**Supplementary Information**

**For**

# Electrochemical detection of interaction between Thioflavin T and Acetylcholinesterase

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## Calibration Plots

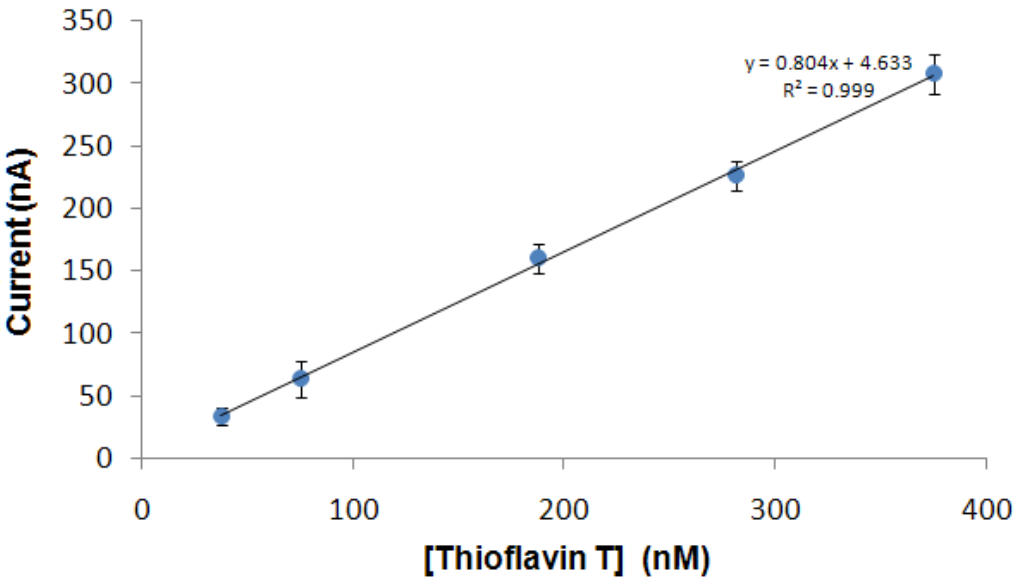


**Figure S1.** The oxidation peak of thioflavin T at pH 7, which appears around 0.86 V on a GCE, exhibits a linear and reproducible response in the concentration range of 40 to 400 nM.

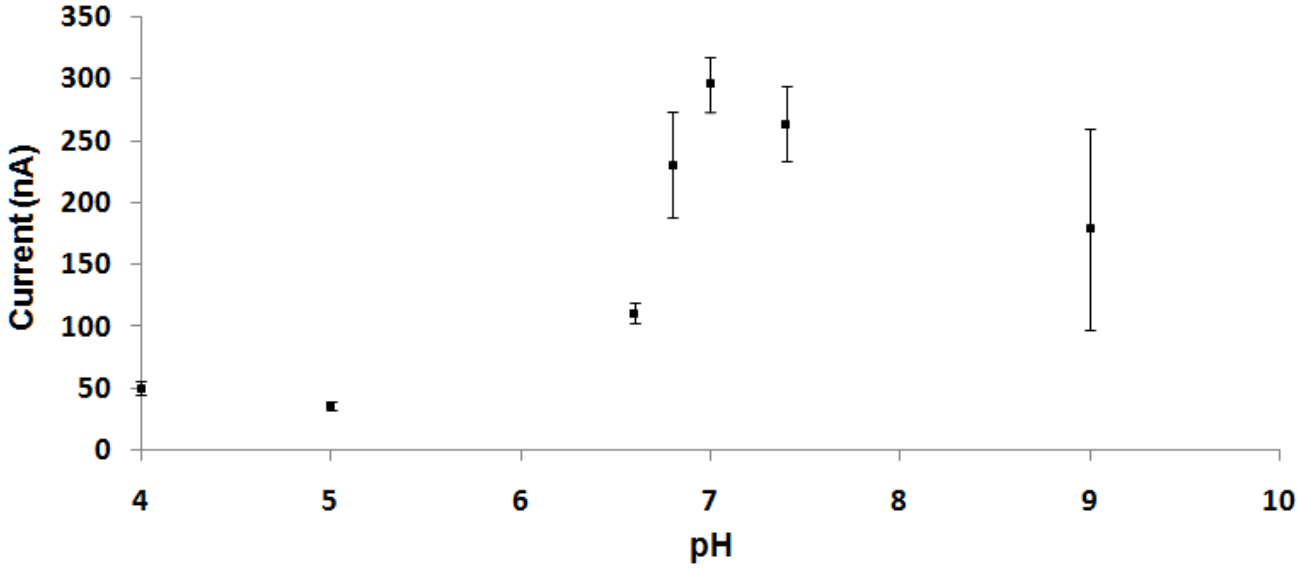


**Figure S2**. The ThT oxidation peak current intensity is pH-dependent. The oxidation peak current seems to show the most reproducibility and sensitivity in the pH range of 7.0 to 7.4.

