Exploring the azimuthal symmetries of electronic transitions in molecular and biomolecular systems by swift electrons

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Abstract

Electron energy loss spectroscopy is consolidating as a powerful tool to explore electronic (as well as vibrational) excitations of matter, including molecules. Performed in a scanning transmission electron microscope, this technique is based on inelastic scattering of fast electrons in a thin specimen. Very recently, new electron optics configuration have been introduced, opening the way to the analysis of the single components of orbital angular momentum of the outcoming electrons, that convey additional information on the spatial features of the investigated excitations: innovative double-dispersed spectroscopic experiments for metallic nanostructures have been therefore suggested. We propose here to extend this technology to probe molecular and supra-molecular systems, devising new kind of experiments: using state of the art quantum chemical methods to describe the molecular system in presence of an electron beam in a configuration that avoid molecular damage, we show that scattered electrons acquire the
different azimuthal components of induced molecular transition potentials. Numerical simulations performed for systems of increasing size, point out that the conceived new technique can open up the possibility of probing the multipolar components and even the chirality of molecular transitions, superseding the usual optical spectroscopies for those cases that are problematic, such as dipole-forbidden transitions, at a very high spatial resolution.

**Introduction**

Understanding the electronic structure of matter is a formidable task that made largely use of optical spectroscopies and their corresponding selection rules; indeed, probing optical excitations with nanometer resolution one can obtain informations on their dynamics and interactions down to the atomic scale. The information acquired can range from the electronic structure and properties of a single molecule to the energy and electron transfer mechanism in complex systems, just to cite a few. The origin of spectral lines is due to the absorption, emission, and scattering of a photon that modify the energy of the system, whereas the line shape can carry out information about the dissipation of the energy absorbed, the interaction with the surroundings and its influence in modulating the microscopic dynamics of chromophores. However, not all the electronic transitions can be probed in optical spectroscopic experiments because of different selection rules: being optically forbidden, the possibility to investigate the role of a given transition in the photophysical and/or photochemical activity of a molecular system is precluded. For instance, a long debate in literature is still ongoing on the possible role of charge transfer (CT) states in photosynthetic mechanisms: being dark, can be only indirectly probed. On the other hand, electron-beam spectroscopies are now emerging as probing techniques to study of optical excitations with combined space, energy and time resolution: nano-photonic structures and their detailed optical responses are now starting to be explored. Between the different type of probe experiments that can be performed in trasmission electron microscopes (TEM) and scan-
ning TEM (STEM), the Electron Energy-Loss Spectroscopy (EELS) can provide insight into the properties of materials at the nanoscale.\textsuperscript{11,12} It is widely used to identify chemical species with atomic resolution\textsuperscript{13–15} through their characteristic high-energy core losses; beside, low-loss EELS can probe the spatial and spectral distributions of plasmons in metallic nanostructures\textsuperscript{16–22} and, more recently, also phonons in polaritonic materials have been investigated.\textsuperscript{23} The main advantage is the possibility to spatially map the fields associated with both bright and dark plasmonic resonances of a given nanostructure. Usually EELS experiments produce swift electrons (from 30 to 300 keV typically) that interact with the sample exchanging energy and momentum. The loss function (i.e. the probability, per unit of transferred frequency, that the swift electron loses energy) is evaluated at the excitation energy of a given plasmonic resonance.\textsuperscript{11} If spectroscopy carried out in the electron microscopes could be extended to the molecular and supra-molecular systems, then this technique could be used not only to determine the overall morphology but also to follow the dynamics of electronic processes inside complex molecular aggregates: for instance, one could find at high spatial resolution where are located the different chromophores within the overall structure in proteins and pigment-protein complexes and then study the processes leading to energy and electrons transfer. In this direction, very recent studies have shown applications of EELS to study vibrations in guanine crystals, resolving their characteristic C-H, N-H and C=O vibrational signatures with no observable radiation damage.\textsuperscript{24} The goal of this study is indeed to explore the possibility of conceive new electron energy loss experiments for molecular and supra molecular systems. To obtain a new electronic excitation fingerprint, we propose to probe the azimuthal symmetry of the molecular transitions, based on the analysis of the different orbital angular momentum (OAM) components of the scattered electrons in TEM and STEM.\textsuperscript{25,26} Indeed, free electrons can carry a quantized OAM value upon free-space propagation: these "electron vortices" are characterized by a spiraling wavefront with a screw dislocation along the propagation axis.\textsuperscript{27,28} As a matter of fact, even if the measure of the OAM spectrum of a light beam has been demonstrated ex-
experimentally ten years ago,\textsuperscript{29} only recently the electronic analogous has been made possible by devices based on electrostatic phase elements for measuring and spatially dispersing the different electrons OAM components.\textsuperscript{30,31} Our work is inspired by what proposed in the field of metallic nanostructures,\textsuperscript{32} here extended to treat molecular and supra-molecular systems. In the following, we describe how to modify the configuration of a TEM-EELS apparatus and how to encode a quantum chemistry treatment of the molecular systems and its interaction with the structured wave of the swift electron to obtain OAM resolved EELS spectra, then simulations of the expected experimental results will be presented in a number of paradigmatic cases considering also the effects of the finite resolutions in both energy and OAM due to a nonideal setup.

