

Supplementary Information

Double Electron-Electron Resonance Reveals Ca²⁺-induced Conformational Changes and
Dimerization of Recoverin†

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Running title: Ca²⁺-induced Structural Changes in Recoverin Probed by DEER

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Figure S1. Overlay of ^{15}N - ^1H HSQC NMR spectra of Ca^{2+} -free myristoylated wildtype recoverin (red) and recoverin $^{\text{N120C}}$ (blue). The sample conditions and procedures for the NMR experiments were the same as used by Ref 44.

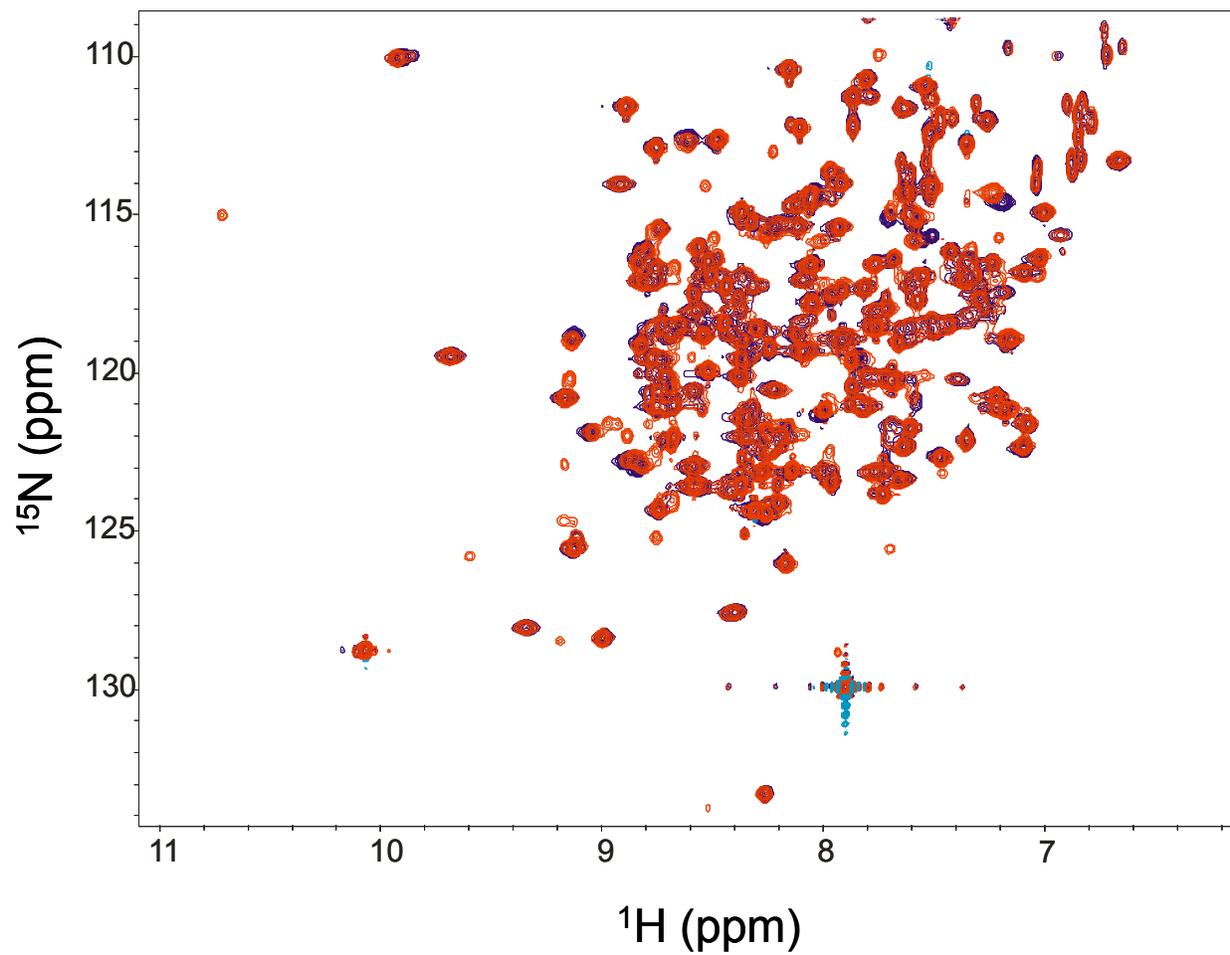


