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A Relationship between the Aggregation Rates of α-Synuclein Variants and the β-Sheet Populations in Their Monomeric Forms

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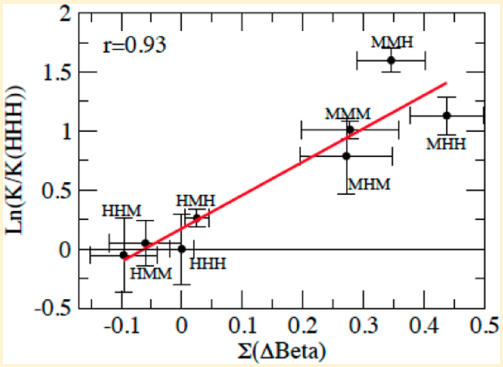
ABSTRACT: Intrinsically disordered proteins constitute a significant part of the human proteome and carry out a wide range of different functions, including in particular signaling and regulation. Several of these proteins are vulnerable to aggregation, and their aberrant assemblies have been associated with a variety of neurodegenerative and systemic diseases. It remains unclear, however, the extent to which the conformational properties of intrinsically disordered proteins in their monomeric states influence the aggregation behavior of these molecules. Here we report a relationship between aggregation rates and secondary structure populations in the soluble monomeric states of a series of mutational variants of α-synuclein. Overall, we found a correlation of over 90% between the changes in β-sheet populations calculated from NMR chemical shift data and the changes in aggregation rates for eight human-to-mouse chimeric mutants. These results provide support to the idea of investigating therapeutic strategies based on the stabilization of the monomeric form of intrinsically disordered proteins through the alteration of their conformational properties.



INTRODUCTION

The process of aberrant aggregation of otherwise soluble peptides and proteins is associated with a wide range of human disorders, including highly debilitating systemic and neurodegenerative conditions such as type II diabetes, and Alzheimer's and Parkinson's diseases.[1−3] Understanding the specific molecular events leading to aggregation would offer novel opportunities for therapeutic intervention, but it has been challenging to make advances in this direction in particular in the cases when the proteins involved are intrinsically disordered, because of the great technical difficulties in characterizing in detail the conformational properties of such proteins. A case of particular relevance is that of α-synuclein, a 140-residue intrinsically disordered protein that can assemble into intracellular inclusions known as Lewy bodies in dopaminergic neurons of patients suffering from Parkinson's disease.[4] Considerable efforts have been devoted to the study of the behavior of this protein,[5−9] in particular its aggregation process,[5,10−17] which involves the formation of potentially neurotoxic oligomers.[10,18−22]

An opportunity to increase our understanding of the fundamental principles that determine the aggregation of α-synuclein is provided by the comparison of the human and mouse forms of this protein. The mouse form, despite being highly homologous to the human form, does not give rise to disease in mice, although it aggregates more readily than the human form.[23,24] The two forms differ at seven positions (A53T, S87N, L100M, N103G, A107Y, D121G, and N122S), which are distributed asymmetrically along the sequence.[23] Single substitutions are present in the N-terminal (A53T) and NAC (S87N) regions, while the five remaining substitutions (L100M, N103G, A107Y, D121G, and N122S) are found in the C-terminal region.

In the present study, we investigate whether, for the various mutant forms of α-synuclein considered here, the differences in the aggregation rates can be associated with differences in the conformational properties of the monomeric states. This study is therefore designed to test the idea that the various steps in the aggregation process of intrinsically disordered proteins can be influenced by the structural properties of their monomeric forms.[25−28] One can expect in particular that if specific regions in the amino acid sequences acquire upon mutation lower intrinsic propensities to form β-sheets, then the aggregation process could be slowed down.[29,30]

METHODS

Mutational Variants of α-Synuclein. We considered here the human and mouse forms and six of their chimeric variants (Table 1) whose aggregation behavior was monitored recently.[23] The human (H) sequence was denoted by the three letters HHH, which correspond, respectively, to the human N-terminal, human NAC, and human C-terminal forms, while the mouse (M) form by the three letters MMM. The six variants were prepared by combining all the possible human and mouse sequences of the three regions.[23] While in the N-terminal and NAC regions only one substitution differentiates the human and mouse forms, in the C-terminal region, there are five substitutions. We grouped these five substitutions

