Temperature-induced collapse of a disordered peptide observed by three sampling methods in molecular dynamics simulations

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The conformational ensembles of a disordered peptide, polyglutamine Q15, over a wide temperature range were sampled using multiple replicates of conventional molecular dynamics (cMD) simulations as well as two enhanced sampling methods, temperature replica exchange (TREM) and replica exchange with solute tempering (REST). The radius of gyration, asphericity, secondary structure, and hydrogen bonding patterns were used for the comparison of the sampling methods. Overall, the three sampling methods generated similar conformational ensembles, with progressive collapse at higher temperatures. Although accumulating the longest simulation time (90 μ s), cMD at room temperature missed a small subspace that was sampled by both TREM and REST. This subspace was high in α -helical content and separated from the main conformational space by an energy barrier. REST used less simulation time than TREM (36 μ s versus 42 μ s), and this gap is expected to widen significantly for larger disordered proteins. We conclude that REST is the method of choice for conformational sampling of intrinsically disordered proteins. *Published by AIP Publishing.*
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I. INTRODUCTION

Since its advent, molecular dynamics (MD) simulation has been a key tool for understanding the structure, dynamics, and function of proteins. This is especially true for intrinsically disordered proteins (IDPs), a class of proteins with a highly dynamic, flexible nature and transient secondary structure.¹ These properties make it difficult to characterize the conformations and dynamics of IDPs at an atomic level, and MD simulation remains one of the few approaches to this end.² Still, it is challenging to adequately sample the full conformational ensemble of an IDP in conventional MD (cMD) simulations, as the protein tends to get stuck in local minima. For this and related reasons, a number of enhanced sampling techniques have been developed to speed up barrier crossing. There are numerous studies applying enhanced sampling methods, but much fewer studies on the direct comparison between conformational ensembles of IDPs generated by different enhanced sampling techniques.^{3–6} In this study, we generated the conformational ensembles of a polyglutamine (polyQ) 15-mer, Q15, over a wide range of temperatures in explicit solvent using cMD and two enhanced sampling methods, temperature replica exchange (TREM)^{7–9} and replica exchange with solute tempering (REST).^{10–14} To our knowledge, this is the first time that REST was tested for its effectiveness at producing temperature-dependent properties of IDPs.

IDPs have been linked to multiple diseases.¹⁵ PolyQ expansion (also known as CAG repeat) diseases arise from

expanded polyQ sequences in proteins. Among the nine known polyQ expansion diseases, the most notable is Huntington's disease.¹⁶ This disease progresses by aggregation through the Huntingtin (Htt) protein's exon-1 domain.^{16–18} This domain consists of a 17-residue N-terminal region, a polyQ track, and a proline-rich region. In crystal structures, the N-terminal region formed an α -helix and the proline-rich region formed a polyproline-II (PPII) helix, but the polyQ track exhibited polymorphism, including full and partial α -helix, extended conformation, and disorder.¹⁹ If the number of glutamine residues exceeds 35,¹⁶ Htt aggregates via a β -sheet fibril formation pathway with a polyQ core.²⁰ It was thought that the flanking regions could not provide sufficient stabilization for expanded polyQ tracks, leading to aggregation.²¹ MD simulations of Lakhani *et al.*²² showed that, with an expanded polyQ sequence, the N-terminal region was less likely to form α -helices and more likely to form β -sheets. Recent simulations of Binette *et al.*²³ using Hamiltonian replica exchange metadynamics demonstrated that the N-terminal and proline-rich regions flanking a 17-residue polyQ track stabilized the secondary structures of the whole exon-1 domain. From these studies, it is clear that polyQs play a key role in Huntington's disease. Studying the behavior of monomeric polyQs will allow us to better understand the conformations and interactions of this sequence in the aggregation of Htt.

In solution, the disordered nature of monomeric polyQs is well established, with evidence from a range of studies, including circular dichroism,^{24,25} fluorescence correlation spectroscopy,²⁶ triplet quenching,²⁷ fluorescence

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resonance energy transfer,²⁵ UV resonance Raman,²⁸ and MD simulations.^{28–35} A variety of secondary structures have been implicated, including α -helices, PPII helices, and β -strands or β -hairpins. PolyQs start to aggregate when the length is 16 or higher.^{25,30,31} Perhaps not coincidentally, shorter polyQs are extended, whereas longer polyQs are collapsed, with Q16 at the transition point.^{25–27} Directly relevant to the present study, polyQ tracks in the Htt exon-1 domain were found in recent temperature jump experiments to show monotonic temperature-induced collapse.³⁶

