# Expanding Electrochemical Bio-sensors to Detect Ricin

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## Abstract

Ricin toxin chain A (RTA) is a byproduct of castor oil production that can be lethal at doses as low as 3 to 5 micrograms per kilogram of body weight[1]. Since there are currently no known antidotes for ricin, efficient detection prior to exposure is essential to avoid death. Current methods typically involve time-consuming ELISA or RIA methods[13] which, on average, require twenty-four hours of wait time. In this project, we have designed and tested an electrochemical bio-sensor that is sensitive enough to detect small and bio-medically relevant concentrations of ricin. Ultimately, this bio-sensor design allows voltammetric interrogation[7] to detect ricin toxin in complex media (such as blood serum and soup). Additionally, it is convenient in that it collects real-time data, offering applications to the monitoring processes of areas involved in castor oil production. Furthermore, it may possess diagnostic potential in assessing ricin exposure. Electrochemical DNA-based (E-DNA) sensors have the potential to be used in a variety of situations. This project exemplifies a strategy for how they may expanded to incorporate the detection and quantification of hazardous toxins.

## Ricin Facts

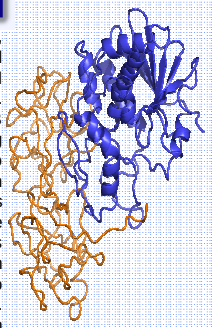
### Background

Ricin is a naturally occurring protein toxin found in castor beans[1]. It is of particular interest as it has previously been studied for use as a biological warfare agent[1] and no known antidotes exist for ricin toxicity. The most common currently used detection methodologies involve Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods, both of which are time-consuming[13].

Castor Bean Plant

### Mechanism of Action

Ricin is composed of two chains, A and B, which create the lethal threat of ricin when bound together by a disulfide bond[1,12]. Once ricin enters the body, the B chain acts as the binding component, facilitating entry into the cytosol[1]. The A chain then inactivates eukaryotic ribosomes through removal of a single adenine residue from the 28s rRNA loop, thus inhibiting protein synthesis and leading to inevitable cell death[1,12]. Ultimately, ricin toxicity results in death within 72 hours after exposure to a high enough dose.

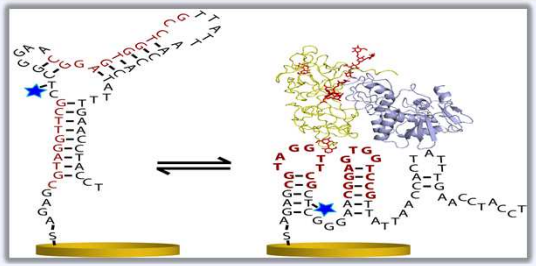


Ricin Protein Structure[11]

## Materials and Methods

Bio-sensors are a recent solution to the need for rapid diagnostic tools specific to a desired molecular target. These sensors depend on biological interactions as the basis for their sensing element. The present study focused on E-DNA bio-sensors, which have previously been demonstrated in the sensitive detection of DNA-binding proteins[2], antibodies[12], and small molecule drugs[10]. E-DNA bio-sensors present an attractive option for toxin detection, as they function in complex media and provide quantitative detection in minutes[6]. By designing an E-DNA bio-sensor that is sensitive to ricin or other biological toxins, the length of time needed to detect the presence of these toxins in diagnostic samples may be greatly reduced.

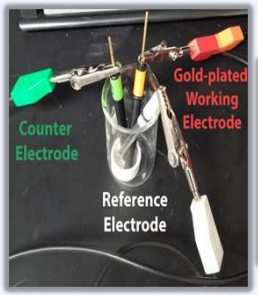
### Bio-sensor Design



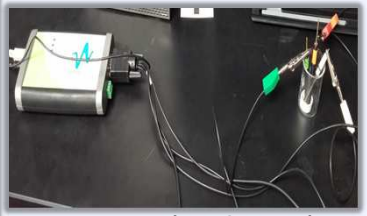
Our bio-sensor was designed based on a known aptamer that binds to the hydrolase protein in ricin toxin[14]. The aptamer was used as the core sequence of a rationally designed DNA oligonucleotide scaffold to allow for the coupling of RTA binding to a conformational change. This change was predicted using Quikfold[16]. Final selection was based on satisfying criteria including energy difference between state, number of folded states, and positioning of bases from the 5' end of the sequence. This biosensor incorporated a 5' disulfide and an internal appended methylene blue dye.

### Data Collection

The DNA bio-sensor was bound to a gold electrode surface to allow electron transfer between methylene blue dye and the electrode. Samples of 0.5 mL were used and experiments were conducted in phosphate-buffered saline (10 mM $PO_4^{3-}$, 137 mM NaCl, and 2.7 mM KCl). Square wave voltammetry was conducted using a Pine Instruments WaveNano portable potentiostat. Equilibrium binding measurements were obtained from sequential addition of ricin toxin. Peak current from methylene blue electron transfer was analyzed using custom peak fitting software[5], and varies proportionally with target concentration.

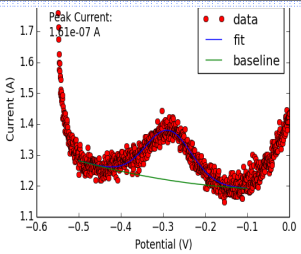


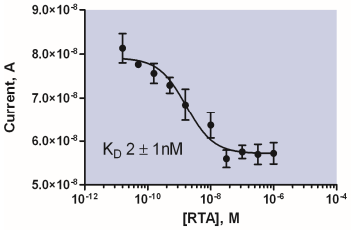

Electrode Close-Up

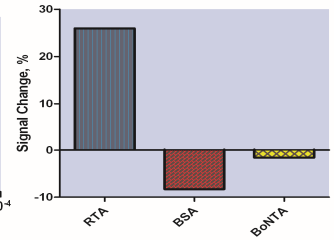


Wavenano Potentiostat Connected to Electrodes in Standard Experiment

## Results




**Example of Data Analysis**

In square wave voltammetry, we observe a peak current from methylene blue redox. This data was fit with a custom peak-fitting algorithm[8].

**Binding Curve Data Representation:**

Peak current response versus RTA concentration fits a saturation binding curve with an apparent binding affinity of 2 ±1 nM, demonstrating sensitive detection of the toxin.

**Determination of Sensor Specificity:**

When tested against off-target proteins (botilunum neurotoxin var. A & bovine serum albumin) at 100nM the bio-sensor shows minimal cross-reactivity.

## Conclusions

We have successfully designed an E-DNA bio-sensor that is sufficiently sensitive to accurately detect and quantify ricin toxin chain A (RTA) at nanomolar concentrations, with an apparent dissociation constant of 2 ± 1 nM. Additionally, this bio-sensor has robust specificity, as the measured current was not significantly affected upon the addition of off-target proteins including bovine serum albumin (BSA) and botulinum neurotoxin variant A (BoNT/A). Although sensor response was decreased in the complex media of bovine blood serum (data not shown), strategies have recently been demonstrated which allow E-DNA bio-sensors to function in whole blood via a laminar flow separation methodology[5]. In the future, these types of optimizations will be explored to allow this sensor to function well in biological media.

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