JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

New Insight into the IR-Spectra/Structure Relationship in Amyloid Fibrils: A Theoretical Study on a Prion Peptide

Laura Zanetti Polzi,[†] Andrea Amadei,[‡] Massimiliano Aschi,[§] and Isabella Daidone*[,§]

[†]Department of Chemistry, University of Rome "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy

[‡]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy

[§]Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila, Via Vetoio (Coppito 1), 67010 L'Aquila, Italy

Supporting Information

ABSTRACT: Molecular-level structural information on amyloid aggregates is of great importance for the understanding of protein-misfolding-related deseases. Nevertheless, this kind of information is experimentally difficult to obtain. In this work, we used molecular dynamics (MD) simulations combined with a mixed quantum mechanics/molecular mechanics theoretical methodology, the perturbed matrix method (PMM), in order to study the amide I′ IR spectrum of fibrils formed by a short peptide, the H1 peptide, derived from residues 109 through 122 of the Syrian hamster prion protein. The PMM/MD approach allows isolation of the amide I′ signal arising from any desired peptide group of the polypeptide chain and quantification of the effect of the excitonic coupling on the frequency position. The calculated single-residue signals were found to be in good agreement with the experimental site-specific spectra obtained by means of isotope-labeled IR spectroscopy, providing a means for their interpretation at the molecular level. In particular, our results confirm the experimental hypothesis that residues ala117 are aligned in all strands and that the alignment gives rise to a red shift of the corresponding site-specific amide I′ mode due to strong excitonic coupling among the ala117 peptide groups. In addition, our data show that a red shift of the amide I′ band due to strong excitonic coupling can also occur for amino acids adjacent in sequence to the aligned ones. Thus, a red shift of the signal of a given isotope-labeled amino acid does not necessarily imply that the peptide groups under consideration are aligned in the β-sheet.

It is well-known that a number of proteins and peptides form large, fibrous structures that are often related to diseases (e.g., Creutzfeldt−Jakob, Alzheimers', Parkinson's). The peptides/proteins involved in these diseases (e.g., β-amyloid proteins[1] and Prion proteins[2]) have been extensively studied, and it has been shown that the overall morphologies of the amyloid fibrils have a high degree of similarity exhibiting a cross-β structure. The cross-β structure constitutes the spine of an amyloid fibril and consists of a stack of two β-sheets.[3] The different orientations of the β-sheets relative to one another have been also effectively analyzed.[4] Nevertheless, there is very little molecular-level structural information on amyloid aggregates because their fibrous nature and large size prevent the acquisition of satisfactory results from X-ray crystallography and NMR methods.

FTIR spectroscopy has been widely used over the past years to study amyloid and fibril formation,[5] as it can be used to probe changes in the secondary structure content on time scales ranging from nanoseconds to hours.[6] More detailed information can be obtained using the combination of IR spectroscopy and site-specific isotope labeling of the polypeptide backbone. With this approach, individual amide I′ modes of different residues within the secondary structure can be identified using isotopic substitution. In fact, when a backbone carbonyl is labeled with ^{13}C, the amide I′ band for that residue is shifted to a lower frequency by 20−30 cm^{-1}, creating a new feature in the IR spectrum. This ^{13}C amide I′ band can then be used as a site-specific probe of protein conformation and dynamics.[7] Nevertheless, the structural information obtained often does not have an unequivocal interpretation. The coupling of site-labeled IR spectroscopy and techniques for simulating vibrational spectra can be then very useful in order to better understand the structural characteristics of the aggregation process of amyloid fibrils. However, although a huge number of theoretical and computational studies focusing on the interpretation of amide I′ bands of secondary structure elements have been reported,[8−10] only very recently have the first results on the reconstruction of IR spectra of amyloid aggregates been obtained.[11,12]