Methods

A problem one can face out to an EELS experiment performed on molecular systems is the avoid of direct interaction of such highly energetic electrons with the specimen, that may substantially alter and destroy the structure of interest during observation. The use of aloof beam electron energy-loss spectroscopy as a non-destructive nanoscale surface characterization tool is one of the most powerful recent advances in this technique.\textsuperscript{24,33,34} For instance, an aloof configuration of the beam, positioned tens of nanometres away from the sample, have been recently used for the detection of electronic and vibrational peaks in guanine crystals extracted from the scales of the Japanese Koi fish.\textsuperscript{24} by controlling the distance of an external narrow electron probe from the edge of the specimen, the authors selectively probe vibrational modes without exceeding the energy thresholds that potentially lead to radiation damage. Here, we propose that the control over beam-sample interaction can be performed by an annular electron beam.\textsuperscript{35-38} One can imagine the experimental setup as depicted in Fig.1: an electron gun (equipped, for instance, with an annular CsI-coated carbon fiber cathode\textsuperscript{36}) or a phase hologram in the condenser system\textsuperscript{37,38} produce an annular shaped...
Figure 1: Scheme of a OAM-resolved EELS experiment to investigate a molecular system. The electron beam source system produces an annular shaped electron beam. The Coulombic interaction of the annular electron beam with the induced molecular transition potentials (inset, right panel) give rise to the scattered electrons, processed in the EELS system, after the passage through an OAM sorting device. This last consist of two electrostatic phase elements in the electron column as detailed in refs. 30, 39 and 40.

electron beam, that interact with the molecular specimen without hitting it. The inelastically scattered electrons are sorted as a function of the different orbital angular momentum components using a set of two electrostatic phase elements in the electron column.

Interested readers can found a detailed description of this type of devices in refs 39 and 40. Finally, the separated OAM components are processed by the EEL spectrometer system that produces the diffraction image observed on a fluorescent screen, giving rise to a double disperse spectrum as a function of the energies and angular momenta, that are determined...
by the azimuthal symmetry of the molecular transition density probed (inset of Fig.1), as
detailed below.

Let us now describe how to properly model the electron-molecule interactions, to determine
the final expected spectra. Atomic units are assumed to simplify the notation. A swift
electron propagating in a homogeneous medium generates an electromagnetic field that can
probe matter with a high spatial resolution. This field can be regarded as an evanescent
source of radiation which permits exploring regions of momentum-energy space around the
beam inducing electronic transitions in the target specimen, from its ground state \( |0\rangle \) of
energy \( E_0 \) to a generic excited states \( |n\rangle \) of energy \( E_n \). The incoming electron, described
by its wave function \( |\psi_i\rangle \) and energy \( \varepsilon_i \), exchange energy and momentum during the target-
probe Coulomb interaction (that give rise to the coupling term) with the specimen, acquiring
components \( |\psi_f\rangle \) of lower energy \( \varepsilon_f \) (see Fig.1 inset). The total wave function of the entire
super-system (i.e. electron beam plus molecular system) can be separated as the tensor
product of the sub-systems wave functions, i.e. for a generic state \( |\Psi_j\rangle \):

\[
|\Psi_j\rangle = |\psi_j\rangle \otimes |j\rangle
\]