Figure S2. Scatchard analysis for Ca^{2+} binding to wildtype myristoylated recoverin (solid squares) and recoverin^{N120C} (cross marks). The sample conditions and experimental procedure were the same as used by Ref 19. The recoverin^{N120C} binding data were fit to the Hill model (dotted line) using apparent dissociation constant of $20 \pm 2 \mu\text{M}$ and Hill coefficient of 1.5. Wildtype myristoylated recoverin has an apparent dissociation constant of $17 \pm 2 \mu\text{M}$ (ref 19).

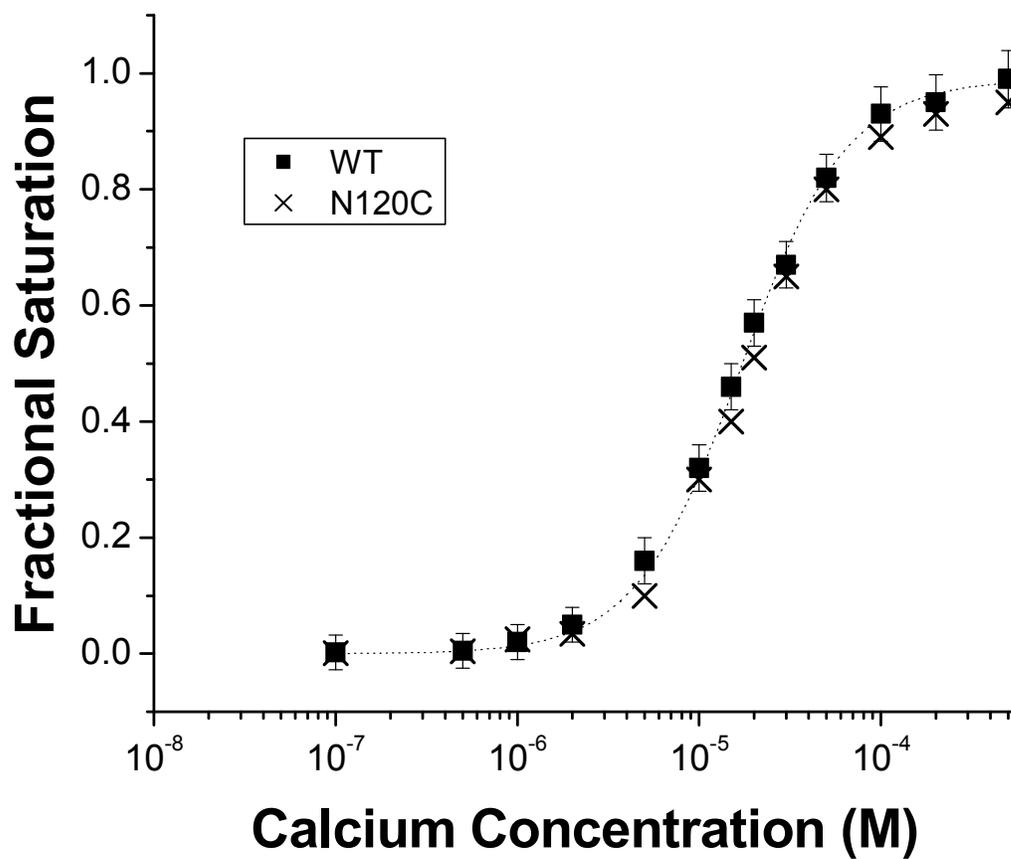
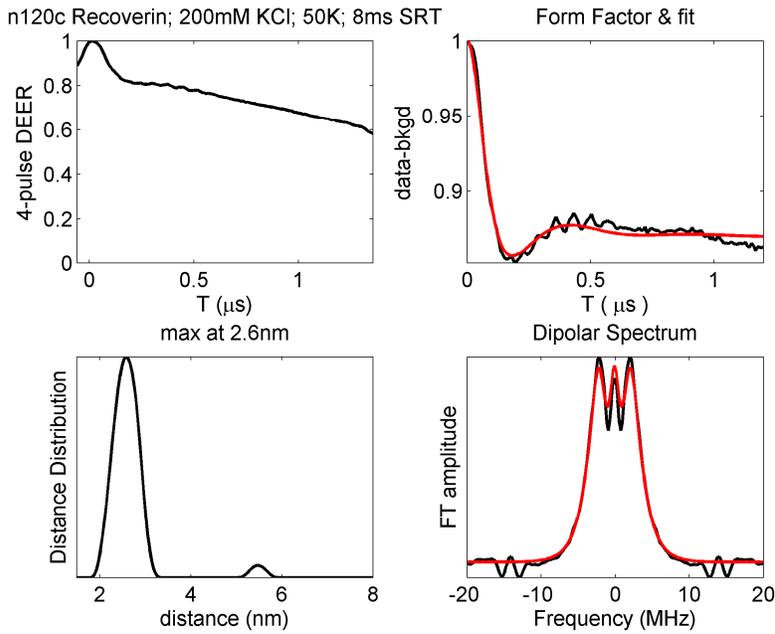


