MOAG-4 promotes the aggregation of α-synuclein by competing with self-protective electrostatic interactions

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Aberrant protein aggregation underlies a variety of age-related neurodegenerative disorders, including Alzheimer’s and Parkinson’s diseases. Little is known, however, about the molecular mechanisms that modulate the aggregation process in the cellular environment. Recently, MOAG-4/SERF has been identified as a class of evolutionarily conserved proteins that positively regulates aggregate formation. Here, by using nuclear magnetic resonance (NMR) spectroscopy, we examine the mechanism of action of MOAG-4 by characterizing its interaction with α-synuclein (α-Syn). NMR chemical shift perturbations demonstrate that a positively charged segment of MOAG-4 forms a transiently populated α-helix that interacts with the negatively charged C terminus of α-Syn. This process interferes with the intramolecular interactions between the N- and C-terminal regions of α-Syn, resulting in the protein populating less compact forms and aggregating more readily. These results provide a compelling example of the complex competition between molecular and cellular factors that protect against protein aggregation and those that promote it.

Insoluble filamentous aggregates, commonly referred to as amyloid fibrils, are associated with many human neurodegenerative disorders, and are typically composed of disease-specific aggregation-prone proteins, including amyloid β (Aβ)^5 in Alzheimer’s disease, and α-synuclein (α-Syn) in Parkinson’s disease (1). The presence of these aggregation-prone proteins may lead to failure of protein homeostasis and loss of normal biological function (2–4). Although such aggregates are key pathological hallmarks of neurodegenerative disorders (5), much remains to be understood about the structural details of the molecular mechanisms leading to aggregate formation and those that protect against it, as the transient nature of species populated during the aggregation process makes it difficult to observe these in experiment.

It has been recently shown that a class of evolutionarily conserved proteins promotes aggregate formation (6). A genetic screen in Caenorhabditis elegans models led to the identification of a positive regulator of protein aggregation, called “modifier of aggregation 4” (MOAG-4). Inactivation of MOAG-4 suppresses protein aggregation and associated toxicity in C. elegans models for aggregation of polyglutamates, Aβ, and α-Syn (6). It has also been shown that a human ortholog of MOAG-4, small EDRK-rich factor (SERF) 1A, accelerates the aggregation of a broad range of amyloidogenic proteins in vitro (7). The ensuing biophysical analysis of the interaction between SERF1A and α-Syn suggested that the acceleration of α-Syn aggregation originates from a transient interaction in the initial stage of the process (7). However, structural details of MOAG-4/SERF proteins, and the molecular mechanism by which they promote aggregate formation have so far remained elusive.

To address this problem, we investigate here the effects of MOAG-4 on the aggregation of α-Syn by monitoring the kinetics of this process using thioflavin T (ThT), a dye known to exhibit enhanced fluorescence upon binding amyloid fibrils (8, 9). The aggregation assay was carried out at 37 °C under agitation (10). We found that MOAG-4 accelerates aggregate formation of α-Syn, which is consistent both with in vitro studies showing that aggregation of α-Syn is promoted by the human ortholog SERF1A (7), as well as with cellular studies attesting that loss of MOAG-4 suppresses aggregation and toxicity of α-Syn (6).

We then use NMR spectroscopy to determine the structural ensemble of MOAG-4, and provide structural evidence for the interaction between MOAG-4 and α-Syn. Although spontaneous (i.e. unseeded) in vitro formation of α-Syn amyloid fibrils at neutral pH under quiescent conditions is slow (11) and requires at least a few weeks (12), all NMR experiments were carried out...
at low temperature to avoid the possible formation of small oligomeric aggregates during the measurements (13). At pH 6.0 and 10 °C, the protein did not form detectable aggregates in the NMR tube during the NMR experiments. Our results show that MOAG-4 affects the intra-molecular interactions within α-Syn that bring together its N- and C-terminal regions. The presence of MOAG-4 is thus relevant as the transient interactions between the terminal regions of α-Syn result in rather compact conformations that appear to be relatively resistant to aggregation (14–18). In this way, MOAG-4 effectively competes with this self-protective mechanism, such that α-Syn takes on a less compact form, and may thereby become poised for aggregation and subsequent amyloid formation.

