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Tracking conformational dynamics of polypeptides by non-linear electronic spectroscopy of aromatic residues: a first principles simulation study

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Abstract

The ability of non-linear electronic spectroscopy to track folding/unfolding processes of proteins in solution by monitoring aromatic interactions is investigated by first-principle simulations of two-dimensional (2D) electronic spectra of a model peptide. A dominant reaction pathway approach is employed to determine the unfolding pathway of a tetrapeptide, connecting the initial folded configuration with stacked aromatic side chains and the final unfolded state with distant non-interacting aromatic residues. π -stacking and excitonic coupling effects are included via ab-initio simulations based on multiconfigurational methods within a hybrid QM/MM scheme. We show that linear absorption spectroscopy in the ultraviolet (UV) is unable to resolve the unstacking dynamics characterized by the three-step process: T-shaped $\rightarrow$ twisted offset stacking $\rightarrow$ unstacking. Conversely, pump-probe spectroscopy can be used to resolve aromatic interactions by probing in the visible (Vis) the excited state absorptions (ESA) that involve charge transfer (CT) states. 2DUV spectroscopy offers the highest sensitivity to the unfolding process, providing the disentanglement of ESA signals belonging to different aromatic chromophores and high correlation between the conformational dynamics and the quartic splitting.

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Supporting Information.

Details of the Dominant Reaction Pathways approach; a comparison between LA, PP and 2DUV spectroscopy techniques; a comment regarding the calculation of the quartic splittings; a table with the energies of the lowest eight charge transfer states in the dimer; LA and time-resolved PP (both pump-UV probe-UV and pump-UV probe-Vis), as well as 2DUV spectra (both pump-UV probe-UV and pump-UV probe-Vis) for all snapshots; Cartesian coordinates of the CYFC QM/MM refined tetrapeptide geometries; Figures 1, 2 and 8 in color. Supporting information for this article is available on the WWW under <http://www.chemphyschem.org> or from the author.

Keywords

CASSCF/CASPT2; SOS//QM/MM; Dominant Reaction Pathway; quartic coupling; charge transfer signals

Introduction

Proteins are complex systems whose functions, such as ligand binding, enzymatic reactivity, or allosteric signaling, are regulated by their thermal fluctuations. The characterization of the dynamic properties is, therefore, fundamental for understanding protein function and for numerous applications such as rational drug design and protein engineering. Two-dimensional (2D) optical spectroscopy using ultrashort laser pulses^{1–3} can be used to investigate dynamics in complex biological systems with high temporal and spectral resolution. Extending the technique to electronic transitions in the ultraviolet (UV) spectral regime (2DUV) has been achieved only very recently.^{4–8} In particular, the $\pi\pi^*$ transitions of protein residues with aromatic side chains (Phenylalanine (Phe), Tyrosine (Tyr) and Tryptophan (Trp)) generate signals in the near-UV (NUV) range (250–300 nm)⁹, providing native “local” probes for tracking protein dynamics in solution with femtosecond time resolution. 2DUV electronic spectroscopy can target the $\pi\pi^*$ transitions of aromatic residues in oligopeptides and proteins without isotopic labeling. We have recently shown how 2D electronic spectra in the NUV distinguish between two different configurations of a model peptide, i.e. Cys-Tyr-Phe-Cys (CYFC, where Cys=Cysteine, Figure 1), with distant and vicinal aromatic side chains¹⁰. While our earlier simulations of 2D spectra in solution have clearly indicated that 2DUV spectroscopy is able to distinguish between a “folded” (i.e. cyclic peptide with disulfide bridge, Figure 1I) and an “unfolded” (open peptide with broken disulfide bridge, Figure 1III) structure of the small oligopeptide, the capacity of this technique to resolve aromatic interactions during the dynamics of a folding/unfolding process remains unexplored. In this study we investigate the sensitivity of 2DUV spectroscopy to aromatic interactions in proteins by following the unfolding/unstacking of the cyclic CYFC tetrapeptide using simulations based on the Dominant Reaction Pathway (DRP) approach.^{11–13} DRP allows for an efficient exploration of the reactive path space and offers a variational basis for the determination of the most probable pathway in the unbiased dynamics. Simulations of linear absorption (LA) and time-resolved non-linear spectroscopy are used to correlate the relative positions of the aromatic side chains of Tyr (Y) and Phe (F) amino acids during the unfolding process with the corresponding electronic spectra. The electronic spectra were obtained using our SOS//QM/MM method,¹⁴ which combines a hybrid quantum mechanics/molecular mechanics (QM/MM) scheme based on wave function method that explicitly accounts for environmental effects and inter-chromophores coupling with the nonlinear response theory. This approach allows to characterize the solvent effect and to directly associate the time-dependent fluctuations of the electronic spectra with the atomistic details of the conformational changes occurring during the unfolding process. The capability of ultrafast electronic spectroscopy to follow folding/unfolding processes of proteins in solution is demonstrated.

