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Exceptional service in the national interest

Sandia National Laboratories

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A Revolutionary Platform for Rapidly Countering Emerging and Gentically-Enhanced Biological Threats

PROBLEM

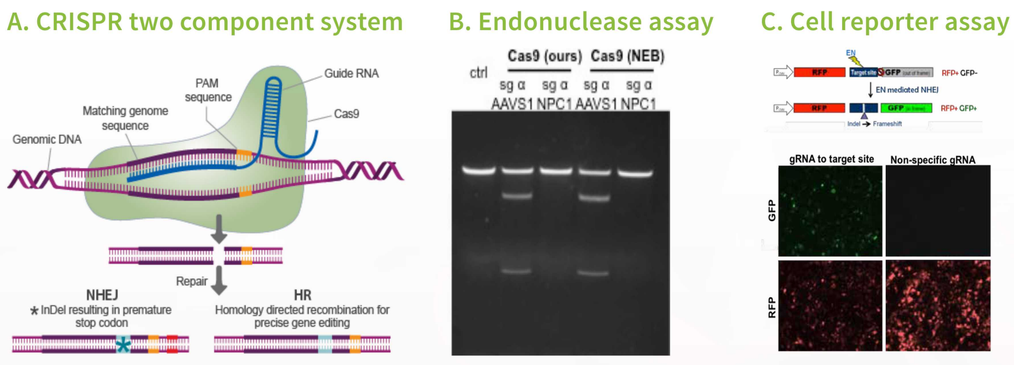
Two urgent problems currently threaten national and global biosecurity: (1) the accelerating emergence of highly virulent, transmissible, drug-resistant pathogens and (2) globally-available, low-cost tools for creating and re-engineering organisms, which greatly increase the odds of accidental or intentional manufacture and release of deadly pathogens. The hallmark of these biothreats is genetic novelty that evolves naturally or is introduced deliberately to enhance virulence and multi-drug resistance, rendering existing countermeasures ineffective. To address the looming threat of these organisms, our nation must have the nimble, flexible capacity to produce medical countermeasures rapidly in the face of any attack or threat, known or unknown, including a novel, previously-unrecognized, naturally-occurring emerging infectious disease.

APPROACH

To solve this Grand Challenge, we propose to develop what we term 'nanoCRISPR,' a rapid, cost-effective, universal technology for identifying and delivering potent new medical countermeasures against emerging and engineered biological threats. We will utilize CRISPR, a recent, revolutionary discovery that was voted Science magazine's '2015 Breakthrough of the Year due to its ability to edit target genes in a highly controlled manner, to develop novel pathogen- and host-directed countermeasures. We will then package CRISPR components within state-of-the-art nanoparticle delivery vehicles developed at Sandia and modulate various nanoparticle properties, including size and surface modifications, to deliver them to specific organs, promote their uptake by pathogen-infected cells, and release them within appropriate intracellular locations in order to achieve targeted cleavage of pathogen RNA/DNA or targeted disruption of pathogen-host interactions.

