Absorption Spectra of Estradiol and Tryptophan Constructed by the Statistical and Elongation Methods

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The statistical quantum chemical/molecular dynamical method is developed and employed to reproduce optical spectra. This technique includes quantum-mechanical calculations on energy states and photophysical properties of molecular conformers obtained during molecular dynamical simulation. Polycyclic organic molecule estradiol surrounded by solvent particles and protein structure including tryptophan fragment under thermodynamical conditions are considered. A wide absorption spectrum over several excited electronic states of estradiol is constructed. First longwave absorption band of tryptophan-cage mini protein is built involving the elongation method. These statistical spectra reflect the main features of the corresponding experimental ones.

Introduction

A theoretical investigation of spectral-luminescent properties such as electronically excited-state energies, molar absorption coefficients (absorptivity), and rate constants (probabilities) of decay conversions is required for better understanding and interpretation of the photoinduced processes proceeding in polyatomic organic compounds. The present work is an attempt to describe experimental absorption spectra using statistical curves constructed by a hybrid method that is based on molecular dynamics (MD), quantum-mechanics, and statistical theory. The quantum chemical (QC) approach is applied to study molecular photophysical and photochemical properties.[1] Excited electronic states are treated for individual molecules fixed as equilibrium structures in gas phase. It gives narrow energy peaks while spectral wings are not included into the consideration. To obtain more realistic pictures and to build optical spectra, computationally inexpensive ways should be employed at the level including QC mass calculations on states of oscillating compounds or complexes where molecular flexibility under thermodynamical (TD) impact is stimulated by MD simulation.[2] Then vibronic (vibrational−electronic) processes responsible for spectral profiles are reproduced due to changeable spatial and therefore electronic structures of chromophore compounds seeded in a MD cell with surrounding molecules.

The following modern QC/MD methods to calculate excited electronic states could be mentioned. The nonadiabatic and adiabatic quantum MD simulations of an excess electron in water under various TD conditions have been performed to obtain absorption spectra when the hydrated electron is a ubiquitous transient species in irradiated aqueous systems.[3] The solvatochromic effect has been studied by using the supermolecule approach with the averaged solvent electrostatic potential (ASEP)/MD treatment; the geometry and charge distribution of the solute and solvent molecules have been fixed along the MD modeling, however, the total structure or the chromophore surrounded by hydrogen-bond shells has been optimized after several steps for calculating excited states.[3f−h] Also other approaches for obtaining spectral widths in hybrid QC/MD schemes were reported in recent years. [3i,j]

In this work the statistical technique involves MD simulation including fully flexible molecular structures where QC calculation of excited nonequilibrium states is focused to construct spectral shapes. Such theoretical spectrum can be built as an envelope over statistically averaged intensities of electronic transitions. This method might be applied to study complicated photoprocesses proceeding under intermolecular interactions. For instance, there are several samples such as the transfer or exchange energy between unbound compounds (anthracene−naphthalene complex),[4] the spectral-luminescent properties of molecules strong dependent on environment behavior (tryptophan in different solvents),[5] or influence of high temperature and/or high pressure on absorption and emission spectra.[6]

Excited states are calculated by configuration interaction singles (CIS) method after applied intermediate neglect differential overlap in sp-basis (INDO/sp) with a special spectroscopic parametrization.[7] This semiempirical implementation is incorporated into the GAMESS system as a chemistry of organic photonics (QOP) code.[8,9a] MD simulation is carried out by the Tinker software.[10a] Different quantum mechanical formalisms such as the time-dependent density functional theory (TDDFT), coupled cluster, or other post-Hartree−Fock levels[10] might be in use in the same manner. Modern techniques to facilitate the calculations can be applied as well. Nevertheless, implementation of high level theories can meet limitation by computer system capability.

