

Electronic Supplementary Information for

**Role of Conformational Heterogeneity in Regulating the Apoptotic Activity of
BAX Protein**

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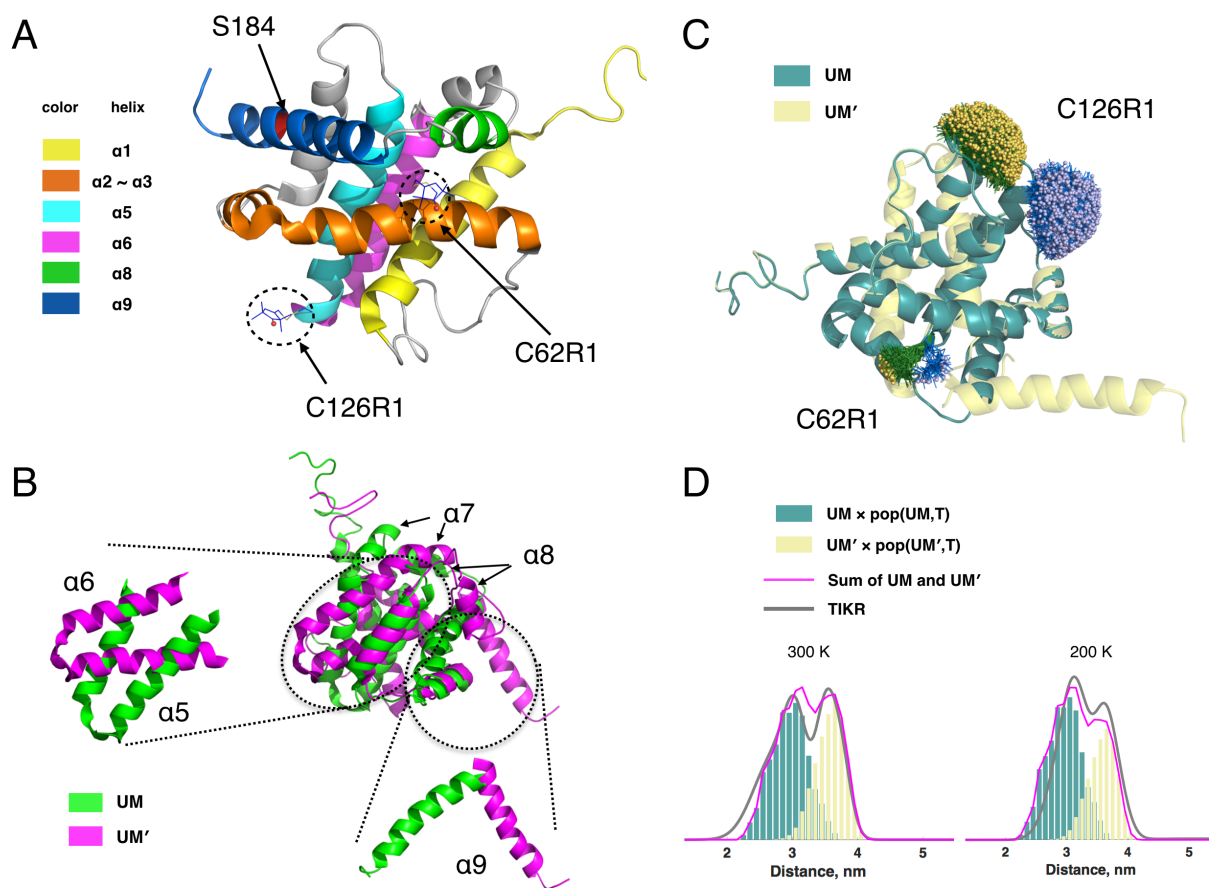


