Internal Dynamics of Dynactin CAP-Gly Is Regulated by Microtubules and Plus End Tracking Protein EB1*

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Background: The role of microtubules on conformational dynamics of microtubule-associated proteins remains poorly understood.

Results: Magic angle spinning NMR studies indicate significant differences in the dynamics of CAP-Gly going from the unbound to the MT-bound or EB1-bound state.

Conclusion: Environment-dependent motions occurring on multiple time scales are functionally (biologically) relevant.

Significance: Conformational dynamics of a MT-associated protein bound to microtubules at atomic resolution is established for the first time.

CAP-Gly domain of dynactin, a microtubule-associated activator of dynein motor, participates in multiple cellular processes, and its point mutations are associated with neurodegenerative diseases. Recently, we have demonstrated that conformational plasticity is an intrinsic property of CAP-Gly. To understand its origin, we addressed internal dynamics of CAP-Gly assembled on polymeric microtubules, bound to end-binding protein EB1 and free, by magic angle spinning NMR and molecular dynamics simulations. The analysis of residue-specific dynamics of CAP-Gly on time scales spanning nanoseconds through milliseconds reveals its unusually high mobility, both free and assembled on polymeric microtubules. On the contrary, CAP-Gly bound to EB1 is significantly more rigid. Molecular dynamics simulations indicate that these motions are strongly temperature-dependent, and loop regions are surprisingly mobile. These findings establish the connection between conformational plasticity and internal dynamics in CAP-Gly, which is essential for the biological functions of CAP-Gly and its ability to bind to polymeric microtubules and multiple binding partners. In this work, we establish an approach, for the first time, to probe atomic resolution dynamic profiles of a microtubule-associated protein assembled on polymeric microtubules. More broadly, the methodology established here can be applied for atomic resolution analysis of dynamics in other microtubule-associated protein assemblies, including but not limited to dynactin, dynein, and kinesin motors assembled on microtubules.

Microtubules (Fig. 1), one of the three essential eukaryotic cytoskeletal structures, are responsible for multiple biological functions, ranging from cell differentiation to mitosis to cell migration (1). Microtubules are assembled from α/β-tubulin heterodimers and are highly dynamic structures, undergoing continuous cycles of polymerization/dem polymerization driven by the hydrolysis of GTP (2, 3). Intracellular transport along the microtubules is accomplished by microtubule-associated motor proteins from dynein and kinesin families (4). Dynein-based retrograde transport is mediated by dynactin, an activator and dominant cofactor of dynein (5). Dynactin is a multisubunit assembly (molecular mass, 1.2 kDa) that binds both dynein and microtubules directly and whose role is to bridge vesicles to the microtubule network and to assist dynein in its long range directed transport along the microtubules (5). The p150Glued subunit is the central component of the dynactin complex (Fig. 1). CAP-Gly (cytoskeleton-associated protein glycine-rich), an 89-residue domain of the p150Glued subunit, is in part responsible for binding to the microtubules (6, 7). CAP-Gly domains are conserved in eukaryotes and constitute, in addition to dynactin, a number of other microtubule-associated proteins (8). Mutations in the CAP-Gly domain are associated with various neurological disorders, such as distal spinal bulbar muscular atrophy (9, 10) and Perry syndrome (11). Despite biochemical and structural studies by us and by others, the underpinnings by which the CAP-Gly mutations are related to neurological disorders remain poorly understood. For example, our work has demonstrated that in the Perry syndrome mutants, despite significant conformational changes with respect to the wild type CAP-Gly, the binding affinity to microtubules is not affected by the mutations to any significant extent; however, the binding of CAP-Gly to an auxiliary end-binding protein EB1 (microtubule end-binding protein; shown in Fig. 1) is abolished (12).

The three-dimensional structures of CAP-Gly free (13, 14) and in complex with the various binding partners (8, 15–17) reveal unusually high loop content and significant conformational flexibility in the immediate vicinity of these loop regions (14, 18). To understand these observations, we examine here the role of conformational dynamics occurring on multiple time scales for the ability of CAP-Gly to interact with its binding partners and with microtubules.

We demonstrate, for the first time, atomic resolution dynamic profiles of dynactin CAP-Gly assembled on polymeric...
microtubules, in complex with end-binding protein EB1 and alone. We utilize magic angle spinning (MAS)\(^2\) NMR spectroscopy and \(^1\)H-\(^{15}\)N and \(^1\)H-\(^{13}\)C dipolar order parameters to obtain a comprehensive and site-specific view of the motional behavior of CAP-Gly in these different contexts. The results reveal that CAP-Gly undergoes motions on the time scales spanning nano- to milliseconds and that the backbone mobility, particularly in the loop regions is strongly dependent on temperature. These observations are recapitulated qualitatively by the molecular dynamics trajectories. Remarkably, the dynamic profile of CAP-Gly is uniquely dependent on the environment, i.e. its mobility is different when CAP-Gly is assembled on microtubules, in complex with EB1 and free.

We further demonstrate that the presence of EB1 attenuates the loop mobility of the CAP-Gly domain. On the contrary and surprisingly, we find that the extent of fast motions occurring on nano- to microsecond time scales increases in CAP-Gly assembled on polymeric microtubules, whereas the extent of slower, micro- to millisecond motions decreases. These results provide further evidence for our hypothesis that such conformational dynamics spanning multiple time scales, viewed over a range of temperatures, and modulated by the microtubule surfaces and by the binding partners of CAP-Gly, play an important role in the biological function of CAP-Gly and its interaction with microtubules.

Our work is the first example of atomic resolution analysis of conformational dynamics occurring on multiple time scales in any microtubule-associated protein assembled on microtubules. In a broader context, our work establishes methodological grounds for atomic resolution analysis of dynamics in microtubule-associated protein assemblies, including but not limited to dynactin, dynein, and kinesin motors assembled on microtubules.

