Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supplementary Information

Spin-label ESR with Nanochannels to Improve the Study of Backbone Dynamics and Structural Conformations of Polypeptides

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I. Dipolar Broadening Functions Obtained from the Analyses of the Distance Measurements.

The quantitative estimation of interspin distances from dipolar interactions of the nitroxides at/above room temperatures is an important improvement demonstrated in the present study. This improvement is potentially valuable for exploring conformational changes associated with function in biomolecules. Therefore, the analyses to extract the distance information from the cw-ESR spectra were performed rigorously. In Fig. S1(a) the black lines denote the dipolar Pake functions M (**B**) that were obtained by deconvolution of the experimental spectra of the singly- and doubly-labeled n3 peptides (Fig. 2) in the bulk-vitrified solutions using the Tikhonov-based deconvolution method[1]. The revealed distinct differences in the respective dipolar Pake functions were ready to provide supporting evidence for the changes in the interspin distances (Fig. 3) of the 144/150-α-bs and the 144/150-β-bs over the studied temperatures. We lay stress on that the deconvoluted curves (black lines) were not possible to be obtained and revealed in such a recognizable Pake pattern if the traditional Fourier-deconvolution method was used[2]. It is because that in Fourier frequency space, it is very difficult to select an appropriate cutoff frequency from weakly broadened spectra[2]. The Tikhonov-based deconvolution made it easily possible to obtain the deconvoluted Pake functions in an improved resolution, thereby obtaining the interspin distance distributions. The gray lines represent the "recovered" dipolar Pake functions, which were simulated using the distance distributions P(r) in Fig. 3. Since the P(r) was not solved in a straightforward way by directly inverting the linear equation[1] connecting the spectra of the doubly- and singly-labeled peptides, the recovered M(**B**) function need not necessarily fit the deconvoluted. The similarity in the black vs. gray lines simply proves that the Tikhonov analysis is a mathematically reliable method for the ill-posed problems, i.e., for recovering the solution from the noise-contaminated data. Figure S1(b) shows the comparisons for the experimental spectra (solid blue lines) and the simulated cw-ESR spectra (dashed green lines), which were calculated using the recovered dipolar Pake functions (gray lines) shown in Fig. S1(a). The *b* value used to account for the fraction of the monoradicals in

real experimental measurements was 10%. The recovered cw-ESR spectra overlap very well with the experimental spectra.

Figures S2(a) and S2(b) show the results for the n3 peptides encapsulated within the mesopores. In Fig. S2(a), the recovered dipolar Pake functions M(**B**) (black lines) for the 144/150-α-meso are distinctly different from those for the 144/150-β-meso, wherein the separations of the dipolar frequencies are greater for the latter than the former. It indicates that the interspin distances are greater for the 144/150-β-meso than the 144/150-α-meso, which is in consistency with the P(r) results shown in Fig. 3. The recovered Pake functions (gray lines in Fig. S2a), which were simulated using the P(r) results, fit very well with the experimental spectra, as shown in Fig. S2(b).

II. Circular Dichroism Characterization of n3 Peptides.

Circular dichroism (CD) experiments were recorded on an Aviv 410 spectrometer at temperatures 25 ^{o}C and 4 ^{o}C to confirm that the secondary structures of the studied n3 peptides are unchanged and approximately the same. Samples were studied in quartz tube cells with 1 mm of path length, and scanned over the wavelength range 190-260 nm by recording values every 1 nm with a 6 nm/min scan rate and a bandwidth of 1 nm. To obtain the CD spectra of the n3 peptides as encapsulated within mesopores, we first prepared the encapsulated n3 as described in Methods. Then the mesoporous materials (12mg) containing n3 were dispersed within a 2.5 ml PB buffer. A cuvette of 10 mm pathlength was employed with a small stirring rod. Other experimental parameters were unchanged from the bulk solution study. After the CD measurement, the solution mixture was washed with buffer and then stirred for 3 minutes prior to centrifugation. No absorption at 280 nm was detected by UV-Vis spectroscopy in the supernatant of the solution mixture after centrifugation. It indicated the n3 peptides remained inside the mesopores.

Figure S3 shows the CD spectra of the n3 peptides in the α-helical form at (a) 4 ^{o}C and (b) 25 ^{o}C. All of the CD spectra in Figs. S3(a) and (b) showed two negative peaks at 208 nm and 218 nm and a positive peak at 195 nm, typical of α-helix structure and in consistency with the reported study of the n3 peptide[3]. This result indicates that the secondary structure of the n3 remain in the α-helical form in both the two experimental conditions, i.e., in bulk solutions and within mesopores.

