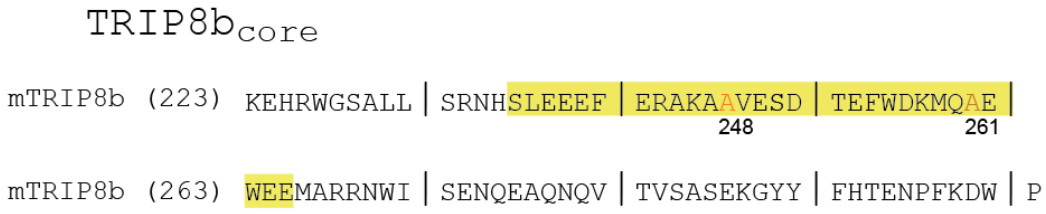
Supplemental Data

A



B

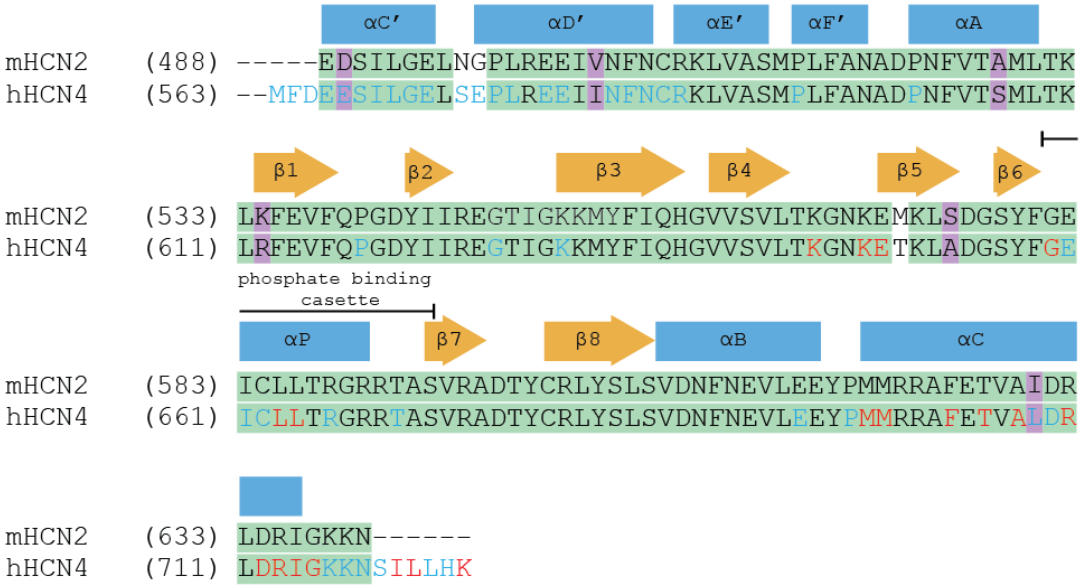


Figure S1, Related to Figure 1: A) Sequence of the human TRIP8b$_{core}$ construct. Yellow highlighted residues are identical in all orthologues of TRIP8b. The residue numbering system for TRIP8b can vary from study to study because TRIP8b is heavily alternatively spliced. For this study, the numbering system is based on TRIP8b isoform 1a-4. Residues that are mutated to cysteines for the DEER studies are colored in orange. B) Alignment of the two HCN channel constructs used in this study. A green background indicates that the region is 100% identical and a pink background indicates the residues are similar. The blue boxes above the sequence show the positions of the alpha helices and the orange arrows indicate the positions of the beta sheets. The residues colored red were affected by binding of 25 μM TRIP8b in the NMR experiments and the residues colored cyan were not assigned.