\(^2\) The abbreviations used are: MAS, magic angle spinning; MD, molecular dynamics; DARR, dipolar assisted rotation resonance; RF, radio frequency.
EXPERIMENTAL PROCEDURES

Sample Preparation—U-^{13}C,^{15}N CAP-Gly, U-^{13}C,^{15}N CAP-Gly/n.a. EB1 complex, and U-^{13}C,^{15}N CAP-Gly/MT assembly have been prepared as described previously (14, 19). Hydrated precipitates of U-^{13}C,^{15}N CAP-Gly from PEG were packed in a 1.6-mm Varian rotor (9.8 mg) and a 3.2-mm Bruker rotor (24.4 mg). Samples of U-^{13}C,^{15}N CAP-Gly/n.a. EB1 complex were packed in a 1.6-mm Varian rotor (8.6 mg) and a 3.2-mm Bruker rotor (23.5 mg). In these U-^{13}C,^{15}N CAP-Gly/n.a. EB1 samples, the molar ratio of CAP-Gly and EB1 is 1:1. Sample containing 15.3 mg of hydrated U-^{13}C,^{15}N CAP-Gly/n.a. microtubule assembly was packed in a Bruker 1.9-mm rotor.

MAS NMR Spectroscopy—The NMR spectra of free CAP-Gly and CAP-Gly/EB1 complex were acquired at 14.1 and 19.9 T; the spectra of CAP-Gly/MT assembly were collected at 19.9 T. The 14.1 T data sets for U-^{13}C,^{15}N CAP-Gly and U-^{13}C,^{15}N CAP-Gly/n.a. EB1 samples were collected using a narrow bore Varian InfinityPlus instrument equipped with a Varian 1.6-mm triple-resonance HXY MAS probe. The MAS frequency was set to 10 kHz controlled within ±10 Hz by a Varian MAS controller. The temperature was calibrated with a KBr temperature sensor, and the temperature variation at the sample was maintained to within less than ±0.1 °C. For the direct excitation and CP MAS temperature cycling experiments of the U-^{13}C,^{15}N CAP-Gly, the actual temperature at the sample was first gradually decreased from +17 to −19 °C (+17, +12, +8, +6, +4, 0, −2, −4, −8, and −19 °C) and then gradually increased from −19 to +32 °C (−19, −8, −2, 0, +2, +4, +17, +27, and +32 °C). At each temperature, one-dimensional ^{13}C direct excitation, ^{1}H-^{13}C and ^{1}H-^{15}N CP MAS spectra were collected. Four two-dimensional DARR experiments of U-^{13}C,^{15}N CAP-Gly were acquired at −18, −2, +5, and +17 °C. The three-dimensional DIPSHIFT experiments were conducted to record the ^{1}H-^{15}N dipolar couplings for U-^{13}C,^{15}N CAP-Gly and U-^{13}C,^{15}N CAP-Gly/n.a. EB1 at T = −2 and −19 °C. The R18,7 DIPSHIFT dipolar recoupling period was incorporated into a basic two-dimensional NCA sequence, as reported previously (21).

For most of the 14.1 T NMR experiments, the 90° pulse lengths were 2.5–3 μs for ^{1}H, 3.0 μs for ^{13}C, and 4.0 μs for ^{15}N. The ^{1}H-^{13}C and ^{1}H-^{15}N CP employed a linear amplitude ramp of 80–100%; the ^{1}H RF field was 95 kHz for ^{13}C CP and 75 kHz for ^{15}N CP; and the center of the ramp on the ^{13}C or ^{15}N was Hartmann-Hahn matched to the first spinning side band. The band-selective magnetization transfer from ^{15}N to ^{13}C was realized using a 3.5-ms SPECIFIC-CP (22) with a tangent amplitude ramp, and the RF field strengths were 45, 35, and 95 kHz for ^{15}N, ^{13}C, and ^{1}H channels, respectively. In the DARR experiments, the RF field on the proton channel was matched to the spinning frequency of 10 kHz during the DARR mixing time at 50 ms. For the R18,7 block, the RF field strength on ^{1}H was 90 kHz for the recoupling of ^{1}H-^{15}N dipolar coupling. The typical ^{1}H decoupling power was 90–100 kHz during the acquisition and evolution periods in all experiments.

The 19.9 T data sets for U-^{13}C,^{15}N CAP-Gly, and U-^{13}C,^{15}N CAP-Gly/n.a. EB1 sample were acquired on a 19.9 T Bruker Avance III instrument equipped with 3.2-mm EFree HCN probe. The MAS frequency was 14 kHz controlled within ±10 Hz by a Bruker MAS controller. The temperature was calibrated with a KBr temperature sensor, and the temperature variation at the sample was maintained less than ±0.1 °C. For the direct excitation and CPMAS temperature cycling experiments on the U-^{13}C,^{15}N CAP-Gly/n.a. EB1, the actual temperature at the sample was first gradually decreased from +7 to −19 °C (+7, −2, −9, −14, −16 and −19 °C) and then was gradually increased from −19 to +17 °C (−19, −16, −14, −9, −2, +7, and +17 °C). At each temperature point, one-dimensional ^{13}C direct excitation, ^{1}H-^{13}C and ^{1}H-^{15}N CPMAS, and ^{1}H-^{13}C, ^{15}N-^{13}C double cross-polarization spectra were collected. The three-dimensional ^{1}H-^{15}C DIPSHIFT experiments for U-^{13}C,^{15}N CAP-Gly were acquired at −2 °C. The R12,4 DIPSHIFT dipolar recoupling period was incorporated into a basic one-dimensional ^{1}H-^{13}C CP sequence, as reported previously (18).

The 19.9 T data sets for U-^{13}C,^{15}N CAP-Gly/MT assembly were acquired on a 19.9 T narrow bore Bruker Avance III instrument equipped with 1.9-mm HCN probe. The MAS frequency was set to 14 kHz controlled to within ±10 Hz by a Bruker MAS controller. The temperature was calibrated with a KBr temperature sensor, and the temperature variation at the sample was maintained to within less than ±0.1 °C. For the direct excitation and CP MAS temperature cycling experiments of the U-^{13}C,^{15}N CAP-Gly/MT assembly, the actual temperature at the sample was first gradually decreased from +17 to −29 °C (+17, −2, −9, −19, and −29 °C) and then was increased gradually from −29 to +27 °C (−29, −19, −9, −2, +7, +17, and +27 °C). At each temperature point, one-dimensional ^{13}C direct excitation, ^{1}H-^{13}C and ^{1}H-^{15}N CP MAS, and ^{1}H-^{15}N, ^{15}N-^{13}C double cross-polarization experiments were collected. The two-dimensional DIPSHIFT experiments were used for the ^{1}H-^{13}C dipolar coupling measurements for U-^{13}C,^{15}N CAP-Gly/MT at −2 and −19 °C. The R12,4 DIPSHIFT dipolar recoupling period was incorporated into a basic one-dimensional ^{1}H-^{13}C CP sequence, as reported previously (18).

