Nanolipoprotein Particles as Delivery Vehicles for Infectious Disease Countermeasures

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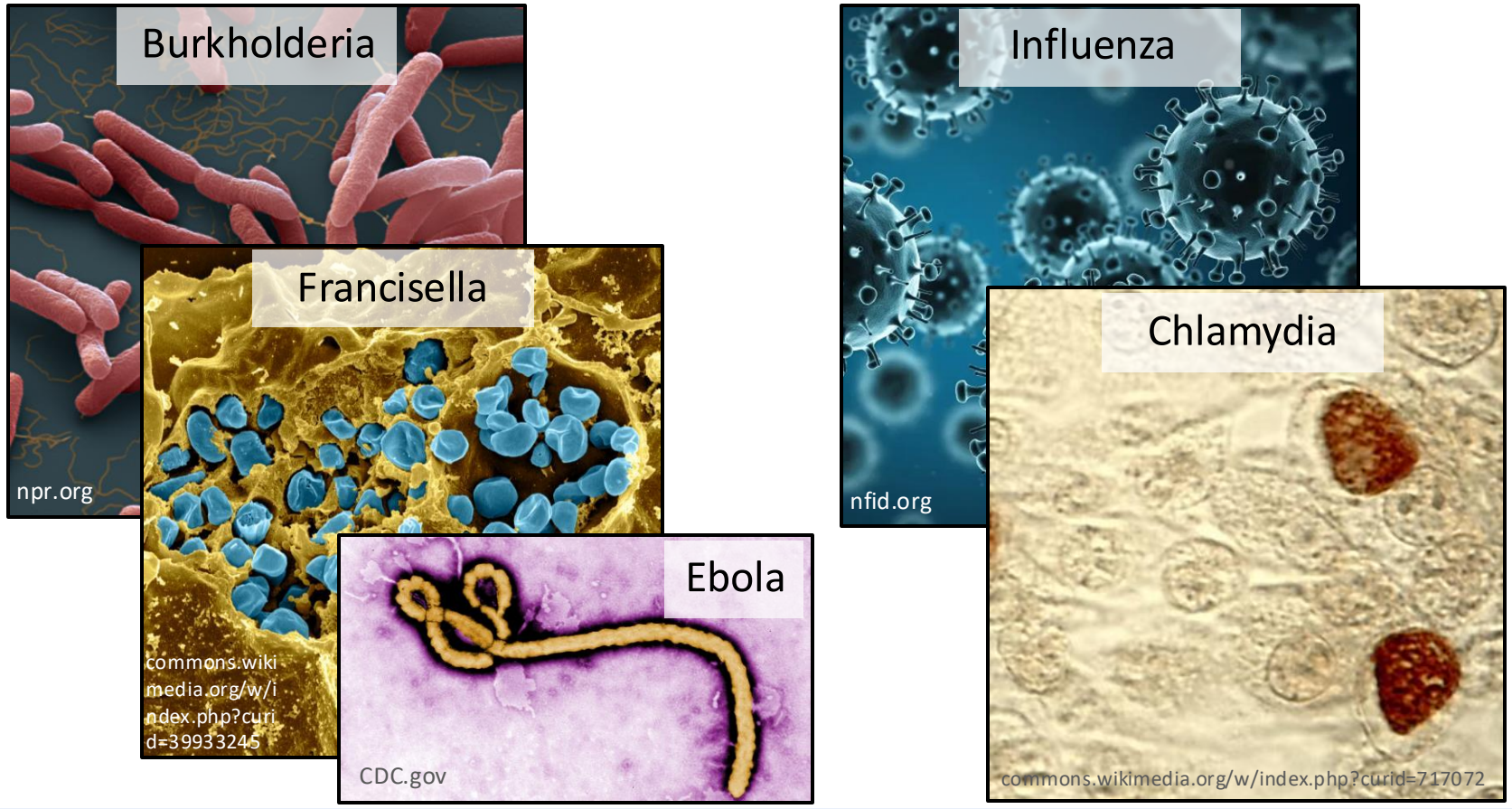
Disclaimer: All opinions expressed in this paper are those of the authors and do not necessarily reflect the policies and views of DOD. The opinions, interpretations, conclusions, and recommendations presented are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense.
The facility where this research was conducted is accredited by the AAALAC International and adheres to the principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011.

