Supporting Information

Open and Closed Form of Maltose Binding Protein in Its Native and Molten Globule State as studied by EPR Spectroscopy

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1. An illustration to PDS data, mostly DQC and some DEER used in this work

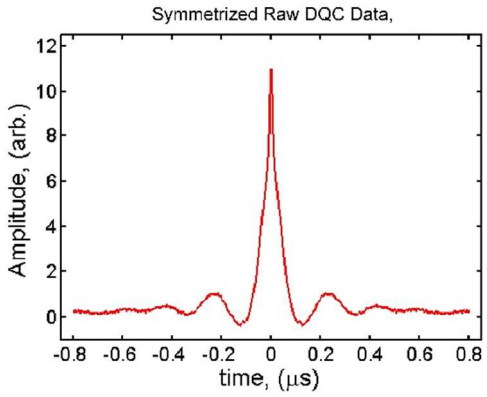

Figure S1. An example of a typical 6-pulse DQC time-domain signal. The as-recorded DQC signal, $V(t)$, was collected to have both halves and symmetrized as $V_s = [V(t) + V(-t)]/2$. Then a half of it was used to obtain $P(r)$. The raw signal however is highly symmetric, so that data symmetrizing merely improves S/N by the factor of $2^{1/2}$ As a matter of this signal symmetry, the "better" half is usually recorded, which has fewer crossing by the residuals of unwanted coherence pathways, suppressed by the phase cycling that is applied to extract the DQC signal.

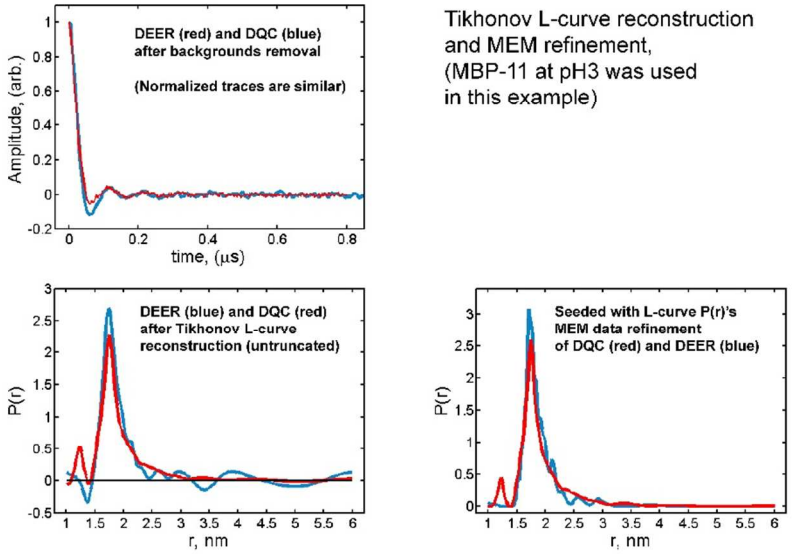

Figure S2. PDS data processing into distances, $P(r)$. After background subtraction L-curve Tikhonov regularization is applied. The resulting $P(r)$ usually contains some negative points, which are then set to zero and the positive P(r) is then refined by using the Maximum Entropy Method (MEM). The background is much smaller in DQC than in DEER, therefore the artifacts of distance reconstruction after its removal are smaller. Also stronger B_1 of microwave (MW) pulses in DQC reduces any orientational effects, further improving the quality of reconstruction.

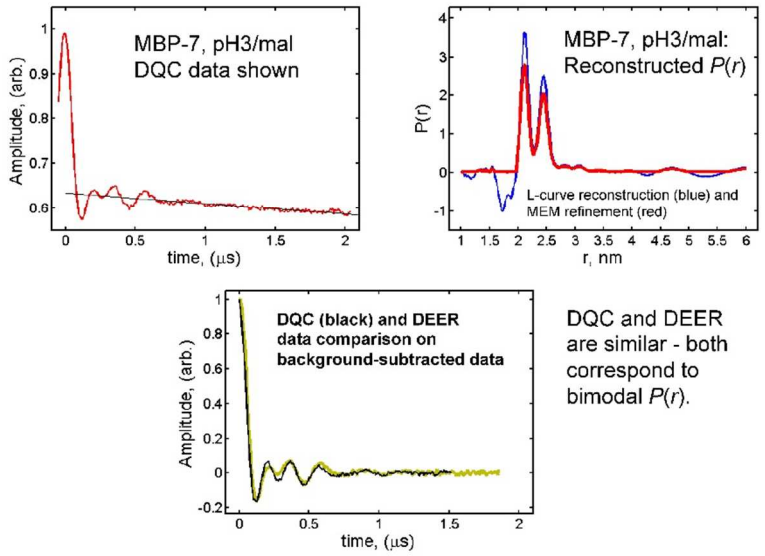

Figure S3. (Top left) Raw DQC data are shown with the background approximated by a straight line. (Bottom) The DQC and DEER time-domain data are compared. They are very close in this case, indicating that if there were orientational effects, they must be very small. (Top right) DQC data processed into distances as described in Fig. S2. The peculiaritiesof the time-domain data are most likely caused by the presence of two distinct spin-label side-chain rotamers.

