## **Supporting Information**



**Figure S1:** *p85ni DEER data.* Spin labeling position are indicated on the plots. Raw data (red points) and inter molecular background contribution (blue line) is shown in the left panel. The background was calculated by fitting an exponential curve between the vertical blue and cyan lines. Data cut-off lines are shown in green. Background subtracted and intensity normalized data are shown in the middle-left column (blue points). The fit (red line) is the dipolar evolution which corresponds to a distance distribution function shown in middle-right column. The error surface of the fit is shown in the right most column, where a large error in determining the width or mean distance of the distribution would be indicated by a large contour surface.



Figure S1 (cont.)



**Figure S2:** DEER control experiments. Panels **A** and **B** demonstrate the absence of a dipolar evolution in pure spin label solution and in single labeled p85ni. Synthetic peptide Trich(1,8 TOAC) in **C** exhibits 1.95 nm distance and shows the capability of measuring distances near 2.0 nm. The biradicals in **D** and **E** have expected distances of 2.8 and 3.6 nm respectively, agreeing with our experimental results of 2.79 and 3.56 nm. Dipolar evolution of a two spin labels attached on nSH2 domain of p85ni in **F** show a clear modulation in the raw data and exhibit a distance distribution with a width of 1.37 nm. **G** and **H** are example data corresponding to extended delay times between second and third observer pulses (same samples' data with shorter evolution times are shown in Figure S1).



Figure S3: Continuous wave EPR spectra of double spin labeled p85ni.