Various enhanced sampling techniques have been applied to the study of IDPs.^{3,37–41} One of the most notable is TREMD,⁷ which involves a set of replicate simulations that spans a temperature range between the temperature of interest and an artificially high temperature. By exchanging between replicas at different temperatures, TREMD allows the system at the temperature of interest to cross energy barriers that would be too high for cMD. TREMD has been used to produce a temperature-induced collapse of CspM34 and ACTR³⁷ as well as several other IDPs³⁸ in explicit solvent. In simulations with explicit solvent, the total energy is dominated by solvent contributions, and hence exchanges between replicas with large differences in energy may not involve large differences in solute conformation. More importantly, with explicit solvent, the total energy may span a vast range so that a large number of replicas may be required to create a finely spaced temperature ladder in order to achieve an adequate exchange rate. Indeed, a major potential limitation of TREMD with explicit solvent is the rapid increase in the number of replicas required as the system size increases.^{3,42,43}

TREMD has been applied to the study of polyQs in both implicit solvent and explicit solvent. Implicit-solvent TREMD was implemented to study the aggregation mechanism of polyQs³⁴ and to compare the conformations of polyQs at different lengths and temperatures.³³ The latter study found polyQ expansion at increasing temperatures. Subsequent studies suggest that, to get correct temperature-dependent effects in implicit-solvent simulations, temperature-dependent parameterization may be required.⁴⁴ Recently, TREMD with explicit solvent was used to compare the conformational ensembles of Q30 in various force fields.³⁵

REST^{10–14} is a form of Hamiltonian replica exchange designed to overcome some of the limitations of TREMD, by treating the solvent and solute separately. Specifically, solute-solute and solute-solvent interactions are scaled to mimic a rise in the temperature of the solute, while maintaining the temperature of the solvent. Accordingly, REST requires fewer replicas than TREMD. REST has been used to study lipid bilayers⁴⁵ and various protein systems.^{41,46–48} Recently, the original version of REST^{10,11} was compared with TREMD in a study of a β -amyloid peptide bound to a bilayer membrane.⁴ REST has also been compared to partial tempering well-tempered ensemble metadynamics (PT-WTE).⁶ Both REST and PT-WTE were found to enhance the conformational sampling of an IDP compared to TREMD, but each method offered its own advantages. One criticism of REST was that it could not produce temperature-dependent data since only the replica with the same

temperature for both the solute and solvent corresponds to a real system.

While the number of studies applying enhanced sampling methods on IDPs keeps growing and new methods are continuously being developed, there are still relatively few studies comparing different methods on IDPs. Such comparative studies enable cross-validation on the conformational ensembles from different methods and provide the necessary information for selecting a method that offers the optimal compromise between sampling accuracy and efficiency. Here we compare three methods in sampling the conformational ensembles of Q15 over a wide range of temperatures in explicit solvent: multiple replicates of cMD, TREMD, and REST. We conclude that REST may be the method of choice for conformational sampling of IDPs.

II. METHODS

A. Simulation setup

All the simulations of Q15 were run in GROMACS 4.6.7.⁴⁹ The initial structure was generated in PyMOL in a fully extended form with acetyl and N-methylamine caps. Following Best *et al.*,³⁷ the Amber ff03ws force field with TIP4P/2005⁵⁰ water was chosen. In this modification, the Lennard-Jones interactions between water oxygen and all protein atoms were scaled by a factor of 1.1, leading to expansion of otherwise overly compact IDPs. The initial structure of Q15 was first energy minimized by 50 000 steps of steepest descent and then simulated for 2 ns in vacuum. The last snapshot was inserted into a box with 1803 water molecules and simulated for 20 ns at constant NPT ($T = 298$ K). Clustering was performed on the resultant trajectory, and a conformation with an intermediate compactness was selected. The selected Q15 structure was added to a cubic box with a 75-Å side length and solvated with 5386 water molecules. Steepest descent energy minimization was performed to remove any clashes. The resulting snapshot was used to prepare the cMD simulations (see Subsection II B). All temperature regulation was performed using velocity rescaling,⁵¹ and all pressure equilibration, targeting 1 bar, was performed using the Parinello-Rahman barostat.⁵²

In all the simulations, the leap-frog integrator was used with a 2 fs time step. Cutoffs of 10 Å were set for both the Lennard-Jones and electrostatic interactions. Particle mesh Ewald summation^{53,54} was used for long-range electrostatic calculations with periodic boundary conditions. Other details of the three sampling methods are given below.