Figure S1. Models of inactive BAX protein. **(A)** Structural model of the UM BAX (PDB: 1F16). Wild-type BAX consists of two endogenous cysteine residues 62 and 126, ready to react with spin probes to have doubly labeled wtBAX (denoted by C62R1 and 126R1). Site S184 is within $\alpha 9$ helix and highlighted in red. **(B)** The two distinct conformational states, UM and UM', were previously determined by Pulsed DEER technique¹. However, the exchange and equilibrium of the two states have not been studied. There are three major differences between the two states; they are the helical hairpin ($\alpha 5 - \alpha 6$), the highly mobile C-terminal helix ($\alpha 9$), and the displacement of the helix $\alpha 8$. As illustrated in Figure 2A, measuring distances between C62R1 and C126R1 (located within $\alpha 2$ and $\alpha 5$, respectively) provides a good indicator of the changes in the relative populations of the UM and UM'. **(C)** Models of the two conformations of the doubly labeled wtBAX by the MtsslWizard. **(D)** Comparison of the TIKR P(r) and the MtsslWizard-generated P(r) (magenta). The magenta line is sum of the respective contributions of the UM and UM' for the given pre-equilibrated T (i.e., $UM \times pop(UM, T) + UM' \times pop(UM', T)$). The corresponding populations are $pop(UM', 300\text{ K}) = 44\%$ and $pop(UM', 200\text{ K}) = 37\%$, as given in main text. The MtsslWizard calculations indicate that average of the interspin distances between 62R1 and 126R1 is 3.0 nm for the UM and 3.6 nm for the UM'. These values are used as a constraint in the multiple Gaussians analysis to extract the two components from the TIKR P(r) results.

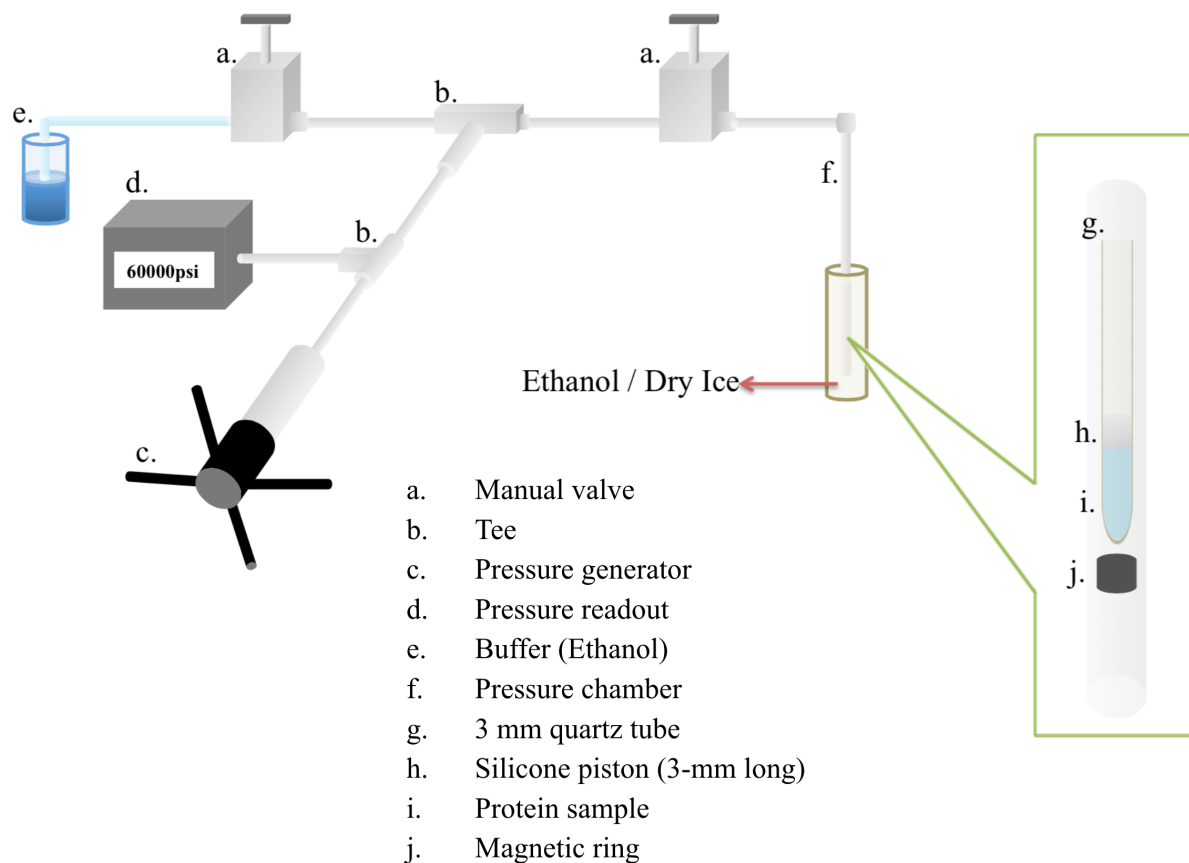


Figure S2. Schematic of the high-pressure DEER system. Details of the system are given in Experimental sections and are similar to the reported².