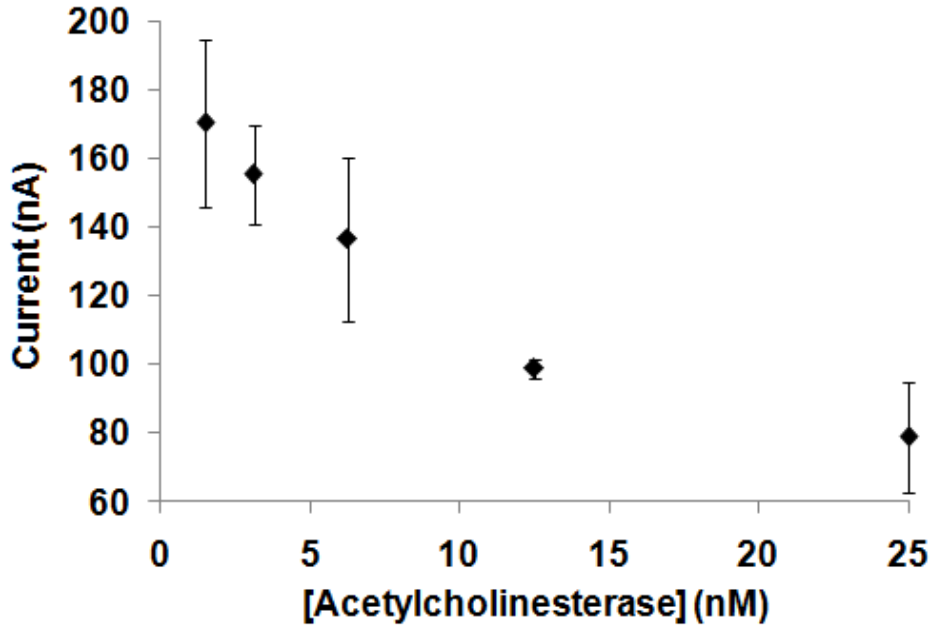


**Figure S3.** The intensity of ThT's oxidation peak current depends on the concentration of AChE in solution. The plotted data represents the current intensity of 280 nM ThT that was incubated for 5 min in solution with variable concentrations of AChE.

**Control Experiments: Electrode Surface Adsorption and Fouling**

*Impact of Paraoxon and Carbachol Concentrations on ThT Oxidation Peak Current Intensity*

In this experiment, 280 nM ThT was incubated for 5 minutes with varying concentrations of paraoxon and carbachol before DPV measurement. No AChE was added at any point.

Carbachol showed no significant effect on the ThT oxidation peak current even at concentrations as high as 100 ppm. Paraoxon showed no significant effects on the ThT peak current when present at low concentrations approaching 20 ppm. At concentrations higher than 20 ppm, paraoxon had the effect of decreasing the intensity of the ThT peak current substantially. This explains the drop in ThT oxidation when AChE is pre-treated with higher paraoxon concentrations as seen in Figure 2 of the main article. The inhibition of ThT's oxidation peak current by ppm levels of paraoxon is worth investigating in the future for a simple detection method of toxic levels of this insecticide.

*ThT Adsorption and Oxidation Peak Current Intensity*

The glassy carbon (GC) electrode was incubated for four minutes in a 280 nM ThT solution. The electrode was then rinsed with PBS buffer and placed in a sample of clean PBS buffer solution. The DPV voltammogram was immediately collected to see how much of the ThT peak current was due to ThT aggregation at the GC electrode surface. The ThT peak intensity of the DPV measurement in clean buffer solution was 80% as big as the measurement taken in ThT solution. This shows that there is a high level of ThT adsorption taking place at the GCE. Most of the recorded current is due to the adsorption of ThT on the electrode surface.

