Vaccine Chemistry:
A Platform for Optimizing Adaptive Immunity

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1/11/2016

Hurdles for Vaccines Against Infections and Tumors

- **HIV-** Broadly neutralizing antibodies (*high somatic mutation*): Requires immunogen design and novel approaches to vaccine delivery
- **Malaria/TB-** Requires *high and sustained levels* of antibodies, tissue resident T cells (liver), TB (lung)
- **Cancer-** Personalized vaccines based on neo-antigens to induce T cells: Rapid and scalable process to make vaccine
- **Infections of infants and elderly** (RSV, Flu, Pertussis, Zoster): Immunogens and adjuvants

Tool Box of Vaccine Vectors in Clinical Studies for Infections and Tumors

- DNA, RNA
- Adenovirus (Ad5, Ad26, Ad35, ChAd3)
- Poxvirus (MVA, NYVAC, ALVAC)
- Protein/Adjuvant

Focus of this Presentation:

- Formulation and delivery of proteins and adjuvants for optimizing antibody and T cell immunity

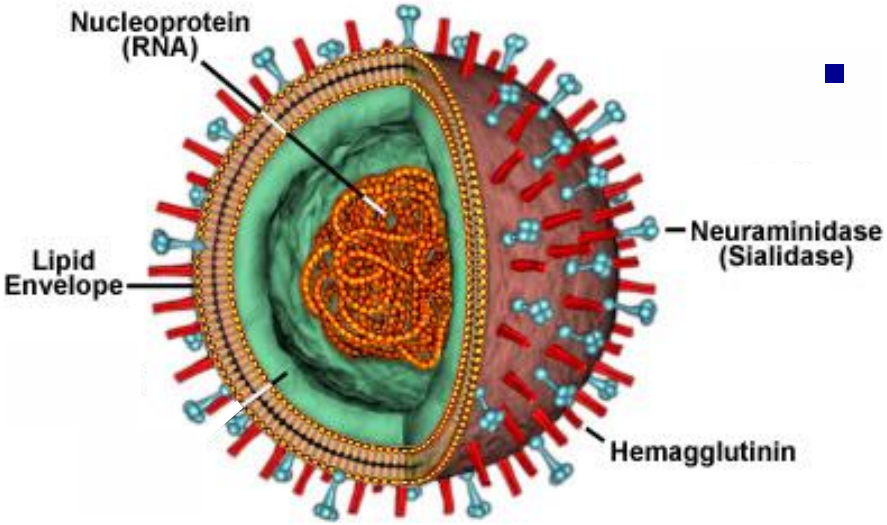
Rationale for Protein Based Vaccines

1. Protein vaccines induce broad-based immunity
   - Antibody
   - Th1 *but low level* CD8+ T cell responses
2. Protein vaccines used in heterologous prime-boost regimens with other platforms
3. Protein vaccines are not limited by pre-existing immunity and can be used repeatedly

Viruses as a Guide to Vaccine Design

ADJUVANT

- Innate activation by RNA or DNA



~ 100 nm

ANTIGEN

- Multivalent array of antigen

DELIVERY PLATFORM

- Synchronous delivery of antigen particle with innate stimulation

Adjuvant Selection:

TLR Agonists Activate Distinct Human Dendritic Cell Subsets

	CD141/BDCA-3 DCs	Conventional DCs	Plasmacytoid DCs
Cytokines:	IL-12	IL-12	Type I IFN
Ag Presentation:	CD8	CD4	?

TLR Expression				TLR Ligand
TLR 4	–	+	–	LPS (MPL)
TLR 3	+	+	–	dsRNA (Poly IC)*
TLR 7	–	–	+	ssRNA (Imiquimod)
TLR 8	+	+	–	(R-848)
TLR 9	–	–	+	CpG DNA

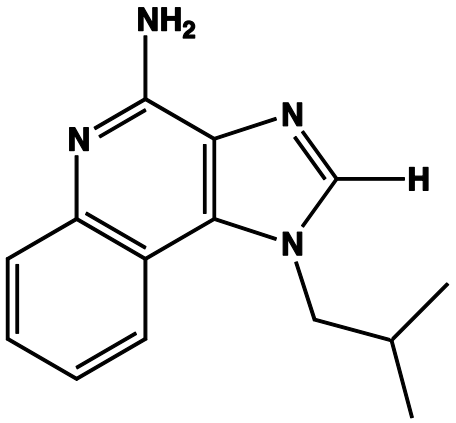
*Poly I:C can induce IFN-a via non-TLR independent pathways (MDA-5)

