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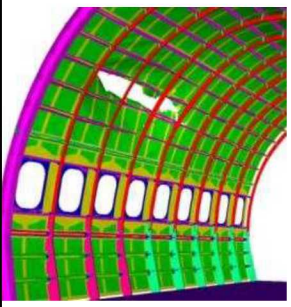

Monitoring the Revolution: Anti-CRISPR Based Biosensing Platform for CRISPR-Cas9 Detection

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New Mexico Tech Biology Seminar
Socorro NM
September 30, 2019

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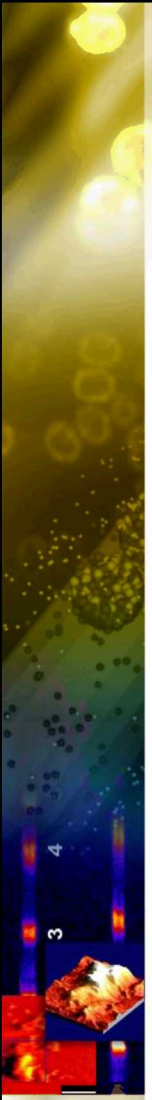
Introduction

- Introduction
  - CRISPR-Cas9
    - Biology
    - Clinical Applications
  - Anti-CRISPR proteins
- Anti-CRISPR based biosensor
  - Motivation
  - Approach
  - Data



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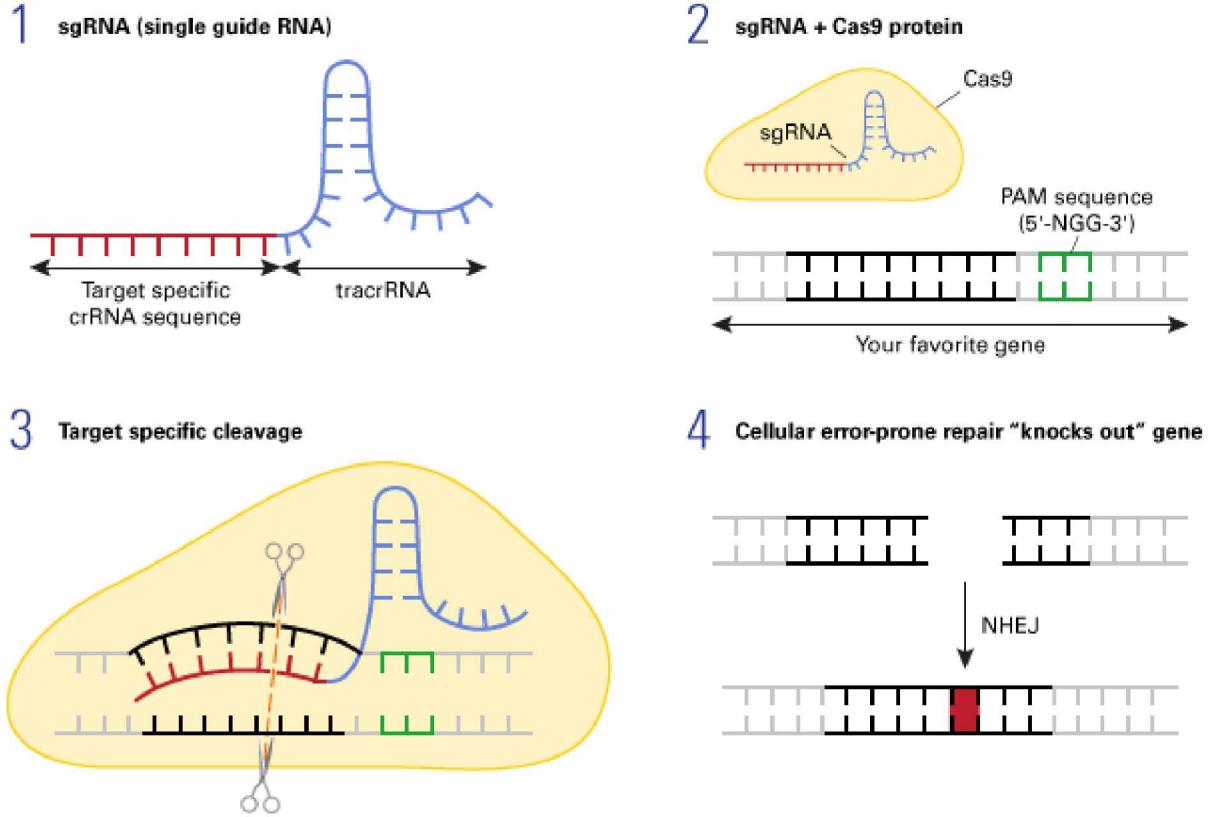
CRISPR-Cas9: A Biotechnology Revolution

- CRISPR-Cas9 is a bacterial adaptive immune system reengineered for targeted genome editing



