HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2021 June 11.

Published in final edited form as:

Methods Mol Biol. 2012 ; 895: 127–138. doi:10.1007/978-1-61779-927-3_10.

Distance Information for Disordered Proteins from NMR and ESR Measurements Using Paramagnetic Spin Labels

David Eliezer

Department of Biochemistry and Program in Structural Biology, Weill Cornell Medical College, New York, USA

Abstract

The growing recognition of the many roles that disordered protein states play in biology places an increasing importance on developing approaches to characterize the structural properties of this class of proteins and to clarify the links between these properties and the associated biological functions. Disordered proteins, when isolated in solution, do not adopt a fixed structure, but can and often do contain detectable and significant residual or transient structure, including both secondary and long-range structure. Such residual structure can play a role in nucleating local structural transitions as well as modulating intramolecular or intermolecular tertiary interactions, including those involved in ordered protein aggregation. An increasing array of tools has been recruited to help characterize the structural properties of disordered proteins. While a number of methods can report on residual secondary structure, detecting and quantifying transient long-range structure has proven to be more difficult. This chapter describes the use of paramagnetic spin labeling in combination with paramagnetic relaxation enhancement (PRE) in NMR spectroscopy and pulsed dipolar ESR spectroscopy (PDS) for this purpose.

Keywords

Intrinsically unstructured; Natively unfolded; Unfolded state; Denatured state; Random coil; NMR; ESR; Paramagnetic relaxation enhancement; Pulsed dipolar spectroscopy

1. Introduction

The use of paramagnetic spin labels for the study of protein structure has experienced a dramatic resurgence over the past decade. PRE experiments are now performed routinely to assist with high-resolution structure determination for both isolated proteins and proteins in larger complexes. ESR distance measurements are also playing an increasing role in structural studies, especially of larger complexes where long-range constraints are difficult to obtain. In addition to the application of these methods to well-ordered systems, they have found increasing use in studies of more dynamic systems, including studies focused on protein–protein interactions that occur prior to the formation of stable complexes (1) and, of particular interest for the subject of this monograph, studies focused on disordered protein states. Early applications included studies of unfolded forms of proteins that can fold into

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

native structures (2–4), but more recent efforts have focused on intrinsically disordered proteins.

Both PRE and PDS measurements rely on the interaction between magnetic dipoles, with PRE detecting the interaction of nuclear magnetic moments with those of unpaired electrons, and PDS detecting interactions between pairs of unpaired electron spins. PDS experiments measure the magnitude of the dipolar coupling, allowing for the dipole–dipole interaction to be considered as a perturbation to the energy levels engendered by the Zeeman interactions of the individual spins with the external magnetic field and for nonsecular terms to be ignored. PRE experiments measure the effect of the dipolar interaction on the relaxation properties of the transverse component of the proton magnetic moment, necessitating the consideration of nonsecular "spin flip" terms. In both cases, a point dipole approximation is used for each spin. The relevant equations governing the interpretation of the observed data in terms of interdipole distances are not reproduced here but are easily found in the literature (5, 6).

The experiments required for the application of these methods to disordered proteins are not particularly different from experiments used in the study of more ordered systems and are fairly standard for practitioners of NMR or ESR spectroscopy The purpose of this work, therefore, is not to describe their implementation. Rather, this chapter is intended to illustrate, to those without previous experience in this area, the basic requirements for preparation of samples suitable for measurements, and for the analysis and interpretation of the data obtained, which on a qualitative level is typically straightforward, but for which quantitative analysis is more complex and remains an area of active research and development.

2. Materials

Protocols for spin labeling of protons involve standard buffers and reagents, with the exception of nitroxide spin labels such as MTSL (*S*-(2,2,5,5-tetramethyl-2,5-dihydro-1 *H*-pyrrol-3-yl)methyl methanesulfonothioate) or metal chelating groups such as *N*-[*S*-(2-pyridylthio)cysteaminyl]EDTA. Concentrated stock solutions of MTSL can be prepared in organic solvents such as dimethyl sulfoxide (DMSO) while *N*-[*S*-(2-pyridylthio)cysteaminyl]EDTA stock solutions can be prepared in either organic solvents or aqueous solutions.