B. cMD simulations

Similar to the simulations of Wang *et al.*,²⁹ we ran 60 replicate simulations each at three temperatures: 298 K, 354 K, and 410 K. To prepare the simulations at each temperature (T_i), first a single short simulation was run, with 100 ps at constant NVT to equilibrate temperature to T_i followed by 1 ns at constant NPT (the latter again at T_i) to adjust water densities. From the last snapshot at each temperature, 60 independent production simulations at constant NVT were

started with different random seeds for selecting velocities from the Maxwell-Boltzmann distribution and continued for 0.5 μ s.

C. TREMD simulations

42 replicas were chosen with temperatures ranging from 298 K to 410 K based on the algorithm of Nadler and Hansmann,⁴² with replica temperatures given by $T_i = T(T_{\max}/T)^{(i-1)/(n_{\text{rep}}-1)}$, where $n_{\text{rep}} = 42$, $T = 298$ K, and $T_{\max} = 410$ K. Starting from the last snapshot of the aforementioned 1 ns constant-NPT simulation at 298 K (Subsection II B), all the replicas were each prepared in a 100 ps simulation at constant NVT to equilibrate temperatures to the desired values. From there, exchanges between replicas were attempted every 2 ps, and the simulations were continued at constant NVT for 1 μ s. For analysis, only the three replicas with the same temperatures as those in the cMD and REST simulations were used.

D. REST simulations

GROMACS 4.6.7 was patched with PLUMED-2.2.1-hrex for the REST implementation by Bussi.¹⁴ All the 267 atoms in Q15 were selected as the “hot” solute region. Solvent temperatures of 298 K, 354 K, and 410 K were chosen to match those in the cMD simulations. The last snapshot of the cMD 1 ns constant-NPT simulation at each temperature was used for starting the REST simulations. In different replicas, the solute-solute and solute-solvent interactions were scaled to generate an effective temperature ladder for the solute, with the solvent temperature as the lowest rung. (Note that, by adapting the ladder to the solvent temperature, the “physical” replica where the solute had the solvent temperature could exchange only with replicas with hotter solutes. We also experimented with a fixed temperature ladder for all the solvent temperatures, meaning that the “physical” replica could exchange with replicas having colder solutes. These simulations covered similar conformational spaces as the simulations with adaptive temperature ladders but, not unexpectedly, were not as robust, as evidenced by comparing repeat simulations.) The interaction scaling values were determined through trial and error in short simulations. Six replicas were sufficient at each solvent temperature to achieve an exchange probability of approximately 0.3 between all replicas. The final values for the effective temperature ladders were 298.0, 317.0, 336.7, 359.0, 384.5, 413.9; 354.0, 378.6, 406.9, 439.8, 475.2, 513.0; and 410.0, 440.9, 476.7, 515.7, 561.6, 616.5. For each solvent temperature, two repeat simulations were run at constant NVT for 1 μ s each. Analysis was performed on the “physical” replicas where the solute and solvent had matching temperatures.

E. Analysis

The first 100 ns were discarded from all production runs as an equilibration period. Snapshots were then saved every 10 ps for analysis. In total, for each of the three solvent temperatures, the numbers of snapshots collected were 2.4×10^6 for cMD, 90 000 for TREMD, and 180 000 for REST.

The radius of gyration (R_g) was calculated using the GROMACS tools *g-polystat*. R_g was specifically defined as the mass weighted root mean square displacement from the center of mass, using all the Q15 atoms. In addition to R_g , *g-polystat* also produced the eigenvalues, $\lambda_{1,2,3}$, of the moment of inertia tensor, which were used to calculate the asphericity, $\delta = 1 - 3 \frac{\lambda_1^2 \lambda_2^2 + \lambda_1^2 \lambda_3^2 + \lambda_2^2 \lambda_3^2}{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)^2}$. The 1-dimensional distribution function in R_g and 2-dimensional (2D) distribution function in (R_g , δ) were obtained as histograms (with bin sizes at 0.1 Å for R_g and 0.025 for δ). The 2D distribution function was converted into a potential of mean force (PMF) according to the Boltzmann relation.

Errors in mean R_g were estimated using a *Python* code, written by J. Fung, R. W. Perry, and T. G. Dimiduk (<https://github.com/manoharan-lab/flyvbjerg-std-err/>), which implemented the block decorrelation technique of Flyvbjerg and Petersen.⁵⁵ Confidence intervals for the distribution function in R_g were estimated using a bootstrapping method in *Python*. From each collection of snapshots, 1000 (denoted by N) bootstrapped sets were randomly drawn; each set contained the same number of snapshots as the original collection but with allowance for duplication of snapshots. A histogram was calculated from each set. For each histogram bin, the 95% percent confidence interval was calculated according to the formula $\bar{x} \pm t(\frac{s}{\sqrt{N}})$, where $\bar{x}$ and s are the frequency mean and standard deviation, respectively, of the bootstrapped sets and t is the 95% t -statistic, which is approximately 1.96 for $N = 1000$.