Takarabio.com

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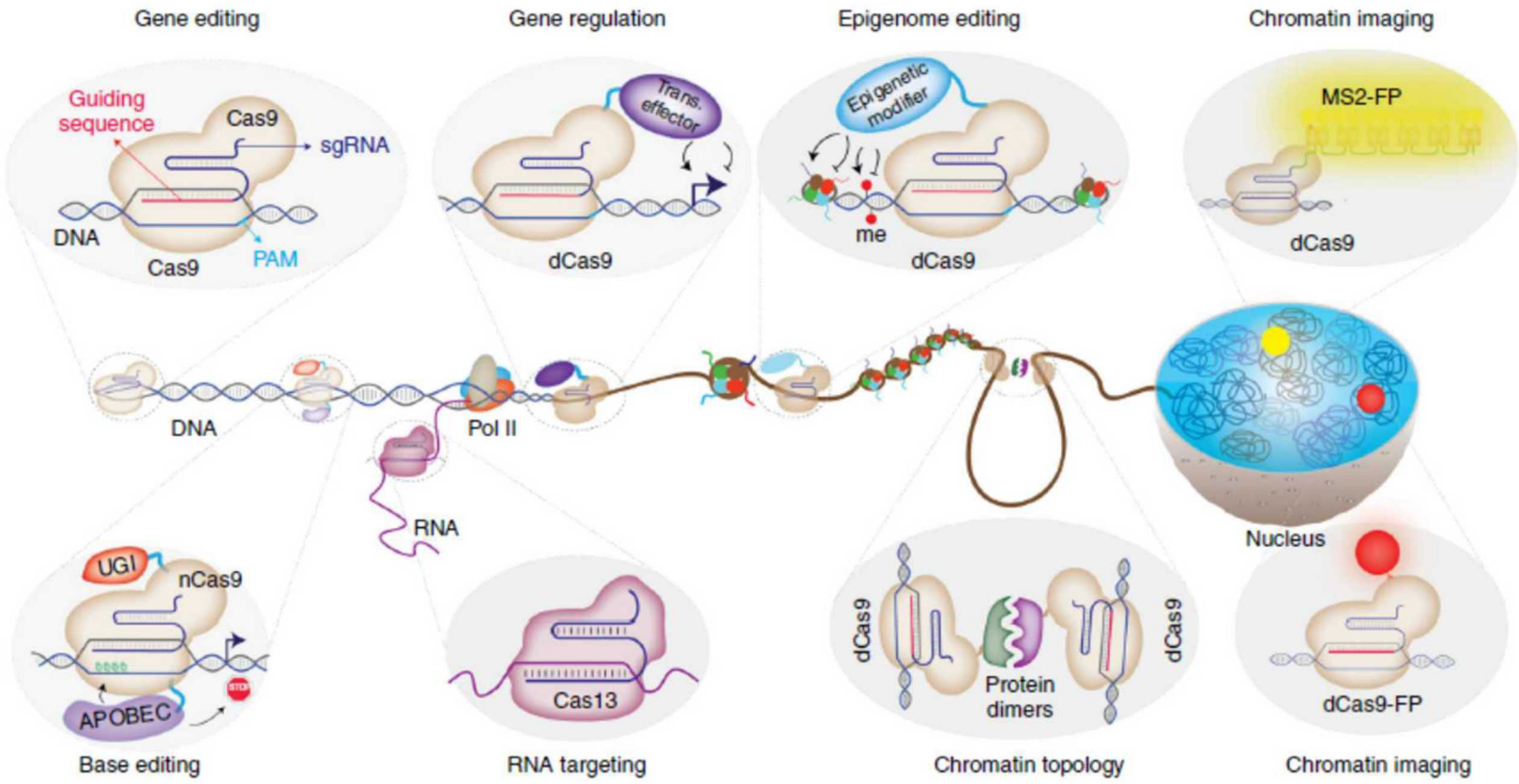
CRISPR Toolbox-More than a Pair of Scissors



SpyCas9 serves as the backbone for engineered protein for:
Epigenetic editing
Single base editing
RNA editing

Adli *Nature Comm.* 2018

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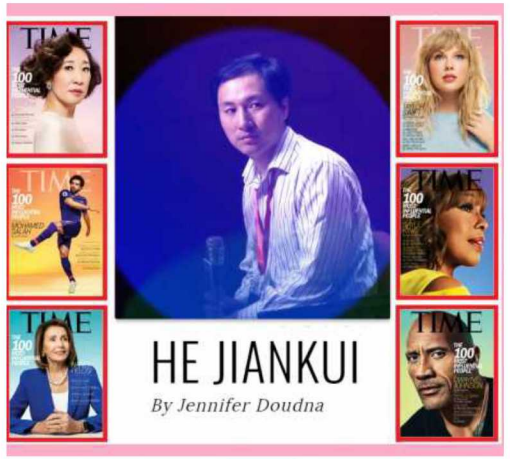
CRISPR-Beyond the Bench

- CRISPR-Cas9 Clinical Trials
  - 28 registered trains on clinicaltrials.gov
    - Sickle Cell Anemia
    - Beta-Thalassemia
    - HIV
    - Cancer

- CRISPR-Cas9 Human Embryo Editing
  - *MYBPC3*
  - *Oct4*
  - *CCR5*



Somatic Cell Editing = Non-heritable

Germline Editing = Heritable

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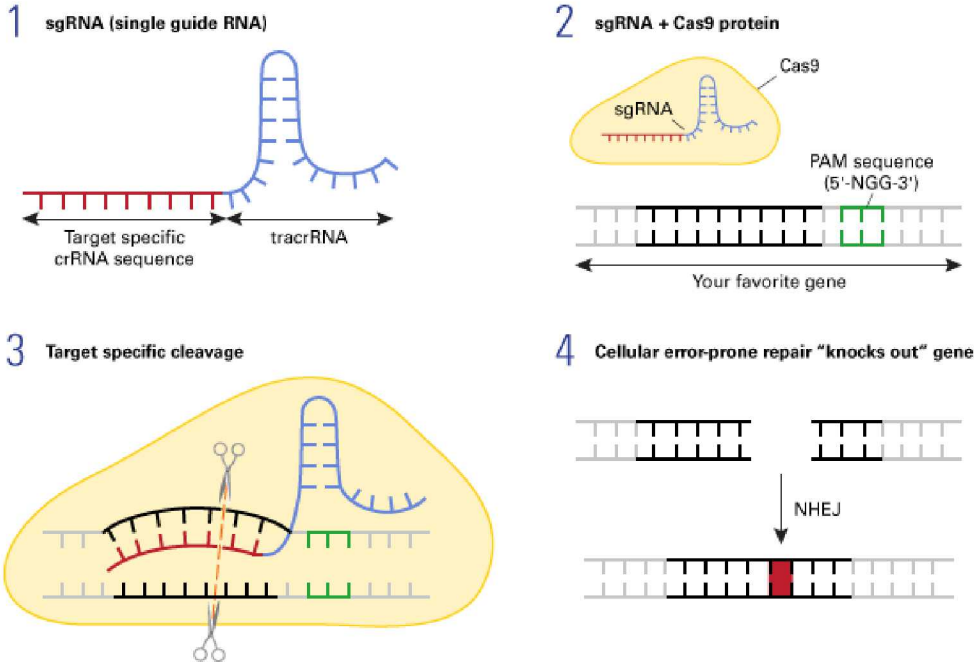
Robust Editors Require Robust Detectors