Methodology

The time-dependent spectroscopic signals were obtained using our recently developed SOS//QM/MM¹⁴ protocol which consists of the following steps: (i) configurational space sampling using molecular dynamics simulations, (ii) snapshot selection, (iii) refinement of the selected geometries at mixed QM/MM level, (iv) calculation of excitation energies and transition dipole moments, and (v) generation of linear and non-linear electronic spectra. The application of the individual steps to CYFC is discussed below.

Molecular dynamics simulations and snapshot selection

Trajectories for the folded to unfolded transition were generated according to the Dominant Reaction Pathways (DRP) approach.^{11–13} The reaction pathway connecting given initial and final molecular configurations X_i and X_f is chosen by maximizing the Onsager-Machlup functional S_{OM} , which can be shown to be proportional to the probability of the pathway:

$$S_{OM}[X, t] = \int_0^t dT \sum_{i=1}^{N_m} \frac{\beta}{4\gamma_i m_i} [m_i \ddot{x}_i + m_i \gamma_i \dot{x}_i + \nabla_i U(X)]^2 \quad (1)$$

where $\beta = (k_B T)^{-1}$, $X = (x_1 \dots x_N)$ is the configuration vector of the N -atom molecule, $U(X)$ is the molecular potential energy, m_i and γ_i are the mass and friction constant of the i -th atom. This functional strictly applies to systems treated in the implicit solvent approximation. In the present work a generalization of the approach described in ref. 13 to explicit solvent was used. Details are available in the Supporting information.

The initial X_i and the final X_f geometries were obtained as the global minima of 50ns classical molecular dynamics simulations of the folded cyclic (i.e. with a -S-S- bond) CYFC with a T-shaped arrangement of the aromatic side chains (Figure 1I) and the unfolded (i.e. with saturated S-atoms) CYFC with distant non-interacting aromatic residues (Figure 1III), as described in ref. 10. We assume that the unfolding is initiated by the photoinduced cleavage of the -S-S- bond¹⁵. Multiscale simulations by Nieber et al.¹⁶ indicate that ultrafast intramolecular recombination in the sub-picosecond range¹⁷ may be the dominant path in solution, which would prevent the unfolding. On the other hand, successfully dissociated S-S bonds can participate in intermolecular H-transfer with the solvent¹⁶ which would facilitate the unfolding¹⁵. In this work we assume that both sulfur radicals are immediately saturated by hydrogens from the waters in their close vicinity and the saturation is fast compared to the unfolding/unstacking dynamics. To achieve this we broke the disulfide bond in the folded CYFC geometry and saturated the sulfur atoms with hydrogens. Subsequently, a loose geometry relaxation at molecular dynamics level was conducted in order to reduce the repulsion between the sulfhydrylic groups. This geometry was then used as initial molecular configuration X_i . It must be pointed out, though, that intramolecular recombination of the sulfur radicals will aggravate the spectroscopic detection of the unstacking/unfolding. Fingerprint signals of the T-shaped CYFC coming from recombined (i.e. still folded) species will obscure the unfolding.