Since the electrons are very energetic and the interaction can be considered generally
small (at least compared to the kinetic energy of the beam electrons), the transition rate can
be properly described within a first-order perturbation theory (Fermi’s golden rule-like).
Taking into account that the total energy before the interaction is \( E_0 + \varepsilon_i \) (\( |\Psi_i\rangle = |\psi_i\rangle \otimes |0\rangle \))
and after the interaction is \( E_n + \varepsilon_f \) (\( |\Psi_f\rangle = |\psi_f\rangle \otimes |n\rangle \)), and assuming that the molecule-
swift electron interaction can be treated as purely electrostatic, the probability for unit
time $\Gamma_{EELS}(\omega)$ for the swift electron to lose an energy $\varepsilon_i - \varepsilon_f = \omega$ is given by:

$$
\Gamma_{EELS}(\omega) = 2\pi \sum_f \left| \langle \Psi_f | \hat{H}_{int} | \Psi_i \rangle \right|^2 \delta(E_n + \varepsilon_f - E_0 - \varepsilon_i) = 
$$

$$
= 2\pi \sum_f \left( \int dr \int dr' \psi_f^*(r) \frac{\langle n | \hat{\rho}(r') | 0 \rangle}{|r - r'|} \psi_i(r) \right)^2 \delta(E_n + \varepsilon_f - E_0 - \varepsilon_i) 
$$

(2)

where $\hat{\rho}(r')$ is the electron density operator acting on the molecular electrons. Setting $\Delta \varepsilon fi = \varepsilon_i - \varepsilon_f = \omega$ and $\omega_{n0} = E_n - E_0$, we obtain:

$$
\Gamma_{EELS}(\omega) = 2\pi \sum_f \left( \int dr \int dr' \psi_f^*(r) \frac{\langle n | \hat{\rho}(r') | 0 \rangle}{|r - r'|} \psi_i(r) \right)^2 \delta(\omega - \omega_{n0}) 
$$

(3)

The coulombic coupling, $\hat{H}'(r)$, acts on the swift electron wavefunctions and is given by the interaction between an electron of the beam and the electrostatic potential due to the ground to excited state transition, $V_{0n}(r)$:

$$
\hat{H}'(r) = \int d^3 r' \frac{\langle n | \hat{\rho}(r') | 0 \rangle}{|r - r'|} = \hat{V}_{0n}(r) 
$$

(4)

In our simulations, the molecular transition potential is calculated by adopting a linear response (LR) approach in the TD-DFT framework (as detailed in the supporting information), but one can of course apply any appropriate electronic structure method that gives access to this quantity.

Free-electron sources in electron microscopy generate unpolarized particles, which are described by the scalar wave function (in sharp contrast to optics) and are highly-paraxial, i.e. the fields propagate along the direction of the free-electron motion $z$, and spread out only slowly in the transverse direction. In these cases the wavevectors $\mathbf{k} = (k_x, k_y, k_z)$ in the angular spectrum representation are almost parallel to the $z$-axis and the transverse
wavenumbers \((k_x, k_y)\) are small compared with \(|k| \approx k_z\). In the following, we apply this paraxial approximation to find a description of the individual electron wave functions:

\begin{align*}
\psi_i(r) &= \phi_i(r_\perp) e^{ik_{iz}z} \\
\psi_f(r) &= \phi_f(r_\perp) e^{ik_{fz}z}
\end{align*}

The annular component (in the plane perpendicular to the beam axis) of the incident electron beam is set therefore:

\begin{equation}
\phi_i(r_\perp) = \frac{1}{N} e^{-\left(\frac{|r| - r_0}{\Delta r}\right)^2}
\end{equation}

\(N\) being the normalization constant, while the transverse component of the final individual electron state is expressed in cylindrical coordinates as:

\begin{equation}
\phi_f(r_\perp) = J_{|l|}(k_{f\perp}r)e^{-il\phi}
\end{equation}

