Supporting Information

Enzymatic Spin Labeling of Protein N- and C-Termini for Electron Paramagnetic Resonance Spectroscopy

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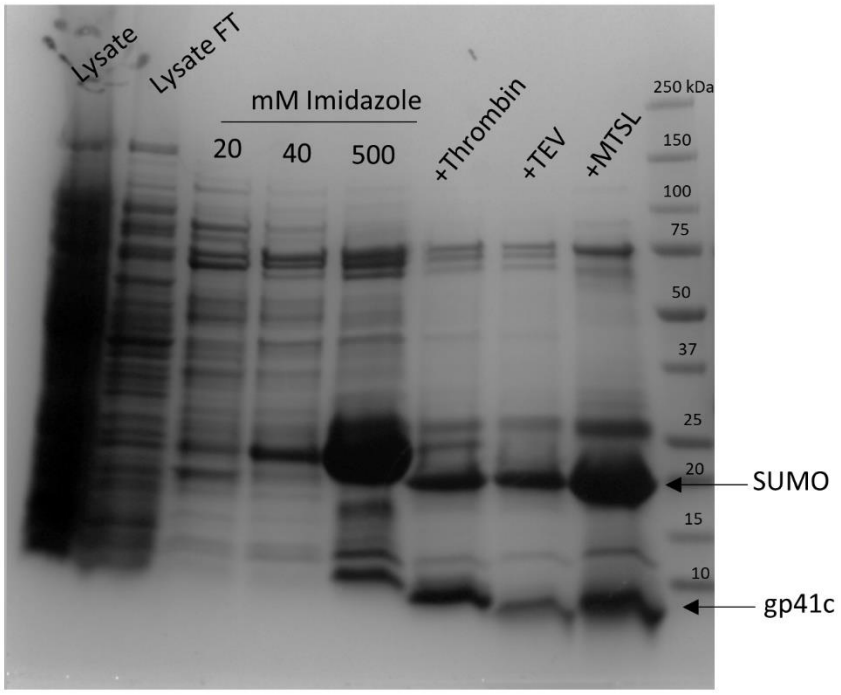
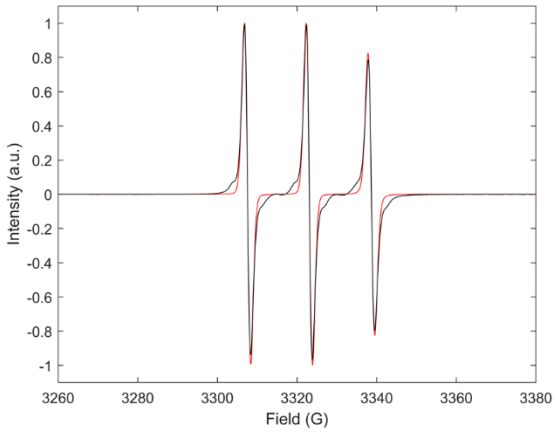


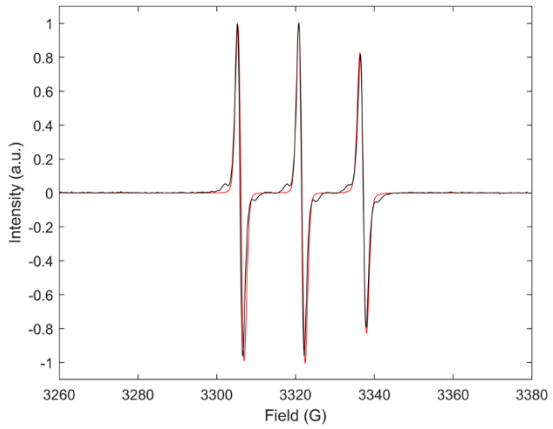
Figure S1. Purification of His-TEV-gp41c-SSSDVC-Thrombin-SUMO