In order to find the optimal pathway an ensemble of trajectories compatible with the boundary conditions is generated by means of ratchet-and-pawl biased molecular dynamics

(rMD)¹⁸. Using the rMD algorithm we produced an ensemble of 48 trial unfolding trajectories. The least-action condition was then used to select a single unfolding pathway. 31 snapshots were extracted out of the unfolding pathway covering the dynamics up to an inter-chromophore separation of 8.5 Å. We note that due to the nature of the DRP the time interval between consecutive snapshots is not known.

QM/MM refinement

A two-step QM/MM refinement was performed with our software package Cobramm¹⁹: an initial Hartree-Fock energy minimization run for 30 steps was followed by a complete active space self-consistent field (CASSCF)²⁰ minimization. An active space of eight electrons and eight orbitals (i.e. CAS(8,8)) was selected for the CASSCF optimization. The software MOLPRO 2010²¹ was employed through an interface with Cobramm. The 6–31G* basis set²² was used for the optimizations. Electrostatic QM/MM interactions were treated via an electrostatic embedding scheme. The link-atom technique and redistribution of residual charges among nearest neighbors were used²³ with both aromatic side chains included in the QM layer and the remaining atoms treated classically. The H-atom link was located along the C_α-C_β bond axis of the aromatic side chains. To preserve the intrinsic conformation of each snapshot the peptide backbone and the solvent were kept frozen during optimization. Only the water molecules involved in hydrogen bonds with the hydroxyl group of the phenol were allowed to move. The suitability of the QM/MM refinement for capturing the instantaneous conformation coded in each snapshot is discussed in the results section.

Excited state calculations

Excited state calculations of the refined snapshots were performed with Molcas 7.7²⁴ at the state averaged (SA)-CASSCF level including all valence π -electrons and π -orbitals of both chromophores in the active space (i.e. CAS(14,13)). The generally contracted ANO-L basis set was utilized²⁵ and the following contraction scheme was adopted: C,O/[3s2p1d] and H/[2s1p]. Subsequent energy refinement was done perturbationally with the multi-configurational counterpart of the Møller–Plesset method, denoted as CASPT2²⁶, in its single state (SS) version. An imaginary shift of 0.2 was used²⁷. An IPEA shift²⁸ of 0.0 was found to give better agreement with experimental data than the default value of 0.25¹⁰. A set of 13 extravalence orbitals with higher angular momentum (six π^* -orbitals in benzene, seven π^* -orbitals in phenol) were localized among the virtual orbitals via a procedure described elsewhere¹⁰ and discarded in the perturbation treatment. This approach was found to reproduce experimental data for valence transitions and was also validated against state-of-the-art calculations for benzene and phenol¹⁰. 70 states were included in the state-averaging procedure. The number of roots were chosen to include excitations which upon CASPT2 correction lie in the energy ranges reported in the electronic spectra. Only states with significant transition dipole moment magnitude were selected for the CASPT2 correction and the threshold was set to 0.03 a.u. This is possible as transition dipole moments are calculated at the CASSCF level. The pre-selection reduced by half the number of states to which CASPT2 was applied. The MM part of each snapshot was treated as external point charges in both CASSCF and CASPT2 calculations. The RICD approximation was used to speed up the calculation of two-electron integrals²⁴.

Electronic spectroscopy

Using the computed SS-CASPT2 energies and the SA-CASSCF transitions dipole moments, linear absorption spectra (LA), pump-probe spectra (PP) and quasi-absorptive two-dimensional UV spectra (2DUV) were computed for each snapshot by the sum-over-states approach²⁹ in the dipole approximation with Spectron 2.7³⁰. The experimental set-ups for PP and 2DUV use three pulses with wavevectors k_I , k_2 and k_3 and a local oscillator k_{LO} . In the paper we report quasi-absorptive spectra obtained by summing signals recorded for the rephasing k_I ($k_{LO} = -k_I + k_2 + k_3$) and non-rephasing k_{II} ($k_{LO} = +k_I - k_2 + k_3$) conditions^{30–32}. This can be achieved experimentally via the partially collinear pump-probe set-up with two collinear pump pulses and a non-collinear self-heterodyne probe pulse (i.e. $k_I = k_2$ and $k_{LO} = k_3$). A constant line broadening of 200 cm⁻¹ is used throughout. All calculated signals use the non-chiral xxxx pulse polarization configurations and are plotted on a logarithmic scale. GSB and SE contributions appear as negative (white) peaks, ESA appear as positive (black) peaks in the 2D spectra. A compendium on LA, PP and 2DUV spectroscopies is provided in the Supporting Information.

