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Molecular Dynamics

Force–Spectrum Relations for Molecular Optical Force Probes**

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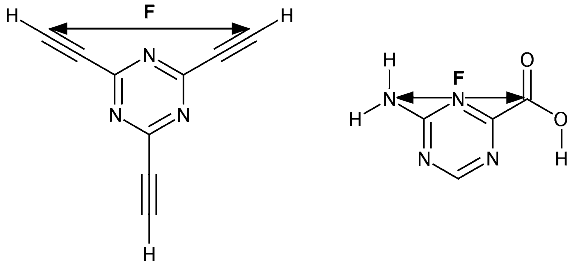
Abstract: *Force probes allow real-time monitoring of forces acting in different regions of large molecules and are potentially suited for the investigation of structural changes occurring in macromolecules during, e. g., folding processes.*[1–10] *Such information is crucial for the understanding of mechanochemical reactivity.*[2,3,6,7] *To this end, small molecular force probes can be incorporated into large molecules.*[2,3,11–13] *Some of the available systems are based on mechanochromism, the change of the UV/Vis absorption spectrum of a molecule under mechanical stress.*[1,14] *Herein we propose the idea of using molecular force probes in which the point-group symmetry is reduced as a result of mechanical deformation. This effect leads to significant and characteristic changes in the UV/Vis, IR, and Raman spectra of the deformed molecules, which were determined using computational methods. Beneficially, these changes are reversible and occur even if the applied forces are small.*



1 **2**

Figure 1. Structures and EFEI coordinates of 2,4,6-triethinyl-1,3,5-triazine (**1**) and 4-amino-1,3,5-triazine-2-carboxylic acid (**2**). In **1**, the force was applied to the outer carbon atoms of the ethinyl groups. In **2**, the nitrogen atom of the amino group and the carbon atom of the carboxyl group were subjected to the force.

As first examples, the mechanochemical behaviors of 2,4,6-triethinyl-1,3,5-triazine (**1**) and 4-amino-1,3,5-triazine-2-carboxylic acid (**2**) were investigated theoretically by applying forces to the molecules by the external force explicitly included (EFEI)[15] method along the coordinates shown in Figure 1. The ethinyl groups in **1** and the amino group and the carboxylic acid in **2** are linkers between the 1,3,5-triazine scaffold and larger molecules, and were chosen to preserve the scaffold's D_{3h} symmetry (**1**) or to break it (**2**) to compare the results. When incorporated into large molecules by two ethinyl linkers, the symmetry of **1** can be preserved by connecting the third, vacant ethinyl group to a small, inert organic group. The experimental investigation of mechanochromism in single molecules is not possible, since the combination of AFM with spectroscopic absorption experiments is not feasible. Hence, the sensitive influence of mechanical force and the molecular point group on the spectroscopic properties of **1** and **2** can be explored only by theoretical calculations. A complete report on the computational methods used in the present work can be found in the Supporting Information (S1).

Mechanical deformation—stretching or compressing—causes a change of excitation energies of the four lowest excited singlet states of **1** (Figure 2a): In the equilibrium ($F = 0$ nN), the second and the third excited singlet states (S_2 and S_3) are degenerate (4.14 eV), since all orbitals which play a role for the excitations into the S_2 and S_3 states in the equilibrium geometry belong to two-dimensional irreducible representations of D_{3h}, the molecular point group of **1** in the equilibrium geometry. As soon as the molecule is stretched or compressed, however, the original D_{3h} symmetry is broken and the molecule assumes a C_{2v} symmetry. As a consequence, the degeneracy of S_2 and S_3 is lifted, since there is no two-dimensional irreducible representation in C_{2v}. As the deformation increases, the energies of almost all the states decrease in energy. An exception is the S_4 state, which increases in energy if compressive forces are applied.

In contrast to **1**, the excited singlet states of **2** are not degenerate in the equilibrium geometry (Figure 2b), since the C_s symmetry of the molecule, which includes no two-dimensional irreducible representations, is never broken during the entire deformation. The electronic excitation spectrum of **2** is influenced only slightly by mechanical stress: The S_2 state increases minimally in energy when the molecule is compressed, while the energies of the S_3 and S_4 states increase slightly as a result of tensile stress. A complete list of the excitation energies of **1** and **2** under constant external forces is given in the Supporting Information (S2).

While considerations about the symmetry of a molecule allow the prediction of the change of energetic degeneracies in the course of the deformation, no reliable assumptions can be made in advance whether the electronic excitation energies increase or decrease as a result of the mechanical deformation in either of the two cases. The particle in a box model is—despite its oversimplification documented in literature[14]—often applied for the description of the spectroscopic changes of a molecule under tensile stress. In this