R1-CNGL



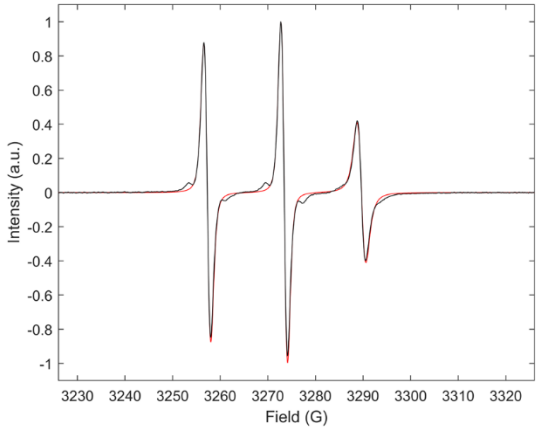
A (MHz)	[2.7453 18.6602 109.5000]
g	[2.00692, 2.00626, 2.00079]
l_w (mT)	0.1841
τ_c	51 ps

GGGGC-R1



A (MHz)	[2.9578 18.5552 109.116]
g	[2.00667, 2.00582, 2.0009]
l_w (mT)	0.1818
τ_c	53 ps

gp41c-SSSDVC-R1



A (MHz)	[1.9604 22.5518 111.8470]
g	[2.00664, 2.00830, 2.00182]
l_w (mT)	0.1331
τ_c	255 ps

Figure S 2: Fitting of CW Spectra using EasySpin. Experimental spectra are shown in black, with fits in red. A represents the principal values of the hyperfine interaction tensor, g represents the principal values of the g tensor, l_W gives the FWHM of the Gaussian broadened linewidth and T_C is the rotational correlation time.