For most of the above experiments acquired at 19.9 T, the 90° pulse lengths were 3 μs for ^{1}H, 3.2 μs for ^{13}C, and 4.0 for ^{15}N. The ^{1}H-^{13}C and ^{1}H-^{15}N CP employed a linear amplitude ramp of 80–100%; the ^{1}H RF field was 85 kHz; and the center of the ramp on the ^{13}C or ^{15}N was Hartmann-Hahn matched to the first spinning side band. The band-selective magnetization transfer from ^{15}N to ^{13}C was realized using a 5-ms SPECIFIC-CP with a tangent amplitude ramp, and the RF field strengths were 63, 49, and 90 kHz for ^{15}N, ^{13}C, and ^{1}H channels, respectively. For the R12,4 block, RF field strength of ^{1}H was 84 kHz for the recoupling of ^{1}H-^{13}C dipolar coupling. The typical ^{1}H decoupling power was 90–100 kHz during the acquisition and evolution periods in all experiments.

Processing and Analysis of NMR Spectra—All data were processed with NMRPipe (23). The chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate, using the ^{13}C methylene peak in solid adamantane as an external standard. Peak assignments were performed in Sparky (24). For all the one-dimensional temperature-dependent spectra, no apodization was used. For the two-dimensional DARR spectra, 30°
shifted sine bell followed by a Lorentzian to Gaussian transformation functions were applied in both dimensions. For the $^{1}H$-$^{15}N$ dipolar coupling spectra acquired at 14.1 T, 45° shifted sine bell followed by a Lorentzian-to-Gaussian transformation functions were applied in both dimensions of the NCA plane. For the three-dimensional DIPSHIFT spectra for $^{1}H$-$^{13}C$ dipolar coupling measurements, a 60° shifted sine bell followed by a Lorentzian to Gaussian transformation functions was applied in both dimensions of the NCA plane. For the two-dimensional DIPSHIFT spectra for $^{1}H$-$^{13}C$ dipolar coupling measurements, a 45° shifted sine bell followed by a Lorentzian to Gaussian transformation functions was applied in the direct dimension.

The $^{1}H$-$^{15}N$ and $^{1}H$-$^{13}C$ dipolar line shapes were extracted from the three-dimensional and two-dimensional spectra using a home written C++ program based on the peak list resulting from the resonance assignments of the two-dimensional NCA plane output from Sparky. All the dipolar coupling constants were obtained by the numerical simulations of the dipolar line shapes using SIMPSON (25), as reported previously (18). The $^{1}H$ CSA parameters of the CAP-Gly domain ($\beta$, $\eta$, $\theta$, and $\phi$) reported by us previously (26) were used for the numerical simulations of $^{1}H$-$^{15}N$ dipolar coupling constants.

**Molecular Dynamics Simulations**—The structure used in the MD simulation is the CAP-Gly structure determined by us from MAS NMR (PDB ID: 2m02) (14). The simulations were performed in NPT ensemble for CAP-Gly spanning residues Leu-27 to residue Phe-97, using NAMD 2.9 (27) and the force activity (Fig. 3). The lack of $^{15}N$-$^{13}C$ dipolar-based signals indicates considerable mobility on the time scales of the N-C dipolar couplings (milliseconds).

To assess the possible presence of motions occurring on the time scales of $^{1}H$-$^{13}C$ dipolar couplings (nano- to low microseconds), we have acquired direct excitation $^{13}C$ MAS spectra at different temperatures. As illustrated in Fig. 2a, at temperatures below $−2^\circC$, the overall spectral envelope is similar in the direct excitation and CPMAS spectra, and a $\sim$2-fold signal enhancement is observed for the aliphatic peaks in the CPMAS data. This result indicates that in this temperature regime, CAP-Gly does not exhibit substantial dynamics on the nano- to low microsecond time scales. On the other hand, at temperatures above $−2^\circC$, the CPMAS spectra broaden, and no signal enhancement is observed with respect to the direct excitation spectra, indicating the presence of motions on the nano- to low microsecond time scales that interfere with cross-polarization. Interestingly, the intensity and the spectral resolution of the aliphatic peaks in the direct excitation spectra are strongly temperature-independent. For carbonyl peaks, signal intensity in the cross-polarization spectra is attenuated at temperatures between $−4$ and $+27^\circC$, as shown in Fig. 5. Most likely, this signal loss is due to the interference between protein dynamics and cross-polarization to carbonyls, which takes place from distant protons because there are no directly bonded hydrogens.

Another interesting and unanticipated observation is that the spectral intensity and resolution depend on whether a particular temperature was reached by heating or cooling the sample, in the range of $−8$ to $0^\circC$ (appropriate time was given for the temperature to equilibrate prior to each measurement). Temperature cycling gives rise to remarkably different spectra, as illustrated in Fig. 5. The highest spectral resolution in the CPMAS data is attained when the sample is first cooled down to $−19^\circC$ and then warmed up to $−2^\circC$. This effect is fully reversible and reproducible when multiple temperature cycles are