We recently proposed and applied a mixed quantum mechanics/molecular dynamics (QM/MD) theoretical computational methodology based on the perturbed matrix method (PMM)[13−15] to calculate amide I′ IR spectra of peptides and proteins [see Theory and Methods in the Supporting Information (SI)]. The main aim of this approach is the reproduction of the essential physics underlying IR excitations without involving phenomenological parameters. In PMM calculations, as in other QM/MM procedures,[16] a portion of the system (the quantum center) is treated at an electronic level, while the rest of the system is described at a classical atomistic level and exerts an electrostatic perturbation on the quantum-center electronic states. As the method is based on classical MD to provide the phase space sampling, a statistically relevant sampling of the quantum-center/environment configurations can be achieved, which is necessary for a proper calculation of the spectra of complex systems, such as amyloids or peptide/protein unfolded states.[13,15] Other

Received: March 29, 2011
Published: June 22, 2011

useful characteristics of this approach are that the amide I′ signal arising from any desired peptide group of the polypeptide chain may be isolated and that the effect of the excitonic coupling on the spectral features can be quantified. However, since our methodology does not make use of empirical/adjustable parameters, it might be less accurate than other methodologies in reproducing some of the details of the spectroscopic signals.[9,10,12] The main differences between the PMM approach and other available methodologies for the calculation of IR spectra have been previously discussed in more detail.[14,15]

Here we used the PMM/MD approach to calculate the spectrum of an amyloid system, a fibrillar structure of the H1 peptide (see below), and to separate the contribution of specific residues to the amide I′ band. This allowed for a direct comparison with the experimental isotope-edited spectra, providing a means for their interpretation at the molecular level. The H1 peptide is a short 14-residue peptide with a sequence derived from residues 109−122 (Ac-MKHMAGAAAGAVV-NH_2) of the Syrian hamster prion protein. This peptide has been extensively studied[17] also by means of IR spectroscopy.[18−21] In particular, Silva et al.[20] reported an isotope-edited FTIR study on H1 peptide amyloid fibrils. They concluded that at equilibrium the strands form β-sheets in which only residues 112−122 (the hydrophobic core) participate in the sheet structure, resulting in the alignment of residue 117 in all of the strands. This hypothesis was based on the observation that the frequency of the isotope-shifted ^{13}C amide I′ band was similar for every position substituted within the hydrophobic core except for position 117, for which the resultant ^{13}C amide I′ band was further shifted to lower frequencies by ∼10 cm^{-1} relative to the other positions. This additional shift was attributed to strong excitonic coupling among the ala117 peptide groups due to the proximity of the corresponding carbonyls arising from a possible alignment of the ala117 residues in all strands; contrarily, the coupling was considered to be too weak to give rise to a frequency shift for the nonaligned positions.

In the construction of a model of the H1 peptide fibril, we thus aligned the ala117 residues in the strands of each β-sheet. Then, to arrange the sheets into a fibril, we followed the recent hypothesis that the first step in fibril formation can be identified as the tight mating of two sheets at a completely dry interface.[22] At this interface, the residue side chains intermesh with close complementarity in what the authors termed a "steric zipper". The same authors[22] identified eight classes of such steric zippers (i.e., eight possible arrangements of the mutual orientation of two closely interacting sheets). On the basis of previous experimental and computational results,[21] we assigned to the H1 peptide the class-6 steric zipper, in which the two antiparallel sheets are oriented "face-to-back" and both sheets have the same edge of the strand "up".[22] A schematic representation of the class-6 steric zipper as defined by Sawaya et al.[22] is shown in SI Figure 1. On the basis of the experimental hypothesis of a fibril height of ∼3 nm,[19] we constructed a four-sheet structure in which the initial intersheet distance was set at 7 Å, giving rise to a 2.8 nm thick fibril. An infinitely long fibril was finally modeled by replicating a structure made up of four sheets, each containing eight strands, along the axis of the fibril by means of periodic boundary conditions (Figure 1). The box dimensions were chosen to make the spacing between the edge strands of the central box and the edge strands of the periodic replica the same as the spacing between adjacent strands of the central-box sheets. A similar protocol was previously used to construct an infinite