\(J_{|l|}(k_{f\perp}r)\) is a Bessel function of the first kind of order \(l\) (the angular quantum number), with transverse wavevector \(k_{f\perp}\) (i.e. the projection of on the xy plane perpendicular to the TEM axis). The azimuthal component, \(exp(-il\phi)\), describes the amount of OAM carried by the beam \((L_z = \hbar l)\). The modes with \(l \neq 0\) are also called vortex beams. The solutions of the free electron Schrödinger equation in cylindrical coordinates are a convenient basis due to the symmetry of the problem and to express the different OAM components of the scattered electron spectrum. In this way, the sum over the final electronic beam states appearing in eq.\(^3\) can be now performed over an ensemble of such final states characterized by a fixed \(l\) and transverse wave vector \(k_{f\perp}\) in the range \([0, k_{max}]\). Here, \(k_{max}\) is related to the collection angle of the detector \((\alpha)\) by de Broglie probe electron’s wave length \((\lambda)\) as \(k_{max} = \alpha\lambda\).

Concerning the molecular transition potentials associated to the electronic excitations, one can expect a sinusoidal (or cosinusoidal) azimuthal dependence, such as \(sin(m\phi)\) or \(cos(m\phi)\) (inset in fig.\(^\text{1}\)). Making use of the Fourier transform (FT) of transition potential \(V_{0n}(r)\) along
the longitudinal direction \((p = k_{fz} - k_{iz})\), we can therefore write down a transverse component of type:

\[
\tilde{V}_{p}^{0n}(r, \varphi) = \int V_{0n}(r)e^{-i(k_{fz} - k_{iz})z}dz = \int V_{0n}(r)e^{-ipz}dz \equiv \sum_{m=-\infty}^{+\infty} \tilde{V}_{p,m}^{0n}(r)(e^{-im\varphi} \pm e^{im\varphi}) \tag{9}
\]

Eq.\((9)\) makes use of the multipole expansion of the transition potential transverse component, expressed in cylindrical coordinates: \(m = 0\) corresponds to the monopole, \(m = \pm 1\) to the dipolar term, \(m = \pm 2\) to the quadrupole and so on (in general, the \(2|m|\)-pole). The final energy loss rate per unit of angular momentum can be therefore conveniently re-expressed in cylindrical coordinates:

\[
\Gamma_{l}^{\text{EELS}}(\omega) = \pi \int_{0}^{k_{\text{max}}} dk_{f} \left| \sum_{m=-\infty}^{+\infty} \int r dr \int_{0}^{2\pi} J_{|l|}(k_{f}r)e^{-il\varphi}\tilde{V}_{p,m}^{0n}(r)(e^{-im\varphi} \pm e^{im\varphi})e^{-\frac{|r| - m}{\Delta r^{2}}}d\varphi \right|^{2} \\
\times \delta(\Delta \omega - \omega_{0n}) \tag{10}
\]

It is now easy to derive the selection rule for this double resolved electron spectroscopy, i.e.:

\[
\int_{0}^{2\pi} e^{-i(l \pm m)\varphi}d\varphi \neq 0 \implies l = \mp m \tag{11}
\]

The eqs. \((10)\) and \((11)\) show that performing an OAM-EELS experiment one can simultaneously probe the energy and the different azimuthal components of the transition potential related to the different electronic excitations, due to the exchange of energy and momentum between individual electrons of the beam and the molecular system. The scattered electrons acquire all the different azimuthal components of induced transition potentials: they are structured waves containing a multitude of vortices (one for each \(l\) component acquired).

The different OAM components can be sorted by the electrostatic optical elements.\(^{30,31,39}\) As a consequence, also optically dark (i.e. dipole forbidden) transitions became detectable due to the signals of the higher angular momenta, i.e. those with \(l \neq \pm 1\).
Results and discussion

In the following, we present some proof of concept simulations, performed at TD-DFT level, as detailed in supporting information and based on the theoretical description presented in the previous section. Electronic structure simulations have been performed with the Gaussian 16 version of the Gaussian suite of programs. The transition potentials, stored on cubic grids, have been used to numerically integrate eq(10) by a Matlab script. To be useful as a spectroscopic tool, the supporting substrate should not perturb significantly the electronic structure of the molecular systems: therefore all the linear response calculations have been performed in vacuo. All simulations have been carried out keeping fixed the collecting angle to 200 mrad and the beam energy to 60 keV, whereas the loss probability have been determined in the angular momentum range of $[-3 : 3]$. In order to obtain doubled resolved spectra that simulate the limited instrumental resolution ($\Delta E = 0.1$ eV, $\Delta l = 0.5\hbar$), we have performed a convolution of OAM-EELS rates with the product of two Gaussian functions, following the procedure outlined in supporting information of ref. 32.