- Multiple methods of detecting CRISPR edits
  - TIDE
  - T7E1
  - NGS
  - Functional Analysis
- Corresponding lack of methods for detecting the "scissors"
  - Western Blot
  - ELISA



Problem: Off-target edits and Immune Response correlated with dosing

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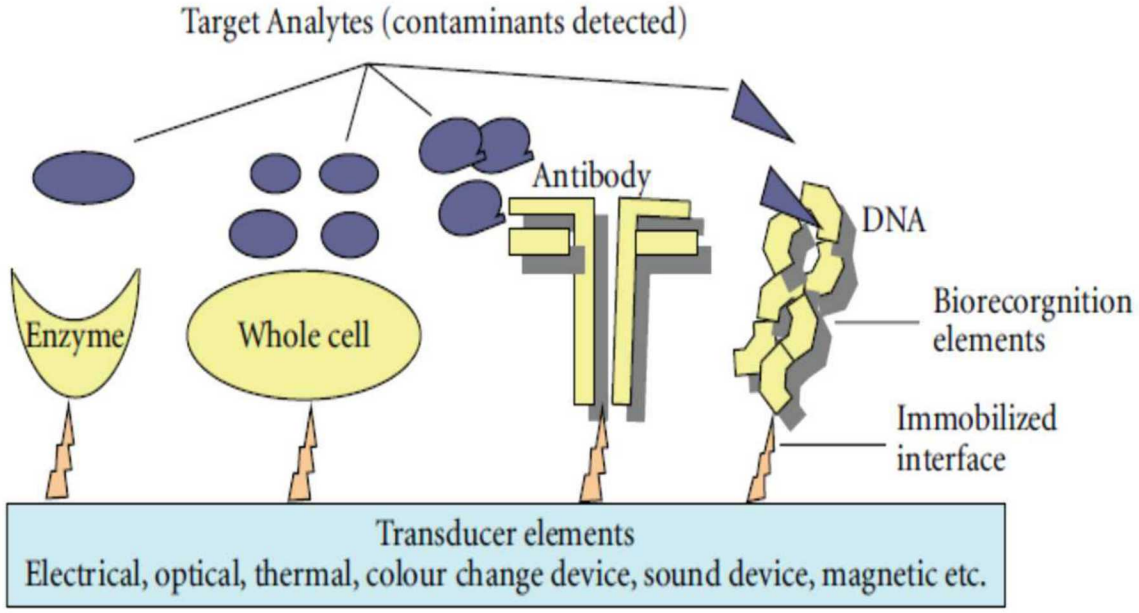
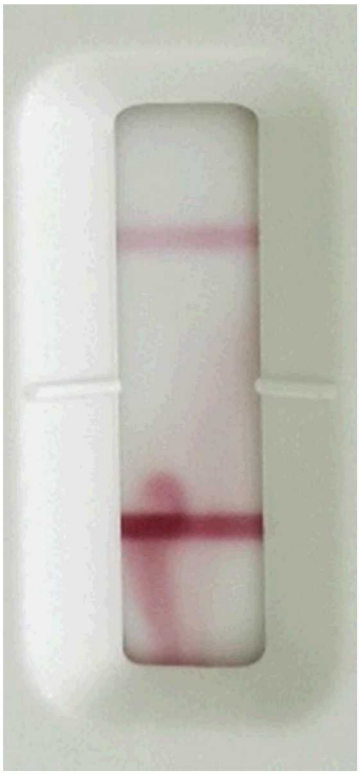
Biosensors Can Fill the Detection Gap





Antibody based lateral flow assay for in-field detection of *B. anthracis*

- Biorecognition elements provide selectivity and specificity
- Transducer element provides signal amplification and transduction
- Rapid, low-cost, portable