Analysis on spectral-luminescent protein characteristics is one of the important aspects to study their structures and behaviors. The female natural hormone estradiol and amino acid tryptophan as part of protein chain are applicable in pharmaceutics and as

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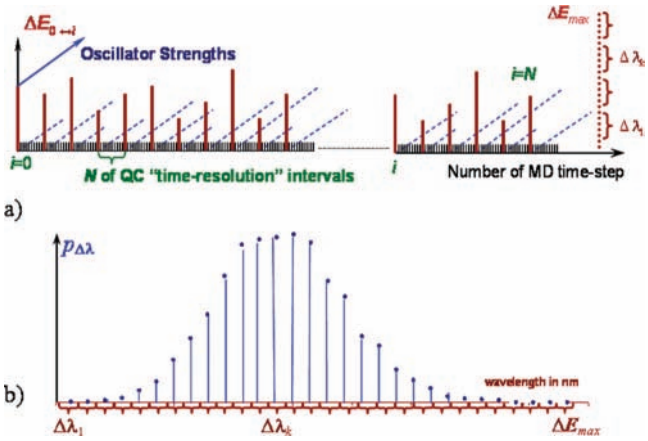

Figure 1. The scheme to construct a statistical spectrum (a) calculation of energies and oscillator strengths along MD run; (b) distribution of energy probability over wavelength intervals.

luminescent detectors in different biological objects. The broad statistical absorption spectra of estradiol in ethanol solvents are considered. First longwave band of the tryptophan cage miniprotein (trpcage-mp) is built. These curves are compared with experimental data.

Methodology and Computational Details

Absorption and emission spectra are formed owing to many electronic transitions in molecular systems of different configurations. This is a very intricate task to estimate intensities and positions of excited states along with their vibration contours. To build a simple theoretical model for obtaining spectral characteristics, several statistical agreements are accepted.

1. The whole of MD run is divided into N periods of "time resolution" and QC calculation is repeated after each period to find an instantaneous excited-state and transition probability in Nth conformer that is a snapshot of the molecule oscillating under impact from solvent particles as well as other external and internal forces (Figure 1a).

2. To obtain reliable results and informative data such as width, profile, and intensity of the spectral bands, the selected N conformers turn into N noninteracting molecules. Time evolution can be replaced by the statistical ensemble according to the Ergodic Hypothesis which says that, "over long periods of time, the time spent in some region of the phase space of microstates with the same energy is proportional to the volume of this region, i.e., that all accessible microstates are equally probable over long period of time and equivalently." It says that time average and average over the statistical ensemble are the same.[11]

3. The complete spectral width is divided into $\Delta\lambda$ wavelength intervals interpreted as "frequency resolution" since frequency is the inverse proportion of light speed to wavelength $\Delta\nu = c/\Delta\lambda$. This value has to exceed the minimal energy gap restricted by Planck's constant to capture one or several allowed vibrational sublevels for applying the quasiclassical method. Choice of an appropriate "frequency resolution" should correspond to the real spectral curves and spectroscopic "device" resolutions as precisely as possible.

4. The averaged absorption band $\Delta\lambda$ efficacy is defined through the relative absorptivity $\epsilon_{\Delta\lambda} = p_{\Delta\lambda} f_{\Delta\lambda}$ when $p_{\Delta\lambda} = N_{\Delta\lambda}/N$ is probability. There are $N_{\Delta\lambda}$ conformers of all N molecules where transitions between states lie within the $\Delta\lambda$ interval (Figure 1b). The oscillator strength $f_{\Delta\lambda}$ is accepted to be an invariable volume on the chosen short segment and currently defined as an average over this interval.

