Electronic Supporting Information

Is DAPI assay of cellular nucleic acid reliable in the presence of protein aggregates?

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Experimental Methods

JASCO UV-Visible spectrophotometer (Model: V530, Japan). Shimadzu spectrofluorimeter (Model: RF-6000, Japan) were used to record ground state absorption and steady state fluorescence spectra, respectively. For emission measurements, all samples were excited with 340 nm light. Time-correlated single photon counting (TCSPC) technique was employed for measuring time dependent changes in the fluorescence intensity. All time-resolved measurements were performed under magic angle polarization condition and samples were excited with 375 nm laser diode having instrument response function (IRF) of ~140 ps. Decay time constants were obtained by fitting the fluorescence decays with multi exponential decay function by non-linear least square method. The average lifetime (τ_{avg}) was calculated using the following equation.[1]

$$\tau_{avg} = \frac{\sum_i a_i \tau_i^2}{\sum_i a_i \tau_i} \quad \text{(S1)}$$

where τ_i and a_i represents the lifetime and amplitude of i^{th} decay component respectively.

The changes in the emission intensity due to the addition of fibrils have been analyzed using following equation for 1:1 complex.[2]

$$\Delta I_f = \Delta I_f^{\infty}\left(1 - \frac{\{K_b[DAPI] - K_b[fibril] - 1\} + \sqrt{(K_b[DAPI] + K_b[fibril] + 1)^2 - 4K_b^2[DAPI][fibril]}}{2K_b[DAPI]}\right) \quad \text{(S2)}$$

Where ΔI_f^{∞} is the change in the fluorescence intensity when all DAPI binds to fibrils. [DAPI] and [fibril] are the total concentrations of DAPI and fibril in the solution, respectively. K_b is the binding constant.

Fluorescence images of DAPI stained insulin fibrils were obtained by using a Zeiss confocal fluorescence microscopic setup (LSM 710). A clean glass slide is used as a substrate for the deposition of DAPI stained fibrils. Cleaning of the glass slides were carried out by sonication in iso-propanol bath for 5 minutes.

The molecular structure of DAPI in its ground electronic state was optimized by DFT theory using B3LYP functional in combination with 6-311++g(d,p) basis function as implemented in Gaussian 03 package.[3-5] Conductor like polarizable continuum model (CPCM) was used to consider the contribution of water as solvent.[6] The optimized structure thus obtained (cf. figure S3) along with its Mullikan atomic charges have been used for the molecular docking studies using the AutoDock4 suites.[7] Default values for all parameters as implemented in AutoDock 4 were used for the docking studies. The maximum energy evaluation used for the present work was 5×10^6 and with 500 runs. The grid volume used in the present study was such that the whole protein aggregate can be accessible to DAPI. Fibril of a variant of $A\beta_{1\text{-}40}$ peptide which is associated with the early onset of Alzheimer's disease (pdb id 2MVX) has been used for docking studies. The peptide corresponding to 2MVX is more prone to fibrillation and also more neurotoxic compared to its wild type variant.

Insulin from bovine pancreas, $A\beta_{1\text{–}42}$ Calfthymus DNA (CTDNA), DAPI, NaCl were purchased from Sigma-Aldrich and used as received. Nanopure water with resistivity of 18.2 MΩ cm (at 25°C) was used for all studies. Analytical grade acetic acid was used for the insulin fibril preparation. The insulin fibrils were prepared by heating 2 mg/ml bovine insulin solution in 20% acetic acid (pH 1.6) at 70°C for 24 hours with continuous agitation.[8] ThT fluorescence assay was used to confirm the fibril formation.[9] The fibrils were further diluted 12 times by distilled water and the pH was adjusted with dilute NaOH solution to physiological pH, 7.4. The stock solution of CTDNA was prepared by adding solid sample in water and kept it for 24 h under stirring condition. The final concentration of DNA was determined by recording its absorption spectra and using its reported molar extinction coefficient value of 6600 M^{-1} cm^{-1} at 260 nm.[10]

Lyophilized A$\beta_{1\text{-}42}$ was dissolved in HFIP and kept at room temperature for 2 h to dissociate all preformed protein aggregates. HFIP was removed by N_2 gas purging and the peptide was dissolved in 50 mM phosphate buffer (50 mM, pH 7.4) to a final concentration of 2 μM and incubated for 15 hours to ensure complete fibrillation. The fibril formation was confirmed by Thioflain T assay.