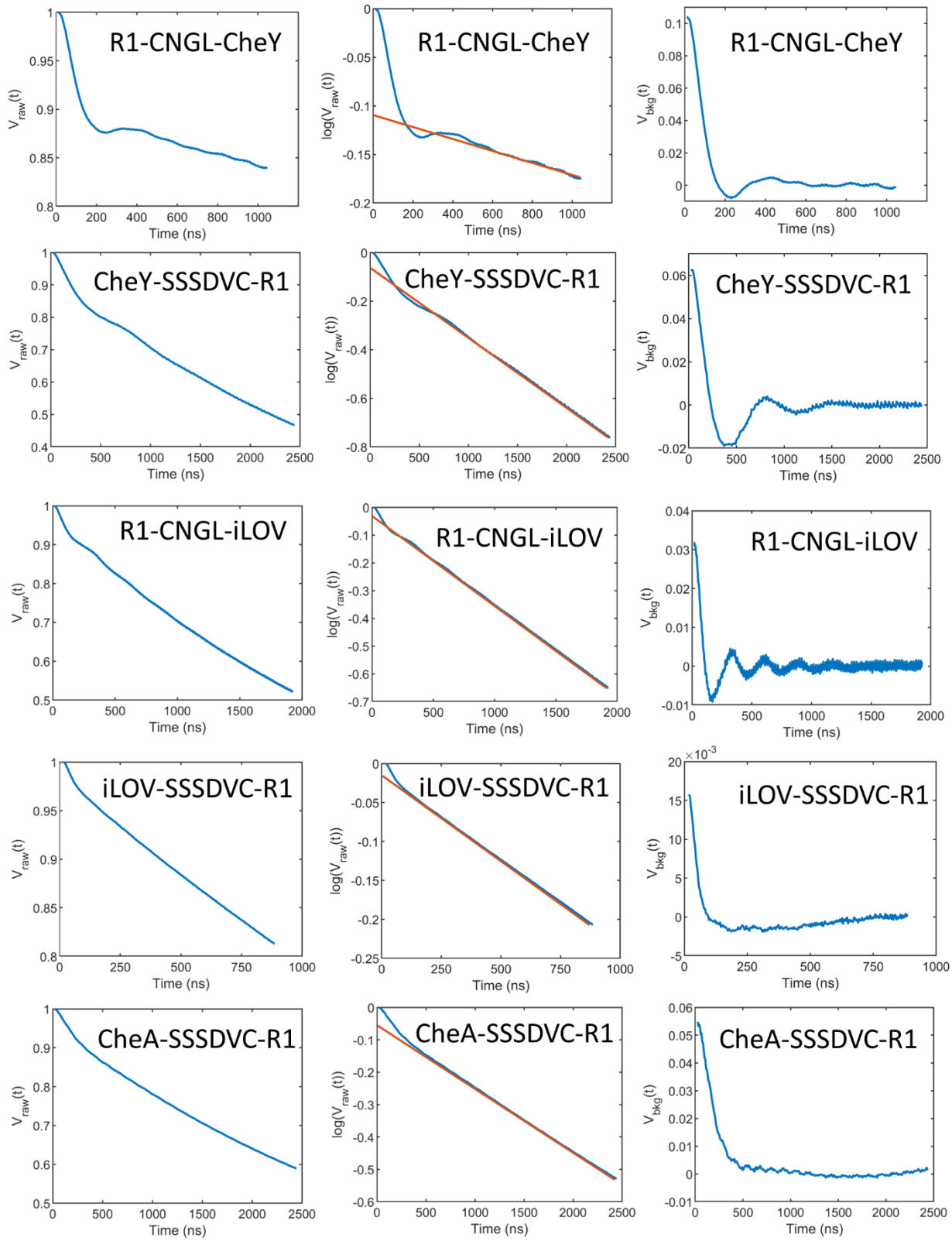

R1-CNGL-CheY
R1-CNGL-CheY
R1-CNGL-CheY
CheY-SSSDVC-R1
CheY-SSSDVC-R1
CheY-SSSDVC-R1
R1-CNGL-iLOV
R1-CNGL-iLOV
R1-CNGL-iLOV
iLOV-SSSDVC-R1
iLOV-SSSDVC-R1
iLOV-SSSDVC-R1
CheA-SSSDVC-R1
CheA-SSSDVC-R1
CheA-SSSDVC-R1
Time (ns)
V_raw(t)
log(V_raw(t))
V_bkg(t)
×10^-3

Figure S 3. Background Subtraction for Select DEER Spectra. For iLOV samples, higher background was observed likely due to blue light irradiation reducing a significant portion of nitroxides present in the sample. Additionally, we have observed increased background for NSQ radicals measuring at 60 K (T_2 maximum ~150 K). For intein labelled samples, background is due to free gp41c-SSSDVC-R1 in solution; low molecular weight proteins (10-15 kDa) are difficult to separate from gp41c-SSSDVC-R1 in size exclusion chromatography.

