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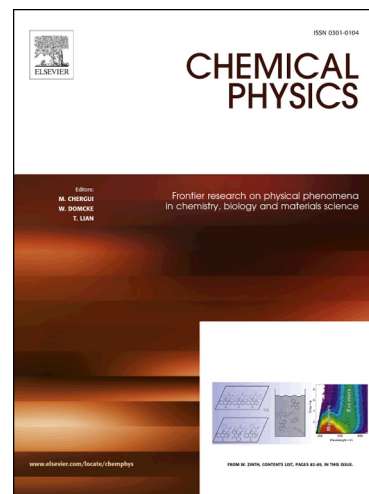
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Extensive stacking of DHI-like monomers as a model of out-of-plane complexity in eumelanin protomolecules: chemical and structural sensitivity of optical absorption spectra

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Abstract

In this work, we study the optical absorption spectra of extensive stacking forms of four molecules known to be among eumelanin basic building blocks: 5,6-dihydroxyindole (DHI), 5,6-dihydroxyindole-2-carboxylic acid (DHICA), indole-quinone (IQ) and quinone-methide (MQ). Stacked monomers can be considered as a minimal model of out-of-plane complexity in eumelanin, as complementary to in-plane oligomerization. The choice of a plane wave density functional theory (DFT) approach allows us to address extensive, *i.e.* several-layer, stacking of these DHI-like monomers at a relatively low computational cost, by treating stacked systems as periodic along the stacking direction. Absorption spectra of stacked monomers display interesting trends in terms of chemical and structural sensitivity, which can shed light on the role of extensive stacking in the transition from the optical properties of small molecules such as DHI to the typical broadband and monotonic absorption spectrum of the eumelanin pigment.

Keywords: eumelanin; extensive stacking; 5,6-dihydroxyindole-like monomers; absorption spectra: plane-wave density functional theory

1. Introduction

Despite the still incomplete knowledge on the three-dimensional structure of eumelanin, due to its amorphous character, almost complete insolubility in all solvents, and molecular heterogeneity (as pointed out, *e.g.*, by d'Ischia et al.[1]), there is a general agreement on the fact that eumelanin contains 5,6-dihydroxyindole (DHI), 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and

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their various redox forms, such as indolequinone (IQ) and quinone-methide (MQ), as its basic building blocks. Data obtained from X-ray scattering, scanning tunneling microscopy (STM) and transmission electron microscopy (TEM) experiments also suggested that larger protomolecules, resulting from the in-plane polymerization of the above-mentioned DHI-like molecules, form π -stacked arrangements of lateral size around 15-20 Å, 4-5 layer thickness, and 3-4 Å interlayer distance[2, 3, 4, 5].

Eumelanin displays a characteristic continuous UV and visible absorption spectrum, with intensity increasing with frequency (*e.g.*[6]), in agreement with its function as a photoprotective pigment. This has drawn attention on eumelanin also in view of possible applications in photovoltaics [7, 8]. Clearly, these spectral features are strongly qualitatively different from the ones expected for monomeric forms of small molecules such as DHI. The broadband spectrum of eumelanin must thus result from the complex arrangement (including both stacking and in-plane polymerization) of the elementary DHI-like building blocks, and/or from their chemical heterogeneity. Chemical disorder, *i.e.* the possible presence of several different monomeric and oligomeric chemical species within eumelanin[9], can only partially explain the transition from a simple absorption spectrum, with few well-separated peaks, to a more broadband one, resulting from a superposition of absorption spectra of the different chemical species. However we must keep in mind that both computed[10, 11] and measured[12, 13] absorption spectra of DHI-like monomers - and therefore also simple superpositions of them - have null or negligible intensity in the visible range, in disagreement with experimental eumelanin spectra. Therefore the effects of intermolecular interactions, involved both in in-plane oligomerization through covalent bonds and in out-of-plane non-covalent stacking, must play an essential role, together with chemical heterogeneity, in the origin of the typical features of experimental eumelanin spectra. A non-negligible effect of intermolecular interactions on the electronic and optical properties of stacked structures is actually to be expected, for molecules arranged at interlayer distances of 3-4 Å.

Several models of oligomers, and stacked arrangements thereof, have been proposed and computationally studied in the literature[10, 14, 15, 16, 17, 18, 19], in terms of possible geometries, energetics, and optical properties, in an attempt to identify possible supramolecular arrangements of DHI-like building blocks able to yield absorption spectra resembling the one of eumelanin. Most of these works used density functional theory with localized bases, or post-Hartree-Fock methods (such as MP2[20] or coupled cluster[21]), generally for the smallest investigated systems. Upon going beyond two- or at most three-layer stacking, especially if in combination with in-plane oligomerization, in order to obtain realistic models, such systems rapidly become computationally intractable with the above-mentioned methods, in particular in terms of calculating the full absorption spectrum. In some of the cited works, in order to somehow overcome these limitations, authors have either restricted their study to absorption onset only[16], or resorted to semiempirical methods for calculating absorption spectra of larger systems[10], and to classical molecular dynamics simulations[10, 14, 15]

both for optimizing the geometries of intermediate-size stacked systems, and for generating very large aggregates.

Apart from the possible computational limitations, the spectra of few-layer stacked forms obtained in these works confirm the above-mentioned consideration on the important role of intermolecular interactions, being indeed not reducible to spectra of the individual molecules undergoing stacking (*e.g.*[14]).