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Table 1. List of the Eight α-Synuclein Variants[23] Considered in This Work[a]

α-synuclein variant	name	$\Sigma(\Delta\beta)$	$\ln(K/K(\mathrm{HHH}))$
human	HHH	0	0
human A53T	MHH	0.42	1.15
human S87N	HMH	0.02	0.26
human L100M-N103G-A107Y-D121G-N122S	HHM	−0.09	−0.05
human A53T-S87N	MMH	0.35	1.58
human A53T-L100M-N103G-A107Y-D121G-N122S	MHM	0.26	0.80
human S87N-L100M-N103G-A107Y-D121G-N122S	HMM	−0.06	0.05
mouse A53T-S87N-L100M-N103G-A107Y-D121G-N122S	MMM	0.28	1.00

[a]The second column reports the name of the mutants, according to our previous notation.[23] The third column reports the change in secondary structure population with respect to the human form (HHH). The fourth column reports the logarithm of the ratio between the aggregation rates of the mutant and human wild-type forms.[23] The correlation between the values in the third and fourth columns is illustrated in Figure 2.

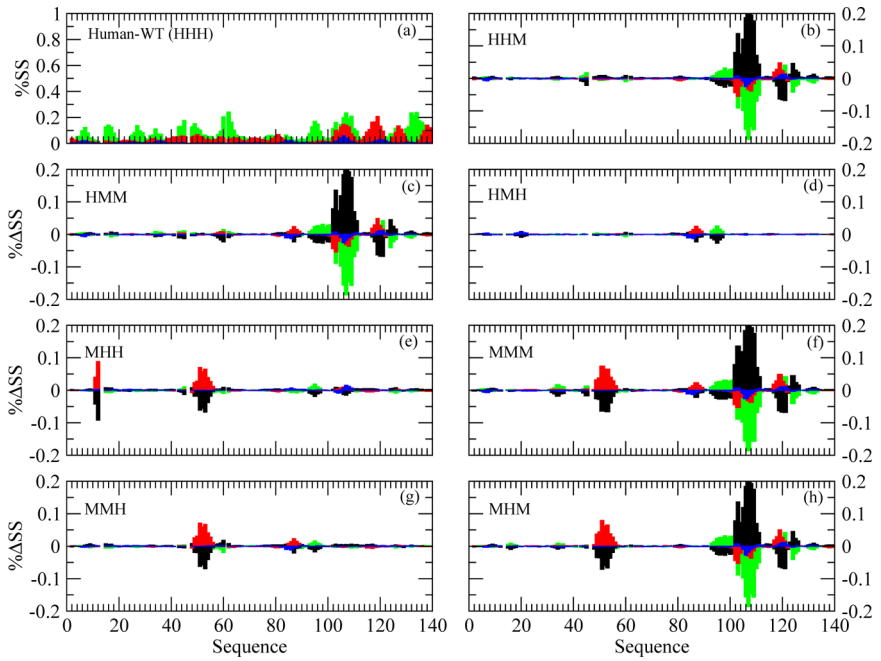

Figure 1. Secondary structure populations estimated from chemical shifts using the δ2D method.[31] (a) Wild-type human form (HHH); β-sheet populations are shown in red, α-helical populations in blue, polyproline II populations in green. (b–h) Differences in the secondary structure populations between the wild-type human form (HHH), the wild-type mouse form (MMM), and the six other chimeric variants considered in this work; changes in random coil populations are shown in black.

together, as the C-terminal region as a whole is thought to play a protective role against aggregation.[5] Specifically, these variants contained either one human-to-mouse substitution (MHH, HMH, or HHM) or two substitutions (HMM, MHM, or MMH), with the sequence with all three substitutions (MMM) corresponding to the wild-type mouse sequence. All the variants were found to form amyloid fibrils within eight days in a plate reader at 37 °C under linear 600 rpm shaking.[23] Typically, for human α-synuclein (HHH) as well as all the other variants, fibril widths were observed to be around 10 nm, with lengths varying from around 20 nm to 2 μm.[23] Up to 5-fold differences in the aggregation rates were observed for the eight variants.[23] The presence of a T residue at position 53 is balanced in the mouse by C-terminal mutations,[23,24] so that the mouse form of α-synuclein (MMM) is less aggregation prone than the MHH and MMH forms. These results are important to understand why the mutant form A53T is associated with early onset of the disease in humans,[23,24] although a T residue at position 53 represents the wild-type form in mice.

