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Rapid Electrochemical Detection of CRISPR/Cas9 Components using anti-CRISPR Protein AcrIIA4 as Capture Ligand

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Sandia National Laboratories

Exceptional service in the national interest

Detection of CRISPR/Cas9 Gene Editing Components

The demonstration of RNA-guided DNA editing using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system (Cas) has revolutionized the field of molecular biology. The facile, programmable nature of gene editing afforded by CRISPR/Cas9 has led to a multitude of *in-vitro* and *in-vivo* applications from basic science to clinical applications including development of model cell lines, discovering mechanisms of disease, identifying disease targets, development of transgene animals and plants, and transcriptional modulation [1].

Within these applications, quantification of the individual CRISPR/Cas9 components needed for optimal gene editing activity is vital in order to optimize genomic editing, while minimizing the potential for off-target or other deleterious effects. Traditional biochemical-based detection methods (Western blot, ELISA), while effective, are either time consuming or are only semi-quantitative, necessitating development of more rapid and precise analytical methods for CRISPR components detection. Further, development of effective capture ligands (e.g. antibodies, peptides) against these new targets is laborious, costly and time consuming.

[1] Lino, Harper, Carney, Timlin et al., *Drug Delivery* **2018,** *2*, 1234.

Natural Anti-CRISPR (Acr) Proteins as Novel Capture Ligands

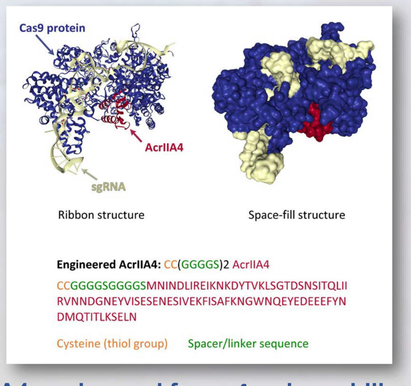
Recently discovered anti-CRISPR (Acr) proteins are naturally occurring peptides that bind tightly to CRISPR proteins, deactivating the DNA cutting properties of the protein.

Here, we show for the first time use of an anti-CRISPR protein, AcrIIA4, as a capture ligand for specific detection of Cas9 and Cas9-ribonucleoprotein (Cas9-RNP). The AcrIIA4 protein was engineered with a twelve amino acid spacer sequence containing two thiol groups to allow for covalent immobilization to an electrode surface.

Quantifiable electrochemical detection of Cas9 protein and Cas9-RNP with picomolar detection limits, in whole cell lysates, and a total assay time of less than three hours is demonstrated.

Successful use of naturally occurring anti-CRISPR proteins as biorecognition elements opens intriguing opportunities for bioanalytical assay development that are challenging, or not possible, using antibody based-systems.

AcrIIA4 bound Cas9-RNP Complex

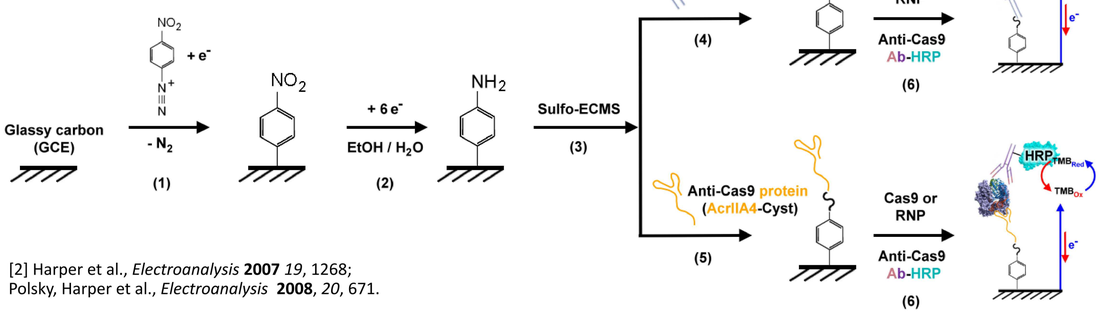


AcrIIA4 engineered for surface immobilization

Electrochemical Assay for Rapid and Quantitative Detection of Cas9 and Cas9-RNP

Immobilization of Anti-Cas9 Antibodies or Anti-CRISPR/Cas9 AcrIIA4 onto Glassy Carbon Electrodes

Aryl diazonium chemistry allows for 1) controlled surface conjugation, 2) well defined amino group density, and 3) provides a robust and stable covalent bond between the capture ligand and electrode surface [2]

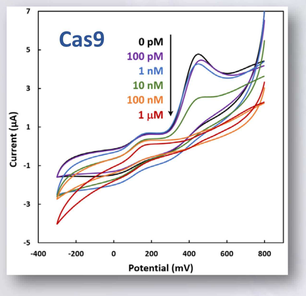


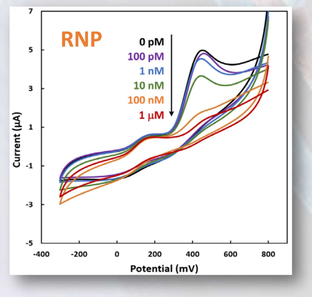
[2] Harper et al., *Electroanalysis* **2007** *19*, 1268;
Polsky, Harper et al., *Electroanalysis* **2008**, *20*, 671.