We work within time dependent density functional theory, with a plane-wave basis set. The use of plane waves, though possibly counterintuitive when dealing with molecules, proves useful for describing extensive stacking, *i.e.* systems consisting of more than 2-3 stacked molecules. It provides a “natural” way to describe several-layer stacked systems (infinite-layer ones, indeed), at a reasonable computational cost, treating them as periodical along the stacking direction, by using a single-molecule unit cell with primitive character along the stacking direction and a suitable mesh of k-points to sample the Brillouin Zone. Moreover this approach makes basis set convergence tests straightforward (*e.g.* [22]), and it allows us to address intermediate cases between the isolated molecule limit and the stacked system, by simply varying the cell size along the stacking direction.

In this work we provide a computational first-principles investigation of the effects of extensive (*i.e.* several-layer) stacking on the optical properties of the elementary constituents of eumelanin, DHI-like molecules. Although not fully sufficient for the interpretation of experimental eumelanin spectra, stacked arrangements of DHI-like *monomers* can be interesting as simple models of out-of-plane complexity in the pigment, considered as complementary to in-plane oligomerization, and of its role in the transition from the optical properties of small molecules such as DHI to the peculiar absorption spectrum of eumelanin.

We consider the DHI, DHICA, IQ and MQ monomers, analyzing the effects of molecular stacking on the main features of absorption spectra. We focus on the sensitivity of such spectra to the chemical species, the interlayer distance, the stacking geometry, the approximation inherent in our describing multi-layer stacked systems as infinite-layer ones, and the type of approximation used for the exchange-correlation kernel when calculating the dielectric function of the system. The present results may provide hints on possible trends in the optical properties of extensive stacking forms of larger and more complex and computationally demanding systems, more directly relevant for the interpretation of eumelanin experimental spectra, *i.e.* oligomers of DHI-like molecules.

2. Methods

We consider the following four monomeric DHI-like species, shown in the top left panel of Fig. 1: DHI (5,6-dihydroxyindole), DHICA (5,6-dihydroxyindole 2-carboxylic acid), IQ (indolequinone), and MQ (quinone-methide).

We performed self-consistent electronic structure calculations within DFT[23, 24] in the local density approximation (LDA) for the exchange-correlation functional, using the Quantum ESPRESSO suite[25, 26]. To describe the ion-

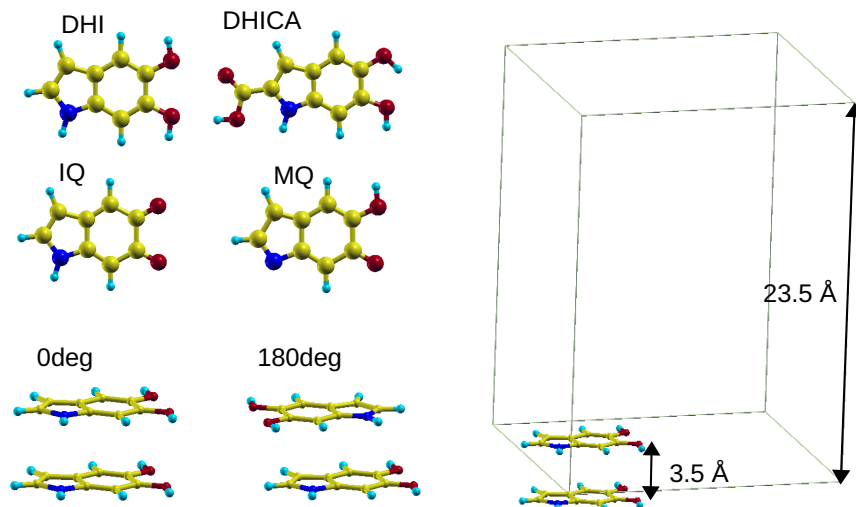


Figure 1: Top left: geometry of the four investigated monomers. Bottom left: geometry of the following stacked models: parallel adjacent molecules with the same orientation (“0deg” model) or rotated by 180° with respect to each other (“180deg” model), for the DHI case. Right: geometry and cell used for the 2-layer stacking system, showing the values of the interlayer distance and of the cell size along the stacking direction. Color code as follows: carbon, yellow; nitrogen, blue; oxygen, red; hydrogen, smaller cyan.

electron interaction we used norm-conserving pseudopotentials of the Trouiller-Martins type generated via the FHI98PP[27] code.

Thanks to the planarity of all the investigated monomers and their high similarity in terms of chemical structure and spatial size, we adopt a tetragonal unit cell having a length of 17 Å in the molecular plane along the longer molecule axis (x), and 16 Å along the shorter one (y). With this choice, we allow for at least 8 Å distance between atoms in replicated cells within the molecular plane in all cases.

In order to simulate isolated monomers, we adopt a large cell size also along the z direction (normal to the molecule plane), setting it to 20 Å; periodic boundary conditions are inherent to this scheme, and the Brillouin zone (BZ) is sampled at the Γ point only. For “standard” stacked systems (“0deg” model in the bottom left panel of Fig 1) we set the cell thickness L_z to 3.5 Å, with one molecule per cell; in these cases we sampled the BZ along the stacking direction with a 12 k-points shifted Monkhorst-Pack mesh[28]. In the “180deg” model (also shown in the bottom left part of Fig 1) adjacent molecules along the stacking direction are rotated by 180° with respect to each other: in this case the unit cell, of thickness $L_z = 7$ Å, contains two monomeric molecules

with the above-specified relative orientation, with an inter-molecule distance of 3.5 Å along z . In the two- (three-) layer stacking models (the right panel of Fig 1 shows the geometry and supercell for the two-layers case), two (three) molecules, with identical orientation and lying in parallel planes, were placed at a reciprocal distance of 3.5 Å along z , in a unit cell of thickness $L_z = 23.5$ Å ($L_z = 27$ Å), thus resulting in an isolated “stacked dimer” (trimer) with the same distance of 20 Å between replicas along z as for the isolated monomer case. Accordingly, the BZ was sampled at the Γ point only. We also considered models where one molecule per cell was placed in a cell with L_z assuming intermediate values between the isolated case value and the “standard stacking”: namely, we considered the $L_z = 7$ Å and $L_z = 5$ Å cases. The number of k -points for sampling the BZ along the z direction was rescaled according to the inverse of L_z scaling, yielding 6 and 9 k -points for the two cases, respectively. The kinetic energy cutoff for the plane wave expansion was set to 30 Ha in all cases.

