Curcumin Prevents Aggregation in α-Synuclein by Increasing Reconfiguration Rate*\\n
Received for publication, November 18, 2011, and in revised form, January 19, 2012. Published, JBC Papers in Press, January 20, 2012, DOI 10.1074/jbc.M111.325548

Basir Ahmad and Lisa J. Lapidus

From the Department of Physics and Astronomy, Michigan State University, East Lansing, Michigan 48824

**Background:** α-Synuclein is an aggregation-prone protein that reconfigures more slowly under aggregating conditions.

**Results:** Curcumin binds to monomeric α-synuclein, prevents aggregation, and increases the reconfiguration rate, particularly at high temperatures.

**Conclusion:** Curcumin rescues the protein from aggregation by making the protein more diffusive.

**Significance:** The search for aggregation inhibitors should account for changes in chain dynamics by the small molecule.

α-Synuclein is a protein that is intrinsically disordered in vitro and prone to aggregation, particularly at high temperatures. In this work, we examined the ability of curcumin, a compound found in turmeric, to prevent aggregation of the protein. We found strong binding of curcumin to α-synuclein in the hydrophobic non-amyloid-β component region and complete inhibition of oligomers or fibrils. We also found that the reconfiguration rate within the unfolded protein was significantly increased at high temperatures. We conclude that α-synuclein is prone to aggregation because its reconfiguration rate is slow enough to expose hydrophobic residues on the same time scale that bimolecular association occurs. Curcumin rescues the protein from aggregation by increasing the reconfiguration rate into a faster regime.

α-Synuclein aggregation is involved in, and likely the cause of, Parkinson disease (1). Although α-synuclein is commonly thought of as intrinsically disordered, a recent report demonstrated that, in human cells, it exists in a helical tetramer that does not easily aggregate (2). This suggests that the physiological pathway for aggregation is first unfolding of the tetramer to kinetically trapped monomers and then reassociation to a disordered aggregate and eventually fibrillar Lewy bodies. Therefore, preventing reassociation of the monomers is a useful therapeutic strategy. Many researchers in the past several years have investigated the interaction of potential aggregation inhibitors with oligomers of various sizes and fibrils, but there have been few observations of inhibitors with monomers, primarily because spectroscopic detection is difficult (3–7).

We have recently investigated the chain dynamics of disordered monomeric α-synuclein under a variety of aggregation conditions and found that the internal reconfiguration rate (or the rate of intramolecular diffusion) is fast under conditions in which aggregation is inhibited and slows when aggregation is more likely (8). We interpreted these observations with a model in which the first step of aggregation is kinetically controlled by the reconfiguration rate of the disordered monomer. When intramolecular diffusion is fast compared with bimolecular association, aggregation is unlikely because exposed hydrophobes quickly reconfigure, but if intramolecular diffusion slows to the same rate as bimolecular association, aggregation becomes more likely. A logical extension of this model is that aggregation inhibitors prevent bimolecular association by raising the reconfiguration (or the rate of intramolecular diffusion) of the disordered protein.

Intramolecular diffusion is the random motion of one part of the protein chain relative to another. To measure intramolecular diffusion, we used the Trp-Cys contact quenching method by which tryptophan is excited to a long-lived triplet state that is quenched on contact with cysteine within the same protein chain. Measurement of this rate of quenching at various temperatures and viscosities allows the extraction of the rate of diffusion between these two points in the chain.

In this work, we investigated the effect of the small molecule curcumin on the intramolecular diffusion of α-synuclein. Curcumin, a compound found in the spice turmeric, has been shown to have many medicinal properties and inhibits aggregation of the Alzheimer amyloid-β peptide (9). In α-synuclein, curcumin has been shown to inhibit fibril formation and increase solubility, but the physical basis of the aggregation inhibition is not known (10). We found that curcumin strongly bound to the monomer and completely inhibited aggregation, and with curcumin, intramolecular diffusion of α-synuclein was increased by >10-fold at 40 °C compared with the protein alone.

