

Silver Cluster Chromophores for Absorption Enhancement of Peptides[†]

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Received: October 15, 2008; Revised Manuscript Received: December 11, 2008

We present a theoretical study of the structural and optical properties of tripeptide–silver cluster hybrid systems which shows that silver clusters induce significant absorption enhancement in the spectral region between 225 and 350 nm with respect to the pure peptide. This allows the use of clusters as chromophores for absorption enhancement of peptides and proteins and offers a potential for different applications in biosensing. Furthermore, we demonstrate that cluster binding can change the conformational preference for the secondary structure type leading possibly to new functional properties.

Introduction

The combination of the unique optical properties of silver clusters with the unique functional and recognition properties of biomolecules makes their hybrid systems attractive for sensorics, bioanalytic, and bioelectronic devices.^{1,2} This active and fascinating research area was mainly based on the idea of similar dimensionality between metallic nanoparticles and proteins. The reduction of the size of nanoparticles leads to the transition from the collective plasmonic excitations to the molecular-like excitations as recently demonstrated theoretically by Schatz et al.^{3–5}

Since the small silver clusters embedded in different environments were found to exhibit light emission under UV–vis excitation^{6,7} and the molecular nature of excitations in the cluster size regime in which “each atom counts” was theoretically identified,^{8–10} such molecular-like cluster chromophores become increasingly important as optical probes for the biomolecular environment.^{11–13} For example, Dickson et al. have demonstrated that in the absence of large plasmon supporting nanoparticles few atom silver clusters encapsulated in biocompatible environment produce strong scaffold specific single molecule Stokes and anti-Stokes Raman scattering.¹² Furthermore, Dickson et al. reported the silver nanocluster emission within living cells where silver clusters were bound to nucleolus proteins.¹³ The advantage of small silver clusters as cell imaging agents lies in their very bright emission, which takes place on a much faster time scale than the emission from the organic dyes. Recently, DNA microarrays were used to create new silver cluster fluorophores with specific spectral and photophysical properties which offer a perspective for optimization of new nanocluster based emitters for single molecule spectroscopy and imaging which are superior to commonly used organic dyes.^{14,15}

To gain a fundamental understanding of the structural and optical properties of cluster–biomolecule hybrid systems the gas phase approach on small prototypes is valuable since it provides experimental and theoretical information on well-defined molecular-like systems without the influence of the environment. Our joint experimental and theoretical work on Trp–Ag_n⁺ and Trp–Gly–Ag₃⁺ allowed us to demonstrate that silver clusters can induce absorption enhancement and conformational control of model peptides.^{16–18} We have chosen to

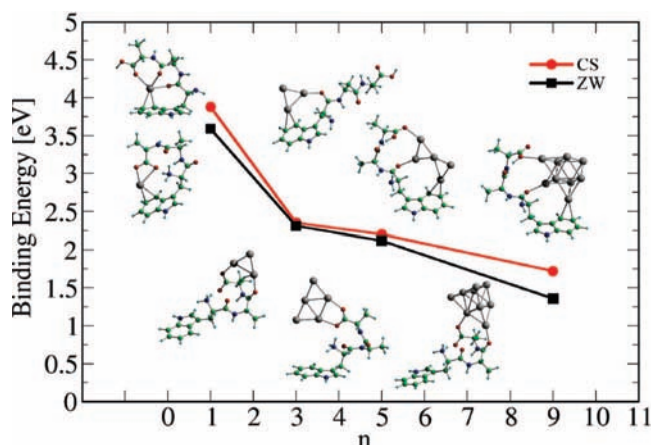


Figure 1. Binding energies (in eV) of Trp-(Ala)₂-Ag_n⁺ ($n = 1, 3, 5, 9$) as a function of the cluster size for the lowest energy charge solvated (CS) structures. The binding energies for the selected zwitterionic (ZW) structures are also shown. Optimized structures for both classes are also given.

investigate cationic systems since they are naturally formed in a solution of AgNO₃ due to the nucleation process starting with Ag⁺. In fact, recent experiments have shown that the Ag₃⁺ species is also present in solution and serves as a nucleation center for the growth of larger species.^{19,20} Moreover the binding energy of cationic clusters is stronger than that for neutral and anionic clusters.

