Supplemental Information

Direct Observation of the Intrinsic Backbone Torsional Mobility of Disordered Proteins

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Supporting Material

Direct Observation of the Intrinsic Backbone Torsional Mobility of Disordered Proteins

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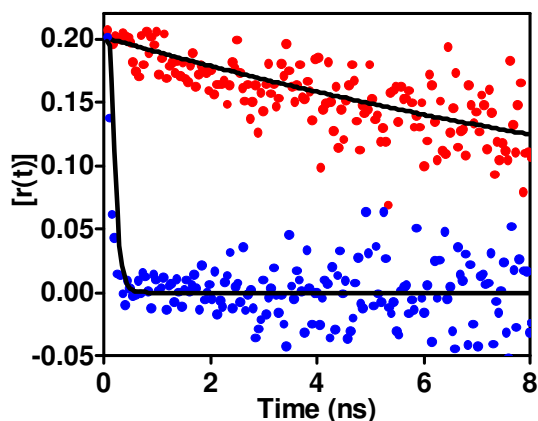
Fig. S1. The time-resolved fluorescence anisotropy decays of the tryptophan analog (NATA) in water (blue) and in 80% glycerol-water mixture (red). The black solid lines are fits. NATA in water exhibits a fast depolarization in water with a rotational correlation time of ~ 0.1 ns. The anisotropy decay in highly viscous medium (80% glycerol-water) shows a slow depolarization with an initial anisotropy (r_0) of ~ 0.2.
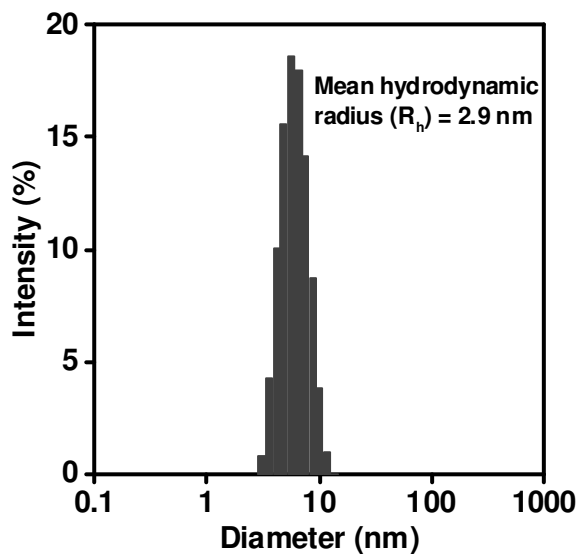

Fig. S2. The size-distribution of α-synuclein obtained using dynamic light scattering experiments. The distribution is devoid of any higher order oligomers and the mean hydrodynamic radius (R_h) is ~ 3 nm that is close to previously reported R_h of α-synuclein (35).
