

Pyrene-Based Probe with Tunable AIE-ICT Emissions for Amyloid Sensing and Imaging

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Summary:

Oligomers and amyloid fibrils are central biomarkers in neurodegenerative diseases such as Alzheimer's. Early detection of toxic oligomers remains challenging, as most dyes preferentially recognize fibrils. Dual-responsive probes capable of distinguishing both species are therefore essential for monitoring aggregation dynamics. We present pyrene-based dye, P1, enabling selective detection of HEWL oligomers and fibrils via distinct aggregation-induced emission (AIE) and intramolecular charge transfer (ICT) channels. P1 exhibit oligomer-specific AIE emissions at 660 nm, while fibril binding induces LE (Locally Excited state) and ICT peaks at 490 nm and 540 nm, respectively, facilitating super-resolution confocal imaging of both aggregation states.

Keywords: Pyrene donor, cyano acceptor, AIE and ICT, sensing of amyloid oligomers and fibrils.

Headlines

Dual-responsive pyrene dye enabling selective visualization of amyloid oligomers and fibrils, advancing early diagnosis and mechanistic insights in neurodegeneration.

Background and Motivation

Misfolded proteins form amyloid aggregates linked to Alzheimer's and Parkinson's. While fibrils mark pathology, short-lived oligomers are more neurotoxic yet difficult to detect due to heterogeneity and instability [1]. These challenges highlight the urgent need for novel, non-invasive, cost-effective diagnostics targeting early oligomeric species during protein aggregation.

Organic π -conjugated materials with aggregation-induced emission (AIE) are widely studied for optoelectronic and biological applications [2]. Pyrene, a planar, electron-rich aromatic molecule with long fluorescence lifetime and high quantum yield, is a versatile scaffold in electronics, self-assembly, and biosensing. Here, we present a pyrene-based ICT dye, P1, synthesized via Knoevenagel condensation, investigated the photophysical properties like solvatochromism, potential AIE behaviour, and biological utility in detecting and imaging amyloid oligomers and fibrils.

Description of the New Approach

We present engineered donor–acceptor fluorophore P1, capable of simultaneously tracking oligomeric and fibrillar protein aggregates via

distinct AIE and ICT emissions, enabling spectral discrimination and super-resolution imaging of distinct aggregation states.

Excitation at 430 nm allows P1 [2-(pyren-1-ylmethylene)malononitrile] to distinguish amyloid species: oligomer binding yields a strong 660 nm AIE band, while fibril interaction produces weaker LE and ICT emissions at 490 nm and 540 nm, requiring higher fibril concentrations for detection.

Results

The probe P1 was synthesized (*Fig. 1a*) via Knoevenagel condensation of 1-pyrenecarboxaldehyde (Py-CA) with malononitrile in ethanol/piperidine, yielding P1 (92%) with high purity.

To assess ICT properties, absorption spectra (*Fig. 1b*) of P1 in acetonitrile was compared with that of the precursor Py-CA. Compared to absorption maximum of Py-CA at 393 nm, P1 shifted strongly to 430 nm elucidating that P1 shows better charge transfer than Py-CA. Fluorescence emission spectra (*Fig. 1c*) of Py-CA and P1 illustrated emission maxima at 430 nm and 540 nm respectively, marking that P1 exhibits more charge transfer than Py-CA. These results confirm the presence of efficient excited-state ICT and strong polarity sensitivity suitable for specific amyloid protein aggregate detection.

The photophysical properties of P1 such as solvatochromism and AIE (aggregation induced

emission) in THF:water mixture were also studied to elucidate the pronounced ICT and AIE effects.

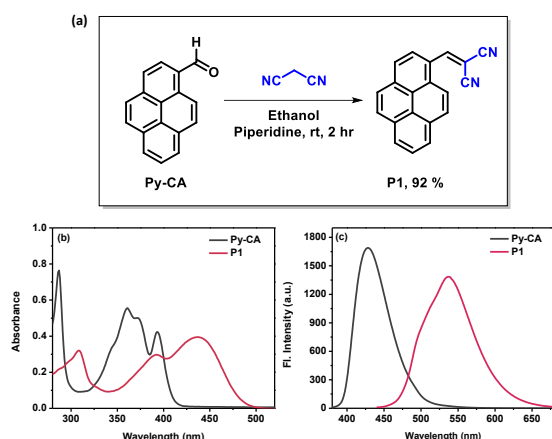


Fig. 1. (a) Synthesis of P1 from precursor Py-CA. Absorption (a) and emission spectra (b) of 20 μM solution of Py-CA, and P1 in acetonitrile (λ_{ex} for Py-CA and P1 are 390 nm and 430 nm).