The aim of this contribution is to study theoretically structural and optical properties of silver cluster–peptide hybrid systems at the molecular level on a prototype example of the interaction between the Trp-(Ala)₂ tripeptide containing the most important chromophoric amino acid tryptophan with small cationic silver species (Ag_n⁺, $n = 1, 3, 5, 9$). The results provide conceptual understanding of the absorption enhancement and change of the conformational landscape in silver cluster–peptide hybrid systems.

Computations

The structural properties and stationary absorption spectra of Trp-(Ala)₂-Ag_n⁺ ($n = 1, 3, 5, 9$) have been determined by using density functional theory with the hybrid B3LYP functional.^{21,22} The search for structures was performed exten-

[†] Part of the “George C. Schatz Festschrift”.

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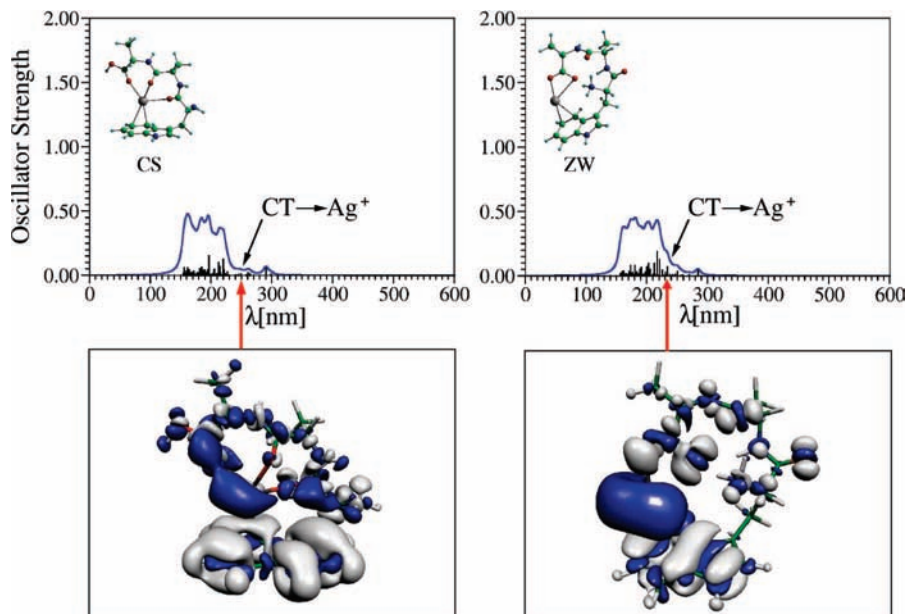


Figure 2. Calculated absorption spectra for the lowest energy CS and ZW structure of Trp-(Ala)₂Ag⁺. In the lower part the density difference between the selected electronic excited state and the ground state is shown. The broadening of the lines is simulated by a Lorentzian function with the width of 10 nm (blue curves).

