PCCP

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pccp

PCCP

RSCPublishing

ARTICLE

On the near UV photophysics of a phenylalanine residue: conformation-dependent ππ* state deactivation revealed by laser spectroscopy of isolated neutral dipeptides

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Yohan Loquais,[a,b] Eric Gloaguen,[b,a] Mohammad Alauddin,[a,b] Valérie Brenner,[a,b] Benjamin Tardivel,[a,b] and Michel Mons[a,b]

The primary step of the near UV photophysics of a phenylalanine residue is investigated in one- and two-color pump-probe R2PI nanosecond experiments carried out on specific conformers of the Ac-Gly-Phe-NH_2 molecule and related neutral compounds isolated in a supersonic expansion. Compared to toluene, whose ππ* state photophysics is dominated by intersystem crossing with a lifetime of ~80 ns at the origin, the first ππ* state of Phe in the peptide environment is systematically found to be shorter-lived. Lifetime at the origin transition is found to be significantly shortened in presence of a primary amide (-$CONH_2$) group, (20-60 ns, depending on the conformer considered), demonstrating the existence of an additional non-radiative relaxation channel related to this chemical group. The quenching effect induced by the peptide environment is still more remarkable beyond the origin of the ππ* state, since vibronic bands of one of the 4 conformers observed (the 2_7-ribbon conformation) become barely detectable in the ns R2PI experiment, suggesting a significant conformer-selective lifetime shortening (below 100 ps). These results on dipeptides, which extend previous investigations on shorter Phe-containing molecules (*N*-Ac-Phe-NH_2 and *N*-Ac-Phe-NH-Me), confirm the existence of conformer-dependent non-radiative deactivation processes, whose characteristic timescales range from tens of ns down to hundreds of ps or below. This dynamics is assigned to two distinct mechanisms: a first one, consistent with an excitation energy transfer from the optically active ππ* state to low-lying amide nπ* excited states accessed through conical intersections, especially in presence of a C-terminal primary amide group (-CONH2); a second one, responsible for the short lifetimes in 2_7 ribbon structures, would be more specifically triggered by phenyl ring vibrational excitations. Implications in terms of spectroscopic probing of Phe in a peptide environment, especially in presence of a quenching amide group, are discussed.

Introduction

Photophysics of biomolecules is an important issue, since exposure to UV radiation can potentially lead to harmful photochemistry effects, affecting their structure and hence their function.[1-8] The existence of ultrafast dynamics in the excited states of these molecules, facilitated by the ubiquitous presence of conical intersection between the various excited states, as evidenced by theoretical investigations, is sometimes considered as an active component of the strategy deployed by nature to preserve the functionality of its building blocks and therefore a potential doorway to photochemical phenomena.[1, 6-8] In proteins, the three aromatic aminoacid residues absorb in the near UV, explaining the recent efforts for theoretical search of fast excited state dynamics.[1-8] Besides these fundamental issues, photophysics also plays a crucial role in more applied issues, such as proteomics, for example with the recently developed coupling of peptide mass spectrometry with optical UV excitation of aromatic residues.[9-11]

Fast dynamics has been suspected for a long time from gas phase studies on neutral peptides. Trp-containing chains, isolated and cooled in a supersonic expansion, have been studied by IR and UV nanosecond experiments.[12] The absence of conformers, that were expected under classical jet cooled conditions, because predicted among the most stable forms by a careful theoretical modelling of the conformational landscape, suggested the existence of a conformer-selective fast dynamics, preventing the excited state short-lived forms to be observed by nanosecond spectroscopy.[13] From the theoretical point of view,

this interpretation was backed up by excited state calculations, focusing on the role of backbone excited states in the deactivation process of the optically excited states and further in the relaxation to the ground state.[4, 5, 14, 15] Phenylalanine (Phe), the third chromophore remained in contrast much less documented, although sparse data on neutral molecules containing both phenyl ring and an amide group have been collected, with excited state lifetimes reported in the tens of ns-range [16, 17] and indirect hints for population of triplet states.[18] In contrast to tryptophan, Phe is often considered as a more gentle chromophore, less inclined to a fast photophysics, and therefore often chosen as a convenient tag, used to detect molecules of interest.[12, 19] Photophysics of isolated toluene, the UV chromophore of Phe, is indeed characterized by a lifetime of 86.4 ns, at the origin of its weakly allowed ππ* transition,[20] essentially controlled by intersystem crossing (ISC) (70%, τ ~ 120 ns [21]) and fluorescence (30%, radiative lifetime ~290 ns [22]). This slow basic photophysics, in contrast to tyrosine or tryptophan chromophores, makes Phe quite sensitive to the existence of slow non-radiative relaxation processes, in particular those due to the peptide environment. As a matter of a fact, excited state investigations of the smallest peptide chains containing Phe, i.e., the *N*-acetyl-Phe-amide and -methylamide molecules (NAPA and NAPMA, in short),[23-25] have shown that the lifetime critically depends on the backbone conformation and the orientation of the Phe side chain, with values ranging from 70 ns down to 1.5 ns. This behavior was assigned to a Dexter-like excitation energy transfer between the ππ* state of the phenyl ring and the nπ* states of the backbone amide groups as suggested by quantum chemistry excited state calculations.

In contrast to neutral system studies, which are essentially sensitive to the primary step of the photophysics, investigation of charged species brings a complementary view on relaxation mechanisms by focusing attention on subsequent dynamics, in particular through observation of fragmentation channels. Protonated aromatic aminoacids have thus been studied either at room temperature or under cooled conditions (in a cold ion trap), with emphasis put on excited state dynamics, and/or on the existence of specific fragmentation channels subsequent to photoexcitation (e.g. side-chain loss).[9-11, 14, 15, 26-28] Larger protonated Phe-containing helical peptides have also been studied under cold conditions.[29-31] These species are closer to peptide chain models with a Phe side chain neighboring the capped N-terminal of the helix, giving rise to a NH-π interaction with the first amide bond; the whole structure being stabilized by a protonated lysine on the C-terminal end.[29-31] Their fragmentation properties, in particular the specific cleavage of the Cα-Cβ side chain bond (which is not the weakest in the system), have been found to be greatly enhanced by subsequent delayed multiphoton IR excitation,[32] suggesting the involvement of long-lived excited states in this dynamics (dissociation from the ππ* triplet state[33] or from a biradical complex formed after photoexcitation.[34]

