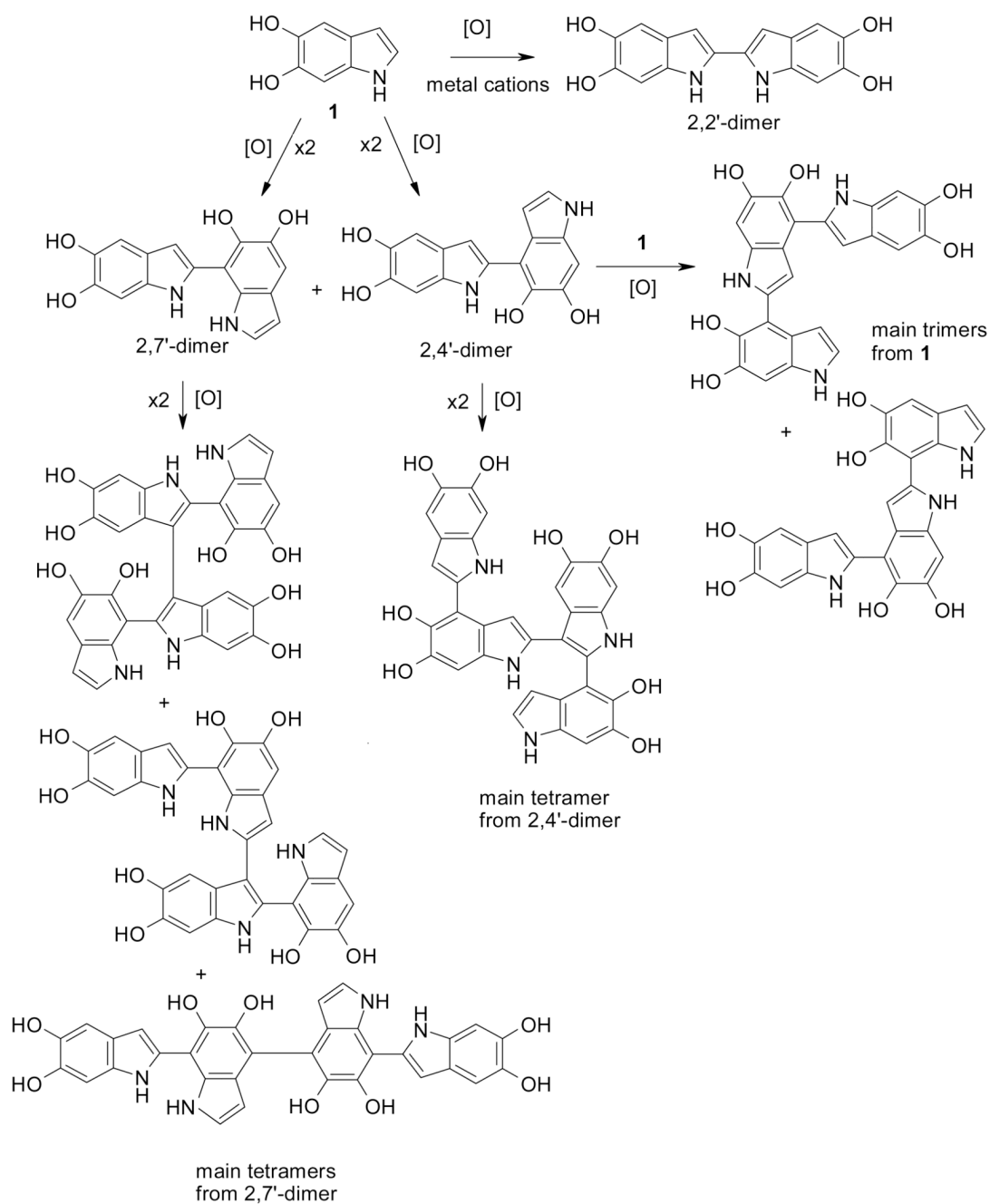


Figure 3.
Structures of main oligomers isolated by oxidation of **1** and its dimers.[5,40,41]

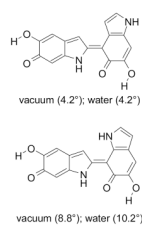


Figure 4.

Extended quinone methide structures proposed to be formed by 2-electron oxidation of dimers from **1**.^[42] Computed interring N-C-C-C(O) dihedrals (degrees) are reported in parentheses.



Figure 5.
Structures of main oligomers isolated by oxidation of **2** and its dimer.[5,44]

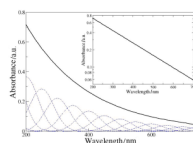


Figure 6.