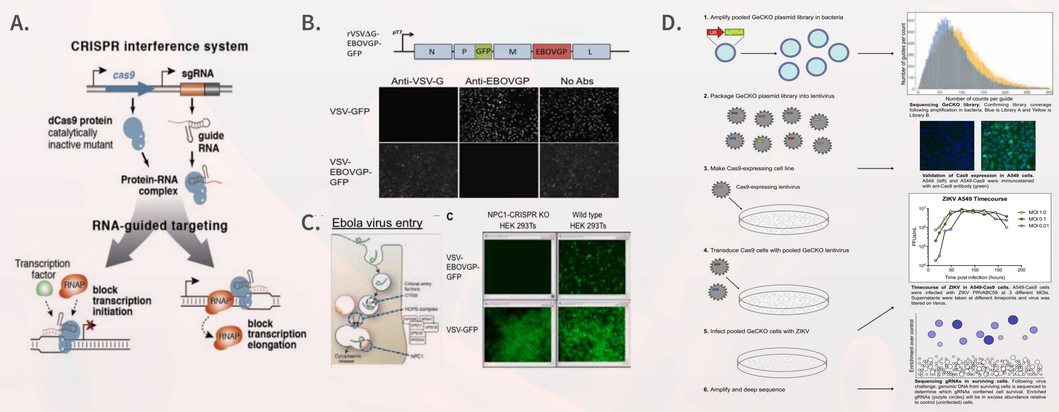
CRISPR TECHNOLOGY

Figure 1. CRISPR/Cas9 schematic and assay development. (A) CRISPR/Cas9 gene editing tools have been adapted from a prokaryotic immune system to provide sequence specific genome targeting of any species. The Cas9 endonuclease introduces double stranded breaks in the genome that can be repaired by one of two mechanism. The non-homologous end joining (NHEJ) pathway creates random insertions or deletions (indels) at the targeted site. (B) Digestion of plasmid DNA containing the AAVS1 site and PAM sequence using commercially available and Sandia produced Cas9 plus gRNA ribonucleic protein (RNP) complexes. (C) The design of a CRISPR reporter for cell-based testing places a target CRISPR site between RFP and GFP genes with the GFP gene out of frame. Endonuclease activity targeted to the CRISPR site will induce indels which moves the GFP in frame. Examples of reporter efficiency with a non-specific gRNA control are shown.



ANTIVIRAL CRISPR DESIGN

Figure 2. Targeted and discovery based approaches to host-directed CRISPR antiviral designs. (A) Direct targeting of RNA viruses such as Ebola or Zika viruses is not currently possible with Cas9. However, CRISPRi is a alternative use of the CRISPR/Cas9 tool to repress host gene expression and can indirectly inhibit pathogen infection by disrupting critical pathogen-host interactions. Vesicular stomatitis virus (VSV) expressing the Ebola Zaire glycoprotein and GFP (VSV-EBOVGP-GFP) was designed and tested as shown in (B) and used to infect cells (C) where the NPC1 Ebola receptor was knocked out through CRISPR gene editing. (D) Methods for conducting a genome-wide knockout screen using the two vector GeCKO (Genome-scale CRISPR Knock-Out) Library (V 2.0). Following amplification of the library and generation of Cas9-expressing cells, pooled GeCKO lentivirus is made and used to transduce Cas9 cells. After allowing gene editing to take place, pooled GeCKO cells are infected with virus until cytopathic effect has occurred and surviving cells have formed pools. The cells are harvested and genomic DNA amplified and sequenced for gRNAs.



