Supporting Material

Induced Conformational Changes in the Activation of the *Pseudomonas aeruginosa* type III Toxin, ExoU

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Supporting information

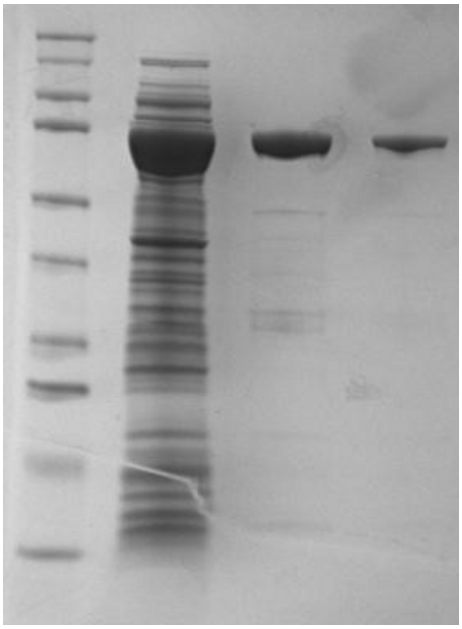

Figure S1. SDS-PAGE analysis showing the progression of ExoU purification. From left to right are (1) molecular weight standards, (2) crude extract, (3) cobalt column eluate, and (4) purified final product separated on a 12% polyacrylamide gel (BioRad) and stained with Coomassie blue. ExoU migrates just below the 74 kD standard. Comparable results were observed for all of the mutants described in this study.

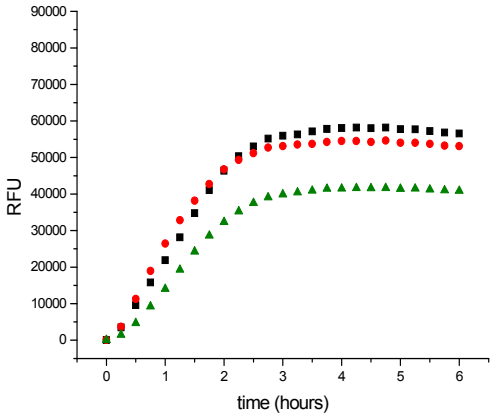

Figure S2. PED6 fluorescence activity assay comparing wt ExoU (black squares), S643C (red circles), and S137C (green triangles). Results are from one of several comparable experiments. RFU, relative fluorescence units. Relative activities given in the text are based on the maximum slopes.