Let us start with testing a single molecule experiment, using guanine as a pedagogical case. Even if it will be probably difficult to experimentally perform a single molecule measure - at least in the first implementations of the experiment here proposed - still we found instructive to start with this case and then increase the dimension of the specimen studied. The structure of guanine is shown in fig.2 panel a, together with the transition potentials (integrated along z) of the first five singlet excitations (panel e). Assuming that the sample is illuminated by an annular beam with a radius of 7 Å and a width of 1.5 Å, we obtain the simulated OAM-EEL spectra for different values of OAM as reported in panel b, c and d of fig.2, using a Gaussian convolution with a broadening of 0.1 eV. As one can expect from the transition potential projections, all the transitions - except the dark singlet $S_4$ - show a large dipolar component ($l = \pm 1$) that is obviously proportional to the corresponding optical oscillator strength (panel e, fig.2). A small quadrupolar contribution is shown for the $S_5$ excitation and, to a lesser extent, also for those involving the $S_1$, $S_2$ and $S_3$ singlets (panel c, fig.2). On the contrary,
Figure 2: a) Structure of optimized guanine. b) Simulated OAM-resolved EEL spectra for different OAM values: black solid line $l = \pm 1$, red dashed line $l = \pm 2$, green dashed line $l = \pm 3$. Enlarged spectra for $l = \pm 2$ and $l = \pm 3$ are shown in figure insets c) and d). For each spectrum, the stick components are reported. All spectra are normalized and have been convoluted with a Gaussian function simulating the limited instrumental resolution ($\Delta E = 0.1$ eV). e) TD-DFT oscillator strength and transition potential projected along the $z$-direction for the first five singlet transitions.

all the octupolar components are almost negligible (panels b and d, Fig.2). We note that the $S_4 \leftarrow S_0$ transition does not show any active component in the OAM range here considered ($[-3 : 3]$). Moving to more extended systems, we considered a tetramer of guanine bases, arranged in a planar configuration (Fig.3, panel a). The geometry of such an arrangement have been obtained optimizing a monolayer of guanine on top of a gold slab with four layers of Au(111), as detailed by Rosa et al.; then, the first 8 singlet transitions were determined by CAM-B3LYP/6-311G(d) TD-DFT calculations. The geometry of each guanine monomer is slightly different from the optimized one used in the previous case, therefore we also performed simulations at the same level of theory on a monomer extracted from the tetramer. The states of the tetramer can indeed be described as excitonic combinations of the first two transitions of each monomer (at 5.06 eV and 5.36 eV respectively) as evident inspecting the
Figure 3: a) Structure of a guanine tetramer, disposed in a planar configuration. b) Simulated OAM-resolved EEL normalized spectra for different OAM values: black solid line \( l = \pm 1 \), red dashed line \( l = \pm 2 \), green dashed line \( l = \pm 3 \). Enlarged spectra for \( l = \pm 2 \) and \( l = \pm 3 \) are shown in figure insets c) and d). For each spectrum, the stick components are reported. e) Simulated OAM-resolved EEL spectra (not normalized) for different OAM values of monomer (dashed lines) and tetramer (solid lines). The same color code of panels b-d have been applied. f) TD-DFT oscillator strength and transition potential projected along the z-direction of the first five singlet transitions. g) 2D representation of the OAM-EEL spectra simulating a realistic experiment. All spectra have been convoluted with a Gaussian function simulating the possible instrumental resolution (\( \Delta E = 0.1 \text{ eV}, \Delta l = 0.5 \hbar \)).