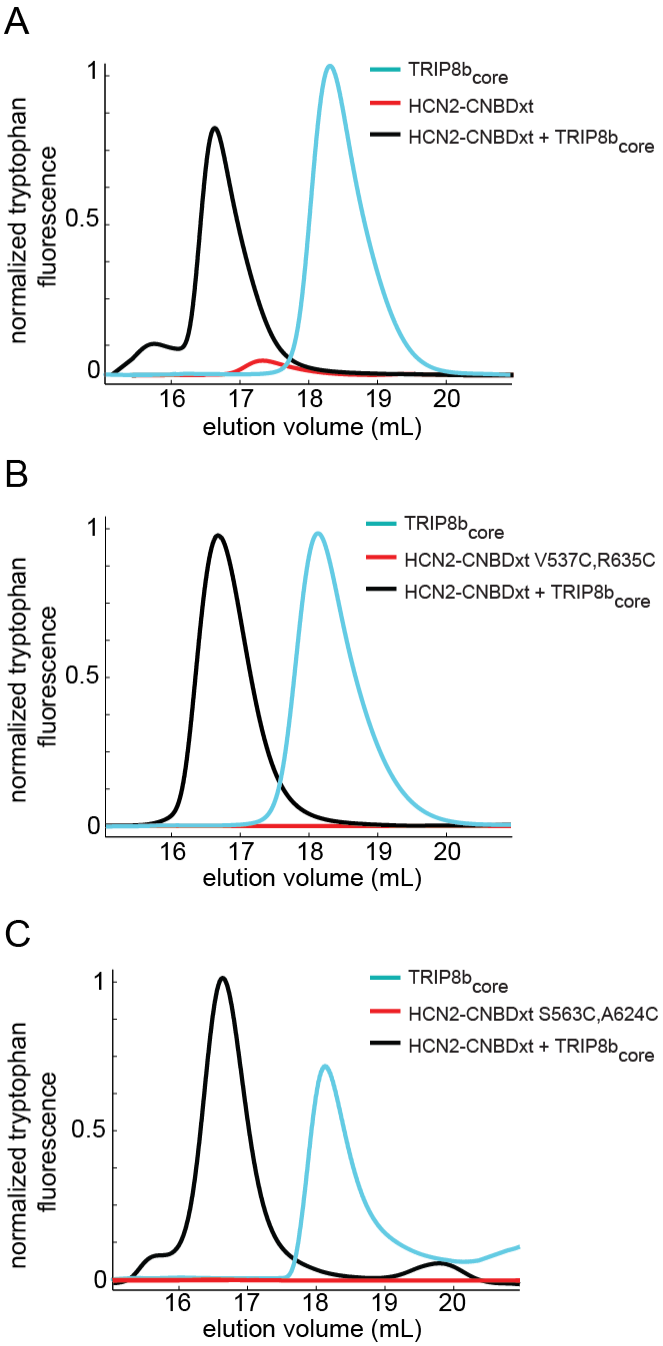


Figure S2, Related to Figure 2: A) Fluorescence size-exclusion chromatography (FSEC) results from purified TRIP8b$_{core}$ (cyan), HCN2-CNBDxt (red), and the complex of the two (black) showing a decrease in elution volume of the fluorescent TRIP8b$_{core}$ indicating a stable complex. B) FSEC results from purified TRIP8b$_{core}$ (cyan), HCN2-CNBDxt V537C,R635C (red), and the complex of the two (black). C) FSEC results from purified TRIP8b$_{core}$ (cyan), HCN2-CNBDxt S563C,A624C (red), and the complex of the two (black).