In this context, the aim of the present work is to further document the photophysics of the Phe chromophore in presence of a series of controlled peptide environments, beyond the NAPA and NAPMA systems, by using nanosecond laser spectroscopy. Phe-containing neutral chains are laser-desorbed, isolated and cooled in a supersonic expansion, allowing us to perform conformation-selective investigations. The present paper is focused on the photophysics of capped Gly-Phe dipeptides (Ac-Gly-Phe-NH_2 and Ac-Gly-Phe-NH-Me, shortened as GFa and GFm in the following; *a* and *m* standing respectively for amidated and methyl-amidated C-terminal), whose structure, known from IR/UV double resonance experiments, has been reported elsewhere[35, 36] and refined in detail more recently.[37] The present work, complemented by studies on Ala-Phe and Val-Phe dipeptides (see Supplementary Information), aims at proposing a synthesis of the excited state behavior of Phe in these small neutral peptides. In addition to the origin band, the first intense 6a/6b vibronic bands have also been investigated in order to document the role of the nature and amount of excess energy on the excited state dynamics, in particular in the perspective of the presence of intramolecular vibrational redistribution (IVR) in these medium-sized isolated molecules.[38-40]

Experimental Methodology

The experiment is based on a pulsed molecular beam, equipped with a home-built laser-desorption module coupled to the supersonic expansion, already described elsewhere, [24, 35] and designed to collect conformer-selective information on the excited state dynamics of the Phe-containing molecules, isolated in the gas phase. UV spectra are obtained from one-color resonant two-photon ionization; excited state lifetime measurements are carried out in a ns pump-probe experiment. The corresponding two-color resonant two-photon detection scheme is carried out using nanosecond frequency-doubled dye lasers (Radiant, narrowline and Lambda Physik FL3000), pumped by excimer (Lambda Physik EMG 103 MSC) or YAG (Continuum) lasers. The photoions are collected in a home built time-of-flight mass spectrometer (TOF MS). Alternatively fluorescence measurements have also been carried out on a second chamber equipped with a dual ion/photon detection scheme, in which both monitoring schemes can be compared. The near resonant fluorescence was detected through a monochromator centered at 280 nm.

Results

3.1 UV spectroscopy from one-color resonant two-photon ionization

First, the UV spectroscopy of the GFa and GFm molecules have been recorded using the mass-selected one-color R2PI technique in order to determine the spectral features of the conformers present in the expansion and to assign them in terms of structure (Fig. 1). Previous IR/UV experiments in the spectral region of Phe origin[35, 41] have shown that isolated GF sequences give rise to two types of backbone (BB) conformations, either extended forms, of the 2_7 ribbon type,

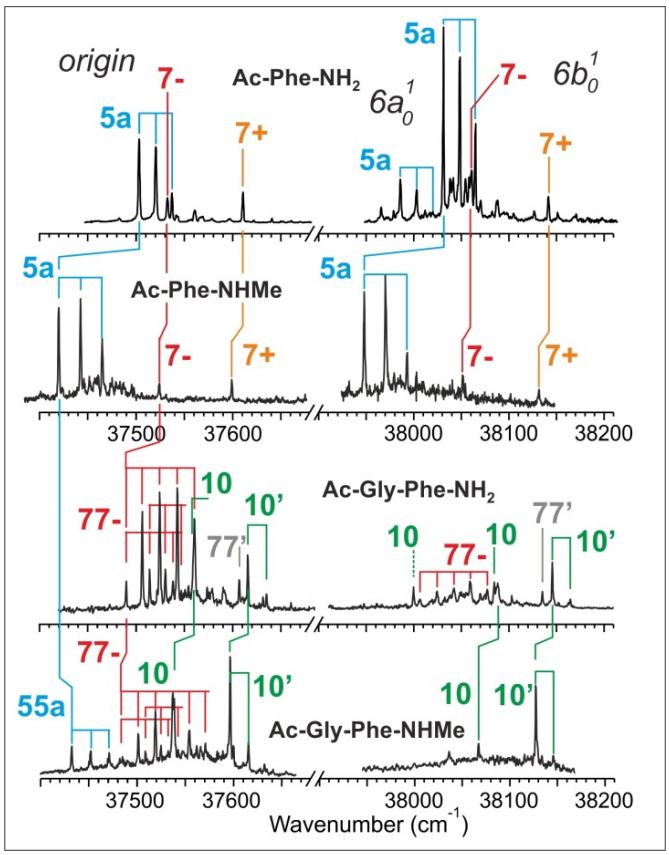

Figure 1 : Mass-selected one-color resonant two-photon ionization spectra in the spectral regions of the origin and of the 6a/6b vibronic bands of the first electronic transition of the phenyl ring for the GFa and GFm peptides, together with the Ac-Phe-NH_2 and Ac-Phe-NHMe compounds for comparison (NAPA and NAPMA). The labels refer to the origins, the 6b vibration (and the 6a vibration when observed) of the conformations detected in a IR/UV spectroscopic study.[37] The bars indicate Franck-Condon activity due to low frequency backbone modes. The belonging of each band in the *6* vibronic region is identified from the corresponding spectral shift (~440-480 cm^{-1} for *6a*; ~530 cm^{-1} for *6b*) specific of each conformer.

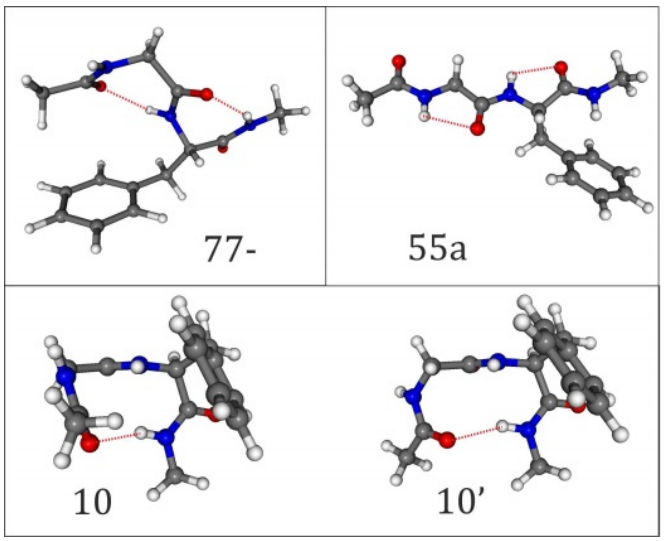

Figure 2: DFT-D (B97-D/TZVPP) structures of the relevant most stable conformations of the GFm molecule, belonging to several secondary structures (77-: 2_7 ribbon; 10 and 10': β-turns of type I and II' respectively; 55a: β -strand). The same structures are also present in the C-terminal non-methylated molecule GFa.

stabilized by sequential C7 H-bonds along the backbone, or folded β-turn structures, stabilized by C10 H-bonds.

