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Supplemental Information

Femtosecond Hydration Map of Intrinsically Disordered α-Synuclein

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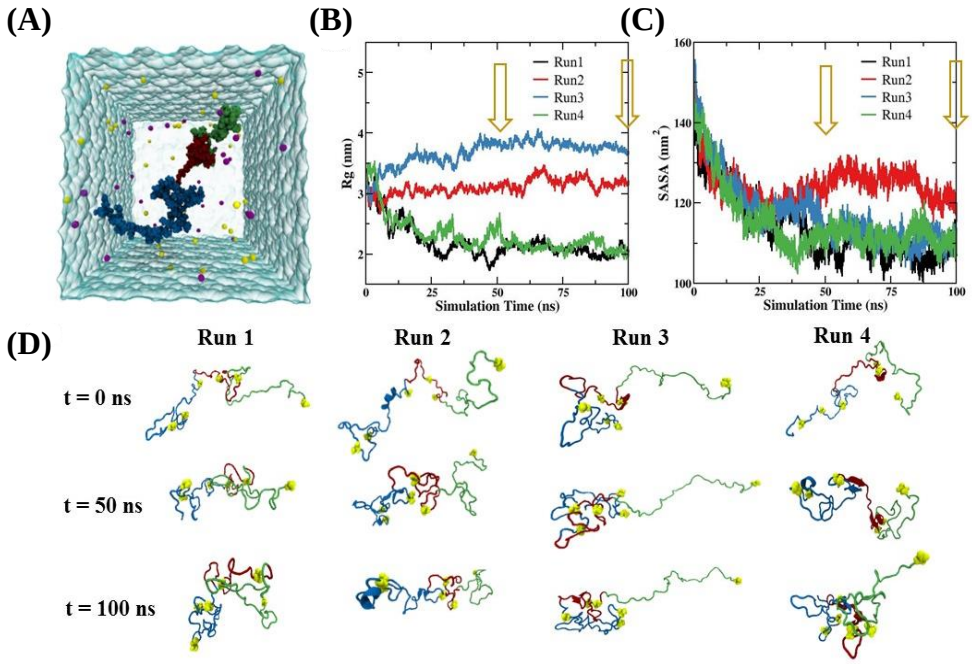

Figure S1 (A) The simulation system of α-syn in water. The protein chain and ions are shown using van der Waals (vdW) spheres (N-domain in blue, NAC-domain in red, and C-domain in green,). The water box is shown in cyan using molecular surface representation. Sodium ions are shown in yellow and chloride ions are shown in purple. (B-C) Evolution of R_g (nm) and SASA (nm^2) during four 100-ns long MD trajectories. Yellow arrows indicate the starting points for shorter 10-ns runs used for hydration water analysis. (D) Snapshots of protein structures (shown in cartoon representation) at 0 ns, 50 ns, and 100 ns from four different runs. Six residue positions used in our ultrafast experiments are shown using yellow vdW spheres.

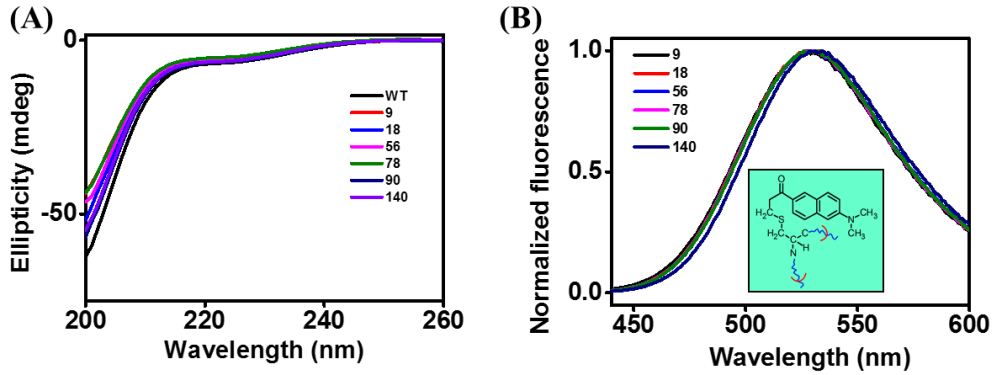

Figure S2 (A) CD spectra of wild-type and cysteine variants of α-synuclein. The CD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics, UK) at room temperature using 1 mm path length quartz cuvette. The protein concentration was 25 µM. (B) The steady-state fluorescence spectra for all acrylodan labeled single cysteine variants of α-synuclein.