Results and Discussion

Analysis of the unfolding pathway

Two representative coordinates are chosen to visualize the most characteristic deformations during the unstacking of the Phe-Tyr aggregate: the distance **d** between the centers of the aromatic rings and the angle **α** between the normals to the planes of the rings (Figure 2). These coordinates have been used in previous studies to characterize aromatic-aromatic amino acid interactions³³.

Starting from a slightly twisted T-shaped (**α** = 65°) stacked conformation with an inter-chromophore distance of ~5.0 Å we observe variations in both **d** (5.0–6.0 Å) and **α** (60–90°) (snapshot 1–10). **α** finally drops down in the range 40–60°. The twisting is accompanied by a parallel displacement of the two rings which leads to an increase of **d** (5.5–6.5 Å) (snapshots 11–21). Subsequently, the rings continue to drift away (**d** > 6.5 Å) which allows for **α** to decrease down to ~0° (snapshots 22–31). Eventually the rings completely separate and the distance/angle correlation is lost (not shown). Thus, the unstacking dynamics can be summarized as a three-step process: T-shaped → twisted offset stacked → unstacked. An interesting observation is the recurrence of close contact interactions (snapshots 5, 8, 14 and 20). It is, therefore, of particular interest to find out if our electronic spectroscopy simulations can reveal these recurrences.

An important issue we need to address before discussing the electronic spectra is the capability of the QM/MM refinement procedure to preserve the intrinsic conformation of the rMD snapshots. Figure 3 compares the rMD (black) and QM/MM (gray) structures with respect to the distance **d**. Although the distance changes by 0.1–0.2 Å upon energy minimization the global tendency is retained. Only snapshots 4, 5 and 7 have deviations larger than 0.2 Å, but also in these cases the trend is conserved. It appears that freezing the environment is a strong enough constraint which allows to retain characteristic information about the configurational dynamics during the structural refinement.

Electronic spectroscopy

Several spectroscopic signals were simulated for the selected snapshots: linear absorption (LA), pump-probe spectroscopy (PP) and quasi-absorptive two-dimensional UV spectroscopy (2DUV). These techniques characterize the electronic structure of the aromatic residues. The relevant states detectable with the simulated experiments are shown in Figure 4. The spectra are analyzed in this section. Although we know *a priori* the nature of the different bands observed spectroscopically thanks to the underlying CASSCF/CASPT2 calculations, we begin with a phenomenological analysis of the relative spectral shifts and intensities and only later discuss the origin of the bands in terms of wavefunctions.

The first excited states of Phe and Tyr (i.e. the L_b states) which absorb in the NUV are selected as targets for the pump pulses. These states have lower oscillator strengths than higher lying states. However, they are spectrally well separated from the DUV electronic transitions of the backbone peptide bonds. As demonstrated in our previous study of the CYFC tetrapeptide, setting the incoming pulses in resonance with the L_b transition of Tyr will cover any Phe signal¹⁰. It is therefore essential to use narrowband pump k_1 and k_2 pulses centered near the frequency of the L_b transition of Phe in order to enhance it. On the other hand, broadband probe k_3 and LO pulses are required to cover a meaningful fraction of the transient absorption spectrum. Therefore, we used k_1 and k_2 pulses centered on Phe absorption (i.e. 39000 cm^{-1}) with a full width at half maximum (FWHM) of 733 cm^{-1} (corresponding to a Fourier limited pulse of 20 fs), a FWHM of 2932 cm^{-1} (corresponding to a 5-fs pulse) for pulse k_3 and the LO, unless specified otherwise. Probing was performed in two spectral windows: NUV (i.e. 35000–43000 cm^{-1}) and visible (Vis, i.e. 24000–32000 cm^{-1}). Probing in the NUV resolves correlated transitions and weak “quartic” couplings in coupled aggregates.^{31,32} Probing in the Vis covers the spectral region below the ionization potential and permits the collection of background-free signals of charge transfer transitions (CT) in coupled chromophore aggregates¹⁰ which are relatively weak and require regions free of the background given by intense local absorptions.