The main conformation of the GFa molecule has been identified as a 2_7 ribbon structure exhibiting two H-bonds, and described as f-7L-7L(g-) (77- in short), according to a terminology indicating the interactions in which the sequential NH bonds along the backbone are engaged ; f, for free, π for a π H-bond, n for Cn H-bonds forming a n-membered atom ring (Fig. 2), and between brackets the orientation of the Phe side chain, gauche +/- or anti (shortened as g+, g- and a). In this main conformation, the phenyl ring interacts with the *N*-terminal acetyl moiety, giving rise upon excitation to a rich Franck-Condon activity in two backbone torsion low frequency modes. The UV spectrum at the origin bears the signature of this most stable form and of minor, less intense forms labelled 10, 10' and 77', which have been identified, from their IR/UV double resonance spectroscopy,[37] as corresponding respectively to two types of β-turns (f- π-10I (g+), type I, and f- π-10 II'(g+), type II') and an alternative 2_7 ribbon structure, f-7D-7L(g-), with a direct γ_D turn on the Gly residue (made competitive due to the achirality of Gly) and the same phenyl ring orientation (g-) as in the 77- conformer.

Spectral region of the phenyl origin band. According to the extensive conformational population, the phenyl origin region of the GFa molecule (Fig. 1) exhibits a dense pattern due to the progression of the 77- form and isolated bands of the 77', 10 and 10' forms. A very similar pattern is also obtained for the GFm molecule, with however two new features: i) the disappearance of the 77' conformer and ii) the appearance of a new conformation 5-5-π (a), assigned to an extended form (55a in short), with the Phe side chain having the same orientation (*anti*) as in the 5a main conformer of NAPMA[25, 42] ; a close inspection of the GFa spectrum combined with IR/UV spectra shows that the 55a form is present as a weak doublet being very close to members of the 77- progression at 37506 and 37521 cm^{-1}. [37]

6a/6b spectral region. Both GFa and GFm spectra are expected to mimic that of the toluene chromophore, with, in the region up to 700 cm^{-1} above the origin, the presence of two active 6a and 6b vibrations (according to Wilson notation,[43] respectively located at 460 and 532 cm^{-1} in toluene). The intensity of these bands is known to be significantly larger than the origin due to a component of the transition dipole moment arising from a Herzberg-Teller-type vibronic coupling between the first and second ππ* states of the phenyl ring.[40, 44, 45] The expected behavior is illustrated in the case of the NAPA and NAPMA molecules, also displayed in the top panel of Fig. 1 for the sake of comparison: the origin pattern, which is due to the conformational population (3 conformers labelled 5a, 7+ and 7- in NAPA/NAPMA, corresponding to conformers A, B and C in

the previous papers) is reproduced at higher excitation energies for the 6a and 6b vibrations (at 482 and 529 cm^{-1} respectively).[23, 42] In contrast, the GFa spectrum in the 6a/b region strongly differs from that of NAPA and NAPMA. Contributions of the 10, 10' and 77' conformers are indeed still found with a significant 6b component at 532 cm^{-1} above the origin, and a weaker 6a, visible for the most populated forms (10 or 10') at ~ 446 cm^{-1}. The most striking point, however, is that the low-frequency progressions of the 77- form are barely visible in this regionand definitely much less intense than expected from NAPA/NAPMA intensities. This weak signal matches well the origin progression, with a shift of ~ 520 cm^{-1}, slightly broadened relative to the origin (1-2 cm^{-1} increase in linewidth) and exhibiting a slightly distorted pattern, compared to the origin progression. Although this latter point can be explained by vibrational couplings within the manifold of backbone vibrational modes in this region (like for VFa -See Fig. S1- or FFa -not shown-, for which line splittings have been observed at the 6b vibration), the broadening of individual lines suggests a significant shortening of the optically excited state lifetime, down to the 10 ps time scale. This is consistent with the weakness of the 77- signal obtained with ns lasers, the short lifetime making difficult the excited state photoionization during the laser pulse. This behavior has been checked against the effect of methylation on the C-terminal: the GFm spectrum in this spectral region (Fig. 1) exhibits the same behavior as GFa, even more marked however, since the 77- signature, undetected, is missing in this case.

In short, R2PI experiment suggests the existence of a fast deactivation channel of the excited state in the extended conformers 77-, at least when excited on vibronic bands of the phenyl ring. This is observed whatever the methylation status of the C-terminal. This fast dynamics, however, does not seem to be shared by the other conformers (in particular the β-turns, 10 and 10'), whose spectral signatures are still observed in this region. This overall behavior (long-lived turns vs. short-lived 77- forms) seems to be a general features of the Ac-Xxx-Phe-NH(H/Me) species, with Xxx = Gly, Ala, Val, as illustrated from the UV spectroscopy data, shown in the Fig. S1 of Supplementary Information.

3.2 Excited state lifetimes

The lifetime of the excited states has been first measured by 2C-R2PI, in ns pump-probe experiments for the most intense spectral features of GFa and GFm. On their origin, all the conformers observed in the methylated species GFm exhibit a long lifetime, in the 70 ns range, comparable to that of toluene (Table 1). In non-methylated GFa, the lifetimes are found to be shorter, in the 10-30 ns range, apart from the origin features of the 77- forms, which turn out to be longer-lived (~ 30-60 ns), with a smooth but significant decrease along the low frequency mode progressions (Figure 3, Table 1). A similar behavior is observed on the 77- form of AFp (see Supp. Info. Figure S2). Alternatively, the laser-induced fluorescence diagnostics was also used to characterize the excited state in GFm and GFa species. As a matter of fact, quasi resonant fluorescence ($\lambda_{collected}$ ~280 nm) was easily detected for all the 4 forms of GFm, and the lifetimes measured (on the most intense bands) are consistent with the 60-87 ns, long lifetime measured by 2C-R2PI. A similar long-lived fluorescence was collected for the 77- conformer of the GFa molecule demonstrating that in both cases the excited state probed by 2C-R2PI is the optically excited ππ* state, whose lifetime is in the 30-60 ns range. One can notice that no long-lived state is observed in the pump probe experiment, demonstrating that the present ns 2C-R2PI experiment (ν_2 = 37380 cm^{-1}) is not able to detect the triplet state, in agreement with earlier experiments on isolated toluene which showed that its detection requires a much shorter wavelength probe laser.[21, 46]

