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# Development of an Antibody Based Electrochemical Platform for Cas9 Detection

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## Motivation and Strategy

- In 2011 the demonstration of RNA guided DNA editing using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system spawned a new era in biotechnology.
- Quantification of CRISPR/Cas 9 components needed for optimal gene editing activity, with minimal off target or other deleterious effects, is of great importance in achieving therapeutic gene editing
- Traditional antibody based detection methods (Western blot, ELISA), while effective, are either time consuming or are only semi quantitative.
- Electrochemical biosensors have shown highly robust and selective detection capability of a variety of analytes, with detection limits in the picomolar and femtomolar range[1]
- In an effort to develop more robust analytical platforms for Cas9 detection, we are investigating a rapid electrochemical bioassay for Cas9 detection utilizing commercial Cas9 antibodies and aryl diazonium electrochemistry.

## Gold Electrode Functionalization

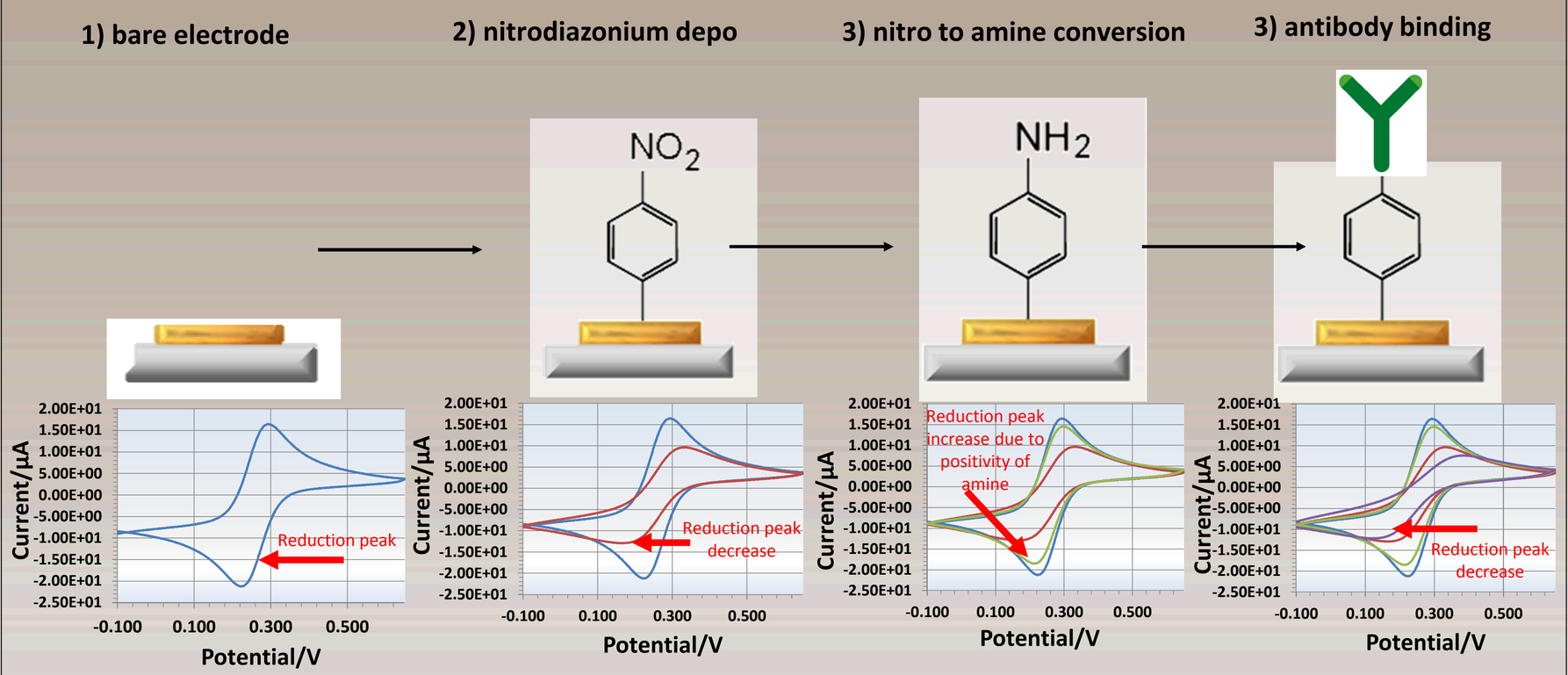
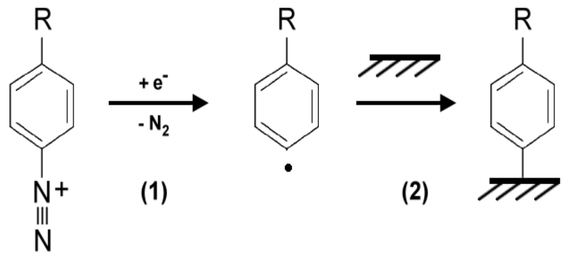



Figure 1. Cyclic voltammogram (CV) of gold electrode surface modification with nitrodiazonium and Cas9 antibody. CV were taken of the 1) bare electrode, 2) after nitrodiazonium deposition, 3) after nitro to amine conversion, and 4) after Cas9 antibody conjugation. CV are overlaid with CV of the preceding steps. Decrease in the peak height after antibody binding (green curve compared to purple curve) shows antibody binding to nitrodiazonium modified electrodes