3. Methods

Both PRE and PDS methods require the introduction of unpaired electrons into the proteins of interest. This is most commonly achieved through the conjugation of either a radical group spin label, or a transition-metal containing paramagnetic tag to the protein. While many possibilities exist for conjugation methods, in practice the most popular and easily achievable approach, often referred to as site-directed spin labeling (7) involves the introduction of cysteine residues at the desired labeling sites (and removal of endogenous cysteines, if any, at other sites) using site-directed mutagenesis, followed by the conjugation of the spin label or paramagnetic tag using one of several cysteine-modifying chemistries,

the most popular being alkylthiosulfonate-mediated disulfide bond formation. The most common spin labels contain nitroxide radicals, with the most popular of these being MTSL, although similar reagents with a relatively reduced degree of side chain mobility are also being used (8, 9). Paramagnetic metal tags are typically introduced by conjugating a chelating group to the desired cysteine residue followed by loading with the paramagnetic ion of choice. The most commonly used reagent is *N*-[*S*-(2-pyridylthio) cysteaminyl]EDTA.

Both PRE and PDS experiments require the use of control samples. In the case of PRE experiments, the control sample provides a measure of the proton transverse relaxation rate (R2) in the absence of the paramagnetic reagent. In the case of PDS, the control samples provide a means of testing for intermolecular contributions to the measured distances.

3.1. Preparation of Paramagnetic Samples

1. Mix an excess (5- to 30-fold is typical) of spin label or metal chelator with the protein (see Note 1).
2. Incubate (as short as 30 min at room temperature can suffice, although many protocols call for overnight incubation at 4 °C).
3. Remove excess label subsequent to the labeling reaction through buffer exchange (see Notes 2 and 3).
4. If a chelating group is used, load the desired paramagnetic ion by adding at slightly superstoichiometric concentrations.
5. Remove excess ions, which can bind nonspecifically to protein sites, through thorough buffer exchange.
6. Samples prepared for PDS measurements must be frozen prior to data acquisition (see Note 4).

3.2. Preparation of Control (Diamagnetic) Samples

An ideal PRE control experiment should involve a sample that is identical to the spin-labeled sample, but in which the paramagnetic effect is eliminated.

---

[1]. Depending on the conditions used to purify and store the protein prior to spin labeling, reduction of the cysteine groups prior to labeling may be advisable and can be achieved using standard reducing agents such as dithiothreitol or TCEP (tris (2-carboxyethyl)phosphine), which should be removed through a rapid buffer exchange prior to addition of the label.

[2]. When introducing a single label into the protein, as is typically done for PRE experiments, a very low level of intermolecular disulfide-bond formation can be expected. Dimeric material can be removed using chromatography if desired.

[3]. When producing doubly labeled proteins, required for intramolecular PDS distance measurements, the potential for intramolecular disulfide bond formation is greater due to the increased effective local concentration of sulfhydryl groups and a greater excess of the conjugating reagent is recommended to maximize labeling efficiency. While any cross-linked multi-meric species can be separated chromatographically, intramolecularly cross-linked molecules may be more difficult to remove. In the case of PDS, such molecules will be spectroscopically silent, but may affect the apparent protein concentration, and may also influence the behavior of the properly labeled protein molecules (for instance, by causing aggregation).

[4]. To minimize damage to or aggregation in protein samples, some form of cryoprotectant typically needs to be added to the samples. Glycerol is often used at around 30 % w/v, but sucrose can also be employed. Potential effects of the presence of cryoprotectants on the behavior of the protein sample are typically minimal, but should be investigated. Freezing is typically accomplished by immersing samples in liquid nitrogen, which leads to freezing times on the order of seconds for typical sample volumes (~50 μl). Faster freezing times can be obtained using cryogens with higher heat capacities and heat transfer rates. The influence of freezing rate on the resulting conformational ensemble remains to be more fully investigated.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. PRE control for nitroxide spin labels: The control is typically produced by reducing the nitroxide radical to its hydroxylamine using a reducing agent such as ascorbic acid. This produces a sample that is chemically nearly identical, but diamagnetic. Such a control can be produced by directly reducing the actual sample used for the PRE measurement. Alternately, an originally prepared sample can be split in two, one of the samples reduced via addition of ascorbic acid, and an equivalent volume added to the other to maintain matched protein concentrations. This allows for preservation of the paramagnetic sample for future experiments. An alternative to reduction by ascorbic acid is to separate the control prior to spin labeling and conjugate to it a diamagnetic analogue of the spin label to be used (such as N-acylated MTSL). However, this may result in an imperfect match in protein concentration, since manipulations to remove excess spin label or diamagnetic analogue may result in irreproducible losses. If single time point PRE measurements are used (see below), closely matched protein concentrations are desirable (see Note 5).