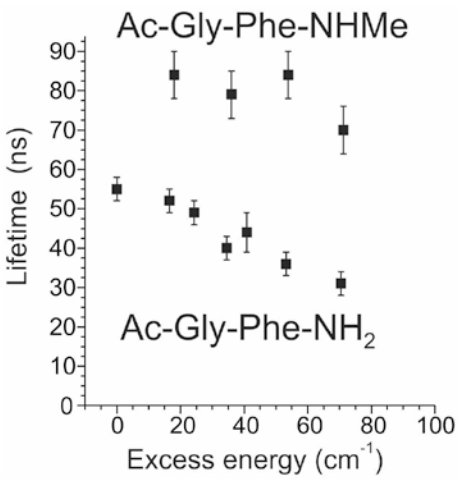

Figure 3: Lifetimes as measured in two-color resonant two-photon ionization resonant pump-probe experiments along the vibrational progressions (Fig. 3) of 77- conformers of GFm and GFa. The horizontal axis corresponds to the excess energy above the origin in the S_1 state. The 30 ns value measured at 71 cm^{-1} from the origin for GFa can be affected by the partial overlap of this band with the origin of the 10 form, also measured to be in this range (see Table 1).

IV. Discussion

The analysis of the present experimental data on Gly-Phe and Ala-Phe sequences together with previous results on NAPA/NAPMA[23-25] molecules illustrates the large diversity in lifetimes for the **optically ππ* excited state of Phe, as well as in the underlying dynamics:**

- **At the phenyl origin**, a continuum of lifetimes is observed along this series of species. For all the methylated species (both NAPMA and GFm), toluene-like ππ* lifetimes (in the 80-48 ns range) are observed. A clear evidence is provided from the detection of fluorescence (from all forms of GFm), with a lifetime corresponding to that measured by R2PI. In non-methylated species, however, a larger diversity of lifetimes is observed, ranging from toluene-like behaviors (NAPA 5a: 70 ns), shortened lifetimes in a few tens of ns (NAPA 7- and the 4 GFa conformers), down to the ns- time scale (NAPA 7+ : 1.5 ns).
- **On the 6 vibronic bands**, generally speaking, shorter ππ* state lifetimes are observed compared with the origin. The 77-

Table 1 : Excited state lifetime (ns) of the first ππ* excited state of conformers 77-, 77', 55a, 10 and 10' of the capped GFa and GFm dipeptides, as measured by ns 2C-R2PI in the region of the origin band and the 6 vibrations. The frequency of the second color, off-resonance, was 37383 cm^{-1}.

	Origin				
	77-	**77'**	**10**	**10'**	**55a**
Ac-Gly-Phe-NH_2 / GFa	55 – 30* ± 3 *(a)*	19 ± 2	30 ± 3	19 ± 2	*(c)*
Ac-Gly-Phe-NHMe / GFm	85 - 70 ± 5 *(a)*	*(c)*	67 ± 4	75 ± 4	75 ± 10
	6a / 6b vibrations				
	77-	**77'**	**10**	**10'**	**55a**
Ac-Gly-Phe-NH_2 / GFa	*(b)*	8 ± 2 *(d)*	14 ± 2 *(e)*	9 ± 2 *(d)*	*(c)*
Ac-Gly-Phe-NHMe / GFm	*(b)*	*(c)*	*(b)*	59 ± 3 *(d)*	*(b)*

(a) Range of values obtained along the vibrational progression in the low frequency modes of conf. 77- (see Fig. 3). In GFa, the last measurement along the progression can be affected by the partial overlap with the origin of the 10 form, also measured in this range.
(b) Band too weak compared with the background to ensure reliable measurements.
(c) Conformer not detected for this molecule
(d) 6b vibration
(e) 6a vibration

conformers set apart, methylated species remain long-lived although slightly (but significantly) shorter lived than on the origin. Similarly, non-methylated species are still in the 10-ns range and shorter-lived than on the origin. In contrast, the 77- conformers, whatever their C-termination, are so short-lived that their 6a/6b vibronic bands are barely observed, precluding reliable lifetime measurements. The short lifetime of their ππ* state indeed limits their detection in the ns R2PI experiment on GFa to weak residual bands, suggesting a lifetime shorter than 100 ps. This time scale is in line with the increased linewidth of these individual weak bands compared to the origin (by 1-2 cm^{-1}), which suggests a 5-10 ps range dynamics. This short dynamics behavior seems to be specific of the 77- conformers, and in particular is not shared by the β-turn species, for which the 6 vibronic bands are observed with the expected relative intensity.

Photophysics of the Phe residue

The longest lifetimes observed, in the methylated species ***at the phenyl origin,*** are all within the 60-80 ns range, i.e., close to the 86.4 ns toluene value. This suggests that, like for toluene, ISC remains the main deactivation process at play in the methylated peptides, even if the slight shortening measured (60-80 instead of 86 ns) could be due to either a slight dependence of ISC relative to the chromophore environment and/or the existence of a weakly efficient additional deactivation mechanism. In contrast, the observation of much shorter lifetimes (by nearly one order of magnitude) in some conformations strongly suggests that a new, efficient deactivation process is at play on the phenyl chromophore when embedded in a peptide environment. A close examination of experimental data requires a separate discussion of origin and vibronic bands.

At the phenyl origin, the ππ* dynamics is found to depend on the methylation of the C-terminal. This is reminiscent of what is observed on the NAPA/NAPMA molecules:[24, 25] whereas the methylated species typically behaves as toluene, significantly shorter lifetimes are observed in two out of the three conformers of NAPA, with a lifetime dropping down to 1.5 ns. Assuming the same ISC rate in NAPA as in toluene, this leads to a non-radiative rate ca. 80 times more efficient than ISC in the shortest-lived conformer of NAPA (γ_L(g+), referred to as 7+ in short notation of Fig. 1). Based on excited state quantum chemistry calculations,[24] the existence of conical intersections (CI) between the ππ* state and backbone (BB) locally excited (LE) states of nπ* nature (whose minima lies significantly lower than the ππ* minimum) was proposed to account for the enhanced NAPA dynamics in a weakly interacting regime (see Fig. 4). This $(\pi\pi^*)_{Phe} \rightarrow (n\pi^*)_{amide}$ excitation energy transfer (EET) can be viewed as a Dexter-type mechanism made possible due to a relatively short distance between the Phe and the excitation acceptor, i.e. the amide group.[47] This EET step (Fig. 4, black horizontal arrow) is thought to be followed by a subsequent fast dynamics due to the existence of conical intersections (CI) between BB LE states and the ground state or other BB excited states, including inter-amide charge transfer states (white arrow to CT_{amide} in Fig. 4) found by calculations.[3, 24] Additionally the effect of methylation on NAPA is accounted for [25] by assuming that the excitation acceptor BB LE nπ* state is localized on the primary amide next to the chromophore (C-terminal): the primary character of this amide (-$CONH_2$ termination) enhances the dynamics, through both a slightly more favorable energetics together with a facilitated access from the ZPE content of the vibrationless level to the CI, which leads to significant distortions of the corresponding amide bond.