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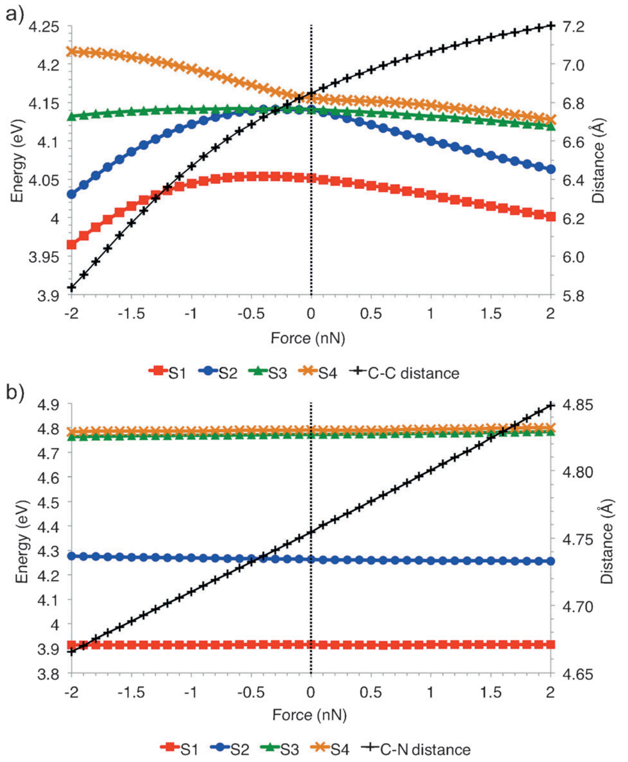

Figure 2. Electronic excitation energies into the four lowest excited singlet states (S_1–S_4) of **1** (a) and **2** (b) as well as the distances between the atoms which were subjected to the force, each as a function of the appropriate force. Compressive forces are denoted with a negative sign. a) In the equilibrium geometry of **1** ($F = 0$ nN), the S_2 and S_3 states are degenerate. This degeneracy is lifted as soon as the original D_{3h} symmetry is broken and a C_{2v} symmetry is assumed as a result of mechanical deformation. b) The excited states of **2** are not degenerate in the equilibrium and the C_s symmetry of the molecule is never broken during the entire EFEI coordinate.

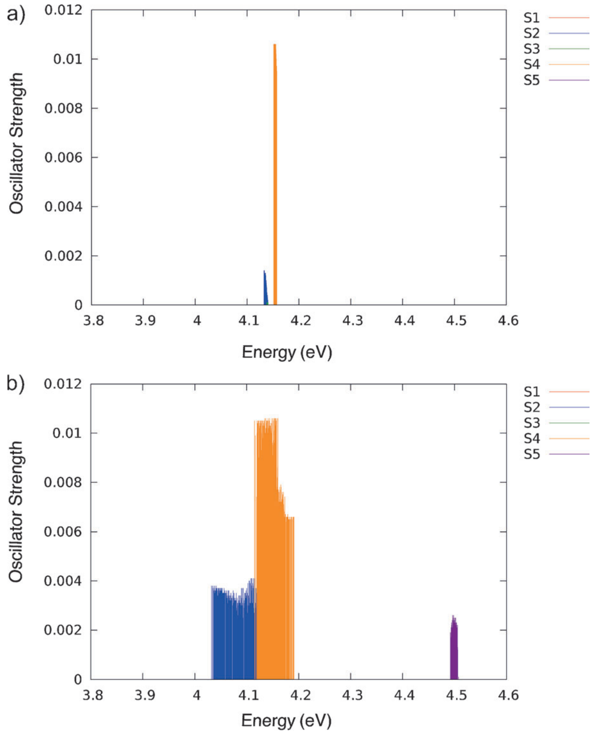

Figure 3. Absorption spectra including the five lowest excited singlet states of **1** for constant external forces of a) 0.1 nN and b) 1 nN. The calculations of the electronic excitation energies were performed on the basis of a BOMD simulation, so that thermal peak broadening is included.

model, the compression of a molecule, which is equivalent to a decrease in the size of the box, is predicted to bring about an increase in the energies of the electronic eigenstates. Consequently, the energy differences between the states are predicted to increase, resulting in a blue shift of the absorption spectrum. The opposite, however, can be observed in the S_1, S_2, and S_3 states of **1** as well as in the S_3 and S_4 states of **2**. Analogously, stretching a molecule is predicted to bring about a red shift of the absorption spectrum. On the contrary, the S_3 and S_4 states of **2** slightly increase in energy upon stretching. Hence, the particle in a box model is too simplistic to describe the changes in the electronic excitation spectra occurring in the course of the mechanical deformation of **1** and **2**.

To investigate the time-dependence of the influence of an external force on the geometry and the excitation energies of **1**, Born–Oppenheimer molecular dynamics (BOMD) calculations and subsequent calculations of the excitation energies into the five lowest singlet states for every fifth time step were carried out (Figure 3). The structural oscillations featured in BOMD trajectories are caused by the external forces in combination with the thermal kinetic energy and give rise to peak broadening of the absorption spectra. If a constant stretching force of 0.1 nN is applied to the ethinyl groups, the distance between the carbon atoms oscillates around an average value of 6.88 Å (6.85 Å in the equilibrium) with an amplitude of 0.05 Å. Therefore, the peak broadening is relatively weak and the average energies of the S_2 and S_4 states are 4.13 eV and 4.15 eV, respectively. If a force of 1 nN is applied, the distance between the ethinyl carbon atoms oscillates around an average value of 7.05 Å with an amplitude of approximately 0.35 Å, which causes pronounced peak broadenings with the average energies of the S_2, S_4, and S_5 states amounting to 4.09 eV, 4.14 eV, and 4.50 eV, respectively. The absorption spectra for a large number of force values are found in the Supporting Information (S3). The significant peak broadening arising from external forces and the increase in the oscillator strengths of the S_2 and S_5 states with increasing forces are beneficial properties of **1** and can be exploited in the interpretation of experimental spectra obtained with the use of the molecule as a force probe.