S. Ripp et. al, *Environmental Microbiology,* 2010.

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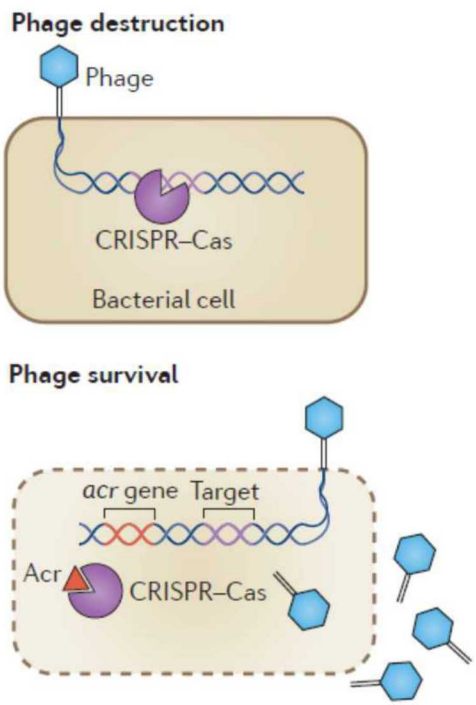
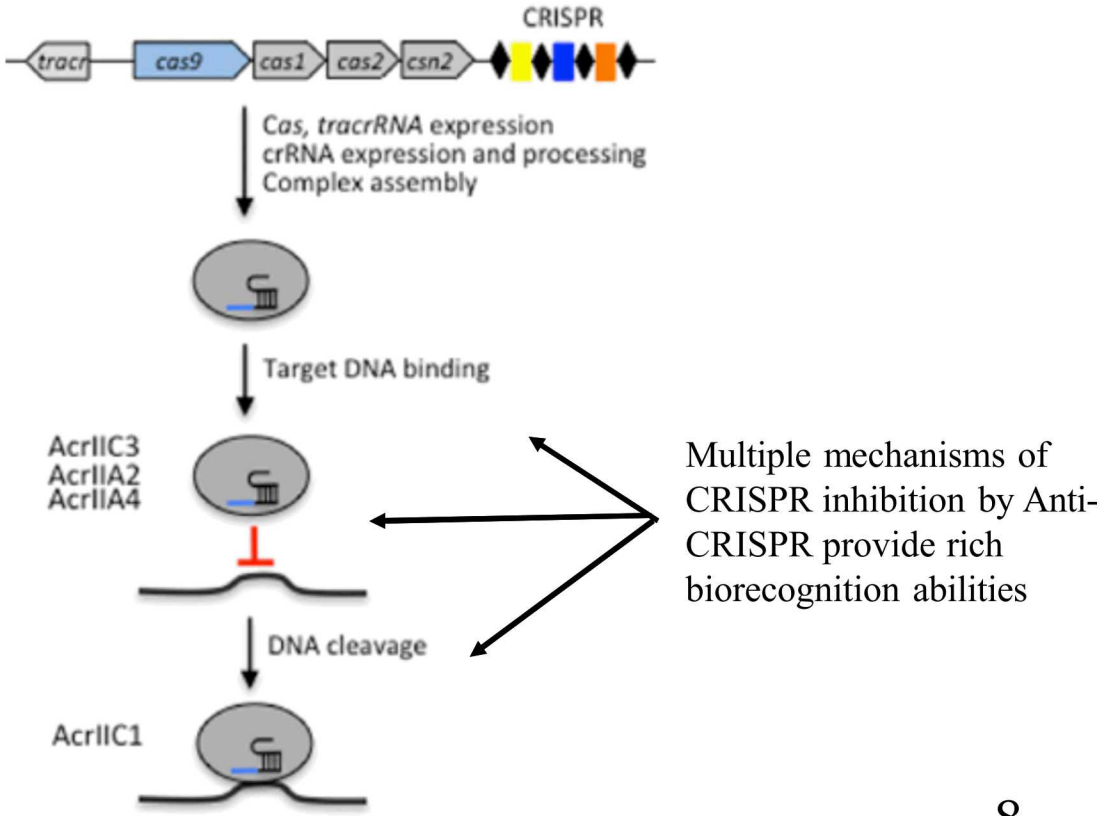
Anti-CRIPR (Acr) Proteins: Natural CRISPR Recognition Elements

- Pace of CRISPR field makes developing antibody based recognition elements difficult
- Answer – Mine natural bacteriophage CRISPR countermeasures





Pawluk et. el *Nat. Rev. Microbiology* 2018
Maxwell, *Molecular Cell* 2018

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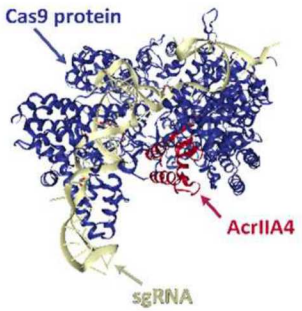
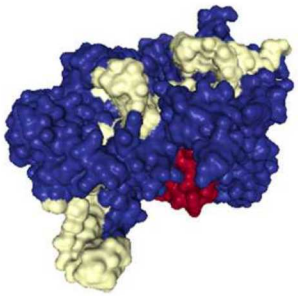
Building an Anti-CRISPR biosensor-Protein Engineering



Ribbon structure

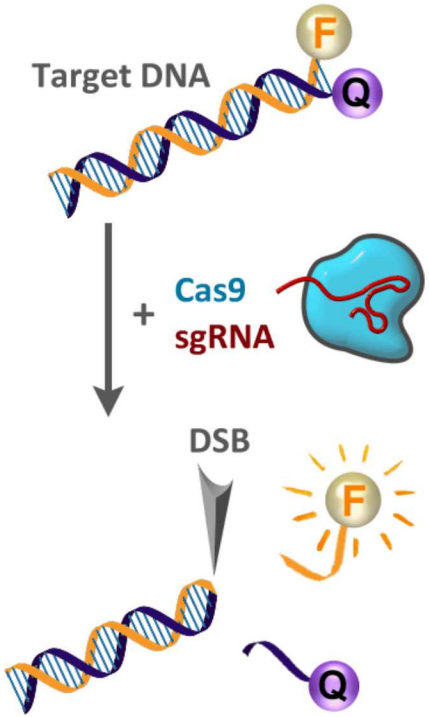


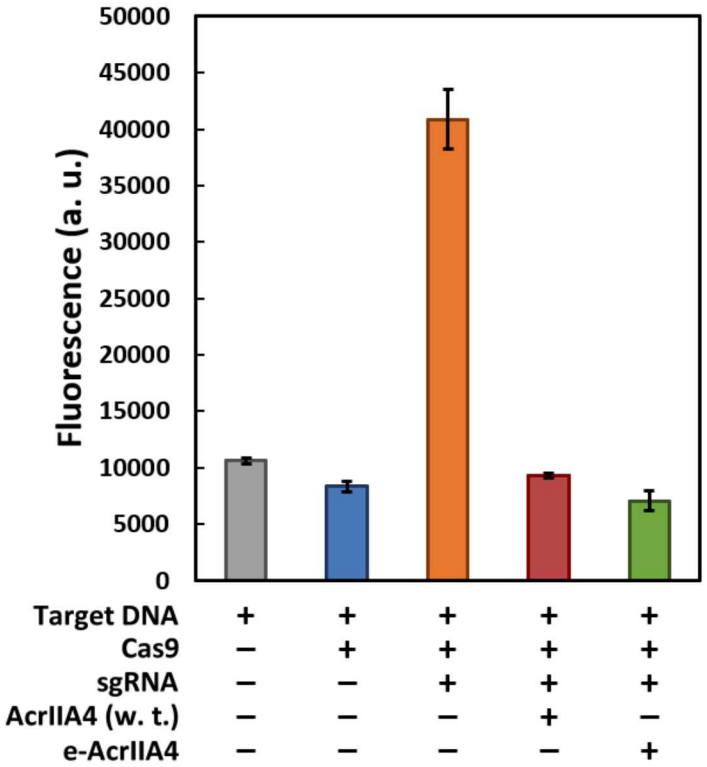
Space-fill structure

Engineered AcrIIA4: CC(GGGGS)2 AcrIIA4

CCGGGGSGGGGSMNINDLIREIKNKDYTVKLSGTDSNSITQLII
RVNNDGNEYVISESENESIVEKFISAFKNGWNQEYEDEEEFYN
DMQTITLKSELN

Cysteine (thiol group) Spacer/linker sequence





- Need a "handle" to covalently immobilize AcrIIA4 onto transducer elements
- Modify wild type AcrIIA4 with 12 aa linker with terminal cysteines for thiol chemistry (e-AcrIIA4)
- Validated activity in SNL developed FRET assay