Finally, it is worth noticing that the deactivation mechanism present at the origin of the non-methylated species seems also to be at play in the methylated species. Evidence for this is revealed by the shortest-lived conformation in both NAPA and NAPMA, i.e., the 7+ form. Even in NAPMA, a significant lifetime shortening compared to toluene (48 ns)[25] indicates an additional non-radiative channel, having a characteristic timescale of ~ 110 ns, i.e., already comparable to ISC. This suggests that the deactivation mechanism, acting in parallel to ISC and radiative decays in these molecules, is already active and significant in a secondary amide environment and therefore more generally in proteins.

In the present triamide molecules, depending on the conformer considered, the longest ππ* lifetimes are toluene-like (methylated species) whereas the shortest are in the 20-ns range (corresponding to an additional non-radiative process ~ 5 times more efficient than ISC). Owing to the dramatic effect of the C-terminal methylation (despite the fact that it takes place at a significant distance from the chromophore, especially in the 77- form of GFa, where phenyl ring and C-terminal point toward opposite directions; see Fig. 2), this additional dynamics can hardly be assigned to an environment-enhanced ISC, and again alternative deactivation processes have therefore to be proposed, involving the C-terminal primary amide -$CONH_2$ group. The same EET mechanism as in NAPA 7+ (an initial excitation transfer from the phenyl ring to the BB C-terminal amide) can be invoked. This is supported by preliminary excited state quantum chemistry calculations, which shows that

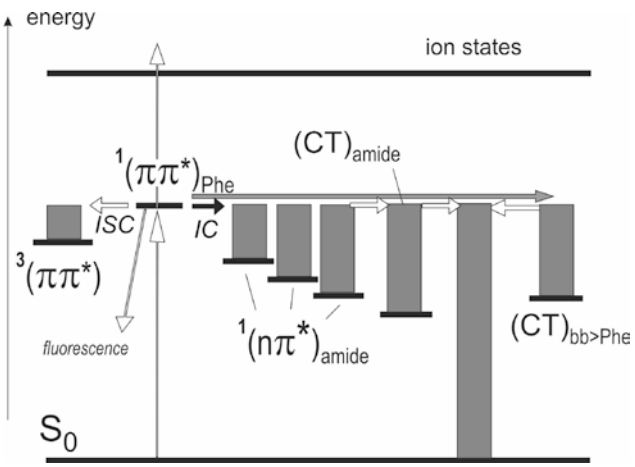

Figure 4 : a) Jablonski-Perrin schematic diagram with the excited states and relaxation processess relevant to the photophysics of Phe-containing isolated peptides and associated pump-probe experiments. The labels CT_{amide} and $CT_{bb>Phe}$ indicate inter-amide and backbone-to-phenyl ring charge transfer states respectively. The horizontal arrows indicate the processes expected to control the lifetime of the states considered, in particular internal conversion (IC) or intersystem crossing (ISC), with emphasis on internal conversion to nπ* amide excited states (black arrow) or to backbone-to-Phe electron transfer state (grey arrow). The vertical arrows illustrate the pump-probe ionization processes used to probe the ππ* state. The absence of arrows for the other states indicates that their probe is expected to be energetically and/or Franck-Condon forbidden, and not observed experimentally.

the three nπ* amide LE states of the 77- conformation of GFa are nearly isoenergetic in the ππ* state geometry (Figure 5) and barely 1 eV higher than this state *in this geometry*. It is also worth considering 10 and 10' GFa forms. These two forms, in which the close environment of Phe is similar (*g*- orientation of the Phe side chain in both cases, see Fig. 1), exhibit similar short lifetimes at the origin (~30 and ~20 ns respectively). Interestingly, these forms are those for which the distance between the C-terminal carbonyl group (O atom) and the phenyl ring (H atom on the proximal Cδ atom) is among the shortest (277 and 269 pm, respectively), to be compared with the close contact (235 pm) occurring in the short-lived NAPA 7+ conformer (1.5 ns).[24] This is in qualitative agreement with the short D-A distances required by such a Dexter-type EET process.[47] By analogy with NAPMA, the lifetime lengthening upon methylation observed in GFm can be assigned to a less easy access to the conical intersection.

One should emphasize that the case of the main 77- conformer is both interesting and puzzling:
First, this 77- form, with its Franck-Condon activity in the UV spectrum, enables us to document the role of internal energy, namely in backbone and backbone-to-side-chain low frequency motions, in the perspective of gaining access to the CI region, as suggested by the earlier work on NAPMA.[25] The significant drop in lifetime from 55 down to 30 ns along the progression in low frequency modes (Fig. 3) suggests that the efficiency of the non-radiative mechanism increases by a factor of ~3, ranging from comparable to ISC at the origin up to ~3 times faster at the end of the progression. This observation corroborates the picture of a weakly interacting regime in which regions of significant coupling close to the CI are made accessible providing relevant modes are excited: as a matter of fact, the low frequency modes calculated (in the ground state) for the 77- conformer exhibit significant distortions of the amide bonds and/or geometric changes of Phe relative to the backbone .[37]
It is moreover interesting to note that, in this 77- conformer, the C-terminal carbonyl lies farther from the phenyl ring than in 10 or 10' forms (with $O_{carbonyl}$-HCγ distance in the 500 pm range). It could be that, in addition to the C-terminal amide (which should be involved as suggested by the methylation effect), a closer amide group (the N-terminal amide at a $O_{carbonyl}$-HCγ distance of 319 pm or the central one at a distance of 437 pm) may also be involved in the mechanism. This could be made easier in such dipeptides by a partial delocalization of the excitation within the three BB amides, as illustrated by the electronic structures of the three nπ* states of GFa 77-, shown in Figure 5. The significant inter-amide delocalization, found for S_3 and S_4 at the ππ* geometry, presumably aided by the presence of amide-bridging H-bonds, could extend the amide excitations to regions of high ππ*/nπ* coupling (where CO and Phe are close) and finally contribute to enhance the dynamics of these 77- forms.