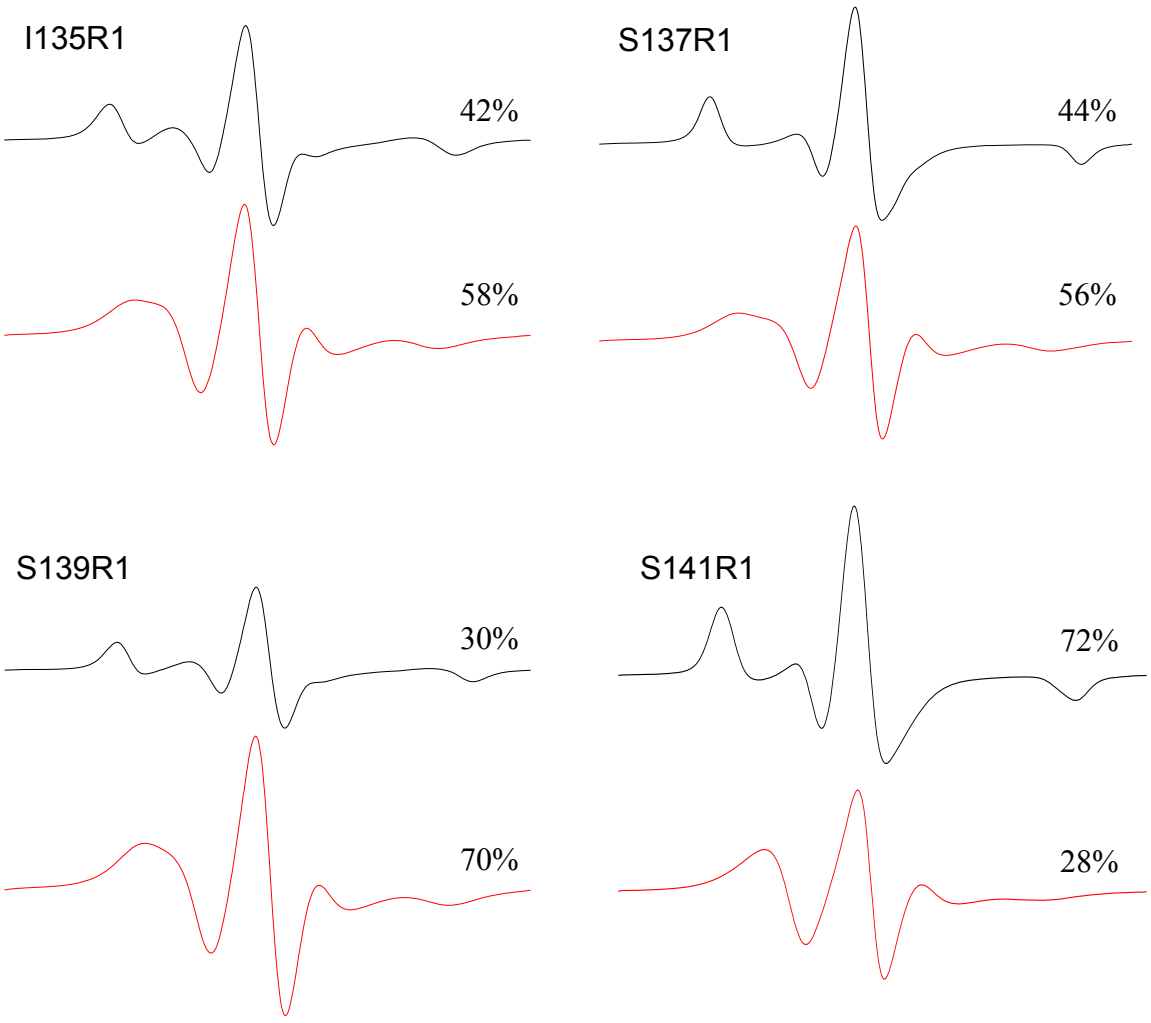

Figure S3. Individual components from two-component fits corresponding to the more immobile (black) and more mobile (red) states of the spin label side chain for labeling sites near catalytic residue S142.

Table S1. Motional parameters for labeling sites near catalytic residue S142.

	$2A_{zz}'$ (G)	$\tau_c(imm)$	$S_{(imm)}$	$\tau_c(m)$	$S_{(mob)}$
I135R1	65.3	12 ns	0.78	4.1 ns	0.23
S137R1	68.2	19 ns	0.85	3.9 ns	0.27
S139R1	66.5	12 ns	0.85	4.0 ns	0.24
S141R1	66.8	7.9 ns	0.85	3.6 ns	0

