SUPPLEMENTARY MATERIALS

A glimpse into the structural properties of the intermediate and transition state in the folding of Bromodomain 2 domain 2 by Φ-value analysis.

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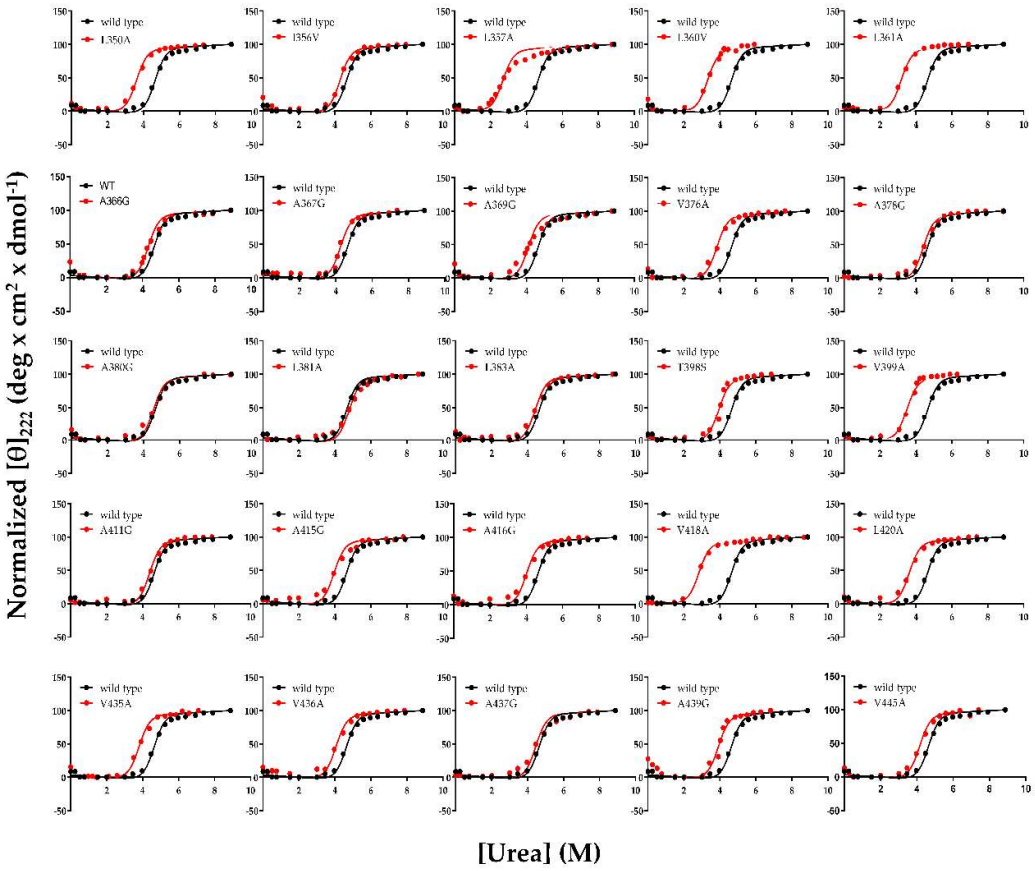
a