The broad band absorption of eumelanin – the spectrum is monotonic and fits an exponential in wavelength space (insert shows the logarithmic-linear plot). The exponential shape can be fitted by a sum of Gaussians with full widths at half maxima characteristic of inhomogeneously broadened chromophores at room temperature.[53] The higher energy transitions with strong transition dipole moment are S_0 – S_1 features of smaller units within the ensemble and are also derived from S_0 – S_2 transitions of larger oligomeric units.

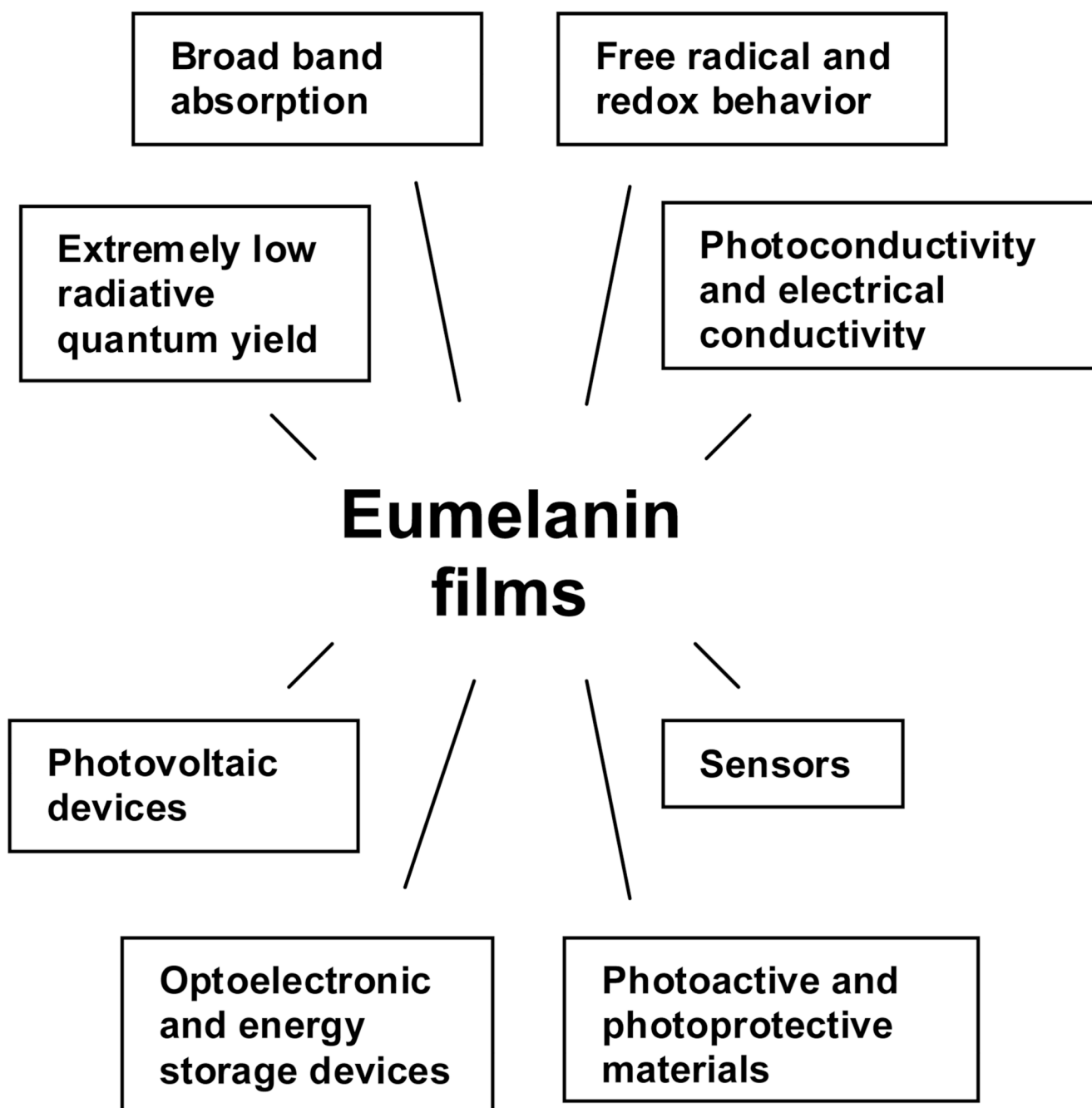


Figure 7.
Summarizing view of physicochemical properties and possible range of applications of eumelanin films.