Example Application: Delivery of Toll-Like Receptor-7/8 Agonists

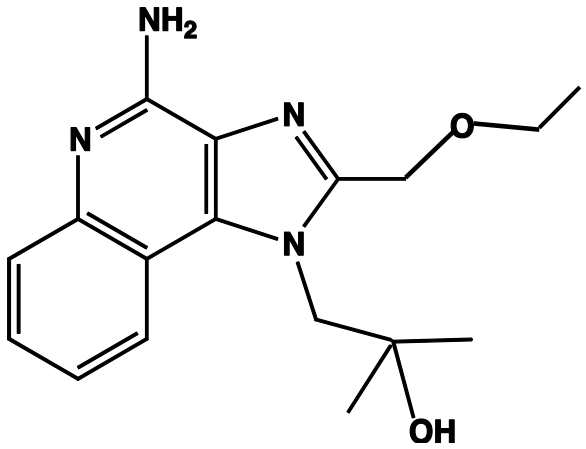
- Activates all human DC subsets, monocytes and B cells
- Receptors are endosomally localized
- Natural ligand is ssRNA
- Synthetic agonists are analogs of nucleotide bases

Imidazoquinolines

Imiquimod (TLR-7a)



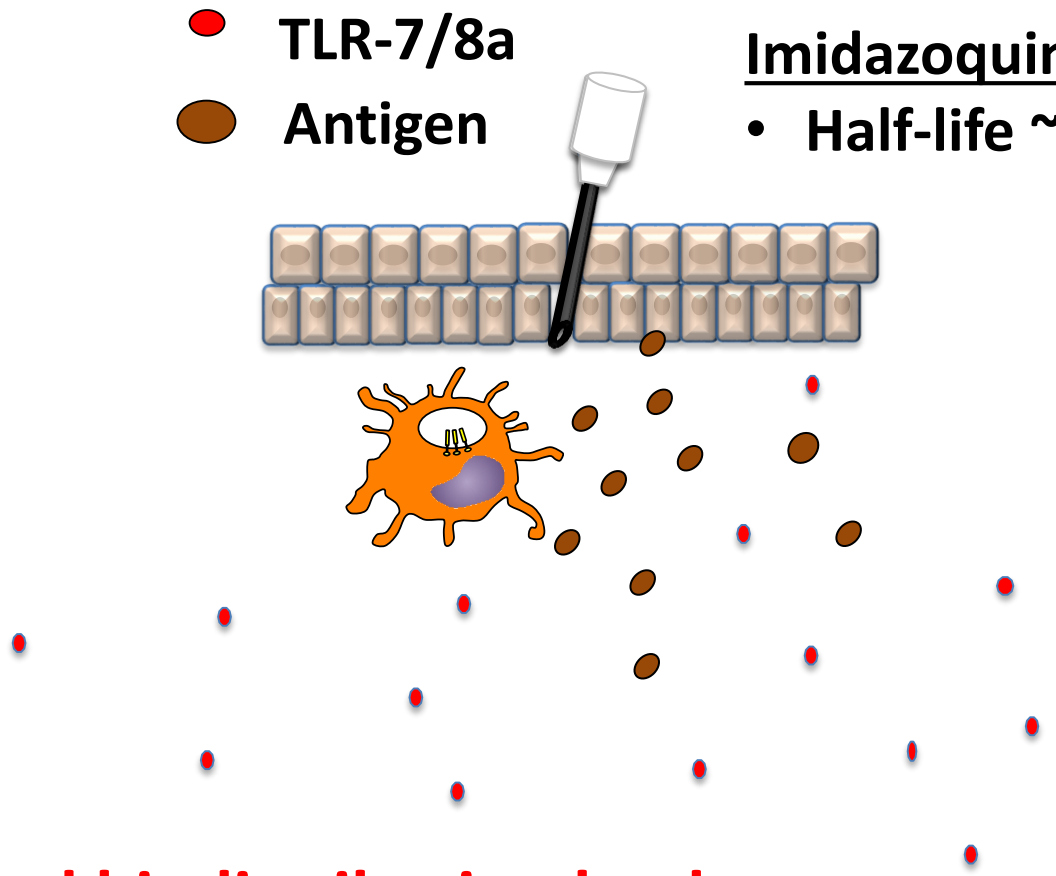
Resiquimod (TLR-7/8a)