Figures S3(c) and S3(d) show the CD spectra of the n3 peptides in the β-hairpin form at 4 and 25 ^{o}C, respectively. Except the CD spectra of the 150/156-β-bs, all of the other spectra in Figs. S3(c) and (d) showed a broad negative Cotton effect at 208 nm with a shoulder around 216 nm, in consistency with the reported study of the n3 in the β-hairpin form[4]. This result indicates that the secondary structure of the n3 remain mostly the β-hairpin form in both the two experimental

conditions of bulk solutions and mesopores. The CD spectra of the 150/156-β-bs displayed a completely different characteristic shape compared to others. It was previously reported that the site 156 is critical to the structural stability of the n3 in the β-hairpin form[4].

Reference

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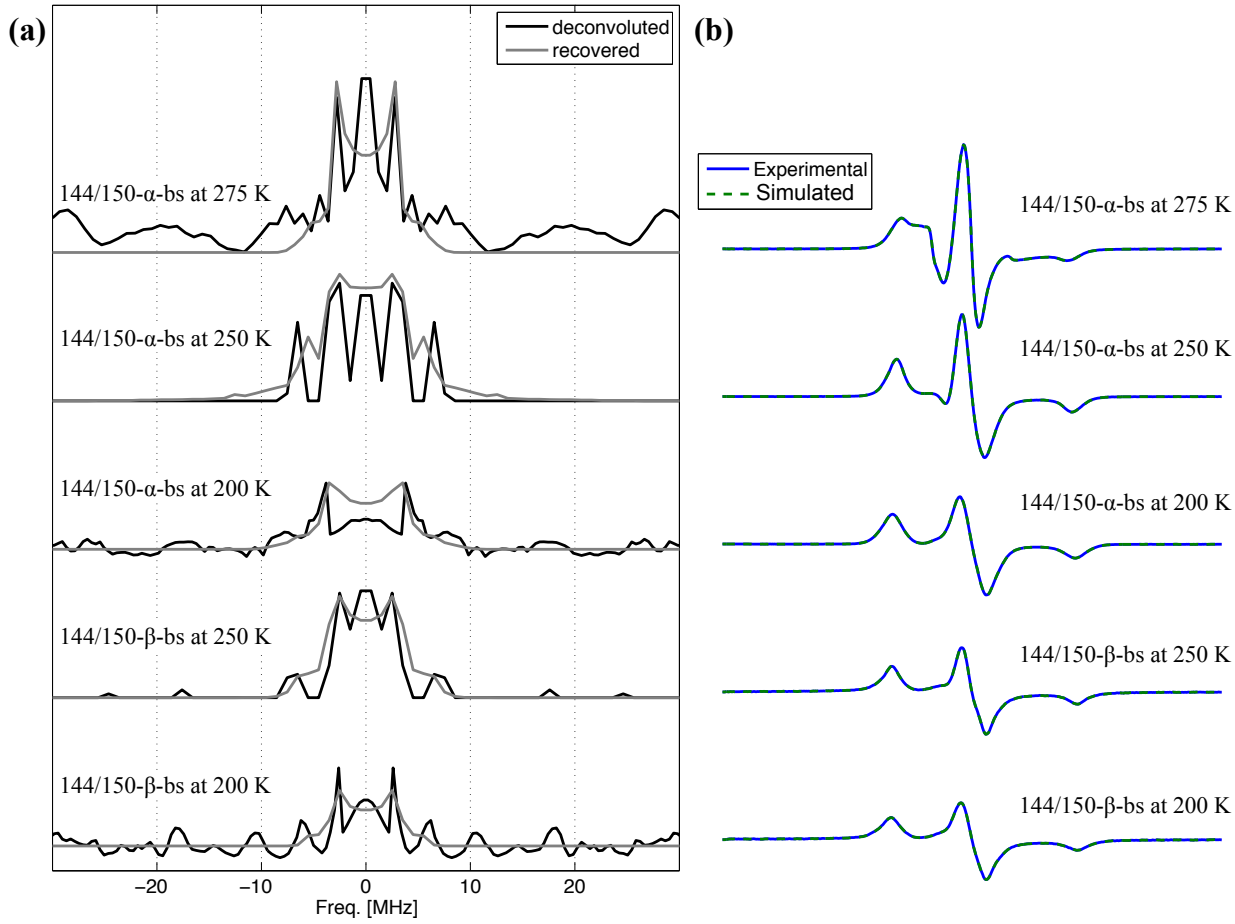

Figure S1. (a) The comparison of the deconvoluted vs. recovered dipolar Pake functions for the results of the bulk-vitrified solution studies. The deconvoluted functions were obtained by the deconvolution of the singly labeled ESR spectra S(B) and the doubly labeled ESR spectra D(B). The recovered functions were simulated using the obtained P(r). (b) The comparison of the experimental and simulated cw-ESR spectra. The simulated spectra were obtained using the recovered dipolar Pake functions (gray lines) in (a).