This model can be extended to several different electronic states. It accelerates QC calculating and building a wide statistical spectrum over several electronic peaks. Each spatial structure of oscillating molecule allows a set of M excited energy levels to be obtained simultaneously. Following the Ergodic Hypothesis, M conformers of the same geometry but in different electronic states can be assumed rather than these M states of one molecular snapshot. It means that the total number of noninteracting conformers is redefined as $L = M \times N$, and a spectrum can be drawn over the M electronic states. All previous deliberated ratios remain the same if the number N is replaced by this L. If there are enough oscillating molecules then all available vibration positions are involved into the spectrum under consideration. Every energy state of these structures modeled by MD simulation is reflected on the statistical curve. For such case electronic states do not need to be distinguished. They are included into the one of defined wavelength intervals depending on their transition energy only. Intensity of every state makes a contribution to the integrated spectrum at the position where the energy is detected. The main values are wavelength and oscillator strength of this state. To a first approximation the type of each spectral maximum can be determined as (0−0) transition from the ground state to the closest intensive energy line treated in equilibrium structure and this contribution is expected to be maximal. Vibration contours overlap each others and the total spectrum is formed as a combined profile.

These assumptions mean there are no interactions between chromophores. All conformers are excited into one of vibration levels with probabilities defined by oscillator strengths.

The following TD conditions and technical details of a MD model are prepared to both considered systems. A canonical NVT ensemble (the number of particles, volume, and temperature are kept constant) at 300 K is applied. The Nose−Hoover thermostat with time relaxation of 1 ps and Ewald summation over the electrostatic interactions are employed. Each MD simulation performs during 0.5 ns with time step of 1.0 fs (500000 steps) and nonbonded cutoff radius of 12 Å. Tinker MD software with the standard MM3 force-field parametrization is applied excepting bond stretching and angle bending parameters on the phenyl cycles. The ideal bond length of carbon hexagonal ring is taken as 1.40 Å; ideal angle is 120°. Then, after a whole MD run completed excited states and oscillator strengths of 5000 conformers are calculated by INDO/s and CIS implemented in the GAMESS package to construct statistical spectra.

Estradiol is seeded in an environment of 56 ethanol molecules where solvent density is 0.33 g/cm^3. The molecular structure is optimized by the ChemOffice software[10c] except for the benzene ring, which is taken with all bonds = 1.4 Å and angles = 120°. One middle H of chromophore is fixed at the initial position to keep the molecule near the center of MD cell. The ethanol solution is constructed from explicit particles to provide direct influence on stereochemical structures of the estradiol during MD interactions through intermolecular and electrostatic potentials. For this consideration, all possible interactions with solution and collisions with other chromophores as well as TD influence are not included into further QC calculation. Molecular moving is restricted by boundary conditions of the box cell with lattice lengths of 24 Å. The solute and solvent molecules are completely flexible according to the force-field potentials. The spectral shapes are built using "frequency-resolution" 4.0 nm

to obtain smoother curve similar to the empiric sample. Once the charge distribution over atoms (atomic partial charges) for the starting equilibrium structure is invariable during whole MD simulation, the charge fluctuation is not considered.

The trpcage-mp is specified as 1L2Y according to the Protein Data Bank (PDB).[12] The present stereochemical configuration is borrowed from Tinker MD package.[10a] Since this compound is a complicated system consisting of 304 nuclei where only the several atoms of phenyl rings are responsible mainly for the lowest excited energy states, the CIS-elongation method can be employed to calculate electronic levels.[9a] The elongation method is one of the techniques that allow huge compounds to be calculated in parts.[13] This CIS-elongation method permits CPU memory and time spent for calculating electronic states to be reduced sharply because much less molecular orbitals (MOs) and atomic orbitals (AOs) are involved into CIS.

For instance, as it was shown earlier,[9a] conventional calculation CIS = 60 × 60 of trpcage-mp in equilibrium position (766 MOs and 766 AOs of basis) has required computer time = 5.1 h and memory = 732.8 MB for convergence. The maximally achieved CIS = 99 × 99 has needed 21.6 h and 5.4 GB. By consideration of the same compounds, the elongation method with truncated AO basis has allowed only selected localized chromophore parts (active MOs of phenol and indole) to be calculated. It has led to decreasing the maximal number of wave functions to 87 MOs and 133 AOs. In this case, the largest CIS = 46 × 41 has taken 1.8 h and 282.4 MB, while a reduced dimension of CIS matrix 20 × 20 (87 MOs and 115 AOs of basis) has spent 0.07 h (4.4 min) and 217.2 MB. The last sample has captured almost all MOs taking part in the lowest excited states.