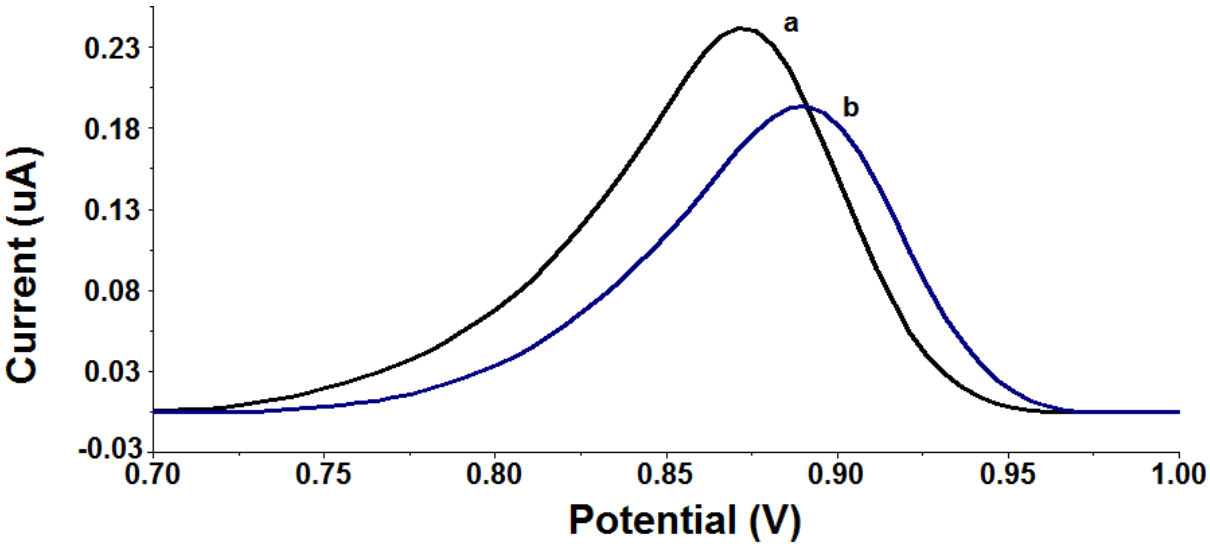


**Figure S4.** DPV oxidation peak current measured in 280 nM ThT solution (a) and in PBS buffer after rinsing the GC electrode (b).

When 12.5 nM AChE and 280 nM ThT are tested in the same way, the same results are found. The DPV measurement taken in a clean PBS solution after electrode incubation with ThT and AChE produces a ThT oxidation peak that is 80% as big as the measurement recorded in solution with ThT and AChE.