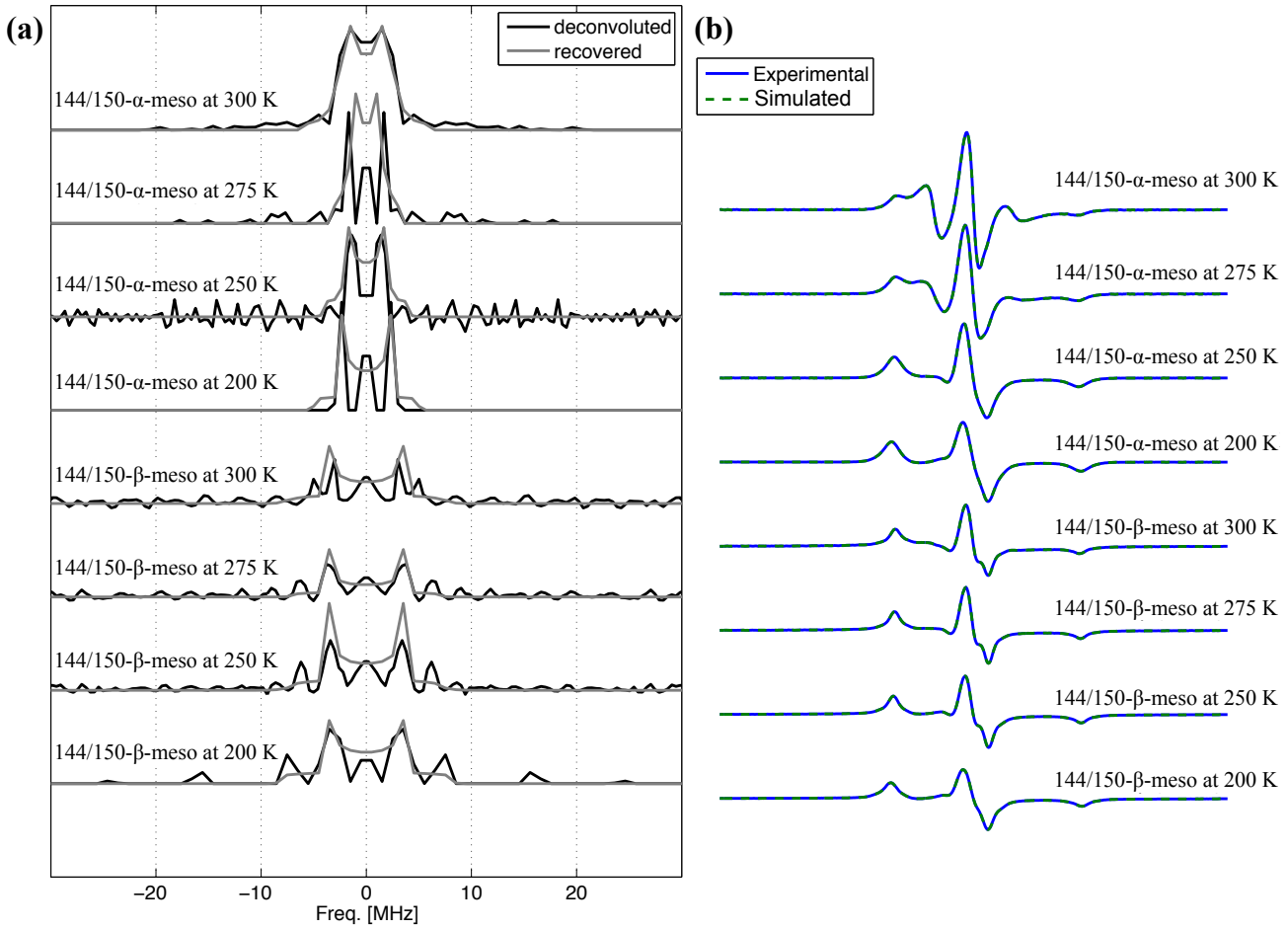

Figure S2. (a) The comparison of the deconvoluted vs. recovered dipolar Pake functions for the results of the mesopore studies. The deconvoluted functions were obtained by the deconvolution of the singly labeled ESR spectra S(B) and the doubly labeled ESR spectra D(B). The recovered functions were simulated using the obtained P(r). (b) The comparison of the experimental and simulated cw-ESR spectra. The simulated spectra were obtained using the recovered dipolar Pake functions (gray lines) in (a).