Then the fluorescence assay (Fig. 2 a, b) of aggregated HEWL protein aggregates in presence of P1 was studied in PBS (pH 7.0). Plotting the LE (490 nm) and ICT (540 nm) emissions of P1 against incubation time (Fig. 2d) yields sigmoidal kinetics similar to ThT (Thioflavin-T) assay, reflecting fibril growth. In contrast, the AIE intensity at 660 nm (Fig. 2c) shows a sharp rise from 0–24 h followed by a decline from 24–120 h. This region appears as a lag phase in ThT assays because ThT cannot detect oligomers, whereas P1's AIE response clearly captures their formation and subsequent depletion during early aggregation.

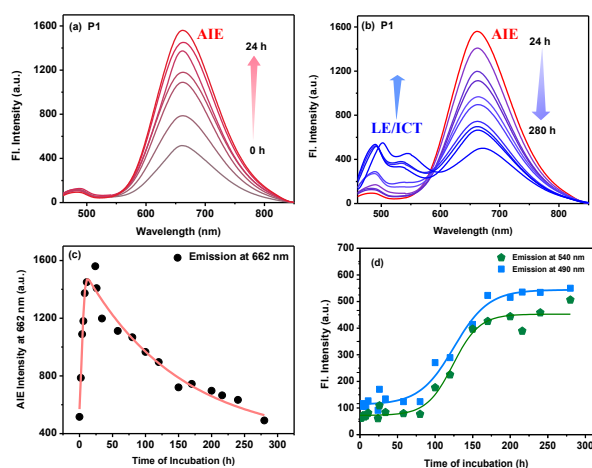


Fig. 2. Fluorescence assay (a, b) and kinetic growth (c, d) of HEWL fibrils and oligomers monitored using 20 μM P1 (λ_{ex} = 430 nm), immediately after mixing with HEWL protein aggregates.

Different protein aggregates of HEWL could be clearly visualized by super-resolution confocal

fluorescence microscopy using P1 following photoexcitation at 445 nm (Fig. 3). Significantly, the super-resolution images of amyloid fibrils (t240) obtained using our P1 dye as staining agent could exhibit clear twisted cross- β sheet structural features of mature amyloid fibrils.

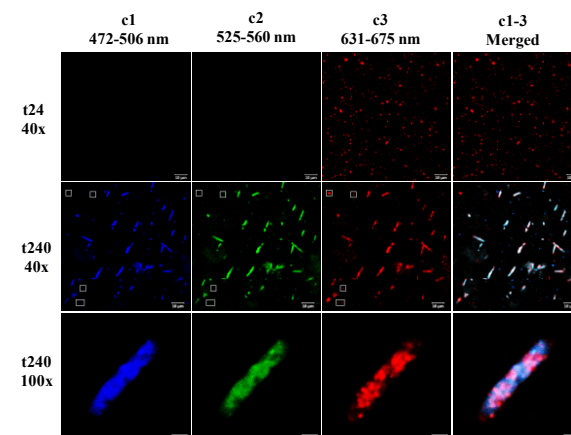


Fig. 3. Super-resolution fluorescence microscopy images of t24 (40x resolution) and t240 (40x and 100x resolution) dry, (λ_{ex} = 445 nm) using 20 μM P1.

Conclusion

Upon binding to oligomers, P1 exhibit AIE peak at 660 nm. In contrast, interaction with mature fibrils induces LE and ICT emissions at 490 and 540 nm respectively. The ability of a single dye to distinguish between oligomeric and fibrillar species based on emission wavelength provides a powerful tool for tracking protein aggregation dynamics and offers promising avenues for early diagnosis and mechanistic studies of amyloidogenic diseases.

Acknowledgements

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References

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