

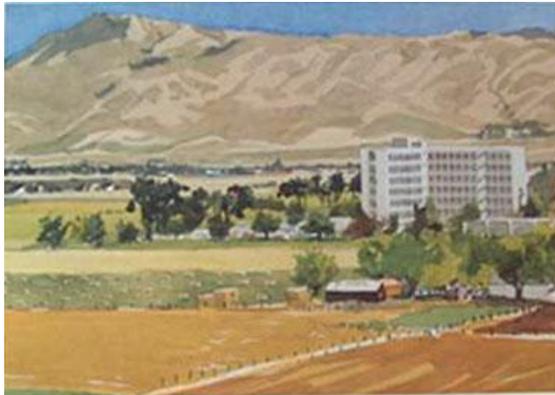


Lawrence Livermore National Laboratory



Immunology, inflammation and immune modulation: an overview at LLNL

*UCD Cancer Center & Lawrence Livermore National Laboratory
Annual Workshop; Livermore, CA
10/17/2011*



**Amy Rasley, Staff Scientist
Host-pathogen Biology Group**

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**Physical and
Life Sciences**

Host-pathogen interactions and development of novel medical countermeasures

LLNL's BSL3 Facility

Single-story AAALAC accredited ABSL3

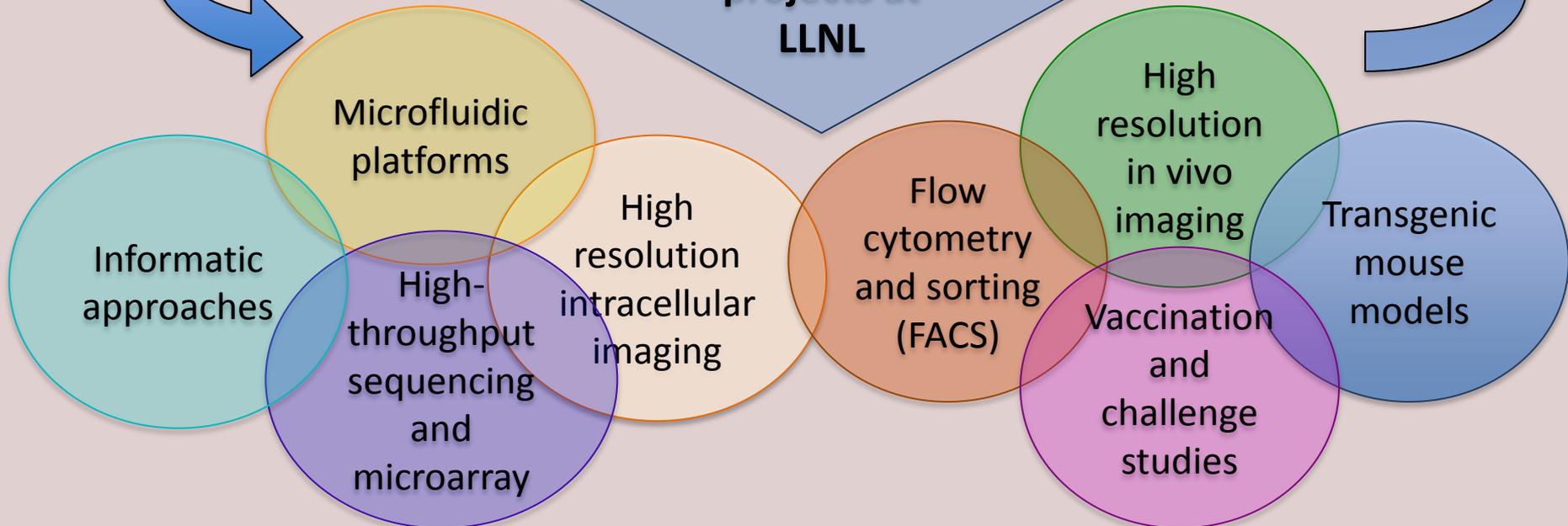
- Cellular autophagy pathways
- Intracellular trafficking in human DCs
- Identification of novel virulence factors
- Vaccine efficacy studies
- Identification of novel phages

Nanolipoprotein (NLP) Technology

Pioneered functionalized NLPs

- Host immune modulation
 - Vaccines
 - Enhancement of innate immune responses
- Cancer targeting molecules