Approved for treatment of cutaneous cancer

Gerster, J.; et al. J. *Med. Chem*. 48, 3481-3491 **(2005)** (3M Pharmaceuticals)

Pharmacokinetics of TLR-7/8 Agonists (TLR 7/8a)



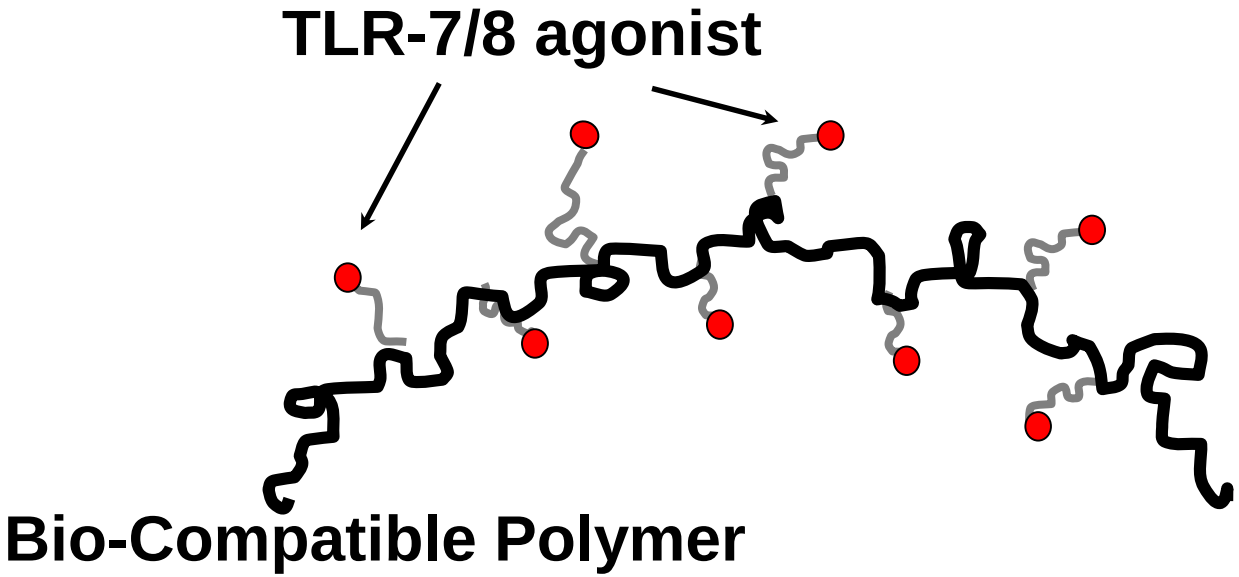
Imidazoquinoline-based TLR-7/8a

- **Half-life ~ 2-8 hours**

Broad biodistribution leads to:

- **Systemic innate immune activation (TOXICITY)**
- **Antigen without TLR-7/8a stimulation (TOLERANCE)**

Polymer Carriers of TLR-7/8a (Poly-7/8a)



<u>Primary Aim</u>: Restrict agonist distribution

- **Enhance local activity**
- **Decrease systemic toxicity**

<u>Secondary Aim</u>: Evaluate how chemically tunable properties permit control over immune activity *in vivo*