### RESULTS

**Temperature Dependence of MAS NMR Spectra**

**Free CAP-Gly**—The $^{13}C$ CPMAS and direct excitation spectra of CAP-Gly exhibit dramatic temperature dependence, as illustrated in Fig. 2a. Similar behavior was found for $^{15}N$ CPMAS and direct excitation spectra (data not shown). The highest resolution and the optimal sensitivity are attained at $−2^\circC$, for all data sets, including two-dimensional DARR (Fig. 3) and NCA spectra (Fig. 4a). At temperatures below $−2^\circC$, the $^{13}C$ and the $^{15}N$ lines broaden, with the highest extent of broadening occurring at $−34.9^\circC$. Comparison of the one-dimensional $^{13}C$ and $^{15}N$ CPMAS spectra acquired at $−2^\circC$ with those recorded at $−19^\circC$ reveals that the signal to noise ratio increases by $\sim 40\%$ for the carbonyl/aromatic regions of $^{13}C$ spectra and by $25\%$ for the amide backbone region of the $^{15}N$ spectra. At temperatures above $−2^\circC$, both the resolution and the sensitivity deteriorate considerably, up to $+17^\circC$, the temperature above which the resolution and sensitivity improve again up to the temperature where the sample deteriorates in the rotor ($+32^\circC$). Interestingly, the degradation of spectral resolution and sensitivity at temperatures between $−2$ and $+32^\circC$ is present throughout the entire spectral region in both $^{13}C$ and $^{15}N$ data rather than being associated with a limited number of amino acids (see Fig. 2a). At temperatures between $−2$ and $+32^\circC$, we could not obtain any signal in the heteronuclear two-dimensional dipolar NC-type spectra (Fig. 4a), whereas the two-dimensional DARR spectra exhibit low sensitivity (Fig. 3). The lack of $^{15}N$. $^{13}C$ dipolar-based signals indicates considerable mobility on the time scales of the N-C dipolar couplings (milliseconds).
performed on the same sample, suggesting that the protein undergoes some kind of conformational annealing en route to the conformer yielding the sharpest spectral lines. At present we do not understand the nature of this process. We note that we have observed this kind of behavior in at least one other protein under study in our laboratory, dynein light chain 8, and it will be interesting to explore this phenomenon in more detail in the future. Overall, the above results corroborate the presence of significant conformational dynamics throughout the protein occurring on time scales from nano- to milliseconds at temperatures above \( \sim 90^\circ C \) and also suggest a large conformational change occurring at approximately \( \sim 2^\circ C \).

**CAP-Gly/EB1 Complex**—We have previously reported an MAS NMR structural study of CAP-Gly/EB1 complex (14). We have identified the CAP-Gly/EB1 interface from the chemical shift perturbations and hypothesized, on the basis of the dramatically higher spectral resolution of the complex versus free CAP-Gly, that CAP-Gly must become less dynamic upon binding to EB1. To address the dynamic behavior of CAP-Gly in complex with EB1, we acquired a series of CPMAS and direct excitation one-dimensional spectra of the complex at different temperatures.

The spectra of CAP-Gly/EB1 complex displayed in Fig. 2b reveal temperature dependence very different from that in the free CAP-Gly. First, the resolution at all temperatures is higher for the complex than for free CAP-Gly. Second, at all temperatures, the CPMAS and SPECIFIC-CP signals are strong, and only mild deterioration of spectral resolution is observed below \( \sim 9^\circ C \). Furthermore, at all temperatures, the \( ^{13}C \) CPMAS and direct excitation spectral envelopes are largely consistent with each other, and there is a \( \sim 2 \)-fold signal enhancement observed in the CPMAS spectra at all conditions. The spectral resolution is the highest at temperatures above \( \sim 9^\circ C \) and does not degrade when the temperature is increased up to \( +17^\circ C \). Finally, only a mild effect of temperature cycling is observed at \( \sim 9^\circ C \), in contrast with the free CAP-Gly behavior. Taken together, these observations suggest that CAP-Gly becomes considerably more rigid when bound to EB1, on time scales spanning nano- to milliseconds.
**CAP-Gly/Microtubule Assembly**—Even more striking are the temperature profiles of the CAP-Gly/microtubule assembly MAS NMR spectra, which are markedly different than those for both free and EB1-bound CAP-Gly. As illustrated in Figs. 2, 4, and 6, at temperatures below −19 °C, the resolution and spectral intensities of the $^{13}$C/$^{15}$N CPMAS, $^{15}$N-$^{13}$C SPECIFIC-CP and two-dimensional DARR data sets are very high. (The $^{15}$N CPMAS data are not shown.) The resolution is comparable with that of free CAP-Gly data sets at −2 °C. Interestingly, at temperatures above −19 °C, the resolution of the $^{13}$C CPMAS spectra does not deteriorate dramatically, and there is never complete loss of $^{15}$N-$^{13}$C SPECIFIC-CP signal, in contrast to free CAP-Gly. Furthermore, the resolution of the $^{13}$C direct excitation spectra is invariably higher than for the corresponding CPMAS data sets, with a number of intense well resolved peaks present throughout the aliphatic, carbonyl, and aromatic regions of the direct excitation spectrum. Finally, the spectral resolution at −19 °C depends strongly on whether the temperature was reached by heating or cooling the sample (data not shown), similar to the free and in contrast to the EB1-bound CAP-Gly.

Taken together, the temperature dependences of the various dipolar-based MAS spectra indicate that there is considerable dynamics in free CAP-Gly and in CAP-Gly assembled on microtubules, occurring on time scales of micro- to milliseconds throughout the entire protein. The temperature profiles of the free and microtubule-assembled CAP-Gly are remarkably different. In contrast, dynamics is significantly attenuated in CAP-Gly bound to EB1.

**Temperature Dependence of $^{1}$H-$^{15}$N and $^{1}$H-$^{13}$C Dipolar Order Parameters: Insights from MAS NMR and Molecular Dynamics Simulations**

To further understand the nature of these motions, we next turn our attention to the temperature dependence of $^{1}$H-$^{15}$N/ $^{13}$C dipolar interactions, which report on dynamics on nano- to microsecond time scales.

**Free CAP-Gly**—The $^{1}$H-$^{15}$N dipolar order parameters of free CAP-Gly are shown in Fig. 7 and Table 1. The experimental findings reveal the following: (i) The $^{1}$H-$^{15}$N dipolar order parameters for the majority of the residues are systematically dependent on the temperature, with the average values being 0.91 at −19 °C and 0.87 at −2 °C. This translates to the average values for the $^{1}$H-$^{15}$N dipolar coupling constants of 10.31 and 9.88 kHz, respectively. The difference of 430 Hz in the average value of the dipolar coupling constant is not trivial and indicates that the backbone mobility on nano- to microsecond time scales is noticeably increased at −2 °C. This is consistent with the temperature dependences of the various spectra discussed in the previous section. (ii) The average $^{1}$H-$^{15}$N dipolar order parameter values for the $\beta$-sheet and the short $\alpha$-helical regions of the protein are 0.91 (dipolar coupling 10.30 kHz) at −19 °C and 0.89 (dipolar coupling 10.14 kHz) at −2 °C. (iii) The average $^{1}$H-$^{15}$N dipolar order parameter values for the loop regions of the protein are 0.91 at −19 °C and 0.86 at −2 °C. This result demonstrates that it is the loop regions of the protein that are largely responsible for the temperature dependence of the backbone mobility of CAP-Gly occurring on the nano- to microsecond time scales.
The $^1$H-$^1$H dipolar order parameters of CAP-Gly recorded at $-5 \, ^\circ C$ at 14.1 T were reported by us previously (18). We found that the $^1$H-$^1$H dipolar order parameters reported here exhibit the same general behavior as the $^1$H-$^1$3C dipolar order parameters recorded in our previous work: the average value for the loops (0.85) was found to be lower than that for the $\beta$-sheet and the short $\alpha$-helical regions (0.92).

