**Electronic Supplementary Information**

# Inhibition and Disintegration of Insulin Amyloid Fibrils: A Facile Supramolecular Strategy with *p*-Sulfonatocalixarenes

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## Experimental section

### Materials and instrumentation

Human insulin was obtained from Sigma-Aldrich and was used as received. *p*-sulfonatocalix[4]arene was purchased from Aldrich and *p*-sulfonatocalix[6]arene was purchased from Alfa Aesar and used without further purification. Thioflavin T (ThT) obtained from Sigma-Aldrich was purified by column chromatography on a silica gel column with mildly acidic methanol (1 mL of 1 N HCl in 500 mL of methanol) as eluent. The purity was further confirmed by the $^1$H NMR, which showed only the corresponding peaks as reported for ThT.[1] Nanopure water (Millipore Gradiant A10 System; conductivity of 0.06 μS cm$^{-1}$) was used throughout for solution preparation. The sample solution was prepared by dissolving human insulin (1.5mg/ml) in 25 mM of HCl, 0.1 M NaCl (pH ~1.7) and ~10 μM ThT was added to this solution, as reported in the literature.[2] The net solution was incubated at 60°C. For the fibril inhibition, SCX was added to the insulin solution before incubation. Initially a white turbid solution was obtained upon addition of SCX which becomes clear after 30 minutes incubation at 60°C. Small aliquots (~200μL) of samples were drawn at regular time intervals, diluted judiciously with blank solvent and fibril formation was monitored by recording the absorption, fluorescence spectra and fluorescence decay traces of the sample solution.

Steady-state fluorescence spectra were recorded using a Hitachi F-4500 spectrofluorimeter (Tokyo, Japan). For steady state fluorescence measurements, the samples were excited at 390 nm. Circular dichroism (CD) studies were carried out on a Biologic spectrometer (MOS-500). The spectra were measured in the wavelength range 200−400 nm using a quartz cuvette with 1.0 cm path length. Fluorescence lifetime measurements were carried out using a time-correlated-single photon-counting (TCSPC) spectrometer (IBH, UK). In the present work, a 374 nm diode laser (~100 ps, 1 MHz repetition rate) was used for excitation and a MCP PMT was used for fluorescence detection. From the measured decay traces, the time constants were evaluated following a reconvolution procedure.[3] The fluorescence decays, I(t) were analyzed using a multi exponential function as

$$I(t) = \sum B_i \exp(-t/\tau_i) \qquad \text{(S1)}$$

where, $B_i$ and $\tau_i$ are the pre-exponential factor and fluorescence lifetime for the i$^{th}$ component, respectively. Reduced chi-square ($\chi^2$) values and random distribution of the weighted residuals among data channels were used to judge the acceptance of the fits. The binding constant value between SCX and insulin has been estimated by following the fluorescence changes of DAPI-SCX6 system[4] at pH 2 with gradual addition of insulin and found to be ~ 4.2x10$^5$ M$^{-1}$.

The atomic force microscopic (AFM) images were recorded in semi-contact mode using a NT-MDT solver model P47 instrument with 50 μm scanner head and silicon nitride tip. The sample for AFM measurement was prepared by drop casting a dilute solution on a mica sheet, followed by drying.

Zeta potentials were determined with a Nanosizer Z (Malvern Instruments, Malvern, UK) by phase analysis light scattering. The light source was He-Ne laser operated at 633 nm operating at 4 mW. The zeta potential (ζ) values were calculated from the electrophoretic mobility data using Smoluchowsky approximation. The experiment was carried out using a quartz cuvette (universal 'dip' cell) with 10 mm light pathway.