The main idea of the elongation method is to extend a polymer chain by adding a monomer unit stepwise to a starting oligomer while keeping the variational degrees of freedom fixed.[9] After a self-consistent field (SCF) procedure is converged and MOs of a starting cluster are obtained, they are divided into "frozen" and "active" regionally lcalized MO (RLMO). Simultaneously the total AO basis is divided on A (frozen AOs) and B (active AOs) regions.[9b] An attacking monomer is attached to the active region, and the new Fock matrix formed on the base of "active" RLMO and MOs of the attacking monomer is to be diagonalized within SCF. Thus, the elongation method works in the RLMO basis rather than in canonical MOs (CMO). New wave functions $|i\rangle_{\mathrm{RLMO}}^{\mathrm{CMO}}$ are expressed in RLMOs ϕ_m that are linear combinations of AOs χ_μ

$$|i\rangle_{\mathrm{RLMO}}^{\mathrm{CMO}} = \sum_m (C_{\mathrm{RLMO}}^{\mathrm{CMO}})_{im}\varphi_m$$

where RLMO basis is

$$\varphi_m = \sum_\mu (C_{\mathrm{AO}}^{\mathrm{RLMO}})_{m\mu}\chi_\mu$$

There is one way to treat the CIS in the framework of the elongation method.[9a] The direct or conventional CIS (CCIS) is applied after all elongation steps completed. In this approach the totality of RLMOs $C_{\mathrm{AO}}^{\mathrm{RLMO}}(\Sigma)$ can be constructed from $C_{\mathrm{AO}}^{\mathrm{RLMO}}(R_g)$ of each gth region that allows the Fock matrix of the whole system to be defined in the integrated RLMO basis

$$C_{\mathrm{AO}}^{\mathrm{RLMO}}(\Sigma) = C_{\mathrm{AO}}^{\mathrm{RLMO}}(R_1) \cup \ldots \cup C_{\mathrm{AO}}^{\mathrm{RLMO}}(R_g) \cup \ldots \cup C_{\mathrm{AO}}^{\mathrm{RLMO}}(R_{\mathrm{last}})_{\mathrm{FRLMO}}$$

$$F_{\mathrm{RLMO}} = C_{\mathrm{AO}}^{\mathrm{RLMO}+}(\Sigma)_{\mathrm{FAO}} C_{\mathrm{AO}}^{\mathrm{RLMO}}(\Sigma)$$

The Fock matrix are diagonalized to obtained the complete set of $C_{\mathrm{RLMO}}^{\mathrm{CMO}}(\Sigma)$ and then CMOs in the AO basis are found according to

$$\widetilde{C}_{\mathrm{AO}}^{\mathrm{CMO}}(\sum) = C_{\mathrm{AO}}^{\mathrm{RLMO}}(\sum) C_{\mathrm{RLMO}}^{\mathrm{CMO}}(\sum)$$

Though this idea is based on elongation method, the last diagonalization procedure is implemented in a conventional manner that defines this approach as CCIS. The obvious advantage is that only one or several small fragments are concerned because the total RLMOs can be constructed by the RLMOs from these regions for CCIS calculation.

To build a statistical absorption spectrum of the trpcage-mp, the spatial structure is frozen during the complete MD run excepting the movable indole chromophore fragment with a short tail. By application of the elongation method, CIS = 12 × 12 reproduces accurately 20 excited states comparatively with the earlier data. In practice, each procedure of both INDO and CIS calculations takes less than one minute.