The LA signal reveals the singly excited states which carry oscillator strength from the GS. Figure 5, left panel shows the LA spectra at selected snapshots along the representative unfolding pathway in the NUV to DUV range (i.e. 35000–60000 cm^{-1}). Two characteristic bands are seen, a weak band in the NUV between 37000 cm^{-1} and 38000 cm^{-1} and a strong band between 47000 cm^{-1} and 56000 cm^{-1} . A closer look at the NUV band reveals that it actually consists of a stronger band around 37000 cm^{-1} and a weaker band around 39000 cm^{-1} . These bands correspond to the L_b absorptions of phenol and benzene (Figure 4). The strong feature between 47000 cm^{-1} and 56000 cm^{-1} includes several intense absorptions of both chromophores. The most red-shifted band (47000–51000 cm^{-1}) belongs to the ground state (GS) $\rightarrow L_a$ absorption in phenol (not shown in Figure 4). The corresponding band for benzene is covered by the more intense GS $\rightarrow B_a$ and GS $\rightarrow B_b$ transitions above 51000 cm^{-1} .^{10,34,35}

It is evident from Figure 5, left panel, that the LA is rather insensitive to chromophore stacking. No correlation between the spectra and the conformational dynamics is observed

neither for the L_b bands, nor for the higher lying L_a or B_a/B_b bands. LA is not a good marker for following the conformational dynamics of the aromatic residues.

Figure 5, middle panel, shows the time-dependent PP spectra for selected snapshots. We used a degenerate PP experiment (the marginal of a 2D experiment, see eq. 10 in the Supporting Information) with both pump and probe pulses centered at 39000 cm^{-1} . This one-color experiment resolves ESA to states at twice the energy of the L_b absorption band (i.e. around 75000 cm^{-1} , see Figure 4) together with the GSB and SE of opposite sign, which coincide when the delay time t_2 is set to 0. Around $37000\text{--}38000\text{ cm}^{-1}$ the GSB of phenol is observed. The corresponding GSB signal of benzene, observed as a weak band around 39000 cm^{-1} is covered by ESA bands which show fluctuations between 38000 cm^{-1} and 40000 cm^{-1} . A second band appears between 40000 cm^{-1} and 42000 cm^{-1} . Each band is a doublet, which show a conformational dependent splitting. The signals are ubiquitous in all snapshots, a clear indication of local transitions belonging to either benzene or phenol. A comparison with the individual chromophore spectra allows to assign bands to each chromophore. Indeed, the $38000\text{--}40000\text{ cm}^{-1}$ band is a transition from the L_b state of phenol to a doublet of super-excited states ($2E_{2g}(Y)$, Figure 4), while the $40000\text{--}42000\text{ cm}^{-1}$ band is the corresponding transition in benzene ($2E_{2g}(F)$, Figure 4)¹⁰. Experimental findings confirm the existence of these “super-excited” states in pure phenol^{36,37} and benzene³⁸. We note the weak transition to a doubly excited state $1D^*(Y)$ and $1D^*(F)$ to each band (Figure 4). *We do not observe a correlation between the relative shifts and splitting of the local signals and the chromophore stacking.*

Figure 5, middle panel, shows that the relative intensity of the bands varies strongly and generally increases for non-interacting chromophores. To understand this we repeated the excited state calculations in the absence of the environment (i.e. backbone and solvent, hereafter referred to as “gas-phase” calculations). Figure 6 shows a comparison of the transition dipole moment (TDM) magnitudes of the L_b transitions with (black) and without (gray) the environment for the first 25 snapshots of phenol (Figure 6, left) and benzene (Figure 6, right). Indeed, the “gas-phase” TDM magnitude is rather constant, regardless stacking or unstacking, while it decreases for stacked conformations when including the environment effect. The decrease of signal intensity is an environmental effect related to peptide conformation and solvent arrangement. As the $GS \rightarrow L_b$ transition of benzene is weak (symmetry forbidden) the fluctuations of its TDM magnitude result in strong variations of the spectral intensity. The effect should be less pronounced when vibrationally induced increase of the TDM magnitude is taken into account. We note that currently available parametrized Frenkel exciton Hamiltonian models neglect the effect of the environment on the TDM⁹.