Optical properties have been computed for all the analyzed molecules and geometries within time-dependent density functional theory (TDDFT)[29, 30], with the time-dependent local density approximation (TDLDA) for the exchange-correlation kernel, using the Yambo code[31]. For the isolated case and the “standard stacking” case only (adjacent molecules with the same orientation, $L_z = 3.5$ Å), spectra have also been calculated with different approximations for the exchange-correlation kernel. In particular, in these cases we have evaluated the dielectric function of the system at the Independent Particle level, and including local fields only (“IP” and “Hartree” datasets, respectively, in the Results section). From the calculated imaginary part of the dielectric function of our systems, we obtained the corresponding photoabsorption cross-sections as

$$\sigma(\omega) = \frac{V}{c} \omega \text{Im}(\varepsilon(\omega)) \quad (1)$$

where V is the volume of the supercell and c the velocity of light. In all cases we used a broadening of 0.2 eV and we averaged spectra over the three polarization directions. Photoabsorption cross-sections are reported in $\text{Megabarn}(Mb) = 10^{-22} m^2$.

The Brillouin zone sampling in optical properties calculations includes the Γ point only for isolated monomers ($L_z = 20$ Å) and for isolated stacked dimers and trimers. For smaller L_z values, we need the following number of shifted k -points within the Monkhorst-Pack scheme[28]: 8 points for the $L_z = 7$ Å case, 12 points for $L_z = 5$ Å, 16 points for $L_z = 3.5$ Å. For each system type (*i.e.* isolated monomer, stacked monomer, “180deg” “rot180” stacking model, cases with intermediate values of the interlayer distance, 2- and 3-layer stacking model) we checked the convergence of the TDLDA spectra with respect to the energy cutoff and to the total number of empty bands included in the calculations.

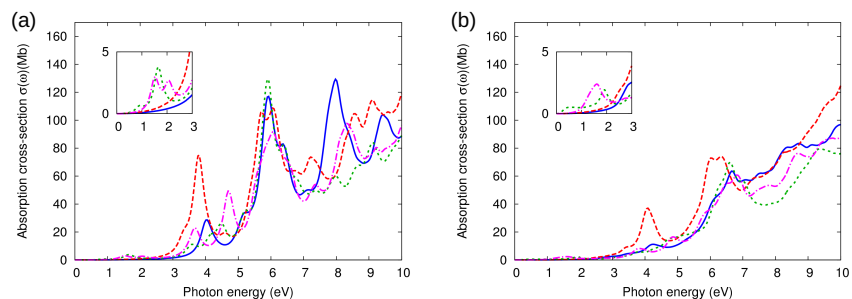


Figure 2: Absorption cross-section for the four investigated monomers, in their isolated (a) and stacked (b) forms: DHI (blue solid curve), DHICA (red dashed curve), IQ (dark green short-dashed curve), MQ (magenta dashed-dotted curve). The insets show a zoom on the 0-3 eV region, with characteristic weak peaks for the IQ and MQ molecules only.

3. Results

3.1. Main features of monomer absorption spectra

In Figure 2 we report absorption spectra for the four investigated monomeric species, DHI, DHICA, IQ and MQ, in their isolated (panel a) and stacked (panel b) forms. For the stacked forms we consider here a simple cofacial geometry where molecules lie in parallel planes, with an interlayer distance of 3.5 Å along the stacking direction, and have the same orientation (bottom left panel of Fig 1, “0deg” geometry). The value for the interlayer distance was chosen in agreement with values of 3-4 Å for stacking of eumelanin protomolecules, reported in the literature, based on both experimental and computational results[2, 3, 4, 5, 10, 15]. Here and in the following of this paper, we are going to show the average of the absorption cross-sections for light polarized along three reciprocally orthogonal directions. Absorption cross-sections for the four above-mentioned monomers in their isolated form, for light polarized along the following three orthogonal directions: the long molecular axis (x axis), the short molecular axis (y axis), the direction perpendicular to the molecular plane (z axis), are instead shown in the Supplementary Data (Figure S1).

In Figure 2, the position of the main absorption peaks is easily analyzed in the isolated case (panel a), where they are in general sharper than their counterparts in the stacked case (panel b). Excluding the very weak low energy peaks of IQ and MQ monomers, for all the four molecules under study the first absorption features are in the region between 3.5 and 5 eV, the most intense of them being the peak at slightly below 4 eV for the DHICA molecule (red curve); we can then identify an intense peak around 6 eV common to the four monomers, while in the region between 7 and 10 eV the situation is more heterogeneous.

The main features of all the reported spectra lie thus in the UV region; interestingly, the two oxidized species, *i.e.* IQ and MQ monomers, display additional very weak structures in the visible region, both in the isolated and stacked form (green and magenta curves in the insets of Fig 2). This confirms

the trend for oxidized *vs* reduced species observed in the literature for isolated or two- or three-layer stacked forms of chosen monomers and oligomers[14, 17, 11], and extends it to extensive stacking configurations (periodic model = infinite number of stacked layers) of DHI, DHICA, IQ and MQ monomers.