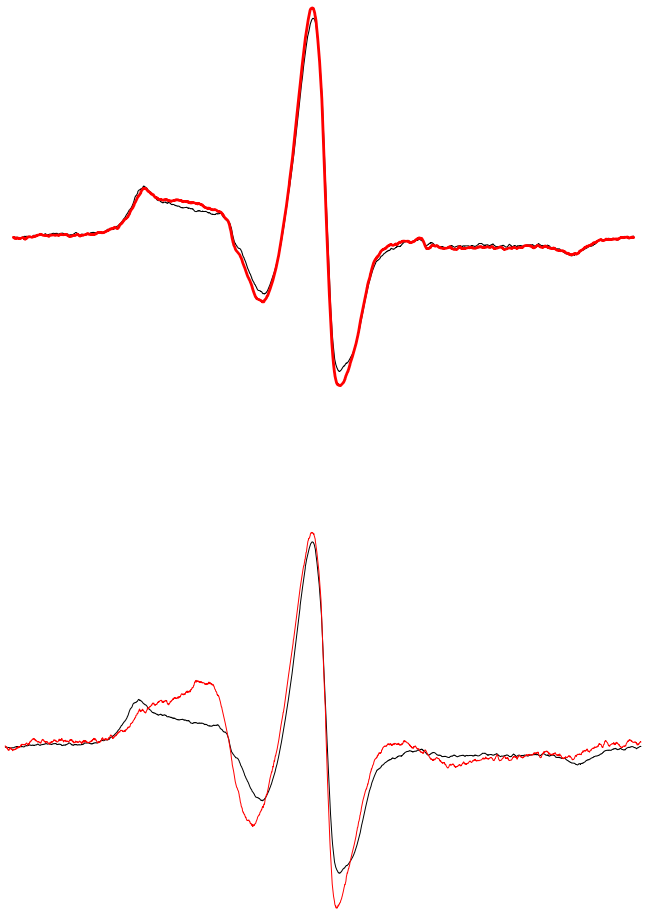

Figure S4. (Top) Overlay of EPR spectra for S137R1 in buffer (black) and in the presence of POPC:POPS (1:1) liposomes (red), showing a very slight increase in the more mobile spin population upon liposome addition. (Bottom) Overlay of EPR spectra for S137R1 in buffer (black) and in the presence of liposomes and SOD1 (molar ratio SOD:ExoU = 22:1) showing a substantial change in the mobility of the R1 side chain. Scan widths 100 G. Spectra are normalized to the same integrated intensity.