Because of the deteriorated spectral resolution and sensitivity at temperatures above $-2 \, ^\circ C$ (see above), we could not record experimental $^1$H-$^1$5N and $^1$H-$^1$3C order parameters under those conditions. Therefore, to understand the nature of the backbone motions and their temperature dependence, we have pursued molecular dynamics calculations of free CAP-Gly at four different temperatures: 250, 263, 278, and 300 K. Even though we cannot compare the temperature in the MD simulations directly to that in the NMR experiments, it is nonetheless instructive to examine the observed trends qualitatively, as discussed below. We note that the $^1$H-$^1$5N and $^1$H-$^1$3C dipolar interactions measured in magic angle spinning NMR experiments provide a direct probe of local motions (fluctuations of H-N and H-C bond vectors, respectively) occurring on time scales spanning nano to microseconds. Therefore, the results of MD simulations can be directly compared with the motions with nano- to microsecond time scale obtained from NMR experiments, provided that the simulations are conducted for long enough time to capture the slow tail of the motions and that the temperature in the simulations is the same as that of the experiments. Indeed, it has been demonstrated by Skrynnikov, Reif, and co-workers (30) and by us (in HIV-1 protein assemblies, to be reported in a forthcoming manuscript) that experimental MAS NMR and MD-derived motional parameters are consistent for several proteins studied so far, even when relatively short MD trajectories were used. CAP-Gly protein under investigation in this report poses unique challenges for this analysis, because of the following: (i) its high loop content (see above), and ii) for temperatures above $-2 \, ^\circ C$, extensive motions in multiple parts of the protein (termini and loops) appear to occur on time scales spanning the entire range of nano- to milliseconds, making it difficult to record site-specifically the dipolar line shapes because of signal disappearance or broadening. For this reason, direct comparison between MD and NMR results is very difficult for CAP-Gly, and we have therefore pursued the qualitative analysis of the trends in the experimental MAS NMR and computational MD data, as a function of temperature.

**FIGURE 5.** $^{13}$C CPMAS spectra (black) overlaid with $^{13}$C direct excitation MAS spectra (red) of U-$^{13}$C,$^{15}$N-CAP-Gly, shown as a function of temperature.
From the MD trajectories, we have extracted the $^1$H-$^1$H and $^1$H-$^1$C dipolar order parameters according to the protocol described under "Experimental Procedures." The computed order parameters are plotted in Fig. 7 (a, b, and c). The overall trends observed in the experimental data are consistent with those captured by the simulations. First, the NMR experiments reveal that at $-19$ and $-2 \, ^\circ\text{C}$ only select few residues in the $\beta1/\beta2$ loop regions, and the termini are mobile. As shown in Fig. 7a, MD simulations conducted at 250, 263, and 278 K qualitatively reproduce this finding and show that these regions are more dynamic than other portions of the protein. According to the MD simulations, at the temperature of 278 K, more loop residues become dynamic, and particularly interesting is the extended mobile region spanning residues 65–85 and encompassing the functionally important GKNL motif (residues 67–70). The mobility of this region becomes even more dramatic at 300 K, revealing that large amplitude motions are present essentially in every residue from positions 67 to 85. As discussed above, at temperatures higher than 271 K, the MAS NMR spectra of CAP-Gly are broadened, which is a manifestation of extensive dynamics in the multiple regions of the protein, consistent with the MD results and hampering site-specific measurement of dipolar order parameters in a large number of residues. Nonetheless, we have recorded the two-dimensional $^1$H-$^1$C DIPSHIFT spectra for CAP-Gly at 279 K,
in our attempt to extract the dipolar line shapes for residues yielding resolved peaks in the spectra. To our surprise and confirming the results of the MD simulations, we could only detect the zero frequency peaks in the dipolar dimension rather than the \textit{bona fide} dipolar line shapes, corroborating that there are extensive motions in multiple residues throughout the protein, which preclude recoupling of the dipolar interaction under these conditions.
To analyze the MD motional trajectories leading to the significantly attenuated computed order parameters at temperatures above 278 K, we have plotted the angular excursions of the H-N/H-13C bond vectors on the surface of a three-dimensional sphere, together with the angular probability distributions for two representative residues, Asp-70 and Val-58, illustrated in Fig. 8. As anticipated, Val-58 located in the rigid region of the protein exhibits canonical Gaussian angular probability distribution and the restricted cone excursions of the H-N/H-13C bond vectors, whereas the angular probability distribution for the mobile Asp-70 residue located in the GKN sequence is rather complex, and the corresponding bond vectors cover significant portions of the three-dimensional sphere, particularly at 300 K.

We have also predicted the generalized order parameter \( S^2 \) (31), indicative of the internal dynamics on nano- to microsecond time scales, using the random coil index method, as implemented in TALOS+ (32). Interestingly, we note the overall qualitative agreement between the computationally predicted \( S^2 \) and the experimental \( ^1\text{H}-^{15}\text{N} \) and \( ^1\text{H}-^{13}\text{C} \) dipolar order parameters, as shown in Fig. 7d.

**CAP-Gly/EB1 Complex**—As discussed above and in our previous report (14), CAP-Gly becomes more rigid upon binding to its partner EB1. To examine the residue-specific dynamics of CAP-Gly in the complex occurring on nano- to microsecond time scale, we have recorded the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters at two temperatures, −2 °C and −19 °C. The findings are summarized in Fig. 9 and Table 2. Close inspection of the results reveals that (i) CAP-Gly bound to EB1 displays systematically higher order parameters as compared with the free CAP-Gly, for the overwhelming majority of the residues, at both temperatures. The average values for the CAP-Gly/EB1 are 0.95 (−19 °C) and 0.92 (−2 °C), whereas the corresponding average values for free CAP-Gly are 0.91 and 0.87. (ii) Interestingly, the changes in the order parameters of CAP-Gly upon binding to EB1 are not restricted to the residues lining the two binding interfaces of CAP-Gly with EB1 (labeled A and B in Fig. 9, according to the nomenclature we employed previously (14)). Instead, the order parameters increase across the majority of the residues upon the formation of the complex. On the basis of the temperature dependences of the various dipolar spectra and the analysis of the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters, we conclude that binding of CAP-Gly to EB1 results in considerably attenuated dynamics of CAP-Gly on time scales spanning nanoseconds to milliseconds.