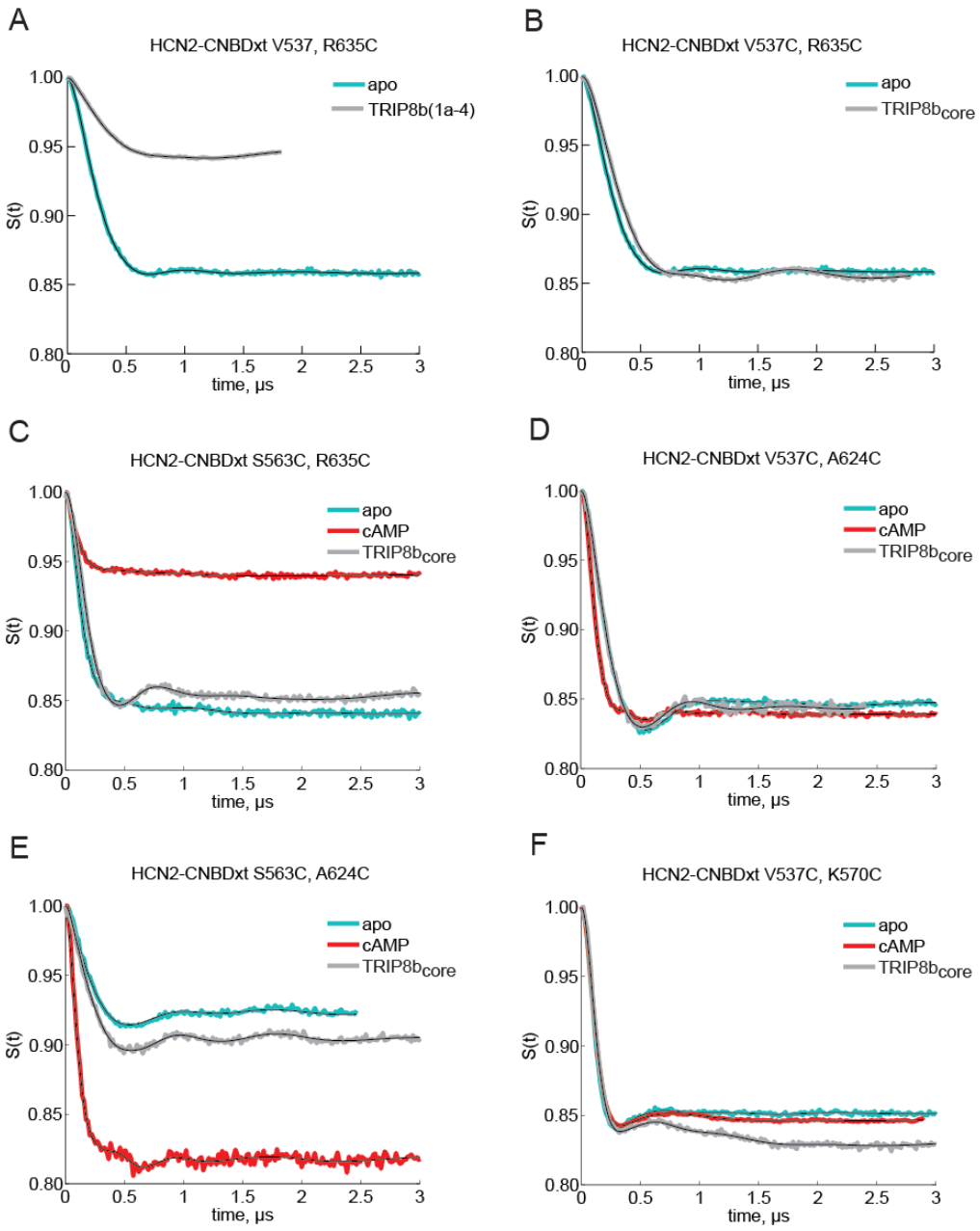


Figure S3, Related to Figures 2 and 3: Background corrected, oscillatory DEER time traces corresponding to data shown in Figures 2 and 3. Raw DEER data is shown for HCN2-CNBDxt bound to TRIP8b$_{core}$ (gray), cAMP (red), and in the apo state (cyan traces). Fits to the data are shown as thin black lines. Traces are displayed for residue pairs A) HCN2-CNBDxt V537C,R635C, B) HCN2-CNBDxt V537C,R635C, C) HCN2-CNBDxt S563C,R635, D) HCN2-CNBDxt V537C,A624C, E) HCN2-CNBDxt S563C,A624C, and F) HCN2-CNBDxt V537C,K570C. In A) HCN2-CNBDxt is bound to TRIP8b(1a-4) and in B-F) bound to TRIP8b$_{core}$.

