Electronic Supplementary Material (ESI) for ChemComm.

Supporting information for

Alteration of water absorption in THz region traces the onset of fibrillation in proteins

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Materials and methods

Bovine serum albumin (BSA) and ethyl alcohol (EtOH) were the products of Sigma-Aldrich and Merck, respectively with >99% purity and were used without any further distillation. Freshly prepared 10 mM phosphate buffer (PBS) with pH~7.4 was used as the solvent throughout the experiment. During the fibril formation the BSA concentration was kept fixed at 200 µM; it was denatured by 60% (V/V) EtOH and then incubated (Innova 42, Brunswick Scientific) at 62 ^{0}C for 6 hours. It was then kept at 20 ^{0}C for 24 hours to form confirm complete fibril formation[1-3]. Prior to every measurement the fibril was centrifuged for 10 minutes and then the supernatant liquid was used for the measurements.

Dynamic light scattering (DLS) measurements were carried out in Nano S Malvern instrument with 632.8 nm laser and the photons are collected at 173^0 scattering angle. Circular dichroism (CD) measurements were performed in Jasco 815 spectrophotometer. The secondary structural analysis was performed in CDNN software in the wavelength range between 190-260 nm. Absorption spectra of concentration dependent BSA were recorded in Shimadzu UV 2600 spectrophotometer. Fluorolog3 (Horiba, Jobin, Yvon, USA) fluorimeter was used to collect the steady state fluorescence spectra. We used time correlated single photon counting (TCSPC) form Edinberg Instrument (Life Spec-II, U.K.) to measure fluorescence lifetime (375 nm excitation with ~80 ps instrument response function, IRF). Morphologies of different BSA structures were studied using atomic force microscopy (AFM) in di INNOVA microscope in tapping mode. Prepared samples were drop casted on thoroughly cleaned silicon wafer substrate which were then kept inside desiccator to facilitate vacuum environment for 14 hours for vaporisation of water before the measurement of AFM images. The size of the various structures, as evident in AFM measurements, are estimated using Gwydion software. Terahertz (THz) frequency domain spectroscopy (TFDS) measurements were performed in Toptica (Tera-scan 1550). Details about this spectroscopy can be found elsewhere[4, 5]. Optical heterodyning technique is used to generate continuous wave (CW) THz radiation. Lights form two CW lasers (The wavelength of one CW laser is kept fixed at 1550 nm and the other one is tuneable having wavelength variation from 1550-1570 nm) are mixed in the photo-mixer to produce THz frequency exactly at the difference frequency of the lasers. Photocurrents, generated upon the application of dc bias voltage to the electrodes present in the photo-mixer, oscillates at

the beat frequency. An antenna near photo-mixer radiates electromagnetic wave in the THz frequency (0.1-1.2 THz). The typical power emitted is about 1 microwatt and beyond 1.2 THz the Signal to Noise is quite low due to the frequency fall-off response of the THz detector. Frequency dependent absorption coefficient was calculated using the following equation[6]

$$\alpha(v) = \frac{1}{d} ln(\frac{I_{ref}}{I_{sample}}) \quad (1)$$

where *d* is the thickness of sample, I_{sample} and I_{ref} are the transmission intensity of sample and reference, respectively. Nitrogen purged air medium is considered here as the reference.

