

# **A Ratiometric Fluorescence Assay for Acetylcholinesterase Activity and Inhibitor Screening Based on Supramolecular Assembly Induced Monomer-Excimer Emission Transition of a Perylene Probe**

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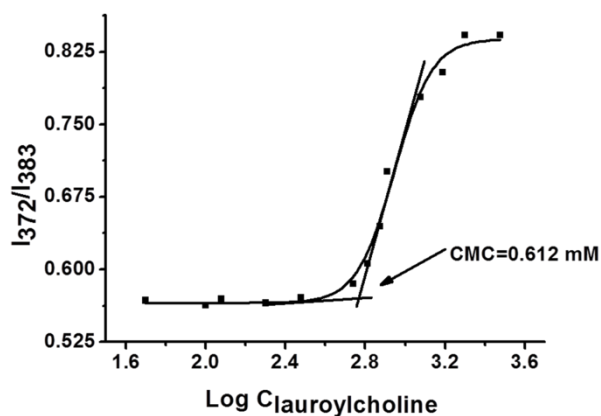
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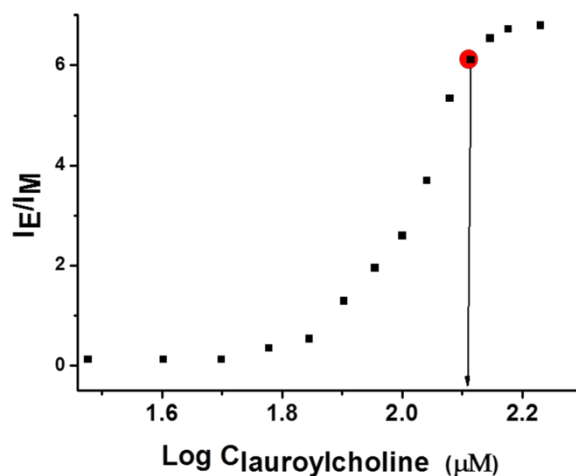
## The inhibition efficiency

The inhibition efficiency ( $I_E$ ) is given by:  $I_E = [I - I_i]/[I - I_0]$

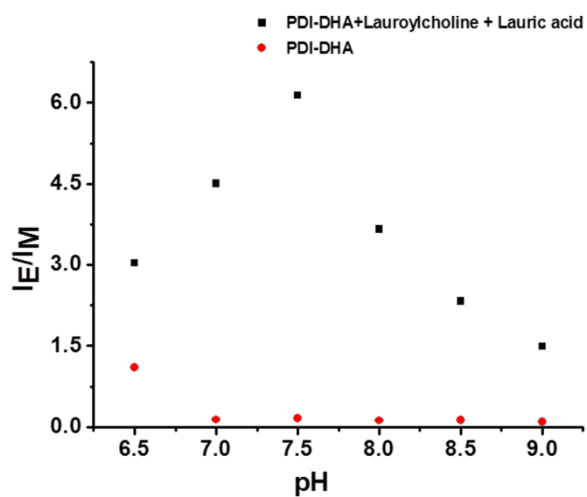
“ $I$ ” is the value of  $I_M/I_E$  at 548 nm and 680 nm in the absence of inhibitor, and “ $I_i$ ” is that in the presence of inhibitor. “ $I_0$ ” is the ratio of  $I_M/I_E$  at 548 nm and 680 nm in the absence of AChE.