The GROMACS tool, *g-cluster*, was used to cluster sampled structures based on root-mean-square-deviation (RMSD). Secondary structures were calculated using the DSSP program,^{56,57} but the results were modified to include the PPII helix. PPII helical residues were defined according to Mansiaux *et al.*,⁵⁸ i.e., their backbone phi and psi angles, as well as those of at least one adjacent residues, must be within $-75^\circ \pm 29^\circ$ and $145^\circ \pm 29^\circ$, respectively. A residue was assigned to PPII only if it was determined to be a coil by DSSP. The GROMACS tool *g-hbond* was used to determine hydrogen bonds.

III. RESULTS

In this study, multiple replicates of cMD simulations and two enhanced sampling methods, TREMD and REST, were implemented to study the conformational ensembles of a disordered peptide, Q15, at three different temperatures, 298 K, 354 K, and 410 K. Below, we compare various observables between the methods.

A. Assessment of simulation convergence

Convergence was first tested for each sampling method by comparing the R_g distribution functions obtained from two “repeat” simulations (Fig. 1). We chose R_g for this test because it represents the overall size of the peptide. For cMD, the two repeat simulations were produced by randomly dividing the 60 replicate simulations into two groups and labeled as Sim1 and Sim2. As shown in Figs. 1(a)–1(c), the R_g distributions

FIG. 1. Probability densities of R_g for the convergence tests of the three sampling methods. [(a)–(c)] cMD at 298, 354, and 410 K. Corresponding results are given in [(d)–(f)] for TREMD and [(g)–(i)] for REST.

from Sim1 and Sim2 are smooth at all the three temperatures and agree with each other closely even at room temperature. The smoothness can be attributed to the large number of snapshots collected. The closeness between Sim1 and Sim2, at first sight, might suggest good convergence. However, it may be an indication that cMD sampled a more restrictive subspace within which convergence was relatively easy to achieve. The latter is indeed the case, as shown by the evidence presented below.

For TREMD, we divided the total of 0.9- μ s production simulation into two halves, with the first and second 450 ns labeled as 550 ns and 1000 ns, respectively [Figs. 1(d)–1(f)]. The R_g distributions from the two halves of the trajectory still superimpose well, although expectedly the discrepancy is somewhat greater at room temperature than at the higher temperatures. Importantly, we note that, at 298 K, TREMD sampled a compact substate around $R_g = 8$ Å that is separated from the main state. This substate was not separately present in the cMD simulations.

For REST, we compared the two actual repeat simulations [labeled as Sim1 and Sim2; Figs. 1(g)–1(i)]. At 410 K, the R_g distributions agree well. At 298 K and 354 K, the R_g distributions still overlap well at high R_g ($R_g > 9$ Å), but discrepancy emerges at lower R_g values. Sim2 sampled compact conformations more frequently than Sim1 at 298 K, but the opposite occurred at 354 K. When combined, the two repeat simulations overall produced R_g distributions that are very similar to those in TREMD, including the existence of a compact substate separate from the main state.

We also tested convergence by estimating the 95% confidence intervals of the R_g distributions, shown as red bands in Fig. 1. The cMD confidence intervals are extremely fine, which, again, we suggest is partly due to sampling in a restricted subspace. For TREMD, the 95% confidence intervals are approximately equal to the deviations between the two halves of the trajectory. The REST confidence intervals are relatively narrow at all the three temperatures, suggesting that the

TABLE I. Aggregated simulation times for the three sampling methods.

	cMD	TREMD	REST
298 K	$60 \times 0.5 \mu\text{s}$		$2 \times 6 \times 1.0 \mu\text{s}$
354 K	$60 \times 0.5 \mu\text{s}$	$42 \times 1.0 \mu\text{s}$	$2 \times 6 \times 1.0 \mu\text{s}$
410 K	$60 \times 0.5 \mu\text{s}$		$2 \times 6 \times 1.0 \mu\text{s}$
Total	$90.0 \mu\text{s}$	$42.0 \mu\text{s}$	$36.0 \mu\text{s}$

two repeat simulations when combined gave robust sampling of the conformational space.

The assessment of whether a simulation has converged is still a matter of debate, especially for IDPs, where complete sampling may be difficult due to a large accessible conformational space with many shallow energy wells possibly separated by high barriers.^{59,60} Apparent convergence between repeat simulations may give a false sense of complete sampling, as we suggest for cMD. This suggestion is further supported by the 2D PMFs given in Subsection III D and by the temperature dependence of secondary structures given in Subsection III E.