NANOCRISPR DELIVERY PLATFORM

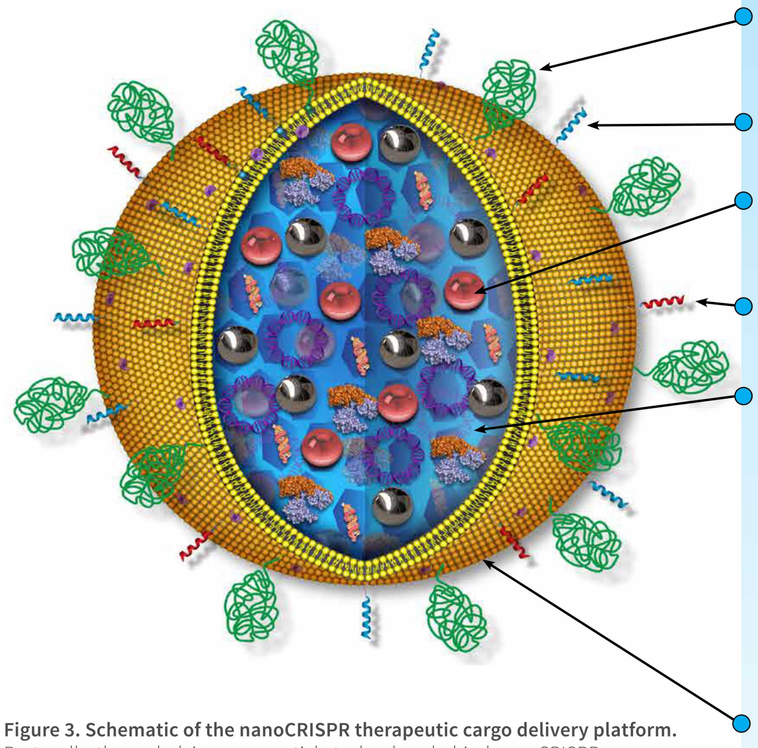
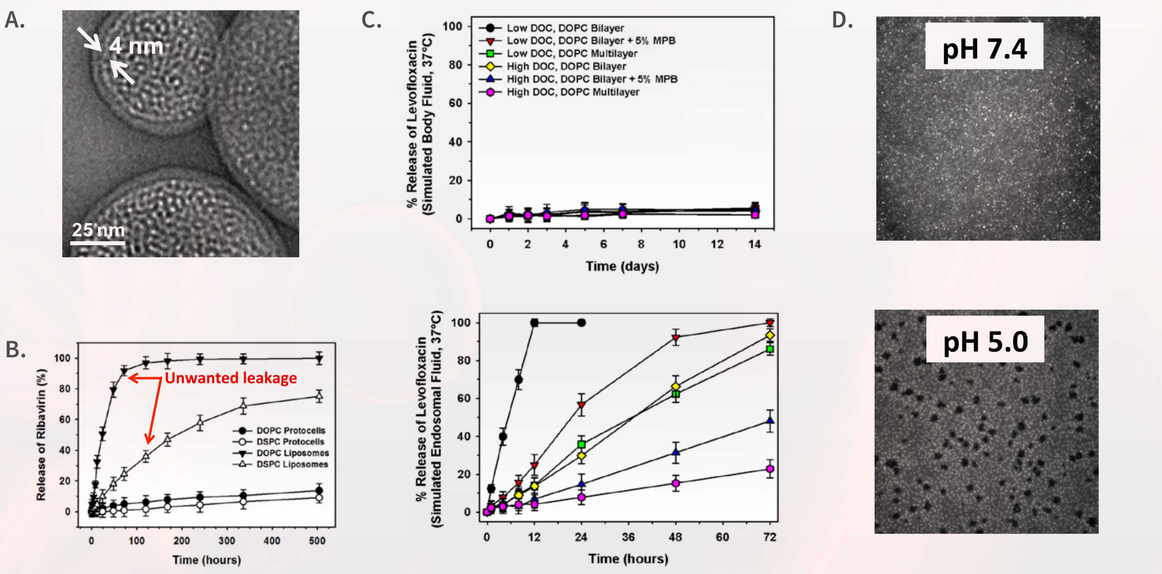


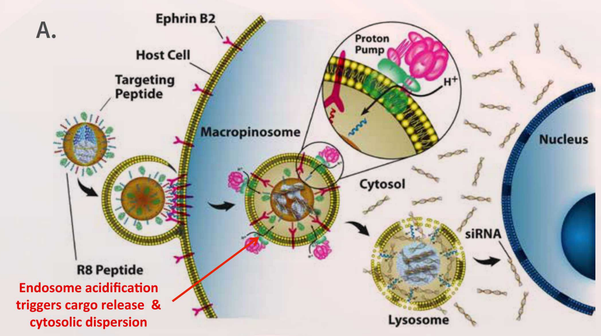
Figure 3. Schematic of the nanoCRISPR therapeutic cargo delivery platform. Protocells, the underlying nanoparticle technology behind nanoCRISPR, are composed of a mesoporous silica nanoparticle (MSNP) core encapsulated within a supported lipid bilayer (SLB) and, therefore, possess advantages of both MSNPs and liposomes, including high loading capacities, tailorable release rates, exquisite targeting specificities, long-term colloidal stability and shelf-life, controllable biodistribution, high biocompatibility and biodegradability, and low immunogenicity.

