Electronic Supplementary Material (ESI) for Molecular BioSystems

Electron spin labeling reveals the highly dynamic N-terminal arms of the SOS mutagenesis protein UmuD

Electronic Supplementary Information

Jaylene N. Ollivierre, David E. Budil, Penny J. Beuning

Department of Chemistry & Chemical Biology, Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston

Protein purification, labeling and analysis. Wild-type UmuD, UmuD-S60A, UmuD3A, and UmuD′-A31C were purified following a reported procedure.[1] The A31C variant of UmuD′ was constructed using a QuikChange site-directed mutagenesis kit (Agilent). Protein with a monomer concentration of 300 μM was utilized for EPR. $UmuD_2$ protein samples (95% purity) were reacted with a thiol-reactive nitroxide derivative, 3-iodomethyl-1-oxy-2,2,5,5-tetramethylpyrroline (iodomethyl spin label, IMSL) for site-directed spin labeling. This was accomplished by adding an appropriate amount of the 100 mM stock solution of spin label to achieve a molar ratio of 1:3 protein to spin label. The mixture was incubated at 37 ºC for 4 hours and then maintained at 4 ºC overnight. The labeled protein was then dialyzed against exchange buffer [25 mM HEPES (pH 7.5), 150 mM NaCl, 0.1 mM EDTA, 5% glycerol] for at least 24 hours using a Slide-A-Lyzer Dialysis Cassette (Thermo Scientific) with a 3500-Da cutoff membrane. Zeba 7K desalting spin columns (0.5 mL) (Thermo Scientific) were then used to remove residual DTT, and the labeled protein was subsequently stored at –80 ºC. Protein samples were analyzed by using a Bruker EMX instrument outfitted with a high-sensitivity cylindrical cavity and variable-temperature module at 24 ºC (297 K) unless noted otherwise, and the temperature was controlled to within 0.3 ºC by a nitrogen stream. The spectra were obtained at a microwave frequency of 9.37 GHz, 6.0 mW microwave power and 0.5 G 100 kHz field modulation amplitude.

Lineshape fitting procedures and best-fit parameters. The LabView-based multicomponent lineshape fitting program of Altenbach[2] was used to analyze the spectra. For the fitting procedure, the magnetic parameters of the nitroxide label were set as follows: the electronic g tensor components were fixed at g_x = 2.0081, g_y = 2.0059, and g_z = 2.0023, and the components of the ^{14}N hyperfine tensor were fixed at A_x/γ_e = 6.6 G, A_y/γ_e = 6.9 G, and A_z/γ_e = 35.1 G, where γ_e is the electronic gyromagnetic ratio.[3] To model the magnetic properties of the remaining nuclei, the calculated spectra were convoluted with a superhyperfine pattern consisting of 12 equivalent ^1H nuclei with isotropic coupling a_H/γ_e = 0.25 G, one ^1H nucleus with isotropic coupling a_H/γ_e = 0.40 G, and a ^{13}C nucleus with isotropic coupling a_C/γ_e = 6.90 G at one of the two carbon atoms alpha to the nitroxide at natural abundance (1.108%).

Except as noted below, each spectrum was fitted using three components, since three species with significantly different mobilities could be visually distinguished in most of the spectra, particularly at the lower temperatures studied. The scale factor for each component and the isotropic rotational diffusion constants R_I, R_{II}, and R_{III}, were varied, together with two Gaussian inhomogeneous line width parameters. The first line width parameter, ΔH_I, was applied to the slowest component, while the second ($\Delta H_{II,III}$) was applied to the components with the two highest R values. The least-squares parameters for each protein variant studied are given in Table 1, for UmuD-S60A at each temperature in Table 2, and the fitting results are plotted in Figure 1 below and Figure 2 in the text.

For the UmuD3A and UmuD′-A31C variants, reliable parameters for a slow component could not be obtained under the assumption of a three-component spectrum. The slow component was therefore eliminated from the UmuD3A and UmuD′-A31C variants.

Parameter uncertainties were estimated from the covariance matrix of the parameters at the least-squares solution according to the procedure described by Budil *et al* [4] The uncertainties were similar for a given parameter in different fits, so for clarity, uncertainties are reported as average values for each class of parameter.

Estimation of rotational correlation time for UmuD dimer. The expected order of magnitude for the rotational correlation time of a protein may be estimated from the Debye Stokes Einstein equation,

$$\tau = \frac{4}{3}\frac{\pi r_e^3 \eta}{kT}$$

where r_e is the effective hydrodynamic radius of the protein (including bound waters), k is Boltzmann's constant, and η is the solvent viscosity, which for water is 8.90×10^{-4} Pa s (0.890 cP) at T= 297 K. Approximating the UmuD dimer as a sphere with radius 25 Å, the above equation gives an average rotational correlation time of 14 ns, quite consistent with the observed rotational correlation times of the slowest component in Table 1 and in Table 2 near room temperature.