b

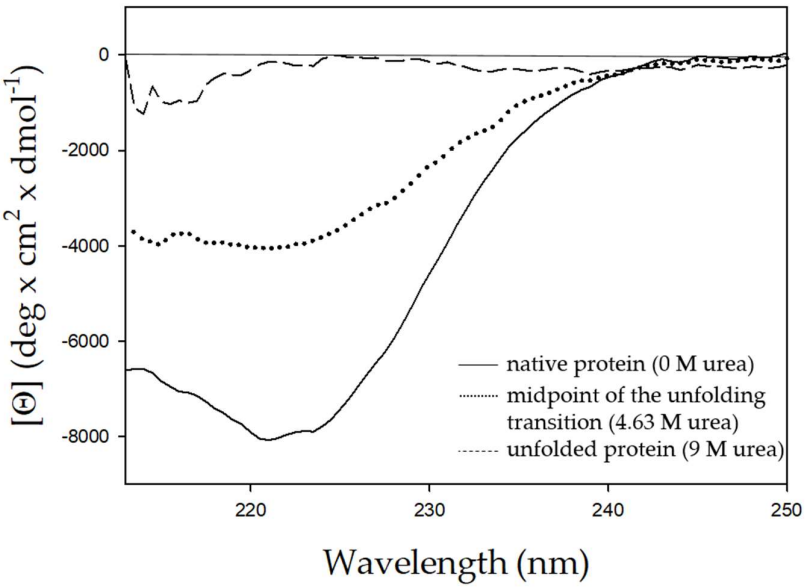


Figure S1. Urea-induced equilibrium unfolding of BRD2(2) wild type and mutants. (a) Plot of the molar ellipticity at 222 nm as a function of denaturant concentration of BRD2(2) wild type and mutants. The continuous lines represent the nonlinear global fitting of the molar ellipticities at 222 nm data ($[\theta]_{222}$) calculated as described in Materials and Methods. Data were normalized between 0 and 100%, where 0 corresponds to the molar ellipticity at 222 nm of the native protein, the smallest value, (at 0 M urea), and 100 corresponds to the molar ellipticity at 222 nm of the unfolded protein, the largest value, (at 9 M urea). Normalization was calculated using GraphPad Prism 5 as follows: $z_i = (x_i - \min(x)) / (\max(x) - \min(x)) * 100$, where z_i is the i_{th} normalized value in the dataset, x_i is the i_{th} value in the dataset, min(x) is the smallest value in the dataset and max(x) is the largest value in the dataset. (b) Far-UV CD spectra of BRD2(2) wild type. The continuous line represents the far-UV CD spectrum of native protein (0 M urea), the dotted line represents the far-UV CD spectrum at the midpoint of the unfolding transition (4.63 M urea) and the medium dashed line represents the far-UV CD spectrum of the unfolded protein (9 M urea). Spectra were recorded between 213 and 250 nm.

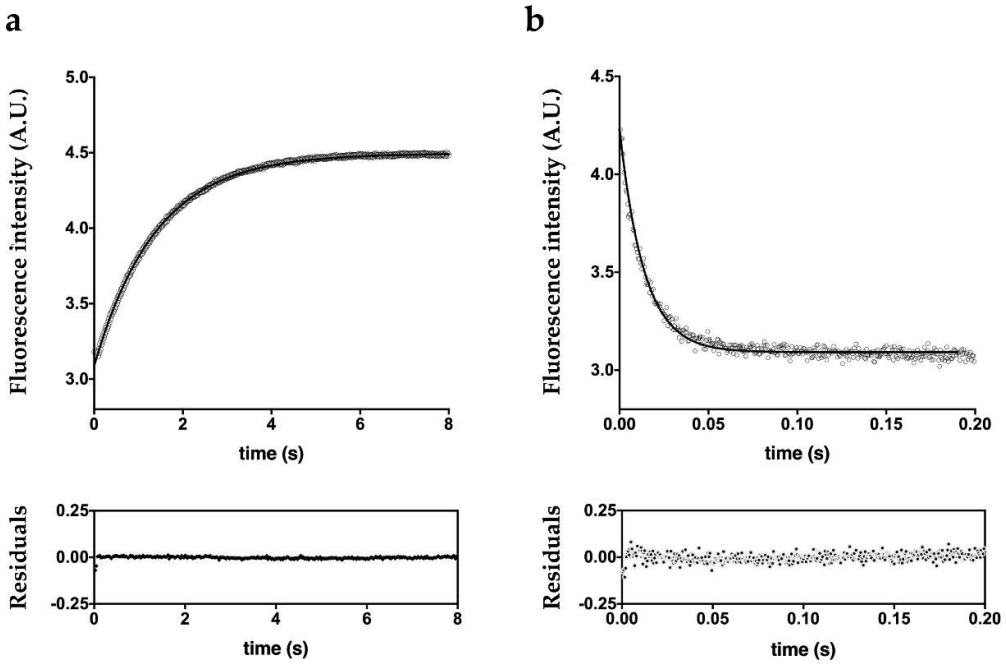


Figure S2. Representative kinetic traces of BRD2(2) A367G mutant for unfolding (a) ([Urea]=8.1 M) and refolding (b) ([Urea]=0.7 M) together with their relative residuals plot. The line represents the best fit to a single exponential decay model ($Y = a*e^{(-k*t)} + c$) without any restrain applied: the parameter obtained for unfolding are $k = 0.72 \pm 0.001$ s^{-1} and $a = 1.41 \pm 0.001$, while for refolding are $k = 72.5 \pm 1.7$ s^{-1} and $a = 1.1 \pm 0.01$. The goodness of fit (R square) is 0.9998 and 0.9979 for unfolding and refolding traces respectively.