Site-Specific Hydration Dynamics in the Nonpolar Core of a Molten Globule by Dynamic Nuclear Polarization of Water

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Supplementary Materials and Methods

EPR simulations. Single- and multiple- component EPR spectra were simulated with the NLSL program assuming a two-component MOMD (microscopic order, macroscopic disorder) model that described the anisotropic motion of the nitroxide (1); the software is available at ftp://ftp.ccmr.cornell.edu/pub/freed. For all simulations, the starting values of the **A** and **g** magnetic tensors were taken as g_{xx} = 2.0078, g_{yy} = 2.0058, g_{zz} = 2.0022, and Ax = 6.2, Ay = 5.9. The Az values (Az in the NLSL software is the same parameter as A_{zz}' in the text) were obtained from spectra recorded in the absence of motion in frozen solutions at -50 ˚C. Az was kept constant during the fitting procedure. The $\log_{10}$ of the three components of the rotational diffusion tensor are given in a modified spherical form as $\langle R \rangle = \frac{1}{3}(R_x+R_y+R_z)$, $N = Rz - \frac{1}{2}(R_x+R_y)$, and $N_{xy} = (R_x-R_y)$, where R_x, R_y, and R_z are the principal components in Cartesian coordinates. The immobile states of E41R1, V66R1 (MG-state), and I142R1 were taken to have isotropic motion with $N=N_{xy}=0$, while the other states have axially symmetric (around the z-axis) anisotropic motion with tilt angles of the diffusion tensor $\alpha_D= 0^\circ, \beta_D= 36^\circ, \gamma_D=0^\circ$, and subject to an ordering potential described by the coefficient C_{20} from which the order parameter is computed. Least-square fits were obtained with $\langle R \rangle$, N and C_{20} as adjustable parameters. After these parameters were optimized, the principal values of the **A** and **g** magnetic tensor (except A_z) were allowed to vary slightly to obtain the final best fit. The effective rotational correlation time for the immobile (τ_i) and mobile (τ_m) components was calculated as $\tau = 1/6\langle R \rangle$, and the order parameter S was computed directly from C_{20} as described (1). A summary of all the above parameters from EPR data fitting is provided in Table S1.

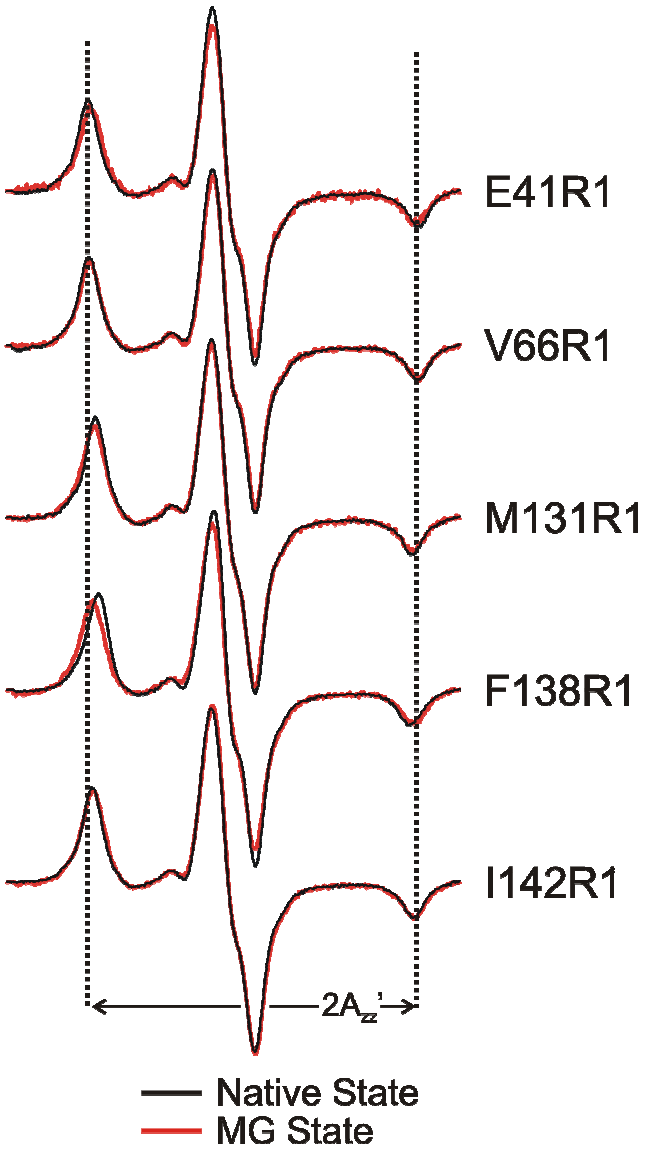

Figure S1. EPR spectra of spin-labeled apoMb variants recorded in the absence of global motions at -50˚C. For each mutant, the spectra of the native and molten globule states are superimposed. The splitting of the resolved hyperfine extrema ($2A_{zz}'$) is labeled in the figure.