**CAP-Gly/Microtubule Assembly**—Similar to the temperature profiles, the heteronuclear dipolar order parameters reveal unexpected dynamic behavior of CAP-Gly assembled on the microtubules. The inherently lower sensitivity of the experiments in the assembly precluded measurements of \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters by three-dimensional correlations. We therefore have recorded \( ^1\text{H}-^{13}\text{C} \) dipolar order parameters in two-dimensional experiments, at two temperatures, −19 and −2 °C. As summarized in Table 3, in these experiments 15 well resolved peaks emerge at −19 °C, and 11 emerge at −2 °C, permitting unambiguous determination of dipolar order parameters for the corresponding residues. Additionally, a total of 28 residues display partial peak overlap in the −19 °C data set, and 18 show partial peak overlap in the −2 °C spectrum, from which we also extracted dipolar order parameters. These residues span the entire CAP-Gly sequence.

As illustrated in Fig. 10, at the temperature of −2 °C, the dipolar order parameters are systematically lower than at −19 °C, with the average values being 0.78 and 0.85, respectively. Surprisingly, the \( ^1\text{H}-^{13}\text{C} \) dipolar order parameters for the assembled CAP-Gly at −2 °C are significantly lower than those recorded under the same conditions (temperature, field strength, and other parameters) for the free protein (average value, 0.86). Interestingly, even at −19 °C, the dipolar order parameters for the CAP-Gly/MT assembly are somewhat lower than or similar to those for free CAP-Gly at −2 °C. These results indicate that, contrary to the conventional wisdom, CAP-Gly becomes considerably more dynamic on nano- to microsecond time scales when associated on microtubules.

**Discussion**

The experimental NMR parameters provide comprehensive view of the residue-specific backbone dynamics in CAP-Gly free, bound to EB1, and assembled on the microtubules, indicating that free and microtubule-assembled CAP-Gly exhibits significant mobility on time scales spanning nano- to millisecond and that motions are strongly temperature-dependent. At the temperature of −34.9 °C, the NMR signals in the DARR spectrum of free CAP-Gly are strong and broad, suggesting that the protein is rigid, and the line broadening is due to the conformational heterogeneity. At temperatures between −20 and −2 °C, the protein becomes dynamic, with predominant motions occurring in the millisecond and submillisecond time scales, with nano- to microsecond time scale motions taking place in a limited number of residues in loops. Under these conditions, the lines are significantly narrowed compared with lower temperatures but still broadened somewhat compared with −2 °C. The slight line broadening is likely due to conformational heterogeneity and finite number of “static” (on the time scale of NMR experiments) conformers with very close

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**TABLE 1**

| T = −19 °C (14.1 T) | T = −2 °C (14.1 T) | T = −2 °C (19.9 T) |
|----------------------|---------------------|---------------------|
| \( ^1\text{H}-^{15}\text{N} \) dipolar coupling S (\( ^1\text{H}-^{15}\text{N} \)) | \( ^1\text{H}-^{15}\text{N} \) dipolar coupling S (\( ^1\text{H}-^{15}\text{N} \)) | \( ^1\text{H}-^{13}\text{C} \) dipolar coupling S (\( ^1\text{H}-^{13}\text{C} \)) |
| \( ^1\text{H}-^{15}\text{N} \) dipolar coupling S (\( ^1\text{H}-^{15}\text{N} \)) | \( ^1\text{H}-^{15}\text{N} \) dipolar coupling S (\( ^1\text{H}-^{15}\text{N} \)) | \( ^1\text{H}-^{13}\text{C} \) dipolar coupling S (\( ^1\text{H}-^{13}\text{C} \)) |
| Secondary structures | Loop/termini | Secondary structures |
| 10.30 | 10.14 | 0.89 | 19.90 | 0.87 | 19.60 |
| 10.31 | 9.75 | 0.86 | 19.47 | 0.85 | 19.60 |
| 10.31 | 9.88 | 0.86 | 19.60 | 0.86 | 19.60 |

**CAP-Gly Dynamics Regulated by Microtubules and EB1**

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**Figure 7d**

**Figure 8**

**Figure 9**

**Figure 10**

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**References**

(14) CAP-Gly becomes more rigid upon binding to its partner EB1. To examine the residue-specific dynamics of CAP-Gly in the complex occurring on nano- to microsecond time scale, we have recorded the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters at two temperatures, −2 °C and −19 °C. The findings are summarized in Fig. 9 and Table 2. Close inspection of the results reveals that (i) CAP-Gly bound to EB1 displays systematically higher order parameters as compared with the free CAP-Gly, for the overwhelming majority of the residues, at both temperatures. The average values for the CAP-Gly/EB1 are 0.95 (−19 °C) and 0.92 (−2 °C), whereas the corresponding average values for free CAP-Gly are 0.91 and 0.87. (ii) Interestingly, the changes in the order parameters of CAP-Gly upon binding to EB1 are not restricted to the residues lining the two binding interfaces of CAP-Gly with EB1 (labeled A and B in Fig. 9, according to the nomenclature we employed previously (14)). Instead, the order parameters increase across the majority of the residues upon the formation of the complex. On the basis of the temperature dependences of the various dipolar spectra and the analysis of the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters, we conclude that binding of CAP-Gly to EB1 results in considerably attenuated dynamics of CAP-Gly on time scales spanning nanoseconds to milliseconds.

**CAP-Gly/EB1 Complex**—As discussed above and in our previous report (14), CAP-Gly becomes more rigid upon binding to its partner EB1. To examine the residue-specific dynamics of CAP-Gly in the complex occurring on nano- to microsecond time scale, we have recorded the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters at two temperatures, −2 °C and −19 °C. The findings are summarized in Fig. 9 and Table 2. Close inspection of the results reveals that (i) CAP-Gly bound to EB1 displays systematically higher order parameters as compared with the free CAP-Gly, for the overwhelming majority of the residues, at both temperatures. The average values for the CAP-Gly/EB1 are 0.95 (−19 °C) and 0.92 (−2 °C), whereas the corresponding average values for free CAP-Gly are 0.91 and 0.87. (ii) Interestingly, the changes in the order parameters of CAP-Gly upon binding to EB1 are not restricted to the residues lining the two binding interfaces of CAP-Gly with EB1 (labeled A and B in Fig. 9, according to the nomenclature we employed previously (14)). Instead, the order parameters increase across the majority of the residues upon the formation of the complex. On the basis of the temperature dependences of the various dipolar spectra and the analysis of the \( ^1\text{H}-^{15}\text{N} \) dipolar order parameters, we conclude that binding of CAP-Gly to EB1 results in considerably attenuated dynamics of CAP-Gly on time scales spanning nanoseconds to milliseconds.