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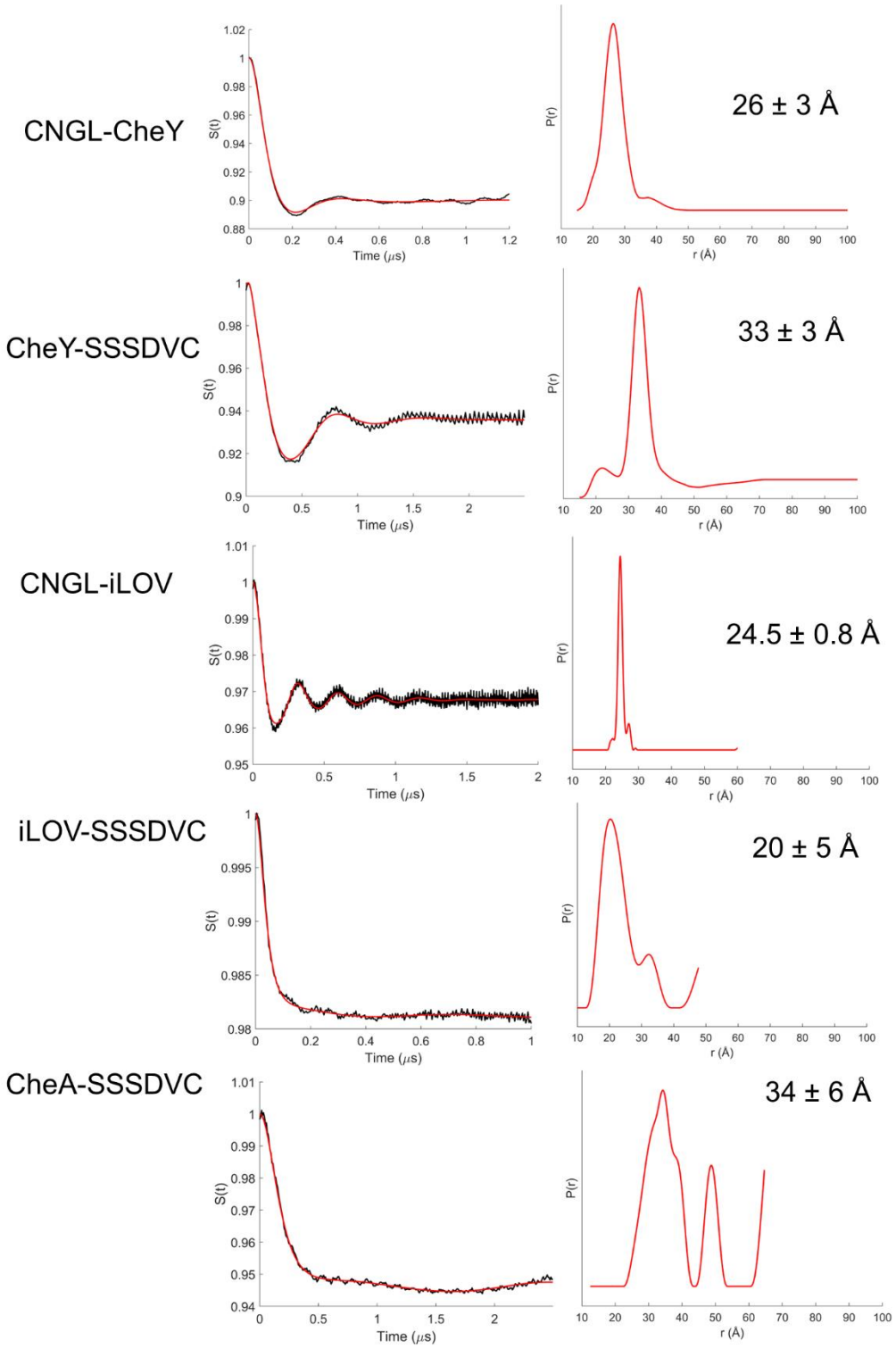

Figure S 4. DEER Spectra of select samples analyzed using DEER Analysis with Tikhonov Regularization. For all samples, a homogeneous (3 dimension) background subtraction was used. The regularization parameter α was chosen using the AIC criteria option in DEER Analysis.