2. Example of DQC data and associated distance distributions

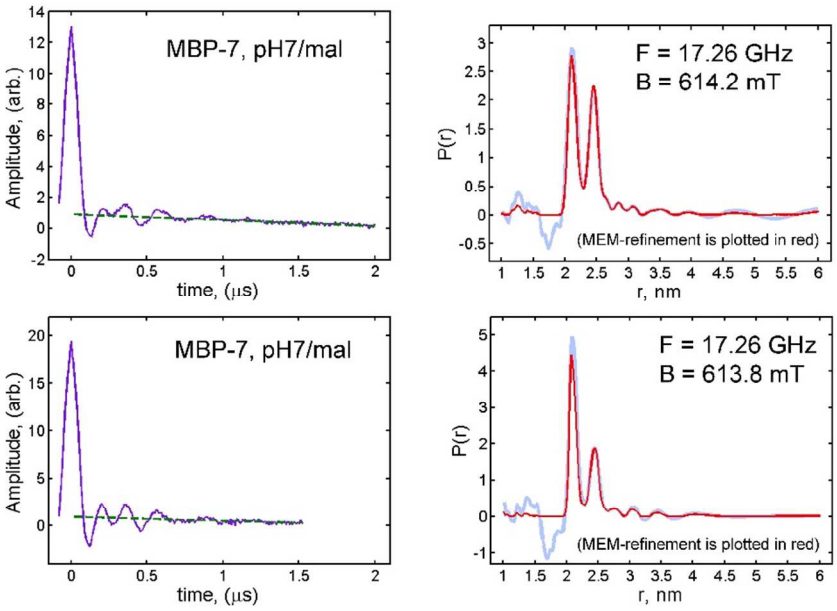

Figure S4. An example of DQC data recorded with the pulse sequence applied at different parts of the nitroxide spectrum. There are some variations in intensities of the two peaks, but otherwise the effects are minor. The smallness of these effects is in part due to a large B_1, which reduces orientation-selection effects.