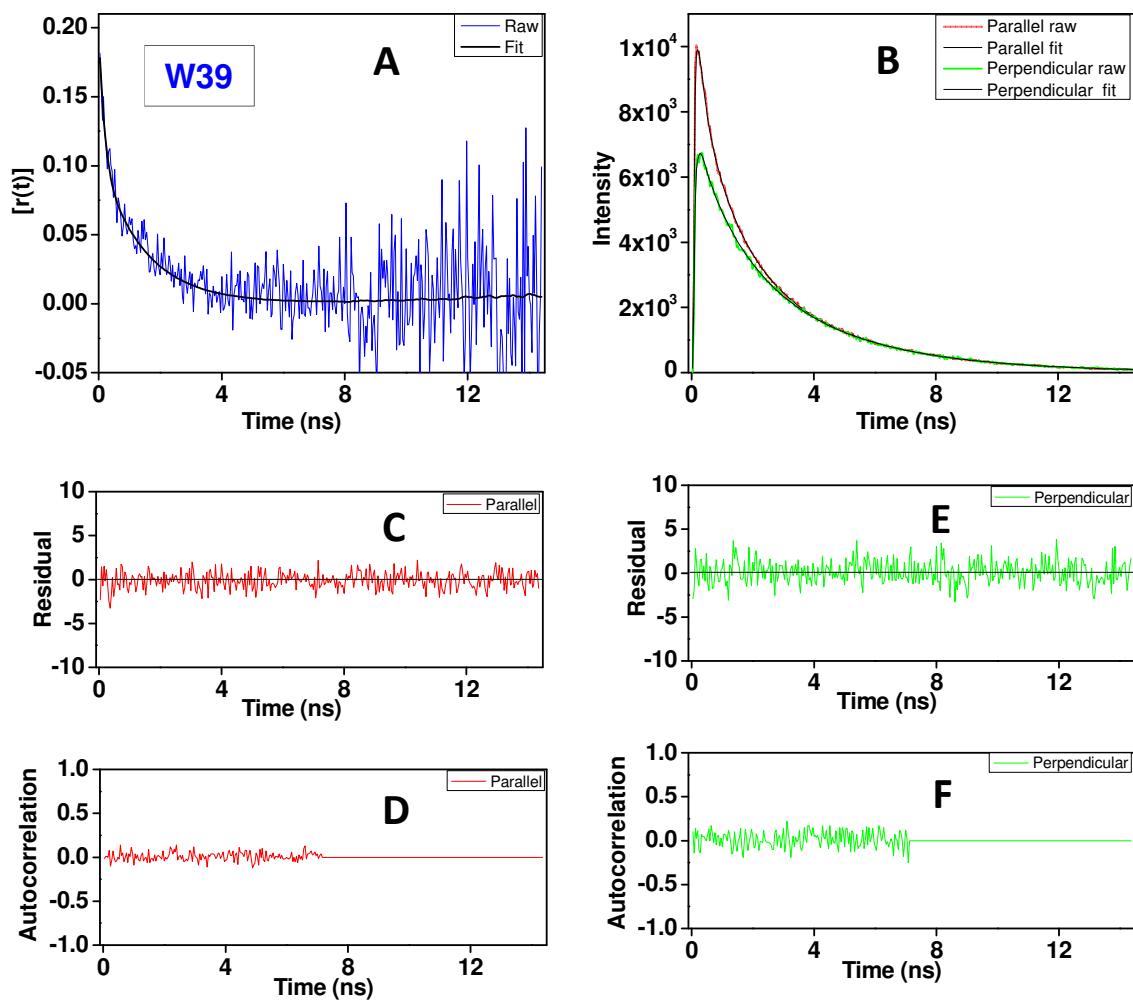
Fig. S3. The time-resolved fluorescence anisotropy decay analysis for Trp-39 variant of α-synuclein. (A) The raw anisotropy data are indicated by blue line, whereas, the fitted decay is shown by solid black line. (B) The global analysis of $I_∥(t)$ (red) and $I_⊥(t)$ (green) and corresponding fits are shown using solid black lines. (C) and (D) are the residual plot and the autocorrelation function for $I_∥(t)$, respectively. (E) and (F) are the residual plot and the autocorrelation function for $I_⊥(t)$, respectively. The recovered parameters are given in Table S1.

Fig. S4. The time-resolved fluorescence anisotropy decay analysis for Trp-78 variant of α-synuclein. (A) The raw anisotropy data are indicated by blue line, whereas, the fitted decay is shown by solid black line. (B) The global analysis of $I_{\parallel}(t)$ (red) and $I_{\perp}(t)$ (green) and corresponding fits are shown using solid black lines. (C) and (D) are the residual plot and the autocorrelation function for $I_{\parallel}(t)$, respectively. (E) and (F) are the residual plot and the autocorrelation function for $I_{\perp}(t)$, respectively. The recovered parameters are given in Table S1.