Our focus is to develop nanotechnology to enhance *in vivo* delivery of vaccines and therapeutics

LLNL's Mission includes countermeasures to biothreat agents and human health pathogens



Vaccines

Immune modulators

Therapeutics

Threat-agnostic approaches ensure broad applicability for countermeasure development

What are the key platform characteristics required for effective delivery?

Loading of diverse biomolecules

- **Antigens**
  - Proteins
  - Carbohydrates
- **Adjuvants**
  - Lipopeptides
  - Oligonucleotides
- **Therapeutics**
  - Small hydrophobic molecules

Favorable physicochemical properties

- **Tunable size**
  - Reproducibility
  - >100 nm for efficient uptake
- **Tailored surface chemistry**
  - Surface charge
  - Functional elements to support biomolecule conjugation

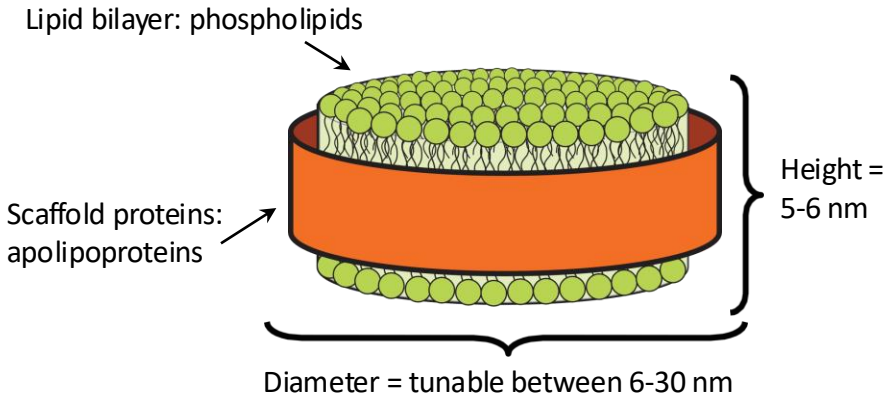
Biocompatibility properties

- **No cytotoxicity**
  - No toxic constituents
- **No reactogenicity**
  - Biological constituents that are well tolerated
- **Immunologically inert**
  - Avoids immune response directed towards platform