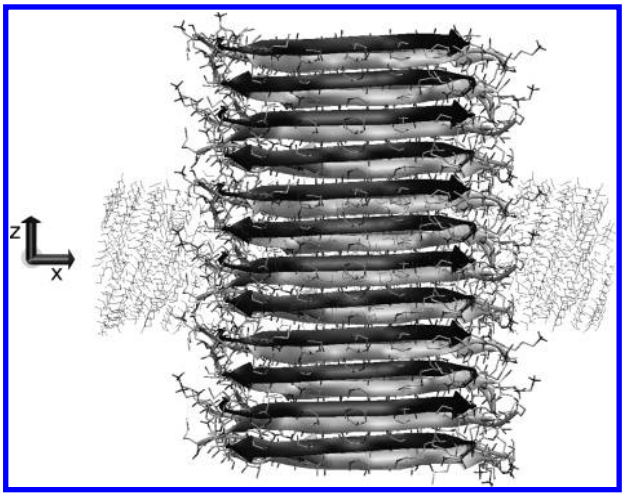

Figure 1. Initial configuration of the H1 fibril model. The simulated structure (four sheets along the *y* axis, each one made up of four strands along the *z* axis) surrounded by water molecules can be seen in the central part of the figure. The "infinite" fibril system was obtained by establishing periodic boundary conditions along the *z* axis. Two periodic images along the *z* axis are also shown.

fibril model of another amyloid peptide.[23] A 20 ns long MD simulation starting from an energy-minimized structure was performed at 300 K in D_2O in the *NPT* ensemble. The fibril structure was well-retained during the simulation (structural details of the fibril along the trajectory are given in SI Figure 2).

We also tested an alternative alignment in which the ala116 residues were aligned, following the same rules in the construction of the fibril model. This alignment was chosen because it results in a tighter packing of the strands with respect to the ala117-aligned case, with residues 110−122 participating in the sheet (see SI Figure 3A).

The PMM/MD procedure[13−15] was used to calculate the amide I′ spectra of the H1 peptide fibril in both alignments, which are shown in Figure 2 along with the experimental spectrum.[20] In the PMM calculations of the spectra, all of the residues belonging to the fibril of the central box [eight strands × four sheets (8 × 4)] were used. Both alignments resulted in calculated spectra that were in good agreement with the experimental spectrum, showing an intense main peak and a high-frequency secondary peak. The frequency shift between the main and secondary peaks in the computed spectra (∼32 cm^{-1}) was lower than in the experimental one (∼70 cm^{-1}). A convergence test revealed that the shift between the main peak and the high-frequency secondary peak depends on the length of the fibrillar sheets (see Figure 2B): when a smaller structure made up of four sheets each containing four strands (4 × 4) was considered, the shift was reduced to ∼20 cm^{-1}. Although longer sheets should be tested in order to achieve full convergence of the peak-to-peak distance, we expect from this trend to get closer to the experimental value of ∼70 cm^{-1} by increasing the length of the participating sheets. Indeed, previous studies of single β-sheets showed the frequency difference between the two peaks to be a function of the number of participating strands.[9,24] However, this was beyond the scope of the present work, as we will focus on frequency shifts of the main peak only (see below).

In order to reproduce and analyze the experimental site-specific data obtained by means of isotope-labeled IR spectroscopy,[20] we isolated the amide I′ signals arising from the different residues.