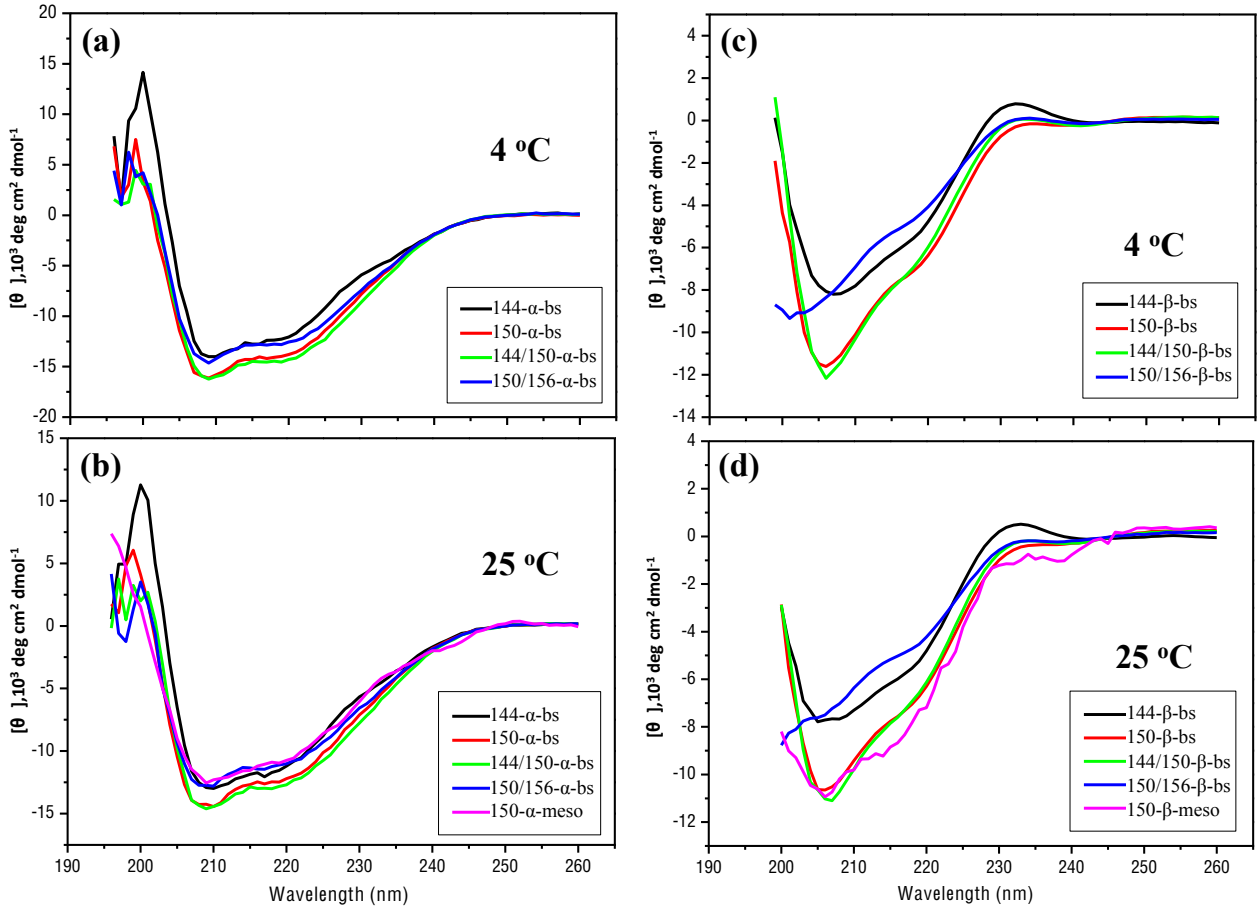

Figure S3. The CD spectra of the studied n3 peptides in the α-helical form at (a) 4 °C and (b) 25 °C, and in the β-hairpin form at (c) 4 °C and (d) 25 °C.