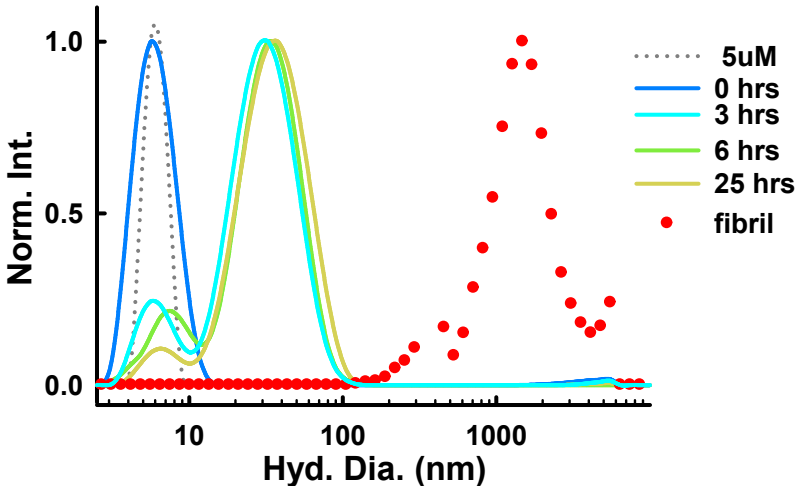

Figure S1. Scattering profile of BSA under different condition

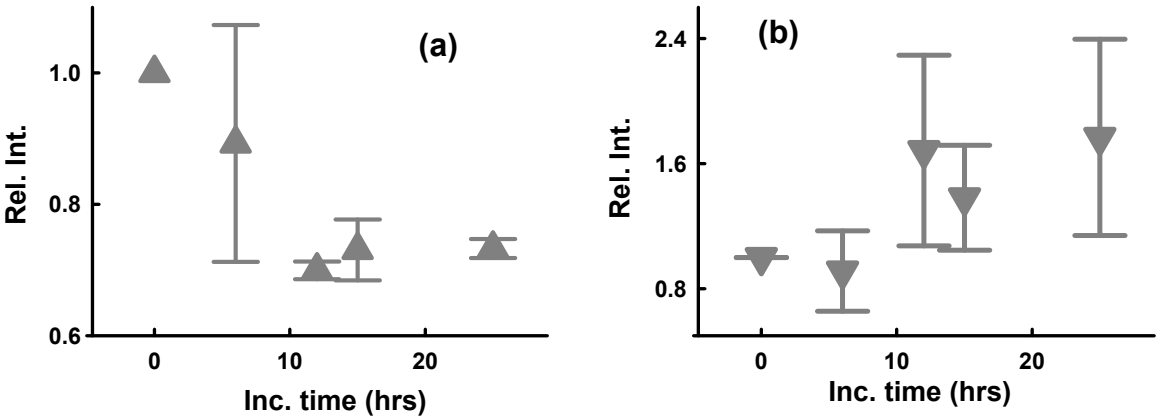

Figure S2. Relative fluorescence intensity (=$\frac{I_t}{I_0}$; I_t is the fluorescence intensity after different incubation time and I_0 is the fluorescence intensity without incubation) of BSA upon excited at (a) 295 nm and (b) 375 nm.

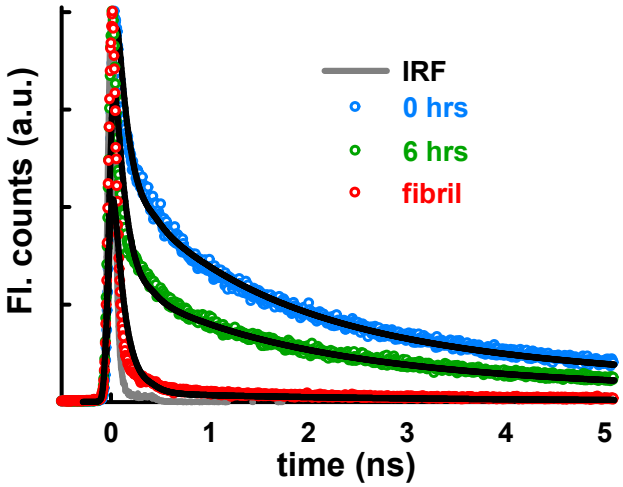

Figure S3. Representative Transient fluorescence decay profiles of different incubated BSA obtained from TCSPC. Excitation laser is 375 nm