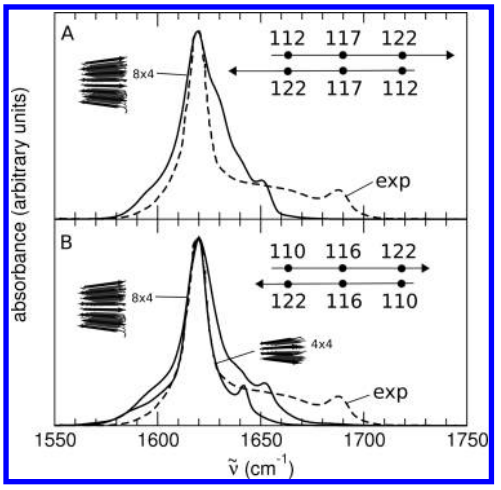

Figure 2. (A) Comparison of the experimental amide I′ spectrum of a fibrillar structure of the H1 peptide in D_2O (dashed line) and the calculated amide I′ spectrum of an 8 × 4 fibrillar structure of the same peptide in which the peptide groups of residue 117 in each sheet were aligned (solid line). (B) Comparison of the experimental spectrum of the H1 peptide in D_2O (dashed line) and the calculated amide I′ spectra (solid lines) of an 8 × 4 fibrillar structure and an additional 4 × 4 fibrillar structure in which the peptide groups of residue 116 in each sheet were aligned. The computed spectra have been shifted to lower frequencies by ∼84 cm^{-1} in order to align the main peak with the experimental one (at ∼1622 cm^{-1}).

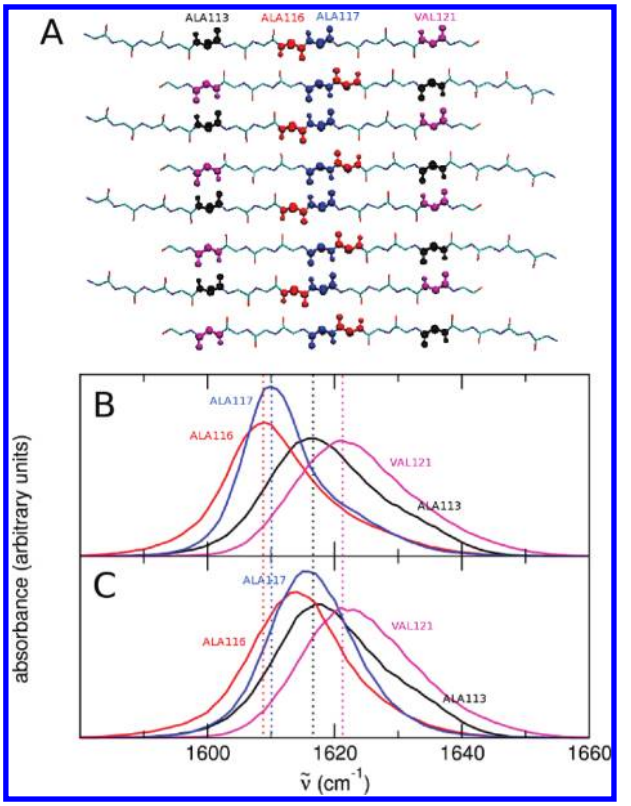

Figure 3. (A) Schematic structure of one β-sheet of the ala117-aligned fibril structure of the H1 peptide. (B, C) Calculated amide I′ modes of the residues highlighted in (A) with (B) and without (C) the inclusion of the excitonic coupling in the PMM calculations. Color code: black, ala113; red, ala116; blue, ala117; magenta, val121. Each dotted colored line points to the frequency of the peak of the corresponding residue when the excitonic coupling was included in the calculations. The same shift of ∼84 cm^{-1} used in the case of the total spectrum (see the Figure 2 caption) has been applied to all of the computed bands.