**EXPERIMENTAL PROCEDURES**

### α-Synuclein Mutation, Expression, and Purification

The α-synuclein plasmid was a kind gift from Gary Pielak (University of North Carolina, Chapel Hill, NC). Mutants Y39W/A69C and A69C/F94W of α-synuclein were created using the QuikChange site-directed mutagenesis kit (Stratagene). The mutations were confirmed by DNA sequencing. The wild-type and mutant proteins were expressed in *Escherichia coli* BL21(DE3) cells transformed with the T7-7 plasmid...
Intramolecular Diffusion in α-Synuclein with Curcumin

and purified as described previously (11). The purity of the mutants was confirmed by SDS-PAGE to be >95%. The protein concentration was determined from the absorbance at 280 nm using an extinction coefficient of 11460 M⁻¹ cm⁻¹. Stock solutions of ~200 μM were stored at −80 °C in 25 mM sodium phosphate buffer (pH 7.4) with 1 mM tris(2-carboxyethyl)phosphine (TCEP). An aliquot was thawed and filtered shortly before each experiment.

Aggregation Inhibition Studies

The effect of curcumin on the inhibition of α-synuclein aggregation was measured under two aggregation conditions. First, fibril formation in the absence and presence of curcumin (curcumin/protein molar ratio of 1.5) was initiated by stirring the protein, at a concentration of 48 μM, in 25 mM phosphate buffer (pH 7.4), 150 mM NaCl, and 1 mM TCEP at 37 °C (11). Second, soluble oligomer formation in the absence and presence of curcumin (curcumin/protein molar ratio of 1.5) was started by incubating 5 μM α-synuclein in 10% (v/v) trifluoroethanol (TFE), 25 mM phosphate buffer (pH 7.4), and 1 mM TCEP at 37 °C (12).

At regular time intervals, individual aliquots of 60 (in one experiment, 10) μl of each sample preincubated without or with curcumin (curcumin/α-synuclein ratio of 1.5:1) were mixed with 440 (in one experiment, 490) μl of 25 μM thioflavin T (ThT) solution and 25 mM phosphate buffer (pH 7.4), and the aggregation kinetics were followed by measurements of ThT fluorescence at 480 nm and far-UV CD at 217 nm, respectively. The ThT fluorescence was measured using a Jobin Yvon SPEX FluoroLog-3 spectrofluorometer equipped with a temperature-controlled cell holder. The excitation and emission wavelengths were 440 and 480 nm, respectively. A 10-mm path length quartz cell and an excitation and emission slit width of 5 nm were used. Far-UV CD data were obtained with an Applied Photophysics Chirascan spectropolarimeter equipped with a temperature-controlled cell holder.

Conformational Studies

Intrinsic Fluorescence—Tryptophan fluorescence measurements were carried out on a Jobin Yvon SPEX FluoroLog-3 spectrofluorometer equipped with a temperature-controlled cell holder. The fluorescence spectra were measured at 25 °C with a 1-cm path length cell, exciting at 295 nm. Both excitation and emission slits were set at 5 nm.

Circular Dichroism—CD measurements were carried out with an Applied Photophysics Chirascan spectropolarimeter equipped with a temperature-controlled cell holder. Spectra were recorded with a 0.5–4 s adaptive integration time and a 1-nm bandwidth. Each spectrum was the average of four scans. Far- and near-UV CD spectra were taken at protein concentrations of 5 and 25 μM with 0.1- and 1.0-cm path length cells, respectively.

Trp-Cys Contact Quenching Studies

Shortly before the experiment, a 300-μl aliquot of the protein with or without the desired concentration of curcumin was diluted 10:1 in 25 mM sodium phosphate buffer (pH 7.4), 1 mM TCEP, and various sucrose concentrations that had been bubbled with N₂O to eliminate oxygen and scavenge solvated electrons created in the UV laser pulse. Triplet lifetime decay kinetics were measured with an instrument similar to one described previously (13). Briefly, the tryptophan triplet was excited by a 10-ns laser pulse at 289 nm created from the fourth harmonic of an Nd:YAG laser (Continuum) and a 1-meter Raman cell filled with 450 p.s.i. of D₂ gas. The triplet population was probed at 441 nm by a HeCd laser (Kimmon). The probe and a reference beam were measured with silicon detectors and combined in a differential amplifier (DA 1853A, LeCroy) with an additional stage of a 350-MHz preamplifier (SR445A, Stanford Research Systems). The total gain was 50-fold. The temperature and viscosity were controlled as described previously (8). The variation of solution viscosity was achieved with the addition of different concentrations of sucrose. Measurement of each sample at five temperatures took ~20 min, so aggregation during this time was minimal. The viscosity of each solvent at each temperature was measured independently using a cone-cup viscometer (Brookfield Engineering).