Results and Discussion

Estradiol. The typical representative of natural estrogens is the luminescent hormone estradiol with a rich π-system of phenyl cycle providing the spectral profile of longwave absorption.[14] First the lowest singlet S_1 = 288.6 nm of the estradiol calculated in the equilibrium position of gas phase corresponds to the statistical spectral maximum on the interval 270−310 nm (Figure 2, the short remote spectrum on right side). This is the sole state that can be controlled during MD simulation easily because the essentially wide energy gap exists between this and the next S = 243.0 nm electronic levels. Energy positions of upper states can be replaced due to the changeable structure. The task to follow each separately distinguished state is not trivial and the simpler way is suggested. The described above methodology for several different electronic states is applied to construct a spectrum of a long range of many maxima.

To find electron excited states of estradiol in frozen equilibrium position, a CIS matrix is composed from all possible 120 configurations obtained by singly electronic excitation employing 10 occupied and 12 vacant MOs. The most intense (minimal magnitude of oscillator strength is 0.015 for the shortest wavelength 146.3 nm) 14 of 40 calculated excited electronic states in the range of 140−310 nm are presented in (Figure 2). High intensities are determined by the $\pi\pi$-type of these states. The height defined by the oscillator strength of the most intensive line is normalized to the corresponding spectral maximum. All other values of oscillator strengths are rescaled

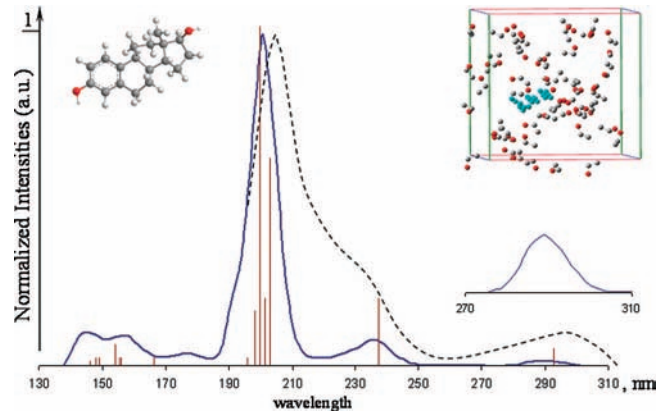

Figure 2. Estradiol: empirical absorption spectrum in alcohol at 20 °C (dashed);[14] statistical profiles obtained in ethanol (solid, a snapshot is presented in the box), are at 300 K and 14 intensive electronic states in equilibrium position (straight vertical lines). The excited states are obtained in the framework of the CIS-INDO approach. Absorption in band 130−190 nm is defined only for the theoretical curve.