The agreement between absorption spectra of the DHI, DHICA, IQ and MQ monomers in their isolated form, obtained here within plane wave TDDFT with the LDA approximation (panel a of Fig. 2), and spectra of the same molecules reported in a previous computational work regarding isolated monomers only, obtained with localized basis TDDFT and the PBE exchange-correlation functional[11], extends to the overall shape of corresponding spectra, and to the positions of the main peaks in the energy region common to the two studies, *i.e.* from 0 to 7 eV. Indeed in this region the isolated DHI molecule (blue curve in panel a of Fig. 2) has the first absorption peak slightly above 4 eV and the main peak at ≈ 6 eV in both datasets, *i.e.* the present one and the PBE one of[11]; for isolated DHICA (red curve) the two main absorption features are (i) within the 3.5-4 eV range, with a weak shoulder on the low energy side, and (ii) at ≈ 6 eV; for isolated IQ (green curve) we find a first very weak peak at ≈ 1.7 eV, followed by other weak peaks in the 3-5 eV region, and the main peak at ≈ 6 eV; for isolated MQ (magenta curve) the first absorption feature is a very weak peak at ≈ 1.5 eV, followed by other peaks of increasing intensity in the 3-5 eV region (generally more intense than the peaks of IQ monomer in the same region), up to a rather broad main absorption feature around 6 eV (resolved into several peaks in[11], which has a smaller broadening). Our absorption spectrum of the isolated IQ monomer is also in good agreement with the one calculated for the same molecule by Meng and Kaxiras[19], regarding both peak positions and relative intensities, on the range common to the two studies, *i.e.* from ≈ 1.5 eV to ≈ 5 eV. **A meaningful comparison can also be done with respect to calculations performed with different functionals, e.g. with B3LYP results of Reference [11], where the inclusion of a fraction of exact exchange yields a spectral blueshift of about 0.5 eV, without dramatically changing the overall spectra¹.**

By comparing absorption spectra for isolated monomers (panel a) with those for stacked forms (panel b), we can already observe that extensive molecular stacking induces a broadening and a decrease in intensity of the main peaks for all the investigated monomeric species. The absorption spectrum of the DHICA monomer is the less affected by stacking, and retains rather sharp features also for the stacked form, most remarkably for the peak at ≈ 4 eV. **In section 3.3, we analyze the transition towards the stacked system by progressively varying the interlayer distance, observing a seamless and progressive change of the resulting spectra.**

¹The extension of this comparison to the case of stacked systems, where Van der Waals interactions may play an important role, is beyond the scope of the present work

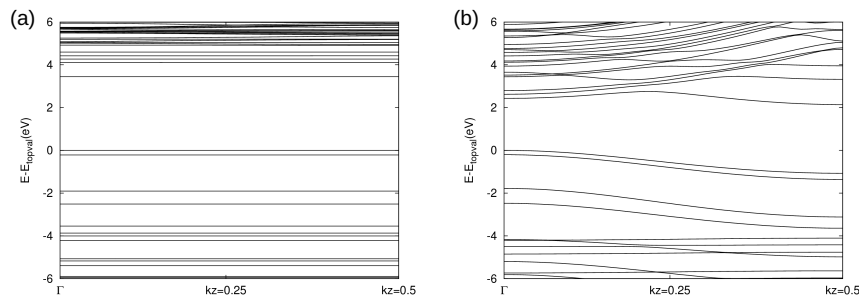


Figure 3: Band structure for the DHI monomer in its isolated (panel a) and stacked (panel b) form. The zero of the energy scale is set at the top of the valence band.

3.2. Band structures

Figure 3 reports the band structures calculated for the DHI monomer in its isolated form (panel a) and in the stacked one (panel b). Whereas for the isolated molecule the bands are flat as expected, in the stacked system the proximity of stacked molecules (3.5 Å interlayer distance) induces band dispersion, resulting in a reduction of the energy gap. The band structure of the stacked form of DHI is in good agreement, both regarding the band gap value and the overall band dispersion and shape along the whole Brillouin zone path, with the results of Oetzel et al.[32] for cofacial stacking of the same molecule (named HQ in that work), despite the different computational details (projector augmented wave pseudopotentials in the cited work) and a slightly different interlayer distance (3.7 Å in that work).

3.3. Stacking: effect of the intermonomer distance

In order to investigate the effects of stacking upon the optical properties in more detail, we have calculated absorption spectra also for intermediate values of the interlayer distance L_z , in addition to the cases with $L_z = 20$ Å (isolated molecule limit) and $L_z = 3.5$ Å (stacked system as considered so far). As explained in the Methods section, this can be easily done in our plane wave approach with periodic boundary conditions, by only varying the cell thickness along the stacking direction, L_z , which coincides with the interlayer distance in the simple stacking geometry considered so far.

Figure 4 shows absorption spectra of the four investigated monomers, considering for each of them four different values of the interlayer distance, namely 20, 7, 5 and 3.5 Å, always keeping the same cofacial stacking geometry, *i.e.* parallel molecules with the same orientation. This allows us to both investigate the dependence of absorption spectra of stacked monomers on the value of the interlayer distance, and simulate the transition from the isolated molecule case to the stacked one.