**MTT Assay:** MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay presents a quantitative colorimetric method for studying cytotoxic agents, where the amount of MTT reduced by cells to its blue formazan derivative is quantified spectroscopically at 570 nm and is equivalent to the number of viable cells. In our experiment we have used Chinese Hamster Ovary CHO cell and DMEM as the cell culture medium. MTT assay has been carried out for the fibril solutions in the absence and presence of pre- and post-added SCXs which were incubated at 37 °C for 48 hours in CHO cells. The CHO cell without sample has been used as the control/reference. The cell viability of the solutions has been determined by considering 100% cell viability for the control CHO cell.

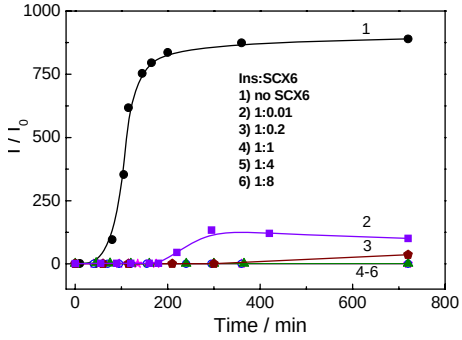


**Figure S1**. Fluorescence titration curve of ThT in insulin (250 µM) with different concentrations SCX6.

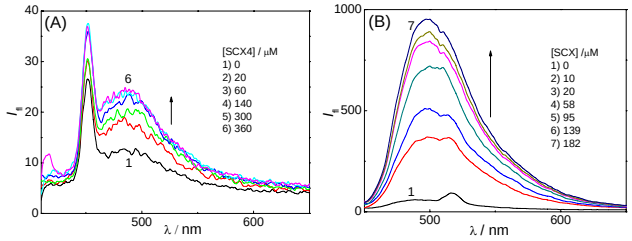


**Figure S2.** The fluorescence spectra of ThT at different concentrations of SCX4 (A) and SCX6 (B).

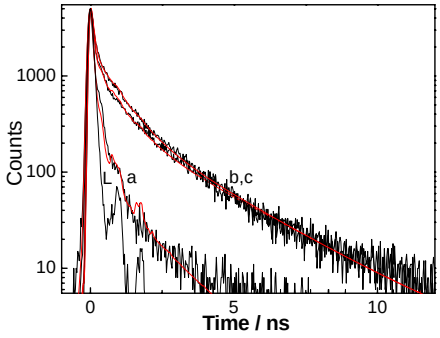


**Figure S3.** Fluorescence decay traces of ThT in human insulin before incubation (**a**) and in 1:1 molar ratio of insulin and SCX4 before (**b**) and after (**c**) incubation.

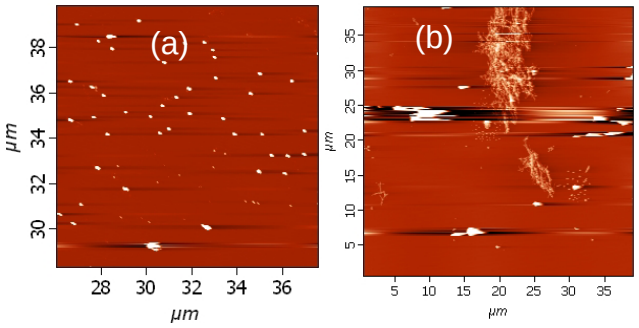


**Figure S4.** AFM images of disintegration mature fibril with SCX4 (**a**) and SCX6 (**b**).

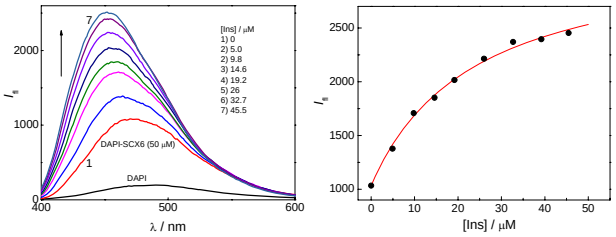


**Figure S5.** (A) The fluorescence spectra of SCX6-DAPI system at different concentrations of insulin at pH 2. (B) Increase in the fluorescence intensity with increasing concentration of insulin. Solid line represents the fitted binding curve.