The OAM-EEL spectrum for \( l = \pm 1 \) is indeed due to the convolution of two nearly degenerate states, that are the results of the excitonic combinations of the first two transitions of each monomer. Each of them give rise indeed to two optically bright (\( S_2 \) and \( S_6 \) levels).
and S₃, S₆ and S₇) and two optically dark (S₁ and S₄, S₅ and S₈) excitons, whose oscillator strengths are reported in panel f of Fig. 3, together with the corresponding transition potential projections along z. A very important result comes out: two of the dark transitions (S₁ ← S₀ and S₈ ← S₀) can be indeed detected probing the different azimuthal symmetries of the corresponding potentials, beside the dipolar component, such as those corresponding to \( l = \pm 2 \). We note however that, as in the case of the single guanine, here the signals for the forth and fifth singlets are too low to be recorded, limiting the sorting to the [-3:3] range. A very low but not negligible \( l = \pm 4 \) component is however observed for the S₅ ← S₀ transition (panel g and table S3 in supporting info). Panel e of fig. 3 point out that, as expected, moving from the monomer to the tetramer cause the increasing of the intensity and the approaching of the different peaks, induced by the aggregation. The enhancement is particularly large in the case of higher angular momenta: two and one order of magnitude for \( l = \pm 2 \) and \( l = \pm 3 \), respectively. This is very encouraging, because it shows that this technique can indeed be very effective to study extended supra-molecular systems, such as those of biological interest.

A double dispersed (energy and angular momenta) spectrum have finally been convoluted in panel g of fig. 3 to simulate how a possible experimental result should look like. Here, we assumed a limited instrumental resolution of \( \Delta E = 0.1 - 0.3 \text{ eV} \) and \( \Delta l = 0.5 \hbar \).

As a final application, we focus on the study of chiral systems: as indeed pointed out in the field of nanoplasmonics, electron OAM dichroism is expected for chiral systems in presence of conventional electron beams. This means that difference in intensities between the loss functions \( \Gamma_{+l}(\omega) \) and \( \Gamma_{-l}(\omega) \) can be detected. The dichroism can be quantified introducing a dichroic figure of merit:

\[
D_{||}(\omega) = \frac{\Gamma_{+l}(\omega) - \Gamma_{-l}(\omega)}{\Gamma_{+l}(\omega) + \Gamma_{-l}(\omega)} \cdot 100\% \tag{12}
\]

We note that this term is analogous (in percentage) to the dyssimetry factor of optical circular dichroism (CD) experiments, apart from a factor 2. As a first chiral paradigmatic
case, we considered an amino acid: alanine, shown in its two enantiomeric forms, L and D, in fig.4 panel a, whose double dispersed (energy and angular momenta) $\Gamma_l(\omega)$ spectrum is reported in panel b of the same figure. From these data, the $D_{|l|}(\omega)$ can be obtained, and the convoluted spectra for $|l| = 1$ (fig.4 panel c) point out how this technique is the electron beam analogue of an optical CD analysis: the two enantiomers give rise to two mirror spectra (this is of course true for all the $|l|$ values, data not shown). More important, one can probe the dichroism not only for the dipolar component, as in the optical CD, but can have access to all the different symmetries of the transitions potentials involved (fig.4 panel d): absolute configurations and conformational analysis of molecular and supra-molecular systems can be investigated taking into account also the contributions from dark transitions.

We therefore tested our protocol toward larger systems of biological interest, such as the G-quadruplex structures that originate in DNA and RNA guanine-rich sequences: the
Figure 5: a) Top and side view of the guanine core in G-quadruplex 2MB2. b) Top and side view of the guanine core in G-quadruplex 143D. c) 2D representation of the OAM-EEL spectra simulating a realistic experiment. Top: guanine core in G-quadruplex 2MB2. Bottom: guanine core in G-quadruplex 143D. d) Simulated spectra (normalized) of OAM percentage of dichroism of guanine cores in 2MB2 G-quadruplex (dashed lines) and in the 143D one (solid lines), for different OAM values: black line $|l| = 1$, red line $|l| = 2$, green line $|l| = 3$, e) Simulated spectra (normalized) of OAM percentage of dichroism for $|l| = 1$ of both G-quadruplexes. In black the 2MB2 guanine core, in red the 143D guanine core. Sticks at the different transitions are also reported. All spectra have been convoluted with Gaussian functions ($\Delta E = 0.1$ eV, $\Delta l = 0.5\hbar$).