Johnston et. al *Biosensors and Bioelectronics* 2019
Seamon et. al *Analytical Chemistry* 2017

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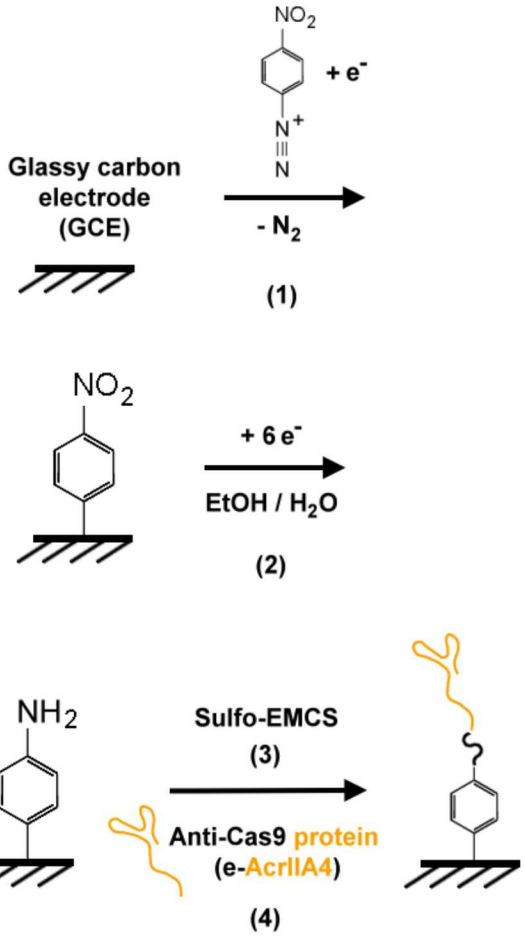
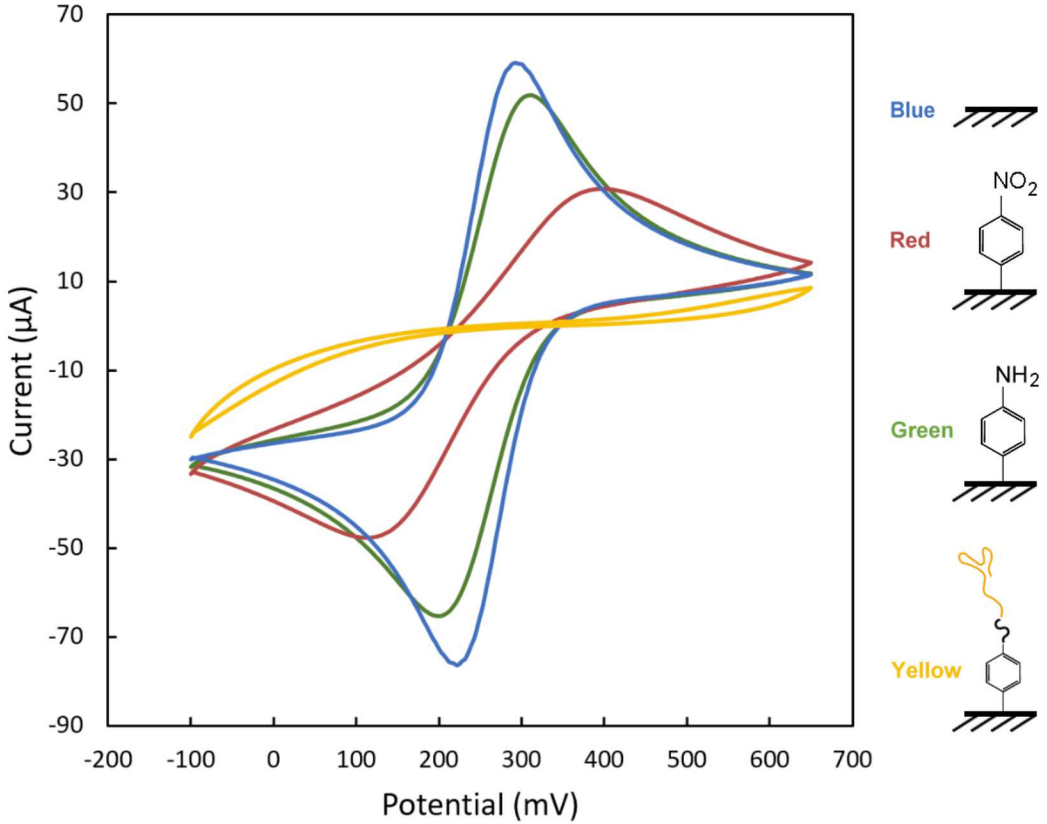
Building an Anti-CRISPR biosensor-Transducer Element

- Utilize electrochemical methods and aryl diazonium chemistry for generating conductive surfaces
- Conversion of NO_2 to NH_2 and treatment with Sulfo-EMCS provide thiol reactive surface for e-AcrIIA4 covalent attachment