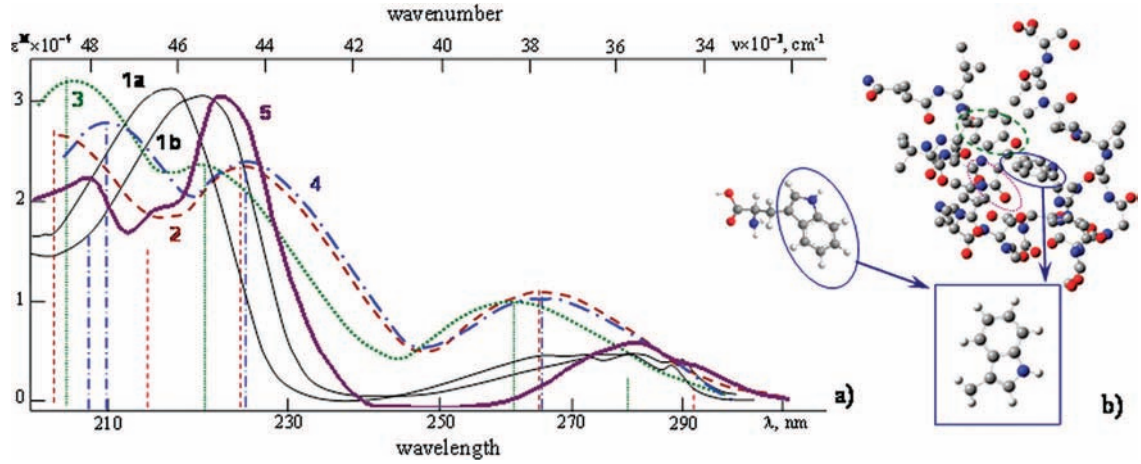

Figure 3. (a) Absorption spectra of indole and tryptophan (two thin solid curves 1a and 1b, consistently) in the phosphate buffer, pH = 6.8 at 20 °C,[15a,b] model-spectra: single molecule (2, dashed),[15b] captured into polyethylene cage (3, doted),[9a] and attached to polyethylene chain (4, dot-dashed),[9a] and statistical spectrum of oscillating indole (5, thick graph) of the unmovable trpcage-mp. The vertical lines mark ε_{max} of equilibrium structures. The excited states are obtained in the framework of the CIS-INDO approach; (b) trpcage-mp, isolated tryptophan, and indole chromophore spatial structures.

TABLE 1: Calculated Intensive Singlets (nm) and Oscillator Strengths (in parentheses) of Several Compounds Containing Indole Chromophore

	fragments of trpcage-mp included in CCIS			isolated molecules	
transition	Phl + Alt + indole	Phl + indole	indole	indole	tryptophan
$^1B_a \leftarrow A$	216.6 (0.13)	218.6 (0.38)	220.1 (0.52)	219.4 (0.49)	219.0 (0.44)
$^1B_b \leftarrow A$	225.5 (0.38)	224.0 (0.68)	225.5 (0.38)	225.3 (0.69)	225.3 (0.69)
$^1L_a \leftarrow A$	269.5 (0.11)	268.1 (0.12)	269.5 (0.11)	263.4 (0.15)	263.4 (0.14)
$^1L_b \leftarrow A$	294.4 (0.03)	295.7 (0.03)	294.4 (0.03)	294.3 (0.02)	294.2 (0.02)

Transitions between ground state A and excited states are labeled according to the accepted classification.[15] These states are calculated in equilibrium positions.

to the strongest one. Single intensive lines provide profile on the intervals (230−250 or 270−310 nm). They are responsible for absorption into these bands. Each peak is higher than the corresponding spectral maximum on the same wavelength. In other words, vibration sublevels of one electronic state define spectral structure of the respective wavelength. Otherwise, groups of electronic levels (140−170 and 190−210 nm) with significant values of oscillator strengths determine the corresponding parts of spectrum collectively. The positions of empirical and statistical peaks coincide pretty well taking into account the rough approximation. Intensities are slightly different excepting the interval 210−250 nm where the experimental curve evidently exceeds the theoretical profile. This disagreement requires further investigation including interactions with environment, molecular collisions, charge redistributions, among other factors. Anyway, it needs to be noted that these empirical and statistical spectral shapes are comparable and they have similar features.

Tryptophan. Tryptophan consists of the indole chromophore C_8NH_6 and alanine subsistent $C_3O_2NH_6$. The optical properties of tryptophan are controlled by the chromophore center, and they strongly depend on the chemical and spatial structure of the environment.[15] The absorption spectrum of trpcage-mp is under consideration as one of the realistic samples. This protein chain is an appropriate example for applying the hybrid technique of CIS and elongation methods along with the statistical MD simulation (Figure 3). This compound contains tryptophan and phenol chromophore fragments that are responsible for longwave absorption and emission spectra. The trpcage-mp is grown up by the elongation method from an initial compound of 278 atoms by successive extension with phenol (Phl), alanine-like tails (Alt), and indole attacking monomers (Figure 3b). Several intensive lowest singlets of the protein as well as the isolated indole and tryptophan are calculated in their equilibrium positions (Table 1), and their values are compared.[12] From Table 1, you can see that energies of the intensive $\pi\pi$ states, indeed, are fully controlled by MOs of π-symmetry localized on the indole ring. This result coincides with the experimental and theoretical data. It proves the assumption of applying CIS to the selected chromophore rather than to the total system.