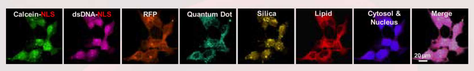
NANOCRISPR DELIVERY PLATFORM

Figure 4. Protocell characterization. (A) TEM images of protocells with a SLB. (B) Rates of ribavirin small molecule antiviral release from protocells with DOPC or DSPC SLBs with comparison to DOPC or DSPC liposomes upon incubation in a simulated body fluid (pH 7.4) at 37ºC for 20 days. (C) Rates of the antibiotic levofloxacin release from protocells with DOPC SLB or SLMs three layers thick upon continuous incubation in a simulated body fluid (pH 7.4), or continuous incubation in a simulated endolysosomal fluid (pH 5.0). At neutral pH, protocells stably encapsulate antibiotics, regardless of the silica degree of condensation (DOC) and the lipid shell composition and thickness. At acidic pH, antibiotic release rates are determined by the silica DOC, the degree of interbilayer crosslinking, and the thickness of the lipid shell. (D) Pore formation observed with SLBs on glass upon lowering of solution pH. SLBs of DOPC/cholesterol/DOPE (65:30:5) were prepared via vesicle fusion (citric acid-PO4 buffer).



CELL CULTURE TESTING





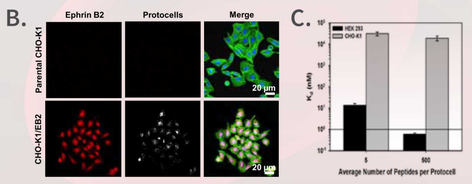


Figure 5. *In vitro* testing in mammalian cell lines. Cell culturing testing of nanoCRISPRs will be performed in permissive and non-permissive cells particular to the pathogen of interest. Shown in (A) is a schematic of nanoparticle binding, internalization and cargo release. In this example, co-displaying targeting and internalization peptide on the SLB enables selective uptake by target cells. A variety of cargos similar to those proposed as CRISPR payloads have been shown to internalize in cell culture. (B) CHO-K1 cells are naturally devoid of ephrinB2 expression but can be transduced to overexpress the protein. Protocells coated with ephrinB2 targeting ligands and internalization peptides were selectively taken up in CHO-K1/EphrinB2 cells and not the parental CHO-K1 cells. (C) Increasing the density of targeting ligands enables highly effective protocell binding and internalization in ephrinB2 expressing cells(HEK293T) versus CHO-K1.

ANIMAL TESTING

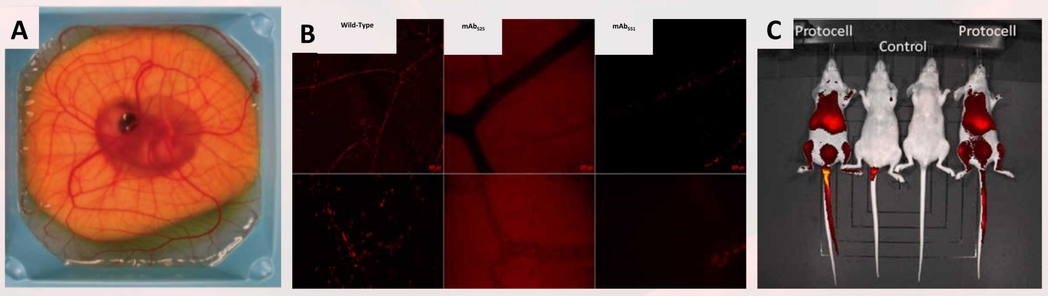


Figure 6. *In vivo* testing in CAM and mouse models. In order to test the nanoCRISPR platform in a cost-effective manner using an *in vivo* system, we will begin testing in the (A) Chorioallantoic Membrane (CAM) chicken embryo model. Ex ovo avian embryos enable cost-effective, high throughput assessment of therapeutic efficacy in a complex environment and are amenable to intravital imaging techniques with single-cell resolution. (B) Nipah virus, a high priority pathogen on the biodefense list, targets cells expressing the ephrinB2 membrane protein high expressed on endothelial cells of the developing vasculature. VSV pseudotyped particles displaying the Nipah glycoproteins were used to infect the chicken embryos and infection was monitored through reporter (mKATE) expression. Neutralizing antibodies prevent infection. (C) Optimized nanoCRISPR formulations will then be tested in mouse models of infection. Protocells that are 20-200 nm in diameter circulate systemically for 24-72 hours. Persistence is controlled by degree of silica condensation; particles are ultimately excreted as silicic acid.

SIGNIFICANCE

Development of a next generation nanomedicine platform for CRISPR-based therapeutics using Sandia patented protocell technology

Target pathogens include Zika virus and Ebola virus, agents of public health and national security concern