

## Time-Resolved Spectroscopy of Thioflavin T and its Building Blocks

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Thioflavin T (ThT) is a benzothiazole (BTA) salt linked to *N,N*-dimethylaniline (DMA). ThT is a widely used marker for studies on protein aggregation and amyloid fibril formation.<sup>[1]</sup> The freely rotatable bond between the BTA and DMA moieties governs the dynamics of ThT, the associated rotation is the main (non-radiative) depopulation channel of the excited state whose lifetime is around 1 ps.<sup>[2,3]</sup> The fluorescence of ThT exhibits a pronounced dependence on excitation wavelength, attributed to the ground-state's pre-rotation angle.<sup>[4]</sup> This suggests that the excited pre-rotated molecule is inhibited from deactivating by further rotation.

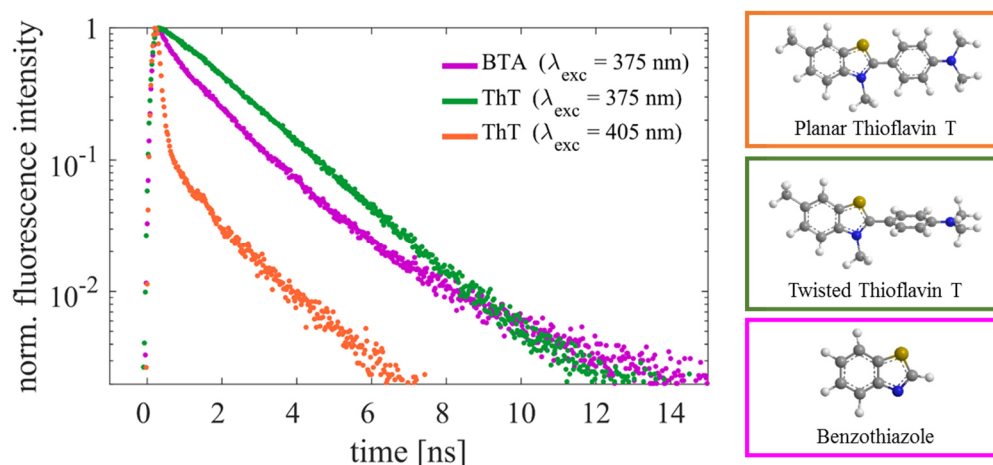


Figure 1. Time-resolved fluorescence at 450 nm for ThT (excited at 405 nm) and its building block BTA (excited at 375 nm) in ethanol.

To test whether pre-rotated molecules exhibit a prolonged excited-state lifetime, we perform time-resolved spectroscopy via fluorescence upconversion, TCSPC and ultrafast transient absorption for ThT and its building blocks. With near-UV excitation, the lifetime is prolonged from  $\approx 1$  ps to 1.3 ns. A comparison of the data for ThT and BTA unveils that the ThT dynamics solely originate from a pre-rotated subensemble for which the two building blocks are spectroscopically independent and no excited-state depopulation through rotation occurs (Fig. 1). Analogous conclusions can be drawn with the DMA building block only. Hence, our studies provide a time-domain corroboration of the pre-rotation model.<sup>[4]</sup>

**Funding:** Deutsche Forschungsgemeinschaft via Cluster of Excellence RESOLV (EXC 1069)

### References:

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