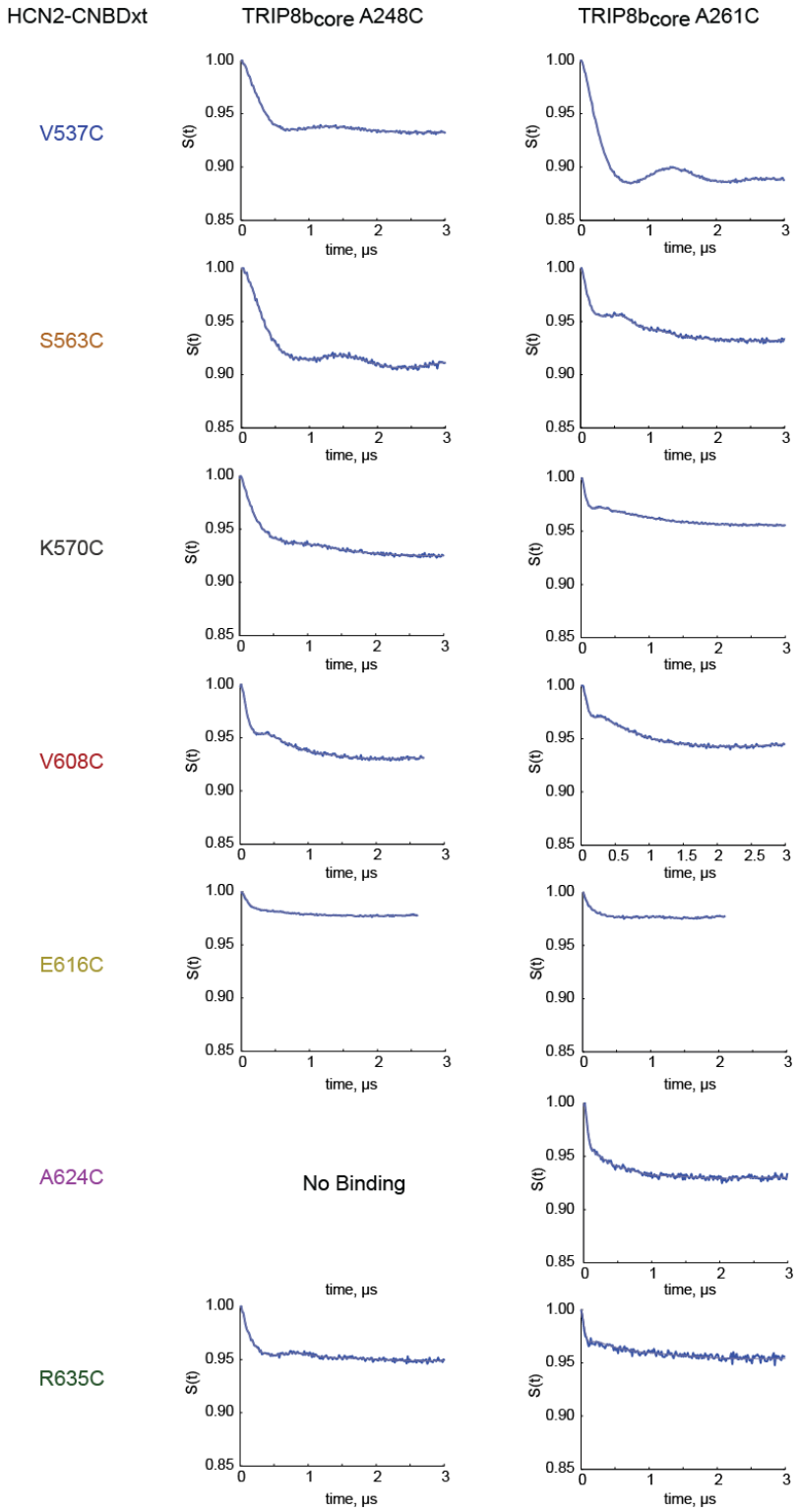


Figure S4, Related to Figure 5: DEER time traces corresponding to the data shown in Figure 5. Background corrected time traces are shown between the indicated residues of singly labeled 150 µM HCN and 40 µM TRIP8b$_{core}$.