Results

MOAG-4 enhances α-Syn aggregate formation

To probe for a direct effect of MOAG-4 on α-Syn aggregation, we monitored the enhancement of ThT fluorescence in an aggregation kinetics assay (Fig. 1A). Amyloid formation of α-Syn was monitored in the absence or presence of an equimolar concentration of MOAG-4 at pH 6.0 and 37 °C. Some of the data displayed biphasic behavior, which cannot be readily explained by simple nucleation-dependent aggregation mechanisms. Although such complex behavior may be an intrinsic and relevant property of α-Syn aggregation induced by MOAG-4, it remains extremely challenging to extract from the data the underlying mechanistic information (19, 20). Thus, although we could not extract from the experimental data the kinetic parameters of the underlying microscopic processes, we found that, whereas α-Syn aggregation without MOAG-4 takes several days (10), MOAG-4 accelerated the process significantly, with a lag time of ~3 h (Fig. 1A).

We then examined whether MOAG-4 is integrated into the α-Syn fibrils (Fig. 1B). The α-Syn aggregation experiment was performed at pH 7.4 and 37 °C in the presence of MOAG-4. α-Syn aggregation was slower at pH 7.4 than 6.0, in agreement with previous studies showing that a decrease in pH enhances the kinetics of α-Syn amyloid fibril formation (21, 22). SDS-PAGE analysis confirmed that the insoluble fraction of the sample did not contain MOAG-4 (Fig. 1B), suggesting that the modulation of the α-Syn amyloidogenesis by MOAG-4 occurred during an early and transient interaction with α-Syn.

MOAG-4 forms a mutable structure with α-helical propensity

The 1H-15N heteronuclear single-quantum correlation (HSQC) spectrum of MOAG-4 (Fig. 2A) has the typical appearance expected for an intrinsically disordered protein, with very limited amide chemical shift dispersion. To characterize the structure in more detail, sequential assignment of backbone and aliphatic side chain 1H, 13C, and 15N resonances was achieved using a set of three-dimensional (3D) triple-resonance experiments (see “Experimental procedures”). Upon attainment of the resonance assignments, an ensemble of MOAG-4 structures was determined using molecular dynamics simulations restrained with backbone NMR chemical shifts (23, 24). The corresponding structural ensemble (Fig. 2B) displays a protein structure with a very high degree of disorder. Despite the global disorderliness, virtually all members of the structural ensemble contain an α-helical segment for residues Asp45–Asn70. When superposing the structures on the coordinates for these residues, the remainder of the protein appears spatially comparatively less well defined. However, a second, lowly-populated transient α-helix is present in these ensembles, comprising residues Asp8–Lys22 (Fig. 2C). Neither significantly populated compact states nor local β-sheet conformations were found. The experimental chemical shifts were well correlated with those back-calculated from the MOAG-4 ensemble that we determined. To validate the MOAG-4 ensemble, we further measured NOE intensities and compared these with the values back-calculated from the ensemble of structures. The good results of this validation attest the accuracy of the ensemble of structures that we describe. Taken together, MOAG-4 is a highly mutable protein containing transiently populated elements of secondary structure, but devoid of tertiary interactions.

As can be seen in Fig. 2A, the peak intensity for the residues between Asp55 and Asn70 is relatively low. The explanation for this observation can now be rationalized with help of the structure: the stretch Asp45–Asn70 forms a long helical structure, in
which amide bond vectors pointing along the direction of the axis of the α-helix experience fast transverse spin relaxation due to slow overall tumbling motion.

**Electrostatic interactions between MOAG-4 and α-Syn**

To determine the binding affinity and the location of the binding site on MOAG-4, a series of 1H-15N HSQC spectra for uniformly 13C,15N-labeled MOAG-4 were recorded with different amounts of unlabeled α-Syn. Fig. 3A shows the chemical shift changes observed for MOAG-4 upon the binding of α-Syn. The fit to saturating binding curves yielded a dissociation constant ($K_d$) value of 10.8 ± 0.8 μM. The backbone chemical shifts of amide proton (1HN), nitrogen (15N), and carbonyl (13C) nuclei of MOAG-4 were obtained from 3D HNCO experiments (25, 26) at [α-Syn]/[MOAG-4] = 0 and 8. The chemical shift perturbations of backbone 15N and 13C nuclei (Fig. 3B) indicate that binding to α-Syn induces significant changes in MOAG-4 for a contiguous stretch of residues between Arg3 and Gly26, corresponding to the first α-helical segment, a region that is rich in positively charged amino acid residues (net charge +6). The structural propensities of MOAG-4, free and bound to α-Syn, were calculated from the backbone 1H, 15N, and 13C chemical shifts using the program ncSPC (27). The α-helical propensity of the N-terminal segment increases slightly upon binding to α-Syn, indicating a direct effect of α-Syn on the MOAG-4 conformational ensemble (Fig. 3C).