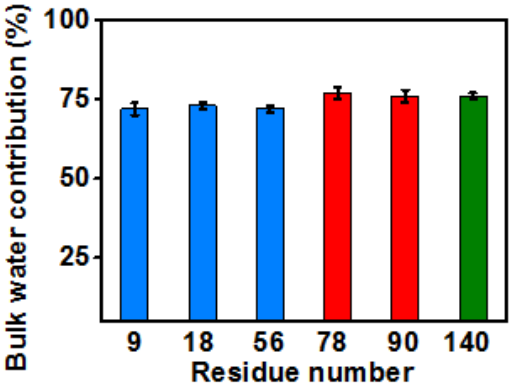

Figure S3 The contribution of bulk water obtained from fitting the solvation correlation function for acrylodan labeled cysteine variants of α-synuclein.

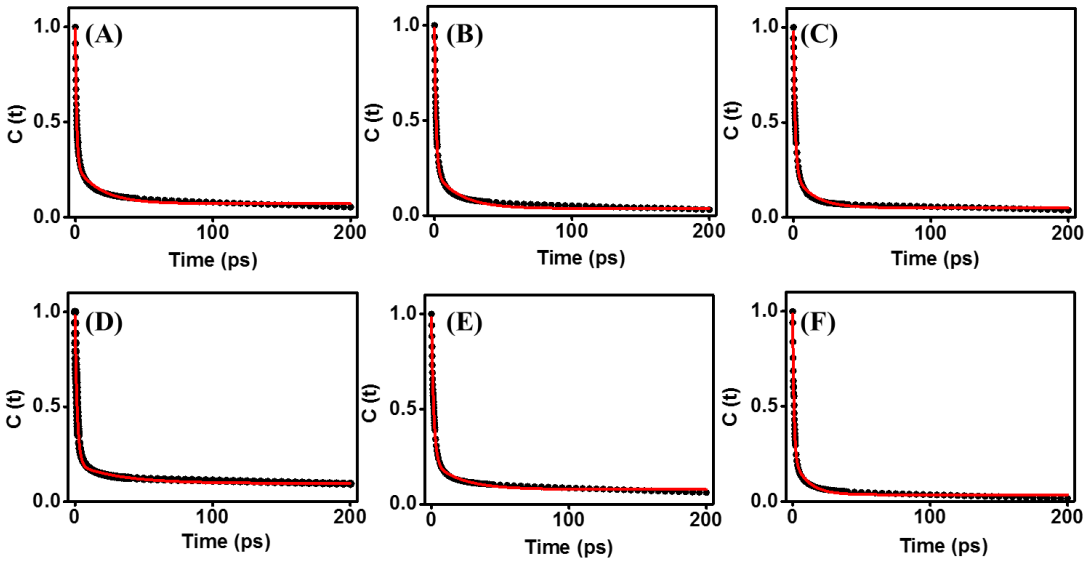

Figure S4 Representative individual C(t) plots (from 0 to 200 ps) for all acrylodan labeled cysteine variants of α-synuclein (A: 9, B: 18, C: 56, D: 78, E: 90, F: 140) are shown.