Before further consideration, it needs to be noted that, in a general way, spectral profiles of the indole, and hence, tryptophan chromophores isolated, solvated, or built in to the protein chain could be deferent; however, under normal TD conditions and in quite neutral environment there is weak influence of surroundings on indole geometrical construction that should lead to similar absorption curves shapes. Currently peculiar strong conditions are not applied to the molecular systems. Spectral contours (Figure 3a) of the compounds under discussion are obtained by applying different procedures. First of all there are experimental curves for indole (curve 1a of 3) and tryptophan (curve 1b of 3) in phosphate buffer at 20 °C.[15a,b] According to many sources, spectral maxima of both molecules do not differ significantly from ones in the gas phase or neutral solvents, but these absorption curves are presented in a broad range between 190 and 310 nm. The simplest model spectra constructed as Gaussian cupolas with averaged half-widths of 5000 cm^{-1} over spectral peak reflect the electronic state of molecules frozen in equilibrium positions as described earlier.[14d] Calculated electronic energy levels and oscillator strengths allow intensities to be estimated according to the empirical ratio for the molar absorption constant $\varepsilon_{max} = f \times 4.63 \times 10^4$ $M^{-1}cm^{-1}$. The spectral profile of isolated tryptophan is built in such manner

(curve 2 of 3).[15b] Complicated systems of this chromophore attached to the polyethylene chain (curve 3 of 3) or captured into polyethylene cage (curve 4 of 3) are calculated by the same method and the CIS-elongation technique. Their absorption reliefs are similar because energies and oscillator strengths of excited states almost coincide.[9a]

Since the elongation method allows the whole system to be calculated once, while a selected fragment is recalculated many times depending on conditions, this technique is employed along with MD simulation to outline the statistical spectrum of trpcage-mp using only localized active MOs of the indole fragment (curve 5 of 3) and leaving the rest of compound to be frozen. By comparison of values of excited energies in the equilibrium positions (Table 1) the Phl and Alt parts can be frozen also. After the elongation procedure, the sole active movable indole segment attached to the cage oscillates near the fixed point during MD simulation. The excited states of the restricted fragment are calculated by CCIS for each conformer. All other atoms frozen in the initial positions provide additional influences on the oscillating structure of this fragment through the classical MD force-field parameters.

The obtained curve is rescaled to the maximal intensity. Each spectral peak can be assigned to the corresponding state of equilibrium structures by comparing their wavelengths. The empirical and statistical spectra are in excellent agreement even in the framework of accepted rough approximations. The indole fragment is responsible for spectral maximum and vibrational profiles over these peaks in the broadband, around 220−310 nm (Figure 3a), which is in accordance to experimental and theoretical investigations. The main result of this study on spectral properties of trpcage-mp is the principle possibility to calculate electronic spectra of huge compounds in parts if several of these fragments are chromophores.

Conclusion

The statistical QC/MD method is successfully applied to construct wide absorption bands of two organic cyclic molecules. The spectrum of estradiol dissolved in many-particle systems is built involving 40 excited electronic states to obtain a curve between 140−310 nm. The indole fragment of tryptophan-cage mini protein provides the spectrum of width 200−310 nm constructed due to this method along with the CIS-elongation technique. Both of the statistical spectral profiles coincide pretty well with the empirical samples taking into account the rough approaches that can be improved in future to achieve a more accurate model. Force-field bonds stretching, bending, and torsion parameters among others should be arranged for photophysical purposes. Solvent influence has to take a bigger part in building the spectral curve. The chromophore with molecules of the closest solvent shell could be calculated as a supermolecule in the QC framework. Charge distribution over the changeable structure should be updated during MD simulation especially when protonic and/or polar solvent is employed. Other coupling and features as well as theoretical technologies can be included into consideration.

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