Supporting Information

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DNA C



Fig. S1. Far-UV circular dichroism spectroscopy of modified EnHD-L16A. Recorded spectra of the individual single point mutants modified with AttoOxa11 at low (black) and high (gray) solution ionic strength. All measurements were performed at identical sample concentration. The spectra at high and low ionic strength resemble those of wild-type and denatured proteins, respectively. The lower right panel shows the measured ellipticities at the absorption maximum of helical secondary structure plotted as function of probe position. The amplitude of the change in ellipticity between low and high ionic strength conditions remains essentially constant. Accordingly, consistent with the kinetic data, there is no evidence for significant probe-induced, position-dependent perturbations of the folding equilibrium.



Fig. S2. Autocorrelation functions (ACFs) of modified EnHD-L16A lacking Trp48. ACFs, normalized to the average number of molecules in the detection focus, recorded from modified mutant A7C where the single tryptophan side chain has been replaced by a phenylalanine (W48F) using site-directed mutagenesis. The dataset recorded at high solution ionic strength (gray) is offset from the one at low solution ionic strength (black) for reasons of clarity. Both ACFs fit well to a model for a single diffusing species without additional relaxations (red lines) showing that the observed relaxations of tryptophan-containing EnHD-L16A can be ascribed exclusively to PET fluorescence quenching of the attached fluorophore upon formation of intrachain contact with W48.



Fig. S3. PET-FCS and far-UV CD spectroscopy of EnHD-L16A in solutions containing the viscogen sucrose. (A) ACF of EnHD-L16A modified with AttoOxa11 at sequence position 7 in buffered solution of 145 mM ionic strength containing 15% (w/v) sucrose. The red line is a data fit to a model for a single diffusing species exhibiting three single-exponential relaxations. (*B*) Far-UV CD spectra of EnHD-L16A recorded in buffered solutions of 145 mM ionic strength containing 0% (black), 10% (red), and 20% (blue) sucrose.