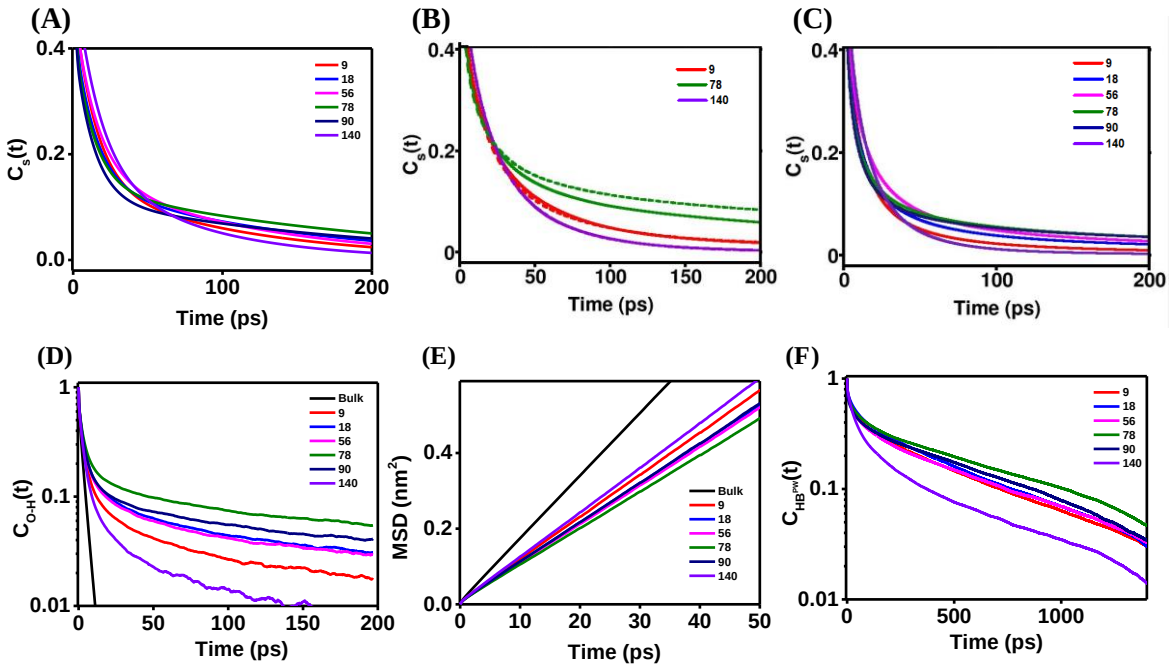

Figure S5 (A) Survival probability functions [$C_s(t)$] of hydration water molecules in the α-syn hydration shell around 6 different sites using SPC/E water model. (B) $C_s(t)$ of SPC/E hydration water around residue 9, 78, and 140, as obtained from two different runs [Run 1 (solid line); Run 2 (dashed line) from the same starting structure). (C) $C_s(t)$ profiles of TIP3P hydration water molecules around 6 different sites. (D) Linear-log plot of second-order re-orientational time correlation function $C_{O\text{-}H}(t)$ for all O-H bonds of hydration shell waters. (E) Mean square displacement, MSD, (in nm^2) as function of time (in ps) of hydration shell water. Black line in (D) and (E) represents data corresponding to bulk water. (F) Linear-log plot of protein-water H-bond time correlation functions, $C_{HB}(t)$.