Cellular studies were performed with SH-SY5Y (neuroblastoma) cells purchased from European Collection of Authenticated Cell Cultures. The cells were cultured in DMEM medium supplemented with FBS (10%), penicillin (100 U/ml), and streptomycin (100 μg/ml). The cells were grown in an incubator (37 ^{o}C, 5% CO_2). Cells (5×10^4 cells/well) were seeded for overnight in a 6-well plate, containing glass cover slips. For cellular uptake of fibrils, cells were treated with A$\beta_{1\text{–}42}$ fibril solution (200 nM) for 24 h as reported by Hu et al.[11] Further, cells were washed thoroughly with PBS and fixed with paraformaldehyde (5%, 10 min) and chilled methanol for overnight. The fixed cells were washed twice with PBS and permeabilized with PBST (0.1% Tween 20 in PBS) for 10 min. Cells were blocked with 5% BSA for 2 h, incubated with primary antibody against A$\beta_{1\text{–}42}$ fibril (1:2000, 2.5% BSA in PBST) (#ab201061, Abcam) for overnight (4 ^{o}C). After washing with PBST, secondary antibody tagged with Alexa Fluor-488 (1:2000, 2.5% BSA in PBST) (# R37118) were added (3 h). Cells were washed thrice with PBST, coverslips were dried and mounted on slides with DAPI solution (10 μM in 80% glycerol). Mounted slides were then analysed with a confocal microscope (LSM 780, Carl Zeiss, Germany). Image analysis was performed using Zeiss Zen software. Approximately 50 nuclei were analysed for each sample

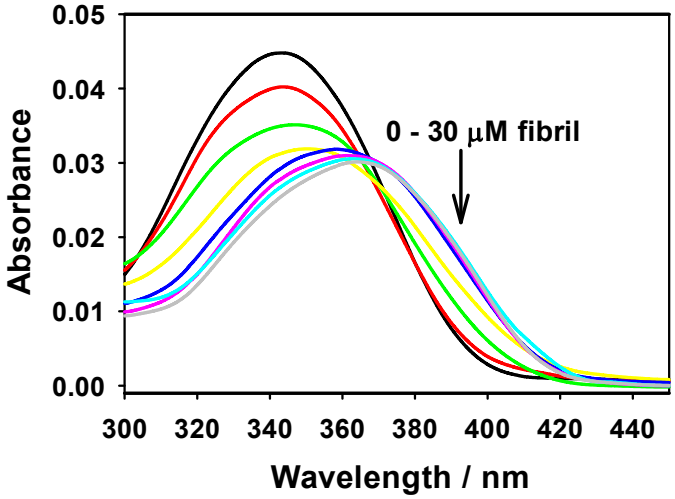

Figure S1 Absorption spectra of DAPI (0.7 μM) in water at different concentrations of insulin fibrils (0-30 μM).

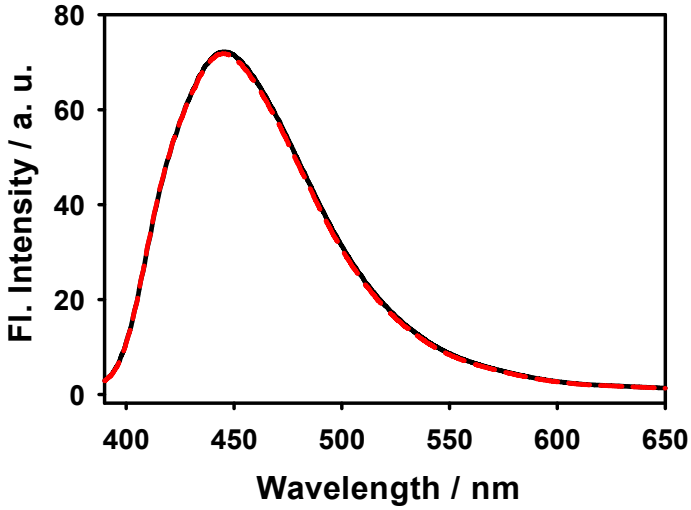

Figure S2 Fluorescence spectra (λ_{ex}=340 nm) of DAPI (0.55 μM) in insulin fibril (30 μM) at 7.4 pH in absence (black solid) and presence of 0.5 M NaCl (red dashed).

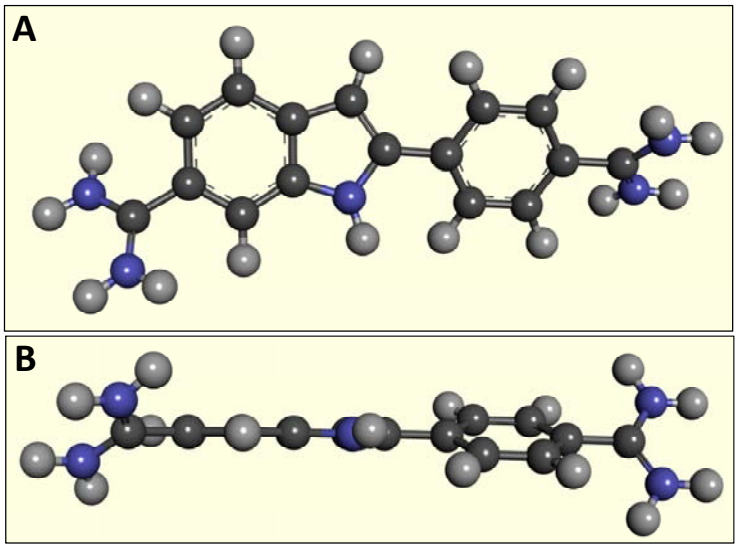

Figure S3 The ground state optimized geometry of DAPI in water: (A) Top view & (B) Side view.