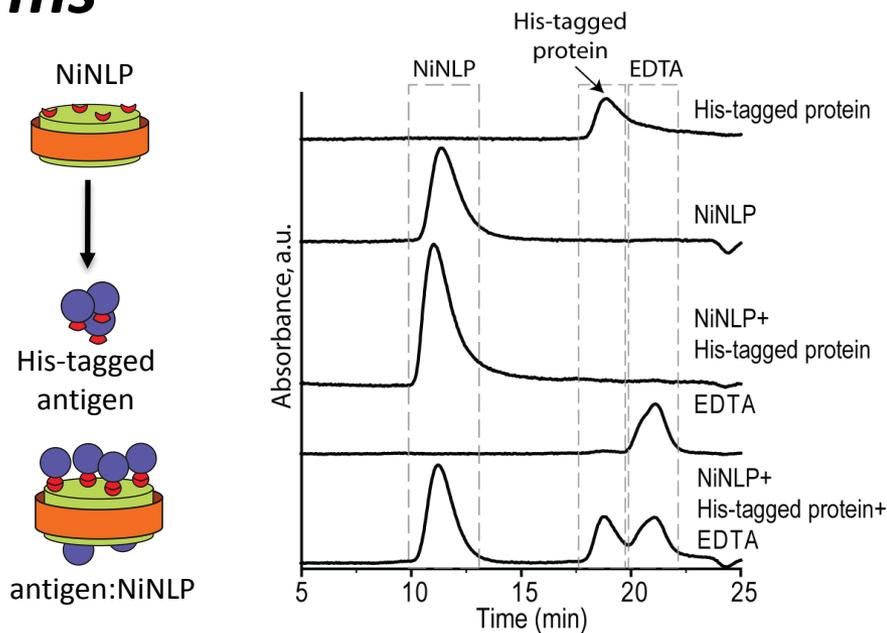
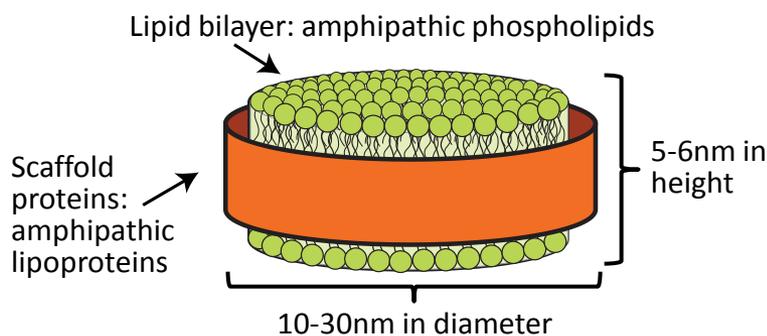
**Immunology
focused
projects at
LLNL**



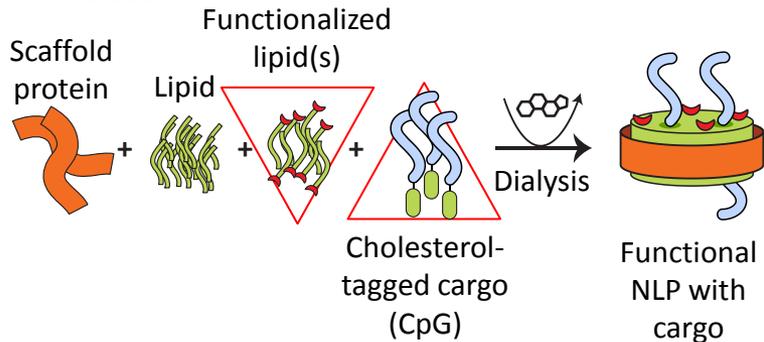
Nanolipoprotein particles (NLPs) are versatile biological platforms

NLPs are biological "nanoparticles"

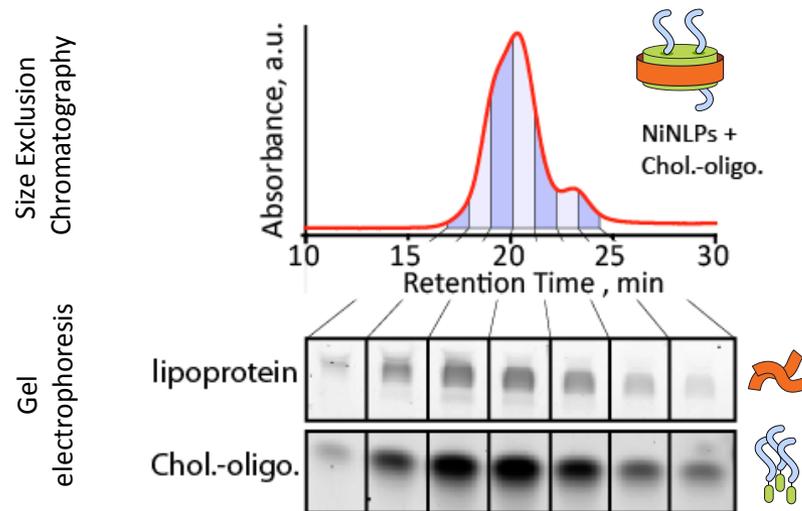
- Biocompatible (analogous to HDLs)
- Self-assembled
- Tunable diameter (10-30nm)