B. Aggregated simulation times

Table I lists the aggregated simulation times across all replicas and temperatures for each method. Despite providing

a less complete sampling, cMD took the most time, totaling $90 \mu\text{s}$. TREMD and REST provided similarly robust sampling, with total times of $42 \mu\text{s}$ and $36 \mu\text{s}$, respectively. So even when covering three temperatures using independent simulations, REST still took less time, highlighting its power in selecting only the solute (or a region thereof) to enhance sampling. Next we compared the sampled conformational spaces of the three methods.

C. Temperature dependence of the mean and distribution of R_g

R_g measures the size of the peptide and provides a simple overview of the conformations sampled. In Fig. 2(a), we display the temperature dependence of the values of mean R_g , $\bar{R}_g$, for each method. At each temperature, the $\bar{R}_g$ values from the three methods are close to each other, although their small differences are beyond the even smaller errors estimated by block averaging (see Subsection II E). The greatest difference occurs at room temperature, with $\bar{R}_g$ at $11.0 \text{ \AA}$ for REST and $11.5 \text{ \AA}$ for cMD. Importantly, $\bar{R}_g$ shows a distinct monotonic decrease with temperature for all the methods, indicating a temperature-induced collapse. The collapse is milder by TREMD and REST, which produced a near constant offset in $\bar{R}_g$ at the different temperatures, suggesting subtle differences in the relative weights of conformations sampled by these two

FIG. 2. Temperature dependence of mean R_g and R_g distribution. The radius of gyration was calculated using all atoms in Q15. (a) Mean R_g plotted against temperature for each of the three methods. Error bars were calculated from block averaging. [(b)–(d)] Probability densities of R_g obtained from the full collection of snapshots from cMD, TREMD, and REST simulations, respectively. The results here are the same as those shown as “Full” in Fig. 1 but grouped here to allow easy identification of temperature-dependent trends.

methods. The steeper collapse by cMD, with a decrease of 0.9 Å on going from 298 K to 410 K, can partly be attributed to the under sampling of compact conformations noted in Subsection III A.

To further examine the difference in $\bar{R}_g$ between TREMD and REST, we plot the average R_g values within 100-ns windows as a function of simulation time (Fig. S1 of the [supplementary material](#)). For each method, the window-averaged R_g values fluctuate randomly around the global mean, demonstrating that there is no systematic dependence on simulation time. Consistent with the very small errors in TREMD $\bar{R}_g$ estimated by block averaging [Fig. 2(a)], the window-averaged R_g values in the TREMD simulations have very low fluctuations. The fluctuations of the window-averaged R_g values in the REST simulations are relatively high at room temperature and are moderated at the higher temperatures. Of the 9 time windows (excluding the first 100 ns), the average R_g values in the two repeat REST simulations are both above, one above and one below, and both below the TREMD counterparts 2, 1, and 6 times, respectively, at 298 K. These numbers are 0, 3, and 6 at 354 K and 0, 0, and 9 at 410 K. Based on these results, we conclude that the differences in $\bar{R}_g$ between TREMD and REST statistically are not significant at 298 K but are significant at 410 K; the case at 354 K is borderline.

One potential source for the differences in $\bar{R}_g$ between TREMD and REST at the higher temperatures is the volumes of the simulation boxes, which were the same at all the temperatures for TREMD but slightly expanded for REST at the two high temperatures. Specifically, the box volumes in the REST simulations increased by 3% at 354 K and 7% at 410 K, with corresponding decreases in water density. We ran additional REST simulations at 354 K and 410 K using the TREMD box, and the $\bar{R}_g$ values increased slightly, in line with the TREMD values. The slight expansion of Q15 at the higher water densities of the TREMD simulations arises from an increased chance of peptide-water interactions and is akin to pressure-induced protein denaturation.⁶¹ Still, the differences in box volume at the higher temperatures and the resulting effects on $\bar{R}_g$ are quite small, so the conclusion that two methods generated nearly identical conformational ensembles stands.

The R_g distributions provide further insight into the temperature-induced collapse [Figs. 2(b)–2(d), the same as the curves labeled as Full in Fig. 1]. Overall, the three methods sampled nearly the same range of R_g , from 7.0 to 15.9 Å. As temperature increases, the R_g distributions in each method shift toward the left (i.e., smaller R_g), giving rise to the temperature-induced collapse shown by $\bar{R}_g$. There is one important difference between cMD on the one hand and TREMD and REST on the other hand. For the former, the leftward shift extends to the smallest R_g , meaning that the extent of sampling for the most compact conformations increases with increasing temperature. By contrast, for the latter two methods, the shift stops at approximately 8.2 Å, meaning that, for the most compact conformations, the extents of sampling either remain nearly constant or decrease with increasing temperature.