As already anticipated above for the comparison between the $L_z = 20$ Å case and the $L_z = 3.5$ Å one, we can observe that a progressive reduction of

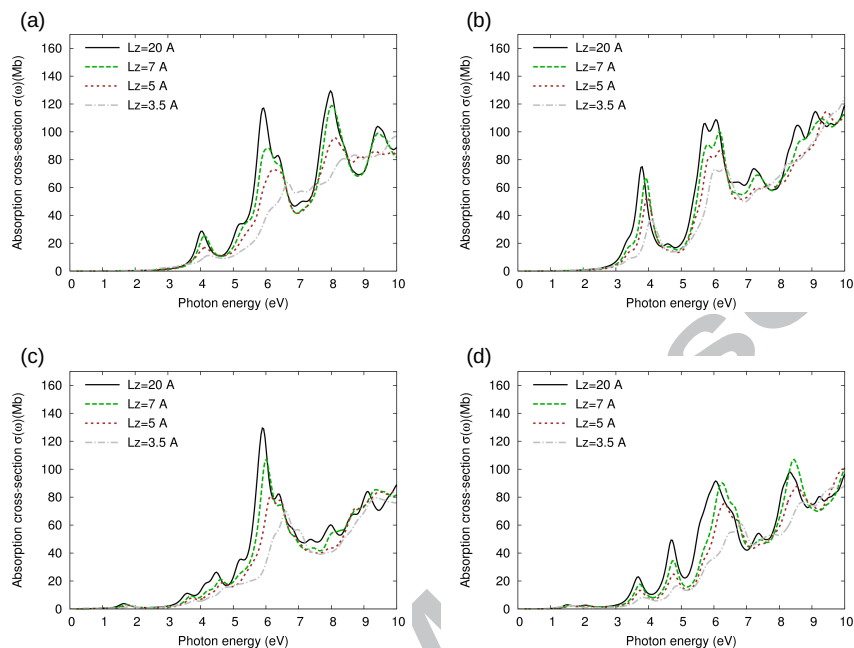


Figure 4: Absorption cross-section for the DHI (a), DHICA (b), IQ (c) and MQ (d) monomers, with different values of the interlayer distance.

the interlayer distance, starting from the isolated molecule case, is accompanied by a broadening and lowering in intensity of all the absorption peaks in the analyzed energy region (0 – 10 eV), together with a weak blueshift, for all the four investigated molecules. This renders spectra more and more similar to the experimental broadband and monotonic eumelanin spectrum, upon decreasing the stacking distance, *i.e.* upon progressively reaching the “standard” stacking geometry as defined so far. DHI and MQ are the molecular species for which this trend is more evident throughout the analyzed 0-10 eV energy range (top left and bottom right panels of Fig. 4, respectively); for IQ (bottom left panel) the same trend holds in the 0-6.5 eV range only, while DHICA (top right) retains relatively sharp absorption features for all the investigated interlayer distances.

Thus, even the present simplified model of the possible arrangement of eumelanin protomolecules in the pigment, limited to out-of-plane stacking of monomers and neglecting in-plane oligomerization, can already yield valuable information on possible mechanisms contributing to the emergence of the typical broadband and monotonic absorption spectrum of eumelanin, starting from spectra of isolated DHI-like monomers, characterized by few rather sharp peaks.

At the same time, our results confirm for the isolated DHI, DHICA, IQ

and MQ molecules and extend to extensive stacking arrangements of each of them the finding, pointed out by Chen et al.[10] for isolated and 2-layer stacked monomers and for large disordered aggregates of monomers, that monomer spectra differ from experimental absorption spectra of eumelanin in that the former display negligible intensity in the visible range. Indeed, in our spectra both of isolated molecules and of stacked ones, IQ and MQ monomers have few very weak features below 2.5 eV, while DHI and DHICA have none.

3.4. Stacking: effect of relative orientation

In the literature several possible stacking configurations have been proposed for few-layer stacking of selected monomers and oligomers[10, 14, 16, 17, 19], therefore we are interested in analyzing the sensitivity of absorption spectra to the stacking geometry. In Fig 5 we show absorption spectra for stacked arrangements of the DHI, DHICA, IQ and MQ monomers, with adjacent molecules along the stacking direction lying in parallel planes with 3.5 Å interlayer distance, and either having the same orientation (black solid curve, cofacial stacking, “0deg” model) or being rotated by 180 degrees with respect to each other (brown dashed curve, “180deg” model). The geometries of the “0deg” and “180deg” models are shown for the DHI monomer in the bottom left panel of Fig 1.

Although the general appearance of absorption spectra remains rather similar in the two geometries for all the four monomeric species, some variations are worth mentioning. Most remarkably, in the spectra of the IQ and MQ monomers, which already display weak features at energies below 3 eV in the “0deg” stacked model (and in their isolated form, too), a rotation of adjacent stacked molecules by 180° with respect to each other results in a higher intensity of low-energy peaks, in particular for MQ. Moreover for DHI, IQ and MQ, the absorption peak at ≈ 6 eV is slightly redshifted in the “180deg” geometry, and, for DHI and MQ only, it is also more pronounced, thus making the spectrum of the “180deg” model less qualitatively similar to the monotonic spectrum of eumelanin in these two cases.