Determination of the Secondary Structure Populations. We calculated the secondary structure populations by applying the δ2D method,[31] which is based on the structural information provided by NMR chemical shifts. We used the backbone chemical shifts (Cα, Cβ, C′, Hα, HN, and N) determined recently for the eight α-synuclein variants[23] considered in this work (Table 1).

RESULTS AND DISCUSSION

In order to characterize the conformational properties of the mutant forms of α-synuclein, we considered the secondary structure populations that can be estimated from the measurement of NMR chemical shifts using the δ2D method.[31] Although these calculations by themselves do not reveal the overall three-dimensional topology of the protein, they enable one to obtain accurate information at the secondary structure level. By using previously reported chemical shifts,[23] our results indicate that there are relatively small but significant changes in the β-sheet populations between the various mutant forms that we have analyzed (Figure 1). In particular, these changes are

more pronounced around position 53 upon the A53T mutation.

The changes in secondary structure populations upon mutation can be put in direct relationship with the corresponding changes in the aggregation rates. Overall, we found a correlation of 93% (with a p value of 0.0002) between changes in β-sheet populations and changes in aggregation rates over eight human to mouse chimeric mutants (Figure 2). More

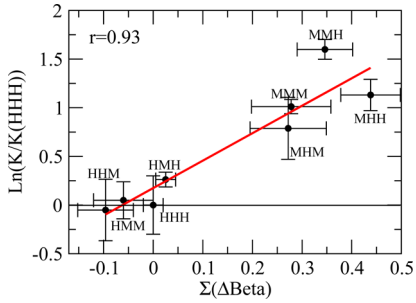

Figure 2. Correlation between the changes in aggregation rates upon mutation, $\ln(K/K(\mathrm{HHH}))$, and the corresponding overall changes in β-sheet populations, $\Sigma(\Delta\mathrm{Beta})$, for the eight α-synuclein variants considered in this work. The coefficient of correlation, r, is 0.93. The error bars on the x-axis are given by assuming an error of 2% in the estimation of the differences in the populations.[31]

specifically, this analysis reveals that the A53T mutation in the MHH variant increases by about 10% the β-sheet population in the region of the mutation with respect to the wild-type human form (HHH), and that this increase corresponded to a 3-fold increase in the aggregation rate of the MHH variant with respect to the HHH form. Since the A53T mutation changes quite substantially the β-sheet population of α-synuclein, we calculated whether the relationship between the variations in the β-sheet population and the aggregation rates holds separately for the two groups of four mutants in which at position 53 there is an A or a T, respectively. The results show that this correlation persists also within these two groups (Table 2), although having only four points for each of them does not result in a strong statistical significance of this finding.

Table 2. Coefficients of Correlation between the Variations in the β-Sheet Populations and the Changes in Aggregation Propensities (See Also Figure 2)

group of mutants	coefficient of correlation
all mutants	0.93
A53 mutants	0.75
T53 mutants	0.42

On the basis of an analysis of the secondary structure propensities estimated from the chemical shifts using the SSP method,[32] we previously suggested that the A53T mutation may increase the α-helical content around position 53.[23] This discrepancy with the present study can be attributed to the different types of results provided by the SSP[32] and δ2D[31] methods. For a given region of the amino acid sequence, the SSP method provides a single propensity score, which is positive for α-helices and negative for β-sheet regions, while the δ2D method provides four populations that quantify the relative weights of the α-helical, β-sheet, polyproline II, and random coil contents along the sequence. An overall positive SSP score may therefore mask subtle variations upon mutations in the β-sheet populations in a given region. Here, we found using the δ2D method only a very weak correlation between the changes in aggregation rates and the overall changes in α-helical populations; the coefficient of correlation was 0.24. We also found that there are quite significant changes in the polyproline II populations between the different mutants but that these changes are only weakly correlated (coefficient of correlation 0.19) with the corresponding changes in the aggregation rates. These results indicate that the changes in the secondary structure populations relevant for describing the aggregation behavior, at least in the cases considered here, are those that concern the β-sheet content.