The sizes of polyQs have been probed in several experimental studies at room temperature. Data from fluorescence

correlation spectroscopy were interpreted as implicating a hydrodynamic radius of approximately 18 Å for Q15,²⁶ which roughly corresponds to $\bar{R}_g = 14$ Å. Triplet quenching data were analyzed by modeling polyQs as worm-like chains, yielding a persistence length of 13 Å.²⁷ With this persistence length, the $\bar{R}_g$ of a worm-like chain would be 11.1 Å. Data from fluorescence resonance energy transfer suggested a smaller persistence length at approximately 9 Å for Q15.²⁵ The latter corresponds to $\bar{R}_g = 10$ Å. So for Q15, the different experimental techniques have produced a range of values for the $\bar{R}_g$ of Q15 at room temperature, from 10 to 14 Å. The $\bar{R}_g$ values from our MD simulations fall within this range.

More interestingly, recent temperature jump experiments showed a temperature-induced collapse for polyQ tracks of various lengths contained in the Htt exon-1 domain.³⁶ This observation provides direct support for our temperature-dependent simulation results.

D. 2D PMFs in size and shape

To further characterize the conformational ensembles across the sampled temperatures, we calculated PMFs in the 2D space of R_g and asphericity. The latter quantity, denoted by δ , measures the overall shape of the peptide; its value ranges from 0 to 1, with 0 representing a sphere and 1 representing a thin rod. As shown by the 2D PMFs in Fig. 3, all the methods sampled a wide range of conformations, from compact globules ($R_g = 7$ Å, $\delta = 0.0$) to extended coils ($R_g = 15$ Å, $\delta = 0.9$). As noted above, at room temperature, relative to TREMD and REST, cMD sampled less frequently compact conformations. On the 2D PMFs, these under sampled conformations fall into a small region around ($R_g = 8$ Å, $\delta = 0.4$). For both TREMD and REST, the 2D PMFs can be characterized as defining a main state with a broad basin and a narrow substate, with minima located at ($R_g = 13$ Å, $\delta = 0.62$) and ($R_g = 8$ Å, $\delta = 0.4$). The latter substate did not appear as a local minimum in cMD due to under sampling. The under sampling, in turn, can be attributed to the fact that cMD was unable to cross an energy barrier separating the substate from the main state. From the 2D PMFs, we can estimate this barrier to be approximately $1.5 k_B T$ for TREMD and $1 k_B T$ for REST, where k_B is Boltzmann's constant. These values are probably underestimates, as hidden barriers may be presented by, e.g., solvent reorganization.

In contrast to our global minimum at ($R_g = 13$ Å, $\delta = 0.62$), cMD simulations of Q15 by Wang *et al.*²⁹ located the minimum at ($R_g = 7.5$ Å, $\delta = 0.1$). Most likely the difference arises from the use of different force fields: the OPLS-AA/L force field with TIP4P water by Wang *et al.* but the Amber ff03ws force field with TIP4P/2005 water by us. The latter combination was parameterized by Best *et al.*³⁷ to correct the over-compaction of IDPs in previous force fields. So it is not surprising that our conformational ensembles are more extended than in the study of Wang *et al.*

To gain a sense of the atomic structures sampled in our simulations, snapshots within 2-Å bins of R_g were clustered based on RMSD to find representatives. For each cluster, R_g and δ were calculated for members to find their locations within the 2D PMF. Representative structures in

FIG. 3. 2D PMFs in R_g and δ . Columns are for the same method at different temperatures, and rows are for the three methods at a given temperature. Representative structures in three regions are shown. A gray circle identifies the location of the starting structure.

three different regions of the 2D PMFs at 298 K are displayed in Fig. 3 (similar structures also dominate in these regions at the higher temperatures). The region around the minimum of the main state is populated by random coils and, to a less extent, PPII helices, whereas the substate around ($R_g = 8$ Å, $\delta = 0.4$) is populated by α -helices. The barrier between them, in some sense, is thus that of a helix-coil transition. Finally the region around ($R_g = 8$ Å, $\delta = 0.1$), near the lower tip of the 2D PMFs, is populated by relatively compact conformations with variable secondary structures.

The 2D PMFs at the higher temperatures are shown as the second and third rows in Fig. 3. As temperature increases, the basin of the main state shifts toward the lower tip, corresponding to the temperature-induced collapse observed above. Meanwhile the population of the compact substate shrinks in the TREMD and REST simulations, but the opposite occurs in the cMD simulations.