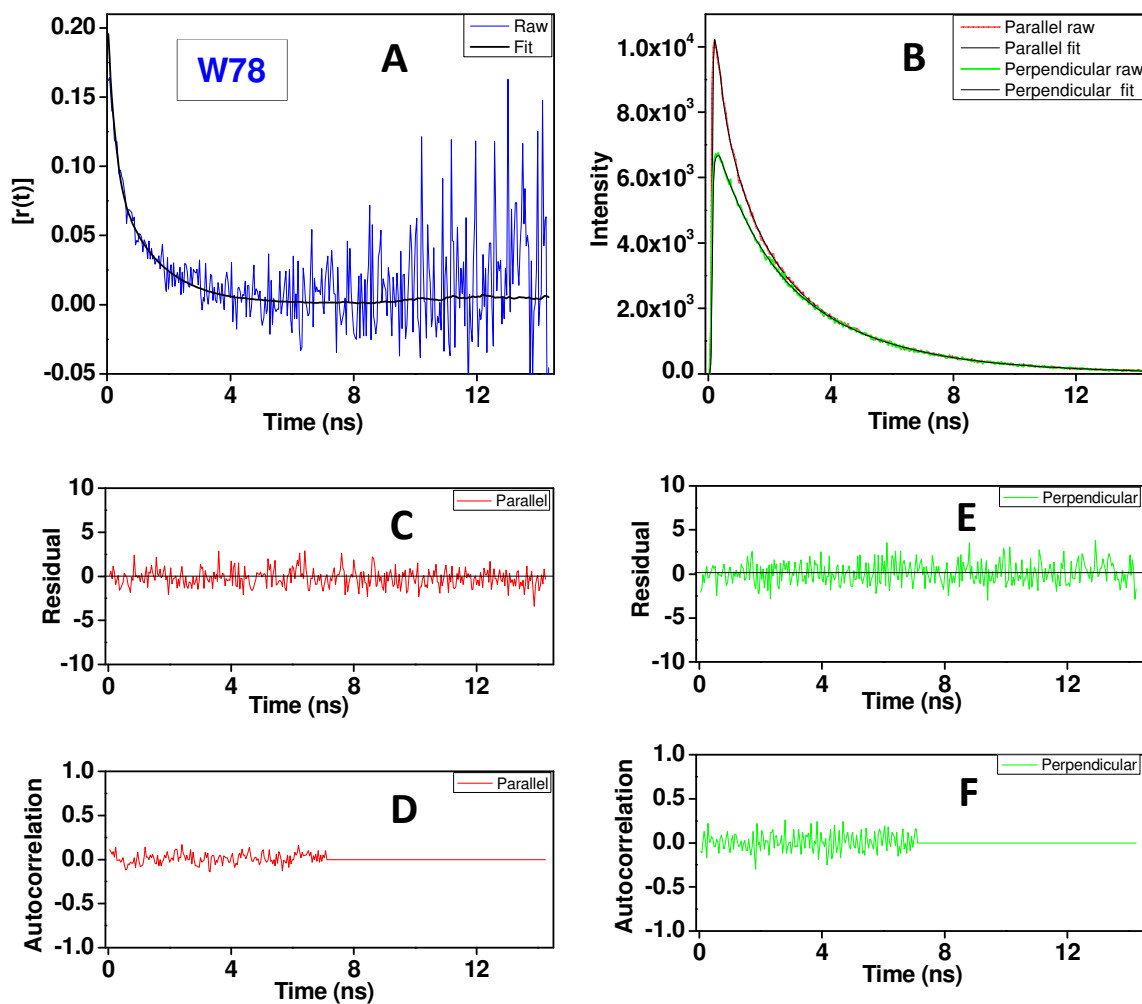
Fig. S5. The time-resolved fluorescence anisotropy decay analysis for Trp-107 variant of α-synuclein. (A) The raw anisotropy data are indicated by blue line, whereas, the fitted decay is shown by solid black line. (B) The global analysis of $I_{\parallel}(t)$ (red) and $I_{\perp}(t)$ (green) and corresponding fits are shown using solid black lines. (C) and (D) are the residual plot and the autocorrelation function for $I_{\parallel}(t)$, respectively. (E) and (F) are the residual plot and the autocorrelation function for $I_{\perp}(t)$, respectively. The recovered parameters are given in Table S1.