Nanolipoprotein Particles (NLPs): Plug-and-play delivery platform

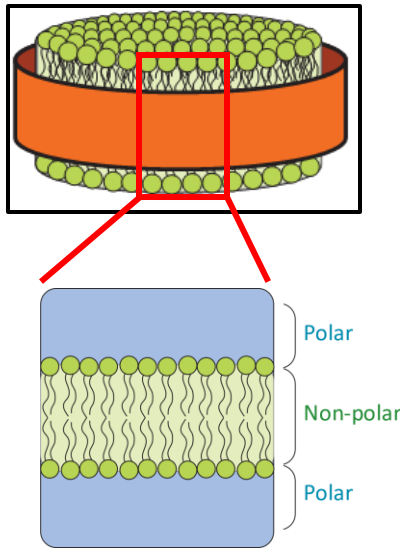
NLPs are a biomimetic nanoparticle

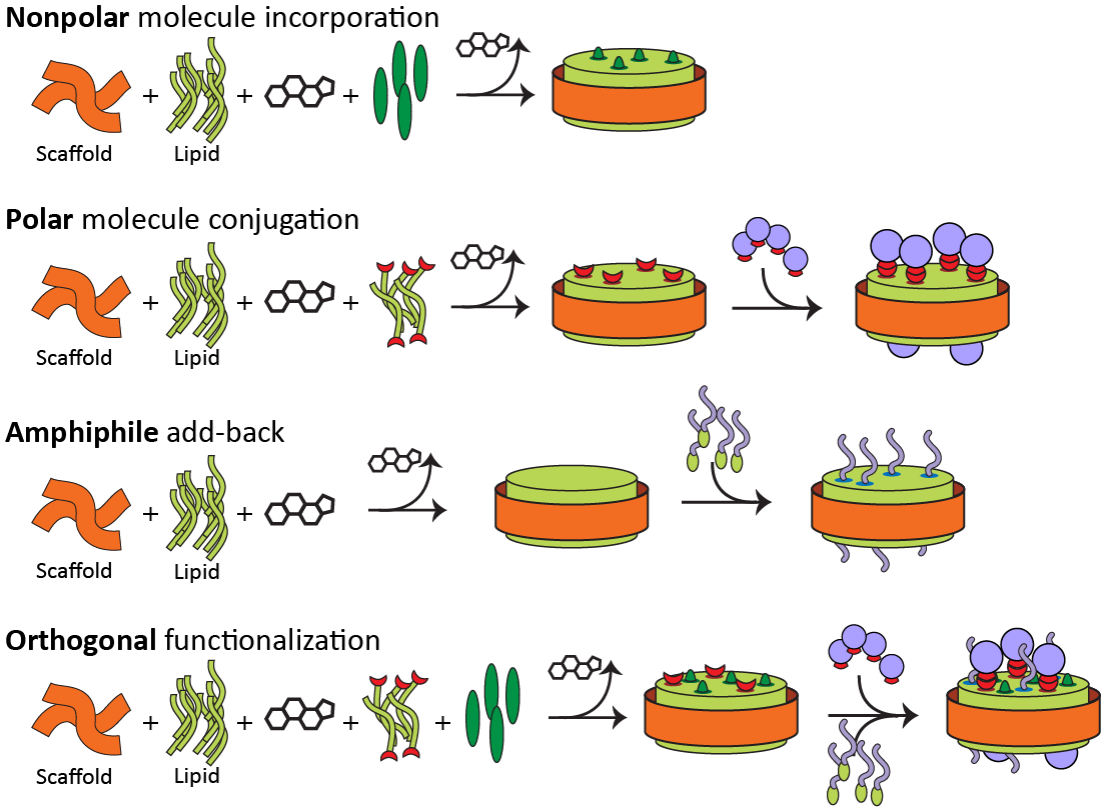
Known as nanodiscs and reconstituted HDLs



- Biocompatible and safe
- Self-assembly process highly versatile to accommodate
  - Nonpolar molecules (e.g. drugs, membrane proteins)
  - Polar molecules (e.g. proteins, peptides)
  - Amphiphiles (e.g. lipidated peptides)

NLPs have defined regions of polarity: versatile functionalization



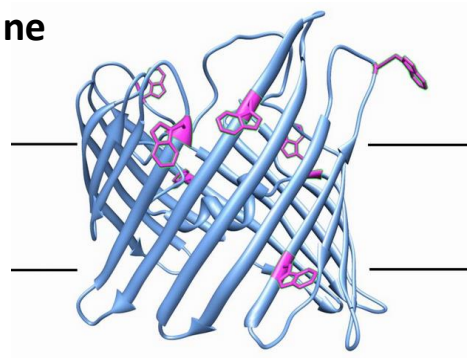


NLPs accommodate molecules with disparate physicochemical properties relevant to countermeasure development

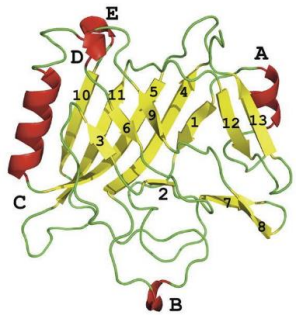
NLPs can be tailored to incorporate polar, hydrophobic, and amphipathic molecules

