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Supplemental Information

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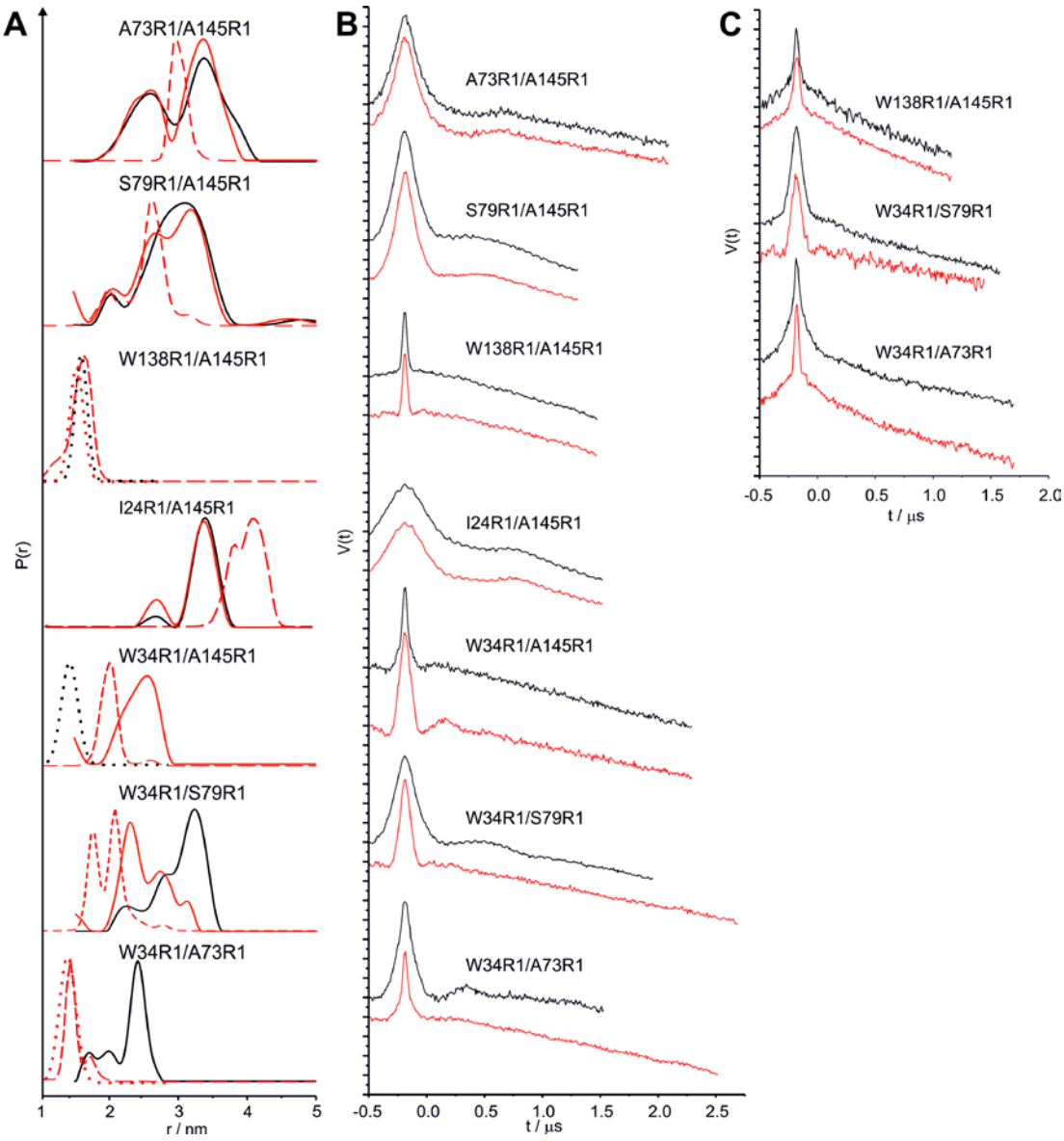
Inventory of Supplemental Information

