

Electronic Supporting Information

Development of a biosensing system for tacrine based on nitrogen-doped graphene quantum dots and acetylcholinesterase

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Supplementary I. Study of concentration and pH of ATCh solution.

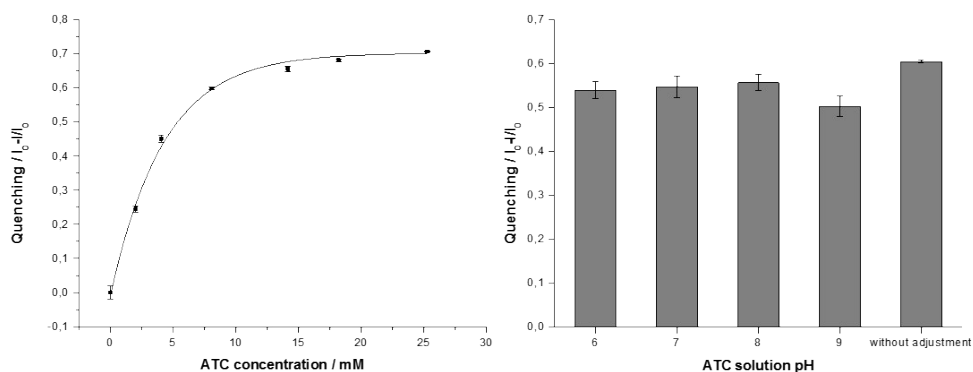


Figure S1. Changes in quenching fluorescence of N-GQDs (a) in presence of different concentrations of ATC (0-25.29 mM) and (b) with different pH values for ATC aqueous solution (6-9). Experimental conditions: AChE (27.4 U·mL⁻¹), ATC (14.16 mM just for graph b) and N-GQDs (1:10 dilution). I₀ and I correspond to the fluorescence intensity recorded in absence and presence of ATC, respectively. $\lambda_{\text{ex}} = 355$ nm and $\lambda_{\text{em}} = 440$ nm. Slit width for excitation and emission was adjusted at 2 nm. Values represent the mean and standard deviation obtained from three separate experiments for each study.

Supplementary II. Influence of N-GQD solution pH.

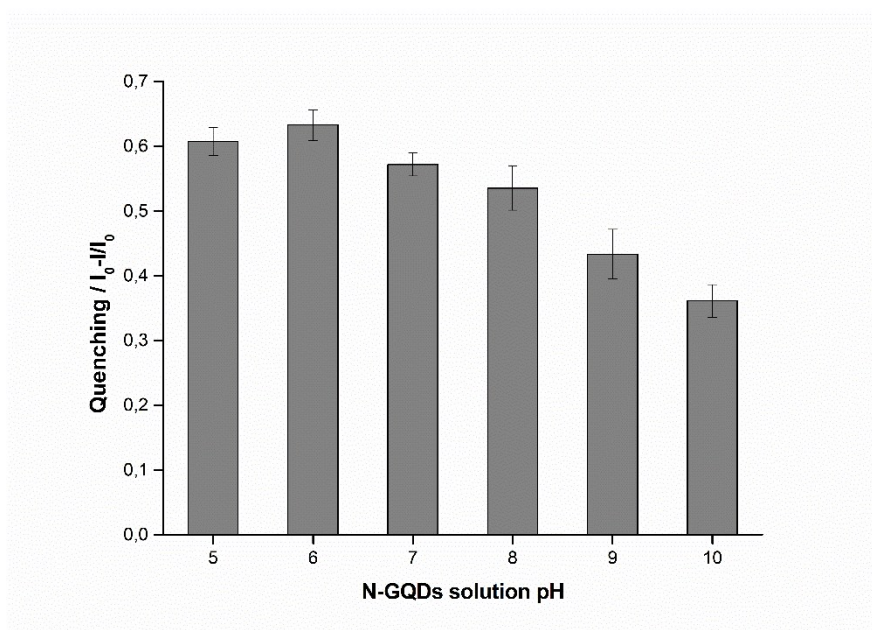


Figure S2. Influence of N-GQD solution pH on fluorescence quenching. Experimental conditions: AChE ($27.4 \text{ U} \cdot \text{mL}^{-1}$), ATC (14.16 mM) and N-GQDs (1:10 dilution). I_0 and I correspond to the fluorescence intensity for N-GQDs single and in presence of hydrolysis reaction products, respectively. $\lambda_{\text{ex}} = 355 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$. Slit width for excitation and emission was adjusted at 2 nm. Values represent the mean and standard deviation obtained from three separate experiments for each pH.

Supplementary III. Study of enzyme and substrate incubation time.