Protein antigens

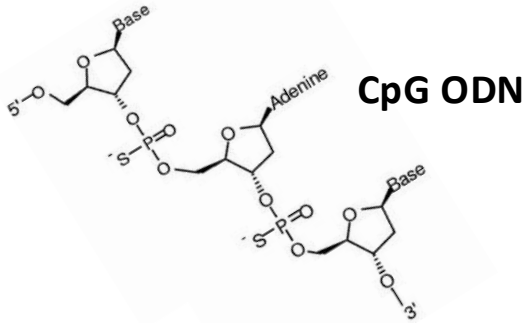
Membrane proteins



Soluble proteins

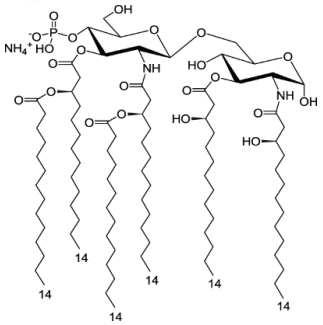


Adjuvants



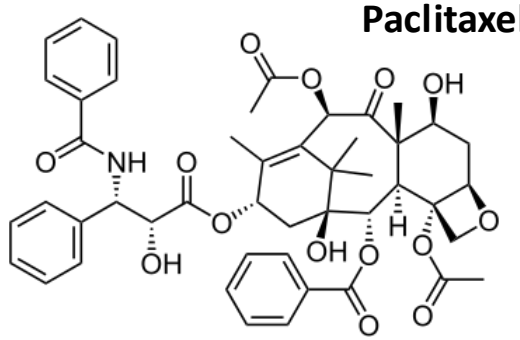
CpG ODN

MPLA

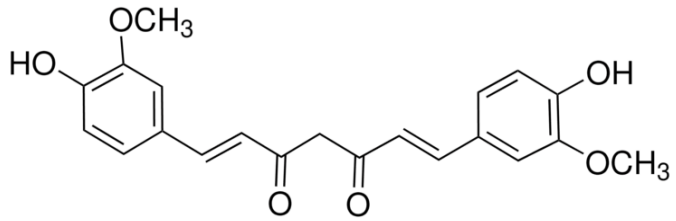


Therapeutics

Paclitaxel

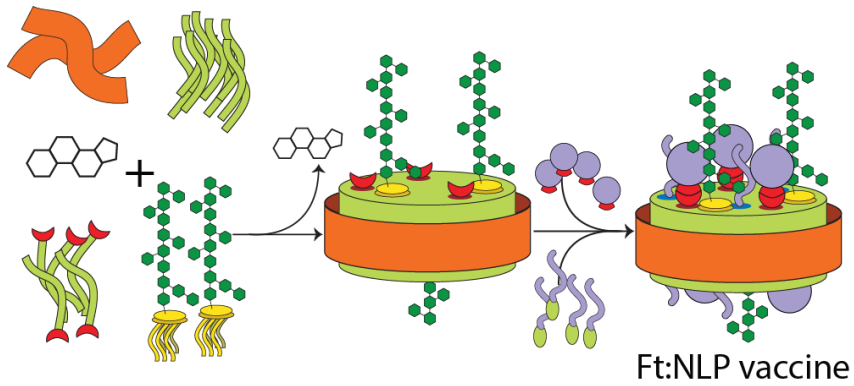


Curcumin



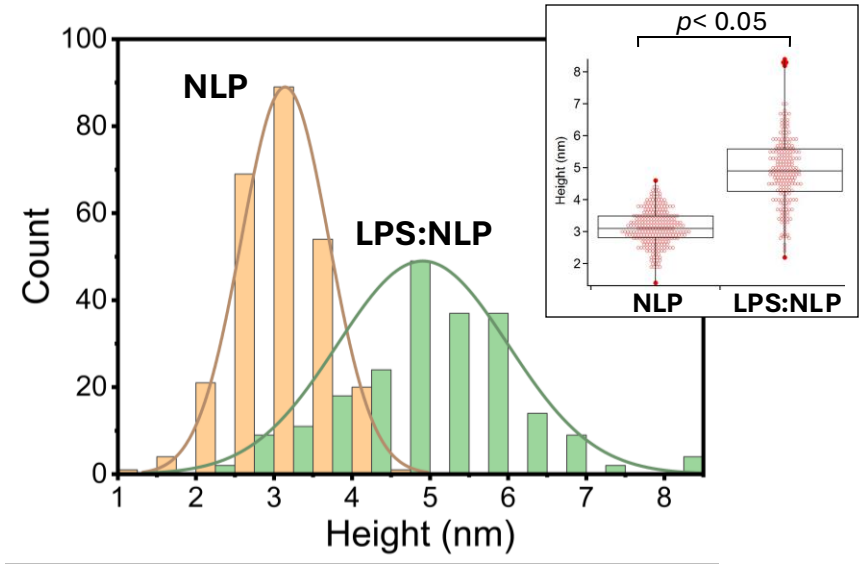
We have developed a novel pre-clinical subunit vaccine that protects against pneumonic tularemia