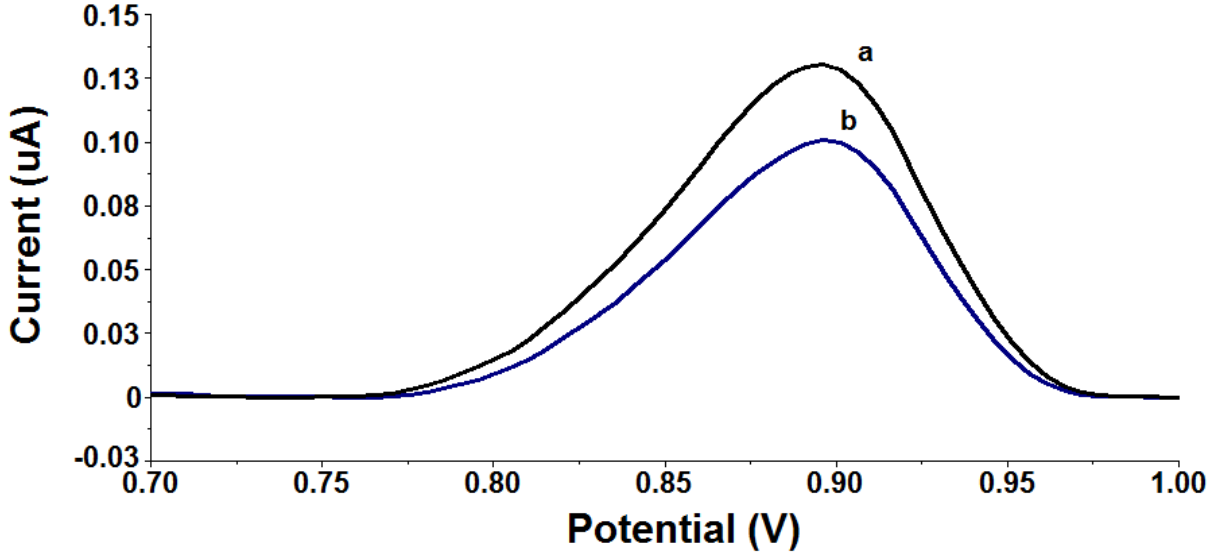


**Figure S5.** DPV oxidation peak current measured in 280 nM ThT and 12.5 nM AChE solution (a) and in PBS buffer after rinsing the GC electrode (b).

These results suggest that most of the ThT oxidation peak current that is observed is determined by ThT's adsorption behaviour at the surface of the electrode during the 4 min incubation step.

*Investigation of Surface Fouling by AChE*

It is possible that the observed drop in ThT oxidation peak current in the presence of AChE is due to surface fouling of the GC electrode by AChE. AChE would block ThT's access to the electrode surface for oxidation. To investigate this possibility, several control tests were performed.

1. *Separate GC electrode incubation steps*

   In this experiment, the GC electrode was incubated first in 12.5 nM AChE solution for 4 minutes. The electrode was rinsed with PBS buffer solution and then incubated for another 4 minutes in 280 nM ThT solution. The DPV measurement was then taken in the 280 nM ThT solution. This produced similar results compared to DPV measurement in a solution of 280 nM ThT and 12.5 nM AChE.

   These results show that the drop in ThT oxidation peak current intensity does not depend on AChE being in solution with ThT. The peak current decrease is seen with AChE adsorbed on the surface of the GC electrode.

2. *Ferri-Ferrocyanide Cyclic Voltammograms*

   Cyclic voltammograms (CVs) with ferri-ferrocyanide allow for the observation of $Fe^{2+/3+}$ redox peak currents at the electrode surface. They are often used to characterise modified electrode surfaces with a drop in ferri-ferrocyanide redox peak currents indicating the presence of modification components on the surface. Our CVs featured 5 standard linear scans from -0.5to 1.25 V, with characteristic $Fe^{2+/3+}$ reduction and oxidation peaks occuring at ~ 0.23 V and 0.27 V respectively.

   i. The GC electrode was incubated for 4 minutes in 12.5 nM AChE solution. It

was then washed with PBS bufer solution and placed into 20 mM ferri-ferrocyanide soluion. CVs were measured and showed the first scan to be very close to the CV measurements in clean 20 mM ferri-ferrocyanide solution. Scans 2-5 showed a progressive decrease in redox peak current intensity, with the fifth scan showing a 20% drop in intensity compared to scan 1.

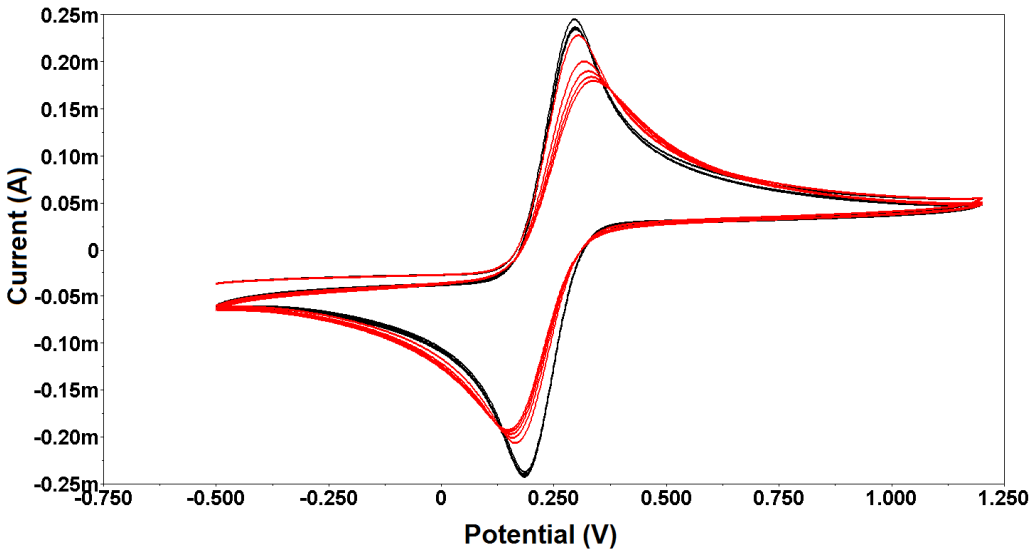


**Figure S6.** CVs of 20 mM ferri-ferrocyanide with a 4-min pre-incubation of the GC electrode in PBS buffer solution (black) and 12.5 nM AChE solution (red).