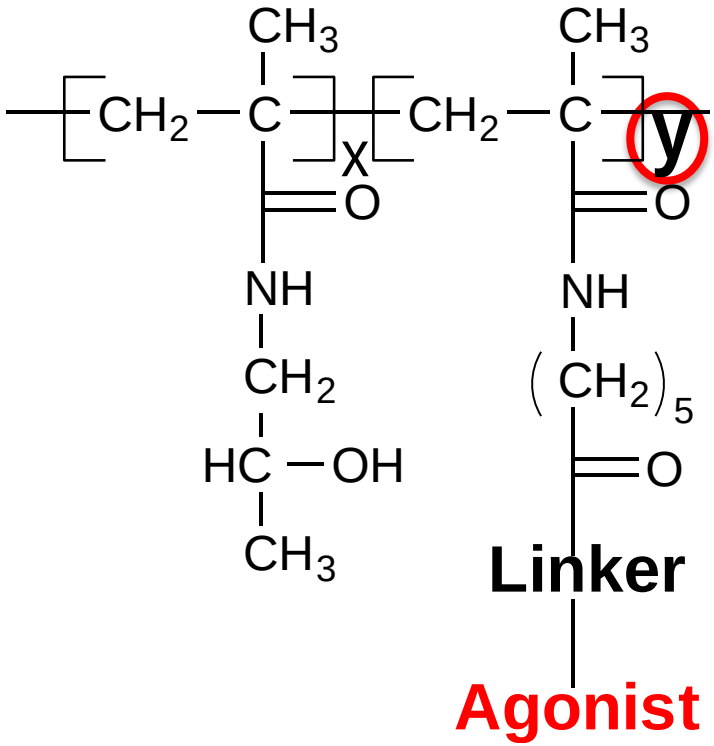
Polymers Used for Medical Applications

- **Polymers are used in food, cosmetics and as "delivery systems" for controlling spatial and temporal activity of drugs**
- **Polymers drug delivery systems can be used to improve activity of antigen and immunostimulants and should be:**
  - Non-toxic, biocompatible and biodegradable (preferred)

Polymer	Application	Product
Polyethylene glycol **(PEG)**	**Used to increase drug solubility, increase half-life**	**Pegasys** (pegylated IFN)
Poly(lactic-co-glycolic acid) **(PLGA)**	**Used in drug delivery, sutures and stents**	**Lupron Depot** (controlled release of Lupron)
Acrylamide-based polymers	**Contact lenses, and chemotherapeutic delivery**	**HPMA-Doxorubicin**