Preparation of functional NLPs is facile and versatile



- Self-assembly is versatile
- NLP preparation complete in 6-24 hours

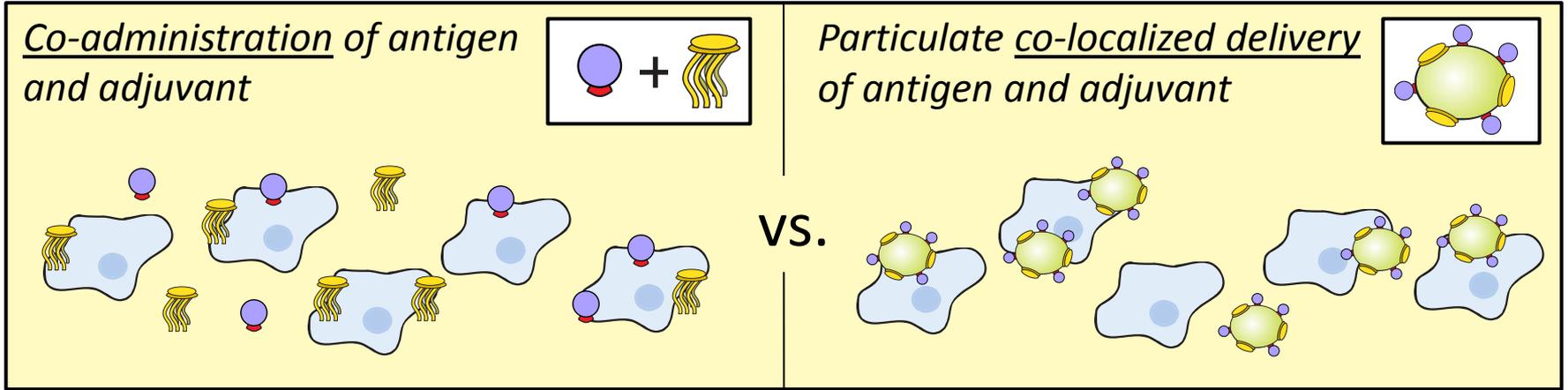


Up to 15 Chol-CpGs are incorporated into a single NLP

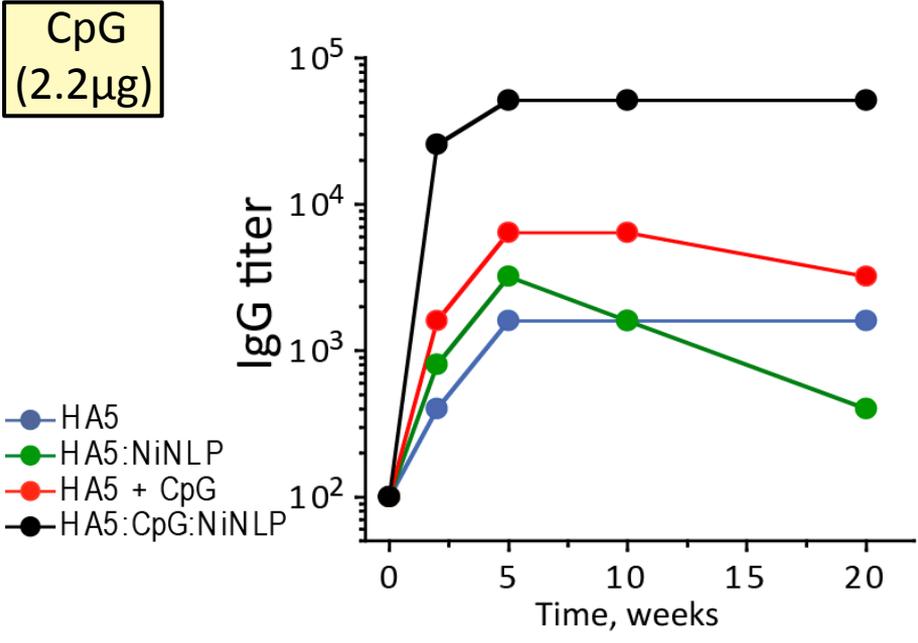
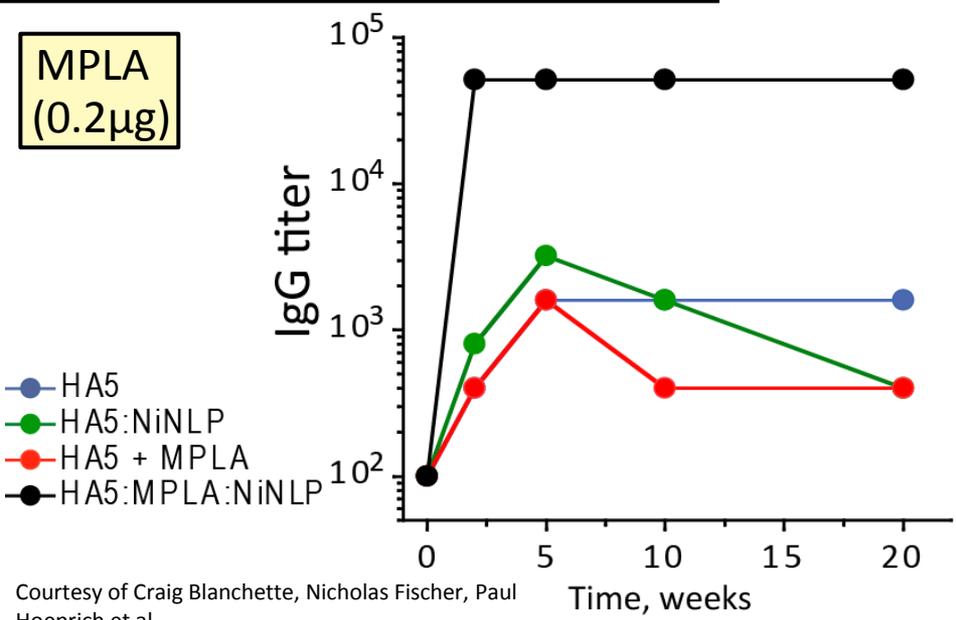
His-tagged antigens and adjuvants can be incorporated into a single NLP