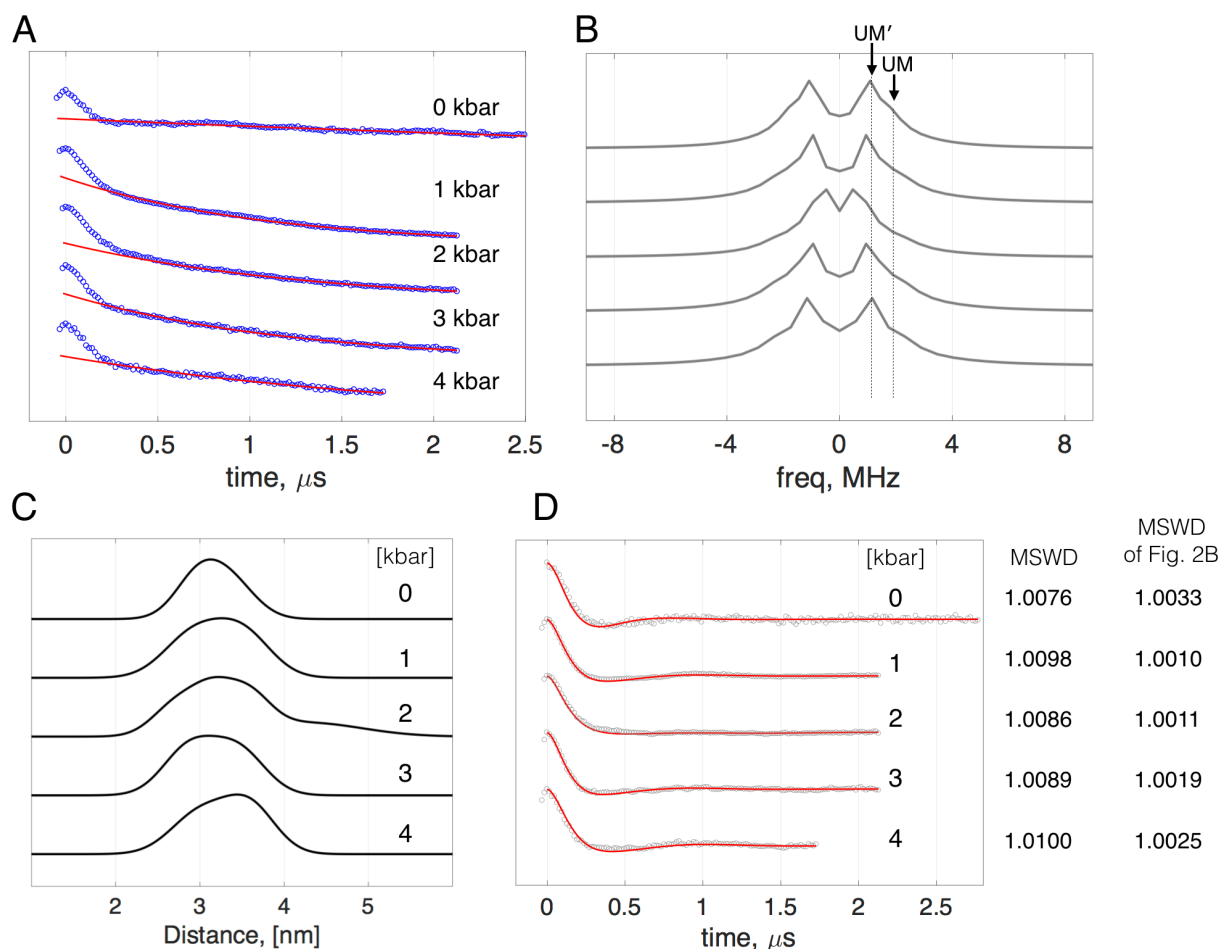


Figure S3. Analysis of the PR DEER data of doubly labeled wtBAX. **(A)** Raw data of the PR DEER measurements. Baselines are shown in red. **(B)** Pake patterns of the Fourier-transformed time-domain PR DEER signals. The components of the UM' and UM are approximately located at 1.1 and 1.9 MHz, respectively, in the frequency domain. The respective components are not well resolved in the plots because the Pake pattern mainly accounts for an average of the ensemble. It is therefore necessary to analyze DEER data using the TIKR method (as shown in Fig. 3A). **(C)** Distance distributions simulated with a constraint forcing the TIKR $P(r)$ results to be homogeneous. **(D)** Time-domain data corresponding to the background-removed experimental data (gray circles) and the simulations (red lines) reconstructed from the homogeneous $P(r)$ (i.e., the result in C). It shows that the red lines fit less satisfactorily, in terms of the MSWD (mean square weighted deviation) values, to the experimental data when compared to the good quality of fits shown in Figure 2B. MSWD values of the data in C and Figure 2B are shown, in which the quality of the fits is clearly poorer in the former. As the equation connecting the distance distributions and the time-domain DEER data is an ill-posed problem^{3,4}, the small deviation from the experimental DEER data would cause a large difference in the data analysis. This numerical study verifies the heterogeneity in the TIKR $P(r)$ results, supporting the existence of the UM' and UM components in the experimental data.