In addition to the electronic excitation spectra, IR and Raman spectra also change as a result of tensile stress, and the decrease of the symmetry of a molecule is again found to be a useful feature. Moreover, IR and Raman spectra are more sensitive to mechanical deformation than electronic excitation spectra (Figure 4): Imposing only minor tensile stress on **1** by the EFEI approach brings about a significant shift of IR and Raman intensities. In the IR spectra, a number of different phenomena occur as a result of applying a stretching force. For example, the signal at 2232 cm^{-1}, which is

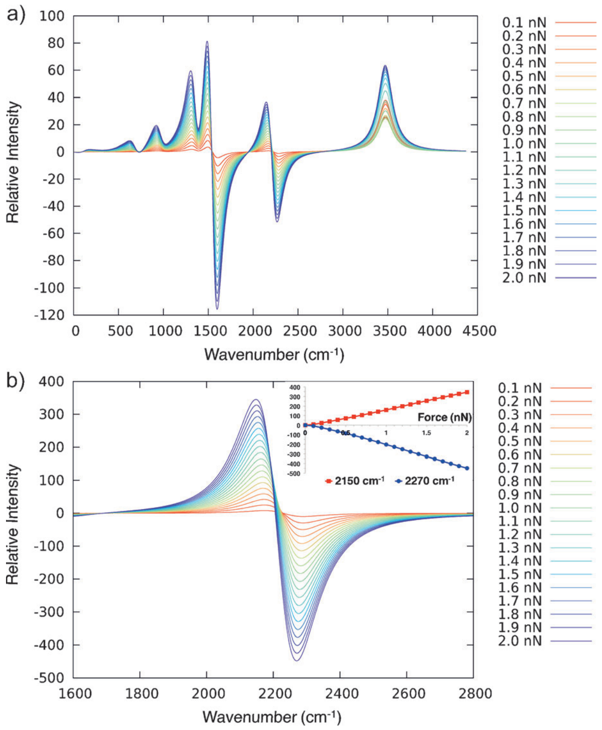

Figure 4. IR (a) and Raman (b) difference spectra of **1** (with Lorentzian fitting) with stretching forces applied to the molecule. At each point of the stretching coordinate, the spectrum at the equilibrium geometry ($F = 0$ nN) was subtracted (see Supporting Information S4). In both IR and Raman spectra, the signal at 2232 cm^{-1}, which is degenerate in the equilibrium, splits and moves to smaller wavenumbers as a result of tensile stress. In the inset of (b), the relative Raman intensities at 2150 cm^{-1} and 2270 cm^{-1} are plotted against the force.

degenerate in the equilibrium state and is characterized by asymmetric stretching motions of the ethinyl moieties, splits and moves in the direction of smaller wavenumbers as soon as minor tensile stress is applied. Hence, in the region above 2232 cm^{-1}, the contribution of this signal to the overall Lorentzian spectrum is negative, while a positive contribution is found below 2232 cm^{-1}. The mechanically induced changes in the Raman spectra are even more pronounced than in the case of the IR spectra. The only significant contribution is delivered by the signal which has already been mentioned in the discussion of the IR spectra and which is strongly Raman active. While the splitting of this signal causes the same qualitative results as in the IR spectra, the change in intensities is much more significant. This useful property makes **1** a particularly promising force probe for experimental studies, since incorporating the molecule in polymers or proteins in, for example, AFM experiments allows a laser tuned to 2150 cm^{-1} to be used to monitor the distinct linear increase in intensity in real-time as soon as minor forces impose tensile stress on **1** (Figure 4b, inset).

In summary, we have introduced a unique approach for the combination of mechanochemistry and optical spectroscopy by proposing a novel kind of molecular force probe that makes use of the change of spectroscopic properties (the electronic excitation energies as well as the IR and Raman spectra) as a result of mechanical deformation which is accompanied by the decrease of the molecular symmetry. The theoretical methodology described herein paves the way for the rational design of new kinds of molecular force probes, which can potentially be used in AFM experiments and in single-molecule spectroscopy. Such probes can be applied to gather information about the forces acting on specific sites of biomolecules, for example, during folding processes in living cells, and is only possible because of the high sensitivity of the force probes: The relevant forces occurring in biological systems are in the magnitude of several hundred pN[1] and the test molecule **1** delivers pronounced spectroscopic responses in this regime. A future aim is to develop a molecular force probe which exhibits characteristic spectroscopic changes that depend on the direction of the external force. Moreover, we will explore new ways of linking the scaffold of the force probe to the macromolecule to minimize the perturbation of the macromolecular structure.

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