**CAP-Gly/Microtubule Assembly**—Similar to the temperature profiles, the heteronuclear dipolar order parameters reveal unexpected dynamic behavior of CAP-Gly assembled on the microtubules. The inherently lower sensitivity of the
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a) Angular probability distributions: H-^13C bond vectors

b) Angular probability distributions: H-N bond vectors

FIGURE 8. Angular probability distributions and angular excursions of the H-^13C bond vectors (a) and H-N (b) bond vectors for two representative residues in CAP-Gly, Val-58 (rigid residue) and Asp-70 (flexible residue constituting the GKND loop motif), plotted on the surface of a three-dimensional sphere. They are shown as a function of temperature. Note the drastic temperature dependence of the Asp-70 mobility, not present for Val-58.

structures contributing to the spectra. At the temperature of -2 °C, the millisecond and submillisecond motions are attenuated, as evidenced by the increased resolution of the DARR spectra, and the mobility of the loop regions on nano- to microsecond time scales increases. At temperatures between -2 and +32 °C, the motions are in the “intermediate” regime (with respect to the time scales of the NMR experiments), i.e. the motional time scales match the magnitude of the ^1H-^13C and ^1H-^15N dipolar couplings, causing interference with the ^1H-^13C/^1H-^15N cross-polarization and heteronuclear decoupling and resulting in severe broadening of spectral lines and signal attenuation. (The most detrimental effect on the spectra is observed at +17 °C.) In this temperature range, the MD simulations reveal the presence of extensive motions on nano- to microsecond time scales throughout the protein, and we could not measure the dipolar order parameters under these conditions because of signal disappearance and severe line broadening. Finally, at the temperature of +32 °C (the highest experimentally attainable temperature before the protein degrades), the motional time scales start entering the “fast” regime as evident by the partial line narrowing and the partial recovery of the signal intensity. Consistent with the experimental results, MD simulations also underscore high mobility of the loops with extensive dynamics observed in loop connecting β-strands 3
and 4 and encompassing the functionally important GKN
motif.

When bound to its +TIP binding partner EB1, CAP-Gly
becomes considerably more rigid on time scales spanning
nano- to milliseconds, and the attenuation of internal mobility
appears to be a global feature of bound CAP-Gly, not limited to
the residues lining the protein-protein interface. We did not
find evidence for any considerable internal mobility on nano- to
microsecond time scales in the temperature range examined.

CAP-Gly assembled on microtubules is found to be more
rigid than the free protein on the time scales of micro- to
milliseconds, as evidenced by the temperature dependences
of the various dipolar-based spectra of the assembly. At the
same time and surprisingly, the assembled protein is more
dynamic than free CAP-Gly on the time scales of nano- to
microseconds.

Whereas we do not understand the underlying basis for the
increased extent of fast motions in CAP-Gly upon binding to
microtubules, we speculate that it may be essential for the abil-
ity of CAP-Gly to interact with microtubules. We note that a
similar discovery of increased internal mobility of a protein
upon binding has been published recently. In this work, Drobny
and co-workers (33) observed that upon binding to a surface of
hydroxyapatite, a salivary peptide statherin, whose function is
to inhibit the nucleation and crystal growth of hydroxyapatite
in the oral environments, becomes more dynamic in the side
chains of Phe residues. Although complete understanding of
the role of this behavior in the surface-bound statherin is cur-
rently lacking, it appears that such modification of the potential
energy surfaces of the statherin side chains by proximal sur-
faces may be important biologically. We note that this result is
consistent with our own surprising finding presented here that
the micro- to nanosecond time scale mobility in CAP-Gly
assembled on polymeric microtubules increases vis-à-vis the
free unbound CAP-Gly. Therefore, it appears that such modu-
lation of the dynamic profile by the binding surfaces is not lim-
ited to CAP-Gly, and we intend to explore this further in the
future to understand whether this behavior is characteristic of
other microtubule-associated proteins as well and what its role
is in the regulation of interactions between microtubule-asso-
ciated proteins and microtubules. We cannot rule out packing
effects in the nanocrystalline samples of free CAP-Gly and
CAP-Gly/EB1 complex, which may contribute to higher rigid-

![Graphical representation of experimental $^1$H-$^{15}$N dipolar order parameters plotted as a function of the residue number for U-$^{13}$C,$^{15}$N-CAP-Gly (green circles) and U-$^{13}$C,$^{15}$N-CAP-Gly in complex with n.a. EB1 (black circles). The data were recorded at 14.1 T and temperatures of $-2$ °C (a) and $-19$ °C (b). The CAP-Gly binding interfaces with EB1 (A and B) are shaded gray. The average dipolar order parameter values for free and EB1-bound CAP-Gly are shown with green and black dashed lines, respectively.](image-url)
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TABLE 3
Experimental NMR $^1$H-$^{13}$C dipolar coupling parameters in CAP-Gly/microtubule assembly