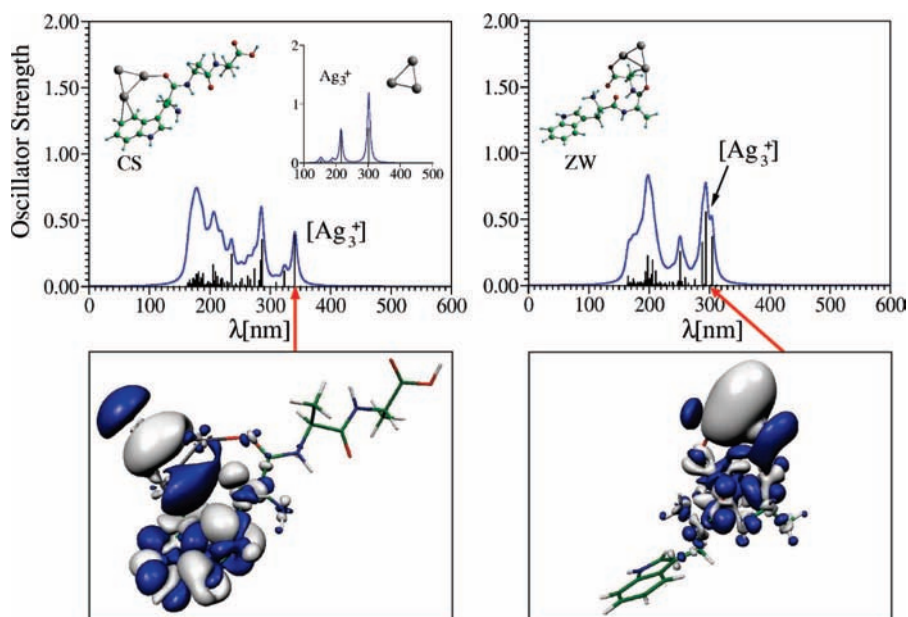


Figure 3. Calculated absorption spectra for the lowest energy CS and ZW structure of Trp-(Ala)₂Ag₃⁺. In the insert the absorption spectrum for pure Ag₃⁺ is given. In the lower part the density difference between the selected electronic excited state and the ground state is shown. The broadening of the lines is simulated by a Lorentzian function with the width of 10 nm (blue curves).

sively by the simulated annealing procedure coupled with molecular dynamics simulations with use of the semiempirical AM1 method.²³ For this purpose, the forces needed for the MD simulations were calculated with the semiempirical AM1 method for the electronic structure. During the MD simulations the initially high temperature (typically around 800 K) has been decreased until the system has been cooled to 50 K. The found structures were subsequently reoptimized at the B3LYP level by using the 19 electron relativistic core potential (19e-RECP) from the Stuttgart group for silver.²⁴ For light atoms the 6-311G** atomic orbital basis set augmented by diffuse function for adequate description of the excited states was used.

The absorption spectra were obtained from time-dependent density functional theory (TDDFT) using the B3LYP functional

and our 11e-RECP with the corresponding AO basis set which has been developed for an adequate description of excited states of silver clusters.^{8,9,25} According to our experience, the absorption spectra using the B3LYP functional for pure silver clusters are in good agreement with the more accurate wave function based EOM-CCSD approach. The lack of an accurate description of the long-range charge transfer transitions within the commonly used functionals is known. However, the transitions in hybrid systems studied in this contribution are mainly due to excitations within the clusters and the charge neutralization between the cluster subunit and the biomolecule is of short-range nature and therefore is not considerably sensitive on the choice of the functional.