E. Secondary structures

We now examine the frequencies of all secondary structure types to assess their possible dependence on the temperature and sampling method (Fig. 4). In line with the disordered nature of Q15, random coils dominate, with approximately 65% frequency, at all the temperatures and in all the sampling methods. Bends occur with approximately 15% frequency, while α -helices, PPII helices, 3-10 helices, and turns each account for approximately 5% frequency, but β -bridges and β -strands have minimal presence.

The comparison in Figs. 4(a)–4(c) between the sampling methods at a given temperature reveals subtle differences. At 298 K and, to a less extent, 354 K, cMD, relative to TREMD and REST, sampled less α -helices and more PPII helices. These differences are in line with the foregoing observations that cMD under sampled α -helices and overestimated $\bar{R}_g$ (recall that PPII helices are relatively extended).

FIG. 4. Relative frequencies of eight types of secondary structures. [(a)–(c)] Comparison of the results from the three methods at each of the three temperatures. [(d)–(f)] Comparison of the results at different temperatures, one method at a time.

The grouping of the results according to method, shown in Figs. 4(d)–4(f), reveals temperature-dependent trends. Across the three methods, with increasing temperature, the frequencies of bends, turns, and 3-10 helices increase, whereas the frequencies of PPII helices decrease. The decrease in PPII helical content with increasing temperature has been observed by circular dichroism on polyQs²⁴ and by a combination of circular dichroism and NMR on other IDPs.⁶² On the

other hand, with increasing temperature, the coil populations increase, while the α -helix populations decrease in TREMD and REST, but the opposite is observed in cMD. The latter result can largely be attributed to the under sampling of the α -helix dominated substate noted above. So the under sampling of a substate in cMD leads to an unphysical trend: increase in α -helical content with increasing temperature. This trend contradicts the prediction of the well-established theory

FIG. 5. The average numbers of hydrogen bonds for five types of partners: backbone-backbone (BB-BB), backbone-side chain (BB-SC), side chain-side chain (SC-SC), backbone-water (BB-water), and side chain-water (SC-water). [(a)–(c)] Comparison of the results from the three methods at each of the three temperatures. [(d)–(f)] Comparison of the results at different temperatures, one method at a time.

of helix-coil transition^{63,64} as well as experimental observations on polyQ tracks in the Htt exon-1 domain⁶⁵ and other IDPs.⁶²

F. Hydrogen bonding patterns

The results on secondary structures presented above are determined by backbone-backbone hydrogen bonds. The hydrogen bonding ability of glutamine, both backbone and side chain, is well known and is thought to be one of the main reasons for the disordered nature²⁹ as well as aggregation propensity^{24,25,31,66} of polyQs. The size of a peptide also depends on the partitioning of hydrogen bonding partners among backbone, side chain, and water. The average numbers of such hydrogen bonds per snapshot ($\bar{N}_{HB}$) are presented in Fig. 5. Overall, the three methods produced similar trends in hydrogen bonding patterns. Each backbone amine or carbonyl on average forms approximately 1 hydrogen bond with water, and for side chains, the average increases to approximately 1.5 hydrogen bonds. The average numbers of intra-peptide hydrogen bonds are one order of magnitude less than those of peptide-water hydrogen bonds.

Grouping of the results at a given temperature [Figs. 5(a)–5(c)] or for a given method [Figs. 5(d)–5(f)] reveals subtle

differences and trends. At room temperature, the number of backbone-backbone hydrogen bonds by cMD is approximately half of those by TREMD and REST; at increasing temperatures, cMD, in contrast to TREMD and REST, produced greater backbone-backbone hydrogen bonds. These differences can be attributed to the under sampling of α -helices by cMD noted above. On the other hand, as temperature increased, all the three methods produced a decrease in peptide-water hydrogen bonds along with a mild increase in backbone-side chain hydrogen bonds. This trend indicates that water becomes a poorer solvent for Q15 at higher temperatures and is consistent with the temperature-induced collapse noted above.

IV. DISCUSSION

In this study, we have investigated the temperature-dependent conformational ensembles of Q15 using multiple replicates of cMD simulations and two enhanced sampling methods, TREMD and REST. Overall, the three methods sampled nearly the same conformational space and produced a similar temperature-induced collapse. Yet, cMD at room temperature under sampled a substate high in α -helical content, leading to an unphysical increase in α -helical content with

increasing temperature. So the two enhanced sampling methods achieved what they were designed for: to cross energy barriers insurmountable by cMD to more completely sample the conformational space. Furthermore, we suggest that REST is the method of choice for larger IDPs, as discussed below.