RESULTS

Curcumin Binds to Monomeric α-Synuclein—Binding of curcumin was measured using a variety of optical absorption and fluorescence methods (see supplemental “Results” for details). As shown in Fig. 1, curcumin bound strongly to monomeric α-synuclein with a dissociation constant (Kᵰ) of ~10⁻⁸ M without making any significant alteration in the unfolded state of α-synuclein (supplemental Fig. S1). By measuring binding at various temperatures, we determined the enthalpy (ΔH = −7.9 kcal/mol) and entropy (ΔS = −0.0081 kcal/mol/K) of binding, indicating that binding is enthalpically driven and suggesting that it is due to non-hydrophobic interactions. These observations are similar to previous reports of binding of curcumin to proteins such as α-1-casein (14), β-lactoglobulin (15), and FtsZ (16). The binding profiles for the two loops investigated here were the same, indicating that the Trp and Cys mutations do not significantly affect curcumin binding. There was no evidence that curcumin significantly altered the conformational ensemble, but the tryptophan emission at position 94 showed significant quenching and a slight blue shift in the spectrum upon binding curcumin (supplemental Fig. S2). No such effect was observed for the tryptophan at position 39. This suggests an affinity for binding in the non-amyloid-β component region.

Curcumin Strongly Inhibits Oligomer and Fibril Formation—We investigated the effect of curcumin on the aggregation of mutant A69C/F94W of α-synuclein under two solution conditions. In 25 mM phosphate buffer (pH 7.4), 150 mM NaCl, and 1 mM TCEP, α-synuclein at high concentration (48 μM) is known to form fibrils in 6 days upon stirring at 37 °C (11), whereas in 10% (v/v) TFE (pH 7.4), α-synuclein at low concentration (5 μM) aggregates into soluble oligomers in 70 min at 25 °C (12). Fig. 2 (a and c) shows the kinetics of mutant A69C/F94W fibrillation and oligomerization, respectively, in the absence and presence of curcumin monitored with ThT fluorescence and far-UV CD at 217 nm. For fibrillation, in the absence of curcu-
Intramolecular Diffusion in α-Synuclein with Curcumin

FIGURE 1. α-Synuclein-curcumin binding as measured by curcumin difference absorption at 404 nm (a), curcumin fluorescence at 512 nm after exciting the protein at 350 nm (b), and tryptophan fluorescence at 355 nm after exciting samples at 295 nm (c and d). In a and b, the curcumin concentration was 10 μM, and in c and d, the α-synuclein concentration was 10 μM. Lines are fits to supplemental Eq. S2 for a and c (n = 1 (black lines) and n = 2 (gray lines)) and for b as indicated. All lines in d are for n = 1.94W, Trp-94.

Fig. 2 (b and d) shows the far-UV CD spectra of the reactants and products formed in the absence and presence of curcumin under two aggregation conditions. Under the fibrillation condition, monomeric α-synuclein was characterized by a deep negative minimum at 198 nm. It has been previously shown that, at very low concentration (~0.5 μM), α-synuclein exists as a monomer between 0 and 60% TFE (12), and we observed a similar partially folded spectrum with and without curcumin in 10% (v/v) TFE. In the absence of curcumin, monomeric reactants incubated under either condition showed a transition from a monomer to a β-sheet structured aggregate with a negative minimum at 217 nm (Fig. 2, b and d). In the presence of curcumin, the spectra of the products under both aggregation conditions were almost unchanged after incubation. Taken together, these results suggest that curcumin at a molar ratio of 1.5:1 completely prevents the fibrillation and oligomer formation of α-synuclein.