TABLE 1

Dynamic parameters and scaling factors for the spin-labeled UmuD variants.

Variant	$\log_{10} R_{(I)}$·s[(a)]	$\log_{10} R_{(II)}$·s[(a)]	$\log_{10} R_{(III)}$·s[(a)]	$\tau_{(I)}$ (ns)[(b)]	$\tau_{(II)}$ (ns)[(b)]	$\tau_{(III)}$ (ns)[(b)]	ΔH_I[(c)] (G)	$\Delta H_{II,III}$[(c)] (G)	ϕ_I[(d)]	ϕ_{II}[(d)]	ϕ_{III}[(d)]
WT UmuD	6.99	8.15	9.40	17.1	1.2	0.066	4.01	0.45	0.47	0.30	0.22
UmuD-S60A	7.01	8.07	9.58	16.3	1.4	0.044	3.11	0.59	0.53	0.30	0.17
UmuD3A	--	7.86	8.76		2.3	0.29	--	0.39	--	0.71	0.29
UmuD′-A31C	--	--	9.57			0.045	--	0.49	--	--	1.00

(a) Log_{10} of isotropic diffusion constant R for components I (slow), II (intermediate), and III (fast) expressed in units of s^{-1}. Average uncertainty in these parameters is 0.05.

(b) Average rotational correlation time for each component, calculated as $\tau = 1/6R$

(c) Inhomogeneous Gaussian derivative line peak-to-peak linewidth for the slowest component (ΔH_I) and the fastest two components ($\Delta H_{II,III}$). Average uncertainty in these parameters is 0.1 G.

(d) Normalized fraction of components I (slow), II (intermediate), and III (fast). Average uncertainty in these parameters is 0.07.

TABLE 2

Dynamic parameters and scaling factors for spin-labeled UmuD-S60A as a function of temperature

T (K)	$\log_{10} R_{(I)}\cdot s$ [a]	$\log_{10} R_{(II)}\cdot s$ [a]	$\log_{10} R_{(III)}\cdot s$ [a]	$\tau_{(I)}$ (ns) [b]	$\tau_{(II)}$ (ns) [b]	$\tau_{(III)}$ (ns) [b]	ΔH_I (G) [c]	$\Delta H_{II,III}$ (G) [c]	$\phi_{(I)}$ [d]	$\phi_{(II)}$ [d]	$\phi_{(III)}$ [d]
275	6.19	7.98	9.50	107.6	1.7	0.05	2.88	0.05	0.616	0.321	0.063
283	6.71	8.08	9.17	32.5	1.4	0.11	2.79	0.09	0.617	0.277	0.105
288	6.82	7.92	9.19	25.2	2.0	0.11	1.55	0.17	0.537	0.283	0.180
293	6.97	7.60	9.18	17.9	4.2	0.11	0.00	0.17	0.511	0.298	0.191
297	6.99	7.86	9.26	17.1	2.3	0.09	0.01	0.14	0.500	0.329	0.171
303	7.08	7.86	9.27	13.9	2.3	0.09	0.01	0.14	0.480	0.350	0.170
308	7.14	7.88	9.29	12.1	2.2	0.09	0.14	0.14	0.464	0.366	0.169
313	7.19	7.89	9.26	10.8	2.1	0.09	0.15	0.15	0.437	0.391	0.172
318	7.22	7.93	9.20	10.0	2.0	0.11	0.20	0.20	0.394	0.399	0.206
323	7.28	7.91	9.26	8.7	2.1	0.09	0.16	0.16	0.374	0.443	0.184

(a) Log_{10} of isotropic diffusion constant R for components I (slow), II (intermediate), and III (fast) expressed in units of s^{-1}. Average uncertainty in these parameters is 0.05.

(b) Average rotational correlation time for each component, calculated as $\tau = 1/6R$

(c) Inhomogeneous Gaussian derivative line peak-to-peak linewidth for the slowest component (ΔH_I). and the fastest two components ($\Delta H_{II,III}$). Average uncertainty in these parameters is 0.1 G.

(d) Normalized fraction of components I (slow), II (intermediate), and III (fast). Average uncertainty in these parameters is 0.07. The individual uncertainties in $\phi_{(II)}$ and $\phi_{(III)}$ were propagated to estimate the uncertainties shown in the van't Hoff plot (Figure 3 in the text).