Excitation of the 6a /6b vibronic bands.

Excitation on these vibronic bands leads to very different initial conditions for the excited compared to excitation at the origin of phenyl, since the initial vibration content of the system is no longer exclusively in the low frequency motion involving side chain and backbone torsions, but also in the phenyl chromophore through an asymmetric in-plane deformation of the ring. It is therefore possible that different mechanisms are at play in the 6 vibrations compared to the origin of Phe. In addition, one should also consider that intramolecular vibrational redistribution (IVR) can also take place in the S_1 state of such medium-sized molecules. Seminal experiments on IVR by Smalley and coworkers[38-40] have indeed demonstrated, in very similar systems (alkylbenzenes), that this phenomenon is expected to occur at the ns timescale as soon as the molecule is large enough, even on the low lying vibrations like the 6b.

Putting again the 77- forms apart, dipeptide dynamics on the 6 bands turn out to be significantly faster than at the origin (Table 1), although remaining in the same order of magnitude whatever the species considered (Table 1). The same trends as at the origin are observed among the conformers, as well as longer lifetimes in the methylated corresponding species. Such a behavior can be rationalized assuming the same mechanism as at the origin and even enhanced by an increased vibrational content, arising from IVR, illustrated by the indirect scheme (1)

$$(\pi\pi^*)_{Phe}\ 6^1[q_i]^0 \xrightarrow{IVR} (\pi\pi^*)_{Phe}\ 6^0[q_i]^{ni} \xrightarrow{CI} (n\pi^*)_{amide}[q'_i]^{n'i} \quad (1)$$

where $[q_i]$ designates the "bath" of low frequency modes of the molecule.

The larger excess energy above the ππ* minimum, distributed among the bath of low frequency modes $\mathbf{q_i}$, would facilitate the access to the CI leading to a faster decay. An alternative more direct process:

$$(\pi\pi^*)_{Phe}\ 6^1\ [q_i]^0 \xrightarrow{CI} (n\pi^*)_{amide}[q'_i]^{n'i} \quad (2)$$

should probably be excluded, since, according to the $(\pi\pi^*)_{Phe} \rightarrow (n\pi^*)_{amide}$ EET model described above, essentially distortions of the backbone moieties are required to approach the CI.[24]

Additionally, one will notice that, despite IVR is suspected, no interconversion takes place in the ππ* state since conformer-specific lifetimes are observed. This is in line with the typical barriers in these systems (at least 3 kcal/mol, for Phe side chain rotation in NAPA, determined at the B97-D/TZVPP level of theory). The diversity of dynamical behaviors as well as the slightly faster dynamics after excitation of the 6 vibronic bands (in species other than 77-) should be ascribed to conformation-dependent couplings at the CI together with a significant sensitivity of the barrier crossing to the population of low frequency modes (through IVR).

The 77- conformers, on the other hand, behave differently with a much shorter-lived ππ* state on the 6 bands than at the origin. Such a sharp contrast with the other species suggests, as already evoked above, that the mechanism responsible for the fast dynamics observed might not be the same as at the origin. Several points plead in favor of a second mechanism:

- the observed dynamics does not depend on the methylation on the C-terminal,
- the short sub-ns ππ* lifetime, qualitatively estimated in the ~100 ps range or less (based on the poor efficiency of the photoionization detection of 77-), which is more than two orders of magnitude shorter than at the origin,
- the line broadening of the 77- progression lines, which corresponds to a ~10 ps time scale, too short to be consistent with that reported for IVR in excited alkylbenzenes on the 6b vibration,[38-40]
- Since mainly amide and backbone/side chain motions are efficient in triggering the Phe → BB EET, assigning the fast dynamics to this EET would require IVR to occur.[24] Owing to the time scale anticipated for IVR (ns range), the dynamics observed should be at longer timescale than ns, at odd with experimental data.

Considering these points, it seems quite probable that a convenient vibrational excitation of phenyl (6b) makes

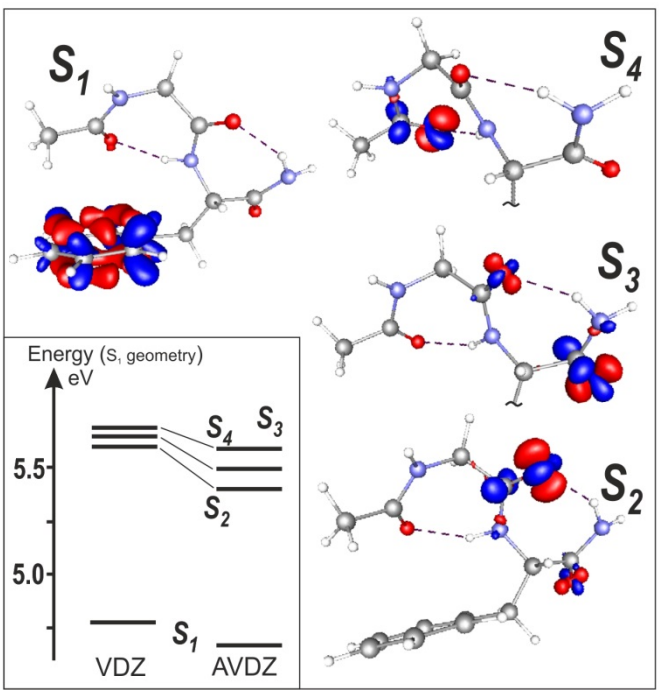

Figure 5: The four excited states of conformer A (77-) of GFa as obtained at the RI-CC2 level of theory, at the geometry of the first excited state S_1 ($\pi\pi^*_{Phe}$). The isosurfaces shown represent the difference in electron density between the excited state considered and the ground state: the blue color (resp. red) corresponds to an increase (decrease) in density. S_1 is assigned to the optically active (and experimentally excited) Phe ππ* state. The three next states correspond to BB LE nπ* states, involving a non-bonding oxygen lone pair n orbital and an anti-bonding π* amide orbital, with some delocalization between the second and third amides observed for the two lowest S_2 and S_3 states. RI-CC2 vertical energies (eV) for the ππ* state geometry, given in the insert for two basis sets : cc-pVDZ (VDZ) and aug-cc-pVDZ (AVDZ), shows that the three BB LE are nearly degenerate.

dominant a second mechanism in the 2_7 ribbon structures, according to a direct scheme (2). In this respect, the mechanism involving a BB → Phe electron transfer followed by a proton transfer from an electron depleted amide NH group, which was previously described theoretically on NAPA (Mechanism I, in Ref. 24) but not observed experimentally so far, could be a possible candidate (grey horizontal arrow in Fig. 4). This is further supported by the analysis of the CI geometry calculated for this mechanism in conformer 7+ of NAPA (Supp. Info. of Ref. 24), which exhibits a distortion of the phenyl ring having qualitatively similar features as the 6b vibration, namely a significant stretching of the carbon ring along the axis containing the Cδ carbon atom, on which proton transfer occurs, and its opposite C counterpart in the ring.