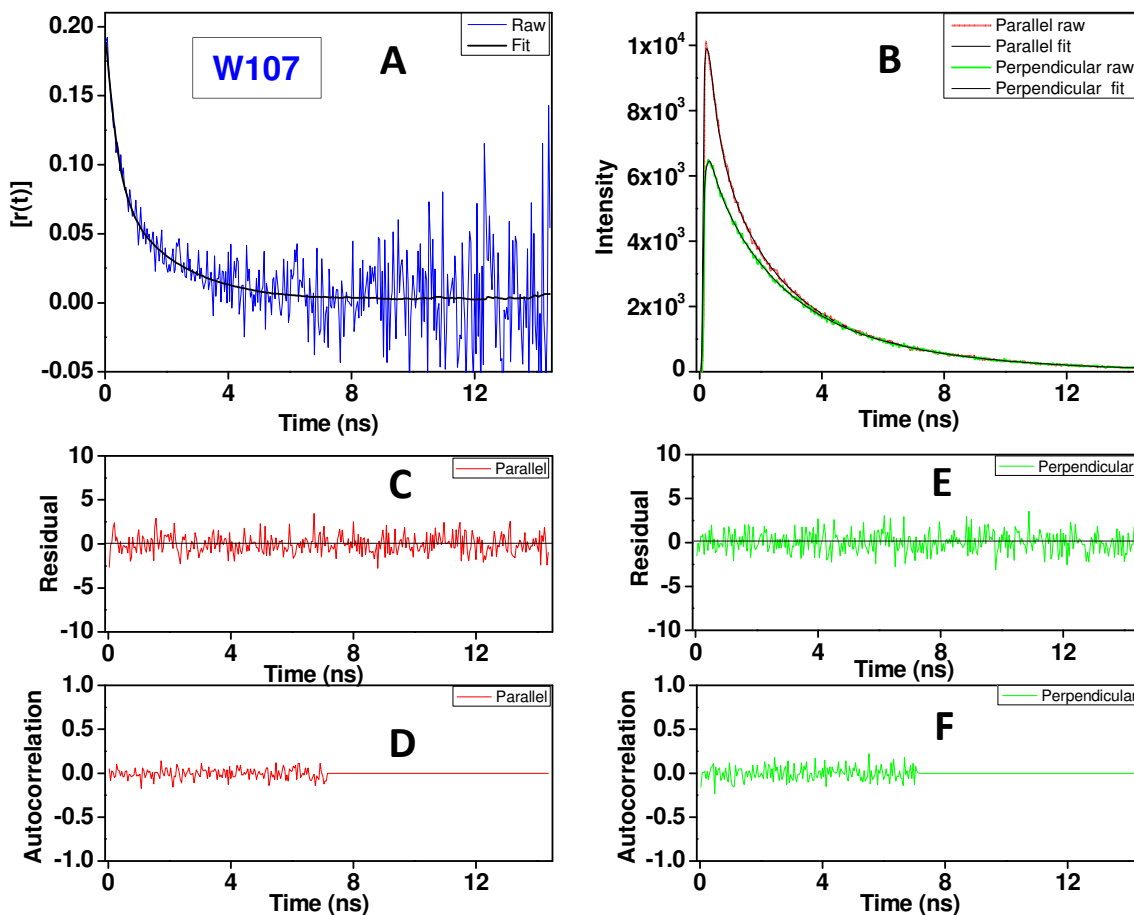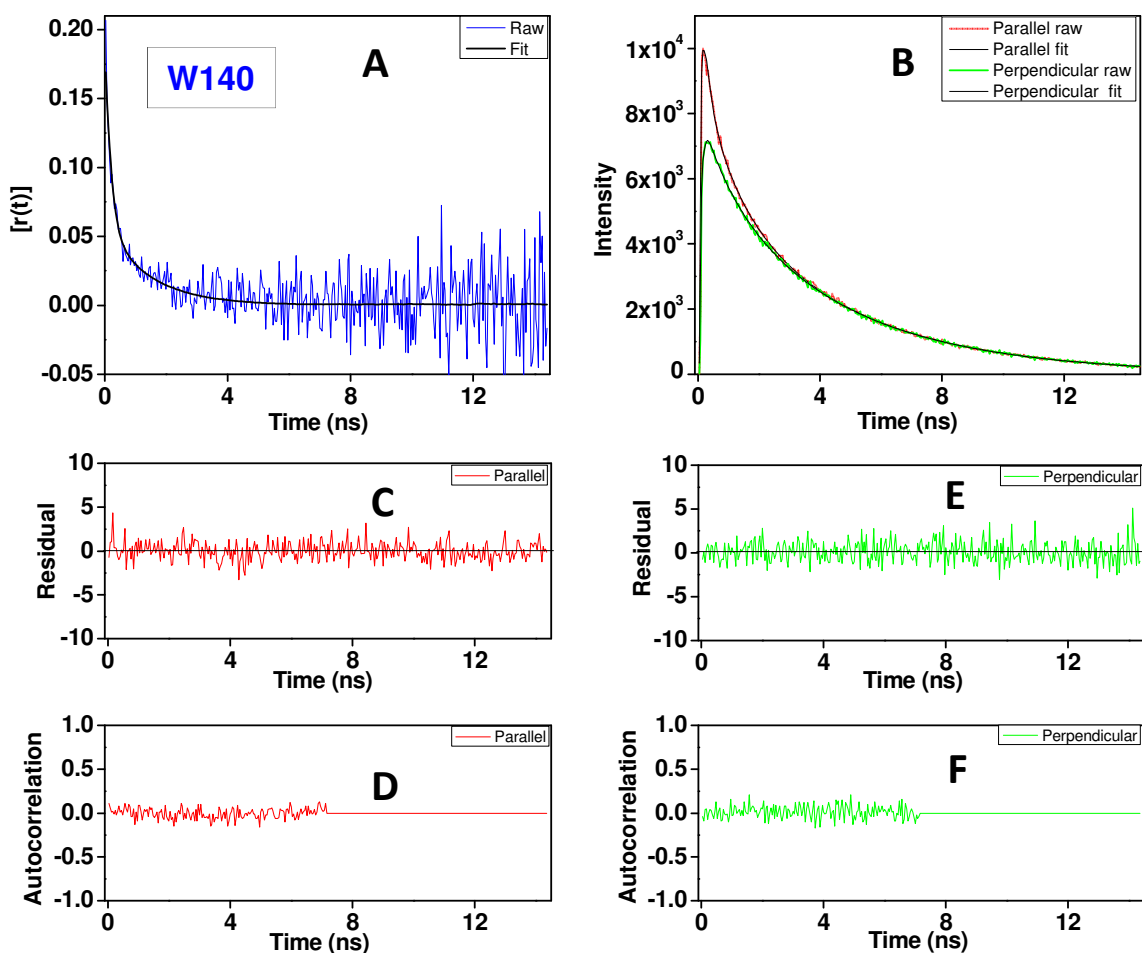
Fig. S6. The time-resolved fluorescence anisotropy decay analysis for Trp-140 variant of α-synuclein. (A) The raw anisotropy data are indicated by blue line, whereas, the fitted decay is shown by solid black line. (B) The global analysis of $I_{\parallel}(t)$ (red) and $I_{\perp}(t)$ (green) and corresponding fits are shown using solid black lines. (C) and (D) are the residual plot and the autocorrelation function for $I_{\parallel}(t)$, respectively. (E) and (F) are the residual plot and the autocorrelation function for $I_{\perp}(t)$, respectively. The recovered parameters are given in Table S1.