Figure S3. Experimental DEER traces at 50K for a sample prepared separately from that in the main text. Amplitude-normalized data, the background-subtraction and the frequency domain are shown. (a) apo-recoverin (b) Ca^{2+} -bound recoverin.

(a)



(b)

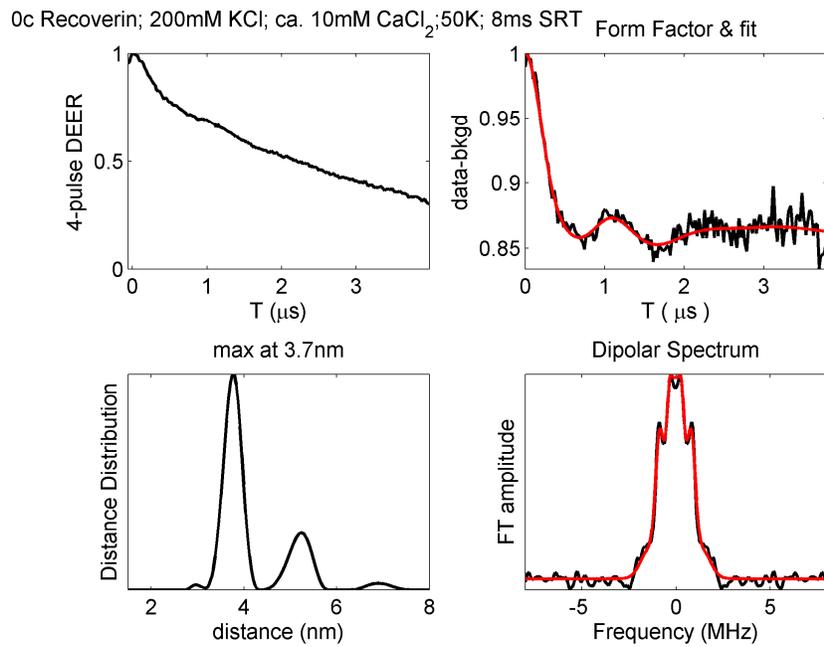


Figure S4. A Q-band DEER trace for $\tau_2=11\mu\text{sec}$, measured at 50K (pump=20ns, probe=16ns and 32ns) from same sample as in main text (transferred to a 1.6mm O.D. tube). The panels are as follows, (a) raw data divided by its max (black) and the optimized 3-D homogenous background subtraction line (red), (b) background subtracted data with a Tikhonov Regularization fit to the data and (c) a distance distribution including validation data. For c., the grey error bars about the mean distribution (in black) indicate the full variation of the probability of a given distance over all trials from starting the background subtraction and varying the noise level. A lower error estimate corresponding to the mean value of the probability minus two times its standard deviation (2σ), and an upper error estimate corresponding to the mean value $+2\sigma$, (red lines).

