Electronic Supplementary Information

PicoGreen: a better amyloid probe than Thioflavin-T

Aruna K. Mora,[a] Prabhat K. Singh,[a] Birija S. Patro,[b] and Sukhendu Nath[a]*

[a]*Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India.*

[b]*Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India.*

Materials and Methods.

Ground state absorption and steady-state emission spectra were obtained from JASCO spectrophotometer (model# V-530) and Hitachi spectrofluorometer (model # F4500), respectively. Time-resolved emission measurements were carried out using a time-correlated single photon counting (TCSPC) technique as described earlier.[1] In time-resolved experiments, samples were excited with 451 nm diode laser and the instrument response function (IRF) of the TCSPC setup was ~170 ps. All spectroscopic measurements were performed at 25 ^{0}C. In vitro fluorescence images were collected using epifluorescence microscope (Carl Zeiss, Germany) coupled to a charge-coupled device (CCD) camera. For recording the fluorescence images, amyloid fibrils was incubated with ThT and PG (both 0.1 μM) for 15 min and smeared on glass slides. Blue filter and same exposure time were used for acquiring images of fibrils with both probes.

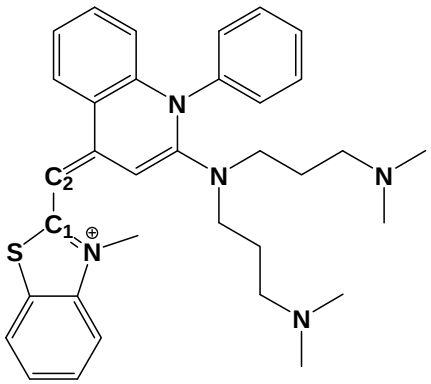
Bovine insulin, ThT, NaCl, was purchased from Sigma Aldrich. ThT was recrystallized twice from water at ambient temperature and its purity was checked by NMR. PicoGreen (PG) in DMSO solution was purchased from Invitrogen and used as received. In all experiments triple distilled water was used. Concentrated ThT stock solution (in water) was added to fibrillar solution to get 0.1 μM ThT in the final solution. In case of PG, its stock solution in DMSO was added to fibrillar solution such that DMSO content in the final solution was <0.1 volume%. The concentration of PG in the present work has been selected through the optimization of the fluorescence enhancement of PG by 30 μM amyloid fibrils, solubilization limit for insulin fibrils at experimental condition. Low concentration of probes were used to avoid the interference of the dye on the fibrillation process and to minimize the self quenching of probes.[2]

Insulin fibrils was prepared following the method reported by Manno et al.[3] Briefly, 2 mg/ml of insulin was dissolved in 20% acetic acid solution and heated at 70 ^{0}C for ~24 hr with constant stirring. The formation of fibril was confirmed by standard ThT fluorescence assay. The

concentrated fibril solution was further diluted 12 times with Tris buffer and the pH of the final fibrillar solution was adjusted to 7.4 using NaOH. For the fibrillation kinetic experiments, insulin fibrils was prepared following the method proposed by Heldt et al.[4] Insulin (2mg/ml) was dissolved in 25mM HCl, 100mM NaCl (pH 1.6) solution and heated at 65^0C for 4 hr. Aliquots of the fibril was collected in 15 minutes interval and the emission spectra were recorded after adding the requisite probe. Fibril concentrations are expressed in terms of total monomeric protein concentrations.

Quantum chemical calculations using Gaussian-03 package was used for the geometry optimization of PG.[5] Density functional theory (DFT) using B3LYP functional[6, 7] and 6-31g basis function were used for structural optimization. The energy optimized structure thus obtained from the quantum chemical calculations was used for the docking studies. Blind molecular docking studies were performed using AutoDock suite (version 4.2)[8] implemented in the AutoDock Tools (version 1.5.6)[9]. Since, the structural information for the insulin fibril is not known, fibril made of 42 residue amyloid-β protein ($A\beta_{1-42}$), which is mainly responsible for the Alzheimer diseases, was used in the present molecular docking studies. The structure of $A\beta_{1-42}$ fibril, determined by NMR spectroscopy, was obtained from pdb data bank (pdb ID=2MXU).[10] For docking studies, PG was used as a flexible ligand and the fibril as a rigid host. The dimension of the grid box (100x100x100 $Å^3$ with 0.6 Å grid spacing) was made sufficiently big such that the ligand can access all sites of the fibril. 200 independent runs, $5x10^6$ energy evaluation and 27000 generations were used for docking studies. The lowest energy docked conformation of the ligand was determined using Lamarckian Genetic Algorithm (LGA) method.[11]