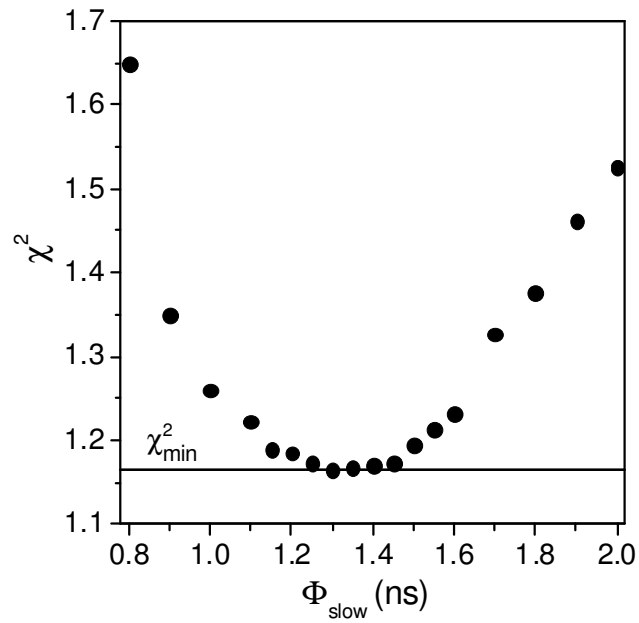
Fig. S7. A representative plot of the rotational correlation time (ϕ) *vs*. the goodness of fit (χ^2) obtained from forced fits indicating the robustness of the recovered rotational correlation times for Trp-39 variant of α-synuclein. The best fits (with χ^2_{min}) are obtained only when ϕ ranges between 1.30 and 1.45 ns.

Table S1. Typical parameters associated with the time-resolved fluorescence intensity and anisotropy decays of single Trp variants of α-synuclein (entry 1-10) and the prion protein (PrP 90-231) containing the (intrinsic) single Trp in the N-terminal segment (entry 11).

Entry	Trp position	τ_m (in ns) (mean lifetime)	ϕ_{fast} (in ns)	β_{fast}	ϕ_{slow} (in ns)	β_{slow}	r_0 (initial anisotropy)	r_{ss} (steady-state anisotropy)	χ^2
1	4	2.4	0.12	0.53	1.18	0.47	0.202	0.034	1.43
2	27	2.2	0.10	0.37	1.15	0.63	0.203	0.042	1.20
3	39	2.4	0.13	0.43	1.26	0.57	0.200	0.041	1.17
4	56	2.5	0.10	0.48	1.53	0.52	0.200	0.039	1.35
5	69	2.2	0.15	0.58	1.30	0.42	0.200	0.037	1.25
6	78	2.3	0.17	0.49	1.31	0.51	0.198	0.041	1.21
7	90	2.5	0.17	0.53	1.36	0.47	0.200	0.038	1.33
8	107	2.3	0.26	0.52	1.86	0.48	0.200	0.048	1.17
9	124	2.2	0.18	0.47	1.54	0.53	0.200	0.047	1.31
10	140	2.3	0.16	0.70	1.39	0.30	0.204	0.023	1.20
11	99 (PrP)	1.5	0.10	0.51	1.29	0.49	0.200	0.048	1.18

Table S2. Mean rotational correlation times and the standard errors estimated from multiple data acquisition and analysis.

Entry	Trp position	Mean ϕ_{fast} in ns (error)	Mean ϕ_{slow} in ns (error)
1	4	0.16 (± 0.04)	1.32 (± 0.12)
2	27	0.18 (± 0.05)	1.47 (± 0.15)
3	39	0.15 (± 0.03)	1.41 (± 0.14)
4	56	0.10 (± 0.02)	1.39 (± 0.18)
5	69	0.13 (± 0.02)	1.24 (± 0.11)
6	78	0.16 (± 0.02)	1.33 (± 0.16)
7	90	0.14 (± 0.02)	1.29 (± 0.16)
8	107	0.26 (± 0.04)	1.79 (± 0.17)
9	124	0.15 (± 0.04)	1.39 (± 0.17)
10	140	0.15 (± 0.02)	1.35 (± 0.17)
11	99 (PrP)	0.14 (± 0.04)	1.39 (± 0.19)