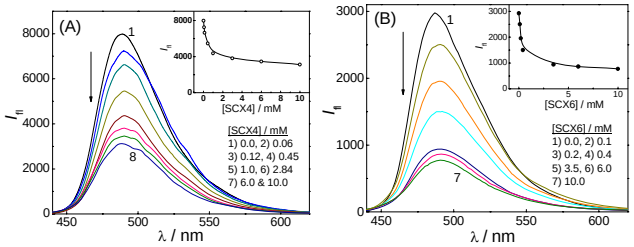


**Figure S6.** The fluorescence spectra of ThT in insulin fibril at different concentrations of SCX4 (A) and SCX6 (B). Respective insets show the decrease in the fluorescence intensity of ThT with increasing concentration of SCXs.

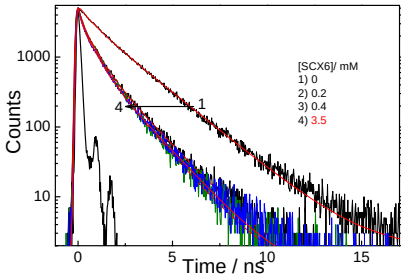


**Figure S7.** The time-resolved fluorescence decay of ThT in insulin fibril at different concentrations of SCX6.

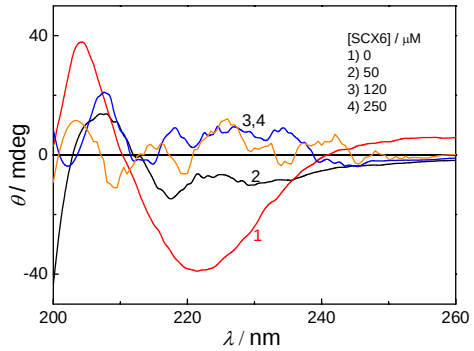


**Figure S8.** The CD spectra of insulin fibril at different concentrations of SCX6. [SCX]/μM: (1) 0, (2) 50, (3) 120 and (4) 250.

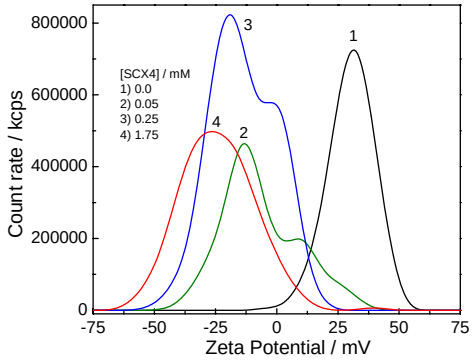


**Figure S9.** Zeta potential curves of mature fibril with and without SCX4.

**Table S1:** Zeta potential values measured for mature fibril with and without different concentrations of SCX.

| System | Zeta potential (mV) |
|---|---|
| Fibril | 30.6 ± 2.00 |
| Fibril-SCX4 (0.05mM) | -5.6 ± 0.1 |
| Fibril-SCX4 (0.25mM) | -13.5 ± 0.2 |
| Fibril-SCX4 (1.75mM) | -22.9 ± 0.2 |
| Fibril-SCX6 (0.05mM) | -8.5 ± 0.4 |
| Fibril-SCX6 (0.25mM) | -13.3 ± 0.8 |
| Fibril-SCX6 (1.75mM) | -24.3 ± 0.8 |

REFERENCES

1 E. I. The Aldrich Library of NMR Spectra, C. J. Pouchert, Vol. 2, p 578.
2 V. Fodera, F. Librizzi, M. Groenning, M. Weert and M. Leone, *J. Phys. Chem. B*, 2008, **112**, 3853-3858.
3 J. R. Lakowicz, *Principles of fluorescence spectroscopy*, Springer, New York, 2006.
4 M. N. Shinde, A. C. Bhasikuttan and J. Mohanty, *Supramol. Chem.*, 2015, DOI:10.1080/10610278.10612015.11126591.