FIGURE 1. Left side: least-squares fits (green lines) to spectra (blue lines) from spin-labeled UmuD-S60A at different temperatures. Right side: Calculated spectra of the slow (green lines) and intermediate motion (blue lines) components from the fits shown on the left. As the temperature increases from 275 K to 323 K, a conversion from the slow component to the intermediate component is observed. Fitting parameters are given in Table 2.

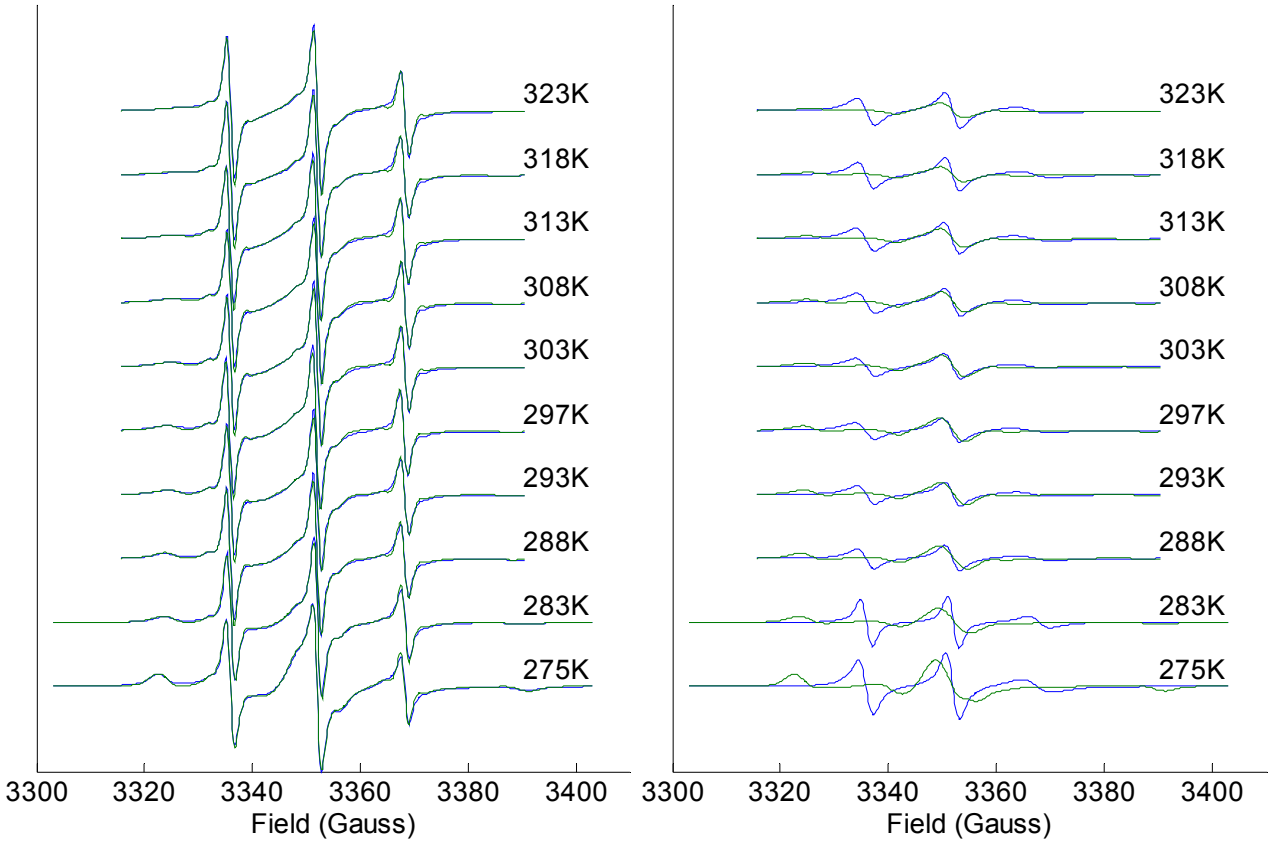


FIGURE 2. Spectrum of free IMSL in aqueous buffer at 297 K (solid line) and least-squares fit to the lineshape (dashed line). The best fit parameters were a_n/γ_e = 16.24 G, ΔH = 0.504 G, and $\log_{10} R{\cdot}s$ = 9.84. Superhyperfine interactions with 13 ^{1}H nuclei and 1 ^{13}C nucleus were included as described in the fitting procedure given above.