Supplemental data



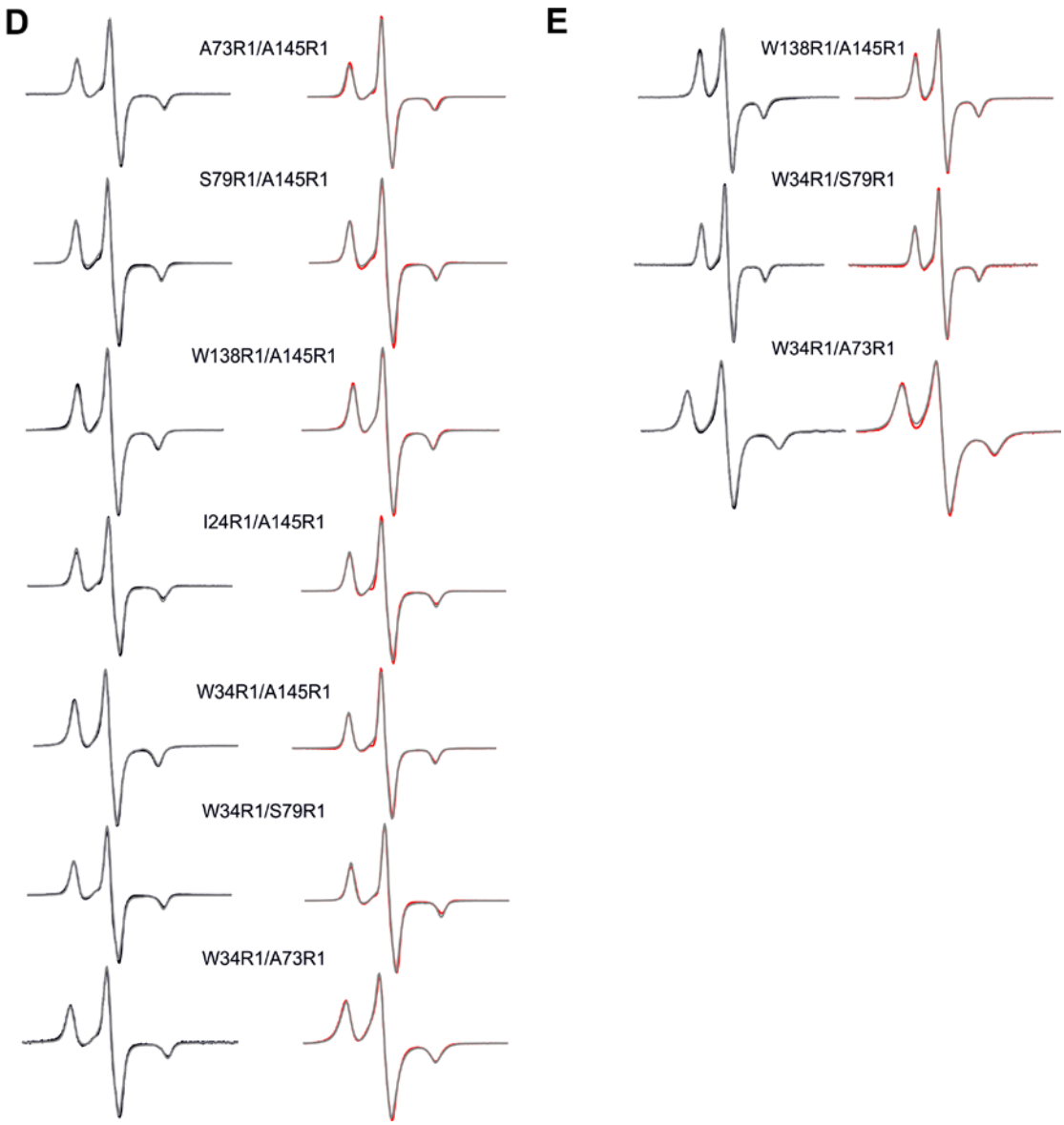


Fig. S1 related to Figure 2. Experimental raw data and data analysis of cw and pulse EPR data. (A) Interspin distance distributions obtained from DEER (P(r), solid lines) (Pannier et al., 2000) or cw EPR (dotted lines) measurements (Steinhoff et al., 1997) in the absence (black lines) and presence of thiamin (red lines) compared to simulated distance distributions obtained from the rotamer library approach (dashed red lines) (Polyhach et al., 2011). Experimentally determined distances were in good agreement with the simulated distances of the labeled protein based on chain A of the crystal structure of thiamin-bound ThiT by Erkens *et al.* (Erkens et al., 2011) except for the ThiT variant spin-labeled at positions 73 and 145. Here, the bimodal experimental distance distribution was not reproduced by the simulated distance distribution. The experimental result indicates either the presence of two conformers of the spin label side chain or of two ThiT conformations. Since a bimodal distance

distribution was not observed for ThiT variants labeled at positions 34 and 73 or 138 and 145 it is likely that there were two conformers of the spin label side chain. (B) Experimental primary data of DEER experiments in the absence (black lines) and presence of thiamin (red lines) for ThiT variants in detergent. (C) Experimental primary data of DEER experiments in the absence (black lines) and presence of thiamin (red lines) for ThiT variants reconstituted in liposomes. (D-E) Experimental low temperature cw EPR spectra in the absence (black lines) and presence of thiamin (red lines) and simulated EPR spectra (grey lines) using the program DipFit (Steinhoff et al., 1997) in detergent (D) and reconstituted in liposomes (E).