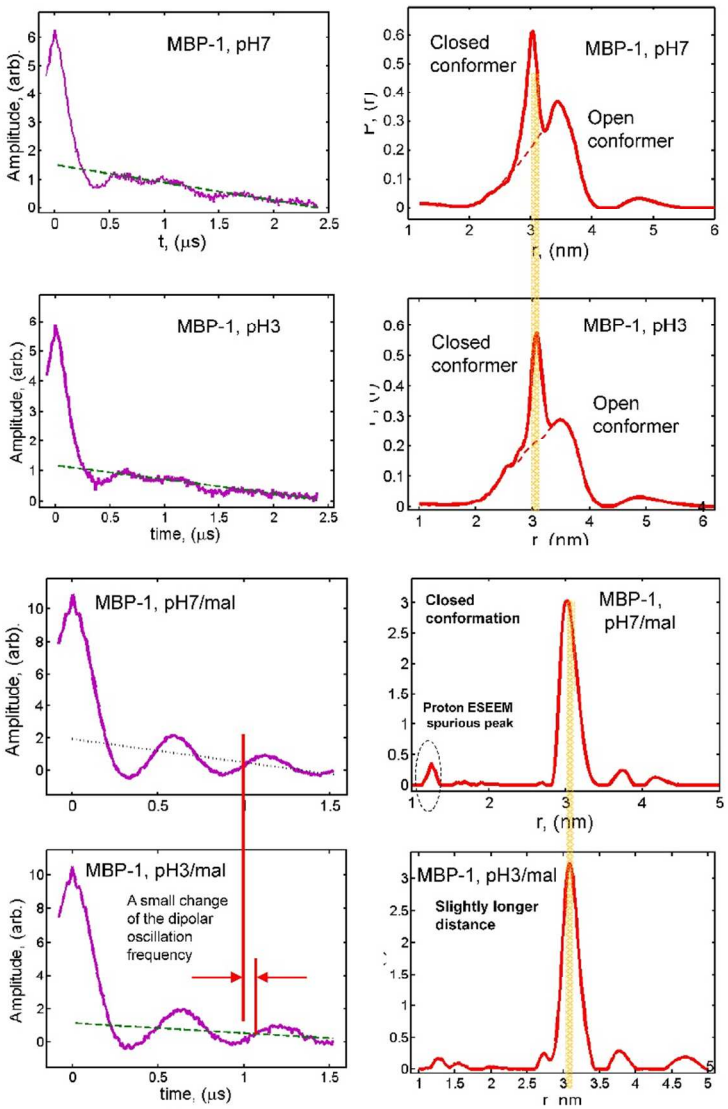

Figure S5. DQC data (left column) and $P(r)$'s (right column) for MBP-1 taken at four conditions: produced by using neutral and acidic pH and then the apo (top two panels) or bound substrate (maltose) states (bottom two panels). Clearly, there are two states in the absence of the substrate. One of the states characterized by narrow distribution apparently is very similar to the case of bound substrate. This state is the"closed" state. The second state is broadly distributed, indicative of a wide range of conformations sampled by the catalytic cleft of the MBP. This is an "open" state

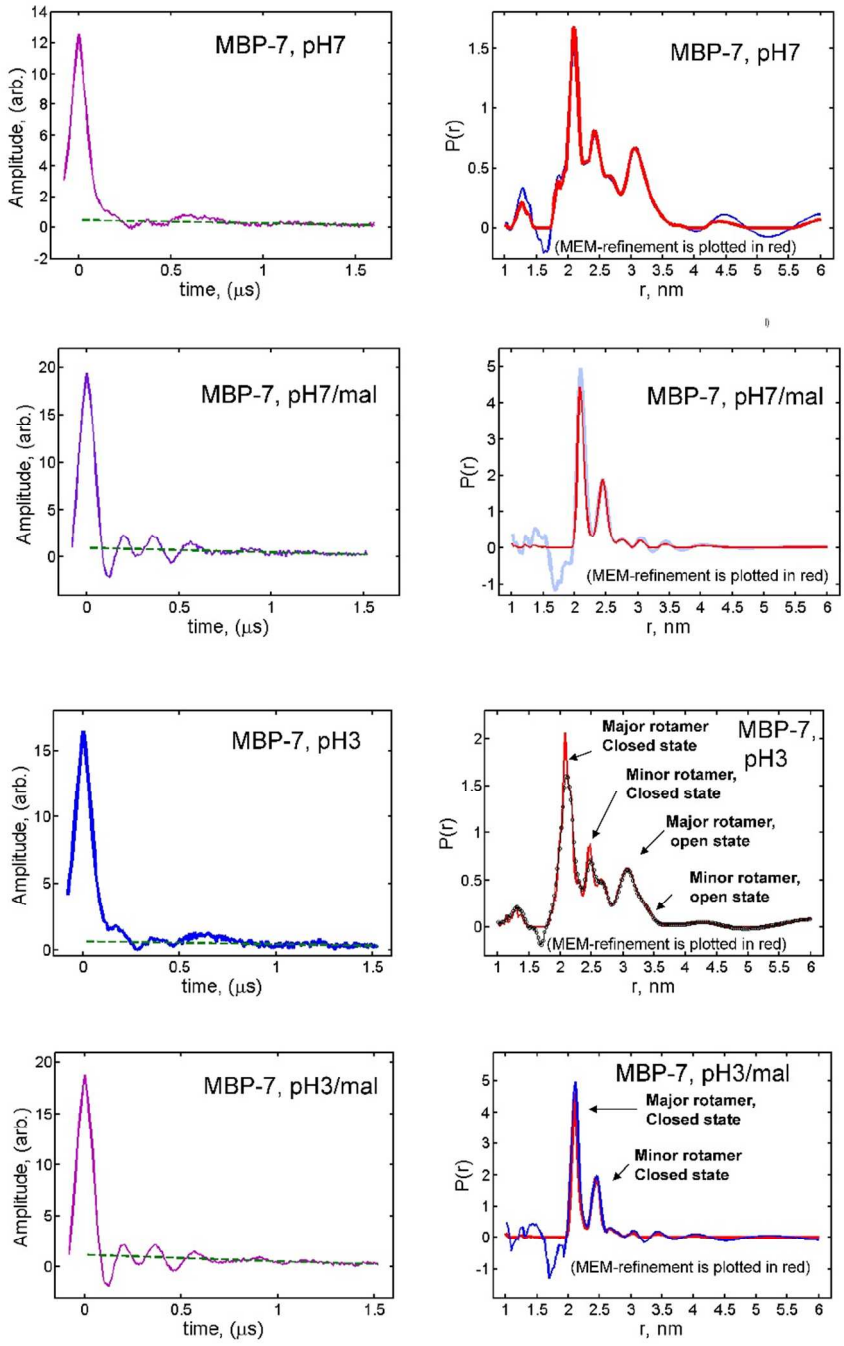

Figure S6. DQC data (left column) and *P*(*r*)'s (right column) for MBP-7 taken at four conditions produced by using neutral and acidic pH and then the apo or substrate-bound state, as indicated in the plots. Clearly, there are two different MBP conformations in the absence of the substrate similar to the previous figure. However there are two distinct conformers of the spin-label side-chain leading to extra peaks in the distance distributions. One of the states, characterized by narrower distributions, apparently is very similar to the case of bound substrate. This state is thus the "closed" state. The second state is an admixture of a broader, less-resolved distributions and of the distinct "closed" state. The closed state experiences only minor variations through all four conditions.