Adjuvants enhance immune responses

NLPs facilitate antigen and adjuvant co-localization and ensure co-delivery

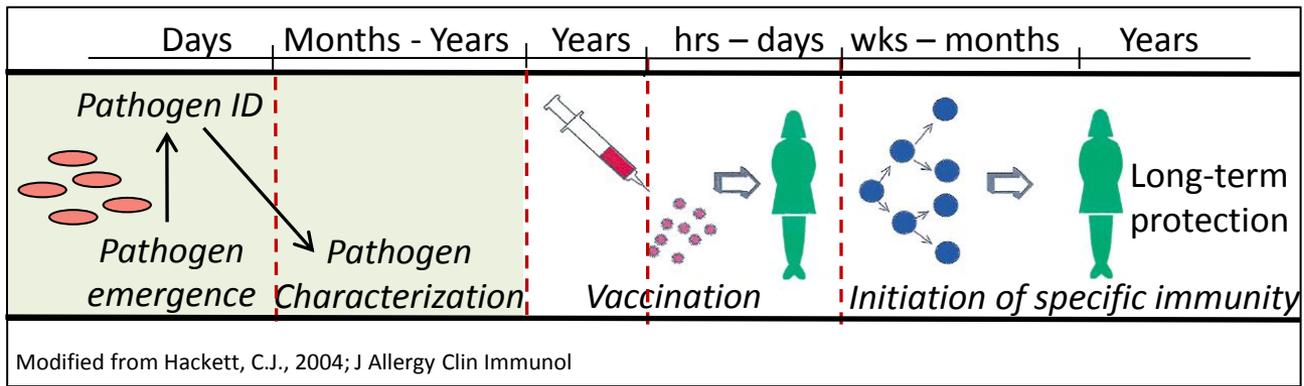


Single i.p. inoculation, 2.5 μ g HA5

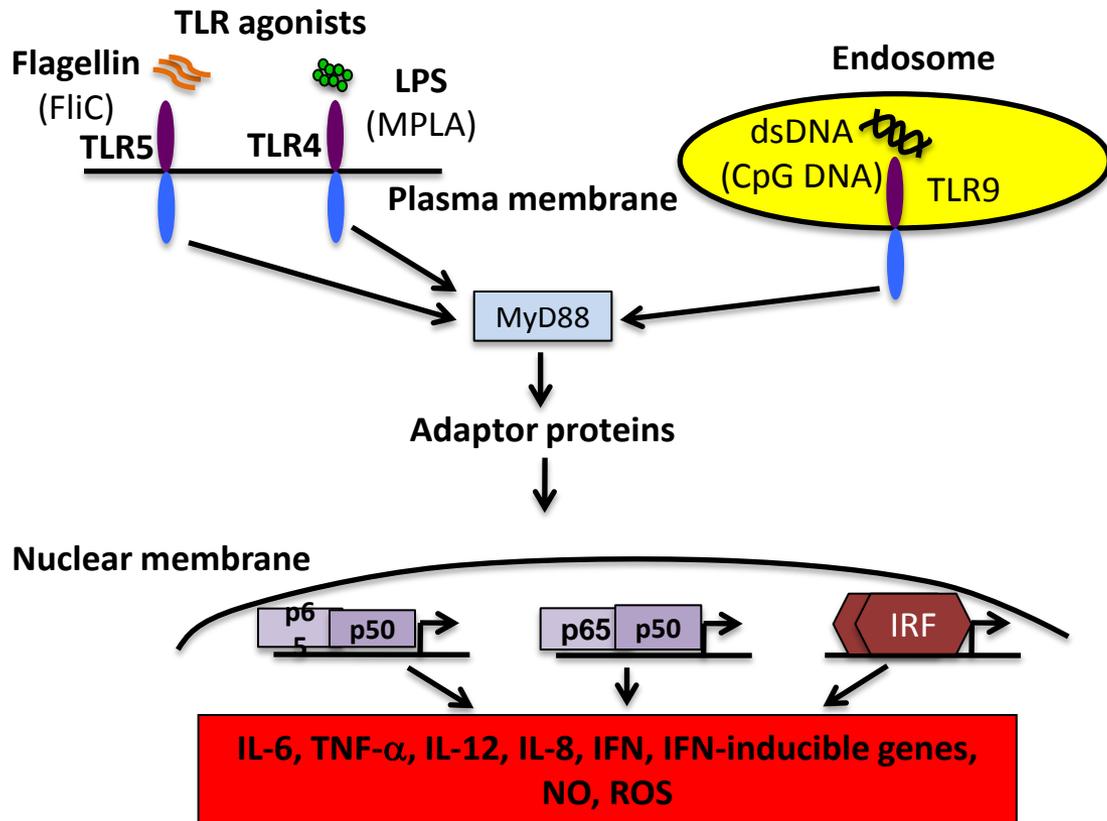


Courtesy of Craig Blanchette, Nicholas Fischer, Paul Hoeprich et al.

Innate immune stimulation can provide protection from infection: can conjugation of agonists to NLPs enhance this effect?