Tunable Properties of Poly-7/8a

Generic structure of Poly-TLRa



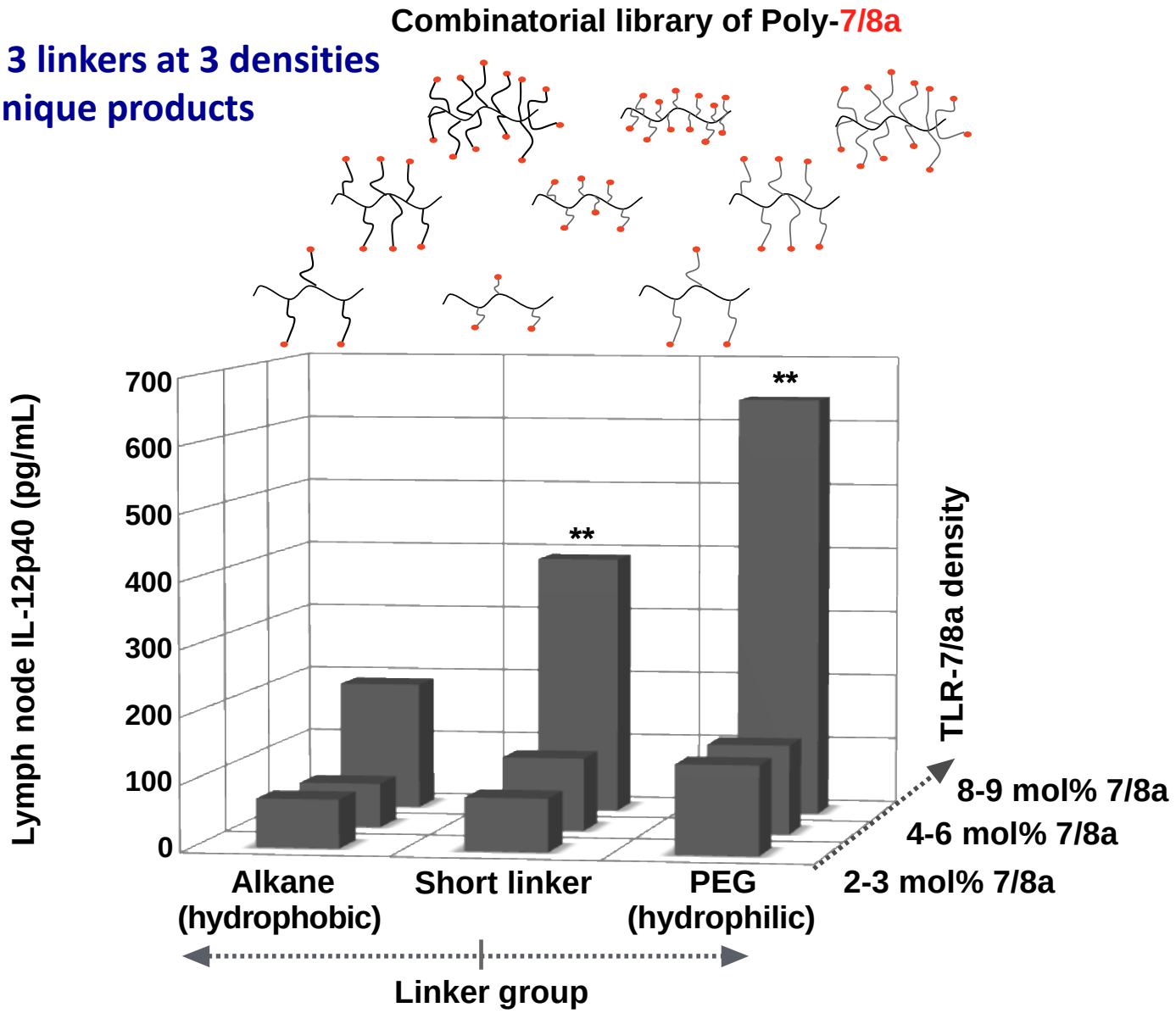
Tunable properties

- **Length and composition**
  - PK and cell uptake
- **Y = agonist density (0 –10%)**
  - Avidity of receptor binding, durability
- **Linker group / attachment site**
  - Rate of release, durability
  - Timing of onset of immune activation
- **Controlled array of ligands**
  - Agonist, antigen, targeting molecules

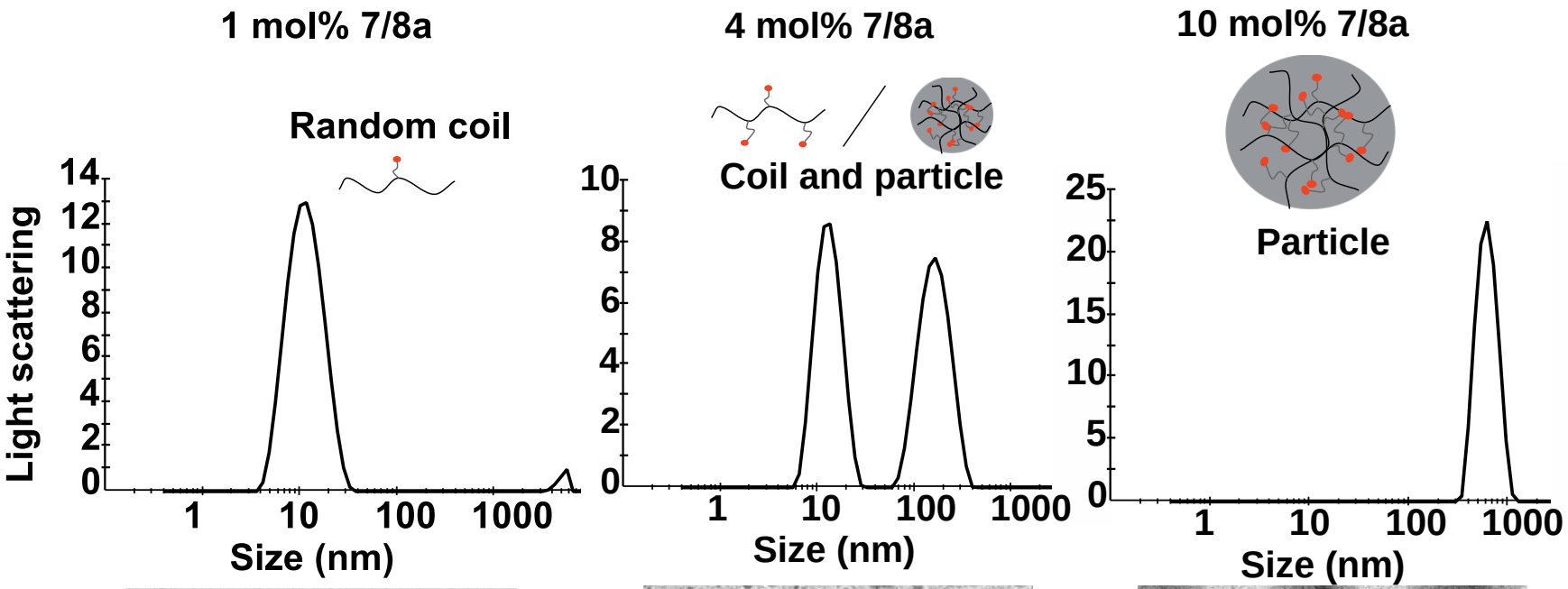
Nature Biotechnology 33, 1201–1210, 2015