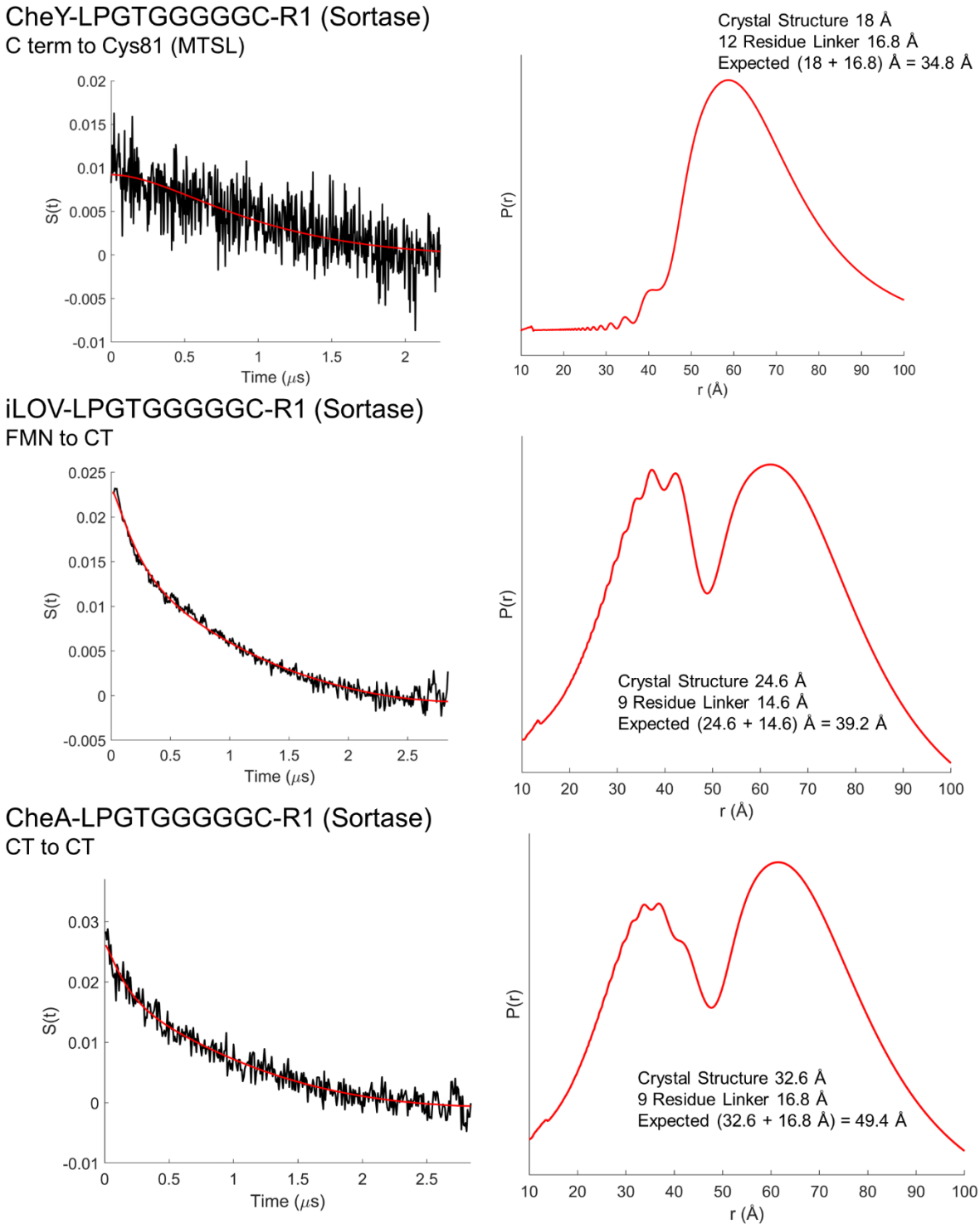

Figure S 5: CheY/iLOV/CheA DEER with C Terminal Sortase Linkers

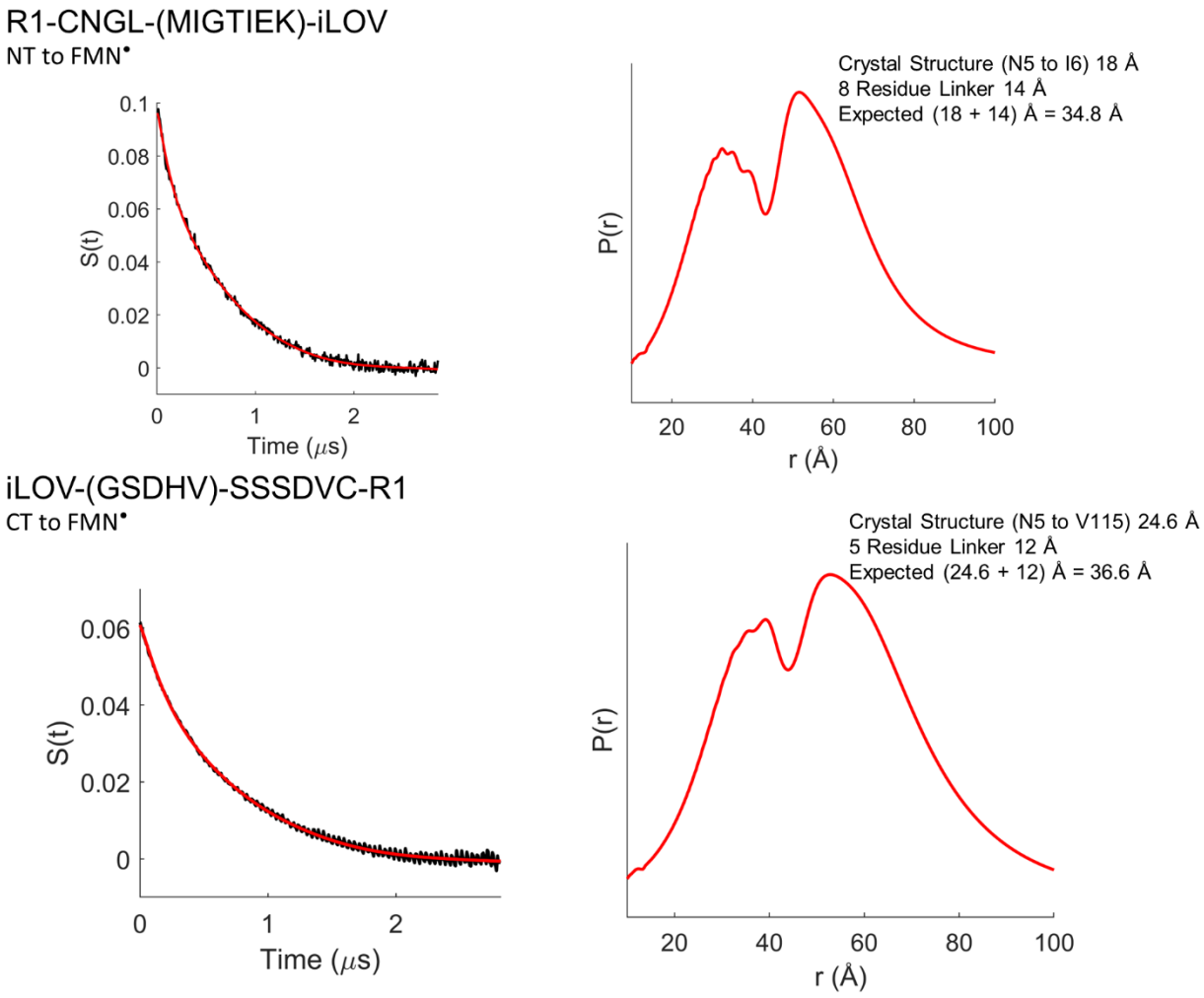

Figure S 6. DEER spectroscopy of iLOV with Extended Linkers. Residues indicated in parenthesis have been added compared to the samples shown in Figure 4.

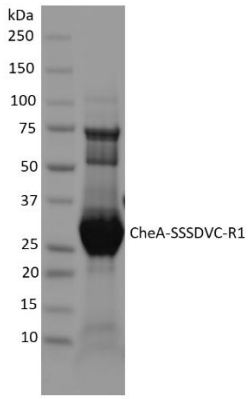

Figure S 7. Nonreducing SDS-PAGE of CheA-SSDVCG (28kDa).

DNA Sequences of Constructs

OaAEP1 (C243A) TEV-His in pET28a

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GGTGGACAGCAAATGGGTCGCGGATCCGCTCGCGATGGTGATTATCTGCATCTGCCGAGCGAAGTGAGCCGTTTC
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His-TEV-gp41c-SSSDVCG-Thrombin-SUMO in pET28a

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His-TEV-L-CheY-gp41n in pET28a

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His-iLOV(Q489D)-gp41n in pET28a

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His-TEV-L-iLOV(Q489D)-gp41n in pET28a

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His-TEV-L-CheA-GP41N

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His-TEV-L-CheA-LPGTGGS

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