Next, we examined the structural changes of α-Syn upon interaction with MOAG-4 by monitoring the corresponding chemical shift changes. Fig. 4A shows a series of BEST-TROSY 1H-15N correlation spectra (28) of uniformly 13C,15N-labeled α-Syn recorded with different amounts of unlabeled MOAG-4. Spectral assignment of α-Syn was taken from BMRB entry ID 18857, and confirmed using BEST-TROSY 3D HNCO and HN(CA)CO experiments (29, 30) before and after the titration. Chemical shift perturbations for backbone 15N and 13C upon binding to MOAG-4 (Fig. 4B) suggest that MOAG-4 interacts with the C terminus of α-Syn. This result parallels the previous finding that the interaction of α-Syn with SERF1A is localized to the C terminus of α-Syn (7). The C-terminal region of α-Syn (residues Glu110-Ala140) is acidic and negatively charged (net charge at neutral pH of −12), constituting a favorable binding location for positively charged proteins, such as MOAG-4 and SERF1A. Although 15N chemical shift changes are also observed for the N-terminal region, and to a lesser extent for the NAC (non-Aβ component of Alzheimer’s disease amyloid) region, as we will show below, we found that these collateral effects on structure are likely to result from the loss of pre-
existing interactions within the \( \alpha \)-Syn ensemble. Fig. 4C shows the structural propensity of \( \alpha \)-Syn in the absence and presence of MOAG-4, calculated from the backbone \( ^1\text{H}^\text{N}, ^{15}\text{N}, \text{and} ^{13}\text{C} \) chemical shifts using the program ncSPC (27). We found that, consistent with previous studies (27, 31), deviations of measured chemical shifts from their random coil values are very small throughout the sequence. We did not observe any significant differences in the structural propensities of \( \alpha \)-Syn in the presence and absence of MOAG-4, but the conformational exchange of \( \alpha \)-Syn with a less compact form upon binding to MOAG-4 was confirmed by an increase of backbone flexibility (see below).

**\( \alpha \)-Syn becomes less compact upon binding to MOAG-4**

To examine the conformational change of \( \alpha \)-Syn upon binding to MOAG-4, we measured the backbone \( ^{15}\text{N} \) transverse relaxation rate constants \( (R_2) \) of \( \alpha \)-Syn in the presence and absence of MOAG-4 (Fig. 5A), and obtained the changes of the backbone \( ^{15}\text{N} \) \( R_2 \) values induced by MOAG-4 (Fig. 5C). The \( R_2 \) values for the C-terminus of \( \alpha \)-Syn are larger in the presence of MOAG-4 than those without MOAG-4, indicative of restricted motion by addition of MOAG-4. These effects can be attributed to direct binding of \( \alpha \)-Syn and MOAG-4 mediated by polyvalent electrostatic interactions. At the same time, a reduction in the \( R_2 \) rates was observed for the N-terminal region (residues Val\(^{15}\)-Thr\(^{39}\)), indicating that the N-terminus of \( \alpha \)-Syn becomes more dynamic upon binding.

These results offer insight into the mechanism by which MOAG-4 enhances \( \alpha \)-Syn aggregation. It has been proposed that, although \( \alpha \)-Syn is highly dynamic as typical of intrinsically disordered proteins, the ensemble of \( \alpha \)-Syn in the native state is more compact than that expected for a random coil polypeptide chain due to the presence of long-range transient intra-molecular interactions (14–18, 32, 33), originating from an electro-
statistic attraction between the slight positive charge in the central region (residues Gly31-Leu100) and the negatively charged C-terminal region (34). These intra-molecular interactions and the associated compaction of $\alpha$-Syn shield the aggregation-prone central region of the protein from forming inter-molecular interactions under physiological conditions in the cell, thereby protecting it against aggregation (35).