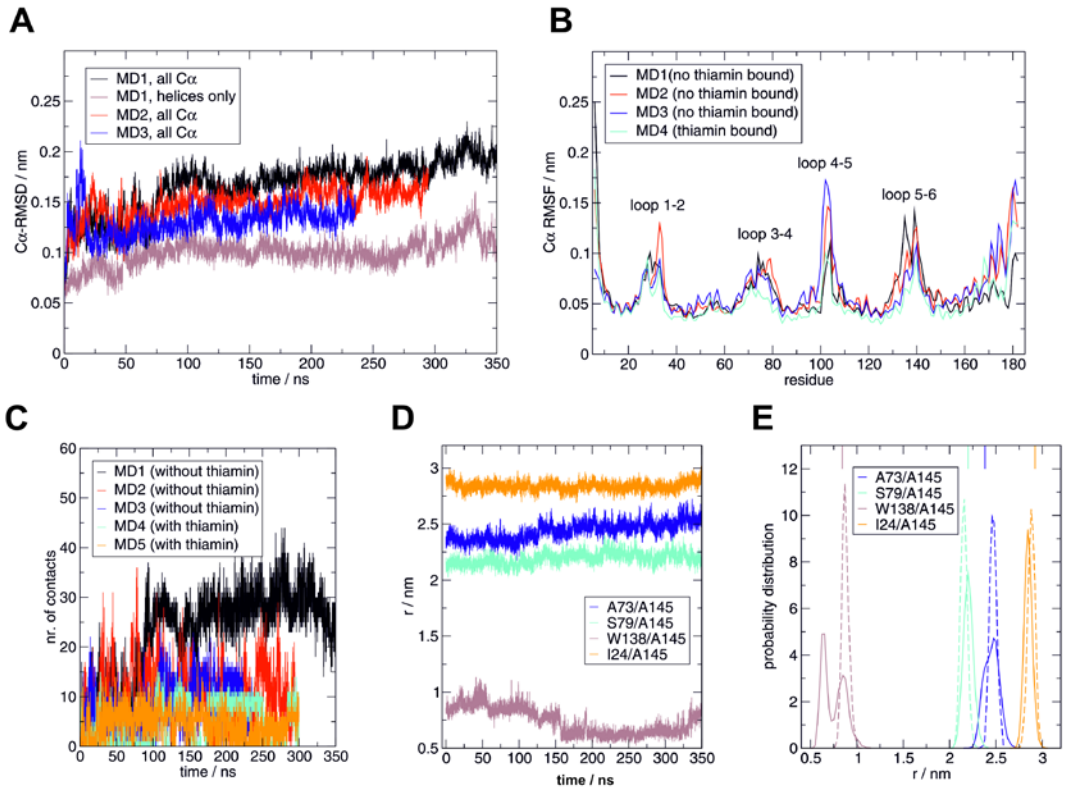


Fig. S2 related Figure 3.

(A) **RMS deviation of C_α-atoms with respect to the starting structure**. The rms deviations during the three MD simulations of *apo*-ThiT, MD1, MD2, and MD3 are shown in black, red, and blue, respectively. For MD1, the curves are shown for the entire protein (black) and for the α-helical scaffold only (brown). Similar curves were obtained from the two simulations of the thiamin-bound form, i.e., C_α-rmsd plateauing at ca. 0.15 nm after 300 ns (not shown for clarity).

(B) **RMS fluctuations of C_α-atoms, averaged over the last 100 ns of the respective MD simulations.**

(C) **Time evolution of the number of contacts between W34 and POPC molecules.** A contact was counted if the distance between the backbone N-atom of W34 and any POPC atom was smaller than 0.8 nm. Although W34 establishes more contacts with POPC during simulation MD1 (black curve) as compared to MD2 (red) and MD3 (blue), the number of W34-POPC contacts is significantly higher in all three simulations of *apo*-ThiT than for simulations MD4 and MD5 of thiamin-bound ThiT (cyan and orange, respectively). The average

number of W34-POPC contacts is 22.6, 10.5, and 8.0 in MD1, MD2, and MD3, respectively, as compared to 4.4 and 4.5 in MD4 and MD5, respectively.

(D) and (E). Time-traces of the distances between the centers of mass (D) and distributions (E) of residue pairs A73/A145 (black), S79/A145 (cyan), W138/A145 (brown), and I24/A145 (orange). Distance distributions represented by solid lines were obtained from MD simulations of the *apo*-ThiT, whereas the dashed curves show the distributions for the thiamin-bound form. Solid lines at the top (D) indicate the respective distances in the X-ray crystal structure.

Table SI related to Figure 1. Thiamin dissociation constants (k_d) of spin-labeled ThiT variants determined by fluorescence titration assays (Erkens and Slotboom, 2010).

Variant	k_d **(nM)**
WT	0.12
A73R1/A145R1	1.85
S79R1/A145R1	0.15
W138R1/A145R1	0.15
W34R1/S79R1	1.10[a]
W34R1/A145R1	0.26
W34R1/A73R1	5.45[a]
I24R1/A145R1	0.15

[a] For the variants W34R1/S79R1, A34R1/A73R1 the dissociation constants were determined for the unlabeled mutants since no fluorescence changes could be detected in the labeled state. For EPR measurements of thiamin-bound ThiT these mutants were spin-labeled after thiamin binding. In all other cases the dissociation constants were determined for the spin-labeled mutants.

Supplemental references:

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