3.5. Stacking: comparison with 2- and 3-layer models

Beyond the finite lateral size of 15-20 Å for eumelanin protomolecules, both Xray [2, 3] and STM[4] experimental studies found evidence for aromatic stacking of 4-5 layers of planar oligomers of DHI-like molecules. In all the results presented so far in this work, we have modeled stacked forms as periodic along the stacking direction. This allows one to address extensive stacking structures, *i.e.* those consisting of more than 2-3 stacked molecules, at a relatively low computational cost, but describes a stacking arrangement with an infinite number of layers, which is clearly not realistic. In order to assess the effect of this infinite-layers approximation on computed spectra, we considered, for the DHI case, additional stacking models, where a sufficiently thick supercell contains two or three stacked molecules only, in the usual cofacial geometry with 3.5 Å interlayer distance, yielding what we can define a “stacking dimer” (or trimer):

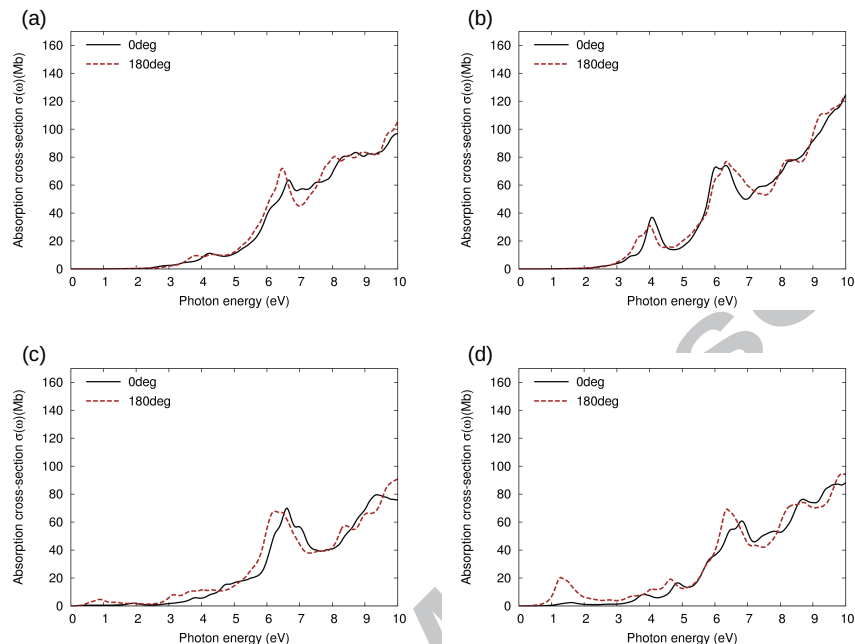


Figure 5: Absorption cross-section for the DHI (a), DHICA (b), IQ (c) and MQ (d) monomers, each one in two different stacking geometries: parallel adjacent molecules with the same orientation (“0deg” model, black solid line), parallel adjacent molecules rotated by 180° with respect to each other (“180deg” model, brown dashed line).

the right panel of Fig 1 shows the geometry and cell of the two-layers case. These “finite-stacking” models are more computationally expensive than the periodic/infinite-layers ones in our plane wave approach, since the former contain a large vacuum region along the stacking direction to ensure the stacking “dimer” or “trimer” can be considered as isolated, resulting in a cell thickness of $[20 + (N - 1) \times 3.5] \text{ \AA}$, where N is the number of layers, to be compared to the 3.5 \AA cell thickness of our standard periodic stacking model.

In Figure 6 we compare absorption spectra of the DHI molecule in its isolated form (black solid line), in the above-mentioned two- and three-layer stacking geometries (violet long dashed line and orange short dashed line, respectively) and in the “standard” periodic stacking geometry (gray solid line with dots). Spectra of the 2- and 3-layer stacking models resemble those of periodic stacking models with intermediate values of the interlayer distance, in that their peaks are more and more broadened and less intense upon increasing the number of layers (Fig 6), or upon decreasing the stacking distance (Fig 4), respectively.

From the point of view of peak shifts, the trends observed upon increasing the number of stacked layers are different from those for periodic stacking systems

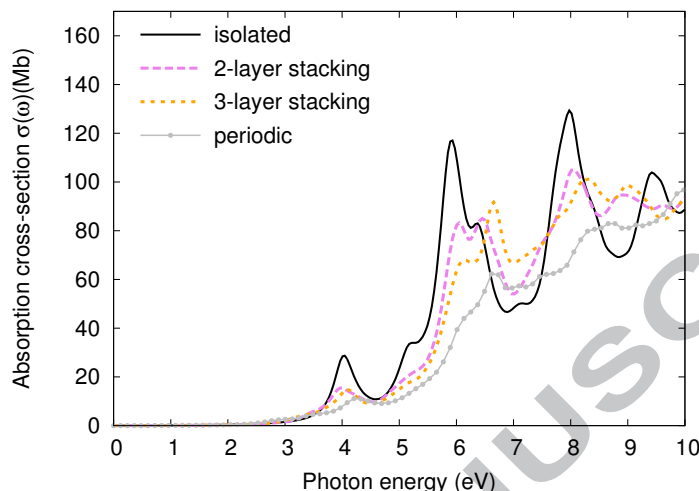


Figure 6: Absorption cross-section for the DHI monomer in its isolated form (black solid line), in the 2-layer (violet long dashed line) and 3-layer (orange short dashed line) stacking geometry, and in an infinite/periodic stacking one (gray solid line with dots).

upon decreasing L_z . In the latter case (Figure 4) we found a weak progressive blueshift of absorption peaks, most pronounced in DHI for the peak at ≈ 6 eV, and less recognizable for the feature at ≈ 9.5 eV due to its large broadening. On the other hand, when comparing spectra of isolated DHI, of 2- or 3-layer stacking models and of the periodic / infinite-layers stacking system (Fig 6), the trend is less well-defined. The peak around 4 eV undergoes a weak redshift switching from the isolated DHI case to the 2-layer stacking model, followed by a weak blueshift upon going to the 3-layer model and then to the periodic stacking system. For the peak around 6 eV the situation is more complex, with a progressive change in the relative intensity of the two components of the doublet. For the features at ≈ 8 and at ≈ 9.5 eV the strong broadening prevents a meaningful discussion on possible shifts in peak positions.

