

SUPPORTING MATERIAL

Temperature dependence of T_{2M}

The temperature dependences of the phase memory times for proteins in water are shown in Fig. S.1, and of proteins or spin labels alone in aqueous glycerol are shown in Fig. S.2. The overall magnitudes of T_{2M} are similar to those found for non-clustered spin-labelled lipids in the low-temperature phases of bilayer membranes ref. 20; and cf. ref. 21). The temperature dependences for the spin-labelled proteins are complex and difficult to unravel completely. Below approximately 180 K for the proteins in water, or the glass transition temperature for samples in aqueous glycerol, the molecular motions are restricted to harmonic vibrations or librations without activation energy that are characteristic of the glassy state (35). Above this temperature, the librational motions become stochastic (i.e., diffusive) and are moderately activated ($E_a \sim 20 \text{ kJ mol}^{-1}$ for proteins in water; see ref. 35). From Figs. S.1 and S.2, it appears that the transition between harmonic and diffusive regimes is accompanied by a broad minimum in T_{2M} . Unfortunately, the temperature range over which the spin echo can be detected in the diffusive regime is rather limited.

Figures for Supporting Material to Guzzi et al. for BJ

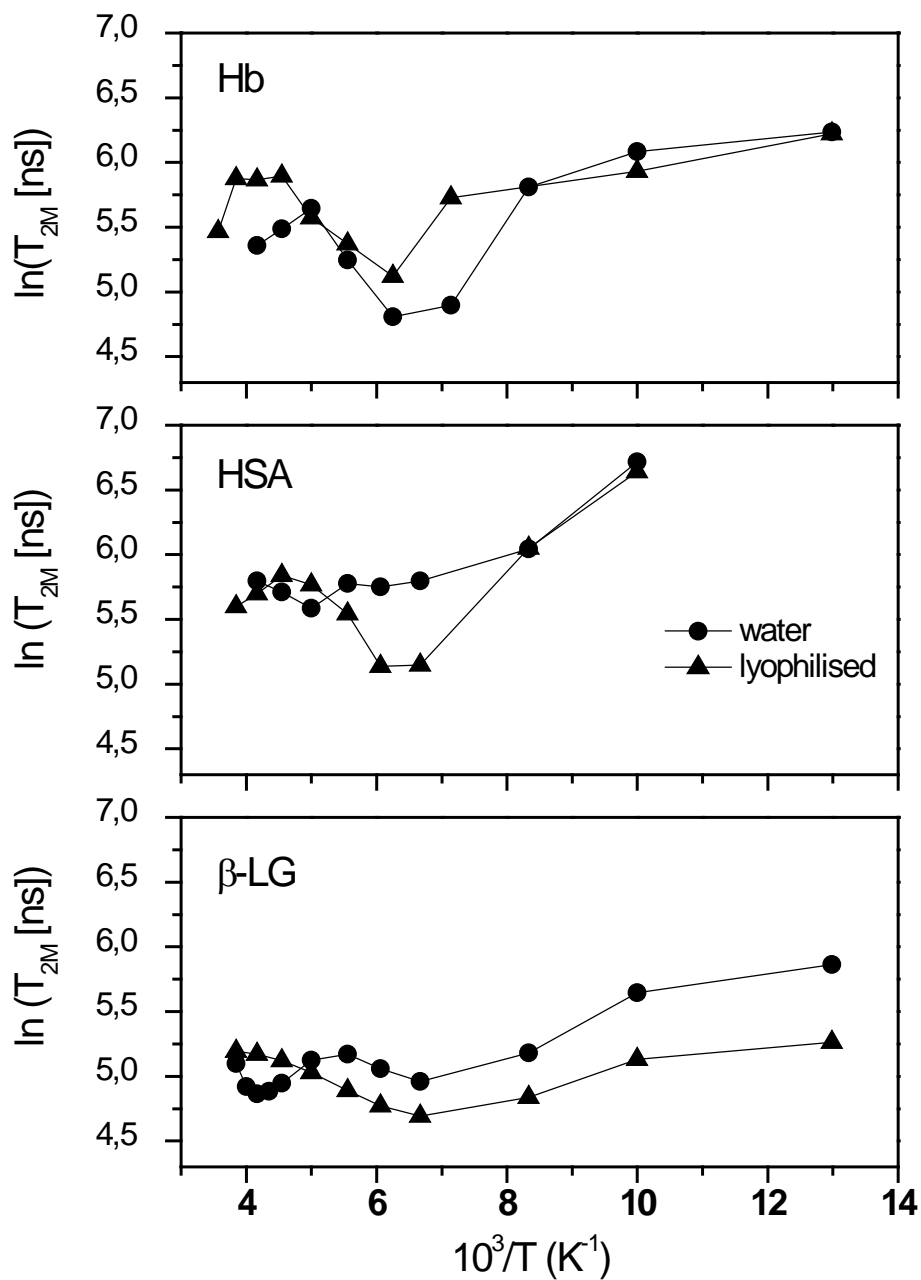


FIGURE S.1: Temperature dependence of the phase-memory time, T_{2M} , for spin-labelled proteins. *Top*: 6-MSL-labelled human haemoglobin; *middle*: 5-MSL-labelled human serum albumin; *bottom*: 5-MSL-labelled bovine β -lactoglobulin. The proteins are either lyophilised (triangles), or dissolved in water (circles).