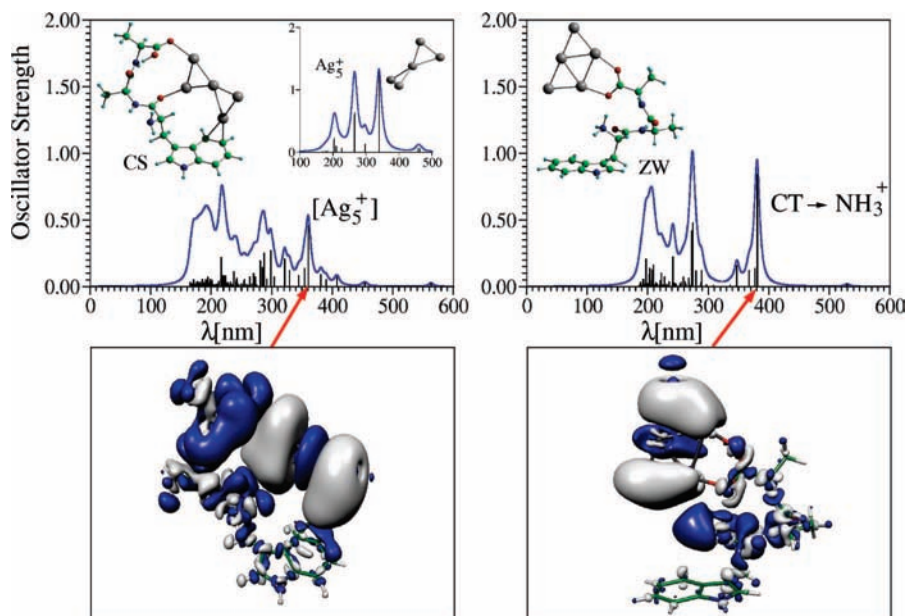


Figure 4. Calculated absorption spectra for the lowest energy CS and ZW structure of Trp-(Ala)₂Ag₅⁺. In the insert the absorption spectrum for pure Ag₅⁺ is given. In the lower part the density difference between the selected electronic excited state and the ground state is shown. The broadening of the lines is simulated by a Lorentzian function with the width of 10 nm (blue curves).

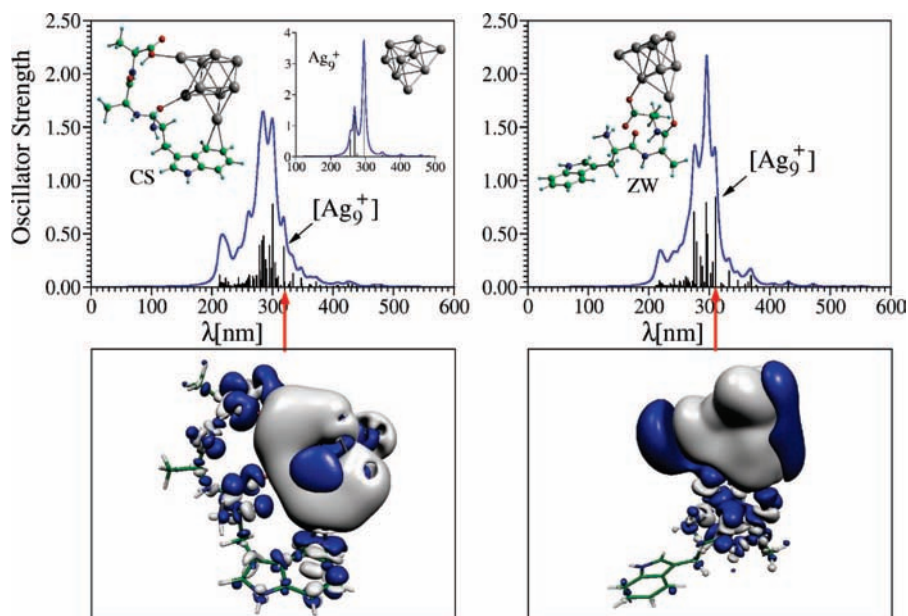


Figure 5. Calculated absorption spectra for the lowest energy CS and ZW structure of Trp-(Ala)₂Ag₉⁺. In the insert the absorption spectrum for pure Ag₉⁺ is given. In the lower part the density difference between the selected electronic excited state and the ground state is shown. The broadening of the lines is simulated by a Lorentzian function with the width of 10 nm (blue curves).

Results and Discussion

The structures of Trp-(Ala)₂-Ag_{*n*}⁺ (*n* = 1, 3, 5, 9) can be classified according to two classes: charge solvated (CS) and zwitterionic (ZW), as shown in Figure 1. The CS type of structure is characterized by the interaction between the positively charged silver species, the indole ring of tryptophan, and free electron pairs which are localized on the carbonyl oxygen atoms belonging to the peptide group. In contrast, the ZW type of structure is characterized by the presence of the negatively charged carboxy group (COO⁻) interacting with the positively charged cluster as well as the positively charged NH₃⁺ group.

The binding energies show slightly higher stability of the CS structures with binding energies ranging from 4 eV for Trp-

(Ala)₂-Ag⁺ to 1.8 eV for Trp-(Ala)₂-Ag₉⁺. Moreover, in the case of Trp-(Ala)₂-Ag⁺ and Trp-(Ala)₂-Ag₉⁺ there are several energetically low-lying isomers belonging to the CS class of structures which are lower in energy than the shown lowest energy ZW structures (cf. the Supporting Information). Notice that the binding is particularly strong for the Ag⁺ cation due to high coordination to the carboxyl and carbonyl group of the peptide bond and the high electron affinity of Ag⁺ in contrast to larger clusters.