Johnston et. el *Biosensors and Bioelectronics* 2019

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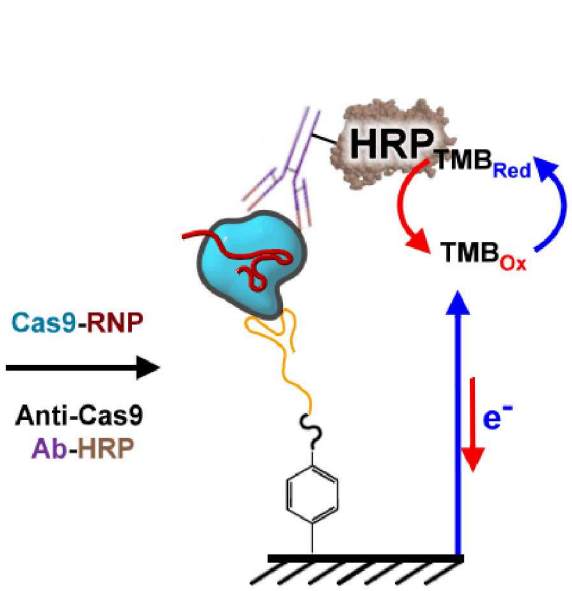
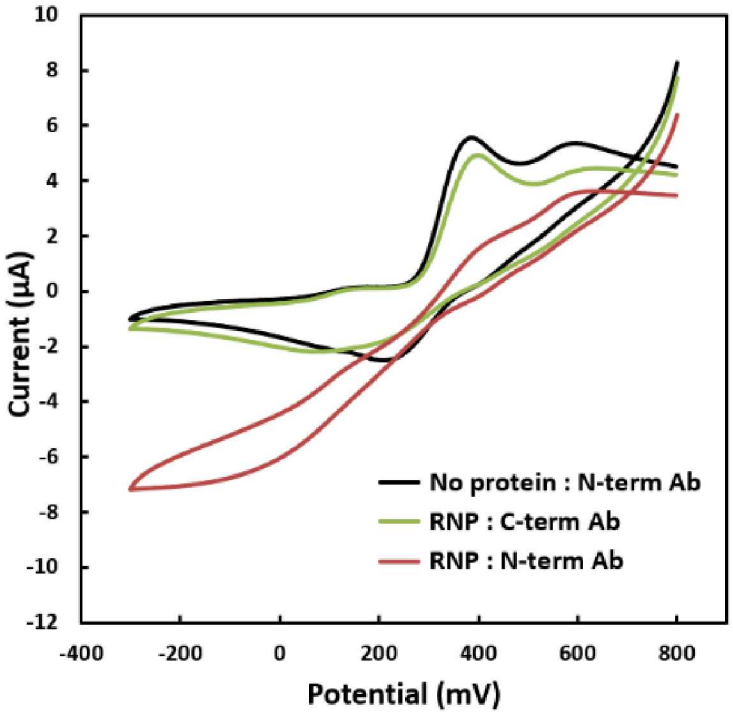
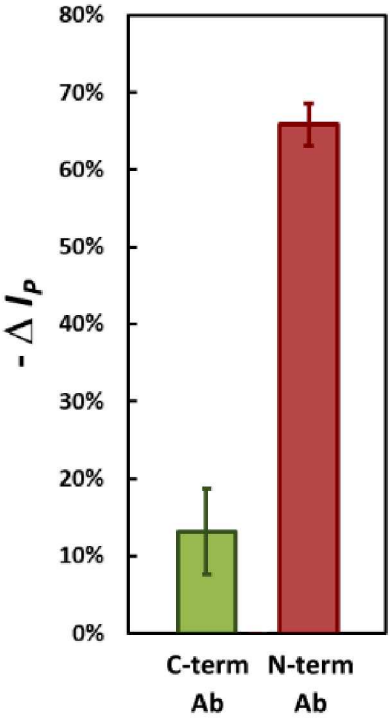
Detection Optimization

- Acr facilitates Cas9 capture, still need a way to generate measurable signal
- Answer – utilize anti-Cas9 antibodies coupled to signaling molecules
- Significant difference between C and N-term targeting Ab







Johnston et. el *Biosensors and Bioelectronics* 2019

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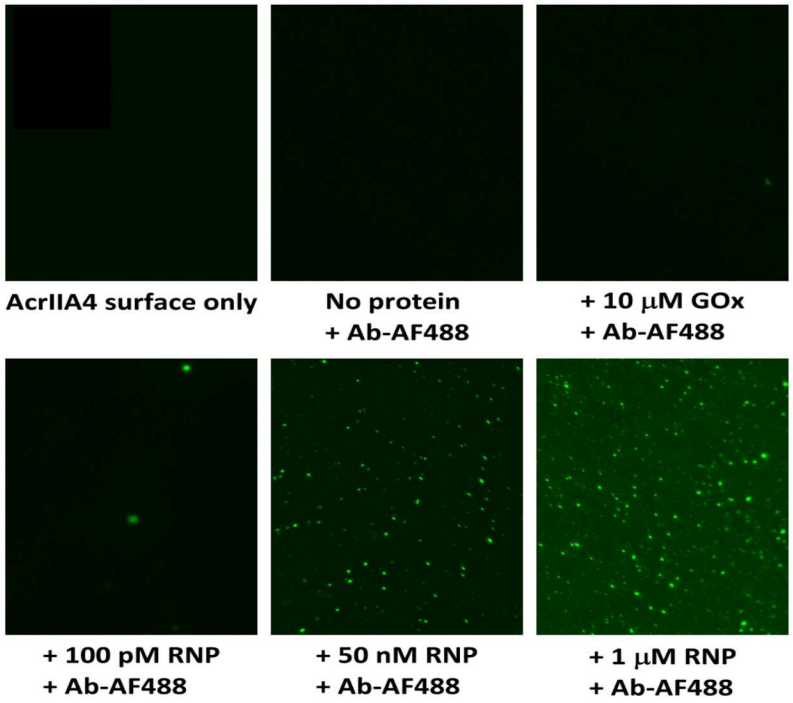
Dose Responsive Signal Orthogonality

