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# Flexibility and Lengths of Bis-peptide Nanostructures by Electron Spin Resonance

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In this communication we demonstrate the use of electron spin resonance (ESR) to determine long-range distances in water-soluble bis-peptide molecular rods. The synthesis of ever larger and more complex molecules with designed shapes and properties is an overarching goal of synthetic chemistry. We have developed an approach to the rapid synthesis of water-soluble nanoscale molecules with designed shapes using conformationally restricted building blocks that we couple through pairs of bonds to create spiro-ladder oligomers.[1,2] To develop these oligomers as rodlike structural elements for applications, such as bivalent display of ligands and as elements of future nanoscale devices, quantitative information on the lengths and flexibility is required. We show that ESR provides a natural spectroscopic method to rapidly assay these structural parameters.

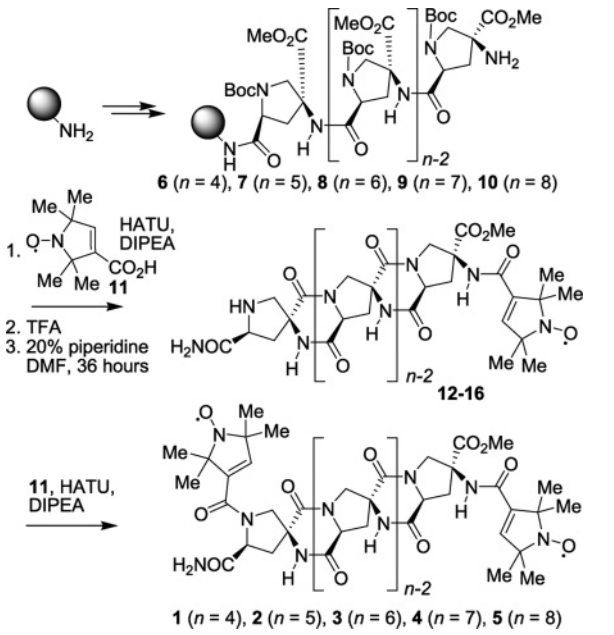
Five bis-peptide molecular rods, **1**−**5**, with $n = 4-8$ monomers were synthesized in parallel and labeled at the ends with nitroxides. Each of the syntheses of compounds **6**−**10** was carried out on solid support following previously described procedures on 5 mg of Rink amide resin.[1,2] While still on solid support, the terminal free amine was acylated with the spin probe 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxylic acid **11** activated with *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′*N*′-tetramethyluronium hexafluorophosphate (HATU). The flexible oligomers were then cleaved from the resin and Boc-deprotected using trifluoroacetic acid (TFA). Intramolecular aminolysis between the secondary amine of each monomer and the methyl ester of the monomer preceding it was catalyzed using 20% piperidine in dimethylformamide at room temperature for 36 h to form the spin-labeled spiro-ladder oligomers **12**−**16**. Finally, a second spin label **11** was coupled to the remaining free secondary amine in solution, and the products **1**−**5** were purified using $C_{18}$ reverse phase high-pressure liquid chromatography. The mass of each oligomer was confirmed by mass spectrometry.

For ESR experiments, 0.2 mM solutions of the double-labeled molecules were prepared in 70% buffer (50 mM phosphate buffer, pH 7.4, 200 mM NaCl, 3 mM $NaN_3$, 1 mM EDTA) and 30% glycerol. The four-pulse double electron−electron resonance (DEER)[3] data on compounds **1**−**5** at 80 K are shown in Figure 1. In the DEER experiment the local dipolar field due to the coupled spin-partner is inverted to lead to a frequency pattern that goes as:

$$\nu_{1,2} = \frac{\mu_0 g_1 g_2 \beta^2 (3\cos^2\theta - 1)}{4\pi h r^3} + J \qquad (1)$$

where $g_1$ and $g_2$ are the isotropic $g$-factors of each electron, $\beta$ is the Bohr magneton, $\mu_0$ is vacuum permeability, $h$ is Planck's constant, $r$ is the interspin distance, and $\theta$ is the angle between magnetic field and the interspin vector. $J$ is the exchange interaction, which is negligible for $r > 15$ Å. At 80 K a "Pake" pattern[4,5] is obtained in which the characteristic turning points corresponding to the parallel and perpendicular orientations [i.e., $\theta = 0°$ and $\theta = 90^0$ in eq 1] of the interspin vectors with respect to the dc-magnetic field are readily observable. The $\theta = 0°$ turning point has much lower intensity, but typically the $\theta = 90°$ peak is clearly visible.