2. PRE control for chelating reagents: The control sample is prepared identically to the paramagnetic sample except that the chelating group is loaded with a diamagnetic metal ion. Samples precisely matched in protein-concentration may be difficult to obtain, and two time point PRE measurements (see below) are recommended.

3. PDS controls: Controls are used primarily to separate intermolecular from intramolecular distances. For this purpose, two primary strategies are employed. First, the effects of magnetic dilution of the sample with unlabeled protein on the measured distances can be determined. Contributions from intermolecular distances should decrease with magnetic dilution and can be thereby identified. A second method that can be used is to prepare controls samples using singly labeled proteins. In this case, any detectable dipolar coupling must necessarily reflect intermolecular distances.

3.3. PRE Experiments

PRE results from the interaction of the unpaired electron with nuclear spins, leading to an increase in the transverse relaxation rate (R2) of the nuclear spins, an effect that can be measurable at distances up to ~30 Å. The PRE contribution to the nuclear R2, typically referred to as Γ2, is simply the difference between the intrinsic R2, measured using a control sample, and the R2 measured for the paramagnetically labeled sample.

---

[5]. A shortcoming of the methods described above for production of PRE control samples can result from a significant affinity of typical nitroxide spin label reagents to aromatic groups in proteins, which can lead to nonspecific binding of unconjugated spin label (5, 24, 25). In cases where such nonspecifically bound spin-label is not efficiently removed during the final removal of excess spin label from the sample, nonspecific PRE effects will be present in the paramagnetic sample, but not in the corresponding control sample. An alternative approach to eliminate this problem involves deconjugation of the spin label from the protein in the control sample through reduction of the disulfide bond linkage. In this case, nonspecifically bound spin labels will be present in both the paramagnetic and the control samples, and their PRE effects will be normalized out. However, this approach results in chemically distinct species in the paramagnetic and control samples, which can lead to chemical shift differences. These are usually confined to sites near the location of the spin label, which typically experience strong PRE effects and are therefore not of particular interest.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. Single time point measurements: Since NMR line widths, and therefore signal intensities, are directly related to R2, a popular approach is to determine the amid proton Γ2 using the ratio of the NMR signal intensities in matched 2D proton-nitrogen correlation (HSQC) spectra collected from the paramagnetic and diamagnetic samples (5). This approach, however, suffers from the unequal longitudinal relaxation rates of nuclear spins in the paramagnetic and controls samples, which can lead to significant errors, especially when metal ions are used (10).

2. Two time point measurements: An improved approach involves direct measurement of the amide proton R2 values (11). Traditionally, such measurements involve multiple experiments at different relaxation times in order to determine the exponential transverse relaxation rate constant, requiring a greatly increased data acquisition time compared to a single intensity measurement. However, in principle two measurements spaced suitable apart can suffice to accurately determine the rate constant, and this two time point approach provides greater accuracy without a dramatic increase in the required data acquisition time (10).

3.4. PDS Experiments

PDS distance measurements rely on the dipolar interaction between two unpaired electrons, which results in a splitting of the spectrum of each individual electron spin and can be detected at distances of up to 90 Å (12). Notably, the contributions of dipolar coupling to line broadening in continuous wave ESR spectra can also be measured and used to extract interspin distances, but typically the presence of substantial inhomogeneous broadening in CW-ESR spectra limits the utility of CW measurements to the characterization of shorter distances of up to ~20 Å. In PDS measurements, the generation of spin echoes removes inhomogeneous broadening, allowing more precise determination of contributions from dipolar coupling (6).