Multi-pulse spectroscopic techniques can utilize pulses with different spectral properties. Probe pulses tunable from the near-IR to the far-UV can be used to cover a broad spectral range (two-color experiments). Figure 5, right panel, shows the time-resolved two-color PP spectra with NUV pump and Vis probe at selected snapshots. As in the one-color experiment the pump pulse is centered at 39000 cm^{-1} , while the probe pulse is at 28000 cm^{-1} . All signals are from ESA type since the probe pulse is not in resonance with $GS \rightarrow L_b$ (GSB) and $L_b \rightarrow GS$ (SE) transitions. In all snapshots we observe an intense band between $24000\text{--}27000$

cm⁻¹. Clearly, this band consists of local absorptions. A comparison with the reference spectra of the monomers³⁸, as well as with theoretical calculations^{34,39,40} reveals that one state with E_{2g} symmetry in phenol (1E_{2g}, Figure 4) and two states with E_{2g} symmetry in benzene (1E_{2g}, Figure 4) absorb in this range (~7.7–7.8 eV). Valence-bond considerations⁴¹ had already predicted that the 1E_{2g} band is the highest band below the ionization limit which lies at ~8.5 eV for phenol³⁴ and at ~9.0 eV for benzene³⁴. This was later confirmed by multi-configuration calculations and by experiment. Indeed, no features are observed above 27000 cm⁻¹ for snapshots 22–31 where the two chromophores are separated by more than 6.5 Å.

Snapshots 11–21 which correspond to the twisted offset stacked conformation of the dimer, exhibit a number of peaks above 27000 cm⁻¹ (Figure 5, right panel, see Supporting Information for remaining PP spectra). *The signals above 27000 cm⁻¹ (i.e. above ~65000 cm⁻¹ / ~8.00 eV from the GS) provide an evidence of chromophore-chromophore interactions.* T-shaped geometries (snapshots 1–10) exhibit fewer peaks in the range 27000–32000 cm⁻¹ (snapshots 1, 2, 5 and 10 in Figure 4 and in the Supporting Information).

Wavefunction analysis shows that the new signals in the 27000–32000 cm⁻¹ regime arise from single electron transitions from the L_b bands to two pairs of CT states with configurations H-1(Y)→L/L+1(F) and H/H-1(F)→L(Y) (see also the Supporting Information). Each CT state can be reached from the L_b band of both chromophores by either electron transfer (transition within virtual orbitals) or hole transfer (transition within occupied orbitals). In non-interacting aggregates (snapshots 22–31) the CT states are dark and often lie above the ionization potential, which renders them inaccessible. Upon stacking, the overlap of the π-orbitals may stabilize the energies by more than 10000 cm⁻¹ and enhances the TDM both from the GS and from the L_b band. *The CT states show a complex dependence on inter-chromophore distance, the orbital overlap and the environment arrangement.*

The two-color PP set-up with probing in the Vis can be used to resolve chromophore-chromophore stacking, albeit not unambiguously, as for particular dimer and environment arrangements the CT signals may lie outside of the 27000–32000 cm⁻¹ window (e.g. snapshots 3, 7, 9, see also Supporting Information). T-shaped conformations seem to be more sensitive to the surrounding than stacked offset ones. Above 6.5 Å the CT transitions are too weak to be detected.