Innate Immune Agonist	Immune Receptor
Monophosphoryl Lipid A (MPLA)	TLR4
CpG Oligonucleotides	TLR9
Flagellin	TLR5
Muramyl dipeptide (MDP)	Nod1/2

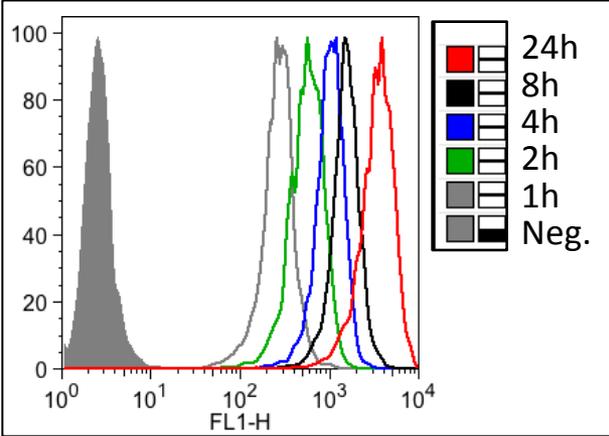


- Bind to known host immune receptors—recognize conserved pathogen motifs
- Immunostimulatory—used as adjuvants in vaccine formulations
- Numerous studies have shown they can ameliorate disease in vivo