NLPs were formulated with two antigens required for protection



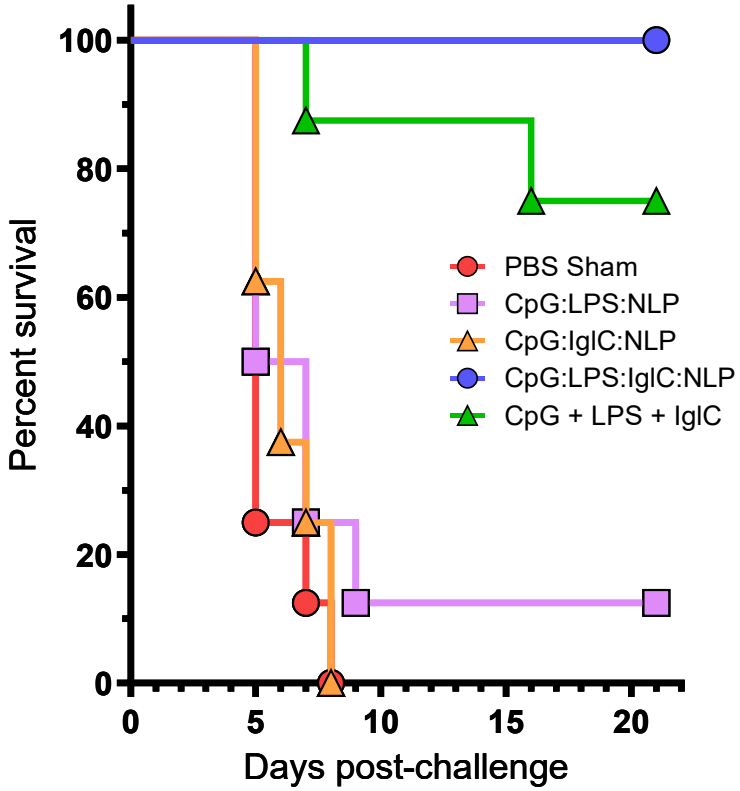
- The lipopolysaccharide (LPS) antigen provide humoral immunological responses
- The protein antigen IglC provides cellular immunological responses

NLPs easily incorporated antigens that are unique in chemical properties and size



- Orthogonal approaches are used to successfully conjugate the protein (<25kDa) and LPS (10-250kDa) antigens

Our NLP vaccine protects against death while minimizing clinical symptoms



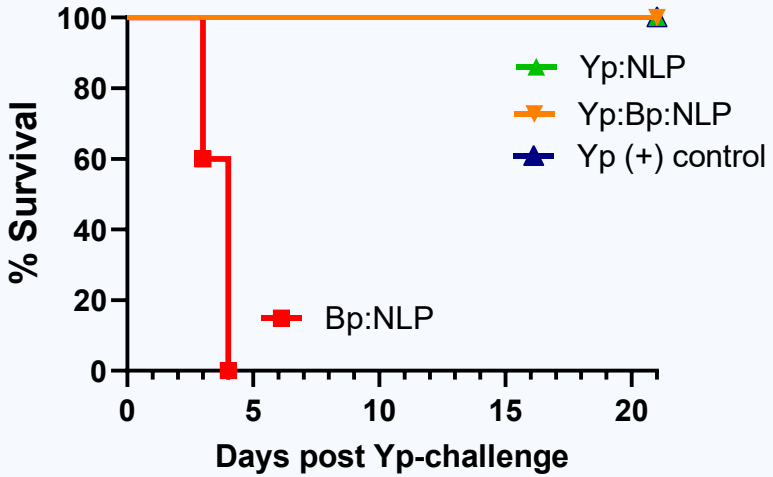
We are now developing combination vaccines that can protect against multiple pathogens

We are developing and characterizing a single vaccine formulation that can protect against three bacterial biothreat agents: *F. tularensis (Ft), Y. pestis (Yp), B. pseudomallei (Bp),* expanding to viral pathogens

Dual vaccine formulations against Ft:Bp and Ft:Yp can independently protect rodent models upon aerosol challenge with protection mirroring single vaccine formulations

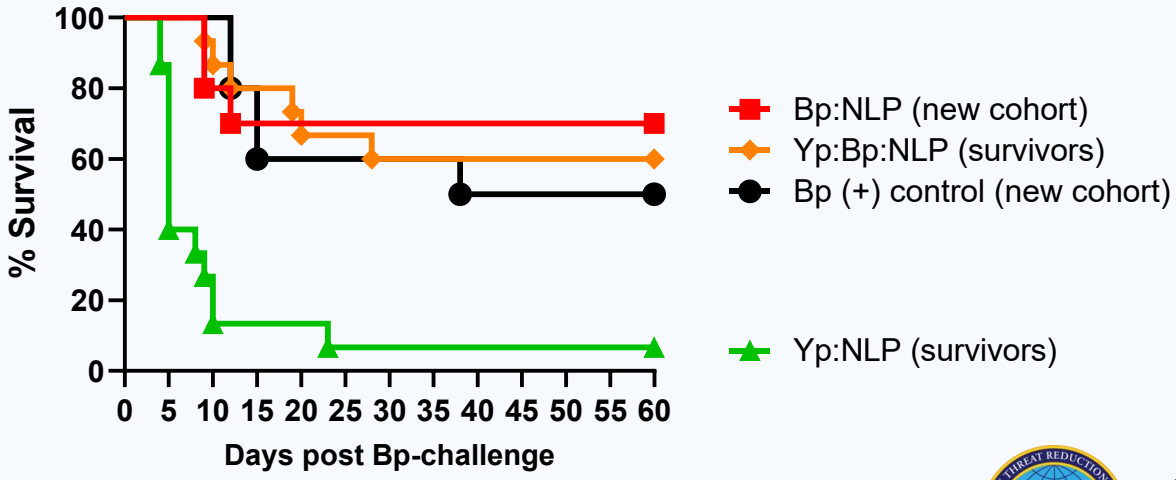
We have demonstrated that a dual-pathogen NLP vaccine protects against serial aerosol challenges of each pathogen