TLR-7/8a Density Increases Lymph Node Cytokines *In Vivo*

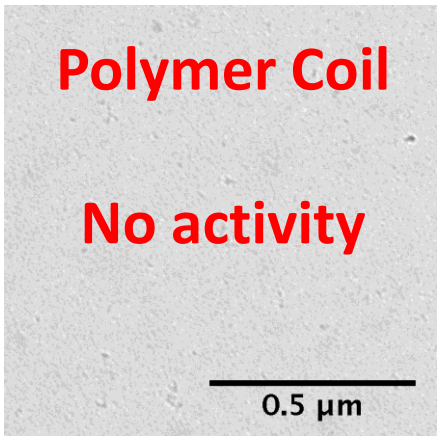
Agonist with 3 linkers at 3 densities
= 9 unique products

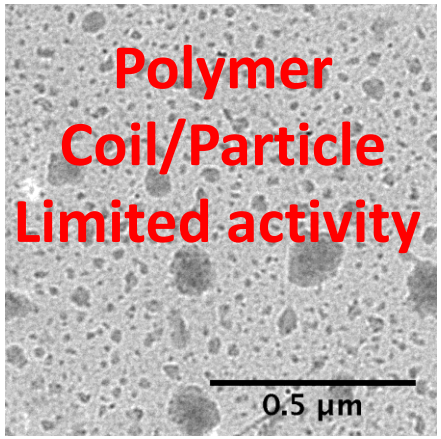


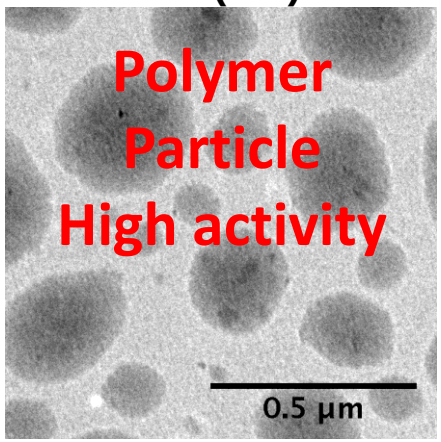
Increasing TLR-7/8a Density Leads to Particle Formation