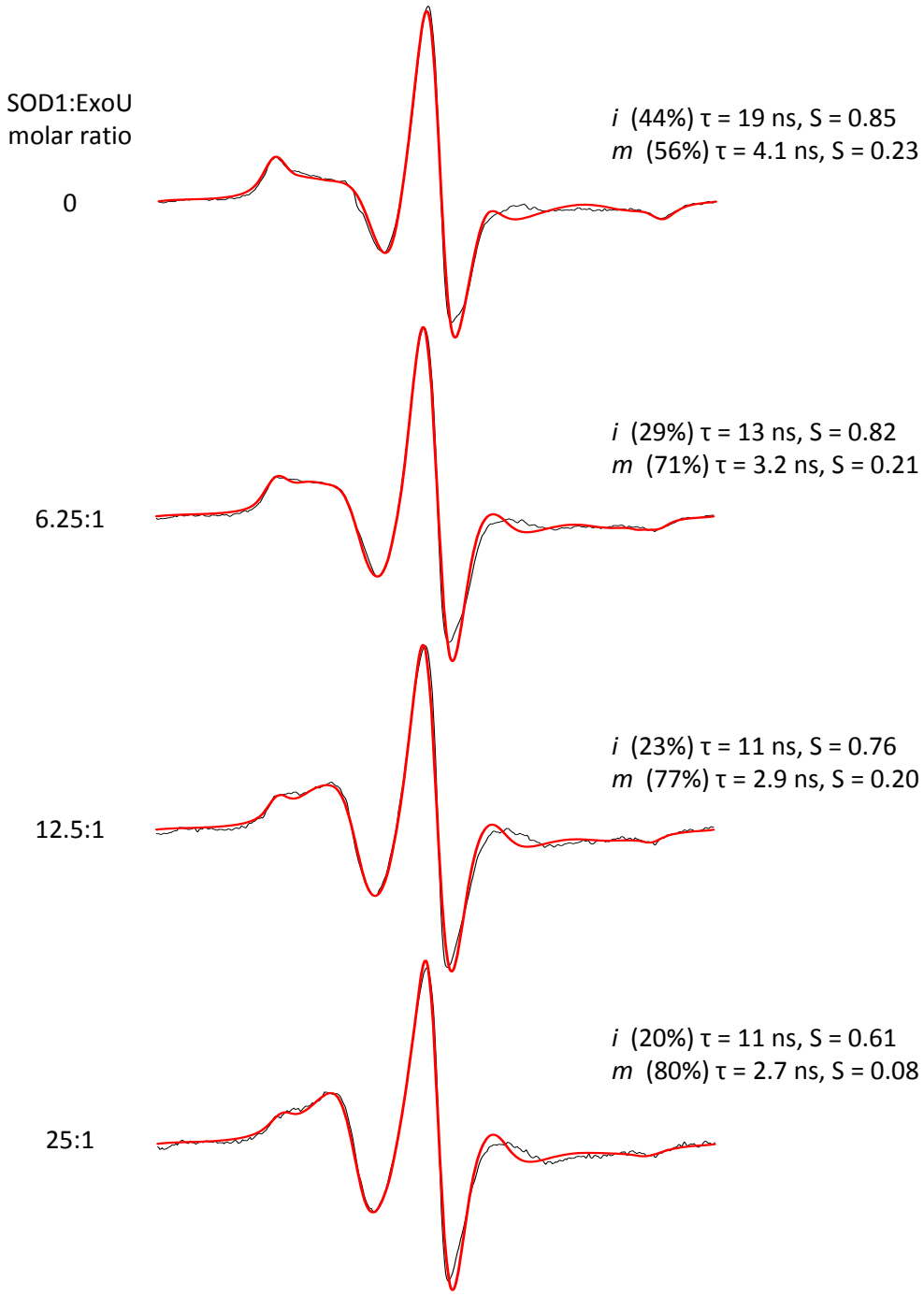

Figure S5. Experimental spectra (black) and two-component fits (red), showing the effects of SOD1 and substrate liposomes on S137R1. Samples also contained POPC:POPS (1:1) liposomes at a lipid:ExoU ratio of 40:1.