Curcumin Significantly Affects α-Synuclein Intramolecular Diffusion—We investigated the effect of curcumin on intramolecular contact rates in two α-synuclein mutants, Y39W/A69C and A69C/F94W, by the Trp-Cys contact quenching method. Tryptophan triplet kinetics observed for these mutants exhibited a rapid decay on the microsecond time scale due to quenching of the tryptophan triplet by contact with cysteine and a second decay on the millisecond time scale due to other physicochemical processes (17). In the presence of curcumin, similar triplet decay kinetics were observed for these mutants. The observed rate of triplet decay consisted of two processes, intramolecular diffusion and irreversible quenching of the triplet by...
cysteine on close contact. In equilibrium, intramolecular diffusion brought the Trp and Cys residues within the same polypeptide together with a diffusion-limited forward rate ($k_{D+}$), where it may be quenched with rate ($q$) or diffuse away with rate $k_{D-}$. The observed rate is given by Equation 1 (18).

$$k_{obs} = \frac{k_{D+}q}{k_{D+} + q} \quad \text{(Eq. 1)}$$

If $q \gg k_{D-}$, then $k_{obs} \sim k_{D+}$, and the observed rate is diffusion-limited. However, cysteine is not a diffusion-limited quencher of free tryptophan in water, so $q \sim k_{D-}$, and Equation 1 can be rewritten as Equation 2.

$$\frac{1}{k_{obs}} = \frac{1}{k_{D+}} + \frac{1}{qK} = \frac{1}{k_{D+}(T,\eta)} + \frac{1}{k_q(T)} \quad \text{(Eq. 2)}$$

We assume that the reaction-limited rate ($k_R$) depends only on temperature ($T$), but $k_{D+}$ depends on both temperature and viscosity of the solvent ($\eta$). Therefore, by making measurements at different viscosities for a constant temperature, we can extract both $k_R$ and $k_{D+}$ by fitting a plot of $1/k_{obs}$ versus $\eta$ at a given temperature to a line in which the intercept is $1/k_R$ and the slope is $1/\eta k_{D+}$.

This measurement typically assumes that the cysteine is the only significant quencher in the sample (19), but it is likely that curcumin is also an efficient quencher. Fig. 3 shows the measured $k_{obs}$ of $\alpha$-synuclein mutant A69C/F94W and $N$-acetyl-$\alpha$-tryptophanamide in various concentrations of curcumin. The rates for $N$-acetyl-$\alpha$-tryptophanamide increased significantly with curcumin, indicating that curcumin is a very efficient quencher, but the rates of Trp-94 actually decreased slightly. This suggests that the curcumin is quite tightly associated with the protein and not free to quench Trp through bimolecular diffusion. Furthermore, although the curcumin is probably bound fairly close to Trp-94 based on the fluorescence measurements (supplemental Fig. S2), it is apparently not accessible to efficiently quench the triplet state, suggesting that it is buried in a hydrophobic pocket within the chain.

Fig. 4 (a and b) shows plots of exponential decay times ($1/k_{obs}$) versus viscosity for various temperatures at pH 7.4 in the absence of curcumin and at a curcumin/$\alpha$-synuclein molar ratio of 1.5:1, respectively. Without curcumin, the intercept decreases, and the slope increases dramatically with temperature. At the highest temperatures, the intercept is consistent with zero, implying that $k_{obs}$ is diffusion-limited. In contrast, in the presence of curcumin, the change in slope is much more gradual, and at no temperature is the observed rate diffusion-limited. This trend is more similar to trends observed in other unfolded peptides and proteins (13, 20).