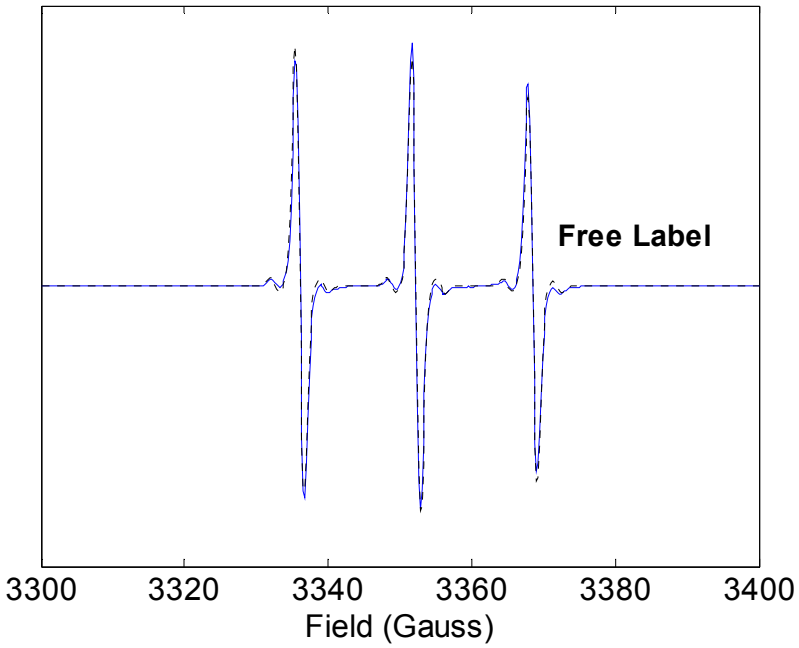
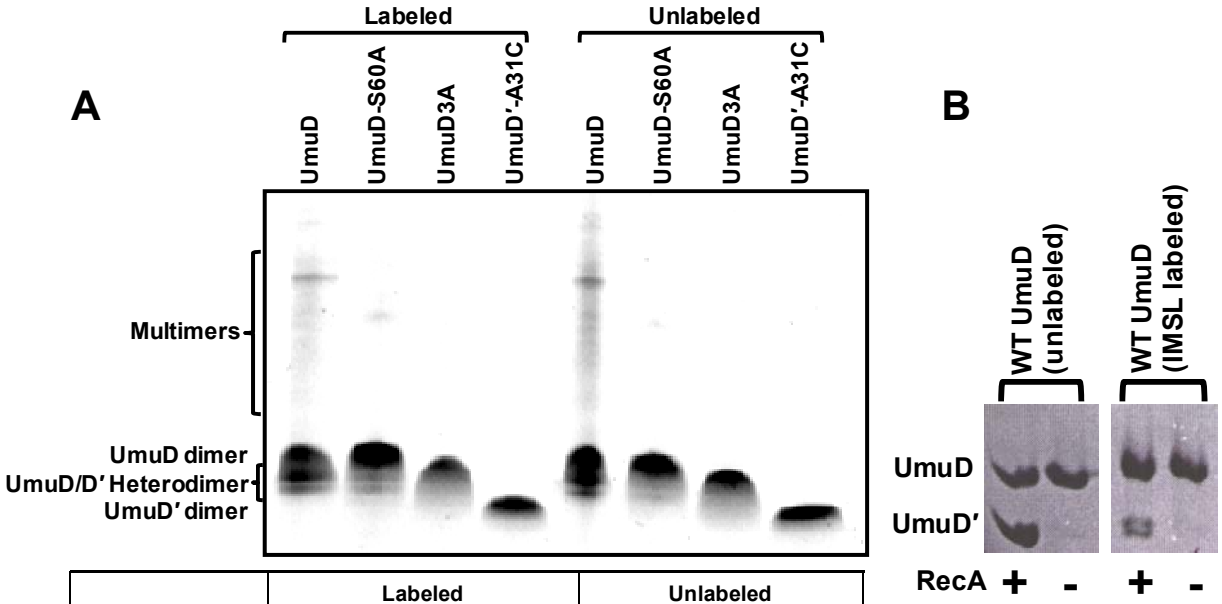
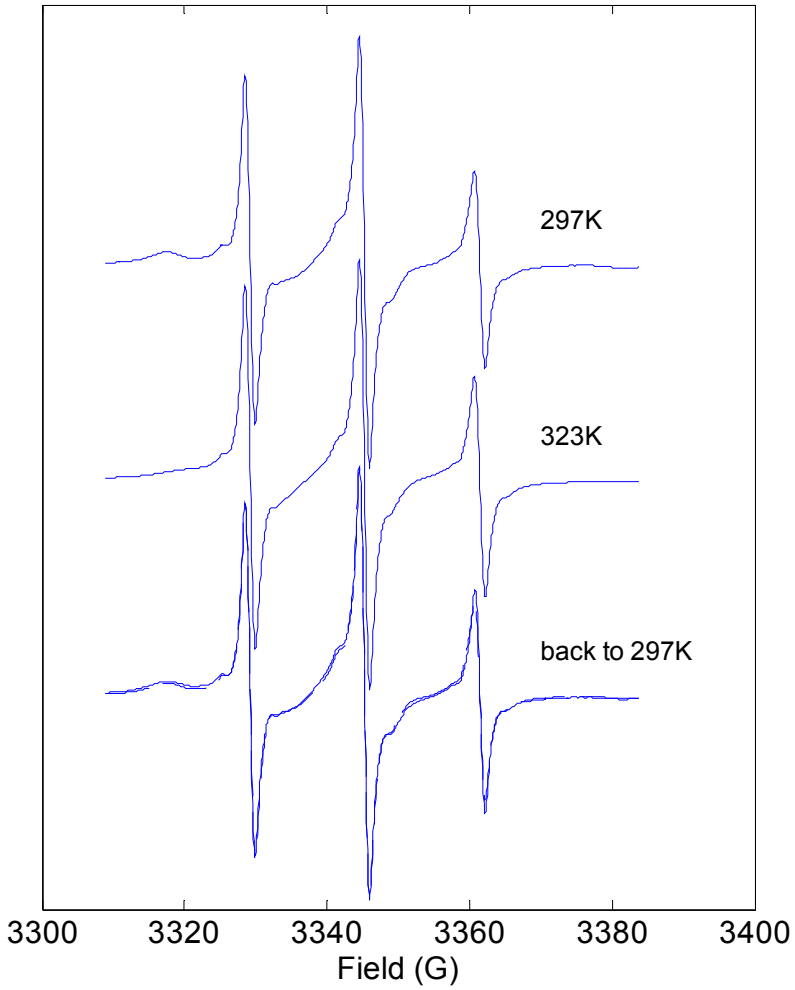