To investigate the influence of silver clusters on the optical absorption of peptides we present for both types of structure classes (CS and ZW) the absorption spectra as a function of the cluster size in Figures 2–5 together with the analysis of the characteristic excitations for selected electronic transitions.

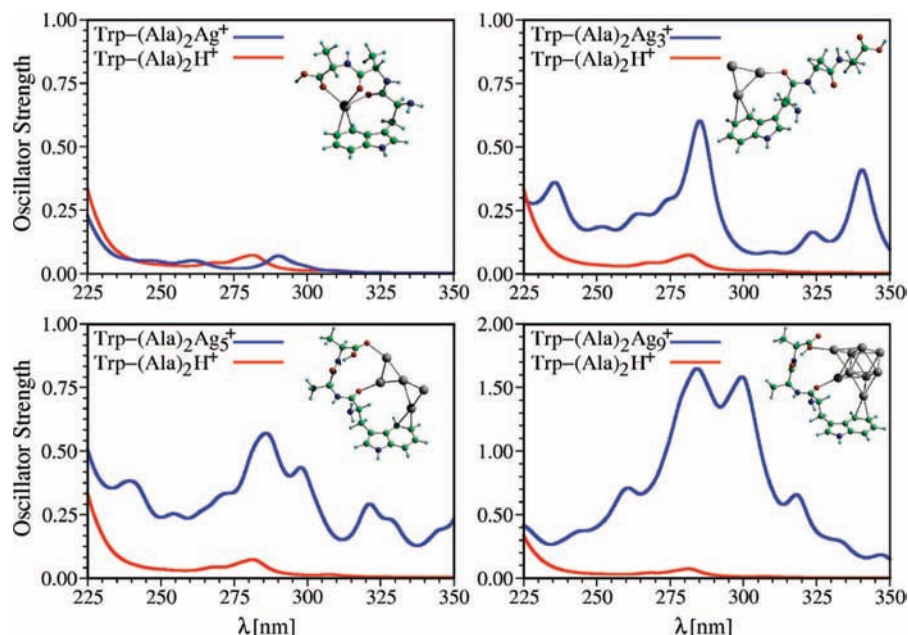


Figure 6. Comparison of the calculated absorption spectrum of the pure $\text{Trp}-(\text{Ala})_2\text{H}^+$ peptide with the absorption spectra of the $\text{Trp}-(\text{Ala})_2\text{Ag}_n^+$ ($n = 1, 3, 5, 9$) hybrid systems. The broadening of the lines is simulated by a Lorentzian function with the width of 10 nm (blue and red curves).

The absorption spectra of $\text{Trp}-(\text{Ala})_2-\text{Ag}^+$ for both types of structures shown in Figure 2 are characterized by low-lying weak transitions which involve the charge transfer from peptide into the empty s-orbital of the Ag^+ ion as can be seen from the electron density difference plots between the ground and excited states presented in the lower part of Figure 2.

In contrast, in the case of $\text{Trp}-(\text{Ala})_2-\text{Ag}_3^+$ the role of the clusters is evident as shown in Figure 3 and leads to strong absorption in the region between 300 and 350 nm for the CS structure and around 300 nm for the ZW structure. This intense absorption results from the excitations within the Ag_3^+ cluster and coupling with the $\pi-\pi^*$ excitations of the indole ring in the case of the CS structure. In the case of the ZW structure the charge transfer between the cluster and the NH_3^+ subunit is not present due to the large distance between the two groups. Consequently, the intense transition located around 300 nm is due to the excitations mainly within Ag_3^+ . The nature of excitations for the selected transitions can be depicted from the electron density differences between the ground state and corresponding excited states shown in the lower part of Figure 3.