In what follows, we discuss our analysis of the ala117-aligned fibril. The contributions to the amide I′ band arising from a series of peptide groups (amino acids), namely, ala113, ala116, ala117, and val121 (see Figure 3A), are reported in Figure 3B. The spectra presented were calculated using the full procedure (i.e., including the excitonic coupling among the given peptide groups while the other residues and the solvent exert the perturbing effect). The data for ala113, ala117, and val121, the residues presented in the experimental work,[20] are in good agreement with the isotope-edited spectra. In fact, the ala117 signal was shifted to lower frequency with respect to the average peak position of the other two bands (ala113 and val121) by ∼9 cm^{-1} (vs ∼10 cm^{-1} in the experimental spectrum). Contrarily, for the ala116-aligned fibril (see SI Figure 3), the peak position of ala117 was approximately in the same frequency range as ala113 and val121. These data support the experimentally based hypothesis of an ala117-aligned fibril model.

To investigate the origin of the relative red shift of the ala117 band, the site-specific signals were recalculated excluding the excitonic coupling among the peptide groups of the residue under consideration. These are reported in Figure 3C. The peak positions of the two nonaligned residues did not vary upon the exclusion of the excitonic coupling, while the signal of the aligned residues (ala117) shifted by 7−8 cm^{-1} to higher frequencies (i.e., to the frequency range of ala113 and val121). Hence, the origin of the red shift of the ala117 residues resides in the coherent excitonic coupling within the ala117-aligned fibril model, in agreement with previous results on single β-sheets.[9,10]

However, the comparison between the data obtained with or without the inclusion of the excitonic coupling revealed an interesting new result. In fact, similar to ala117, a red shift upon the inclusion of the excitonic coupling was also observed for the peptide groups corresponding to residue ala116 (Figure 3B,C), for which, unfortunately, no experimental data are available. Although the peptide groups of ala116 were not aligned in the sheet structure, it can be observed that the corresponding carbonyls were in fairly close contact, the relative distance being just slightly higher than for the ala117 peptide groups (Figure 3A). This can explain the similar excitonic coupling, and thus the similar frequency shifts, observed for ala117 and ala116. Similar results were obtained from the analysis of the ala116-aligned fibril (see SI Figure 3). In fact, the signals of ala116 (the aligned ones) and ala115 (the preceding ones) shifted toward lower frequency upon the inclusion of the excitonic coupling, while the other bands did not change. Hence, our data suggest that the shift to lower frequencies because of the excitonic coupling occurs not only for the amide I′ bands of the aligned residues but also for the signals of the amino acids that precede the aligned residues in sequence.

An interesting aspect emerging from the calculation of the amide I′ bands disregarding any excitonic coupling (Figure 3C) is that the signals of the different residues showed a certain variability (6−7 cm^{-1}) in the frequencies of their main peaks. A similar variability is also present in the experimental site-specific bands of the nonaligned residues, although it is less pronounced (∼4 cm^{-1}). These data indicate that the excitonic coupling is not the only mechanism responsible for frequency shifts of amide I′ modes

and point to a role played by the environmental perturbation of each localized vibrational mode, similar to what we previously observed in the case of small β-hairpins.[13−15]

In conclusion, our results for the H1 peptide have confirmed the experimental hypothesis that residues ala117 are aligned in all strands and that the alignment gives rise to a red shift of the corresponding site-specific amide I′ mode due to strong excitonic coupling among the ala117 peptide groups. However, it appears from our data that a red shift of the amide I′ band of a given isotope-labeled residue does not necessarily imply that the peptide groups under consideration are aligned in the β-sheet. In fact, a strong excitonic coupling was also observed for amino acids adjacent in sequence to the aligned ones. We have also shown that the excitonic coupling is not the only source of frequency shifts of the amide I′ mode.

The combination of isotope-edited IR spectroscopy with the approach for the computation of IR spectra used herein is a particularly promising tool for obtaining insights into the molecular structures of peptides and proteins, particularly amyloid structures. For example, if the isotope-labeled amide I′ band of a given amino acid is red-shifted, to verify whether this implies that the peptide groups under consideration are aligned in the β-sheet, the amide I′ band of the preceding amino acid should also be measured and found to be red-shifted.