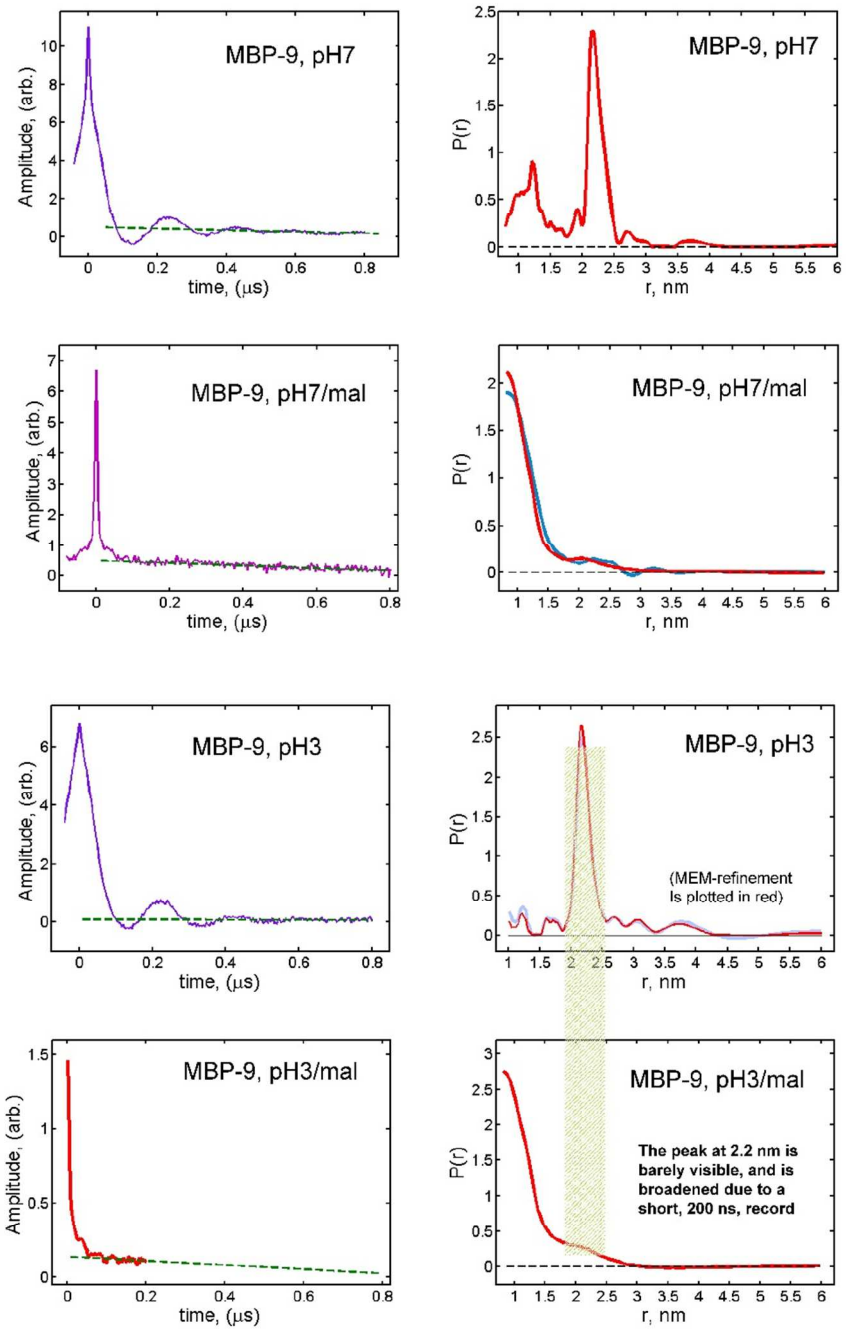

Figure S7. DQC data (left column) and *P*(*r*)'s (right column) for MBP-9 taken at four conditions produced by using neutral and acidic pH and then the apo or substrate-bound state, as indicated in the plots. The closed state has spin-labels very close to each other, leading to the high frequency of dipolar oscillations. The presence of two distinct conformers is apparent just by looking at the time-domain data in the top two rows on the left. The distance distributions contain significant populations at about 1 nm or maybe even shorter, but those distances are outside of the DQC range. In the measurement with maltose some traces of the open state rotamers characterized by the separation of ~2.2 nm can be noticed.