These results are complementary to those recently discussed about the N-acetylated variant of α-synuclein, which has been found to increase the α-helical propensity at the N-terminus,[33−35] and may reduce its aggregation propensity, although further studies in this respect will be required.[33,34] We also found that the balancing effects exerted by the C-terminal mutations in the MHM and MMM forms, which decrease their overall aggregation rates with respect to the MHH and MMH forms,[23] can be put in quantitative correspondence with the changes in the β-sheet populations in the C-terminal region of the protein (Figure 2).

A question that remains open is about the nature of the relationship between the quite sizable changes in aggregation rates and the relatively small changes that we observed in the β-sheet population of α-synuclein in its monomeric state. It is possible that an increased probability of forming β-strands corresponds to a greater probability to form intermolecular β-sheets by direct association of the β-strands themselves. An additional effect could be that an increase in secondary structure populations results in a decrease of the entropy of the protein molecules in their soluble monomeric forms. Hence, the entropic penalty for intermolecular association could be decreased with even small increases in β-sheet populations, and the formation of oligomeric forms is correspondingly promoted. We anticipate that further studies will be required to clarify these aspects. We also observe that the relationship between changes in aggregation rates and changes in β-sheet populations are expected to extend to other cases in which individual mutations do not change significantly other physicochemical properties of the amino acid sequence, such as hydrophobicity and charge, because it is well established that these physicochemical factors strongly affect the aggregation behavior of proteins.[29,30]

■ CONCLUSIONS

We have studied the relationship between the secondary structure populations and the aggregation rates of various mutant forms of α-synuclein. We have reported initial evidence for a quantitative relationship between these two quantities, a result that suggests that the conformational properties of the monomeric state of α-synuclein play an important role in determining the rate of the whole aggregation process. Therefore, our study supports further investigations of therapeutic strategies analogous to those that have already been demonstrated for folded proteins[36] and aimed at modulating the behavior of the monomeric states of intrinsically disordered peptides and proteins.[37]

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Balch, W. E.; Morimoto, R. I.; Dillin, A.; Kelly, J. W. Adapting Proteostasis for Disease Intervention. *Science* **2008**, *319*, 916−919.

(2) Chiti, F.; Dobson, C. M. Protein Misfolding, Functional Amyloid, and Human Disease. *Annu. Rev. Biochem.* **2006**, *75*, 333−366.

(3) Haass, C.; Selkoe, D. J. Soluble Protein Oligomers in Neurodegeneration: Lessons from the Alzheimer's Amyloid Beta-Peptide. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101−112.

(4) Spillantini, M. G.; Schmidt, M. L.; Lee, V. M. Y.; Trojanowski, J. Q.; Jakes, R.; Goedert, M. Alpha-Synuclein in Lewy Bodies. *Nature* **1997**, *388*, 839−840.

(5) Dedmon, M. M.; Lindorff-Larsen, K.; Christodoulou, J.; Vendruscolo, M.; Dobson, C. M. Mapping Long-Range Interactions in Alpha-Synuclein Using Spin-Label NMR and Ensemble Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **2005**, *127*, 476−477.

(6) Thomas, B.; Beal, M. F. Parkinson's Disease. *Hum. Mol. Genet.* **2007**, *16*, R183−R194.

(7) Lees, A. J.; Hardy, J.; Revesz, T. Parkinson's Disease. *Lancet* **2009**, *373*, 2055−2066.

(8) Auluck, P. K.; Caraveo, G.; Lindquist, S. Alpha-Synuclein: Membrane Interactions and Toxicity in Parkinson's Disease. *Annu. Rev. Cell Dev. Biol.* **2010**, *26*, 211−233.

(9) Bartels, T.; Choi, J. G.; Selkoe, D. J. Alpha-Synuclein Occurs Physiologically as a Helically Folded Tetramer That Resists Aggregation. *Nature* **2011**, *477*, 107−U123.

(10) Conway, K. A.; Lee, S. J.; Rochet, J. C.; Ding, T. T.; Williamson, R. E.; Lansbury, P. T. Acceleration of Oligomerization, Not Fibrillization, Is a Shared Property of Both Alpha-Synuclein Mutations Linked to Early-Onset Parkinson's Disease: Implications for Pathogenesis and Therapy. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 571−576.