1. DEER: The most common experiment used to extract interspin distances is the double electron—electron resonance (DEER) pulse sequence, also known as PELDOR (pulsed electron double resonance). In this experiment, the intensity of a standard spin echo signal is modulated by recoupling the dipolar interaction (using frequency selective pulses) for variable times, resulting in a frequency modulation of the observed signal intensity. DEER pulse sequences are usually arranged in a constant-time fashion (the overall length of the pulse sequence is constant) in order to minimize the contributions from relaxation. The most commonly employed sequence is the so-called four-pulse DEER experiment, which improves upon the original three-pulse experiment by using a primary echo to generate the initial signal that is subsequently refocused in a second echo, thereby avoiding the need for simultaneous pulses during short evolution times.

2. DQC: A more recently developed class of PDS experiments employ hard (frequency nonselective) pulses and double quantum coherence (DQC) filters to remove signals that are not modulated by the interspin coupling, resulting in a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

decreased background (13). DQC experiments provide an advantage over DEER for short (<20 Å) distances and at lower protein concentrations (6). At present, PDS experiments are performed most commonly at Ku-band (17.3 GHz).

3.5. PRE Data Analysis

Analysis of PRE data begins with calculation of either the ratio of peak intensities in the control and diamagnetic spectra (single time point data) or with the fitting of the intensities or peak volumes from spectra at each time point to an exponential to extract the apparent R2, followed by the calculation of Γ2 as the difference between R2s from the control and paramagnetic spectra (two time point data). At this point, a qualitative interpretation of the data can be easily made by plotting either the intensity ratio or Γ2 versus residue number and establishing whether any PRE effect is observable at sites outside the window of residues that are covalently restricted to be in the proximity of the paramagnetic label, and therefore always experience PRE. For disordered proteins, the size and shape of this window can be estimated by employing one of several ideal polypeptide random coil models to calculate the average distance for a given residue from the labeling site, and using the appropriate form of the Solomon-Bloembergen equation (5) to calculate a predicted value for Γ2, and in the case of single point measurements, to then calculate the predicted peak intensity ratio (4, 14).

3.6. PRE Data Interpretation

Qualitative interpretation of PRE data can provide general insights into long-range interactions in disordered proteins, and how these may be affected by sequence variations or environmental conditions, with perhaps the best example being that of the Parkinson's disease associated protein alpha-synuclein (Fig. 1). Quantitative interpretation of PRE data is complicated for disordered proteins by the fact that the protein and the paramagnetic label are constantly in motion with respect to one another. Several groups have employed a strategy of converting measured PREs to distances, which are then used to restrain simulations, from which protein conformational ensembles are derived (15–17). An alternative approach involves the generation of an unrestricted ensemble for a given disordered protein, calculation of the PRE effect for each member of the ensemble based on the distance between each nucleus and the spin label, and selection of a subensemble for which the appropriately averaged PRE effect is consistent with experiment. Here it is important to note that the Solomon Bloembergen equation, which links Γ2 to the dipolar coupling and thence to the interspin distance, is derived assuming a fixed distance between the spins. A model free approach to including the effects of local motions of the spin label side chain on the measured Γ2 values has been developed in the context of an otherwise well defined protein structure (18). The motions present in disordered proteins are far more complex than side chain motions and cannot easily be accounted for using this approach alone, but this method can be used to account for spin label mobility when performing distance calculations in the ensemble selection method (19).

3.7. PDS Data Analysis

To obtain structural information using PDS, time domain data need to be transformed into an average distance or a distance distribution. Weak intermolecular background contributions

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

are typically removed by subtracting a linear or polynomial fit to the latter part of the time domain signal. Subsequently, the most straightforward approach is simple Fourier transformation into frequency space, followed by conversion of the observed frequency, which is a measure of the dipolar coupling, to an average distance. In well-ordered systems, this approach can yield distances that are accurate to within the variability introduced by the flexibility of the spin label side chain. In disordered systems, however, the distance is typically not well defined, and recovering a distance distribution is necessary. The observed time domain signal can be modeled as an integral of the probability-weighted contribution from each individual interspin distance, and inversion of the observed signal can be performed to recover the $P(r)$, or distance distribution, function. The problem, however, as is often the case for inverse problems, is not well posed and requires the application of a regularization method. Tikhanov regularization and/or maximum entropy methods are typically employed and require the careful choice of a regularization parameter, for which further algorithms exist (6).