By increasing the cluster size, the connection between the structural properties of $\text{Trp}-(\text{Ala})_2-\text{Ag}_5^+$ for CS and ZW structures and absorption patterns shown in Figure 4 is evidenced by the different nature of the intense low-energy transitions. The spectrum of pure Ag_5^+ given in the insert of Figure 4 serves to identify common spectral features. In the case of the CS structure, the intense transition located at 360 nm is mainly due to excitation within the cluster while the lowest intense transition of the ZW structure located at 380 nm has a typical charge transfer character from the cluster to the NH_3^+ group as shown in the lower part of Figure 4.

Notice that in the cluster size regime in which each atom counts, addition of one or a few atoms changes substantially the structural and optical properties of these species. Transition from planar 2D to 3D structure for cationic silver clusters Ag_n^+ occurs between $n = 6$ and 7. Here we illustrate the influence of the cluster size and the structural properties on the example of $\text{Trp}-(\text{Ala})_2\text{Ag}_9^+$. Since Ag_9^+ assumes a three-dimensional

structure, typically the intense transitions are condensed around 300 nm. In fact this is also the case in the hybrid systems for both CS and ZW structure as shown in Figure 5. For the CS structure the excitations responsible for intense transitions are located within the Ag_9^+ subunits, as expected. In the case of the ZW structure the dominant intense transition at 310 nm is also mainly due to excitations within the cluster, because the cluster is not in the vicinity of the NH_3^+ group. However, the next intense transition at 295 nm is dominated by charge transfer from the cluster to the NH_3^+ group, which is typical for ZW structures in which the vicinity of the cluster and the NH_3^+ group is more pronounced.

The radiative lifetimes estimated for low-energy intense transitions lie in the range from approximately 4 to 1.7 ns for $\text{Trp}-(\text{Ala})_2-\text{Ag}_3^+$ and $\text{Trp}-(\text{Ala})_2-\text{Ag}_9^+$, respectively. They are generally shorter than those estimated for common organic chromophores. This might be one of the factors responsible for the bright fluorescence of silver clusters in the biomolecular environment.¹³

To address the role of the silver clusters on enhancement of the absorption properties of peptides, the comparison of spectra of the pure peptide and cluster-peptide hybrids is given in Figure 6. As can be seen, in the case of the $\text{Trp}-(\text{Ala})_2-\text{Ag}^+$ the single silver atom does not lead to significant enhancement of the overall optical absorption of the peptide. In contrast, in all three cases of $\text{Trp}-(\text{Ala})_2-\text{Ag}_n^+$ ($n = 3, 5, 9$) the enhancement of the absorption of the peptide due to the cluster is not only in the wavelength regime where the cluster absorbs but extends over the whole spectral region between 225 and 350 nm, thus significantly enhancing the absorption of the tripeptide. Such absorption enhancement induced by the silver clusters can be utilized in bioanalytic applications where the cluster serves as an optical marker.

Another interesting issue is the influence of the cluster on the conformational landscape of the peptide. To address this issue we have run constant temperature MD simulations at $T = 300$ K for $\text{Trp}-(\text{Ala})_2$ and $\text{Trp}-(\text{Ala})_2-\text{Ag}_n^+$ ($n = 3, 5, 9$). The results of these simulations have been projected onto the two-dimensional Ramachandran plot for the $\text{Trp}-(\text{Ala})_2$ in order to