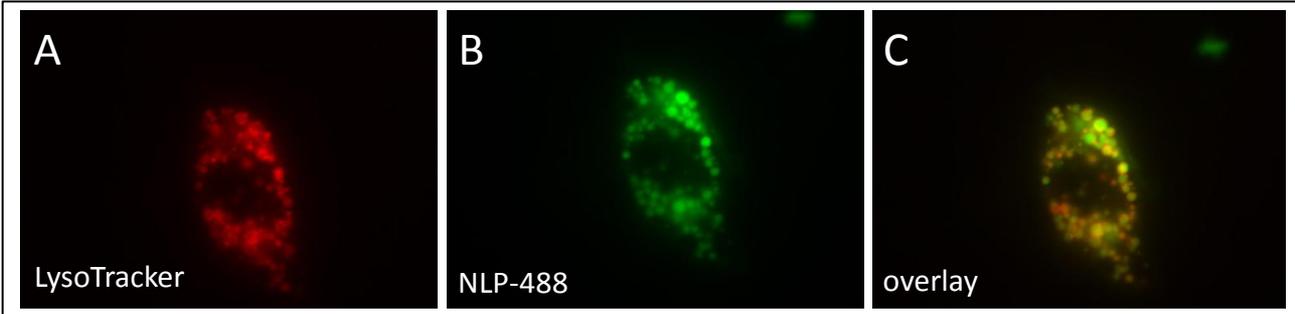
NLP:agonist distribution and inflammatory responses in vitro

Quantification of kinetics of NLP internalization

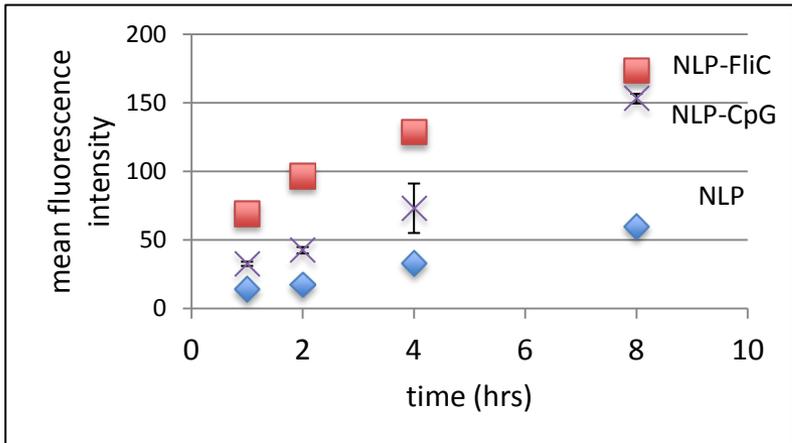
internalization



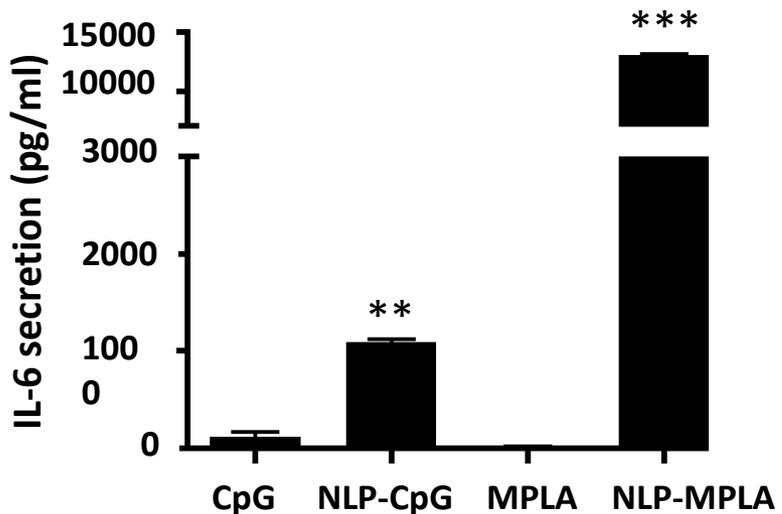
NLPs co-localize with the endosomal marker LysoTracker