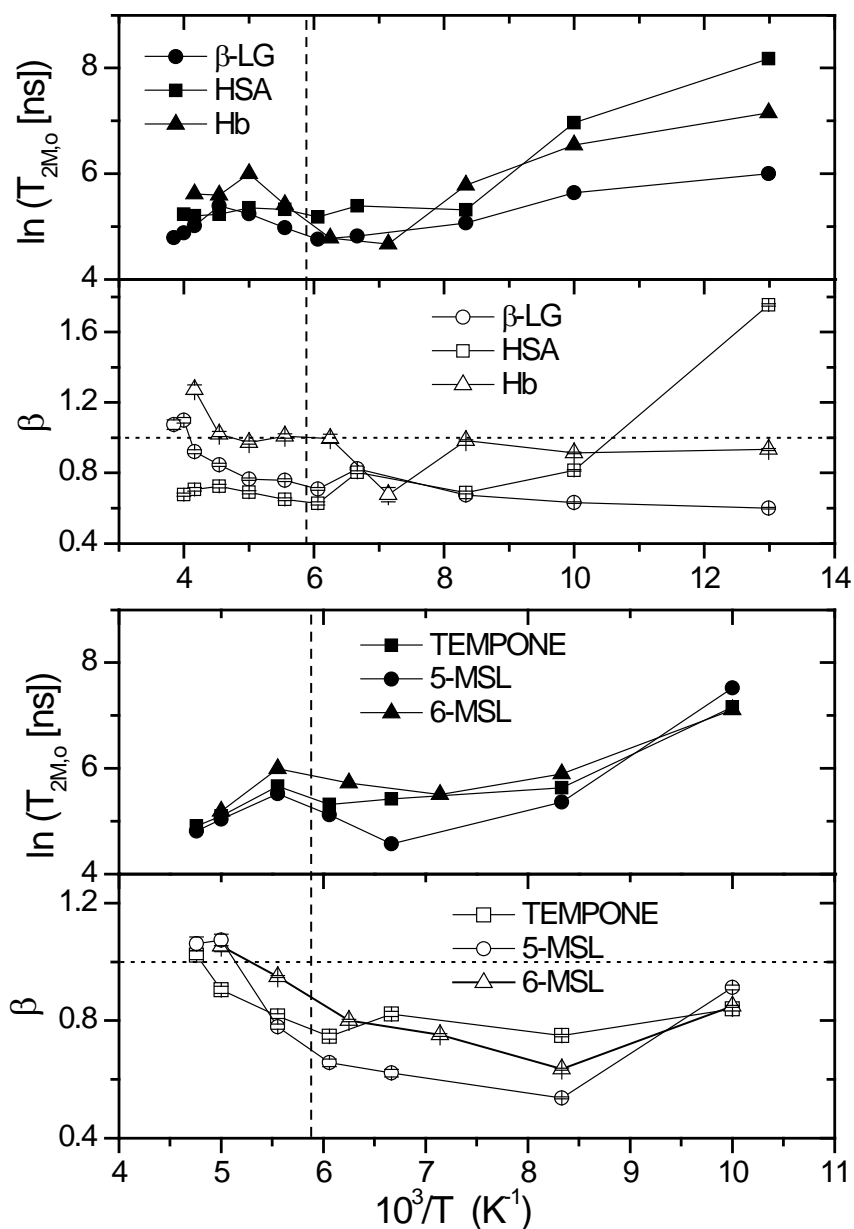


FIGURE S.2: Temperature dependence of the stretched exponential parameters, $T_{2M,o}$ (solid symbols) and β (open symbols), specifying the distribution of phase-memory times according to Eq. 1, for spin-labelled systems in 60% v/v glycerol-water. The upper of each pair of panels gives $T_{2M,o}$ and the lower of each pair gives the exponent β . *Top*: 5-MSL-labelled bovine β -lactoglobulin (β -LG, circles) and human serum albumin (HSA, squares), and 6-MSL-labelled