The strength of 2D spectroscopy lies in the ability to disentangle contributions coming from different chromophores. This is demonstrated in Figure 7, showing the 2D spectra for snapshots 1, 10, 16 and 19, which collapse to the PP spectra in Figure 5, middle and right panels, when integrated over Ω₁ (see the Supporting Information for remaining 2DUV spectra). We observe two distinct bands corresponding to transitions associated with the GS→L_b absorptions in phenol (Ω₁ = ~37000 cm⁻¹) and benzene (Ω₁ = ~39000 cm⁻¹). The 2D spectra confirm the conclusion of the one-color PP experiment that the ESA bands at 38000–40000 cm⁻¹ and 40000–42000 cm⁻¹ are local excitations of phenol and benzene, respectively (Figure 7, left column). As already demonstrated for the PP spectrum, the local signals show no correlation to conformational dynamics.

2DUV spectra reveal additional signals unresolved in the PP spectra. For most stacked geometries (e.g. snapshots 1, 10 in Figure 7, left column) a pair of signals emerges along the benzene band: an off-diagonal bleach signal at $\Omega_1 = \sim 39000 \text{ cm}^{-1}/\Omega_3 = \sim 37000 \text{ cm}^{-1}$ and a red-shifted ESA, belonging to a transition out of the L_b of benzene to a mixed doubly excited state with a wavefunction $L_b^{\text{benz}} + L_b^{\text{phen}}$. These two signals are characteristic signatures of the chromophore-chromophore interactions. The splitting between the GSB and the ESA is known as quartic splitting and measures the strength of the interaction.³⁰

To better resolve the quartic splitting we focus on the spectral region between 35000 cm^{-1} and 39000 cm^{-1} (Figure 8) by using following pulse parameters: a 733 cm^{-1} pump pulse-pair centered at 39000 cm^{-1} and a 733 cm^{-1} probe pulse centered at 37000 cm^{-1} . Figure 8 shows three snapshot sequences which represent recurrences of chromophore-chromophore close contact interactions: 6-7-8-10-11, 13-14-15-16-17 and 18-19-20-21-22. The highlighted snapshots exhibit the shortest inter-chromophore distance d in each sequence. We observe *qualitative correlation between the conformational dynamics and the quartic splitting*. This is confirmed also by the scatter plot in Figure 9, which correlates the quartic splittings for the T-shaped (snapshots 1–10) and twisted offset stacked (snapshots 11–21) conformations to the distance d . The quartic splittings are generally weak (less than 2000 cm^{-1}), between $6.0\text{--}6.5 \text{ \AA}$ they are less than 100 cm^{-1} and vanish completely above $6.5 \text{ \AA}$.

As pointed out earlier, the CT states emerging in stacked conformations are common to both chromophores, i.e. are accessible via a single electron excitation from both L_b states. Thus, transitions to bright CT states result in a characteristic signature in the 2DUV spectra: both L_b bands exhibit identical band patterns with CT peaks in the benzene band red-shifted by the energy difference $L_b^{\text{phen}} - L_b^{\text{benz}}$ (marked with circles in Figure 7, right column). UV-pump Vis-probe 2DUV spectroscopy with cross-polarized pulses¹⁰ can selectively detect the common states and, thus, unambiguously resolve whether the ESA peaks observed in the corresponding PP (Figure 5, right panel) are associated with transitions to local or CT states.

Conclusions

Using the SOS//QM-MM approach for ab-initio simulations of electronic spectra we demonstrate the ability of different linear and non-linear spectroscopic techniques to probe peptide conformation dynamics on a model tetrapeptide using the $\pi\pi^*$ electronic transitions in the aromatic residues Phe and Tyr. The trajectories were obtained in explicit solvent, by means of the DRP approach. DRP made it possible to concentrate the computational effort on the reactive part of trajectories, while reducing the effect of the bias to a minimum. Different stacking arrangements, adopted by the aromatic side chains during peptide unfolding, are found to exhibit characteristic spectral signatures, detectable with tailored experimental set-ups.