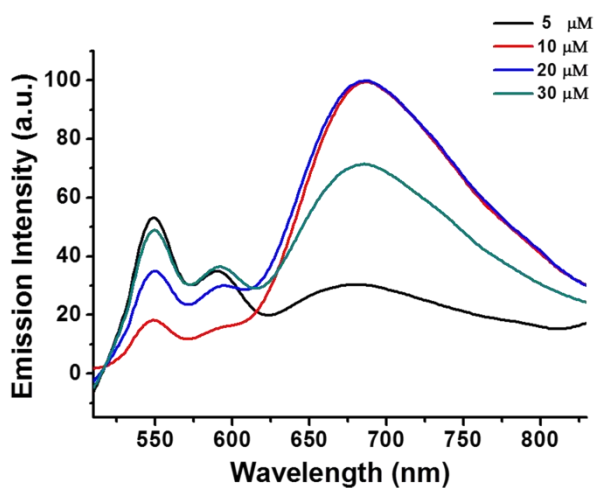
**Fig. S1** Plot of the intensity ratio  $I_{383} / I_{372}$  of pyrene (from emission spectra) as a function of the logarithm concentration of lauroylcholine.



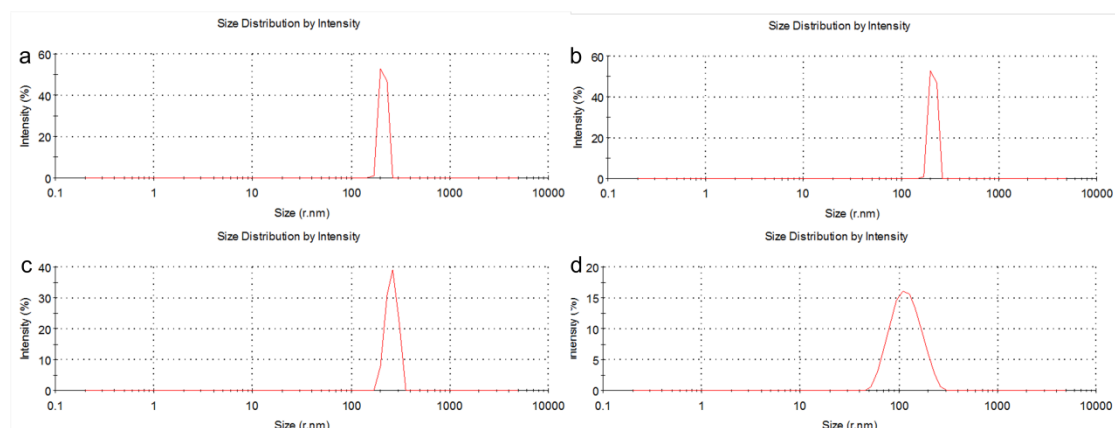
**Fig. S2** The plot of  $I_E/I_M$  value of PDI-DHA (10  $\mu\text{M}$ ) in lauroylcholine-lauric acid assemblies with the fixed ratio of [lauroylcholine] : [lauric acid] = 130  $\mu\text{M}$  : 300  $\mu\text{M}$ .



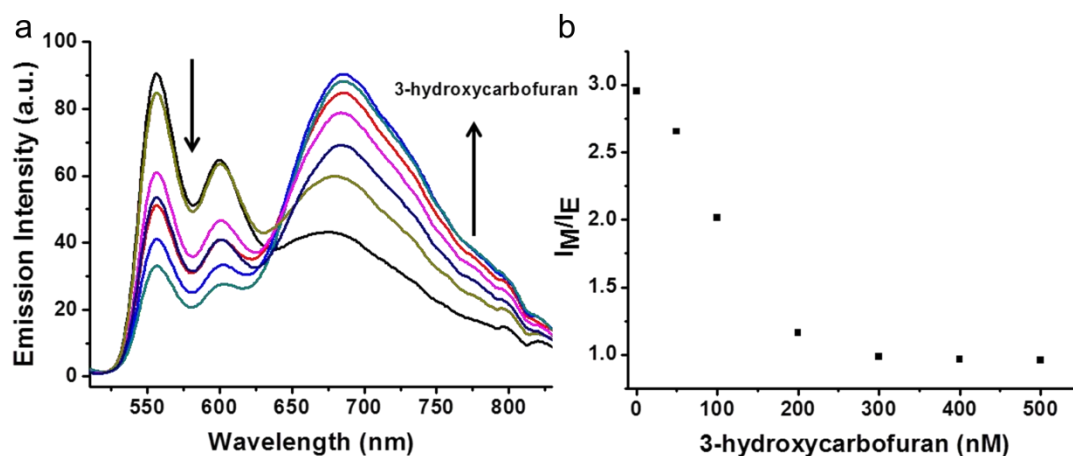
**Fig. S3** Plot of the  $I_E/I_M$  value of PDI-DHA in the presence (black plot) and absence (red plot) of the mixture of lauroylcholine (130  $\mu\text{M}$ ) and lauric acid (300  $\mu\text{M}$ ) under different pH values.