## Methods

- Electro-addressable surface modification of gold electrodes with nitrodiazonium Salts.
- Crosslinking of reduced nitrodiazonium with N-terminal and C-terminal targeting SpyCas9 antibodies
- Measurement of electrochemical kinetics using cyclic voltammetry




## SpyCas9 Electrochemical Detection in Buffer

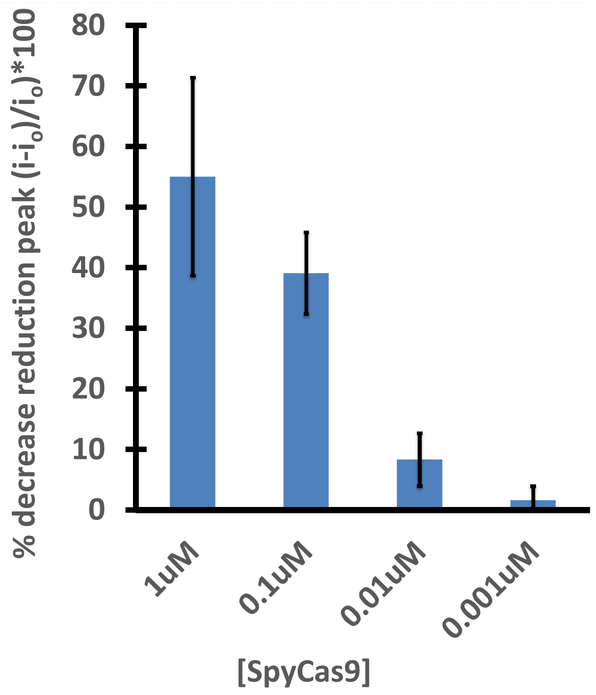



Figure 2. Electrochemical detection of SpyCas9 in simple buffer. Gold electrodes were modified with nitrodiazonium and N-terminal Cas9 antibody. Antibody modified electrodes were exposed to 1× PBS containing SpyCas9 at varying concentrations for 2 hours, washed, and a CV scan performed. Data are presented as the % decrease in the CV reduction peak height after incubation of antibody modified electrodes in samples ($(i-i_o)/i_o*100$, where i = reduction peak height after protein exposure, and $i_o$ = reduction peak height after antibody binding. N = 3 electrodes).

## Electrochemical Detection of SpyCas9 in the Presence of BSA

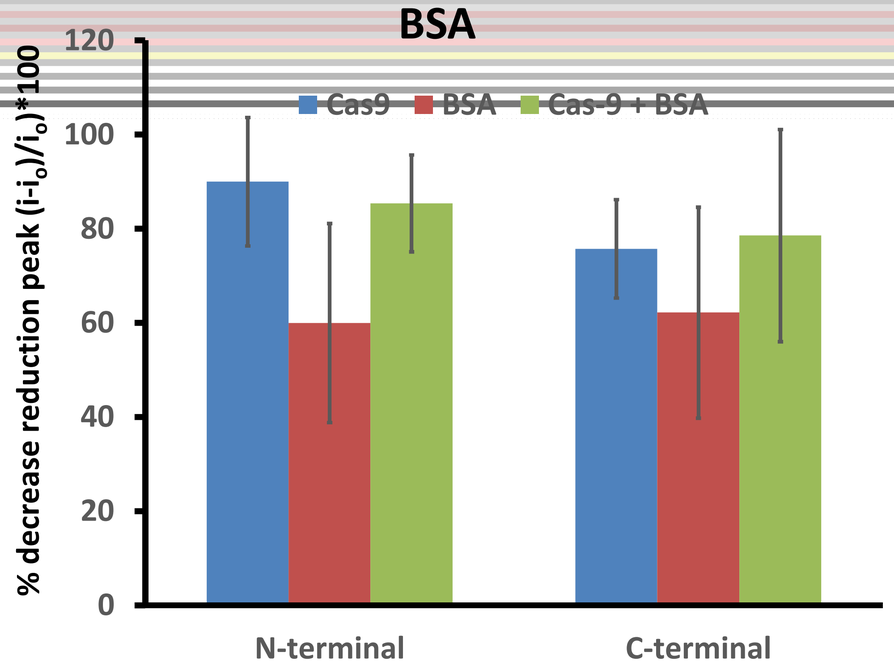



Figure 3. Electrochemical detection of Cas9 in the presence of non-specific protein. Cas9 antibody conjugated gold electrodes were incubated in 1× PBS containing 5 uM BSA, 5 uM SpyCas9, or 5 uM BSA and 5uM SpyCas9 for 2 hours. Data are presented as the % decrease in the CV reduction peak height after incubation of antibody modified electrodes in samples ($(i-i_o)/i_o*100$, where i = reduction peak height after protein exposure, and $i_o$ = reduction peak height after antibody binding N = 3 electrodes).

## Conclusions and Future Work

- Electro-addressable surface modification of gold electrodes allows functionalization of electrodes with SpyCas9 antibodies
- When attached to gold electrodes, both N and C-terminal Cas9 antibodies maintain the ability to recognize and bind SpyCas9, high non-specific binding of BSA necessitates additional blocking steps
- This platform allows detection of SpyCas9 even in the presence of equimolar amounts of BSA, continuing to refine platform for detection of SpyCas9 in complex samples

### Acknowledgements & References

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1. Huang et. al. Disease-Related Detection with Electrochemical Biosensors: A Review. *Sensors*. Oct. 2017

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