3.6. Effects beyond the Independent Particle Approximation

Another interesting issue is the sensitivity of the investigated optical properties to the approximation used when calculating the dielectric function of the system. Fig 7 shows absorption spectra for the four monomers under study in their isolated form, obtained by using different approximations for the exchange-correlation kernel; Fig 8 shows an analogous comparison for the **cofacially** stacked form of the four monomers. Similarly to the trends observed in[33] for silicon clusters, we find that the largest variation in absorption spectra occurs when moving from the Independent Particle (**IP**) approximation (“IP” datasets, blue dashed-dotted curves in the **Figures** Figure) to the inclusion of Local Field (LF) effects (“Hartree” datasets, orange dashed curves). **The**

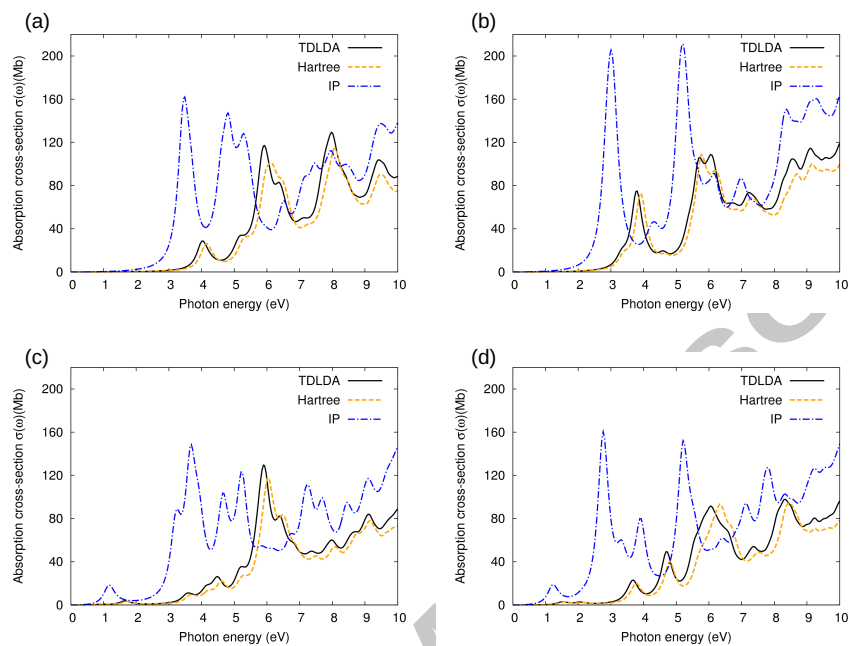


Figure 7: Absorption cross-section for the DHI (a), DHICA (b), IQ (c) and MQ (d) monomers, in their isolated form, obtained at the following three levels of approximation: Independent Particle (blue dashed-dotted line), including local fields (orange dashed line) and including also exchange-correlation effects at the ALDA level (black solid line).

On the other hand, the additional inclusion of correlation effects contained in f_{XC} within the Adiabatic Local density Approximation (“TDLDA” datasets, black solid curves) leads to spectra which retain the overall appearance of the LF ones, differing only for minor changes in peak intensity and position. Local fields effects are indeed expected to be dominant for finite or low-dimensional systems, due to their strong inhomogeneity. On the other hand, the effects of stacking are much better described when LF are included than at the IP level. The almost monotonically increasing absorbance of the stacked systems only appears when LF effects are taken into account. A direct comparison of experimental eumelanin spectra [34] with the average of all computed spectra of stacked systems (Fig 9) displays a good agreement in the whole UV region, including a structure at $\approx 4\text{-}5$ eV, which is present in the experiment. The exact position of this peak may be affected by many-body effects such as self-energy corrections and excitonic effects, which could be described in a more complex and computationally heavy approach, such as in a GW-BSE

scheme [30], and are beyond the scope of this work². The fact that the inclusion of local field effects yields isolated and stacked monomer spectra which are rather similar to those obtained with the additional inclusion of exchange-correlation effects at the ALDA level suggests the possibility of using the former method, less computationally expensive, for further studies involving larger eumelanin constituents, such as extensive stacking of oligomers.

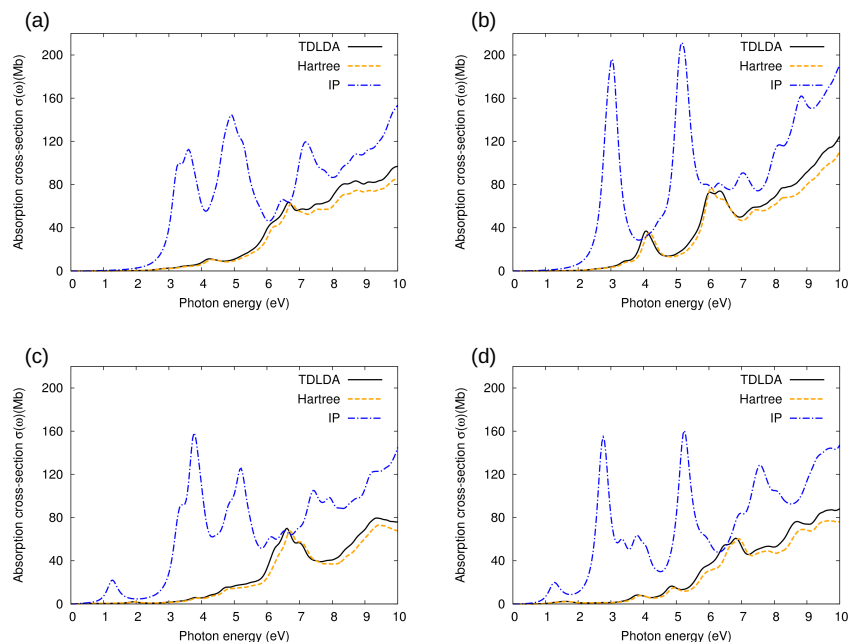


Figure 8: Absorption cross-section for the DHI (a), DHICA (b), IQ (c) and MQ (d) monomers, in their stacked form, obtained at the following three levels of approximation: Independent Particle (cyan dashed-dotted line), including local fields (orange dashed line) and including also exchange-correlation effects at the ALDA level (black solid line).