haemoglobin (Hb, triangles). *Bottom*: TEMPONE (squares), and 5-MSL (circles) and 6-MSL (triangles) alone. The vertical dashed line is the approximate position of the glass transition temperature for 60% v/v glycerol.

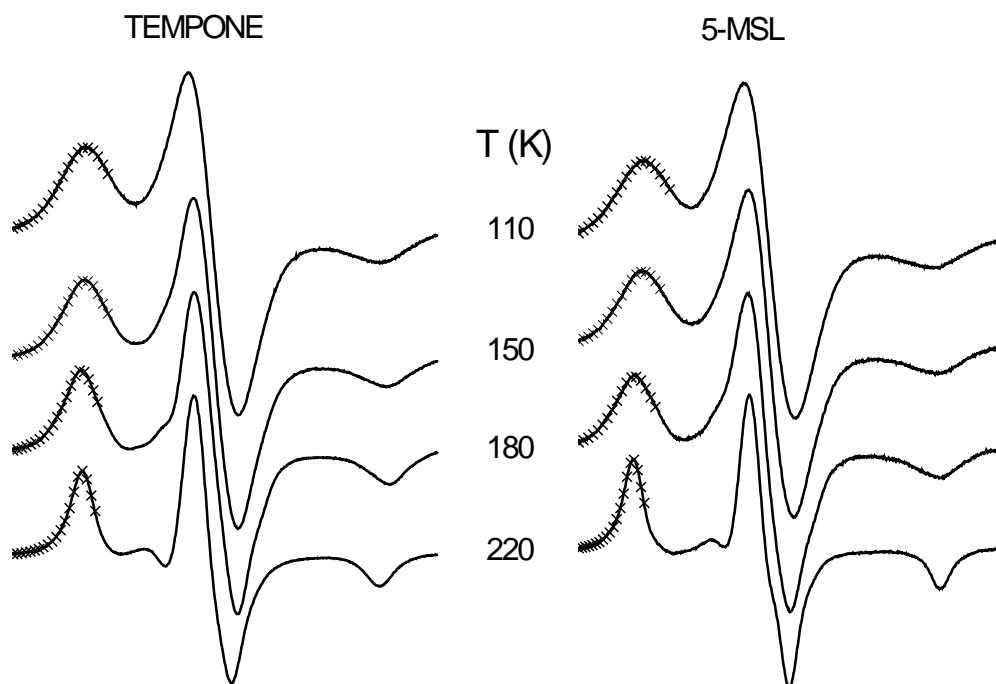


FIGURE S.3: Temperature dependence of the CW-EPR spectra of 5-MSL alone (*right*) and TEMPONE (*left*) in 60% v/v glycerol-water. Crosses represent fitting of the low-field ($m_I = +1$) hyperfine extremum with a Voigt lineshape (Eq. 2). Total scan width = 10 mT.