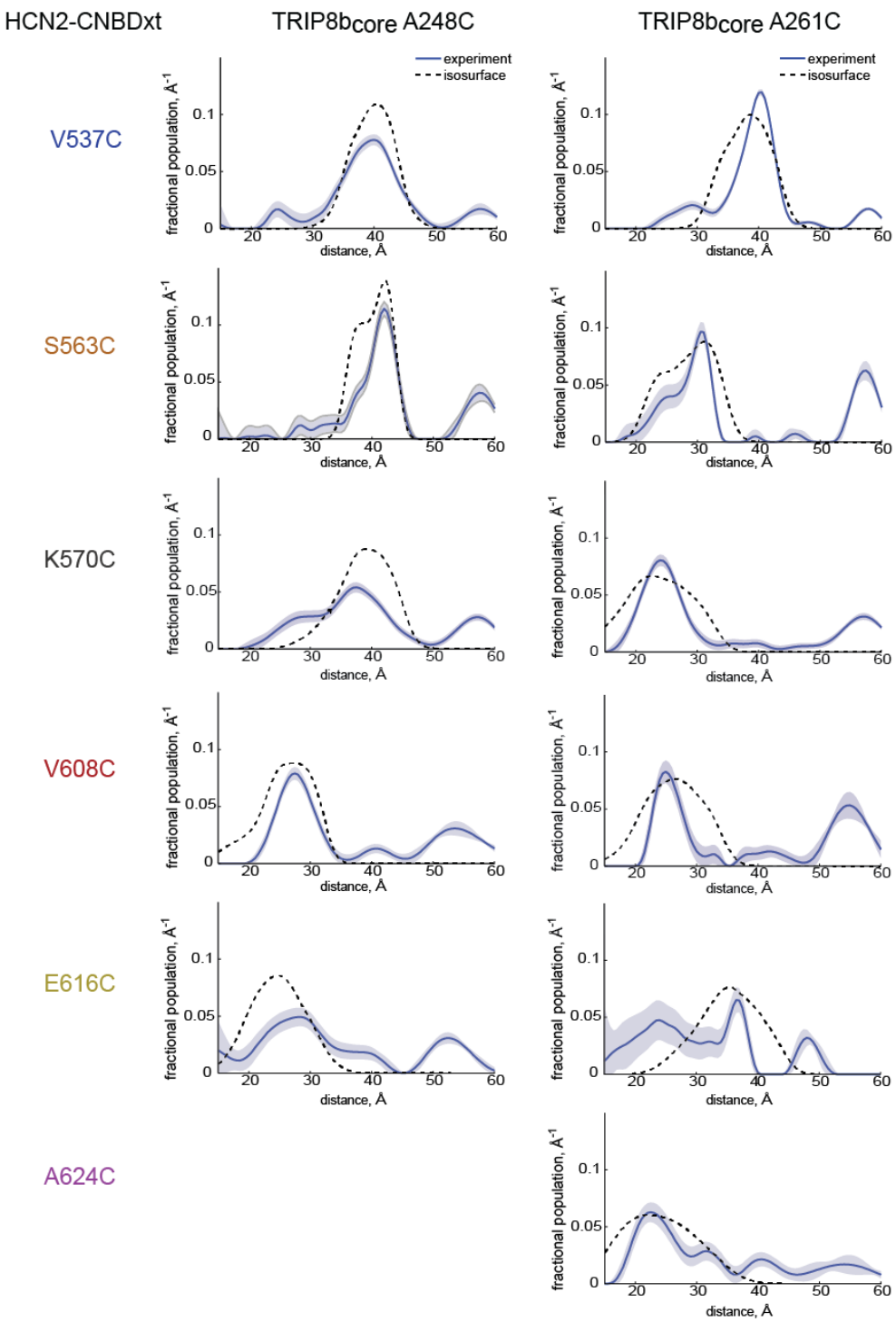


Figure S5, Related to Figure 6: Calculation of expected distance distributions from different isosurfaces of the TRIP8b$_{core}$ A248C and A261C spin label probability densities (black, dashed lines). The experimental distance distributions from Figure 5 are also plotted for comparison (dark blue, solid lines).

