Supporting Information

Sensing and Modulation of Amyloid Fibrils By Photo-switchable Organic Dots†

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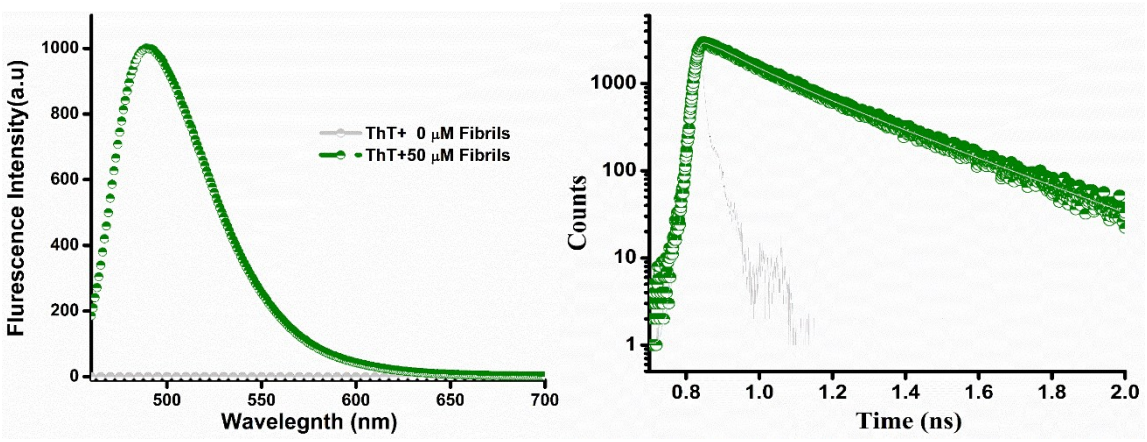


Fig. S1 Confirmation of fibril formation by Thioflavine-T.

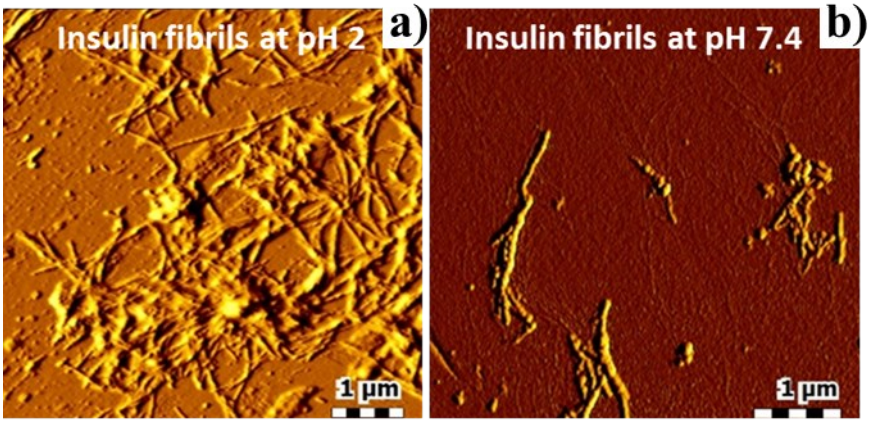


Fig. S2 a) AFM topography images of the a) fibrils at pH 2 and b) fibrils at 7.4.