NaCl reduces intra-molecular interactions within $\alpha$-Syn and enhances aggregation

To test whether the action of MOAG-4 could be emulated by screening the electrostatic interaction between the N and C termini by salt, we measured the backbone $^{15}$N $R_2$ relaxation rates at pH 6.0 and 10 °C in the presence and absence of 100 mM NaCl (Fig. 5B). In full agreement with a previous report (36), addition of NaCl reduced the backbone $^{15}$N $R_2$ relaxation rates in the N- and C-terminal regions (Fig. 5D), indicating that the screening of electrostatic interactions increased mobility in both domains. A positive correlation between the changes in the $^{15}$N $R_2$ relaxation rates at the N terminus of $\alpha$-Syn by addition of NaCl and those upon interaction with MOAG-4 (Fig. 5E) indicates that the interaction of MOAG-4 with the C terminus of $\alpha$-Syn abrogates pre-existing intra-molecular interactions, increasing the dynamics at the N terminus of $\alpha$-Syn. These changes, in turn, affect the chemical shifts for regions outside the C terminus (Fig. 4), explaining the pervasive pattern observed throughout the protein sequence. The study of Bai et al. (36) shows clearly how paramagnetic relaxation enhancement effects can remain restricted to two regions of the protein, whereas leading to chemical shift changes that are transmitted also to regions other than those in direct contact.

Previous studies have shown that increasing the salt concentration accelerates the formation of $\alpha$-Syn amyloid fibrils (37, 38). To examine the effect of NaCl on the formation of $\alpha$-Syn amyloid fibrils, the aggregation kinetics of $\alpha$-Syn at 100 mM NaCl was monitored by measuring ThT fluorescence at pH 6.0 and 37 °C. Overall, and consistent with previous studies (37, 38), the introduction of salt increases $\alpha$-Syn aggregation (Fig. 6), in a way similar to that observed for MOAG-4 (Fig. 1A), suggesting a fundamental link between the modulation of intra-molecular electrostatic interactions and $\alpha$-Syn aggregation.

**Discussion**

In vitro at neutral pH, and under quiescent conditions, spontaneous aggregation of $\alpha$-Syn requires at least a few weeks (12). In this study, soluble $\alpha$-Syn persists without aggregate formation during NMR measurement at 10 °C (Figs. 3–5). On the other hand, aggregation is accelerated upon agitation of the protein solution (10, 12), where the lag time of $\alpha$-Syn fibril formation is reduced from weeks/months to hours/days (10) (Figs. 1 and 6). Although the lag phase preceding assembly was several days in the absence of MOAG-4 or NaCl, $\alpha$-Syn readily self-associated with a lag time of $\sim$3 and $\sim$16 h in the presence of an equimolar amount of MOAG-4 and 100 mM NaCl, respectively.

To understand the role of MOAG-4 on $\alpha$-Syn aggregation, we note that it is well known that electrostatic effects play key roles in the formation of amyloid fibrils (39–42). The effects of
these interactions on protein aggregation have been extensively studied for a variety of amyloidogenic proteins, including insulin (43), α-Syn (37, 38), β2-microglobulin (44), glucagon (45), islet amyloid polypeptide (46), and the mouse prion protein (47). The C terminus of α-Syn is rich in negatively charged amino acid residues, and α-Syn remains soluble for an extended period of time because of inter-molecular electrostatic repulsion. The shielding of charge repulsion by NaCl decreases the free energy barrier of nucleation (39, 40), and enhances the kinetics of α-Syn aggregation (Fig. 6). Neutralization of charge repulsion can also be brought about by polyamine compounds (14, 48, 49) and metal cations (50–52), and these have been shown to enhance the kinetics of α-Syn aggregation. Furthermore, C terminally truncated recombinant α-Syn readily aggregates (53). These observations indicate that the negatively charged C terminus plays an inhibitory role in the formation of α-Syn amyloid fibrils. More recently, it was observed that interactions between the N and C termini persist in cells, despite the high ionic strength of the cytoplasm (35). It was also suggested that hydrophobic residues of the central amyloidogenic region of α-Syn are shielded from interactions with cellular components (35), although experiments with paramagnetic co-solute molecules in vitro have revealed solvent accessibility of the entire chain (54, 55).

Furthermore, it has been suggested that the amyloid-modifying properties of MOAG-4/SERF proteins are evolutionary conserved (6). The binding affinity of MOAG-4 to α-Syn obtained in this study (Kₐ = 10.8 ± 0.8 μM) is comparable with the Kₐ value for the binding of SERF1A, a human ortholog of MOAG-4, to α-Syn (7). The inter-molecular interaction with SERF1A is localized to the C terminus of α-Syn, and the C terminally truncated mutant, α-Syn(1–110), showed a very weak binding affinity to SERF1A (Kₐ > 1 μM) (7). Furthermore, the aggregation kinetics of α-Syn(1–110) was not affected by SERF1A (7). Therefore, the chemical shift perturbations (Fig. 4B) and a reduction in the backbone 15N R₂ rates (Fig. 5C) observed for the N-terminal region of α-Syn upon binding to MOAG-4 is likely due to a combination of effects, as explained below.