1) Animals vaccinated with Bp, Yp, and Yp:Bp NLP formulations were challenge with Yp



2) Surviving animals were challenged with Bp

3) Animals vaccinated with Yp:Bp:NLP that survived Yp challenge also survived Bp challenge

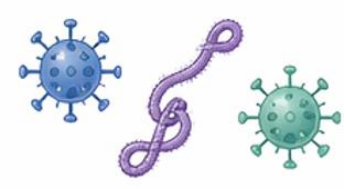


Delivering host- and pathogen-directed therapeutics

Formulation

Pathogen-agnostic approach

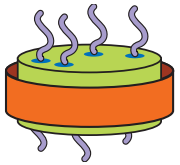
New and emerging pathogens



- High morbidity/mortality
- Limited or no approved therapies
- Need for broad-spectrum treatments

Combination Nanoparticle Therapeutics

Rationally designed combinations



Host-directed nanoparticle

- Immunomodulatory
- Anti-inflammatory

Pathogen-directed nanoparticle

- Antiviral
- Antibacterial

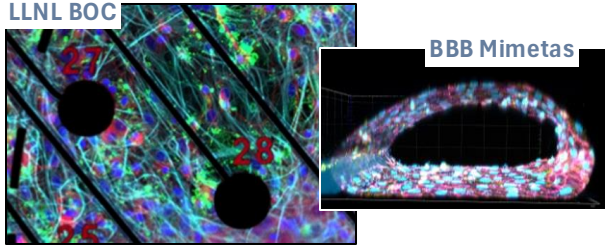
- ✓ Complementary mechanisms
- ✓ Enhanced efficacy
- ✓ Reduced resistance potential
- ✓ Optimized delivery (e.g. brain)

Evaluation

BOC MPS and Animal Models

Human relevant models & traditional animal models

Human relevant MPS



Relevant animal challenge models

- Mimic human neuroimmune and neuronal interactions
- Allow real-time longitudinal assessment
- Huma-relevant data complements efficacy data from relevant animal models

Evaluation & Mechanistic Insights

Multi-dimensional data

<u>Efficacy</u>
- Pathogen load ↓
- Infected cell death ↓
- Survival ↑

<u>Neuroprotection</u>
- Neuron viability ↑
- Neuronal function ↑

<u>Inflammation</u>
- Cytokines/chemokines ↓
- Microglial activation ↓

<u>BBB function</u>
- TEER ↑
- Permeability ↓

<u>Mechanistic insights</u>
- Pathway modulation
- Cell-type specific effects

Goal

Accelerated Translation Pathway

Prioritized lead combinations based on human and animal data

Streamlined preclinical development

Faster progression to clinical evaluation

Rapid response to new & emerging pathogens

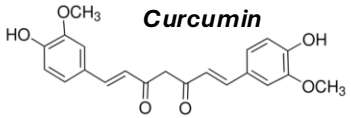
OVERALL IMPACT The integration of combination nanoparticle therapeutics with brain-on-chip MPS platforms and relevant animal models enables a faster, more human-relevant, and data-rich path to discovery and preclinical assessment for delivering effective treatments against new and emerging pathogens

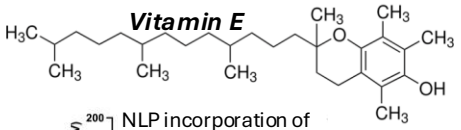
We have successfully formulated host- and pathogen-directed therapeutics: evaluation underway