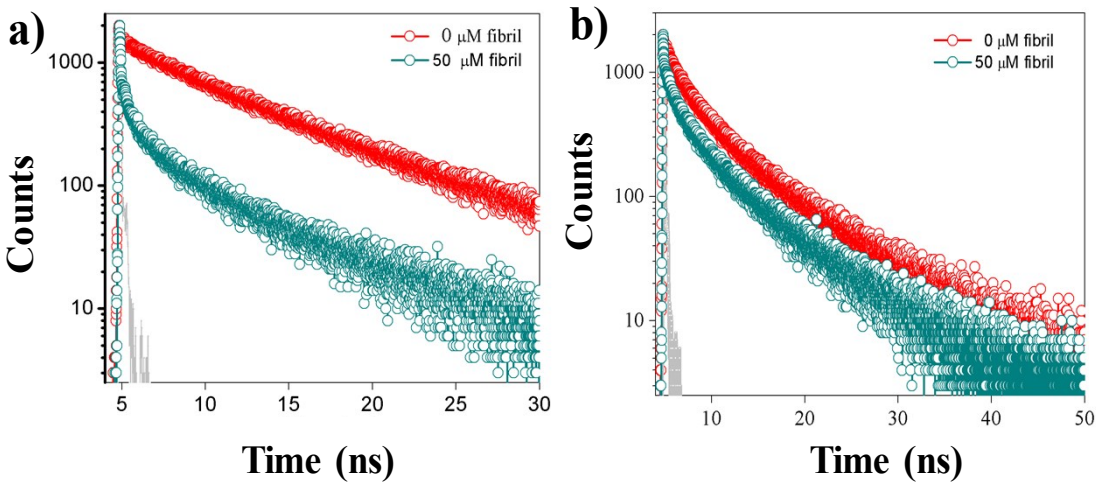
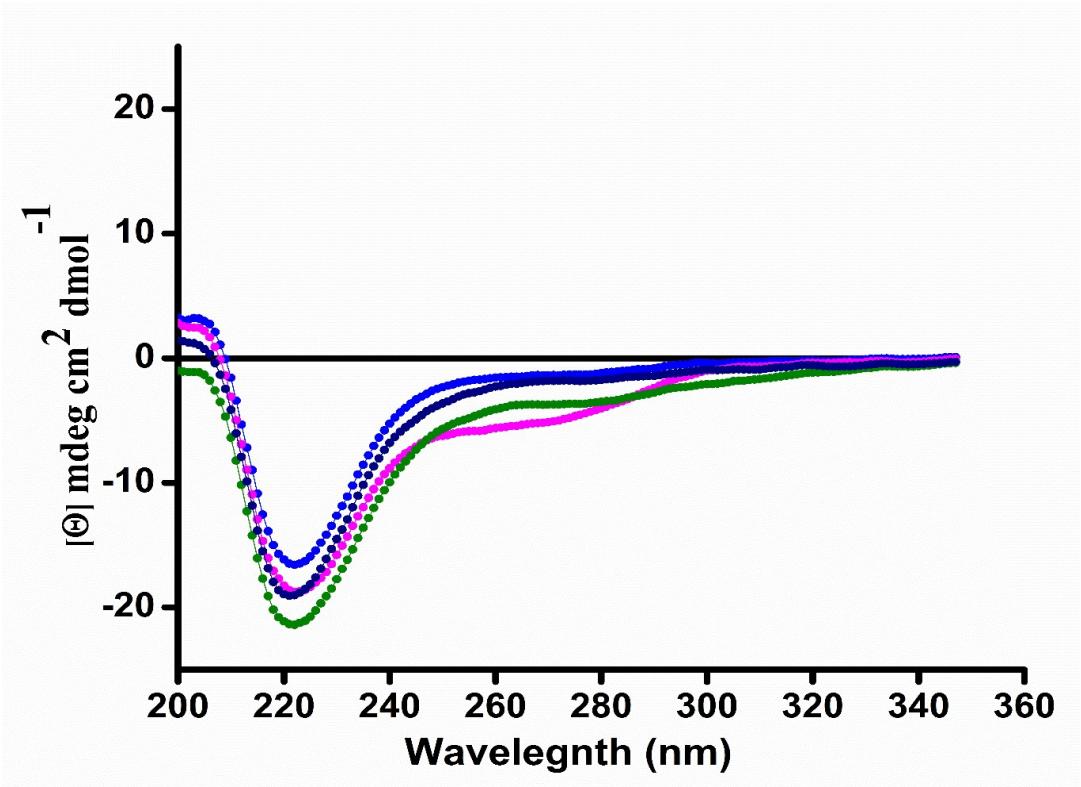


Fig. S3 Emission transient decay for DPAPMI (a, λ_{col} =550 nm) and CPMI (b, λ_{col} =510 nm)



in the presence of 0 and 50 μM of insulin fibrils (λ_{ex} = 375).

Fig. S4 CD spectra of 50 μM insulin fibrils (blue), 50 μM insulin fibrils with CPMI (black), 50 μM insulin fibrils with DPAPMI (green), and 50 μM insulin fibrils with DMAPMI (green) at pH 7.4.

Table S1 The Life time data plot of DPAPMI, CPMI and DMAPMI with and without fibrils.

Probes	[Fibril] (μM)	λ_{ex}	λ_{col}	A_1	τ_1 (ns)	A_2	τ_2 (ns)	A_3	τ_3 (ns)	$<\tau_{av}>^{\#}$ (ns)
DPAPMI	50	375	435	0.53	0.054	0.28	0.547	0.19	3.15	0.770
CPMI	50	375	420	0.40	0.181	0.35	1.834	0.26	6.54	2.38
DMAPMI	50	375	435	0.55	0.053	0.27	0.703	0.19	3.75	0.913

Table S2 The Life time data plot of DPAPMI, CPMI and DMAPMI with and without fibrils.