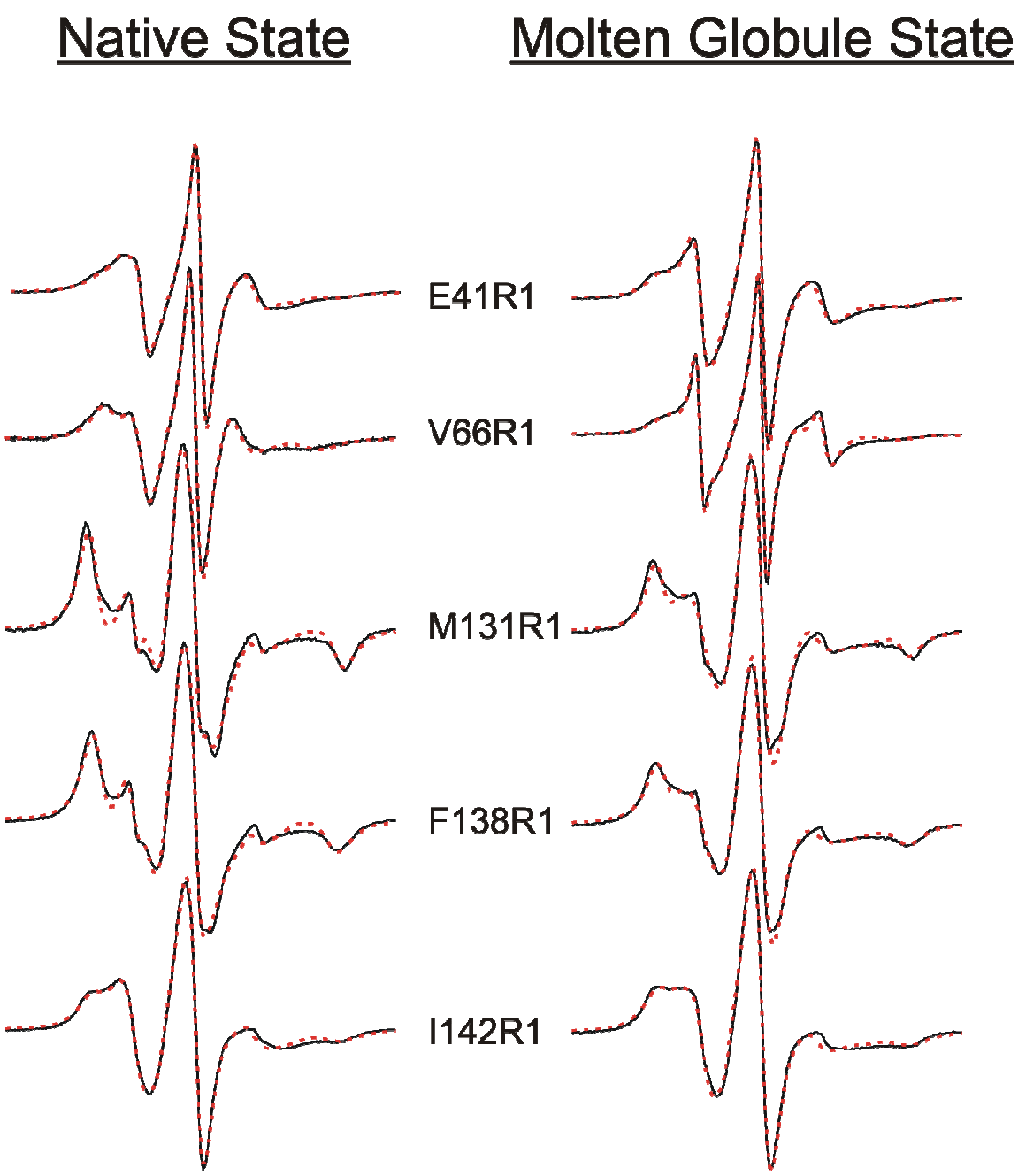

Figure S2. Experimental EPR spectra (solid black lines) and matching curve fits (red dashed lines). All spectra were fit to a two-component model except for V66R1 in the native state, which was fit to a single component.

Sample	A_{zz}' (Gauss)	$\%i$	τ_i (ns)	$\%m$	τ_m (ns)	S_i	S_m
M131R1-N	34.90	95	18.7	5	1.0	0.88	0.25
F138R1-N	34.38	95	14.0	5	1.0	0.85	0.22
I142R1-N	35.40	61	10.0	39	1.5	---	0.31
E41R1-N	36.40	58	5.9	42	1.6	---	0.35
V66R1-N	36.22	---	---	100	3.1	---	0.45
M131R1-MG	35.10	60	18.7	40	2.0	0.88	0.40
F138R1-MG	35.38	59	14.0	41	2.1	0.85	0.41
I142R1-MG	35.40	60	12.7	40	1.8	---	0.43
E41R1-MG	35.74	57	8.5	43	2.1	---	0.14
V66R1-MG	36.13	72	5.2	28	1.1	---	0.07

Table S1. Expanded summary EPR data and fit parameters. A_{zz}' is the effective hyperfine splitting obtained from the EPR spectra at -50˚C (shown in Fig. S1). $\%i$ and $\%m$ are the relative percentages of immobile and mobile components, respectively, of the EPR spectra obtained from the fits. τ_i and τ_m are the effective rotational correlation times of the spin label immobile and mobile components. S_i and S_m are the order parameters of the immobile and mobile component, and are calculated from EPR data fitting as described above.

References

1. Budil DE, Lee S, Saxena S, & Freed JH (1996) Nonlinear-Least-Squares Analysis of Slow-Motion EPR Spectra in One and Two Dimensions Using a Modified Levenberg-Marquardt Algorithm. *J. Magn. Reson. A* 120(2):155-189.