Intrinsically disordered proteins play key roles in a range of biological processes, including cellular signaling, molecular recognition, and transcriptional regulation.1–4 Several members of this protein class have also been implicated in debilitating human diseases, such as the Aβ peptides in Alzheimer’s disease (AD) and α-synuclein (αSyn) in Parkinson’s disease (PD).5 These proteins appear to lack both secondary and tertiary structure, at least in the absence of binding partners, when studied under physiological conditions. However, the absence of fully formed α-helices and β-sheets does not preclude a rudimentary protein architecture, as demonstrated by the discovery of long-range contacts in globular proteins studied under denaturing conditions.5–7 and additionally, many important protein contacts in disordered proteins studied under denaturing conditions,6–11 and such interactions could play a key role in the pathogenic nature of these proteins. Here, a combination of paramagnetic relaxation enhancement (PRE) NMR spectroscopy and ensemble molecular dynamics (MD) simulations11 are used to probe the topology of native αSyn.

The high-resolution characterization of disordered protein states is challenging due to their intrinsic heterogeneity.12 Such states are composed of dynamic ensembles rather than singular structures, and additionally, many important protein contacts in disordered states tend toward distances far exceeding the useful range of conventional NOE effects (≤ 5 Å).13 The PRE method overcomes these difficulties by probing ensemble-averaged, transient contacts over distances as great as 20 Å. As previously described,6,7,9–11 a nitroxide spin-label is attached to a region of a protein, and in its oxidized (paramagnetic) state, enhancements of heteronuclear NOEs suggest the presence of long-range contacts. Nitroxide spin-labels, such as those shown for specific values of 9,6,14 are distributed in the native state of αSyn conformers is significantly narrower than that of a random coil, which is extremely broad with the value of 33.3–36.9 Å expected for a highly denatured 140-residue polypeptide.15

We describe here a PRE study of the intrinsically disordered protein αSyn. Cysteine mutations facilitating attachment of the spin-label, MTSL, were made at positions Q24, S42, Q62, S87, and N103 along the 140-residue sequence of αSyn as described in the Supporting Information (SI). Intensity ratios were obtained from HSQC spectra acquired for each mutant with the spin-label in either its oxidized or reduced state. In addition to the local effects of the oxidized spin-label, which reduce strongly the cross-peak intensity of adjacent residues, long-range contacts between the C-terminal tail and the central region of the protein were also observed, indicating the presence of nonrandom structure (see Figure 1 in the SI).
Experimental and computational simulations of 20 protein replicas were used to generate expanded Residual contact plot for native αSyn. The residual contacts have been averaged locally over ±7 residues. The different regions of the αSyn sequence, as defined previously (refs 16, 17), are shown in the lower bar.

The extent of structure in native αSyn was quantified by analyzing residue–residue contacts in 4000 structures obtained as described above. Contacts in native αSyn were evaluated with respect to contacts in the random coil ensemble of αSyn, with nonrandom structure being designated as a preference for specific interactions relative to their probability of formation in the random coil state.11 Although the native state of αSyn is a highly heterogeneous ensemble, structural analysis reveals the presence of a number of significant long-range interactions. The nonrandom structure in native αSyn is shown in Figure 2 as a topological contact map of $-\ln(p_i/p_o)$, where $(p_i/p_o)$ is the contact probability ratio between the restrained and reference simulations. Darker coloring indicates an increased probability for interactions between two regions of sequence. It is evident that the native αSyn ensemble samples nonrandom conformations involving, in particular, contacts between residues ~120–140 of the C-terminus and residues ~30–100 in the central region of the protein sequence. These results were reproduced consistently in ensemble calculations utilizing only a subset of PRE restraints and also for shorter simulation times. Due to the discrete position of spin-labels in PRE experiments, more distance restraints are available at spin-labeling sites relative to unlabeled sequence locations. While this can potentially result in a calculated structural compaction surrounding the site of labeling, long-range interactions observed here for αSyn do not arise from effects of this type. For example, the most probable contacts occur at positions ~120–140, whereas the nearest site of labeling is residue 103; the large separation in sequence of these regions supports strongly the validity of the PRE method in this study. Considering the broad nature of the native αSyn ensemble (Figure 1) and the absence of any significant secondary structure, the presence of long-range contacts that are ~200 times more probable than in a random coil is a striking topological feature.

We have shown therefore that the native state of αSyn is composed of an ensemble of structures that are, on average, significantly more compact than a random coil. This partial condensation is driven by long-range contacts between residues 120–140 in the negatively charged C-terminal tail and residues 30–100 in the center of the protein. The NAC region (61–95)16 is the most hydrophobic part of αSyn, and since it is known to form amyloid fibrils both in vitro and in vivo,16 the proximity of the highly charged C-terminus may shield this region from aggregation. Indeed, truncation of the C-terminal region greatly accelerates fibril formation in vitro,18 and a large proportion of aggregated αSyn found in vivo in Lewy bodies is truncated in this manner.19 Furthermore, EPR studies20 of αSyn fibrils suggest that the highly ordered amyloid fibril core region is composed of residues ~30–100, precisely the region that we observe to interact with the C-terminal residues. The results presented here are therefore consistent with the idea that the aggregation of αSyn is inhibited by the existence of long-range interactions within the native structure. Moreover, the C-terminal region also contains binding sites for Ca$^{2+}$ ions, heavy metals, and polyamines, all of which increase the rate of αSyn aggregation.20–22 The binding of ligands to the C-terminal region may therefore act to modulate the function and/or aggregational propensity of αSyn. The long-range contacts observed here may therefore affect substantially the role of αSyn in the pathogenesis of PD, and their detection represents a significant step toward an increased understanding of the consequences of intrinsic disorder in proteins.

Acknowledgment. We thank A. Olteanu and G. Pietak (UNC-Chapel Hill) for the αSyn plasmid, and C. Griesinger and M. Zweckstetter (Max Planck Institute, Göttingen) for valuable discussions. This work was supported by the Wellcome and Leverhulme Trusts.

Supporting Information Available: Experimental and computational details, and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

References

JA044834J

Figure 2. Residual contact plot for native αSyn. Darker colors correspond to decreasing values of $-\ln(p_i/p_o)$, indicating contacts that are more probable than in a random coil model of αSyn. The residual contacts have been averaged locally over ±7 residues. The different regions of the αSyn sequence, as defined previously (refs 16, 17), are shown in the lower bar. 