Scheme S1. Molecular structure of PicoGreen (PG)



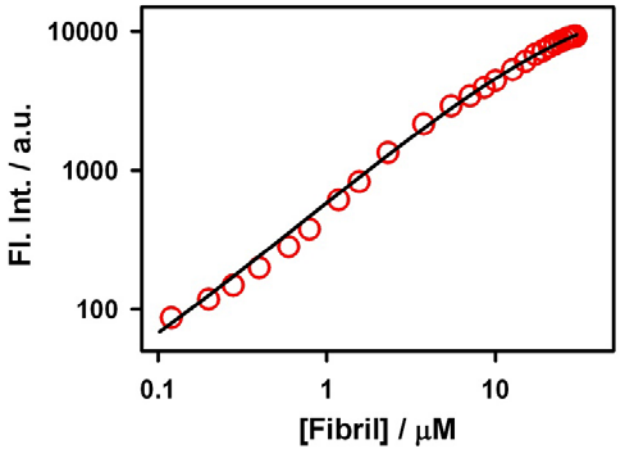


Figure S1. The log-log plot for the variation in the emission intensity of PG with amyloid fibril concentration. The solid line is the fitting of the experimental data by eq. (1) (see main text).

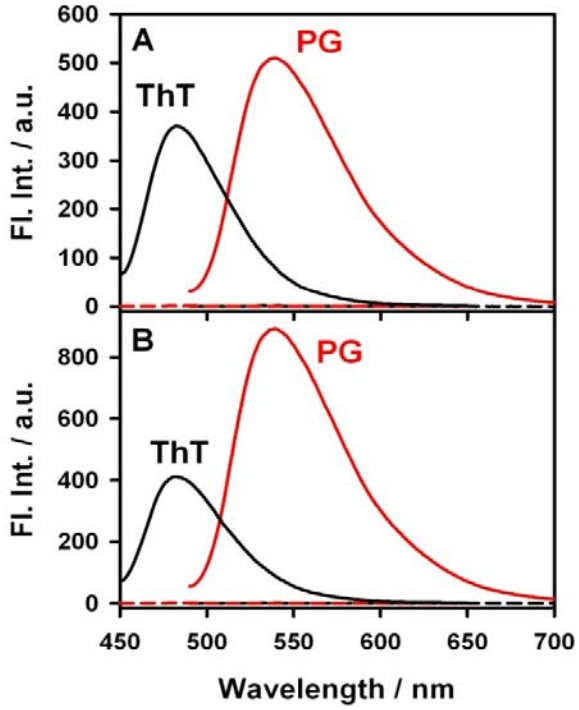


Figure S2. The emission spectra of ThT (λ_{ex}=440 nm) and PG (λ_{ex}=485 nm) in water (- - - - -) and fibrillar solution (———): (A) 10 μM and (B) 20 μM fibrils. The concentration of ThT and PG is 0.1 μM for all measurements.