latter can indeed fold into tetra-helical structures stabilized by hydrogen bonds between guanine tetrads and electrostatic interactions with monovalent cations. Such arrangements, as a function of the specific sequence and folding conditions, can adopt various topologies
classified in parallel and anti-parallel depending on the relative direction of the four guanine strands. One can use optical CD spectroscopy to disentangle the two topologies, thanks to the particular fingerprints of the spectra: indeed, parallel G-quadruplexes are characterized by a positive couplet (two bands of opposite signs and similar amplitude. The sign of a couplet is defined by the longer-wavelength component) at 260 nm, whereas anti-parallel G-quadruplexes have a positive band at 290 nm and a negative band at 260 nm. Here, we selected one parallel (PDB code: 2MB2) and one anti-parallel (PDB code: 143D) G-quadruplex, which core is formed by three guanine planes, as shown in panel a and b of fig. 5. As shown in figure, here only the core of guanine chromophores have been taken into account to perform the simulations: the final geometries have been extracted from the NMR structures of PDB files, once refined by projecting the MP2/cc-pVDZ optimized geometry of the guanine base to the NMR structure, as detailed in ref. 47. The two G-quadruplexes are not the enantiomers of the same compound, therefore the double dispersed OAM-EEL spectra, reported in panel c of fig. 3, are not expected one to be the mirror image of the other (w.r.t the \( l = 0 \) axis), contrary to what observed in the L- and D-alanine cases. We simulated the spectra of \( D_{|l|}(\omega) \), convoluted with a Gaussian function (width=0.1 eV, \( |l| \in [0 : 3] \)), in fig. 5 panel d. Even if for \( |l| = 1 \) no couplets are present, this is not the case of the higher OAM components: for \( |l| = 2 \) the parallel structure does not show appreciable dichroism (at least supposing a bandwidth of 0.1 eV), whereas the anti-parallel configuration (143D) give rise to a positive couplet around 5.02 eV. This last is mainly due to the second excitation in the positive part of the couplet, and to the \( S_6, S_8, S_{10} \) and \( S_{15} \) in the negative one (see panel e of fig. 5). We note that the bands for \( |l| = 3 \) show an opposite trend for the two investigated structures: even if they cannot really be defined as a couplet because the two opposite peaks does not have similar amplitude, however in the case of the parallel structure the positive band (at 5.08 eV) is very large and the negative one (at 5.29 eV) is very small; vice versa, the 143D system have an almost negligible positive peak at 4.93 eV and a large negative band at 5.23 eV (green lines, panel d of fig. 5).
Conclusions

In this contribution we conceive a new type of experiment, based on the extension to molecular and supra-molecular systems of the EELS technique: it made use of an electron beam configuration that avoid the direct interaction with the specimen, and the sorting of scattered electrons as a function of the different electron orbital angular momenta. We have therefore demonstrated theoretically that, combining the evaluation of the energy and the OAM spectra of inelastically scattered electrons by (supra-)molecular systems, it is possible to obtain additional information about the symmetries and also the chirality of electronic excitations induced in these systems, by performing only a single measurement, i.e. without the need of modifying the features of the incoming electron wave. Indeed, thanks to the interaction with the molecular excitations, the scattered electrons acquire different components of helical phase fronts and spiralling currents, carrying a well-defined OAM per particle along the TEM axis. The numerical simulations performed clearly pointed out that this new technique, exploring the azimuthal symmetries of transitions, provide a unique molecular fingerprint that can be used to disentangle near degenerate states, to detect optically dark transitions, or to assign different topologies in extended systems.

As further extensions, we are now actively working to treat even more complex structures, such as pigment-protein complexes in Light-Harvesting (LH) systems. A promising way is the integration of the theoretical framework here described with the excitonic description of the supramolecular system and the inclusion of the effect of environment. Another active field is the study of the effects of plasmonic nanoantennae to enhance the intensity of signal for single molecule applications or to fine-tuning of the coherences in natural photosynthetic systems. The work here proposed can pave the way to many experimental applications in the field of biophysics and biochemistry: for instance, one could probe the role of optically dark charge transfer excitations in LH systems and their contribution in energy and electron transfer processes.
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Supporting Information Available

The following files are available free of charge.

The numerical integration procedure of the rate expression and the computational details of the simulated experiments are reported.

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