3.8. PDS Data Interpretation

Interpretation of PDS data is in some ways simpler than for PRE data in that the ensemble of conformations being observed is invariant in time. Thus, each individual interspin distance in the ensemble is relatively well defined and does not experience motional averaging due to polypeptide disorder, although some degree of side chain motions can be retained even in frozen samples. Consequently, each individual interspin distance appears in the final distance distribution that is derived from the data. Not surprisingly, the large conformational ensemble sampled by disordered proteins invariably leads to very broad distance distributions. Such distributions are in many ways similar to those obtained from FRET or SAXS measurements (20, 21), and computational approaches have been combined with such measurements in order to determine or evaluate representative ensembles (22, 23). In contrast to PRE methods, PDS measurements only reflect distances between the two specifically labeled sites. Thus, information regarding potential long-range interactions involving other sites is not readily extractable. However, information on the presence of subpopulations with distinct distance distributions can be revealed (Fig. 2), whereas such subpopulations may be invisible to, or difficult to detect by, PRE methods.

Acknowledgments

This work was supported by NIH/NIA grants AG019391 and AG025440, by the Irma T. Hirschl Foundation, and by a gift from Herbert and Ann Siegel.

References

1. Clore GM, Tang C, Iwahara J (2007) Elucidating transient macromolecular interactions using paramagnetic relaxation enhancement. Curr Opin Struct Biol 17:603–616 [PubMed: 17913493]
2. Gillespie JR, Shortle D (1997) Characterization of long-range structure in the denatured state of staphylococcal nuclease. I. Paramagnetic relaxation enhancement by nitroxide spin labels. J Mol Biol 268:158–169 [PubMed: 9149149]
3. Gillespie JR, Shortle D (1997) Characterization of long-range structure in the denatured state of staphylococcal nuclease. II. Distance restraints from paramagnetic relaxation and calculation of an ensemble of structures. J Mol Biol 268:170–184 [PubMed: 9149150]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. Lietzow MA, Jamin M, Jane Dyson HJ, Wright PE (2002) Mapping long-range contacts in a highly unfolded protein. J Mol Biol 322:655–662 [PubMed: 12270702]
5. Battiste JL, Wagner G (2000) Utilization of site-directed spin labeling and high-resolution heteronuclear nuclear magnetic resonance for global fold determination of large proteins with limited nuclear overhauser effect data. Biochemistry 39:5355–5365 [PubMed: 10820006]
6. Borbat PP, Freed JH (2007) Measuring distances by pulsed dipolar ESR spectroscopy: spin-labeled histidine kinases. Methods Enzymol 423:52–116 [PubMed: 17609127]
7. Hubbell WL, Gross A, Langen R, Lietzow MA (1998) Recent advances in site-directed spin labeling of proteins. Curr Opin Struct Biol 8:649–656 [PubMed: 9818271]