Probes	[Fibril] (μM)	λ_{ex}	λ_{col}	A_1	τ_1 (ns)	A_2	τ_2 (ns)	A_3	τ_3 (ns)	$<\tau_{av}>^{\#}$ (ns)
DPAPMI	0	375	550	0.44	0.220	0.33	2.10	0.24	6.73	2.350
	50	375	550	0.66	0.043	0.20	0.706	0.14	5.10	0. 863
CPMI	0	375	510	0.40	0.181	0.35	1.834	0.26	6.54	2.38
	50	375	510	0.27	0.403	0.34	2.50	0.39	1.03	4.99

$^{\#}\tau_{av} = a_1\tau_1 + a_2\tau_2 + a_3\tau_3$, we have considered the average lifetime values for explanation.

To investigate the generality over other biocompatible peptide/protein of this fluorescence probes, we have tested our designed probes with BSA fibrils. Which show that these probes are good sensors for the detection of BSA amyloid fibrils (Fig. S6a, b, c).

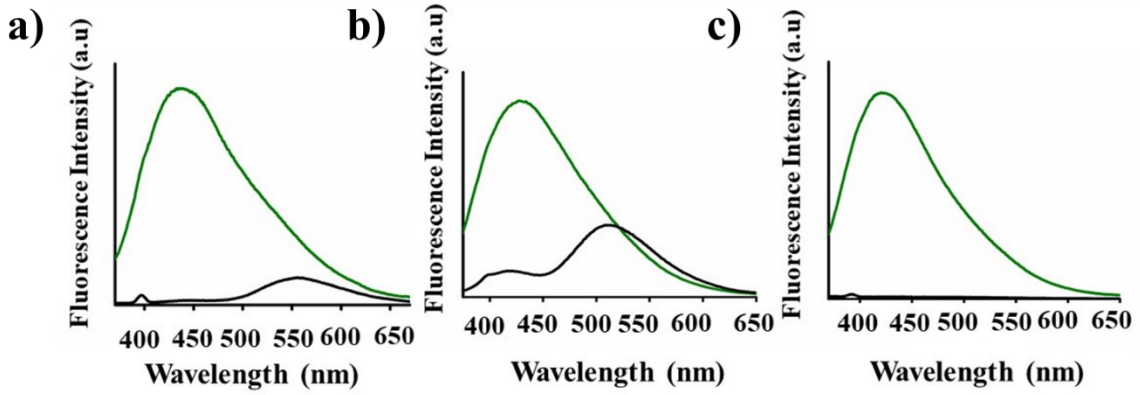


Fig. S5 a), b), c) Emission spectra (λex=350 nm) of DPAPMI, CPMI and DMAPMI, respectively in the presence of 0 (black) and 50 μM BSA fibrils (green).

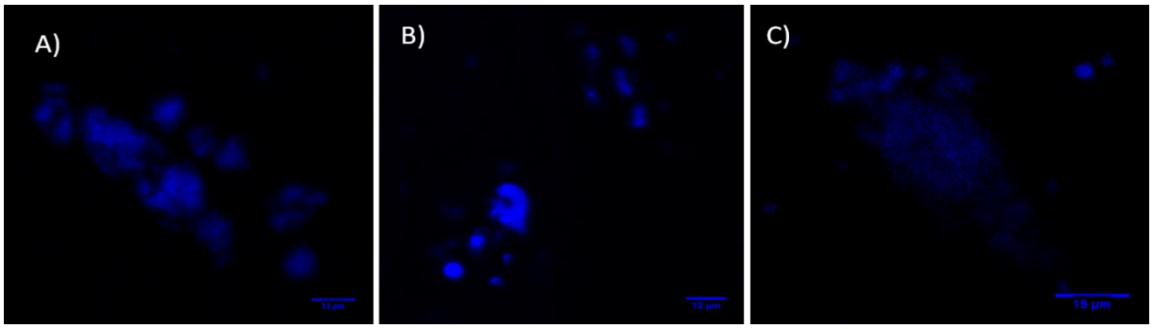


Fig. S6 Confocal images of fibrils stain with probes (λex = 405 nm) A) fibrils stain with DPAPMI, B) fibrils stain with CPMI, C) fibrils stain with DMAPMI.