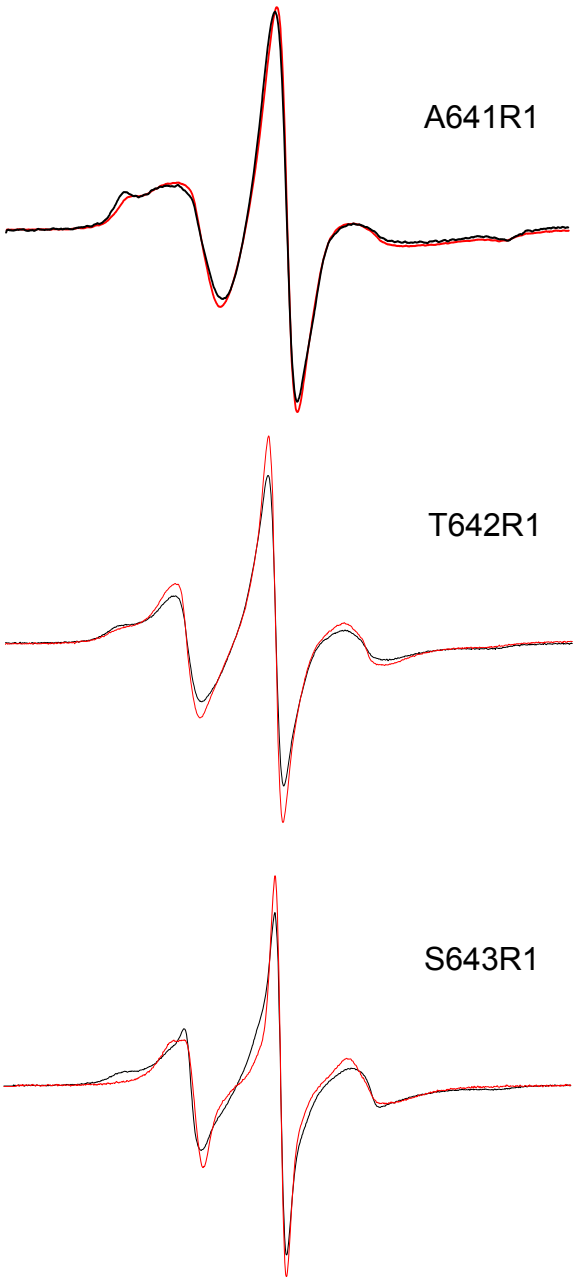

Figure S6. SOD1-induced conformational changes in the C-terminal domain. EPR spectra of A641R1 (top), T642R1 (middle), and S643R1 (bottom) in buffer (black) and in the presence of SOD1 (red). The molar ratio of SOD1:ExoU was 15:1. Spectra were normalized to the same integrated intensity. Scan widths 100 G.