Conclusions

The present paper takes advantage of conformer-selective laser spectroscopy of isolated peptides in a supersonic expansion to document the photophysics of a Phenylalanine residue as a function of its peptide environment. The pump-probe 2C-R2PI experiments allows us to report the observation of a large diversity of lifetimes for the excited ππ* state of Phe as a function of the peptide conformation. Corresponding lifetimes at the origin of the first ππ* state are ranging from toluene-like (~ 80 ns) down to sub-ns range, demonstrating the occurrence of an additional deactivation mechanism, apart from ISC or fluorescence.

Emphasis should be first put on the quenching effect of the primary amide (-$CONH_2$ on C-terminals), which enhances deactivation, through a mechanism assigned to a Dexter-type excitation energy transfer from the phenyl ring to the amide, and makes it comparable to or faster than ISC. Such a process is expected to also be at play in presence of residues possessing a primary amide in their side chain, namely asparagine and glutamine.

Beyond this first mechanism, enhanced in primary amide-containing peptides, the observation of specific conformations exhibiting much shortened lifetimes compared to toluene in *both* methylated *and* non-methylated forms suggest that a second deactivation process should be at play, involving a distortion of the phenyl ring. The observation of deactivation mechanisms active in methylated species (namely in 7+ NAPA/NAPMA or 77- GFa/m conformers) endows them with a more general scope, since they should occur in any peptide environment, in particular in proteins.

The clear occurrence of a fast deactivation process capable of overcoming, in specific conformations, those naturally occurring in the isolated chromophore, i.e., ISC and fluorescence, qualitatively supports the recent findings of excited state quantum chemistry studies,[1-5, 48] which have emphasized the potential role of backbone locally excited states and of conical intersections between the several excited states of peptides and proteins. Obviously quantum chemistry calculations of the excited state of the present model species, currently underway, should allow us to ascertain the present tentative excited state assignments.

Finally the competition between the presently evidenced fast dynamical channel and ISC or radiative relaxation, should lead to various deactivation pathways and subsequent population of excited states, depending on the conformation considered. This point might be of some importance for the spectroscopy of peptide ions,[10, 11] in which absorption is monitored through the observation of specific fragmentation channels. Such specific fragmentation channels, e.g., a local Cα-Cβ bond cleavage within the Phe side chain, which rely on the existence of excited states localized on the phenyl ring, might actually be strongly dependent on the peptide conformation, affecting the general character of the diagnostics considered. The presence of a nearby amide group could for example enhance fragmentation within the amide moiety if long-lived amide LE states are populated, or, in contrast, give rise to a fragmentation pattern similar to that induced by collisions, if these LE states relax to the ground state via conical intersections.

Acknowledgements

MA acknowledges financial support for his post-doctoral fellowship from the Fondation de Coopération Scientifique "Campus Paris-Saclay", through the Inter-Labex PALM-CHARMMMAT Grant "LALIFOLD2013-0272I", under the Program Investing in the Future (Grant n°ANR-11-IDEX-0003-02), funded by the French National Research Agency.

Notes and references

[a] *CEA, IRAMIS, Laboratoire Interactions, Dynamique et Lasers ; CEA Saclay, Bât 522, 91191 Gif-sur-Yvette, France*

[b] *CNRS, INP, Laboratoire Francis Perrin, URA 2453, CEA Saclay, Bât 522, 91191 Gif-sur-Yvette, France*

Electronic Supplementary Information (ESI) available: [Ac-Ala-Phe-NH_2 and Ac-Ala-PheNHMe: UV spectra and lifetimes]. See DOI: 10.1039/b000000x/

1. A. L. Sobolewski and W. Domcke, *PCCP*, 2010, **12**, 4897-4898.
2. A. L. Sobolewski and W. Domcke, *Chemphyschem*, 2006, **7**, 561-564.
3. D. Shemesh, A. L. Sobolewski and W. Domcke, *J. Am. Chem. Soc.*, 2009, **131**, 1374-1375.
4. D. Shemesh, A. L. Sobolewski and W. Domcke, *PCCP*, 2010, **12**, 4899-4905.
5. W. Domcke and A. L. Sobolewski, *Nat Chem*, 2013, **5**, 257-258.
6. K. Kleinermanns, D. Nachtigallova and M. S. de Vries, *Int. Rev. Phys. Chem.*, 2013, **32**, 308-342.
7. V. Sauri, J. P. Gobbo, J. J. Serrano-Perez, M. Lundberg, P. B. Coto, L. Serrano-Andres, A. C. Borin, R. Lindh, M. Merchan and D. Roca-Sanjuan, *J. Chem. Th. and Comput*, 2013, **9**, 481-496.
8. A. L. Sobolewski, W. Domcke and C. Hattig, *PNAS*, 2005, **102**, 17903-17906.