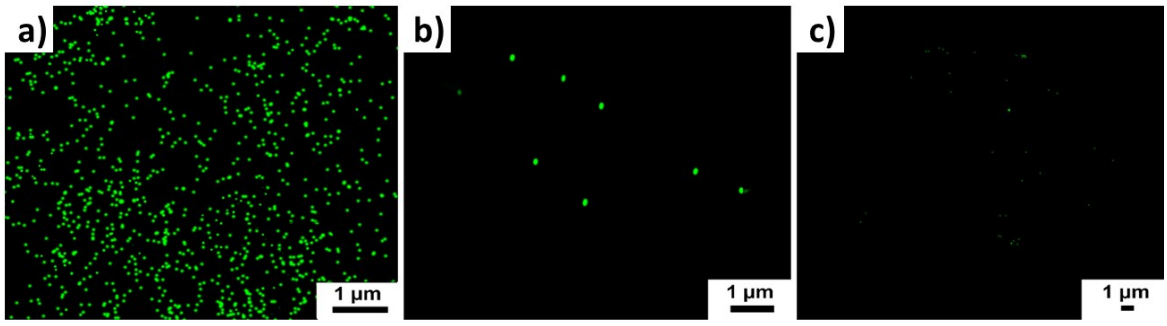


Fig. S7 d-STORM super-resolution microscopic images of insulin fibrils stained with our probe molecules. a) 4 μM DPAPMI in buffer, b) 4 μM CPMI in buffer and c) 4 μM DMAPMI in buffer.

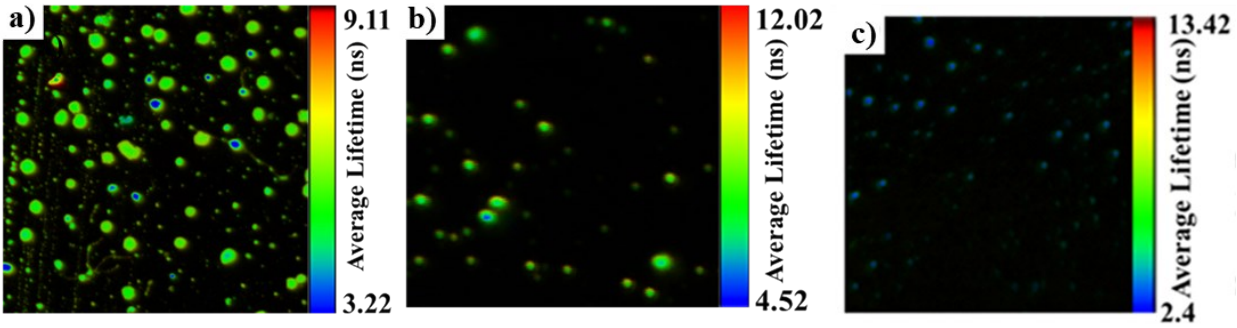


Fig. S8 FLIM-Z stack (400×400 pixel) images (λ_{ex} = 405 nm, λ_{col} =430-700 nm) of fluorescents probes with and without fibrils; a) DPAPMI in buffer b) CPMI in buffer c) DMAPMI in buffer.

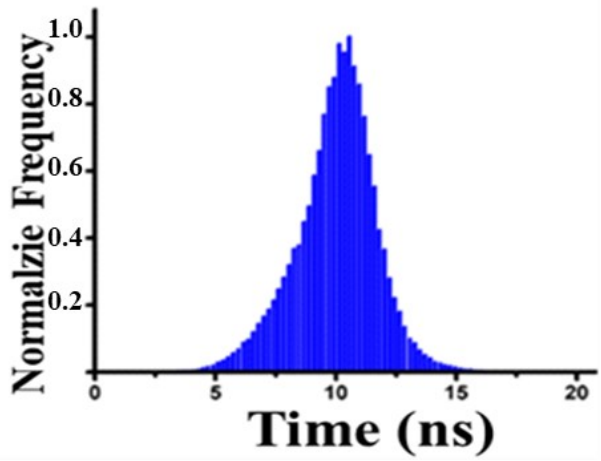


Fig. S9 Lifetime distributions of DMAPMI in the presence of insulin fibrils.

Note S1: Mipar calculation information: Recipe for image processing

Mipar[1, 2] calculation is an image processing tool, which is performed in a right order and with the right setting, in order to identify the hiding features inside an image. Below is elaboration of some typical post processing steps that we have used in this study to create our required images.