Agonists enhance NLP internalization in vitro

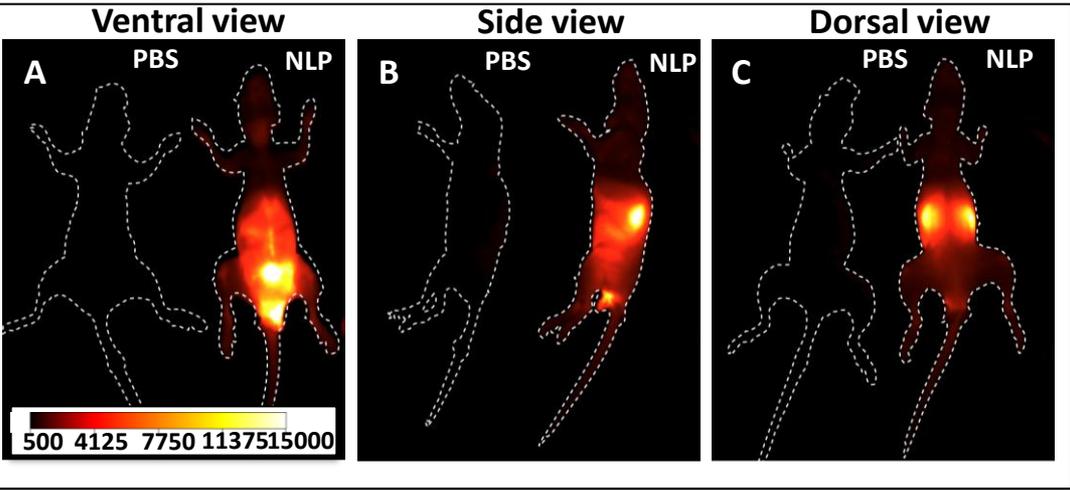


Agonists conjugated to NLPs are significantly more immunostimulatory capacity

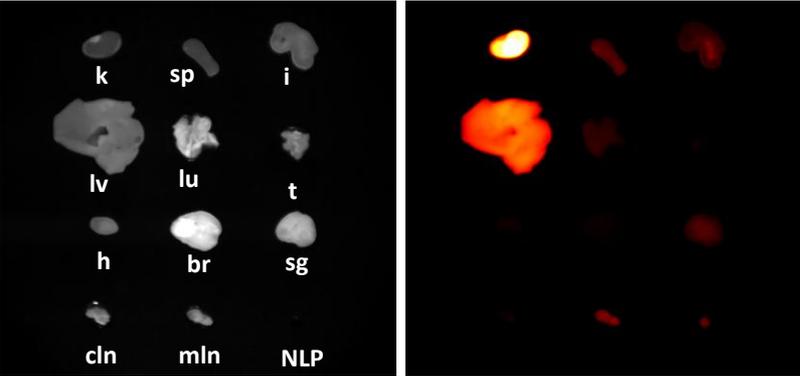


NLP:agonist distribution and inflammatory responses in vivo

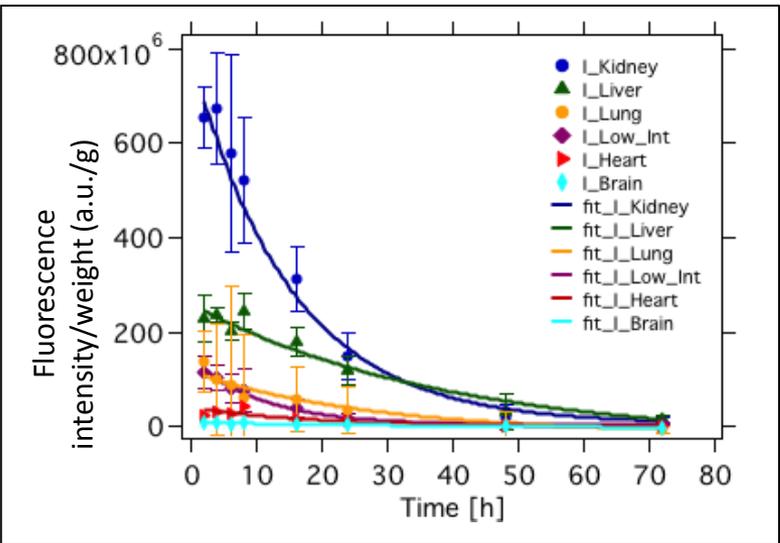
NLPs exhibit broad tissue distribution profiles upon i.p. injection in vivo