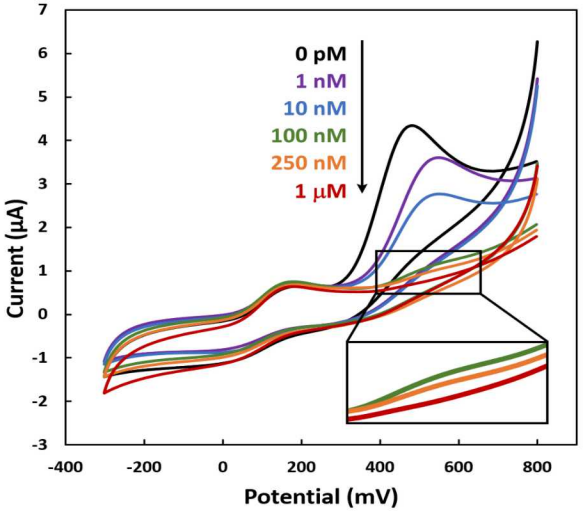
Acr capture surface combined with Anti-Cas9 Ab allows for electrochemical, fluorescent, or colorimetric signal outputs

fluorescent



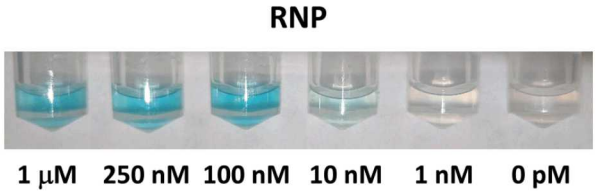
LOD = 3 nM

electrochemical



LOD = 280 pM

colorimetric



LOD between 10 – 100 nM

Johnston et. el *Biosensors and Bioelectronics* 2019

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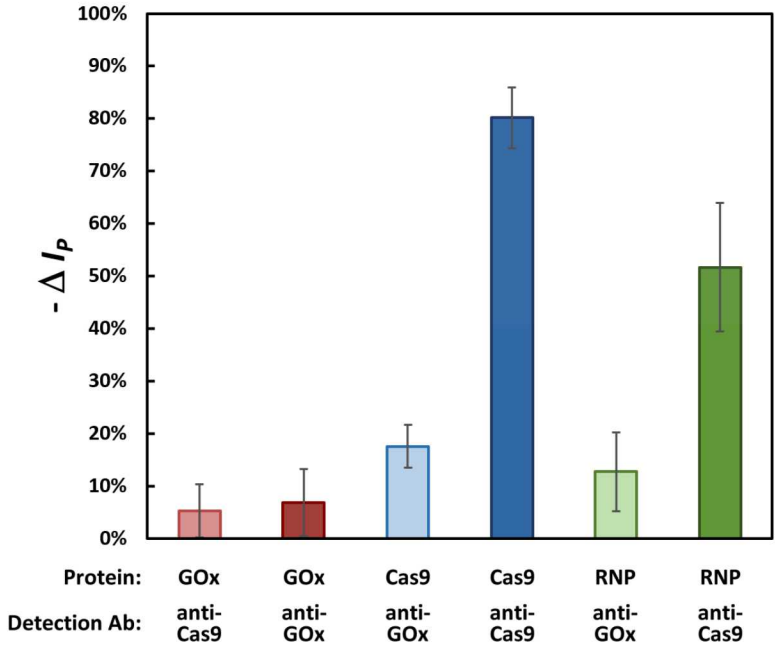
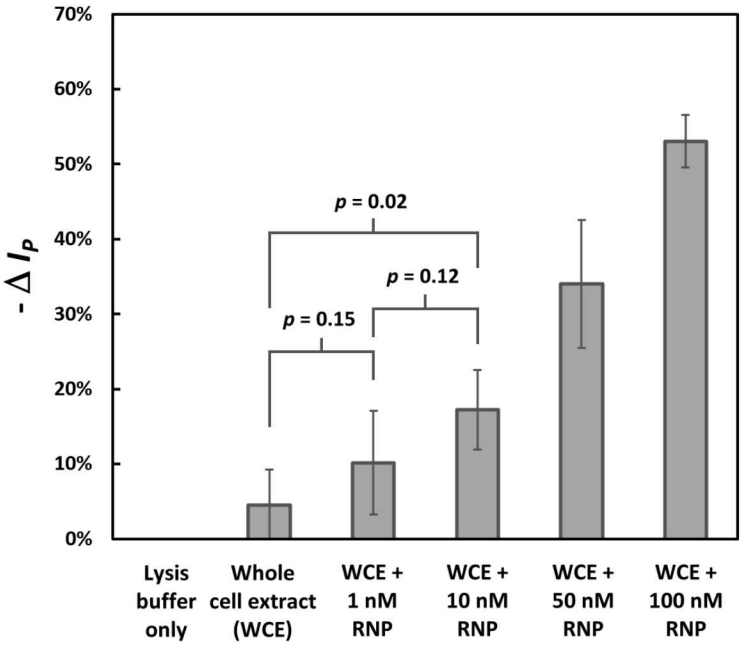
Analytical Sensitivity of Acr based Electrochemical Biosensor





Acr capture surface shows specificity for SpyCas9 over a similarly sized non-specific protein (Gox) when probed with Cas9 Ab