The reaction-limited ($k_R$) and diffusion-limited ($k_{D+}$ at the viscosity of water at each temperature) rates are plotted in Fig. 5 for A69C/F94W and in supplemental Fig. S3 for Y39W/A69C at various concentrations of curcumin. The protein concentration was held fixed at 10 $\mu$M, and the highest concentration of curcumin was 15 $\mu$M, beyond which the curcumin absorbed too much light at 442 nm to measure the Trp triplet absorption accurately. To interpret these rates, we use the Szabo, Schulten, and Schulten theory, which models intramolecular diffusion as diffusion on a one-dimensional potential of mean force determined by the probability of intrachain distances ($P(r)$) (21). The measured reaction-limited and diffusion-limited rates are given by Equations 3 and 4 (22),

$$k_R = \int_{r_0} q(r) P(r) dr \quad \text{(Eq. 3)}$$
Intramolecular Diffusion in \(\alpha\)-Synuclein with Curcumin

\(k_e\) and \(k_{D+}\) are both inversely proportional to the average volume of the chain, and \(k_{D+}\) is directly proportional to \(D\). Therefore, in the absence of curcumin, the large increase in \(k_R\) represents a significant compaction in the size of the protein, and the moderate decrease in \(k_{D+}\) represents a significant slowing in diffusion as temperature is increased. The reconfiguration rate can be defined as the rate to diffuse one part of the chain across the diameter of the protein: \(k_e = 4D/(2R_g)^2\).

To determine the diffusion constant, we assume that the probability distribution is given by a Gaussian chain (Equation 5),

\[
P(r) = \frac{4\pi r^2}{N} \exp\left(-\frac{3r^2}{2R_g^2}\right) \quad (Eq. 5)
\]

where \(\langle r^2 \rangle\), the average Trp-Cys distance, is an adjustable parameter, and \(N\) is a normalization constant such that \(\int P(r) = 1\). For each measured \(k_D\), \(\langle r^2 \rangle\) was found such that it matched the measured rate using Equation 3. These distances are plotted in Fig. 5c. Then, the correct \(P(r)\) was used in Equation 4 with the measured \(k_{D+}\), to determine \(D\), which is plotted in Fig. 5d. Without any curcumin, \(D\) decreased by \(\sim 50\)-fold from 0 to 40 °C. The addition of curcumin had a small effect on the size of the chain, and the diffusion constant at low temperature, but the effect increased dramatically at high temperature. Because the binding curves suggested that curcumin was preferentially binding near Trp-94, we repeated these measurements with mutant Y39W/A69C (supplemental Fig. S3) and found qualitatively similar results, suggesting that curcumin affects the global dynamics of the protein.

**DISCUSSION**

We have previously shown that \(\alpha\)-synuclein, unique among disordered sequences, compacts and diffuses more slowly as temperature is increased (8). Examining other conditions under which aggregation is enhanced (low pH or the familial mutation A30P), we found a good correlation between the rate of intramolecular diffusion and the rate of aggregation. When diffusion is fast (\(D \sim 10^{-5}\) cm\(^2\) s\(^{-1}\)), such as is observed for most intrinsically disordered sequences, the protein reconfigures too fast to make stable bimolecular interactions with another protein chain, but when the reconfiguration rate is about the same as the bimolecular encounter rate, stable interactions are more likely, and aggregation can proceed. This accounts for the dramatic increase in the aggregation rate of \(\alpha\)-synuclein at 40 °C compared with 0 °C.

In this work, we examined the effect of curcumin binding on the intramolecular diffusion of \(\alpha\)-synuclein. There is little difference in \(D\) at \(T = 0\) °C, but the difference widens with increasing temperature. At \(T = 40\) °C and an equal molar ratio of curcumin to protein, \(D\) is 15 times higher than with no curcumin. This difference widens to 30 times at 1.5:1 curcumin/protein, the highest ratio measurable in our instrument, which suggests that multiple curcumin molecules bound to a single protein further increase \(D\).

The Trp fluorescence data suggest that one preferred binding site for curcumin is near position 94. Molecular mechanics simulations of Alzheimer peptides have shown that curcumin preferentially associates with alanine and other aliphatic resi-
Intramolecular Diffusion in α-Synuclein with Curcumin

![Diagram](Image)

**FIGURE 6.** Schematic of the action of curcumin on α-synuclein in bimolecular association and subsequent aggregation steps.

dues (24). Between residues 60 and 100, there are 15 aliphatic residues (alanine and valine plus Leu-100), and in particular, there are three alanes in a row at positions 89–91. We propose this as a possible binding site. Having made one or more bonds between the side chains and the curcumin, the aromatic rings of the molecule are then available to interact with any of the nearby hydrophobic residues, creating a hydrophobic cluster of residues close in sequence.