FIGURE 3. A. Native gel showing IMSL-labeled wild-type UmuD, UmuD-S60A, UmuD3A and UmuD′-A31C, compared to their unlabeled counterparts, under non-denaturing conditions.[5] Wild-type UmuD sample contains residual UmuD′ as well as the UmuD/D′ heterodimer, whereas UmuD-S60A contains less of the lower molecular weight species. $UmuD_2$ multimers are also faintly detected. The relative proportions of the different species are given in the table below the gel. Numbers may not sum to 100% due to rounding. B. IMSL-labeled UmuD is cleavable upon incubation with a RecA/ssDNA nucleoprotein filament,[6] albeit with lower efficiency than unlabeled UmuD.



	Labeled				Unlabeled			
	UmuD	UmuD-S60A	UmuD 3A	UmuD′-A31C	UmuD	UmuD-S60A	UmuD 3A	UmuD′-A31C
Multimer (%)	10	4.8			10	5.5		
Dimer (%)	62	78	100	100	58	86	100	100
Heterodimer (%)	28	17			32	8.2		

FIGURE 4. Study of the temperature reversibility of the EPR spectrum of spin-labeled UmuD-S60A. Shown are the initial spectrum at room temperature (top), exhibiting the broad line at low field corresponding to the low-mobility component, the spectrum at 323 K, the highest temperature studied (middle), and recovery of the spectrum upon returning to 297 K (bottom), with the initial spectrum taken at 297 K superimposed as a dashed line.



References

1. P. J. Beuning, S. Chan, L. S. Waters, H. Addepalli, J. N. Ollivierre and G. C. Walker, *J Bacteriol*, 2009, **408**, 318-340.
2. C. Altenbach, LabVIEW programs for the analysis of EPR data. Web. 15 November 2010 <http://sites.google.com/site/altenbach>. 2009.
3. L. Columbus, T. Kalai, J. Jeko, K. Hideg and W. L. Hubbell, *Biochemistry*, 2001, **40**, 3828-3846.
4. D. E. Budil, S. Lee, S. Saxena and J. H. Freed, *J. Magn. Reson., Ser. A*, 1996, **120**, 155-189.
5. J. N. Ollivierre, J. L. Sikora and P. J. Beuning, *J Biol Chem*, 2011, **286**, 3607-3617.
6. P. J. Beuning, S. M. Simon, V. G. Godoy, D. F. Jarosz and G. C. Walker, *Methods Enzymol*, 2006, **408**, 318-340.