■ ASSOCIATED CONTENT

Supporting Information. Theoretical and computational details of the simulations, further information on the fibril structure, and computed spectra of the different peptide groups of the ala116-aligned fibril. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
daidone@caspur.it

■ ACKNOWLEDGMENT

We acknowledge CASPUR (Consorzio Interuniversitario per le Appplicazioni di Supercalcolo per Università e Ricerca) for the use of its computational facilities and its financial support with the project “Theoretical Study of Electron Transfer Reactions in Complex Atomic−Molecular Systems”.

■ REFERENCES

(1) (a) Masuda, Y.; Uemura, S.; Nakanishi, A.; Ohashi, R.; Takegoshi, K.; Shimizu, T.; Shirasawa, T.; Irie, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3206. (b) Olofsson, A.; Lindhagen-Persson, M.; Vestling, M.; Sauer-Eriksson, A.; Ohman, A. *FEBS J.* **2009**, *276*, 4051. (c) Ahmed, M.; Davis, J.; Aucoin, D.; Sato, T.; Ahuja, S.; Aimoto, S.; Elliott, J.; Nostrand, W. V.; Smith, S. *Nat. Struct. Mol. Biol.* **2010**, *17*, 561.

(2) (a) Damo, S. M.; Phillips, A. H.; Young, A. L.; Li, S.; Woods, V. L., Jr.; Wemmer, D. E. *J. Biol. Chem.* **2010**, *285*, 32303. (b) Walsh, P.; Simonetti, K.; Sharpe, S. *Structure* **2009**, *17*, 417. (c) Wille, H.; Bian, W.; McDonald, M.; Kendall, A.; Colby, D. W.; Bloch, L.; Ollesch, J.; Borovinskiy, A. L.; Cohen, F. E.; Prusiner, S. B.; Stubbs, G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 16990.

(3) (a) Jahn, T.; Makin, O.; Morris, K.; Marshall, K.; Tian, P.; Sikorski, P.; Serpell, L. *J. Mol. Biol.* **2010**, *395*, 717. (b) Nelson, R.; Sawaya, M.; Balbirnie, M.; Madsen, A.; Riekel, C.; Grothe, R.; Eisenberg, D. *Nature* **2005**, *435*, 773.

(4) (a) Tycko, R. *Q. Rev. Biophys.* **2006**, *39*, 1. (b) Paravastu, A.; Leapman, R.; Yau, W.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18349. (c) Nielsen, J.; Bjerring, M.; Jeppesen, M.; Pedersen, R.; Pedersen, J.; Hein, K.; Vosegaard, T.; Skrydstrup, T.; Otzen, D.; Nielsen, N. *Angew. Chem., Int. Ed.* **2009**, *48*, 2118.

(5) (a) Kim, Y. S.; Liu, L.; Axelsen, P. H.; Hochstrasser, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *107*, 7720. (b) Peralvarez-Marin, A.; Barth, A.; Graslund, A. *J. Mol. Biol.* **2008**, *379*, 589. (c) Strasfeld, D.; Ling, Y.; Shim, S.; Zanni, M. T. *J. Am. Chem. Soc.* **2008**, *130*, 6698. (d) Shim, S.; Gupta, R.; Ling, Y.; Strasfeld, D.; Raleigh, D.; Zanni, M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6614.

(6) Siebert, F. In *Infrared Spectroscopy of Biomolecules*; Mantsch, H. H., Chapman, D., Eds.; Wiley-Liss: New York, 1996; Chapter 4.

(7) Decatur, S. *Acc. Chem. Res.* **2006**, *39*, 169.