ii. The same experiment was repeated but with 200 nM AChE. The same pattern was observed, but with an overall 40% drop in the redox peak current intensity after 5 scans.

iii. When this experiment was repeated with 600 nM AChE, there was a 50% drop in the redox peak current intensity after 5 scans. In addition, the first scan now showed a 20% drop in redox peak current intensity compared to the control measurements made on a clean GC electrode in 20 mM ferri-ferrocyanide solution.

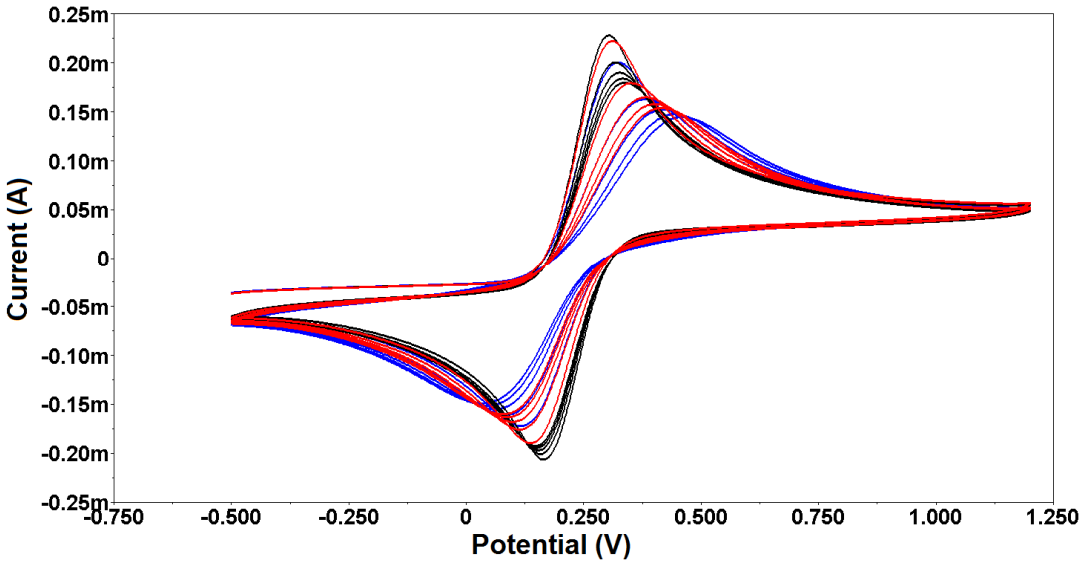


**Figure S7.** CVs of 20 mM ferri-ferrocyanide after 4-minute pre-incubations of the GC electrode in 12.5 nM AChE (black), 200 nM AChE (red), and 600 nM AChE (blue). Even with 600 nM AChE, the GC surface is not saturated with the enzyme at the time of measurement.

In summary, the first CV scan showed that the ferri-ferrocyanide molecules had easy access to the GC electrode at the experimental AChE concentrations. Only when the AChE concentration was 60 times bigger was there a substantial change in the first CV scan intensity when compared to clean GC electrode controls in 20 mM ferri-ferrocyanide solution.

Furthermore, even after 5 scans, the ferri-ferrocyanide species could still undergo their redox processes with 50% of the initial peak current magnitudes even at 600 nM AChE. This shows that the electrode surface is not saturated at this concentration or at the experimental 12.5 nM AChE conditions.

These results suggest that with our experimental conditions of 12.5 nM AChE, the enzyme does not block a substantial amount of the electrode area to justify the observed ~60% drop in ThT oxidation peak current intensity. Instead, the ThT

molecules are preferably interacting with AChE before oxidizing on the surface. The native tetrameric form of AChE may be creating a surface modification that effectively captures ThT from solution near the electrode surface.

iv. In this experiment, the GC electrode was incubated in 100 ppm carbachol for 4 minutes. The electrode was rinsed in PBS buffer solution and then placed into 20 mM ferri-ferrocyanide solution, where CV measurements were taken. The incubation with carbachol showed no effect on the redox peak currents of ferri-ferrocyanide in any of the 5 scans.