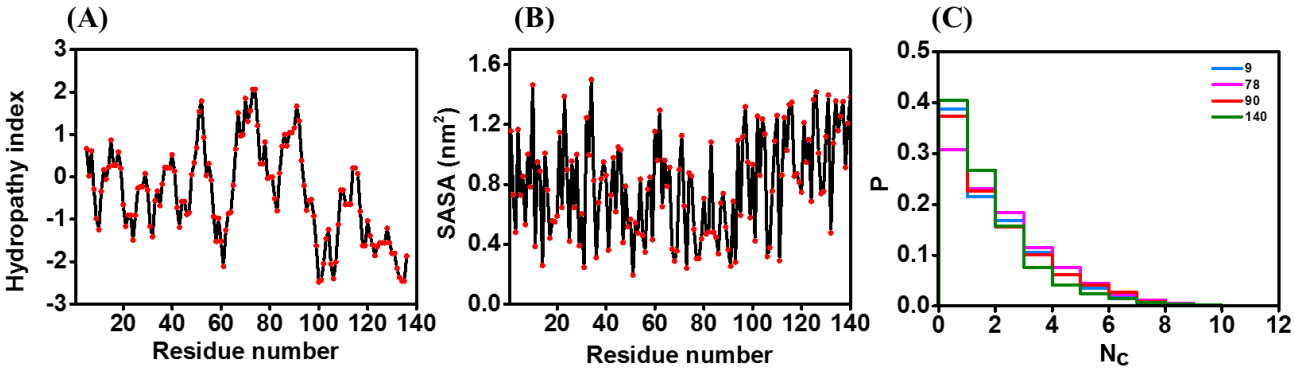

Figure S6 (A) Kyte & Doolittle hydropathy index of α-syn sequence estimated using the ProtScale webserver (http://web.expasy.org/protscale/) with a window size of 9. (B) Solvent accessible surface area (SASA) in nm^2 per residue of α-syn, as obtained from MD. (C). Probability distributions of number of protein sidechain carbon atoms that are within 4.5 Å of hydration water.

Table S1. Both forward and reverse primer sequences for the single cysteine variants of α-synuclein

Primer name	Primer sequence 5'-3'
S9C Fwd	GAAAGGACTTTGTAAGGCCAAGGAGGG
S9C Rev	CCCTCCTTGGCCTTTGAAAGTCCTTTC
A18C Fwd	GGGAGTTGTGGCTTGTGCTGAGAAAACCAAACAGGG
A18C Rev	CCCTGTTTGGTTTTCTCAGCACAAGCCACAACTCCC
A56C Fwd	GGTGTGGCAACAGTGTGTGAGAAGACC
A56C Rev	GCTCTTTGGTCTTCTCACACACTGTTGC
A78C Fwd	GGTGTGACAGCAGTATGCCAGAAGACAG
A78C Rev	CTCCACTGTCTTCTGGCATACTGCTGTC
A90C Fwd	GCAGGGAGCATTGCATGTGCCACTGGCTTTGTC
A90C Rev	GACAAAGCCAGTGGCACATGCAATGCTCCCTGC
A140C Fwd	CAAGACTACGAACCTGAATGCTAAGAAATATCTTTGCTCC
A140C Rev	GGAGCAAACATATTTCTTAGCATTCAGGTTCGTAGTCTTG

Table S2. Recovered solvation time components and their amplitudes obtained upon fitting the hydration correlation function $C(t) = \alpha_{bulk} e^{-t/\tau_{bulk}} + \alpha_{type-I} e^{-t/\tau_{type-I}}$

Residue number	τ_{bulk} (ps)	α_{bulk}	τ_{type-I} (ps)	α_{type-I}	$\alpha_{type-II}$ (Unrelaxed component)
9	1.0 ± 0.1	0.74 ± 0.02	19 ± 2	0.20 ± 0.02	0.06 ± 0.01
18	1.4 ± 0.2	0.74 ± 0.02	18 ± 2	0.20 ± 0.01	0.06 ± 0.01
56	1.4 ± 0.1	0.75 ± 0.04	15 ± 2	0.19 ± 0.03	0.07 ± 0.01
78	1.8 ± 0.1	0.77 ± 0.02	31 ± 4	0.10 ± 0.02	0.12 ± 0.02
90	1.6 ± 0.1	0.76 ± 0.02	21 ± 2	0.15 ± 0.02	0.09 ± 0.02
140	1.1 ± 0.1	0.79 ± 0.03	11 ± 1	0.20 ± 0.02	0.03 ± 0.01

The up-conversion data were fitted up to 200 ps, as shown in Figure 3. The recovered solvation times are referred to as τ_{bulk} and τ_{type-I} and their respective amplitudes (fractional contributions) as α_{bulk} and α_{type-I}. The amplitude of the (unrelaxed) residual solvation component is denoted as $\alpha_{type-II}$ and equals to 1-(α_{bulk} + α_{type-I}).