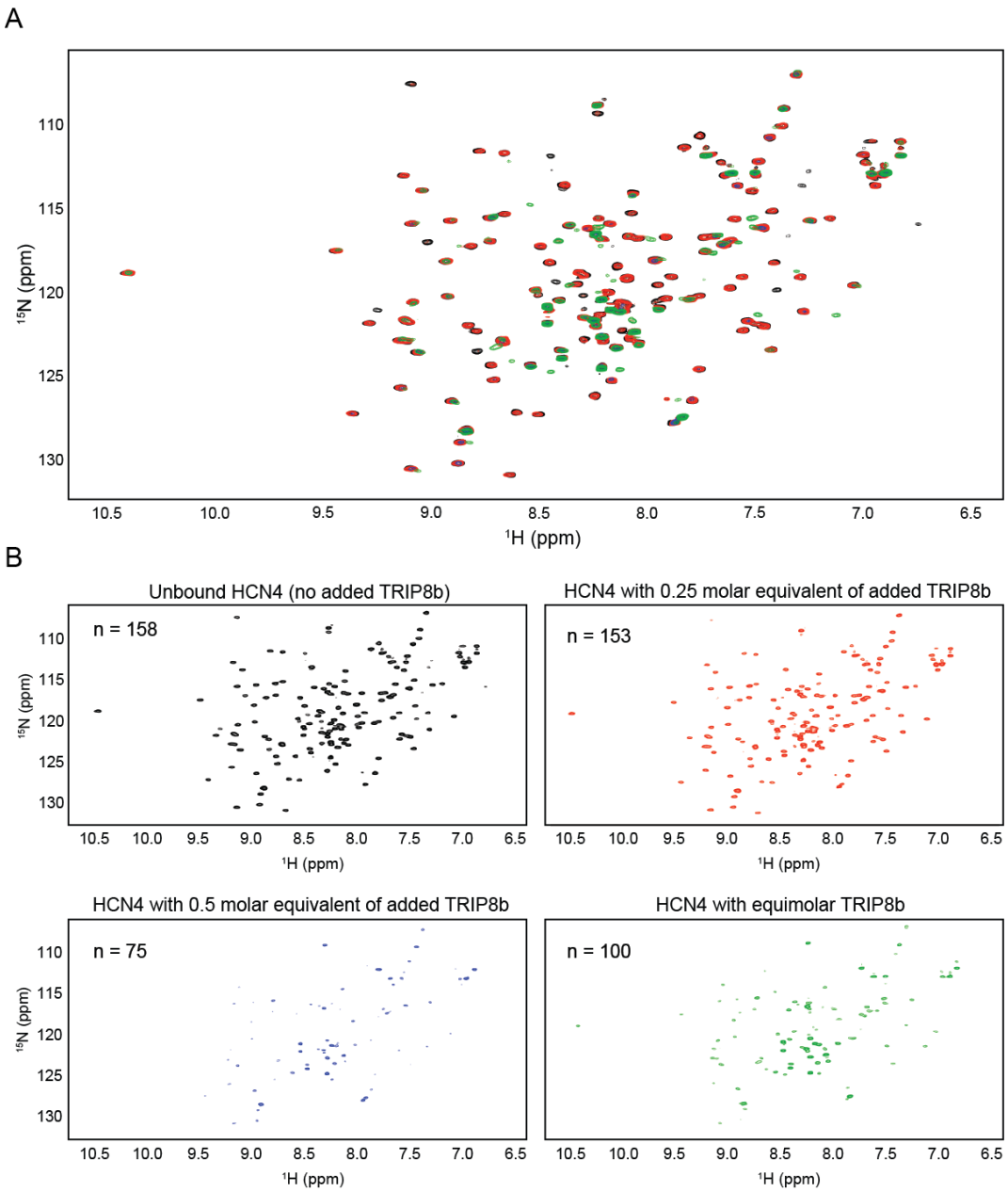


Figure S6, Related to Figure 7: A) Spectral overlay showing HCN4-CNBDxt under increasing concentrations of TRIP8b$_{core}$. Unbound 100 μM HCN4-CNBDxt is shown in black, while spectra of HCN4-CNBDxt in the presence of 25 μM, 50 μM, and 100μM TRIP8b$_{core}$ are shown in red, blue, and green, respectively. B) Each spectrum displayed individually, highlighting the loss of signal intensity characteristic of slow-intermediate exchange. A number indicating the observable peak count is shown with each spectrum.