Linear absorption spectroscopy, as well as one-dimensional one-color pump-probe spectroscopy in the NUV show no correlation to the geometrical changes as neither the $GS \rightarrow L_b$ absorptions nor the local ESA signals show correlated shifts. In contrast, two-color pump-probe and 2DUV spectroscopies are very sensitive to stacking interactions, which manifest through off-diagonal bleach signals and bright CT states. The former are correlated

to the ESA from the L_b band to the mixed doubly excited $L_b^{\text{benz}}+L_b^{\text{phen}}$ state present in the aggregate and give rise to the weak quartic splitting (up to 2000 cm^{-1}). The quartic splitting correlates nicely to the inter-chromophore distance along the unfolding pathway and seems less sensitive to the relative orientation of the chromophores. On the contrary, the energetic position and brightness of CT states depend on the distance, π -orbital overlap and the environment. This complex dependence may only be resolved by accurate quantum mechanical calculations which take the environment explicitly into account. Intense CT signals can be still observed between $6.0\text{--}6.5\text{ \AA}$ where the quartic splitting is very small ($< 100\text{ cm}^{-1}$). Above separation of $6.5\text{ \AA}$ no signature of chromophore-chromophore interaction is observed.

Non-linear 2DUV electronic spectroscopy and its marginal the pump-probe spectroscopy prove to be excellent tools for following coherent dynamics. The former provides a combination of high spectral and temporal resolution while the latter represents a compromise between experimental complexity and informative value. The experiments proposed in this study are within reach of current state-of-the-art ultrafast spectroscopy instrumentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Conformational dynamics of CYFC: after the photoinduced cleavage of the disulfid bond the sulfur radicals get saturated on a ultrashort time scale. Subsequently, the peptide unfolds. The simultaneous unstacking of the aromatic side chains of Tyr and Phe is probed with different spectroscopic techniques. Color in the Supporting Information.

Figure 2.

Representation of the conformational dynamics of the CYFC tetrapeptide in the space of the inter-chromophore distance d and the angle between the normals to the aromatic planes α . Color code labeling according to the three-step unfolding/unstacking process: T-shaped (white on black), twisted offset stacked (gray), unstacked (black on white). Selected snapshots are visualized. Color in the Supporting Information.

Figure 3. Comparison of the rMD snapshot (black) and the QM/MM refined (gray) inter-chromophore distance d .

Figure 4.

Transition frequencies from the GS for the singly excited manifold ($L_b(Y)$, $L_b(F)$) and the bright excited states in the VIS (24000–32000 cm^{-1}) and UV (35000–43000 cm^{-1}) probing windows for selected snapshots. States with transition dipole moment magnitude below the threshold of 0.03 a.u., as well as local states in the range 40000–60000 cm^{-1} (e.g. L_a , B_a , B_b) are not shown, since they do not show in the presented non-linear spectra. Snapshot labeling according to Figure 2.

Figure 5.

left panel: Linear absorption (LA) spectra for selected snapshots. The L_b , L_a and B_a/B_b bands are labeled; middle panel: Time-resolved pump-UV probe-UV (i.e. one-color PP) spectra for selected snapshots. The GSB and ESA bands are labeled; right panel: Time-resolved pump-UV probe-Vis (i.e. two-color PP) spectra for selected snapshots. The ESA and CT bands are labeled. Snapshot labeling according to Figure 2.

Figure 6.

Comparison of the transition dipole moment magnitude for the ground state to L_b transition in phenol (left) and benzene (right) in the presence (black) and absence (gray) of the backbone and solvent.

Figure 7.

Pump-UV probe-UV 2DUV (left column) and pump-UV probe-Vis 2DUV (right column) for selected snapshots together with the marginals (i.e. PP spectra), obtained by integrating over Ω_1 . 2DUV signal intensities are normalized $[-1(\text{white}):1(\text{black})]$. GSB/SE and ESA appear as negative and positive contributions, respectively. CT signals are denoted. Snapshot labeling according to Figure 2. Color in the Supporting Information.

Figure 8.

Pump-UV probe-UV 2DUV spectra for three sequences: 6-7-8-10-11 (upper panel), 13-14-15-16-17 (middle panel) and 18-19-20-21-22 (lowe panel). Signal intensities are normalized [-1(white):1(black)]. GSB/SE and ESA appear as negative and positive contributions, respectively. The quartic splittings are highlighted [unit: cm^{-1}]. Snapshot labeling according to Figure 2. Color in the Supporting Information.

Figure 9. Scatter plot correlating the inter-chromophore distance **d** and the quartic splitting for snapshots 1–21.