9. H. Kang, C. Jouvet, C. Dedonder-Lardeux, S. Martrenchard, G. Grégoire, C. Desfrançois, J. P. Schermann, M. Barat and J. A. Fayeton, *PCCP*, 2005, **7**, 394-398.
10. J. A. Stearns, S. Mercier, C. Seaiby, M. Guidi, O. V. Boyarkin and T. R. Rizzo, *J. Am. Chem. Soc.*, 2007, **129**, 11814-11820.
11. T. R. Rizzo, J. A. Stearns and O. V. Boyarkin, *Int. Rev. Phys. Chem.*, 2009, **28**, 481-515.
12. D. Reha, H. Valdés, J. Vondrášek, P. Hobza, A. Abu-Riziq, B. Crews and M. S. de Vries, *Chem.-Eur. J.*, 2005, **11**, 6803-6817.
13. H. Valdés, D. Řeha and P. Hobza, *J. Phys. Chem. B*, 2006, **110**, 6385-6396.
14. G. Grégoire, C. Jouvet, C. Dedonder and A. L. Sobolewski, *Chem. Phys.*, 2006, **324**, 398-404.
15. G. Grégoire, C. Jouvet, C. Dedonder and A. L. Sobolewski, *J. Am. Chem. Soc.*, 2007, **129**, 6223-6231.
16. T. Hashimoto, Y. Takasu, Y. Yamada and T. Ebata, *Chem. Phys. Lett.*, 2006, **421**, 227-231.
17. Y. H. Lee, J. W. Jung, B. Kim, P. Butz, L. C. Snoek, R. T. Kroemer and J. P. Simons, *J. Phys. Chem. A*, 2004, **108**, 69-73.
18. C.-M. Tseng, M.-F. Lin, Y. L. Yang, Y. C. Ho, C.-K. Ni and J.-L. Chang, *PCCP*, 2010, **12**, 4989–4995.
19. J. P. Simons, R. A. Jockusch, P. Çarçabal, I. Hung, R. T. Kroemer, N. A. Macleod and L. C. Snoek, *Int. Rev. Phys. Chem.*, 2005, **24**, 489-531.
20. C. G. Hickman, J. R. Gascooke and W. D. Lawrance, *J. Chem. Phys.*, 1996, **104**, 4887-4901.
21. T. G. Dietz, M. A. Duncan and R. E. Smalley, *J. Chem. Phys.*, 1982, **76**, 1227-1232.
22. C. S. Burton and W. A. Noyes, *J. Chem. Phys.*, 1968, **49**, 1705-1714.
23. W. Chin, M. Mons, J. P. Dognon, R. Mirasol, G. Chass, I. Dimicoli, F. Piuzzi, P. Butz, B. Tardivel, I. Compagnon, G. von Helden and G. Meijer, *J. Phys. Chem. A*, 2005, **109**, 5281-5288.
24. M. Mališ, Y. Loquais, E. Gloaguen, H. S. Biswal, F. Piuzzi, B. Tardivel, V. Brenner, M. Broquier, C. Jouvet, M. Mons, N. Došlić and I. Ljubić, *J. Am. Chem. Soc.*, 2012, **134**, 20340-20351.
25. M. Mališ, Y. Loquais, E. Gloaguen, C. Jouvet, V. Brenner, M. Mons, I. Ljubić and N. Došlić, *PCCP*, 2014, **16**, 2285-2288.
26. V. Lepère, B. Lucas, M. Barat, J. A. Fayeton, V. J. Picard, C. Jouvet, P. Çarçabal, I. Nielsen, C. Dedonder-Lardeux, G. Grégoire and A. Fujii, *J. Chem. Phys.*, 2007, **127**, 11.
27. O. V. Boyarkin, S. R. Mercier, A. Kamariotis and T. R. Rizzo, *J. Am. Chem. Soc.*, 2006, **128**, 2816-2817.
28. S. R. Mercier, O. V. Boyarkin, A. Kamariotis, M. Guglielmi, I. Tavernelli, M. Cascella, U. Rothlisberger and T. R. Rizzo, *J. Am. Chem. Soc.*, 2006, **128**, 16938-16943.
29. Y. Xie, H. F. Schaefer, III, R. Silaghi-Dumitrescu, B. Peng, Q.-s. Li, J. A. Stearns and T. R. Rizzo, *Chem.-Eur. J.*, 2012, **18**, 12941-12944.
30. J. A. Stearns, C. Seaiby, O. V. Boyarkin and T. R. Rizzo, *PCCP*, 2009, **11**, 125-132.
31. J. A. Stearns, O. V. Boyarkin and T. R. Rizzo, *J. Am. Chem. Soc.*, 2007, **129**, 13820-13821.
32. M. Guidi, U. J. Lorenz, G. Papadopoulos, O. V. Boyarkin and T. R. Rizzo, *J. Phys. Chem. A*, 2009, **113**, 797-799.
33. A. Zabuga, M. Kamrath, O. Boyarkin and T. R. Rizzo, Proceedings of the IBBI-14 Conference on "Isolated Biomolecules and Biomolecular Interactions" , Porquerolles, France, 2014.
34. M. Guidi, Ph. D. thesis, EPFL, 2010.
35. W. Chin, J. P. Dognon, F. Piuzzi, B. Tardivel, I. Dimicoli and M. Mons, *J. Am. Chem. Soc.*, 2005, **127**, 707-712.
36. W. Chin, J. P. Dognon, C. Canuel, F. Piuzzi, I. Dimicoli, M. Mons, I. Compagnon, G. von Helden and G. Meijer, *J. Chem. Phys.*, 2005, **122**, 054317.
37. Y. Loquais, E. Gloaguen, S. Habka, V. Vaquero, B. Tardivel, V. Brenner and M. Mons, *in preparation.*
38. J. B. Hopkins, D. E. Powers, S. Mukamel and R. E. Smalley, *J. Chem. Phys.*, 1980, **72**, 5049-5061.
39. J. B. Hopkins, D. E. Powers and R. E. Smalley, *J. Chem. Phys.*, 1980, **73**, 683-687.
40. J. B. Hopkins, D. E. Powers and R. E. Smalley, *J. Chem. Phys.*, 1980, **72**, 5039-5048.
41. W. Chin, F. Piuzzi, I. Dimicoli and M. Mons, *PCCP*, 2006, **8**, 1033-1048.
42. M. Gerhards, C. Unterberg, A. Gerlach and A. Jansen, *PCCP*, 2004, **6**, 2682-2690.
43. E. B. J. Wilson, *Phys. Rev.*, 1934, **45**, 706.
44. A. Bernhardsson, N. Forsberg, P. A. Malmqvist, B. O. Roos and L. Serrano-Andres, *J. Chem. Phys.*, 2000, **112**, 2798-2809.
45. S. R. Long, J. T. Meek and J. P. Reilly, *J. Chem. Phys.*, 1983, **79**, 3206-3219.
46. E. Sekreta and J. P. Reilly, *Chem. Phys. Lett.*, 1988, **149**, 482-486.
47. Z.-Q. You and C.-P. Hsu, *Int. J. Quantum Chem.*, 2014, **114**, 102-115.
48. D. Shemesh and W. Domcke, *Chemphyschem*, 2011, **12**, 1833-1840.