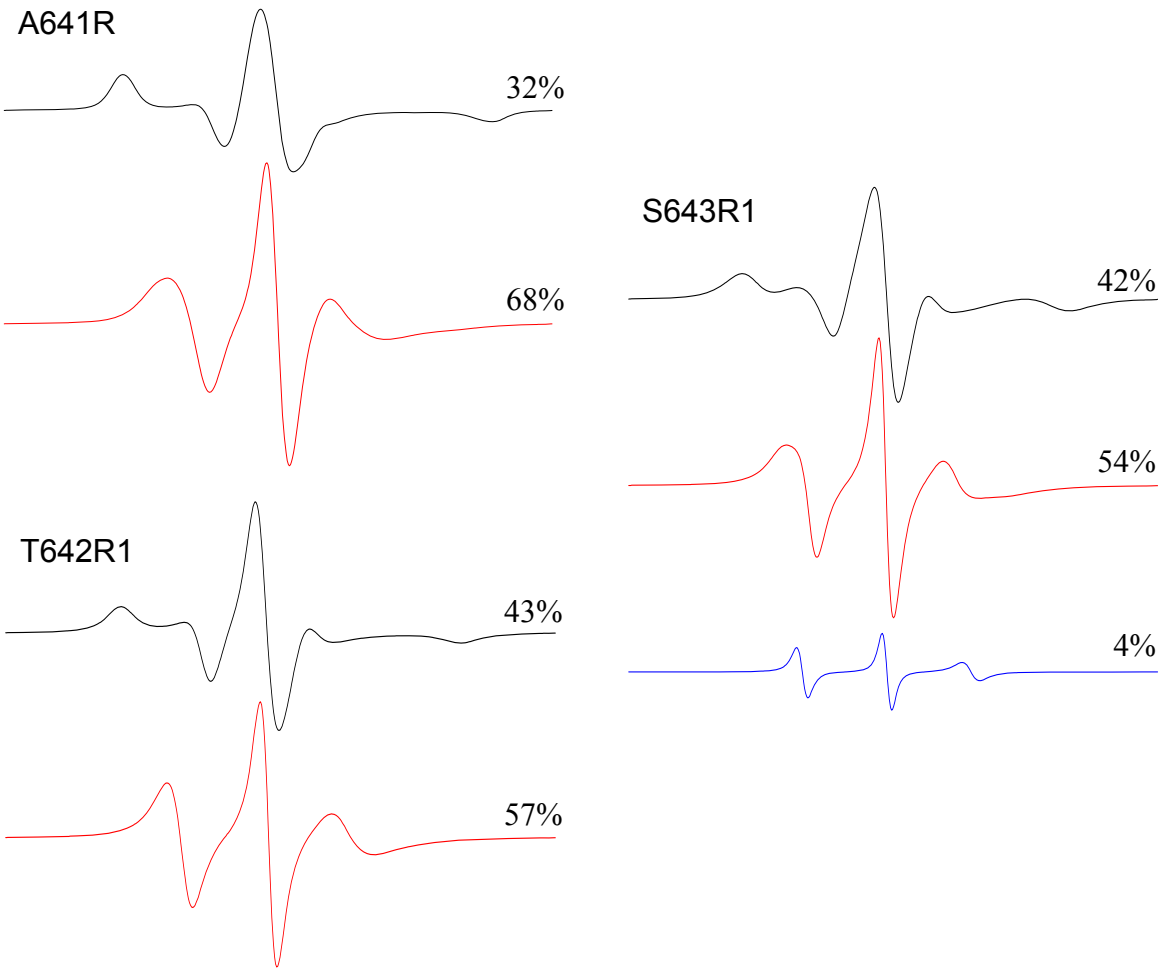

Figure S7. Individual components from multi-component fits corresponding to the more immobile (black) and more mobile (red) states of the spin label side chain at labeling sites in the C-terminal domain. Fitting of S643R1 required incorporation of a small population (< 5%) of highly mobile spin labels (blue).

Table S2. Motional parameters for labeling sites in the C-terminal domain.

	$2A_{zz}'$ (G)	$\tau_c(imm)$	$S_{(imm)}$	$\tau_c(mob)$	$S_{(mob)}$
A641R1	65.2	10 ns	0.48	3.4 ns	0.20
T642R1	63.9	4.5 ns	0.58	1.4 ns	0.32
S643R1[a]	64.7	5.8 ns	0.40	1.6 ns	0.36

[a] Fit with an isotropic third component, τ_c = 0.66 ns (see Figure S7).