8. McHaourab HS, Lietzow MA, Hideg K, Hubbell WL (1996) Motion of spin-labeled side chains in T4 lysozyme. Correlation with protein structure and dynamics. Biochemistry 35:7692–7704 [PubMed: 8672470]
9. Zhang Z, Fleissner MR, Tipikin DS, Liang Z, Moscicki JK, Earle KA, Hubbell WL, Freed JH (2010) Multifrequency electron spin resonance study of the dynamics of spin labeled T4 lysozyme. J Phys Chem B 114:5503–5521 [PubMed: 20361789]
10. Iwahara J, Tang C, Marius Clore G (2007) Practical aspects of (1)H transverse paramagnetic relaxation enhancement measurements on macromolecules. J Magn Reson 184:185–195 [PubMed: 17084097]
11. Donaldson LW, Skrynnikov NR, Choy WY, Muhandiram DR, Sarkar B, Forman-Kay JD, Kay LE (2001) Structural characterization of proteins with an attached ATCUN motif by paramagnetic relaxation enhancement NMR spectroscopy. J Am Chem Soc 123:9843–9847 [PubMed: 11583547]
12. Georgieva ER, Ramlall TF, Borbat PP, Freed JH, Eliezer D (2010) The lipid-binding domain of wild type and mutant alpha-synuclein: compactness and interconversion between the broken and extended helix forms. J Biol Chem 285:28261–28274 [PubMed: 20592036]
13. Borbat PP, Freed JH (1999) Multiple-quantum ESR and distance measurements. Chem Phys Lett 313:145–154
14. Sung YH, Eliezer D (2007) Residual structure, backbone dynamics, and interactions within the synuclein family. J Mol Biol 372:689–707 [PubMed: 17681534]
15. Dedmon MM, Lindorff-Larsen K, Christodoulou J, Vendruscolo M, Dobson CM (2005) Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. J Am Chem Soc 127:476–477 [PubMed: 15643843]
16. Lowry DF, Stancik A, Shrestha RM, Daughdrill GW (2008) Modeling the accessible conformations of the intrinsically unstructured transactivation domain of p53. Proteins 71:587–598 [PubMed: 17972286]
17. Marsh JA, Dancheck B, Ragusa MJ, Allaire M, Forman-Kay JD, Peti W (2010) Structural diversity in free and bound states of intrinsically disordered protein phosphatase 1 regulators. Structure 18:1094–1103 [PubMed: 20826336]
18. Iwahara J, Schwieters CD, Clore GM (2004) Ensemble approach for NMR structure refinement against (1)H paramagnetic relaxation enhancement data arising from a flexible paramagnetic group attached to a macromolecule. J Am Chem Soc 126:5879–5896 [PubMed: 15125681]
19. Salmon L, Nodet G, Ozenne V, Yin G, Jensen MR, Zweckstetter M, Blackledge M (2010) NMR characterization of long-range order in intrinsically disordered proteins. J Am Chem Soc 132:8407–8418 [PubMed: 20499903]
20. Ferreon AC, Moran CR, Gambin Y, Deniz AA (2010) Single-molecule fluorescence studies of intrinsically disordered proteins. Methods Enzymol 472:179–204 [PubMed: 20580965]
21. Mylonas E, Hascher A, Bernado P, Blackledge M, Mandelkow E, Svergun DI (2008) Domain conformation of tau protein studied by solution small-angle X-ray scattering. Biochemistry 47:10345–10353 [PubMed: 18771286]
22. Bernado P, Mylonas E, Petoukhov MV, Blackledge M, Svergun DI (2007) Structural characterization of flexible proteins using small-angle X-ray scattering. J Am Chem Soc 129:5656–5664 [PubMed: 17411046]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