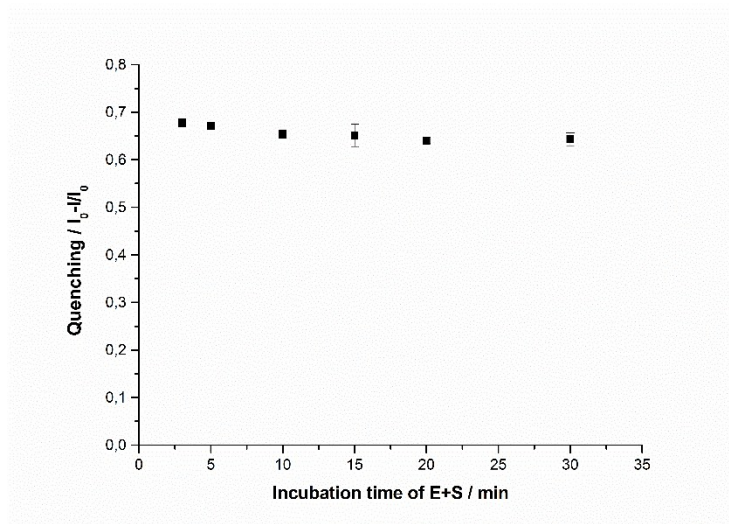


Figure S3. Influence of the incubation time between AChE and ATC. Experimental conditions: AChE ($27.4 \text{ U} \cdot \text{mL}^{-1}$), ATC (14.16 mM), $500 \mu\text{L}$ N-GQDs (1:25 dilution). I_0 and I correspond to the fluorescence intensity of N-GQDs+ATC and N-GQDs in presence of hydrolysis reaction products after each incubation time, respectively. $\lambda_{\text{ex}} = 355 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$. Slit width for excitation and emission was adjusted at 2 nm . Values represent the mean and standard deviation obtained from three separate experiments for each time.

Supplementary IV. Study of enzymatic system and N-GQD solution reaction time.

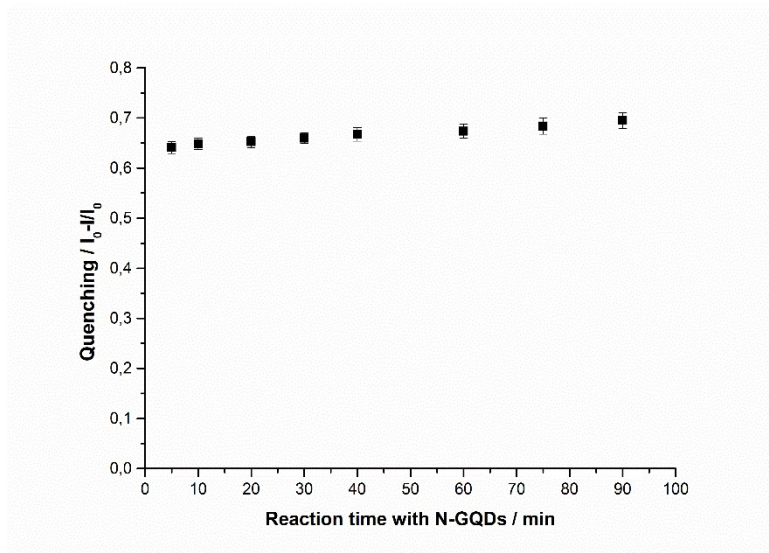


Figure S4. Influence of the time of contact between the N-GQDs solution and enzymatic products. Experimental conditions: $27.4 \text{ U} \cdot \text{mL}^{-1}$ AChE, 14.16 mM ATC, $500 \mu\text{L}$ N-GQDs (1:25 dilution). I_0 and I correspond to the fluorescence intensity of N-GQDs+ATC and N-GQDs in presence of hydrolysis reaction products after each incubation time, respectively. Values represent the mean and standard deviation obtained from three separate experiments for each reaction time.

Supplementary V. Study of enzyme and inhibitor incubation time.

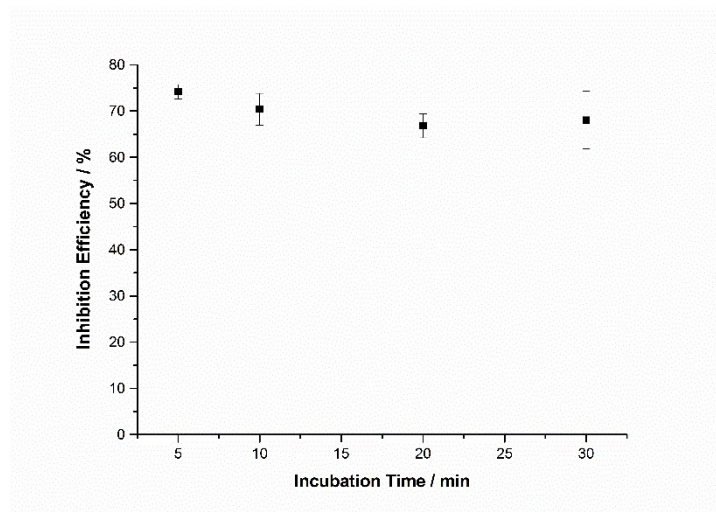


Figure S5. Changes in the inhibition efficiency at different incubation times between enzyme and inhibitor. Experimental conditions: $27.4 \text{ U}\cdot\text{mL}^{-1}$ AChE, 14.16 mM ATC, $20 \text{ }\mu\text{M}$ tacrine, $500 \text{ }\mu\text{L}$ N-GQDs (1:25 dilution). Inhibition efficiency has been calculated following the equation of section 3.4. Values represent the mean and standard deviation obtained from three separate experiments for each incubation time.