(11) Lashuel, H. A.; Petre, B. M.; Wall, J.; Simon, M.; Nowak, R. J.; Walz, T.; Lansbury, P. T. Alpha-Synuclein, Especially the Parkinson's Disease-Associated Mutants, Forms Pore-Like Annular and Tubular Protofibrils. *J. Mol. Biol.* **2002**, *322*, 1089−1102.

(12) Uversky, V. N.; Eliezer, D. Biophysics of Parkinson's Disease: Structure and Aggregation of Alpha-Synuclein. *Curr. Protein Pept. Sci.* **2009**, *10*, 483−499.

(13) Roodveldt, C.; Andersson, A.; De Genst, E. J.; Labrador-Garrido, A.; Buell, A. K.; Dobson, C. M.; Tartaglia, G. G.; Vendruscolo, M. A Rationally Designed Six-Residue Swap Generates Comparability in the Aggregation Behavior of Alpha-Synuclein and Beta-Synuclein. *Biochemistry* **2012**, *51*, 8771−8778.

(14) Rabe, M.; Soragni, A.; Reynolds, N. P.; Verdes, D.; Liverani, E.; Riek, R.; Seeger, S. On-Surface Aggregation of Alpha-Synuclein at Nanomolar Concentrations Results in Two Distinct Growth Mechanisms. *ACS Chem. Neurosci.* **2013**, *4*, 408−417.

(15) Giehm, L.; Oliveira, C. L. P.; Christiansen, G.; Pedersen, J. S.; Otzen, D. E. SDS-Induced Fibrillation of Alpha-Synuclein: An Alternative Fibrillation Pathway. *J. Mol. Biol.* **2010**, *401*, 115−133.

(16) Anderson, V. L.; Webb, W. W.; Eliezer, D. Interplay between Desolvation and Secondary Structure in Mediating Cosolvent and Temperature Induced Alpha-Synuclein Aggregation. *Phys. Biol.* **2012**, *9*, 056005−0560013.

(17) Ladiwala, A. R. A.; Bhattacharya, M.; Perchiacca, J. M.; Cao, P.; Raleigh, D. P.; Abedini, A.; Schmidt, A. M.; Varkey, J.; Langen, R.; Tessier, P. M. Rational Design of Potent Domain Antibody Inhibitors of Amyloid Fibril Assembly. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 19965−19970.

(18) Volles, M. J.; Lansbury, P. T. Zeroing in on the Pathogenic Form of Alpha-Synuclein and Its Mechanism of Neurotoxicity in Parkinson's Disease. *Biochemistry* **2003**, *42*, 7871−7878.

(19) Danzer, K. M.; Haasen, D.; Karow, A. R.; Moussaud, S.; Habeck, M.; Giese, A.; Kretzschmar, H.; Hengerer, B.; Kostka, M. Different Species of Alpha-Synuclein Oligomers Induce Calcium Influx and Seeding. *J. Neurosci.* **2007**, *27*, 9220−9232.

(20) Winner, B.; Jappelli, R.; Maji, S. K.; Desplats, P. A.; Boyer, L.; Aigner, S.; Hetzer, C.; Loher, T.; Vilar, M.; Campionic, S.; et al. In Vivo Demonstration That Alpha-Synuclein Oligomers Are Toxic. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 4194−4199.

(21) Hinault, M. P.; Cuendet, A. F. H.; Mattoo, R. U. H.; Mensi, M.; Dietler, G.; Lashuel, H. A.; Goloubinoff, P. Stable Alpha-Synuclein Oligomers Strongly Inhibit Chaperone Activity of the Hsp70 System by Weak Interactions with J-Domain Co-Chaperones. *J. Biol. Chem.* **2010**, *285*, 38173−38182.

(22) Cremades, N.; Cohen, S. I. A.; Deas, E.; Abramov, A. Y.; Chen, A. Y.; Orte, A.; Sandal, M.; Clarke, R. W.; Dunne, P.; Aprile, F. A.; et al. Direct Observation of the Interconversion of Normal and Toxic Forms of Alpha-Synuclein. *Cell* **2012**, *149*, 1048−1059.