23. Merchant KA, Best RB, Louis JM, Gopich IV, Eaton WA (2007) Characterizing the unfolded states of proteins using single-molecule FRET spectroscopy and molecular simulations. Proc Natl Acad Sci USA 104:1528–1533 [PubMed: 17251351]
24. Bussell R Jr, Ramlall TF, Eliezer D (2005) Helix periodicity, topology, and dynamics of membrane-associated alpha-synuclein. Protein Sci 14:862–872 [PubMed: 15741347]
25. Card PB, Erbel PJ, Gardner KH (2005) Structural basis of ARNT PAS-B dimerization: use of a common beta-sheet interface for hetero- and homodimerization. J Mol Biol 353:664–677 [PubMed: 16181639]
26. Rospigliosi CC, McClendon S, Schmid AW, Ramlall TF, Barre P, Lashuel HA, Eliezer D (2009) E46K Parkinson's-linked mutation enhances C-terminal-to-N-terminal contacts in alpha-synuclein. J Mol Biol 388:1022–1032 [PubMed: 19345692]
27. McClendon S, Rospigliosi CC, Eliezer D (2009) Charge neutralization and collapse of the C-terminal tail of alpha-synuclein at low pH. Protein Sci 18:1531–1540 [PubMed: 19475665]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

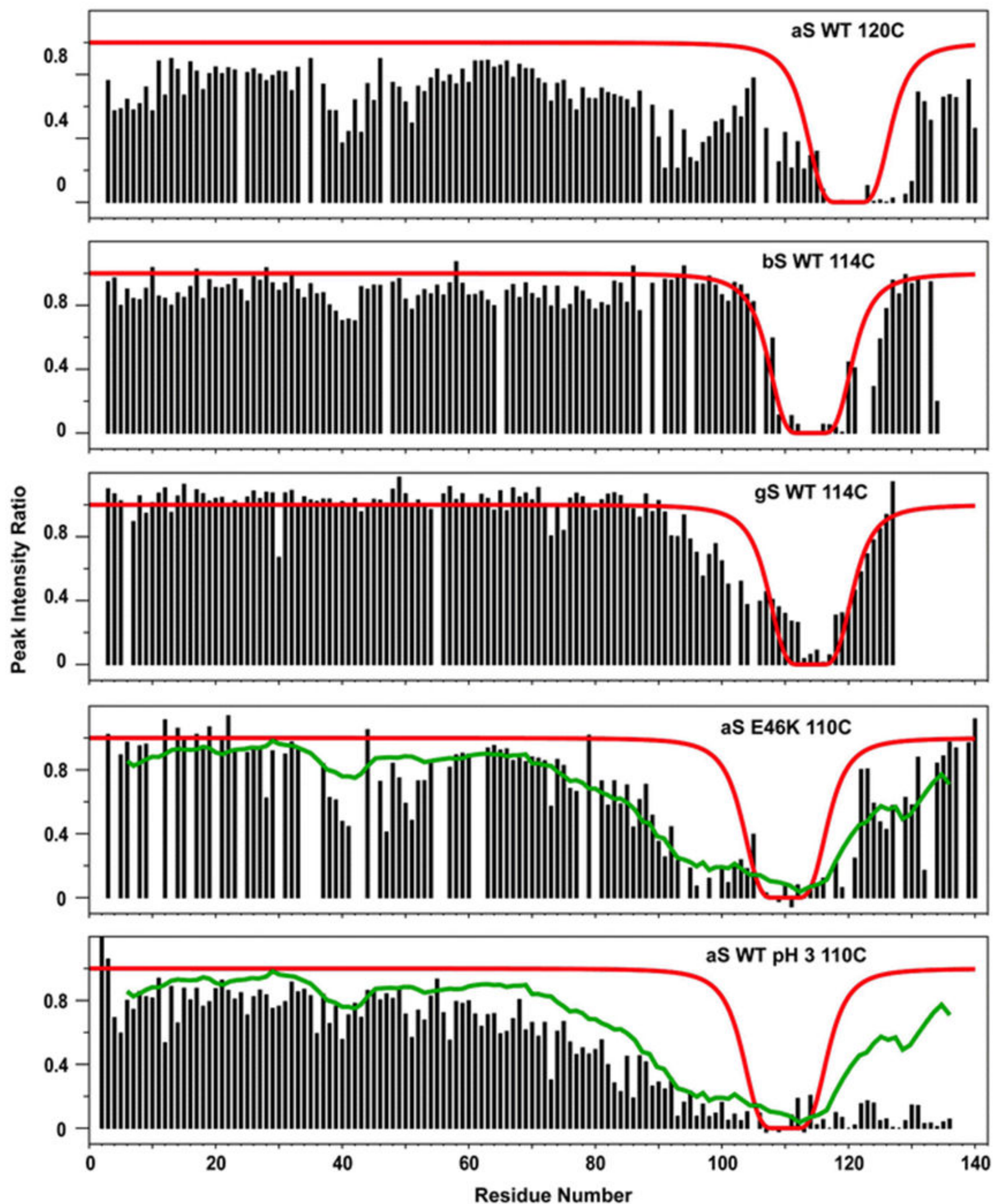

Fig. 1.

PRE data from wild type (WT) alpha-synuclein (aS), family variants beta-synuclein (bS) and gamma-synuclein (gS), the Parkinson's disease associated E46K mutation, and the low pH form of the protein, illustrating that the effects of sequence variations and environment on long-range structure can be evaluated qualitatively (14, 26, 27). *Solid red lines* represent the expected PRE effect based on a Gaussian chain model of the protein. *Solid green lines* in the E46K and pH3 plots are smoothed data from the wild type protein labeled at the same position (110) at neutral pH and are shown for ease of comparison. In this case, there is a

correlation with a decrease (bS and gS) or increase (E46K and low pH) in long-range interactions and a concordant decrease (bS and gS) or increase (E46K and low pH) in aggregation propensity.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