Electrochemical detection employs horseradish peroxidase (HRP) labeled detection antibodies (Ab-HRP) which turnover an electroactive substrate: 3,3',5,5'-tetramethylbenzidine (TMB)

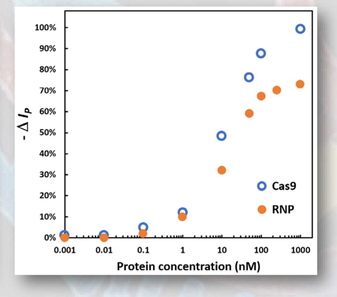
Antibody-based Capture and Detection of Cas9 and Cas9-RNP

Electrochemical Detection





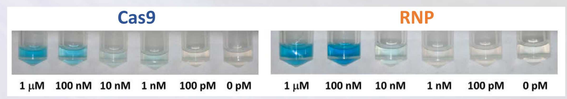
Signal vs. Concentration



Cyclic voltammograms show classic electrocatalytic behavior – decrease in enzyme substrate (TMB) oxidization wave – with increasing protein concentration.

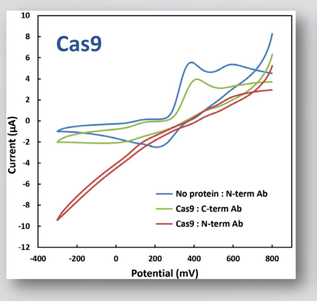
Detection limit (3σ above background) for Cas9 = 235 pM, and Cas9-RNP = 715 pM.

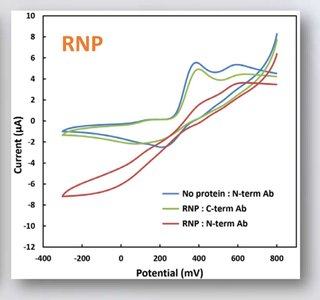
TMB turnover results in visible product, proving an orthogonal colorimetric detection method

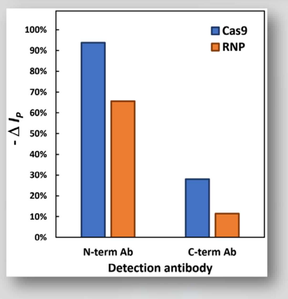


AcrIIA4 is an Effective Cas9 & Cas9-RNP Capture Ligand

AcrIIA4 Capture of Cas9 and Cas9-RNP Hinders Access to Cas9 C-terminus







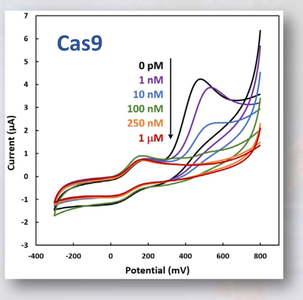
Cyclic voltammograms show less significant reduction in electrocatalytic peak using an anti c-term detection Ab.

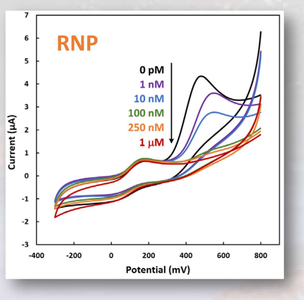
A recent report [3] showed that AcrIIA4 binds to the Cas9/Cas9-RNP c-term, hindering access.

Detection assays subsequently used anti n-term detection Abs.

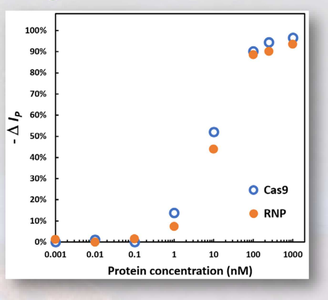
AcrIIA4-based Capture and Detection of Cas9 and Cas9-RNP

Electrochemical Detection





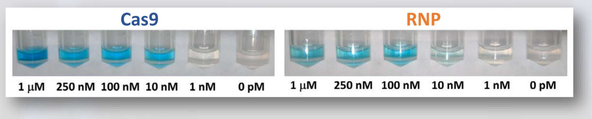
Signal vs. Concentration



Cyclic voltammograms show electrocatalytic behavior similar to Ab capture-based detection indicating AcrIIA4 can serve as an capture/binding ligand alternative to antibodies.

Detection limit (3σ + background) for Cas9 = 480 pM, and Cas9-RNP = 840 pM. These higher detection limits are indicative of the higher K_D for AcrIIA4 [3] vs. IgG.

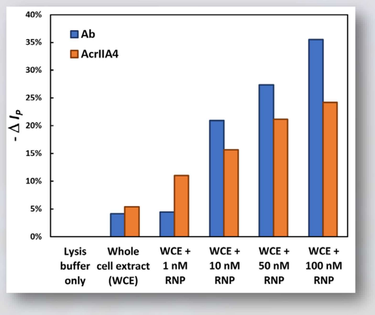
TMB turnover colorimetric detection



[3] Kim et al., *Sci. Rep.* **2018,** *8,* 3883

AcrIIA4 vs. Ab Capture of Cas9-RNP in Whole-Cell Extracts

Electrocatalytic Signal vs. Concentration [4]



Both Ab-based and AcrIIA4-based capture surfaces show small (~5%) non-specific binding signals after treatment with whole-cell extracts (WCE).

Both surfaces also show concentration dependent Cas9-RNP detection in the complex sample matrix.

[4] Johnston, Seamon, Saada, Branda, Timlin, Harper, **2018**, *in prep.*

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NNSA National Nuclear Security Administration

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