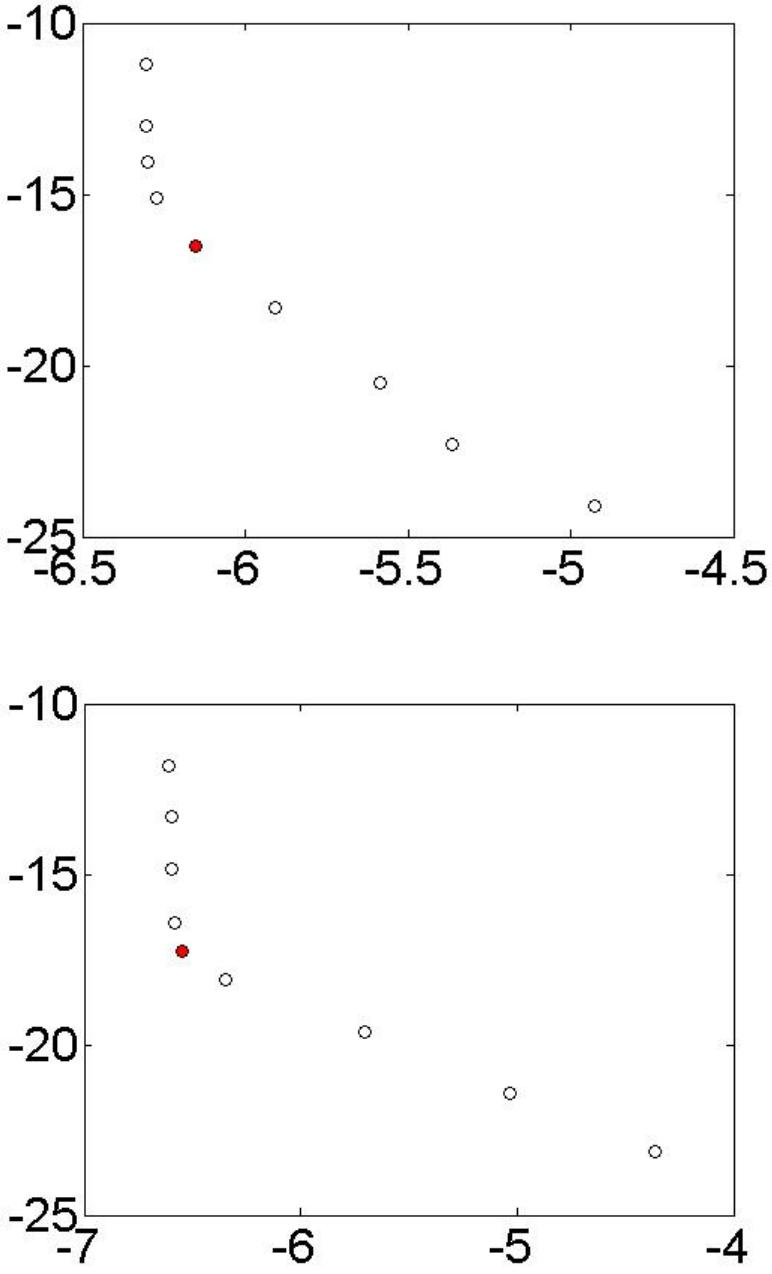

Figure S8. L curves for S137R1-S643R1 buffer control (top) and in the presence of SOD1 (bottom). The points highlighted in red correspond to α = 10.

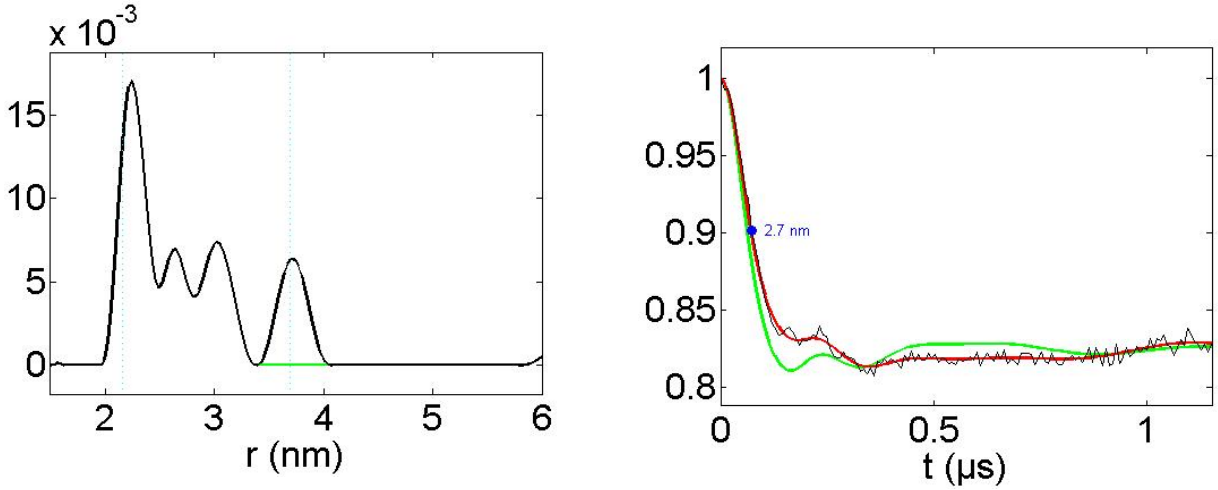

Figure S9. Effect of suppressing the 3.8 nm component in analysis of the S137R1-S643R1 control dipolar evolution. (Left) Distance distribution. The green line indicates that distances between 3.4 and 4.5 nm were suppressed. (Right) Tikhonov regularization analysis of the background corrected dipolar evolution. The black line is the experimental data, the red line is the best fit for all distances between 1.5 and 6.0 nm, and the green line is the fit if distances between 3.4 and 4.5 are suppressed. The fit is well outside the noise level of the experimental spectrum if the 3.8 nm peak is suppressed.