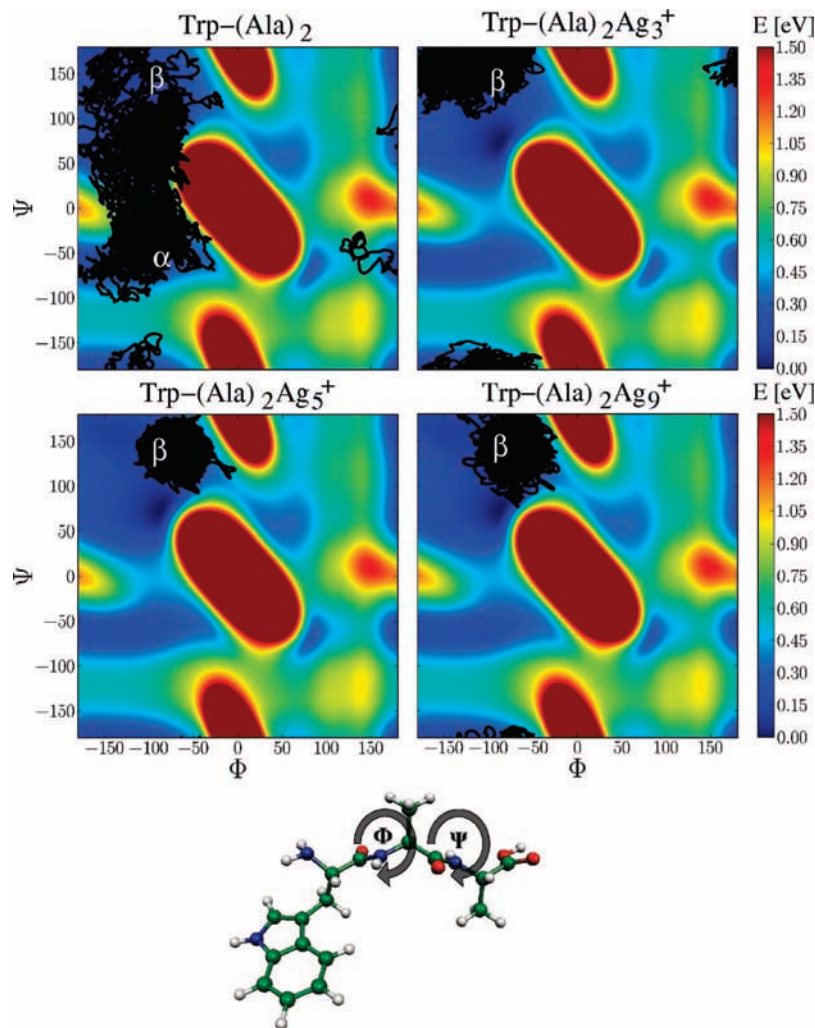


Figure 7. Ramachandran plots for the pure tripeptide Trp-(Ala)₂ and for the Trp-(Ala)₂Ag_n⁺ ($n = 3, 5, 9$) hybrid systems. The black lines in the plots represent MD trajectories at $T = 300$ K projected onto the two-dimensional space of the Φ and Ψ dihedral angles as defined in the lower part. The colors indicate the relative energy of a peptide configuration corresponding to each (Φ, Ψ) pair. The labels α and β represent regions in the plots corresponding to α -helix and β -sheet secondary structure.

identify the secondary structure regions which are populated in the pure tripeptide and in the cluster–tripeptide hybrids (Figure 7). In the case of the pure Trp-(Ala)₂ an equilibrium between the α -helix and β -sheet types of secondary structures exists. Upon binding of the clusters the conformational preference is shifted toward the β -sheet types of secondary structures. This demonstrates that the clusters can locally modify the conformations of peptides by immobilizing the part of the peptide that is involved in the interaction with the cluster. At the same time this local interaction region is also responsible for the enhanced and extended optical properties of cluster–peptide hybrid systems with respect to pure peptides.

Conclusion

Our presented theoretical findings show that the small silver clusters may be suitable for optical labeling as well as for inducing local conformational transitions in proteins. Moreover, the discrete molecular properties of silver cluster chromophores offer an attractive alternative to dyes commonly used for optical labeling. Furthermore, moving from the gas phase to the deposition of small silver clusters attached to functional proteins such as antibodies is an attractive possibility for developing new cluster based biochips.

Acknowledgment. We wish to thank to our experimental partners P. Dugourd, M. Broyer and R. Antoine for fruitful

cooperation. This work was supported by the Deutsche Forschungsgemeinschaft SFB 450.

Supporting Information Available: Figures showing the isomers of Trp-(Ala)₂-Ag_n⁺ ($n = 1, 3, 5, 9$) complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JP809118Q