Thus, it appears that one or more curcumin molecules bound to α-synuclein rescue the protein from the slow diffusion regime that promotes aggregation. Because the reaction-limited rates are correlated with temperature and the diffusion-limited rates are inversely correlated, by extension, the chain volume and diffusion coefficient are inversely correlated. We conclude that curcumin disrupts long-range interactions within the chain, allowing it to more quickly reconfigure. Fig. 6 shows a schematic of this behavior. Typically, α-synuclein is a fairly compact disordered protein with many long-range interactions within the chain (gray circles). This makes reconfiguration fairly slow (upper row) and allows exposed hydrophobes to associate with other chains, making oligomers, which eventually rearrange into larger fibrillar species. With the addition of curcumin (middle row), the chains become less compact, and intramolecular interactions are more short-range, allowing faster reconfiguration. Faster reconfiguration allows the chains to escape from bimolecular association (lower row) and prevents further aggregation steps.

Future work should investigate whether this property is common in aggregation inhibitors. For example, as a control experiment, we measured intramolecular diffusion of the protein in the presence of N-acetylleucine, a hydrophobic amino acid, and found that the diffusion coefficient was unchanged (supplemental Fig. S4), suggesting that the ability of curcumin to affect reconfiguration is somewhat unique.

This assay yields unique information about the mechanism of aggregation inhibition at the first step of the process. More common assays, such as ThT fluorescence, are not sensitive to monomer/monomer interactions, which are the preferred step for an inhibitor to act on. One potential danger with inhibiting a later step of the aggregation pathway is that accumulation of a toxic intermediate could make toxicity worse (25). Therefore, this measurement should become a common assay in the development of new Parkinson drug candidates that prevent aggregation at the first step.

**Acknowledgments—**We thank Charles Hoogstraten for helpful discussions, Gary Pielak for the kind gift of the α-synuclein plasmid, and Terry Ball for mutation and expression of the protein. We acknowledge the support of the Michigan State University High Performance Computing Center and the Institute for Cyber Enabled Research.