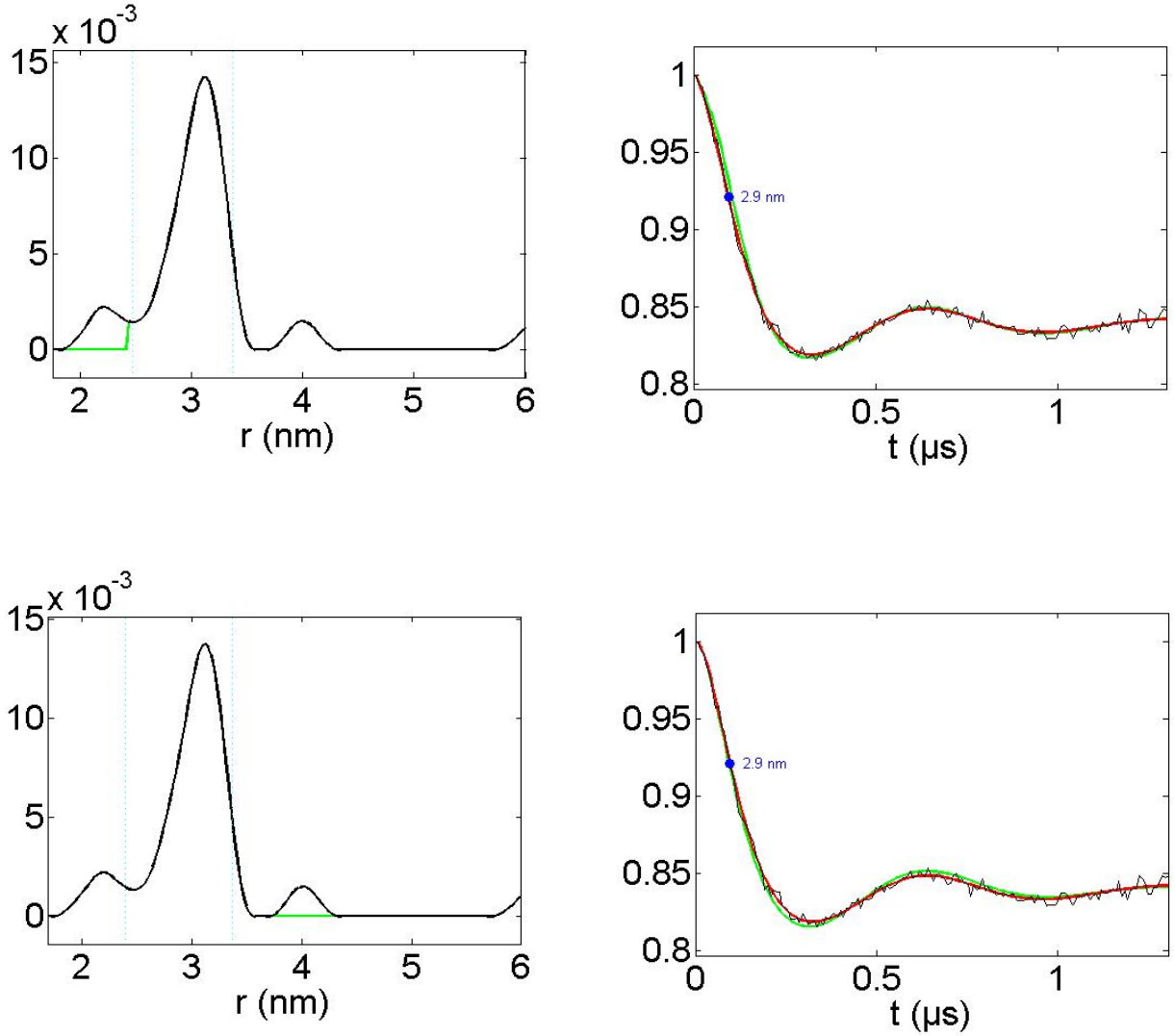

Figure S10. Effect of suppressing minor components in analysis of the dipolar evolution curve for S137R1-S643R1 in the presence of SOD1. In the upper two panels, distances between 1.7 nm and 2.4 nm are suppressed. In the lower panels, distances between 3.6 and 4.5 nm are suppressed. (Left) Distance distributions. The green lines indicate the distance range suppressed. (Right) Tikhonov regularization analysis of the background corrected dipolar evolution. The black line is the experimental data, the red line is the best fit for all distances between 1.5 and 6.0 nm, and the green line is the fit if the indicated distances are suppressed. In both cases, the fits with suppressed distances fall within the noise of the experimental data. Consequently the presence of these two minor components should be considered questionable.