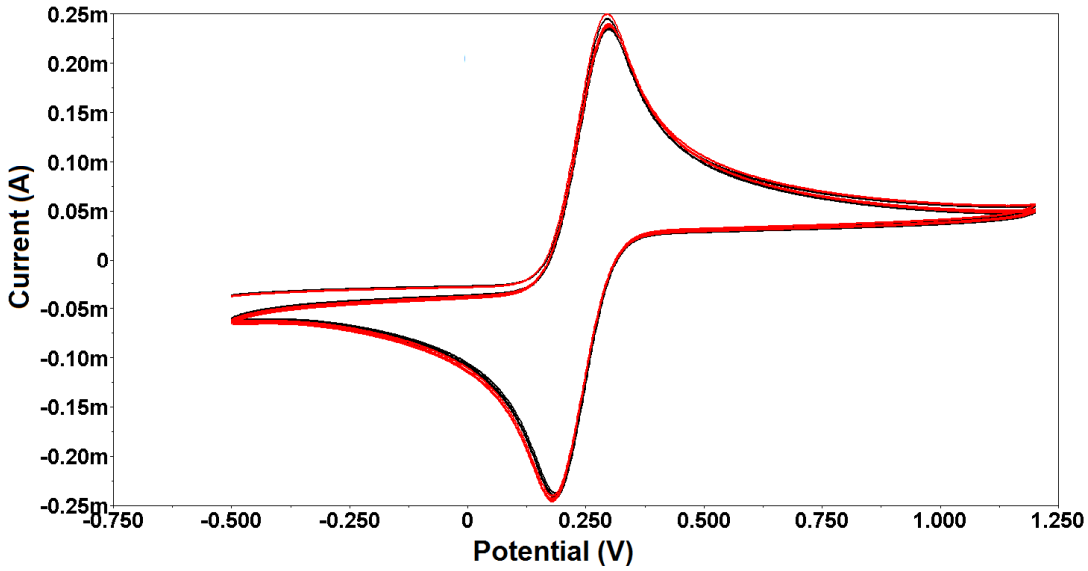


**Figure S8.** CVs of 20 mM ferri-ferrocyanide after a 4-min pre-treatment of the GC electrode in PBS buffer solution (black) and 100 ppm carbachol solution (red).

v. The same procedure was followed as in (iv) to see the effects of incubating the GC electrode in a solution of 12.5 nM AChE and 100 ppm carbachol. The CVs that were measured were not substantially different from the CVs collected in (i) for clean 12.5 nM AChE.

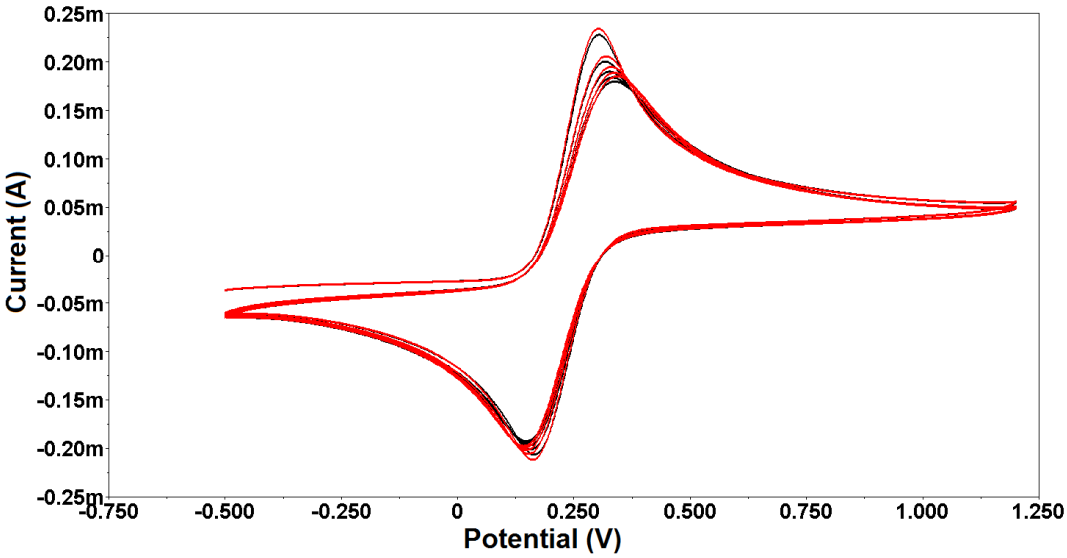


**Figure S9.** CVs of 20 mM ferri-ferrocyanide after a 4-min pre-incubation of the GC electrode in 12.5 nM AChE (black) and 12.5 nM AChE with 100 ppm carbachol (red). No significant differences are observed.

