Supporting Information

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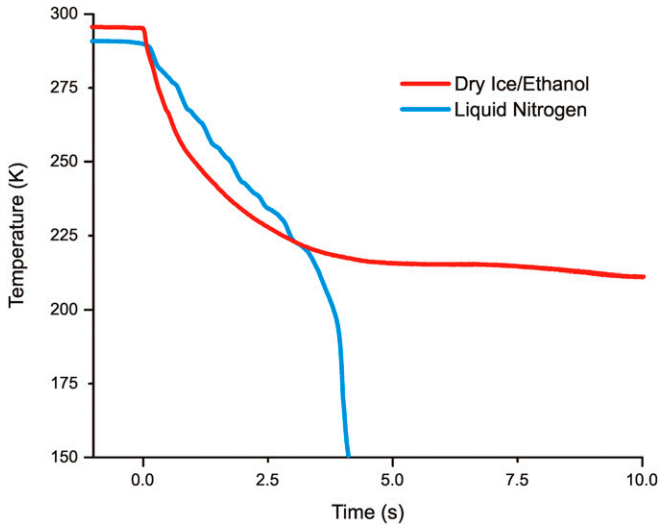

Fig. S1. Freezing rates in dry ice/ethanol and liquid nitrogen. A borosilicate DEER capillary was loaded with ~20 μL 25% (vol/vol) glycerol in water, and a silicone piston was placed above the solution. A thermocouple (Omega COCO-001) with an ~2-ms response time was used to monitor the temperature of the solution as the capillary was frozen in dry ice/ethanol or liquid nitrogen. For the dry ice/ethanol test, the pressure bomb was filled with ethanol and precooled in the dry ice bath, and the capillary was plunged into the cooled bomb exactly as in the DEER experiments reported in this study. The initial temperature was 295 K in the dry ice/ethanol test and was 290 K in the liquid nitrogen test. When the capillary is plunged into the cooling baths, dry ice/ethanol has a faster cooling rate: To cool to 273 K, dry ice takes ~300 ms and liquid nitrogen takes ~700 ms. A crossover occurs at 223 K at ~3 s; after this time, liquid nitrogen cools the sample to a lower temperature at equivalent time points. The temperature in the dry ice/ethanol test stabilizes after ~16 s at 208 K, according to the thermocouple reading. The thermocouple accuracy is limited at low temperature, and it is assumed that the true final temperature is that known for dry ice/ethanol at atmospheric pressure, 200 K.