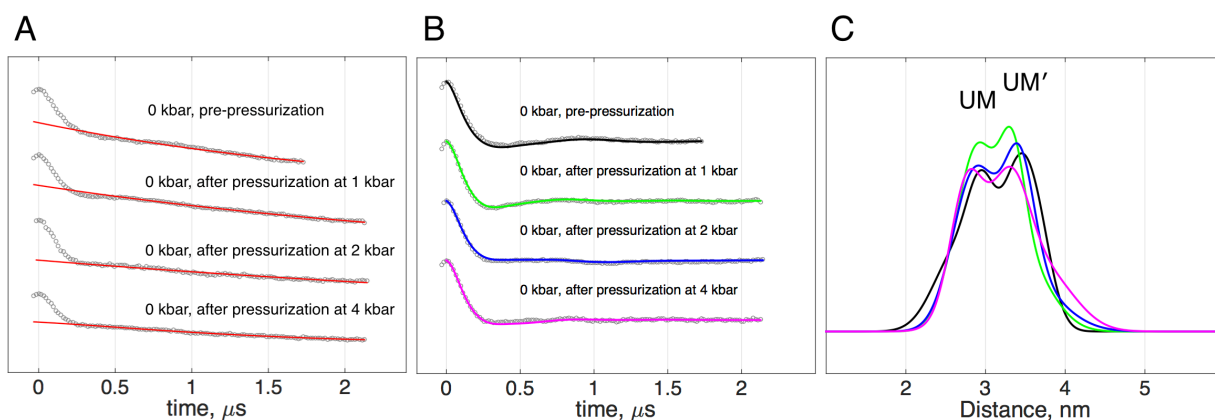


Figure S4. Reversibility of the changes observed with PR DEER. **(A)** Raw experimental DEER data for wtBAX before and after the pressurizations. Red lines are background signals assuming wtBAX proteins are homogeneously dispersed in solution. Pre-equilibrium temperature was 300 K for the data shown here. Samples were pressurized at the given pressure for 5 min, followed by de-pressurization and then 0-kbar DEER measurements. **(B)** Experimental time-domain (background-removed) data (in gray color) and simulations recovered from the TIKR distance distributions. Simulation in black color corresponds to the result of pre-pressurization. Simulations shown in green, blue, and magenta colors correspond to the results for the post-pressurization of 1, 2, and 4 kbar, respectively. The simulated time-domain data fit nicely to the experimental data. **(C)** Distance distributions obtained by the TIKR analysis. Lines are colored according to **B**. The distance distributions for the post-pressurizations are similar to the result of the pre-pressurization, supporting that the two states are in equilibrium and the two conformations of BAX relax back to the pre-pressurization state. The reversibility of the pressure-induced changes in BAX is verified.