(23) Kang, L. J.; Wu, K. P.; Vendruscolo, M.; Baum, J. The A53T Mutation Is Key in Defining the Differences in the Aggregation Kinetics of Human and Mouse Alpha-Synuclein. *J. Am. Chem. Soc.* **2011**, *133*, 13465−13470.

(24) Losasso, V.; Pietropaolo, A.; Zannoni, C.; Gustincich, S.; Carloni, P. Structural Role of Compensatory Amino Acid Replacements in the Alpha-Synuclein Protein. *Biochemistry* **2011**, *50*, 6994−7001.

(25) Citron, M. Alzheimer's Disease: Strategies for Disease Modification. *Nat. Rev. Drug Discovery* **2010**, *9*, 387−398.

(26) Metallo, S. J. Intrinsically Disordered Proteins Are Potential Drug Targets. *Curr. Opin. Chem. Biol.* **2010**, *14*, 481−488.

(27) Yamin, G.; Ono, K.; Inayathullah, M.; Teplow, D. B. Amyloid Beta-Protein Assembly as a Therapeutic Target of Alzheimer's Disease. *Curr. Pharm. Des.* **2008**, *14*, 3231−3246.

(28) Zhu, M.; De Simone, A.; Schenk, D.; Toth, G.; Dobson, C. M.; Vendruscolo, M. Identification of Small-Molecule Binding Pockets in the Soluble Monomeric Form of the Abeta42 Peptide. *J. Chem. Phys.* **2013**, *139*, 035101.

(29) Chiti, F.; Stefani, M.; Taddei, N.; Ramponi, G.; Dobson, C. M. Rationalization of the Effects of Mutations on Peptide and Protein Aggregation Rates. *Nature* **2003**, *424*, 805−808.

(30) Tartaglia, G. G.; Pawar, A. P.; Campioni, S.; Dobson, C. M.; Chiti, F.; Vendruscolo, M. Prediction of Aggregation-Prone Regions in Structured Proteins. *J. Mol. Biol.* **2008**, *380*, 425−436.

(31) Camilloni, C.; De Simone, A.; Vranken, W. F.; Vendruscolo, M. Determination of Secondary Structure Populations in Disordered States of Proteins Using Nuclear Magnetic Resonance Chemical Shifts. *Biochemistry* **2012**, *51*, 2224−2231.

(32) Marsh, J. A.; Singh, V. K.; Jia, Z. C.; Forman-Kay, J. D. Sensitivity of Secondary Structure Propensities to Sequence Differences between Alpha- and Gamma-Synuclein: Implications for Fibrillation. *Protein Sci.* **2006**, *15*, 2795−2804.

(33) Kang, L. J.; Moriarty, G. M.; Woods, L. A.; Ashcroft, A. E.; Radford, S. E.; Baum, J. N-Terminal Acetylation of Alpha-Synuclein Induces Increased Transient Helical Propensity and Decreased Aggregation Rates in the Intrinsically Disordered Monomer. *Protein Sci.* **2012**, *21*, 911−917.

(34) Fauvet, B.; Fares, M. B.; Samuel, F.; Dikiy, I.; Tandon, A.; Eliezer, D.; Lashuel, H. A. Characterization of Semisynthetic and Naturally N-Alpha-Acetylated Alpha-Synuclein in Vitro and in Intact Cells Implications for Aggregation and Cellular Properties of Alpha-Synuclein. *J. Biol. Chem.* **2012**, *287*, 28243−28262.

(35) Maltsev, A. S.; Ying, J. F.; Bax, A. Impact of N-Terminal Acetylation of Alpha-Synuclein on Its Random Coil and Lipid Binding Properties. *Biochemistry* **2012**, *51*, 5004−5013.

(36) Bulawa, C. E.; Connelly, S.; Devit, M.; Wang, L.; Weigel, C.; Fleming, J. A.; Packman, J.; Powers, E. T.; Wiseman, R. L.; Foss, T. R.; et al. Tafamidis, a Potent and Selective Transthyretin Kinetic Stabilizer That Inhibits the Amyloid Cascade. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 9629−9634.
(37) Zhu, M.; De Simone, A.; Schenk, D.; Toth, G.; Dobson, C. M.; Vendruscolo, M. Identication of small-molecule binding pockets in the soluble monomeric form of the A42 peptide. *J. Chem. Phys.* **2013**, *139*, 035101.