Given the same total simulation time (i.e., 30 μ s per temperature), alternative choices might affect the extent of under sampling of cMD. One choice concerns the length of each replicate simulation. In our view, full sampling by cMD requires, at a minimum, multiple replicate simulations that are each longer than the slowest relaxation time in the conformational space of interest. For Q15, a possible slow relaxation is the helix-coil transition, which for helix-forming peptides has an estimated time constant on the order of a few hundred ns.⁶⁷ The latter value motivated our choice of 0.5 μ s for the length of each replicate simulation. It is not entirely clear what is the nature of the slowest relaxation for Q15 (with the force field chosen here) and what is the corresponding time constant. These uncertainties add to the challenges faced by cMD for simulating Q15, let alone larger IDPs.

Another choice concerns the starting conformation. We started the 60 replicates from a single conformation with an intermediate compactness, but after 100 ns, the conformations of Q15 in the replicate simulations spread to almost the entire basins in the 2D PMFs (Fig. S2 of the [supplementary material](#)). Given the disordered nature of Q15, our choice for the starting conformation is well justified. A possible alternative for the starting conformation is a fully formed α -helix. We ran additional REST simulations starting from an α -helix. Even with the enhanced sampling of REST, Q15 at 298 K was trapped in the helical conformation for at least 150 ns (Fig. S3 of the [supplementary material](#)). We can infer that Q15 would be trapped in the helical conformation for even longer in cMD, and therefore, starting from an α -helix would not be profitable for cMD sampling of a peptide known to be disordered.

For IDPs in particular, finding the appropriate force fields, which traditionally have been parameterized for structured proteins, leading to over-compact IDPs,^{37,68} is still an area of active research. The conformational ensembles obtained here for Q15 and in a previous study²⁹ illustrate the impact of IDP-specific force field parameterization. A suitable enhanced sampling method will be essential for testing force fields for IDPs.

The comparison between TREMD and REST presented here both validates and expands previous studies. Similar to our finding on Q15 in water, Smith *et al.*⁴ compared REST (initial version) with TREMD on a short β -amyloid peptide in a membrane environment and concluded that the two methods generated the same conformational ensembles, but REST was substantially faster. In another study, PT-WTE and REST were compared.⁶ Again the same conformational ensemble was produced, but the authors noted that a single REST simulation cannot produce temperature-dependent properties since only the REST replica with matching solute and solvent temperatures represents a physical system. Here we addressed this criticism by running REST simulations at a few different solvent temperatures and found that, even then, REST is faster than TREMD. Of course, if properties at many more

temperatures for small peptides like Q15 are desired, TREMD could be more efficient.

Our simulations over a range of temperatures produced a temperature-induced collapse for Q15, in agreement with recent experimental observations on polyQ tracks in the Htt exon-1 domain³⁶ and on other IDPs. Many hypotheses have been proposed to explain the temperature-induced collapse, including strengthened hydrophobic interactions,³⁶ weakened solvation,⁴⁴ reduced thermal fluctuations,⁴⁰ melting of PPII helices,⁶² and others.^{38,69} In a recent study, Zerze *et al.*³⁸ simulated 5 different IDPs with different amino acid compositions. They found that, as temperature increased, the most hydrophilic, high mean net charge IDPs collapsed, along with a decrease in solvent accessible surface area and burial of negatively charged residues. The more hydrophobic, low mean net charge IDPs all initially collapsed and then re-expanded at high temperatures. Another study attributed the collapse of the Tau IDP to 60% entropic, 25% hydrophobic, and 15% secondary structure change.⁶⁹ For Q15, we found loss of peptide-water hydrogen bonds and gain of intra-peptide hydrogen bonds, along with melting of PPII helix, at higher temperatures. We believe that these are the contributing factors to the temperature-induced collapse of Q15.

We found that REST can produce temperature-dependent conformational ensembles faster than TREMD for a small disordered peptide, Q15. For larger IDPs, we expect the gap between REST and TREMD to widen significantly. As the protein size increases, the number of water molecules required to solvate it in MD simulations will increase rapidly, leading to a substantial increase in the number of replicas needed for TREMD simulations.³ By contrast, because REST limits exchange to the solute alone, the number of replicas should increase much more gradually with increasing solute size. In our preliminary studies, a system size of 45 000 atoms for a 34-residue IDP required only 12 replicas in REST simulations to span a temperature range of 278 K to 463 K and achieve an exchange probability of 0.3. The same system would require approximately 100 replicas according to two different prediction methods.^{42,43} As the IDP size increased to 70 residues, 24 replicas were required in REST simulations, but TREMD would require 200 replicas. We conclude that REST is the method of choice for enhanced sampling of large IDPs.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for Figs. S1–S3.

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