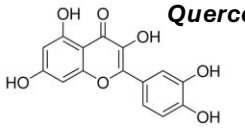
NLP development and formulation

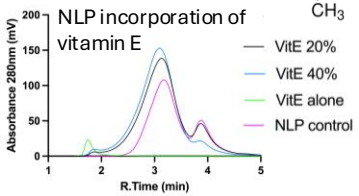
Immune agonists: >10 formulations

Antioxidants



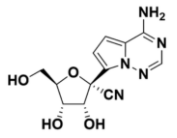




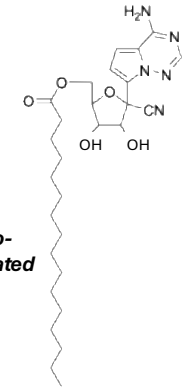


Antimicrobials

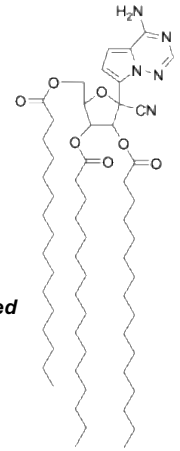
Remdesivir derivatives



Nucleoside analog (parent)



Mono-acylated

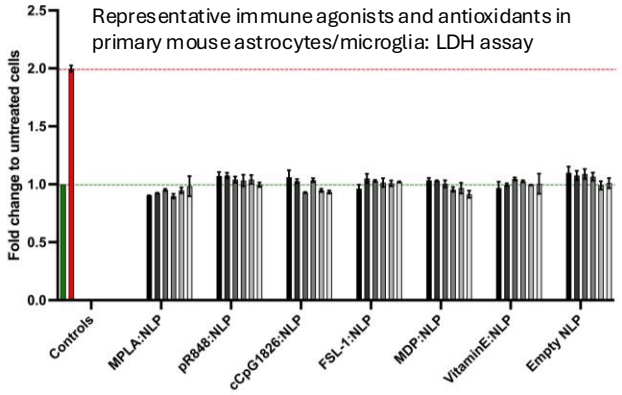


Tri-acylated

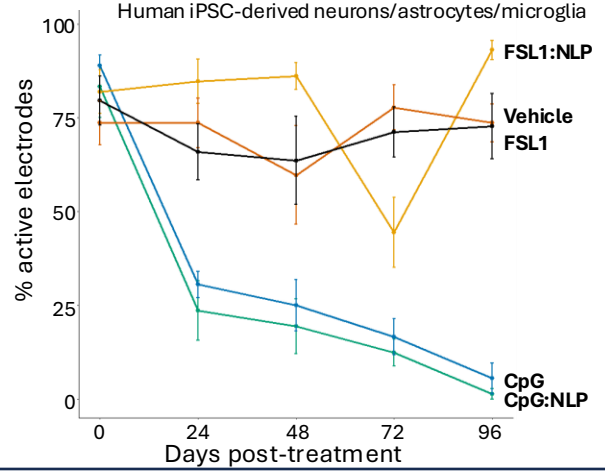
BBB-penetrating elements: Peptides, VHH

Functional and efficacy evaluations

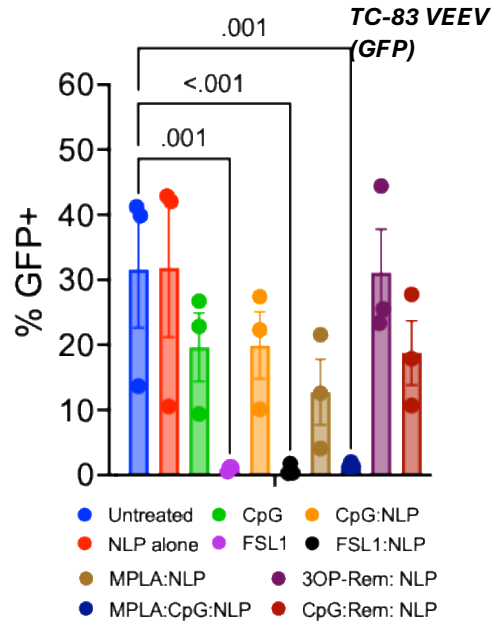
Cellular toxicity assays



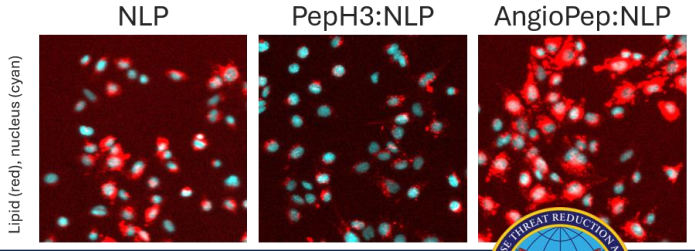
Brain-on-Chip evaluations



Viral inhibition studies



Uptake/BBB penetration



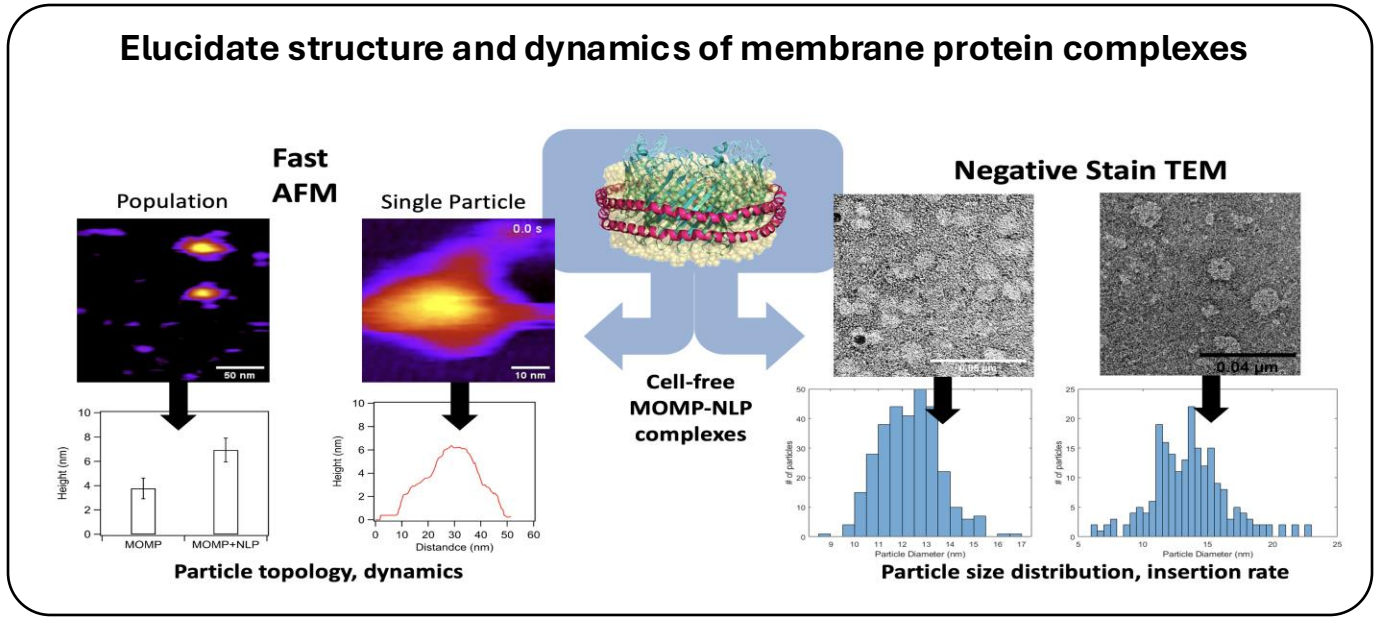
We are expanding our biotechnology scope, key capabilities toolkit, and production pipeline

Applications development at LLNL:

- **Elucidating protein structure** – stabilizing membrane protein structure in native lipid environment
- **Enabling drug discovery** – GPCR & ion channel screening, antibodies, small molecule delivery
- **Exploring precision medicine** – Tyrosine receptor delivery for signaling/cell cancer therapy

Expanding our capabilities toolkit:

- **Expression approaches**: Cell-free synthesis of human receptors GPCRs, ion channels, kinases, porins
- **Biophysical characterization**: FRET/FCS, electrophysiology, AFM/TEM for folding, stability, activity
- **Cargo delivery**: Functional receptors delivered into cells/tissues (CAR-T, signaling, therapies)



Advancing our production pipeline:

- **Scale-up** – leveraging microfluidic technology used for LNP vaccine manufacture to provide scalable production
- **Storage** – spray drying and lyophilization for long-term storage outside of cold-chain
- **Synthetic scaffolds** – evaluating scalable, non-protein alternatives for NLP assembly and in vivo applications (SMALPs, telodendrimers)

Acknowledgements

Key team members

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