Table S3. Residence times (in ps) from tri-exponential fit of survival probability function $C_s(t) = \alpha_1{}^s e^{-t/\tau_1{}^s} + \alpha_2{}^s e^{-t/\tau_2{}^s} + (1 - \alpha_1{}^s - \alpha_2{}^s) e^{-t/\tau_3{}^s}$

Residue number	τ_1^s (ps)	α_1^s	τ_2^s (ps)	α_2^s	τ_3^s (ps)	α_3^s
9	0.84	0.50	14.48	0.35	110.7	0.15
18	0.83	0.55	13.87	0.31	144.47	0.14
56	0.84	0.51	15.14	0.33	117.87	0.17
78	0.83	0.56	13.09	0.30	196.89	0.14
90	0.80	0.57	12.68	0.31	189.11	0.12
140	0.75	0.44	16.38	0.37	76.10	0.18

The residence times (τ_1^s, τ_2^s, and τ_3^s) and corresponding amplitudes (α_1^s, α_2^s, and α_3^s) were extracted by fitting the 200 ps portion of $C_s(t)$ of hydration water to the tri-exponential function, such as $\alpha_1^s + \alpha_2^s + \alpha_3^s = 1$ (see Figure S5A). τ_1^s, τ_2^s, and τ_3^s correspond to the bulk water, type-I bound water, and type-II bound water, respectively.

Table S4. Translational self-diffusion constant [D (in x10^{-5} cm^2s^{-1})] and tri-exponential fitting parameters of O-H reorientational time-correlation function $C_{O-H}(t) = {\alpha_1}^{O-H} e^{-t/{\tau_1}^{O-H}} + {\alpha_2}^{O-H} e^{-t/{\tau_2}^{O-H}} + (1 - {\alpha_1}^{O-H} - {\alpha_2}^{O-H}) e^{-\frac{t}{{\tau_3}^{O-H}}}$

Residue number	D (nm^2s^{-1})	τ_1^{O-H} (ps)	α_1^{O-H}	τ_2^{O-H} (ps)	α_2^{O-H}	τ_3^{O-H} (ps)	α_3^{O-H}
Bulk	2.76	0.16	0.30	2.40	0.70	-	-
9	1.91	0.18	0.34	3.10	0.56	55.10	0.11
18	1.82	0.19	0.33	3.30	0.53	68.10	0.14
56	1.85	0.19	0.34	3.30	0.53	66.80	0.13
78	1.78	0.20	0.33	3.48	0.51	98.44	0.16
90	1.84	0.19	0.34	3.30	0.53	89.60	0.13
140	1.99	0.18	0.33	2.96	0.59	38.07	0.25

The reorientational time constants in ps (τ_1^{O-H}, τ_2^{O-H}, and τ_3^{O-H}) and corresponding amplitudes (α_1^{O-H}, α_2^{O-H}, and α_3^{O-H}) extracted from the tri-exponential fit of $C_{O-H}(t)$, see Figure S5D. τ_1^{O-H}, τ_2^{O-H}, and τ_3^{O-H} represent fast libration motion of water, whereas τ_2^{O-H} and τ_3^{O-H} describe slow water relaxation. Self-diffusion constant (D) was estimated from mean-square displacement plot of hydration and bulk water (See Figure S5E).

Table S5. Protein-water H-bond lifetimes (in ps) from tri-exponential fit of $C_{HB-PW}(t) = {\alpha_1}^{HB} e^{-t/{\tau_1}^{HB}} + {\alpha_2}^{HB} e^{-t/{\tau_2}^{HB}} + (1 - {\alpha_1}^{HB} - {\alpha_2}^{HB}) e^{-\frac{t}{{\tau_3}^{HB}}}$

Residue number	τ_1^{HB} (ps)	α_1^{HB}	τ_2^{HB} (ps)	α_2^{HB}	τ_3^{HB} (ps)	α_3^{HB}
9	1.12	0.27	43.98	0.35	530.6	0.38
18	0.89	0.26	46.12	0.32	534.4	0.42
56	0.72	0.32	48.29	0.33	594.9	0.35
78	0.61	0.31	48.87	0.30	724.3	0.39
90	0.36	0.33	39.41	0.29	637.6	0.38
140	3.30	0.30	50.36	0.48	484.7	0.22

The protein-water H-bond lifetimes (τ_1^{HB}, τ_2^{HB}, and τ_3^{HB}) and corresponding amplitudes (α_1^{HB}, α_2^{HB}, and α_3^{HB}) extracted from the tri-exponential fit of $C_{HB}^{PW}(t)$ (See Figure 5D and Figure S5F).