4. Conclusions

In this work we calculated optical absorption spectra of the isolated and π -stacked forms of four molecules, known to be among eumelanin elementary building blocks, namely 5,6-dihydroxyindole (DHI), 5,6-dihydroxyindole-2-carboxylic acid (DHICA), indolequinone (IQ) and quinone-methide (MQ). We described extensive *i.e.* several-layer stacking within a plane wave DFT approach, with

²Such an high-level approach would also be able to reproduce the possible presence of charge-transfer excitations, which are known to be poorly described at the TDLDA level

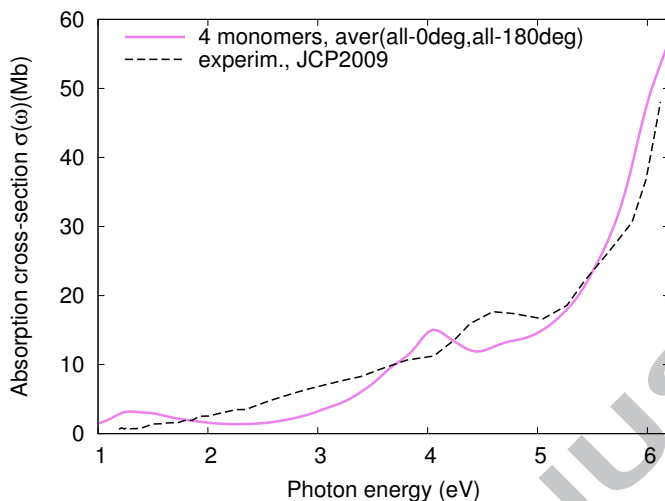


Figure 9: Average of the absorption cross-sections for the four investigated monomers, both in the “0deg” and “180deg” stacking geometries (magenta solid curve), compared with the experimental eumelanin spectrum from ref [34] (black dashed curve) rescaled in order to have comparable absorption intensity.

the system treated as periodic along the stacking direction, and investigated both the effect of stacking on absorption spectra of the molecules under study, and their chemical sensitivity and dependence on both stacking geometry and computational approximations.

Although oligomers rather than monomers of DHI-like molecules are believed to be the units undergoing stacking in eumelanin according to experimental data, the trends found here for extensive monomer stacking can be useful in both predicting and rationalizing the behaviour of stacked forms of larger molecular species such as dimers or tetramers of DHI-like molecules. Stacked DHI-like monomers can be considered as minimal models of out-of-plane complexity in eumelanin (complementary to the in-plane complexity determined by oligomerization) and of its effects in the emergence of the peculiar broadband and monotonic absorption spectrum of the eumelanin pigment, wherein both the three-dimensional arrangement and chemical heterogeneity are expected to play a role.

Upon decreasing the interlayer distance for infinite/periodic stacking geometries, or increasing the number of layers for finite-layers cases, which corresponds to modeling the transition from the isolated molecule case to a stacked arrangement, absorption spectra of the DHI, DHICA, IQ, MQ monomers become more and more similar to the experimental spectrum of eumelanin, *i.e.* continuous and with intensity increasing with frequency. These findings, **beyond confirming that, even for extended stacking geometries, suggest an analogous behaviour of oligomer spectra upon extended monomer spectra alone cannot explain eumelanin absorption in the visible range, show**

that the typical features of the eumelanin experimental absorption spectrum in the UV range between 3 and 6 eV are present already in stacked monomer spectra, and suggest an analogous behaviour for oligomer spectra in an extended *i.e.* more than 2 or 3 layers stacking, which would be more directly relevant for the interpretation of experimental eumelanin absorption. At the same time, our results confirm for extended stacking geometries the conclusion that (*i.e.* more than 2 or 3 layers) stacking geometry, which may be suitable to explain the experimental eumelanin *monomer* spectra alone can not explain eumelanin absorption, in that the former do not display absorption in the whole vis-UV spectral region. visible range.

Along the same line of reasoning, the observed chemical sensitivity of absorption spectra of stacked monomers, in particular the presence of low-energy (visible) absorption features in oxidized forms only, although not bearing direct information for the interpretation of absorption spectra of the eumelanin pigment, can suggest similar trends for spectra of extended stacking arrangements of oligomers. These latter - if confirmed - would provide means to identify the types of oligomers present in eumelanin, in case such chemical sensitivity results in well-defined fingerprint features for the different oligomeric species, oxidation states etc.

A combined chemical/structural sensitivity can also occur: spectra of stacked oxidized species IQ and MQ are more sensitive than those of stacked DHI and DHICA to a change in geometry consisting in rotating adjacent stacked molecules by 180° with respect to each other.

Finally, our analysis shows that, both for isolated and stacked monomers, the inclusion of local field effects yields spectra in good agreement with those obtained at the more computationally expensive TDLDA level, *i.e.* with the further addition of exchange-correlation effects at the ALDA level.

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6. Appendix A. Supplementary data

Supplementary Figure S1 shows the absorption cross-sections of the four investigated monomers **in their isolated form**, for light polarized along three reciprocally orthogonal directions, together with the average of these three components.

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