1. *Scale calibration*: Allows us to calculate the pixel size in units of length rather than pixels.
2. *Highlight Lines*: Uses the Frangi filter[3] to highlight linear-type features in the image. Combine with a threshold afterwards to select certain linear-type features.
3. *Fiber selection*: Balance fiber vs. background selection by selecting pixels based on whether they are above or below a certain pixel value. *Optimization of selection* was performed at this step by selection matching algorithm. It is perhaps the most critical yet less explored area of image processing to quantify the accuracy of the image-processing algorithm in question. MIPAR™ has provide with such facility where we can optimize parameter(s) to produce best similarity of the segmentation between the processed and the selected image.
4. *Uniform erosion*: Shrinks selection, if needed, by a specified number of pixels in all directions.
5. *Reject Features*: Which can remove or keep features based on a variety of shape and size metrics.
6. *Background*: Invert fiber selection.

Feature measurements: It allows one to measure a number of feature properties based on the features selected in the image. This includes, size parameters (area, area fraction, caliper diameter, etc.) mand shape parameters (roughness, aspect ratio, eccentricity, roundness, etc.)

Few important definitions:

Eccentricity: First an ellipse with same second moments as the feature was fitted. Then eccentricity is calculated as, $\sqrt{\left(1-\left(\frac{minor\ axis\ length}{major\ axis\ length}\right)^2\right)}$. It describes how elongated or circular each feature is. 0 is a perfect circle, 1 is a straight line.

Major axis length: Calculated as, $2\sqrt{2}\left(\sqrt{U_{xx}+U_{yy}+common}\right)$

Minor axis length: Calculated as, $2\sqrt{2}\left(\sqrt{U_{xx}+U_{yy}-common}\right)$

Common = $\sqrt{((U_{xx}-U_{yy})^2+4U_{xy}^2)}$

The second moments are calculated as: $U_{xx} = \frac{Sum(x^2)}{N} + 1/12$; $U_{yy} = \frac{Sum(y^2)}{N} + 1/12$; $U_{xy} = Sum(xy)/N$, where, x and y are the coordinate of each pixel in the feature, N is the number of pixels in each feature.

Roundness: Ratio of equivalent diameter to caliper diameter.

Aspect ratio: Ratio of major and minor axis length.

Roughness: Ratio of area of convex hull and area of each feature.

Area fraction: Count all the selected pixels in the reconstructed image and divides by the total number of pixels in the entire image.

Caliper diameter: Largest line length that fits across each feature.

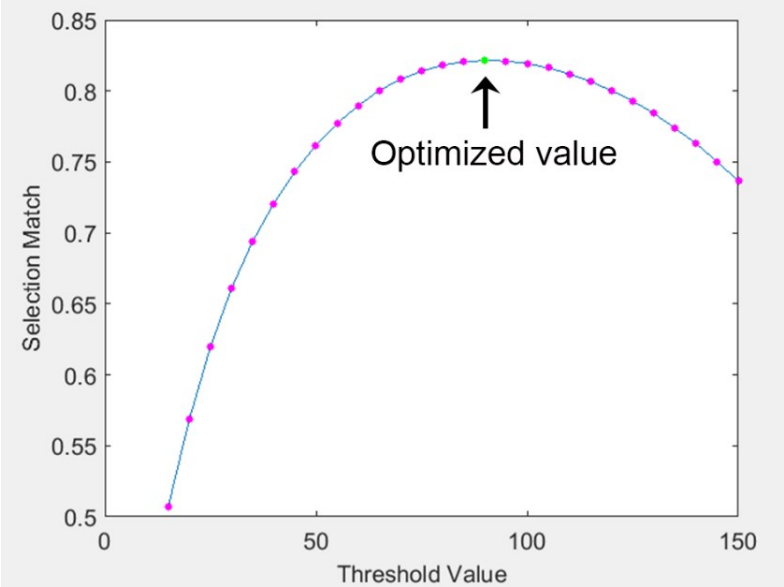


Fig. S10 Typical single parametric optimization of fiber selection strength using selection match algorithm.

References

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2. D. J. Magginetti, J. A. Aguiar, J. R. Winger, M. A. Scarpulla, E. Pourshaban and H. P. Yoon, *Advanced Materials Interfaces*, 2019, **6**, 1900300.
3. I. C. Campbell, B. Coudrillier, J. Mensah, R. L. Abel and C. R. Ethier, *Journal of The Royal Society Interface*, 2015, **12**, 20141009.