Our experimental data suggest that the acceleration of α-Syn aggregation induced by MOAG-4 is due to electrostatic interactions between the two oppositely charged proteins (Fig. 7). Although α-Syn is typified as an intrinsically disordered protein, the long-range nature of electrostatic interactions allows transient intra-molecular contacts within α-Syn to occur (14–18, 32–36). As a result, the ensemble of conformations in the native state, although disordered, remains rather compact. Direct binding of MOAG-4 to the negatively charged C terminus of α-Syn could be facilitated because positively charged residues are distributed evenly throughout the first helical segment of MOAG-4. At the same time, the chemical shift perturbations and a reduction in the backbone 15N R₂ rates were colaterally observed for the N-terminal region of α-Syn (Figs. 4B and 5C). These results reveal that the highly positively charged first α-helical segment of MOAG-4 binds the negatively charged C terminus of α-Syn, effectively competing with pre-existing intra-molecular interactions within α-Syn, thereby reducing the compactness of the protein.

Our NMR data and aggregation assay demonstrate that MOAG-4 directly binds to α-Syn and enhances its aggregation, whereas the resulting insoluble fraction did not contain MOAG-4 (Fig. 1B). The aggregation-promoting activity of SERF1A results from early and transient interactions with α-Syn (7), which act catalytically. Electron microscopic images of the mature α-Syn amyloid fibrils obtained in the absence and presence of SERF1A did not show morphological differences (7). Although it still remains to be elucidated whether the reduction in compactness of α-Syn is required for the formation of α-Syn amyloid fibrils (32, 33), our results now shine light on the structures of the molecular species involved in the early and transient interaction between MOAG-4 and α-Syn, and the mechanism by which electrostatic interactions may prevent or drive protein aggregation. We also note that the type of interactions between MOAG-4 and α-Syn detected in the NMR measurements that we reported are likely to be conserved under the conditions used in the aggregation assays.

In closing, we observe that the competition between inter-molecular and intra-molecular electrostatics may offer ways for cells to cope with aggregation-prone proteins in general. By protecting the hydrophobic regions in proteins through intra-molecular interactions leading to the formation of compact forms, the fraction of proteins susceptible to aggregation can be maintained at a manageable level. In this view, proteins like MOAG-4/SERF would be employed to reduce the self-protection provided by such a compaction, and effectively convert disordered proteins into aggregates when concentrations reach dangerous levels. In addition, proteins like MOAG-4/SERF can
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use this mechanism to support other physiological roles, as in the generation of functional amyloids.

Experimental procedures

Sample preparation

Expression and purification of human α-Syn was described previously (56). For expression of uniformly $^{13}$C, $^{15}$N-labeled and $^{15}$N-labeled α-Syn, Escherichia coli cells were grown in M9 minimal medium supplemented with $^{15}$N-labeled NH$_4$Cl and $^{13}$C-labeled d-glucose as sole source for $^{15}$N and $^{13}$C, respectively. Uniformly $^{13}$C, $^{15}$N-labeled and unlabeled MOAG-4 was obtained from Giotti Biotech (Florence, Italy).

ThT fluorescence assay

Aggregation of α-Syn was performed at a concentration 12.5 μM. Lyophilized α-Syn was dissolved in 10 mM sodium phosphate (pH 6.0) containing 40 μM ThT. A clear-bottom 96-well plate with a final volume of 150 μl of assay solution was loaded into a Genios Pro (Tecan) fluorescence plate reader incubated at 37 °C (10). ThT fluorescence was measured with excitation and emission at 448 and 485 nm, respectively. A 3-mm glass bead was added to each of the wells, and the plate was subjected to cycles of an orbital shaking at 300 rpm for 16 min followed by quiescence for 4 min. Because amyloid fibril formation often varies across sample replicates and low reproducibility of the kinetic assays is attributed to the inherently stochastic nature of the aggregation process (57–59), the experiment was performed in nine replicates, where one solution was divided into nine samples and the assay was run in parallel in the same plate. Binding of MOAG-4 to ThT was negligible.