***Scheme 1*** Synthesis of the Bis-spin Probe-Labeled Oligomers **1**−**5**



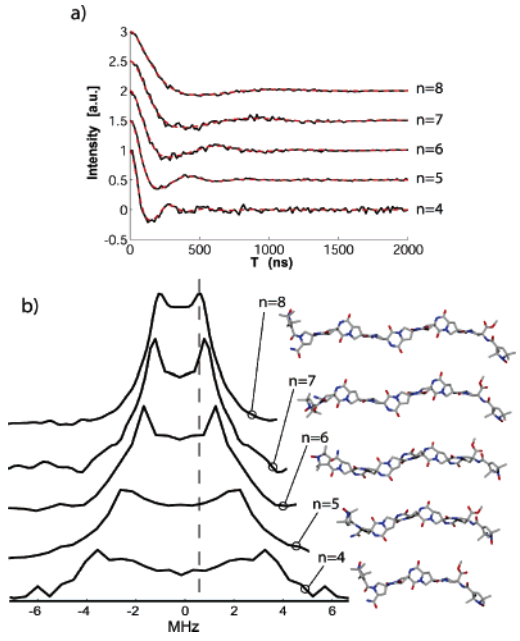




***Figure 1.*** (a) Time domain DEER data (black) with the simulated signal (red). (b) Four-pulse DEER spectra and the structures of compounds **1**−**5** are shown.



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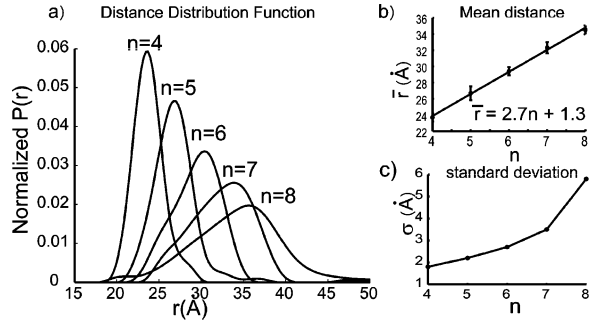


***Figure 2.*** (a) The distance distribution function, $P(r)$, (b) the mean distance, and (c) the standard deviation for compounds **1**−**5** are shown.

Thus the interspin distance, $r$, can be readily obtained from the DEER spectrum.

Qualitatively, the frequency of the $\theta = 90°$ peak in Figure 1b decreases as the number of monomers, $n$, increases, indicating an increase in mean end-to-end length. The spectra were inverted to obtain the distance distribution functions, $P(r)$, using the DEER-Analysis 2004 program.[6,7] In the analysis a Tikhonov regularization method[8] was used. The distance distribution functions for compounds **1**−**5** are shown in Figure 2a.

The mean distance, $\bar{r}$, and the standard deviation, $\sigma$, for these $P(r)$, calculated using a moment analysis (see Supporting Information), are shown in Figure 2b,c. The error in $\bar{r}$, estimated by the spectral resolution, is ∼1.0−1.7 Å. The "linear" rodlike shape of these materials is readily interpreted from the plot of $\bar{r}$ versus $n$. The linear fit to the data of Figure 2b indicates that each building block adds 2.7 Å to the average distance between the spin probes. Five-nanosecond molecular dynamics simulations were carried out in vacuo at 300 K on each oligomer. The DEER experiment inadequately samples conformers with $r$ less than 20 Å (see Supporting Information). Within this experimental limitation, the estimates of $\bar{r}$ from dynamics are in reasonable agreement with the experiments for $n = 4-7$. However, molecular dynamics overestimates the mean distance for $n = 8$ by ∼3.5 Å (see Supporting Information). Distributions from molecular dynamics also progressively overestimate the most probable internitroxide distance for long scaffolds (by ∼2, 2.5, and 7 Å for $n = 6$, 7, and 8).

The flexibility of the molecular rods can be characterized by the standard deviation, $\sigma$, of the distribution function. The standard deviation increases from 1.8 Å for $n = 4$ to 5.8 Å for $n = 8$.

The sensitivity of DEER-ESR[9,10] to the measurement of interspin distances in the ∼15−80 Å[10] has been used to determine global folding patterns in proteins,[11−14] nucleic acids,[15] ionic polymers,[16] and conformational and aggregation states of polypeptides.[17]

We show that we can create water-soluble bis-peptide molecular rods with defined lengths and that the overall shapes and flexibility of these rods can be obtained by using ESR. The key advantage of the use of ESR is two-fold. First, large distance constraints can be measured from which the overall shape of the conformationally restricted material can be rapidly inferred. NMR has been used to determine short-range distances in bis-peptides,[1,2] but the rodlike nature of these materials precludes the measurement of distance between residues far apart in the linear sequence. This can lead to a substantial uncertainty in the modeling of the overall structure. In principle, energy transfer in FRET[2,18,19] is sensitive to distances in these length scales. However, the correct interpretation of the energy transfer in terms of distances requires a careful accounting of molecular dynamics.[20] Second, ESR measures the full distance distribution function, from which the flexibility of the nanostructured materials can be directly assayed. The shape and flexibility are both important criteria for the design of nanostructured materials with targeted functions.

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**Supporting Information Available:** Details about the experimental parameters, analysis procedures, and modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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