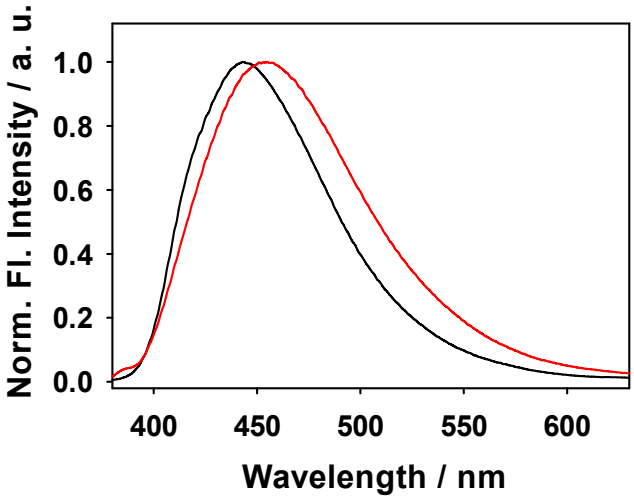

Figure S4 The peak intensity normalized emission spectra of DAPI in 30 μM DNA (red) and 30 μM insulin fibrils (black).

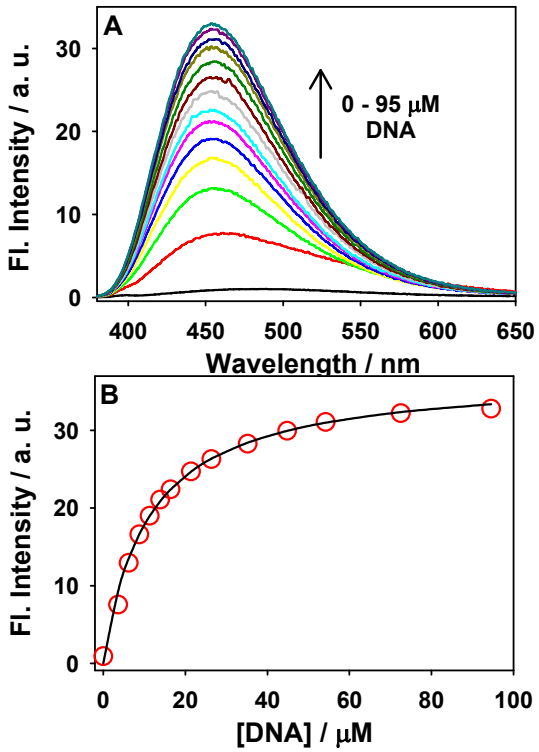

Figure S5 (A) Changes in the emission spectra of DAPI with DNA concentrations. (B) Variation in the emission intensity of DAPI at 458 nm with DNA concentration. The solid line is the fitting of the experimental data by eq. S2. Such fitting provides the binding constant value of 0.92±0.06 x 10^5 M^{-1}

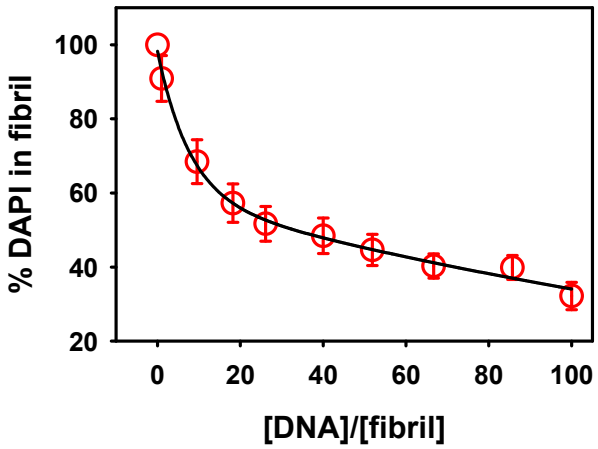

Figure S6 The variation in the percentage of DAPI bound to insulin fibrils with DNA concentrations. The concentration of insulin fibrils has been kept constant at 30 μM.

Table S1: Fitted parameters for emission transient decays s of DAPI-fibrils solutions.

[fibril]/ μM	a_1	τ_1 /ns	a_2	τ_2 /ns	a_3	τ_3 /ns	τ_{avg} /ns
0	82.2	0.15	0.0	0.00	17.8	2.01	0.48
0.18	57.3	0.16	12.9	0.81	29.8	2.29	0.88
0.52	37.1	0.19	24.7	0.79	38.2	2.32	1.15
0.70	27.5	0.25	39.3	0.85	33.2	2.25	1.15
1.38	17.5	0.28	48.2	0.76	34.3	2.38	1.23
2.05	15.1	0.2	41.2	0.69	43.7	2.29	1.32
3.33	7.9	0.32	33.5	0.75	58.6	2.22	1.58
6.32	3.3	0.3	29.5	0.68	67.2	2.18	1.68
9.00	1.1	0.32	22.5	0.72	76.4	2.12	1.79
13.64	0.0	0.00	17.7	0.75	82.3	2.15	1.9
22.22	0.0	0.00	16.9	0.62	83.1	2.13	1.87

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