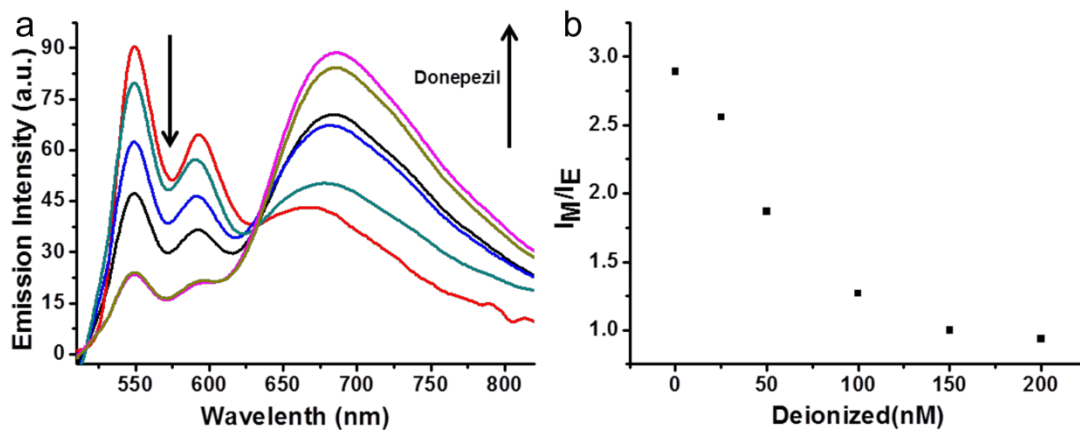
**Fig. S4** Changes in emission spectrum of the sample solutions containing lauric acid (300  $\mu\text{M}$ ), lauroylcholine (130  $\mu\text{M}$ ), and PDI-DHA of different concentrations (5, 10, 20, 30  $\mu\text{M}$ ).



**Fig. S5** Dynamic light scattering results of the supramolecular assemblies, the sample mixture contained: (a) PDI-DHA (10  $\mu\text{M}$ ) and lauric acid (300  $\mu\text{M}$ ), (b) PDI-DHA (10  $\mu\text{M}$ ) and lauroylcholine (130  $\mu\text{M}$ ), (c) lauroylcholine (130  $\mu\text{M}$ ) and lauric acid (300  $\mu\text{M}$ ), (d) PDI-DHA (10  $\mu\text{M}$ ), lauroylcholine (130  $\mu\text{M}$ ) and lauric acid (300  $\mu\text{M}$ ).



**Fig. S6** (a) Changes in emission spectrum of PDI-DHA (10  $\mu\text{M}$ ) upon the addition of increasing concentrations of 3-hydroxycarbofuran (0 - 500 nM). Assay solutions contained 130  $\mu\text{M}$  lauroylcholine and 300  $\mu\text{M}$  lauric acid, and 150 U/mL AChE. (b) Changes in  $I_M / I_E$  value with 3-hydroxycarbofuran concentration.



**Fig. S7** (a) Changes in emission spectrum of PDI-DHA (10  $\mu$ M) upon the addition of increasing concentrations of donepezil (0 - 200 nM). Assay solutions contained 130  $\mu$ M lauroylcholine and 300  $\mu$ M lauric acid, and 150 U/mL AChE. (b) Changes in  $I_M/I_E$  value with donepezil concentration.