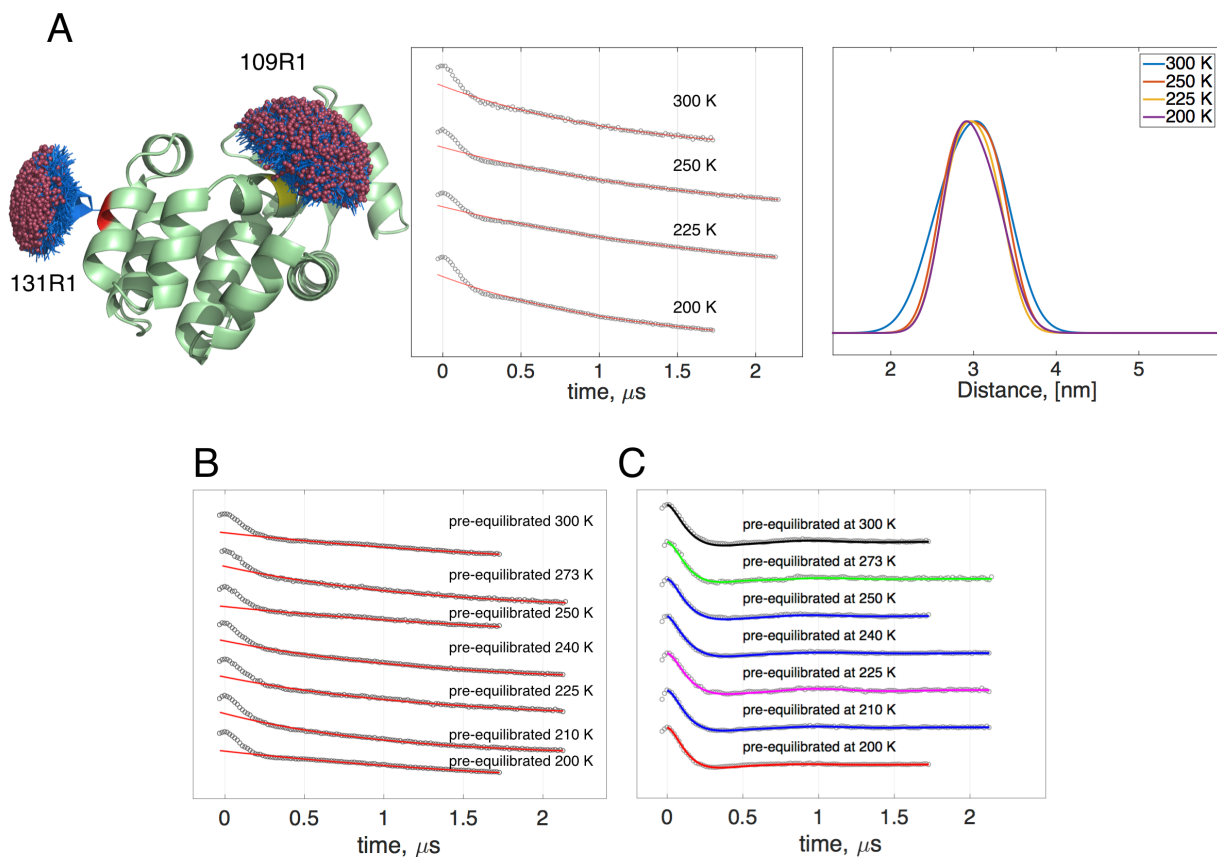


Figure S5. Demonstration of the TR DEER methodology. **(A)** Cartoon model of T4 Lysozyme (T4L) and the two spin-labeled sites 109 and 131 (left). The MtsslWizard calculations indicate an average distance of 3.3 nm between the 109R1 and 131R1. Raw experimental TR DEER data of the doubly labeled T4L 109R1/131R1 (middle). TIKR $P(r)$ results obtained from the TR DEER measurements of the T4L 109R1/131R1 (right). The average distances (ca. 3.1 nm) of the $P(r)$ results are in a good agreement with the reported structure (PDB: 3LZM). The average distances change little with temperature, consistent with our expectation that the conformation of T4L changes little within the temperature range 200–300 K. The result of this T4L study demonstrates the methodology of TR DEER. **(B)** Raw experimental TR DEER data of spin-labeled wtBAX. **(C)** Time-domain (background-removed) DEER data for the TR DEER measurements shown in **B**. Solid lines in colors are the simulations reconstructed from the TIKR results shown in Figure 3A.

References

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2. M. T. Lerch, Z. Yang, E. K. Brooks, and W. L. Hubbell, *Proc. Natl. Acad. Sci. U.S.A.*, 2014, **111**, E1201-10.
3. Y. W. Chiang, P. P. Borbat, and J. H. Freed, *J. Magn. Reson.*, 2005, **172**, 279–295.
4. Y.-W. Chiang, P. Borbat, and J. H. Freed, *J. Magn. Reson.*, 2005, **177**, 184–196.