(8) (a) Schmidt, J. R.; Corcelli, S. A.; Skinner, J. L. *J. Chem. Phys.* **2004**, *121*, 8887. (b) Moran, A.; Mukamel, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 506. (c) Choi, J.; Hahn, S.; Cho, M. *Int. J. Quantum Chem.* **2004**, *104*, 616. (d) Huang, R.; Kubelka, J.; Barber-Armstrong, W.; Silva, R.; Decatur, S.; Keiderling, T. *J. Am. Chem. Soc.* **2004**, *126*, 2346. (e) Brauner, J. W.; Flach, C. R.; Mendelsohn, R. *J. Am. Chem. Soc.* **2005**, *127*, 100. (f) Choi, J. H.; Lee, H.; Lee, K. K.; Hahn, S.; Cho, M. *J. Chem. Phys.* **2007**, *126*, No. 045102. (g) De Flores, L. P.; Ganim, Z.; Nicodemus, R. A.; Tokmakoff, A. *J. Am. Chem. Soc.* **2009**, *131*, 3385. (h) Kubelka, J.; Keiderling, T. *J. Am. Chem. Soc.* **2001**, *123*, 6142. (i) Roy, S.; Jansen, T.; Knoester, J. *Phys. Chem. Chem. Phys.* **2010**, *12*, 9347.

(9) Cheatum, C.; Tokmakoff, A.; Knoester, J. *J. Chem. Phys.* **2004**, *120*, 8201.

(10) Lee, C.; Cho, M. *J Phys. Chem. B* **2004**, *108*, 20397.

(11) Strasfeld, D.; Ling, Y.; Gupta, R.; Raleigh, D.; Zanni, M. *J. Phys. Chem. B* **2009**, *113*, 15679.

(12) Karjalainen, E.; Ravi, H.; Barth, A. *J. Phys. Chem. B* **2011**, *115*, 749.

(13) Daidone, I.; Aschi, M.; Zanetti, L.; Nola, A. D.; Amadei, A. *Chem. Phys. Lett.* **2010**, *488*, 213.

(14) Amadei, A.; Daidone, I.; Di Nola, A.; Aschi, M. *Curr. Opin. Struct. Biol.* **2010**, *20*, 155.

(15) Amadei, A.; Daidone, I.; Zanetti-Polzi, L.; Aschi, M. *Theor. Chem. Acc.* **2011**, *129*, 31.

(16) (a) Gao, J.; Truhlar, D. G. *Annu. Rev. Phys. Chem.* **2002**, *53*, 467. (b) Shurki, A.; Warshel, A. *Adv. Protein Chem.* **2003**, *66*, 249.

(17) (a) Inouye, H.; Kirschner, D. A. *J. Struct. Biol.* **1998**, *122*, 247. (b) Daidone, I.; Amadei, A.; Di Nola, A. *Proteins: Struct., Funct., Genet.* **2005**, *59*, 510. (c) Daidone, I.; D'Abramo, M.; Di Nola, A.; Amadei, A. *J. Am. Chem. Soc.* **2005**, *127*, 14825.

(18) Petty, S. A.; Decatur, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 14272.

(19) Petty, S. A.; Adalsteinsson, T.; Decatur, S. M. *Biochemistry* **2005**, *44*, 4720.

(20) Silva, R. A. G. D.; Barber-Armstrong, W.; Decatur, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 13674.

(21) Lee, S.; Mou, Y.; Lin, S.; Chou, F.; Tseng, W.; Chen, C.; Lu, C. D.; Yu, S. S. F.; Chan, J. C. *J. Mol. Biol.* **2008**, *378*, 1142.

(22) Sawaya, M. R.; Sambashivan, S.; Nelson, R.; Ivanova, M. I.; Sievers, S. A.; Apostol, M. I.; Thompson, M. J.; Balbirnie, M.; Wiltzius, J. J. W.; McFarlane, H. T.; Madsen, A. O.; Riekel, C.; Eisenberg, D. *Nature* **2007**, *447*, 453.

(23) Buchete, N. V.; Tycko, R.; Hummer, G. *J. Mol. Biol.* **2005**, *353*, 804.

(24) (a) Hahn, S.; Kim, S.; Lee, C.; Cho, M. *J. Chem. Phys.* **2005**, *123*, No. 084905. (b) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 12048.