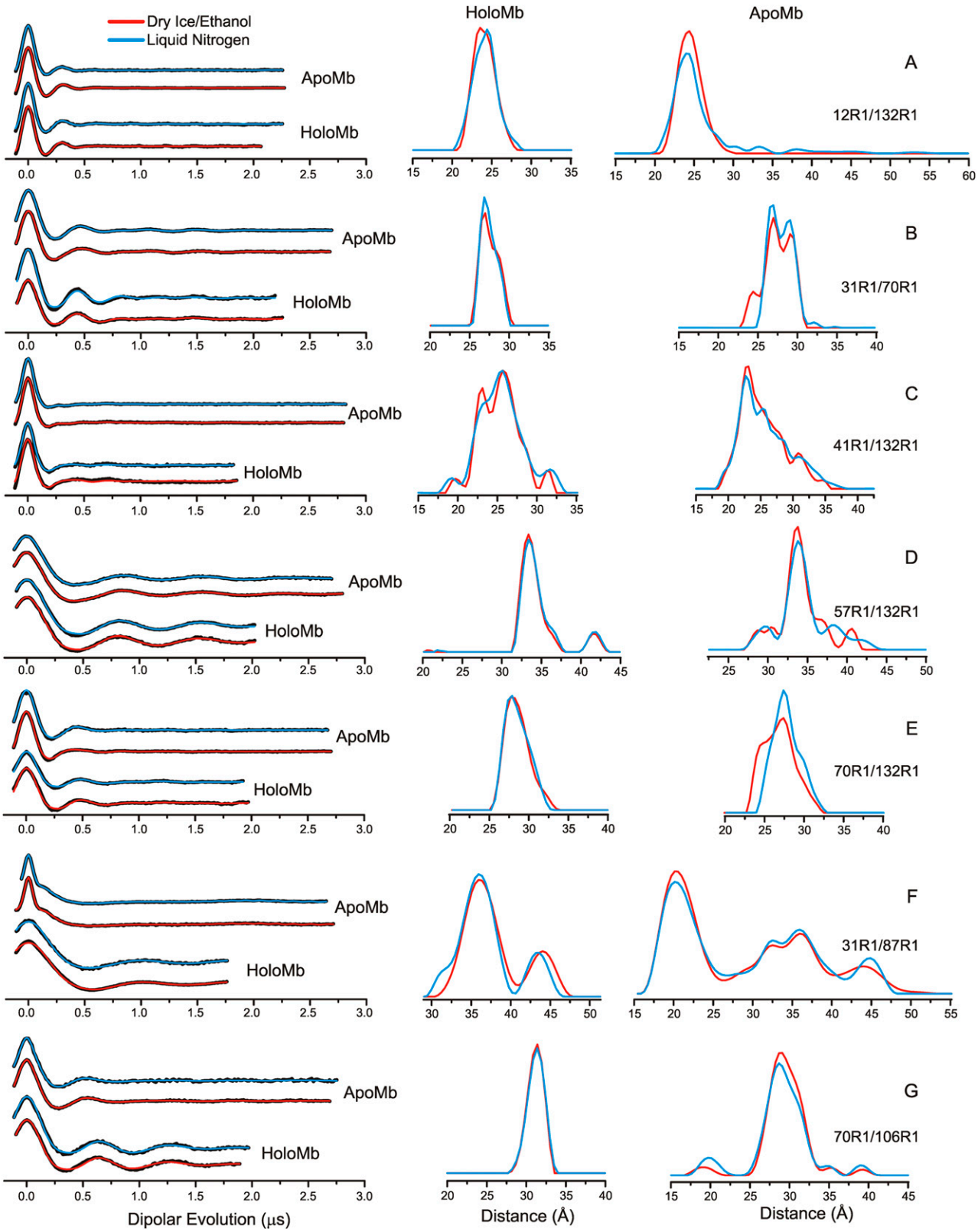

Fig. S2. Double electron–electron resonance distance distributions for native apomyoglobin (apoMb) and holomyoglobin (holoMb) at 0 bar, pH 6, generated from samples prepared using dry ice/ethanol and liquid nitrogen. (*Left*) Background-corrected dipolar evolutions of the indicated mutants are shown in black, with fits to the data (*Methods*) color coded according to freezing method used. (*Center* and *Right*) The area-normalized distance distributions are shown for holoMb (*Center*) and apoMb (*Right*) for (*A*) 12R1/132R1, (*B*) 31R1/70R1, (*C*) 41R1/132R1, (*D*) 57R1/132R1, (*E*) 70R1/132R1, (*F*) 31R1/87R1, and (*G*) 70R1/106R1. The *Inset* indicates the color coding of the distance distributions and fits to the dipolar evolutions. Dipolar evolutions are vertically offset for clarity.

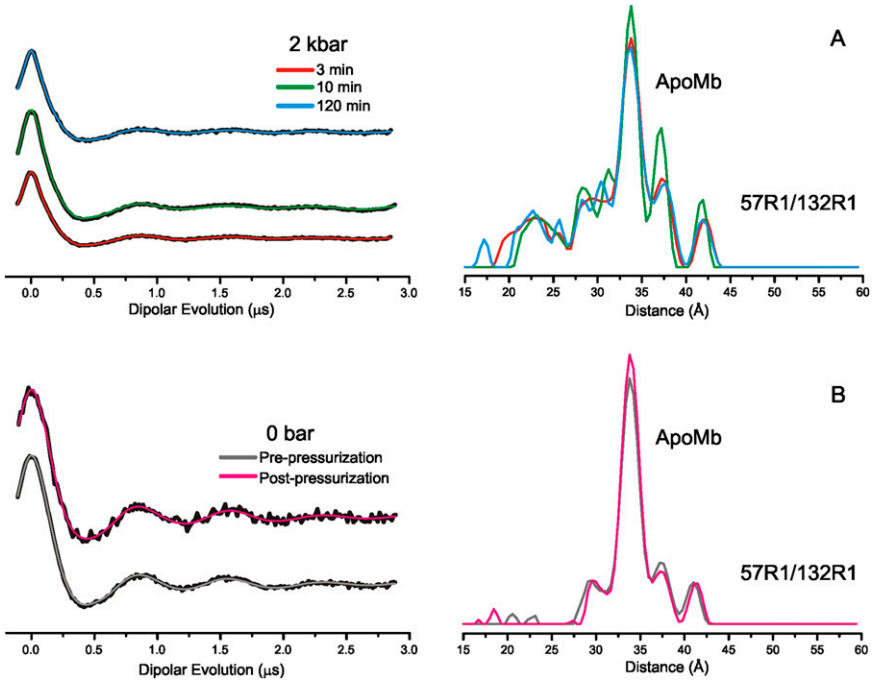

Fig. S3. Reversibility of pressure-populated changes in apoMb and evaluation of conformational relaxation at 200 K. (*Left*) Background-corrected dipolar evolutions are shown with fits to the data (*Methods*) color coded as indicated in the *Inset*. (*Right*) The area-normalized distance distributions are shown for apoMb 57R1/132R1 in (*A*) the pressure-populated MG at 2 kbar, pH 6, for samples held at 200 K for variable times after depressurization of the frozen sample, before further cooling in liquid nitrogen to 77 K for data acquisition, and (*B*) the native state at 0 bar, pH 6, pre- and postpressurization to 2 kbar. Dipolar evolutions are vertically offset for clarity. The *Inset* indicates the color coding of the distance distributions and fits to the dipolar evolutions.