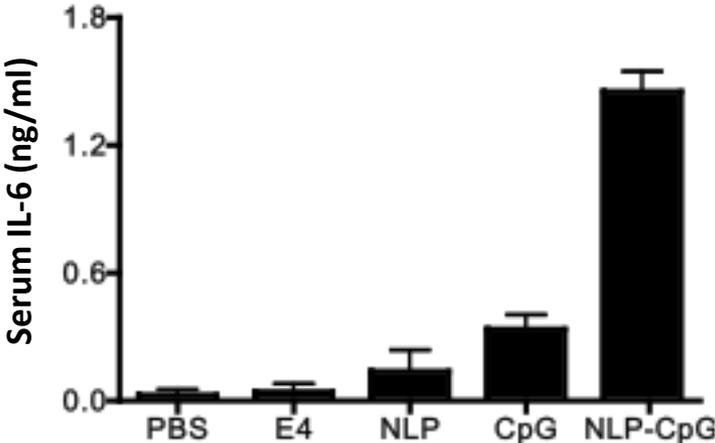
Quantification of kinetics of NLP fluorescence intensities in tissues in vivo



NLPs exhibit prolonged $t_{1/2}$ in a variety of tissues in vivo

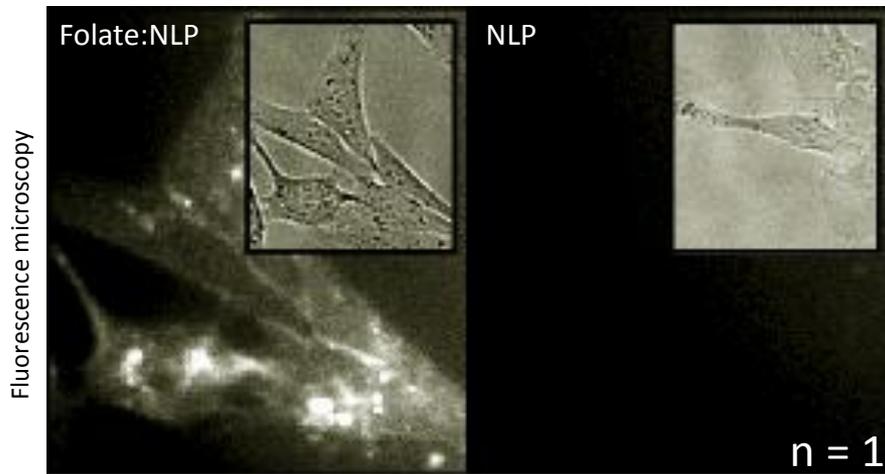


Conjugation of CpGs to NLPs significantly enhances inflammatory cytokine production in vivo



NLPs can easily incorporate targeting moieties

Preliminary data indicates that NLPs can be targeted to cancer cells



Advantages of using NLPs for tumor targeting

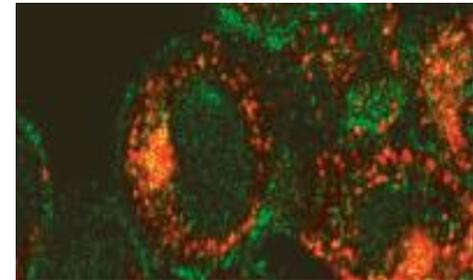
- 1) stable over 24 hours in serum
- 2) specifically bind target cell
- 3) Internalized

Other targeting moieties can also be incorporated into NLPs

Muc1 DNA aptamer

- Many cancer cells over-express MUC1 (up to 20-fold in breast, lung, pancreatic, ovarian, colorectal)
- Successfully used to deliver drugs to cancer cells
- Readily conjugated to NLPs using cholesterol tag

GGCTATAGCACATGGGTAAAACGAC



Red=aptamer

M. Ferreira et al., 2009 *Nucleic Acids Res.* 2009 37, 866.

TLRs, adjuvants, antigens, targeting and cancer therapies: putting it all together

- Modified NLPs can be used for targeted deliver of adjuvants and antigens—significantly enhances immune responses in vivo
 - Administer innate immune agonists alone or in combination with existing therapies
 - Platform for a novel cancer vaccine—combine antigens + adjuvants
 - Potential for targeting—include targeting moiety into NLP

Acknowledgements

Immunotherapeutics NLP Team

- Craig Blanchette
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Original NLP Team

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- Paul Henderson
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- Henry Benner
- Todd Sulchek
- Vicki Walsworth
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Host-pathogen/immunology/inflammation leads (not comprehensive)

- Catherine Lacayo (autophagy and inflammation)
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- Monica Borucki (viral pathogenesis and evolution)
- Brian Baker (POC diagnostics)
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