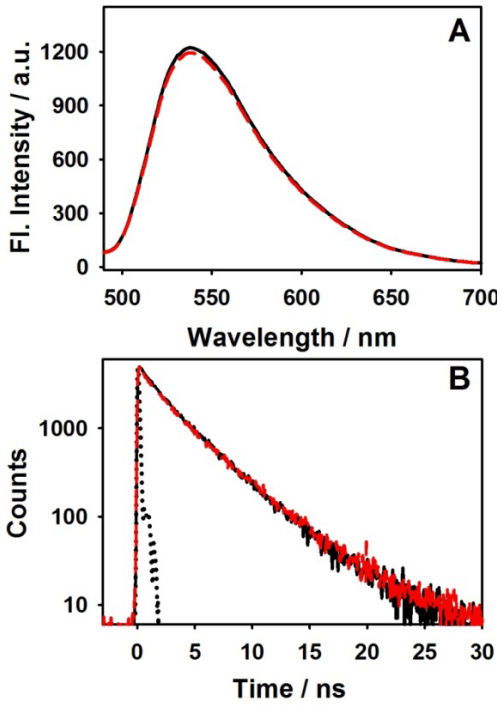


Figure S3. (A) The fluorescence spectrum of PG in insulin fibrillar solution in the absence (———) and in presence of 500 mM NaCl (- - - - - -). (B) Transient fluorescence decays of PG in amyloid fibrils in absence (———) and presence of 500 mM NaCl (- - - - - -). The dotted curve represents IRF.

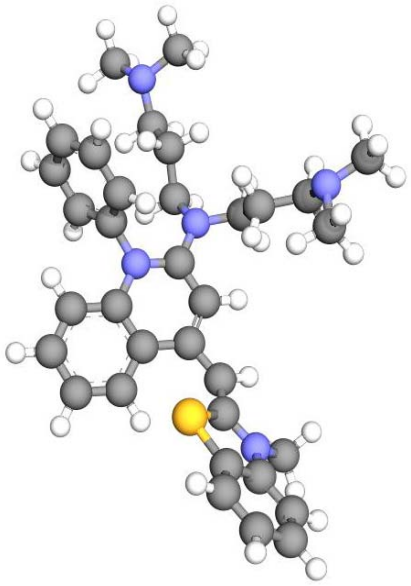


Figure S4: The energy optimized structure of PG obtained from the quantum chemical calculations.

Table S1. Fitting parameters for fluorescence transient decays and average lifetime (τ_{av}) for PG in amyloid fibril solutions

[Fibril]/μM	A_1	τ_1/ ns	A_2	τ_2 / ns	A_3	τ_3 / ns	τ_{av}/ ns
0.30	41	0.05	33	0.40	26	2.24	0.73
0.60	28	0.06	36	0.47	36	2.26	1.00
1.18	13	0.06	35	0.77	52	2.99	1.83
2.31	7	0.07	29	0.94	64	3.30	2.39
5.45	5	0.14	26	1.32	69	3.75	2.94
10.0	4	0.15	22	1.31	74	3.79	3.10
13.8	3	0.17	23	1.45	74	3.93	3.25
20.0	4	0.24	23	1.58	73	3.96	3.26
24.7	3	0.25	23	1.63	74	3.99	3.34
32.7	3	0.25	23	1.65	74	4.05	3.38

References

1. A. K. Mora, P. K. Singh and S. Nath, *J. Phys. Chem. B*, 2016, **120**, 4143−4151.
2. N. D. Younan and J. H. Viles, *Biochemistry*, 2015, **54**, 4297−4306.
3. M. Manno, D. Giacomazza, J. Newman, V. Martorana and P. L. S. Biagio, *Langmuir*, 2010, **26**, 1424–1426.
4. C. L. Heldt, D. Kurouski, M. Sorci, E. Grafeld, I. K. Lednev and G. Belfort, *Biophys. J.*, 2011, **100**, 2792-2800.
5. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *GAUSSIAN 03*, Gaussian, Inc., Wallingford, CT, 2004.
6. A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648-5652.
7. C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1998, **37**, 785-789.
8. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Comput. Chem.*, 2009, **16**, 2785-2791.
9. M. F. Sanner, *J. Mol. Graph. Model*, 1999, **17**, 57-61.
10. Yiling Xiao, Buyong Ma, Dan McElheny, Sudhakar Parthasarathy, Fei Long, Minako Hoshi, R. Nussinov and Y. Ishii, *Nat. Struct. Mol. Biol.*, 2015, **22**, 499-505.
11. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.*, 1998, **19**, 1639-1662.