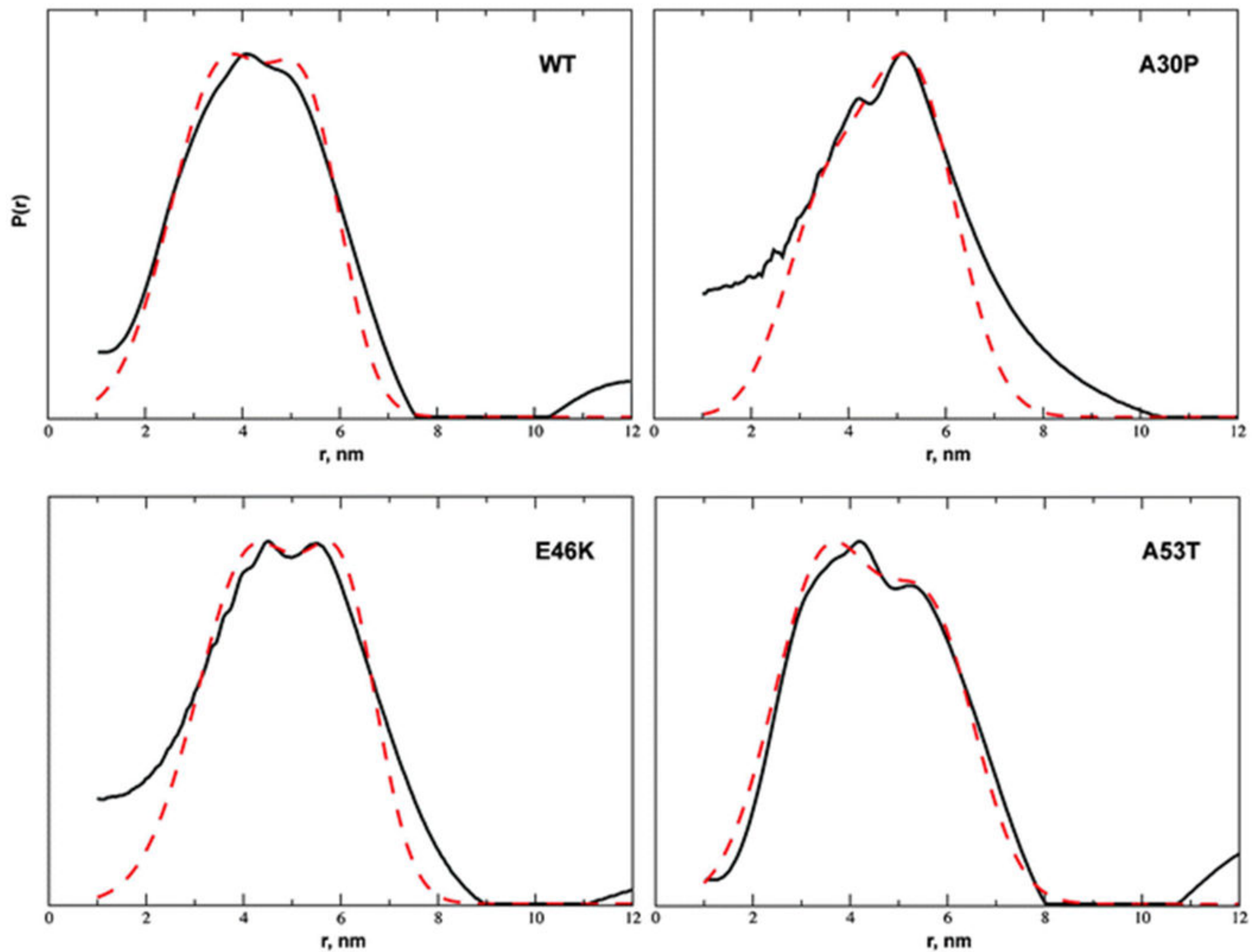

Fig. 2.

PDS-derived distance distribution for WT alpha-synuclein and three Parkinson's linked mutants, A30P, E46K, and A53T, doubly spin-labeled at positions 24 and 72, illustrating the presence of two subensembles, one more compact with an average distance around 3.7 nm and one more extended with an average distance of around 5.7 nm (12). The distributions could be reasonably fit as a pair of Gaussians (*dashed red lines*), allowing for an estimate of their relative populations. While compact conformations can be directly inferred from PRE data such as those in Fig. 1, extended conformations are difficult to detect using PRE approaches.