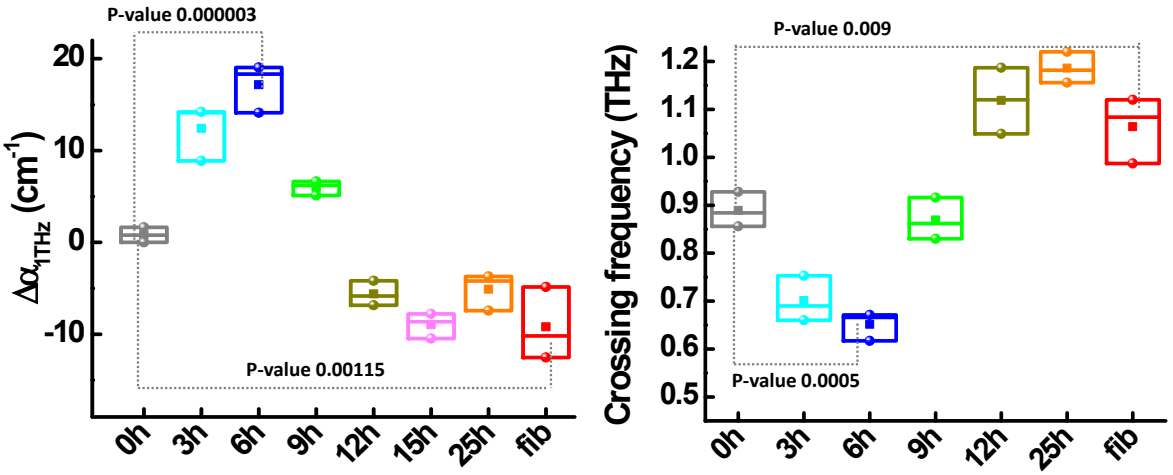

Figure S4. One-way ANOVA significance test (Tukey post hoc test) result for $\Delta\alpha_{1THz}$ (left panel) and crossing frequency (right panel) with significance level of 0.05. The changes (as shown by dotted lines) are significant as P value < significance level.

Table S1: Secondary structure information of BSA in different condition:

System	α-Helix (%)	β-Antiparallel (%)	β-Parallel (%)	β-Turn (%)	Random coil (%)
Buffer	62.83 ± 0.55	2.99 ± 0.42	4.05 ± 0.18	13.30 ± 0.37	16.83 ± 0.36
3 hr	55.94 ± 0.57	4.05 ± 0.43	5.04 ± 0.16	14.57 ± 0.38	20.44 ± 0.35
6 hr	45.23 ± 0.58	5.95 ± 0.36	6.69 ± 0.09	16.00 ± 0.27	26.10 ± 0.42
9 hr	45.18 ± 0.54	6.13 ± 0.30	6.66 ± 0.11	16.07 ± 0.33	25.96 ± 0.39
15 hr	48.87 ± 0.54	5.40 ± 0.33	6.08 ± 0.12	15.65 ± 0.39	24.00 ± 0.38
20 hr	52.28 ± 0.61	4.77 ± 0.37	5.58 ± 0.16	15.18 ± 0.42	22.16 ± 0.37
25 hr	46.22 ± 0.55	5.94 ± 0.30	6.49 ± 0.12	16.00 ± 0.36	25.31 ± 0.42
fibril	12.52 ± 1.37	30.85 ± 3.38	9.57 ± 1.74	15.32 ± 1.03	31.74 ± 4.06

Table S2: Time resolved fluorescence data of BSA after different time of incubation.

time (hrs)	a_1	t_1 (ns)	a_2	t_2 (ns)	a_3	t_3 (ns)	t_{avg} (ns)
0	0.65	0.10	0.23	1.39	0.12	6.53	1.14
1	0.77	0.10	0.16	1.51	0.07	6.82	0.81
3	0.80	0.10	0.14	1.58	0.06	6.98	0.72
6	0.81	0.10	0.14	1.58	0.05	7.07	0.64
12	0.83	0.10	0.12	1.60	0.05	7.05	0.60
15	0.83	0.10	0.12	1.59	0.05	7.05	0.60
25	0.83	0.10	0.12	1.63	0.05	7.24	0.61
fibril	0.98	0.10	0.02	2.65	--	--	0.15

Notes and References:

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