**REFERENCES**

1. Goedert, M. (2001) α-Synuclein and neurodegenerative diseases. Nat. Rev. Neurosci. 2, 492–501
2. Bartels, T., Choi, J. G., and Selkoe, D. J. (2011) α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature 477, 107–110
3. Amer, D. A., Irvine, G. B., and El-Agnaf, O. M. (2006) Inhibitors of α-synuclein oligomerization and toxicity: a future therapeutic strategy for Parkinson disease and related disorders. Exp. Brain Res. 173, 223–233
4. Caruana, M., Högen, T., Levin, J., Hillmer, A., Giese, A., and Vassallo, N. (2011) Inhibition and disaggregation of α-synuclein oligomers by natural polyphenolic compounds. FEBS Lett. 585, 1113–1120
5. Ehrnhoefer, D. E., Bieschke, J., Boeddrich, A., Herbst, M., Masino, L., Lurz, R., Engemann, S., Pastore, A., and Wanker, E. E. (2008) EGCG redirects amyloidogenic poly peptides into unstructured, off-pathway oligomers. Nat. Struct. Mol. Biol. 15, 558–566
6. Lamberto, G. R., Binolfi, A., Oreclet, M. L., Bertoncini, C. W., Zweckstetter, M., Griesinger, C., and Fernández, C. O. (2009) Structural and mechanistic basis behind the inhibitory interaction of PcTS on α-synuclein amyloid fibril formation. Proc. Natl. Acad. Sci. U.S.A. 106, 21057–21062
7. Zhu, M., Rajamani, S., Kaylor, J., Han, S., Zhou, F., and Fink, A. L. (2004) The flavonoid baicalein inhibits fibrillation of α-synuclein and disaggregates existing fibrils. J. Biol. Chem. 279, 26846–26857
8. Ahmad, B., Chen, Y., and Lapidus, L. J. (2012) Proc. Natl. Acad. Sci. U.S.A. 109, 2336–2341
9. Yang, F., Lim, G. P., Begum, A. N., Ubeda, O. J., Simmons, M. R., Ambegaokar, S. S., Chen, P. P., Kayed, R., Glabe, C. G., Frautschy, S. A., and Cole, G. M. (2005) Curcumin inhibits formation of amyloid β-oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J. Biol. Chem. 280, 5892–5901
10. Pandey, N., Strider, J., Nolan, W. C., Yan, S. X., and Galvin, J. E. (2008) Curcumin inhibits aggregation of α-synuclein. Acta Neuropathol. 115, 479–489
11. Uversky, V. N., Li, J., and Fink, A. L. (2001) Evidence for a partially folded intermediate in α-synuclein fibril formation. *J. Biol. Chem.* **276**, 10737–10744
12. Anderson, V. L., Ramblall, T. F., Rospigliosi, C. C., Webb, W. W., and Eliezer, D. (2010) Identification of a helical intermediate in trifluoroethanol-induced α-synuclein aggregation. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18850–18855
13. Singh, V. R., Kopka, M., Chen, Y., Wedemeyer, W. J., and Lapidus, L. J. (2007) Dynamic similarity of the unfolded states of proteins L and G. *Biochemistry* **46**, 10046–10054
14. Sneharani, A. H., Singh, S. A., and Appu Rao, A. G. (2009) Interaction of α-synuclein–1-casein with curcumin and its biological implications. *J. Agric. Food Chem.* **57**, 10386–10391
15. Sneharani, A. H., Karakkat, J. V., Singh, S. A., and Appu Rao, A. G. (2010) Interaction of curcumin with beta-lactoglobulin-stability, spectroscopic analysis, and molecular modeling of the complex. *J. Agric. Food Chem.* **58**, 11130–11139
16. Rai, D., Singh, J. K., Roy, N., and Panda, D. (2008) Curcumin inhibits FtsZ assembly; an attractive mechanism for its antibacterial activity. *Biochem. J.* **410**, 147–155
17. Lapidus, L. J., Eaton, W. A., and Hofrichter, J. (2000) Measuring the rate of intramolecular contact formation in polypeptides. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7220–7225
18. Lapidus, L. J., Eaton, W. A., and Hofrichter, J. (2002) Measuring dynamic flexibility of the coil state of a helix-forming peptide. *J. Mol. Biol.* **319**, 19–25
19. Gonnelli, M., and Strambini, G. B. (1995) Phosphorescence lifetime of tryptophan in proteins. *Biochemistry* **34**, 13847–13857
20. Chen, Y., Parrini, C., Taddei, N., and Lapidus, L. J. (2009) Conformational properties of unfolded HypF-N. *J. Phys. Chem. B* **113**, 16209–16213
21. Szabo, A., Schulten, K., and Schulten, Z. (1980) 1st passage time approach to diffusion controlled reactions. *J. Chem. Phys.* **72**, 4350–4357
22. Lapidus, L. J., Steinbach, P. J., Eaton, W. A., Szabo, A., and Hofrichter, J. (2002) Effects of chain stiffness on the dynamics of loop formation in polypeptides. Appendix: Testing a 1-dimensional diffusion model for peptide dynamics. *J. Phys. Chem. B* **106**, 11628–11640
23. Lapidus, L. J., Eaton, W. A., and Hofrichter, J. (2001) Dynamics of intramolecular contact formation in polypeptides: distance dependence of quenching rates in a room-temperature glass. *Phys. Rev. Lett.* **87**, 258101–258104
24. Kumar, P., Pillay, V., Choonara, Y. E., Modi, G., Naidoo, D., and du Toit, L. C. (2011) *In silico* theoretical molecular modeling for Alzheimer disease: the nicotine-curcumin paradigm in neuroprotection and neurotherapy. *Int. J. Mol. Sci.* **12**, 694–724
25. Ross, C. A., and Poirier, M. A. (2004) Protein aggregation and neurodegenerative disease. *Nat. Med.* **10**, S10–S17