| Residues | $^1$H-$^{13}$C dipolar coupling S($^1$H-$^{13}$C) Residues | $^1$H-$^{13}$C dipolar coupling S($^1$H-$^{13}$C) Residues |
|-----------|---------------------------------------------------------|---------------------------------------------------------|
|           | kHz                                                     | kHz                                                     |
| Val-44    | 20.75 ± 0.01                                           | 0.91 ± 0.01                                             |
| Ile-36    | 19.75 ± 0.74                                           | 0.87 ± 0.03                                             |
| Thr-40    | 19.20 ± 0.08                                           | 0.84 ± 0.01                                             |
| Thr-54    | 19.28 ± 0.26                                           | 0.84 ± 0.01                                             |
| Ser-31/Thr-72/Val-47/Val-73 | 19.20 ± 0.07 | 0.84 ± 0.01                             |
| Thr-43/Val-60/Ile-61 | 19.60 ± 0.09 | 0.86 ± 0.01                             |
| Gly-91/Thr-50/Ser-92 | 18.90 ± 0.11 | 0.83 ± 0.01                             |
| Val-89    | 19.44 ± 0.09                                           | 0.85 ± 0.01                                             |
| Tyr-46    | 19.15 ± 0.06                                           | 0.84 ± 0.01                                             |
| Glu-83/Val-33/Ile-94 | 18.80 ± 0.07 | 0.82 ± 0.01                             |
| Phe-88    | 19.55 ± 0.30                                           | 0.86 ± 0.01                                             |
| Asp-63/Lys-56/Trp-57/Gln-74/Lys-77/His-85/Cys-81/Phe-52/Phe-79/Leu-62 | 19.50 ± 0.11 | 0.85 ± 0.01                             |
|           | 19.00 ± 0.06                                           | 0.83 ± 0.01                                             |
|           | 18.80 ± 0.05                                           | 0.82 ± 0.01                                             |
|           | 19.20 ± 0.08                                           | 0.84 ± 0.01                                             |
|           | 19.05 ± 0.12                                           | 0.84 ± 0.01                                             |
|           | 19.20 ± 0.08                                           | 0.84 ± 0.01                                             |
| Arg-41    | 18.05 ± 0.04                                           | 0.81 ± 0.01                                             |
| Asp-82    | 18.76 ± 0.07                                           | 0.82 ± 0.01                                             |
| Asn-69    | 19.32 ± 0.01                                           | 0.85 ± 0.01                                             |
| Asp-70    | 19.57 ± 0.09                                           | 0.86 ± 0.01                                             |
| Ala-49/Ala-53 | 19.36 ± 0.10 | 0.85 ± 0.01                             |
| Ala-45    | 19.63 ± 0.01                                           | 0.86 ± 0.01                                             |
| Ala-65    | 18.95 ± 0.06                                           | 0.83 ± 0.01                                             |
| Gly-59    | 21.30 ± 0.14                                           | 0.93 ± 0.01                                             |
| Gly-48/84/86 | 19.00 ± 0.13 | 0.83 ± 0.01                             |
|           | 19.32 ± 0.11                                           | 0.85 ± 0.01                                             |
|           | 18.73 ± 0.11                                           | 0.85 ± 0.01                                             |
|           | 19.50 ± 0.01                                           | 0.84 ± 0.01                                             |
|           | 19.20 ± 0.06                                           | 0.84 ± 0.01                                             |
|           | 18.60 ± 0.12                                           | 0.74 ± 0.01                                             |
|           | 19.43 ± 0.01                                           | 0.76 ± 0.01                                             |
|           | 16.30 ± 0.25                                           | 0.73 ± 0.01                                             |
|           | 17.71 ± 0.28                                           | 0.78 ± 0.01                                             |
|           | 16.80 ± 0.12                                           | 0.74 ± 0.01                                             |
|           | 17.25 ± 0.07                                           | 0.76 ± 0.01                                             |
|           | 17.25 ± 0.06                                           | 0.76 ± 0.01                                             |
|           | 16.00 ± 0.16                                           | 0.70 ± 0.01                                             |
|           | 19.72 ± 0.01                                           | 0.86 ± 0.01                                             |
|           | 19.90 ± 0.71                                           | 0.87 ± 0.03                                             |
| Average   | 19.32 ± 0.11                                           | 0.85 ± 0.01                                             |
|           | 18.73 ± 0.11                                           | 0.85 ± 0.01                                             |

FIGURE 10. Experimental $^1$H-$^{13}$C dipolar order parameters plotted as a function of the residue number for U-$^{13}$C,$^{15}$N-CAP-Gly assembled on n.a. microtubules (green circles, $T = −2 °C$; black circles, $T = −19 °C$) and U-$^{13}$C,$^{15}$N-CAP-Gly (purple circles, $T = −2 °C$). The data were recorded at 19.9 T. The average dipolar order parameter values for each sample and condition are shown with dashed lines of the corresponding color.

ity of CAP-Gly in these samples vis-à-vis CAP-Gly assembled on microtubules. At the same time, the results summarized in Fig. 10 suggest that packing alone is not sufficient to explain higher mobility of CAP-Gly assembled on microtubules because increased nano- to microsecond dynamics is found throughout the entire sequence rather than being limited to surface residues.

We also note that the binding affinities of CAP-Gly to EB1 and microtubules are drastically different (high nanomolar versus mid-micromolar (12, 19), and unpublished data), and it will be instructive in the future to examine the possible connection between the thermodynamic binding parameters and the internal dynamics of CAP-Gly bound to EB1, assembled on MTs, and of CAP-Gly/EB1 complex associated on MTs. Such studies were reported in other systems (34–36), where relationships

3 S. Ahmed and J. C. Williams, unpublished data.
between the binding entropies and NMR order parameters have been unequivocally established. Future work will address extended p150Glued subunit in the context of its assemblies with polymeric microtubules and EB1.

CONCLUSIONS

Our work is the first example of atomic resolution analysis of conformational dynamics occurring on multiple time scales, in a microtubule-associated protein assembled on polymeric microtubules. We have demonstrated that dynactin CAP-Gly has unusual dynamic behavior, exhibiting motions occurring on multiple time scales and in a wide temperature range and extending over a significant part of the protein. Furthermore, the dynamic profile of CAP-Gly is dramatically dependent on the environment, i.e. whether the protein is assembled on polymeric microtubules, bound to its binding partner EB1, or free. The analysis of temperature-dependent NMR parameters and MD trajectories yielded a comprehensive view of its backbone dynamics over broad time scales and wide temperature range, which to our knowledge is the first study of this kind. The mobility of CAP-Gly in complex with EB1 is attenuated considerably across all time scales vis-à-vis free CAP-Gly. On the contrary and surprisingly, CAP-Gly assembled on microtubules becomes more dynamic on nano- to microsecond time scales. Taken together, the findings presented in this report reveal that inherent conformational plasticity of CAP-Gly is essential for the interactions of dynactin with microtubules and binding partners and its ability to interact on the microtubules. More broadly, the integrated MAS NMR/MD simulations approach introduced in this work is well suited for analysis of conformational dynamics of a broad range of microtubule-associated proteins assembled on microtubules, including but not limited to microtubule motors of the dynein, dynactin, and kinesin families.

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