Platform can detect SpyCas9-RNP in cell extracts with a LOD of 9 nM

Johnston et. el *Biosensors and Bioelectronics* 2019

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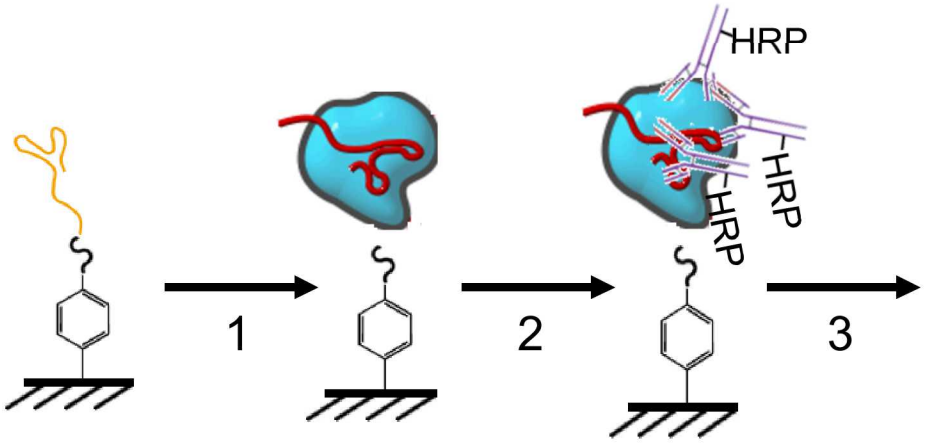
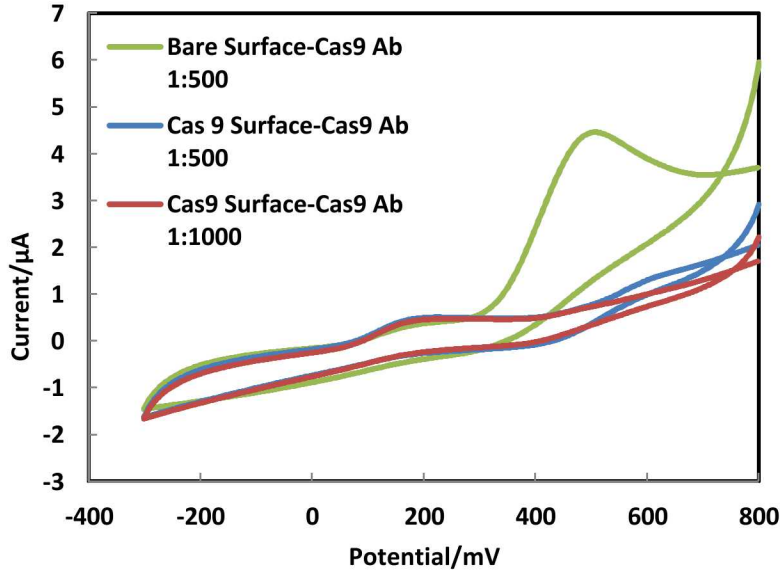
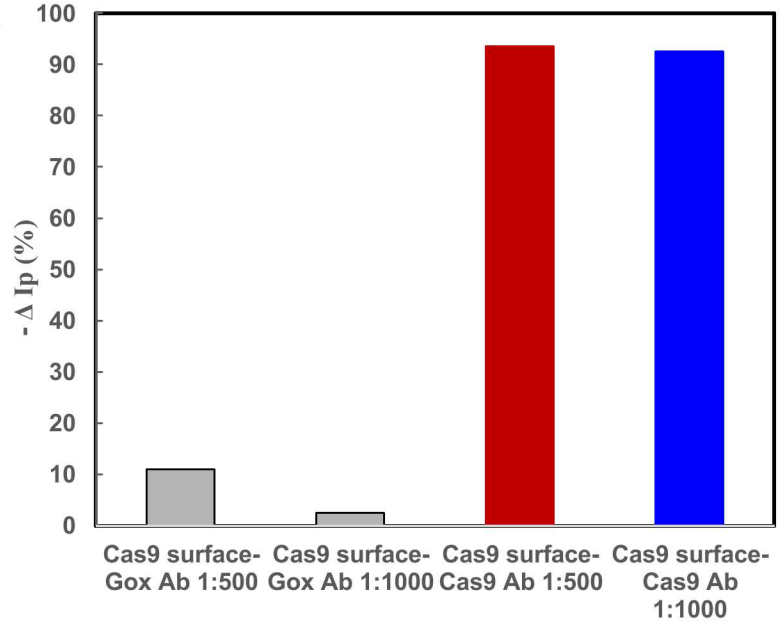
Detecting Orthogonal Clinical Responses to Cas9

- AcrIIA4 modified electrodes facilitate effector identification
- Can we modify electrodes with the effector and detect orthogonal clinical response (i.e. humoral immune response?)



1. Swap e-AcrIIA4 for cys – Cas9
2. Pull down Anti-Cas9 Ab
3. Measure electrochemical response





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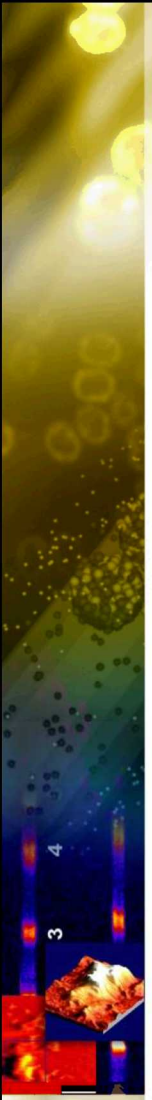
Summary and Future Work

Summary

- Incorporated Anti-CRISPR protein AcrIIA4 onto glassy carbon electrodes
- Demonstrated AcrIIA4 modified electrodes afford electrochemical, fluorescent and/or colorimetric signal outputs for SpyCas9 detection
- Established specific detection of SpyCas9-RNP in whole cell extracts using AcrIIA4 modified electrodes
- Demonstrated antibody pulldown using Cas9 as a capture element

Future Work

- Develop additional Acr based platforms for other clinically relevant CRISPR editors (SauCas9, Cas12, Cas13)
- Develop multiplex system for identification of both gene editing effector and potential immune response



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Acknowledgements

Sandia National Labs – New Mexico

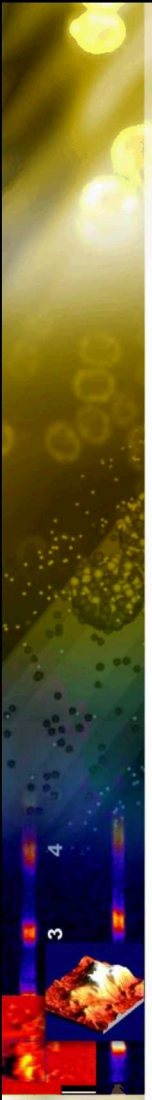
Dr. Jason C. Harper
Dr. Joshua Podlevsky
Dr. Jerilyn Timlin

Sandia National Labs – California

Dr. Edwin A. Saada
Dr. Steven S. Branda
Dr. Kyle Seamon

Funding

Sandia National Labs LDRD Program
DARPA Safe Genes Program



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