These results suggest that the changes in ThT peak current intensity observed in our studies in the presence of insecticides are not a result of the acetylcholinesterase inhibitors somehow detaching the enzyme from the electrode surface.

vi. This experiment featured the incubation steps described in (ii) but also included an extra step where the GC electrode was washed with PBS buffer solution and then incubated for another 4 minutes in 280 nM ThT solution. The GC electrode was then washed again with clean PBS buffer solution and placed in 20 mM ferri-ferrocyanide solution for CV measurements. The ferri-ferrocyanide CVs obtained in (i) showed redox couple current intensities that were 10% greater than those obtained after incubation with 280 nM ThT.

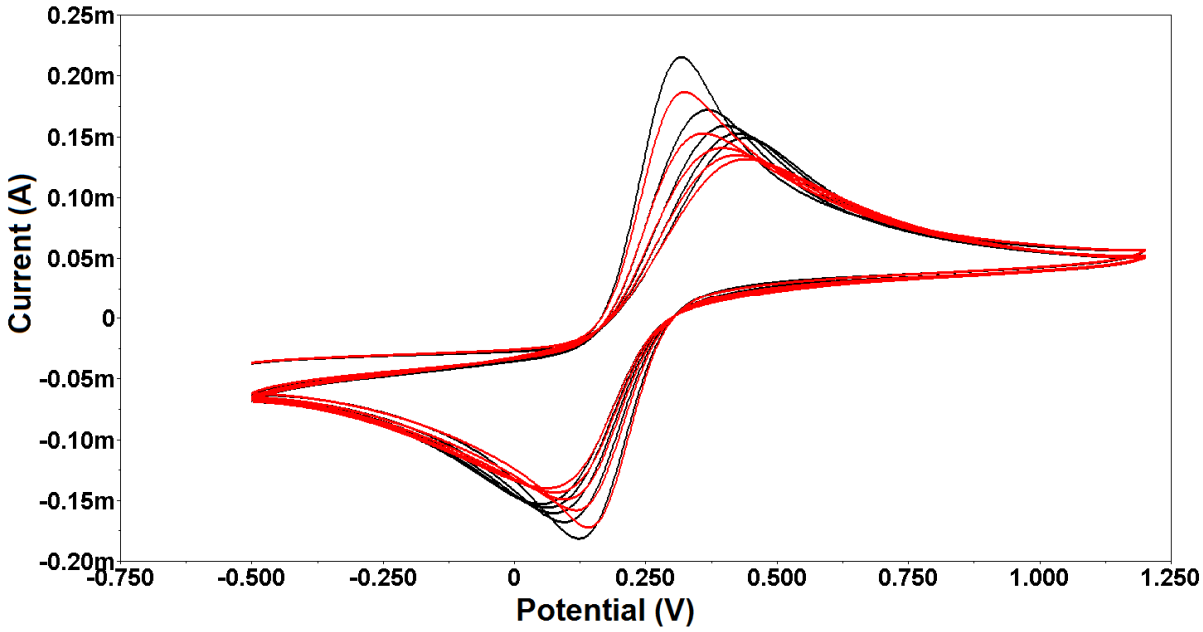


**Figure S10.** CVs of 20 mM ferri-ferrocyanide after a 4-min pre-incubation of the GC electrode in 200 nM AChE solution (black) and with the same pre-incubation in 200 nM AChE followed by another 4-min incubation in 280 nM ThT (red). The ThT adsorbs to the GC electrode surface and blocks ferri-ferrocyanide redox processes by an additional ~10%.

This indicates that the ferri- and ferrocyanide molecules are still easily able to reach the electrode surface even in the presence of adsorbed species of both 280 nM ThT and 200 nM AChE. Experimentally, 12.5 nM AChE was used, so there was more opportunity for ThT to reach the GC electrode surface.