SDS-PAGE analysis

A 250-μl volume of 200 μM α-Syn in 50 mM Tris-HCl, 150 mM NaCl, and 3 mM NaN$_3$ (pH 7.4) was mixed with 250 μl of 200 μM MOAG-4 in the same buffer, tapered to the rack of an Eppendorf themomixer 5436, and agitated at 37 °C and 1,400 rpm. After given time points, 35-μl samples were taken and frozen immediately at −20 °C. Each sample was then centrifuged at 20,000 × g, and the insoluble fraction was washed twice and resuspended in 50 μl of 50 mM Tris-HCl, 150 mM NaCl, and 3 mM NaN$_3$ (pH 7.4). The soluble and insoluble samples (5 μl) were run on a 12% NuPAGE SDS-PAGE gel (Life Technologies, Germany), followed by Coomassie staining.

NMR assignment experiments

For resonance assignment of MOAG-4, all NMR spectra were recorded on a Bruker Avance III spectrometer at 1H frequency of 500 MHz. Backbone resonance assignments of uniformly $^{13}$C, $^{15}$N-labeled MOAG-4 (0.4 mM) in 10 mM sodium phosphate (pH 6.0) containing 2% (v/v) D$_2$O and 40 mM DSS, were recorded with increasing amounts of unlaelembed α-Syn. A 3D HNCO spectrum was recorded after the 3D HNCO-HSQC (3D 1H-15N-1H) were recorded to collect NOEs for structural validation (72). These experiments were performed at a MOAG-4 concentration of 400 μM on a Bruker Avance III 950 MHz spectrometer at 10 °C.

Titration of MOAG-4 with α-Syn

NMR titration experiments (73) were performed at 10 °C on a Bruker Avance III 950 MHz spectrometer equipped with a TCI cryogenic probehead. A series of 2D 1H-15N HSQC NMR spectra of uniformly $^{13}$C, $^{15}$N-labeled MOAG-4 (100 μM) in 10 mM sodium phosphate (pH 6.0), containing 2% (v/v) D$_2$O and 40 μM DSS, were recorded with increasing amounts of unlabeled α-Syn. A 3D HNCO spectrum was recorded after the titration with α-Syn, where [α-Syn]/[MOAG-4] = 8, to obtain the $^{15}$N, $^{13}$C chemical shifts. The binding curves were obtained from chemical shift changes during the titration in a fast-exchange manner, and fitted to the following equation,

$$\Delta \delta_{\text{obs}} = \Delta \delta_{\text{max}} - K_d [\text{MOAG-4}] \left(1 - e^{-[\alpha-\text{Syn}]/[\text{MOAG-4}] K_d}\right)$$

(Eq. 1)

where $\Delta \delta_{\text{obs}}$ is the observed chemical shift change from the free state, and $\Delta \delta_{\text{max}}$ is the maximum chemical shift change upon saturation. $K_d$ is the dissociation constant. Nonlinear least-squares fitting was performed with Igor Pro software.

$^{15}$N R$_2$ relaxation experiment

The transverse relaxation rate constant, R$_2$, of α-Syn was obtained from an $^{15}$N Hahn-echo pulse sequence (74). Two spectra were recorded, one without the relaxation delay and the other with the relaxation delay. The $R_2$ value was obtained by the following equation,

$$R_2 = -\frac{1}{\tau} \ln \left( \frac{I(\tau)}{I(0)} \right)$$

(Eq. 2)

where I(τ) values are resonance intensities at the relaxation delay τ. Temperature compensation was included to minimize
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the effects of heating caused by \(^1\)H decoupling during the \(^{15}\)N chemical shift evolution and relaxation delay periods (75). Proton \(x\) and \(y\) purge pulses were applied prior to the recycling delay to ensure that the amount of water magnetization present at the beginning of each scan is always identical (76). The spectra were recorded at 10 °C on a Bruker Avance III 950 MHz spectrometer equipped with a TCI cryogenic probehead (with/without MOAG-4; Fig. 5, A and C) or a Bruker Avance III 500 MHz spectrometer (with/without NaCl; Fig. 5, B and D).

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References

31. Nielsen, J. T., and Mulder, F. A. (2016) There is diversity in disorder: “in all chaos there is a cosmos, in all disorder a secret order. Front. Mol. Biosci. 3, 4


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