Cryo-EM

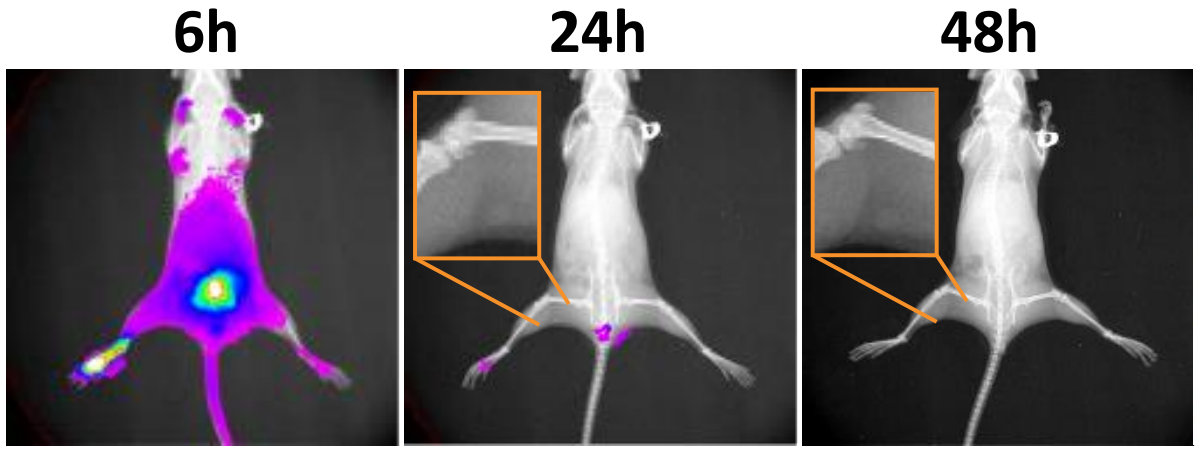






Is particle formation, agonist density, or both critical for activity?

Pharmacokinetics and Bio-distribution of PP 7/8a

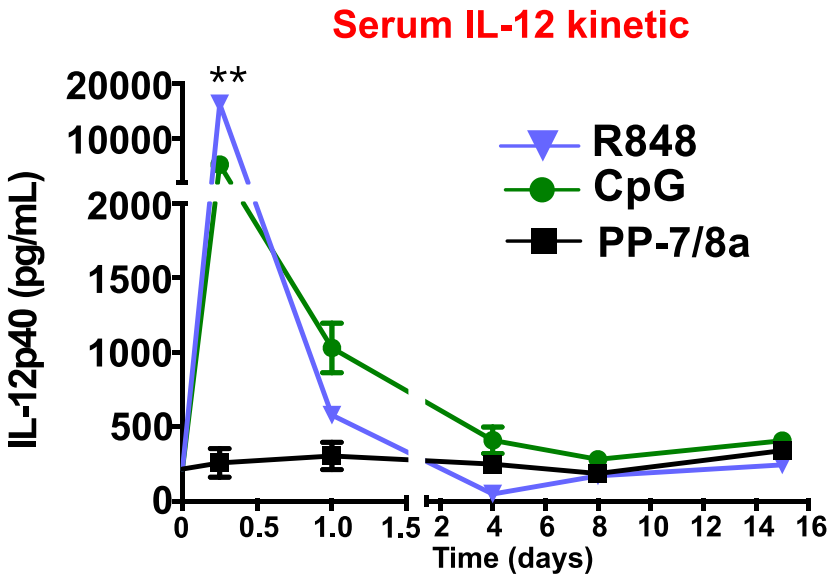


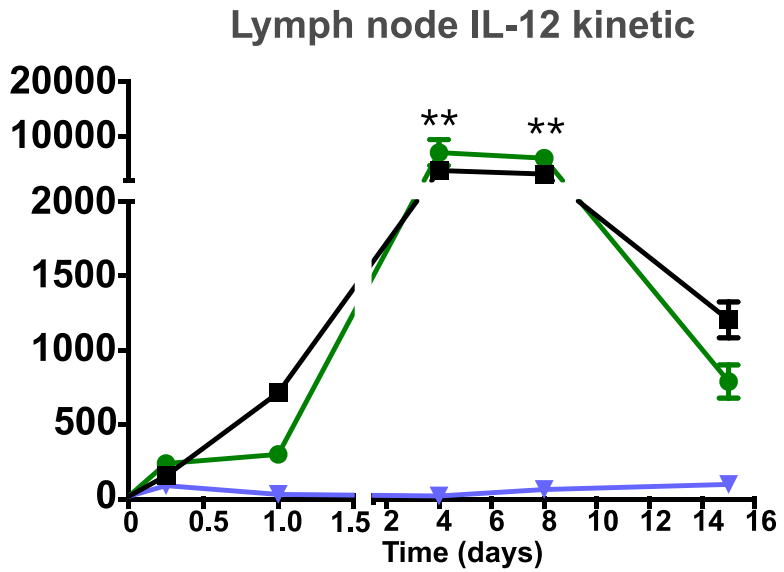
Small molecule (SM)

Polymer coil (PC)

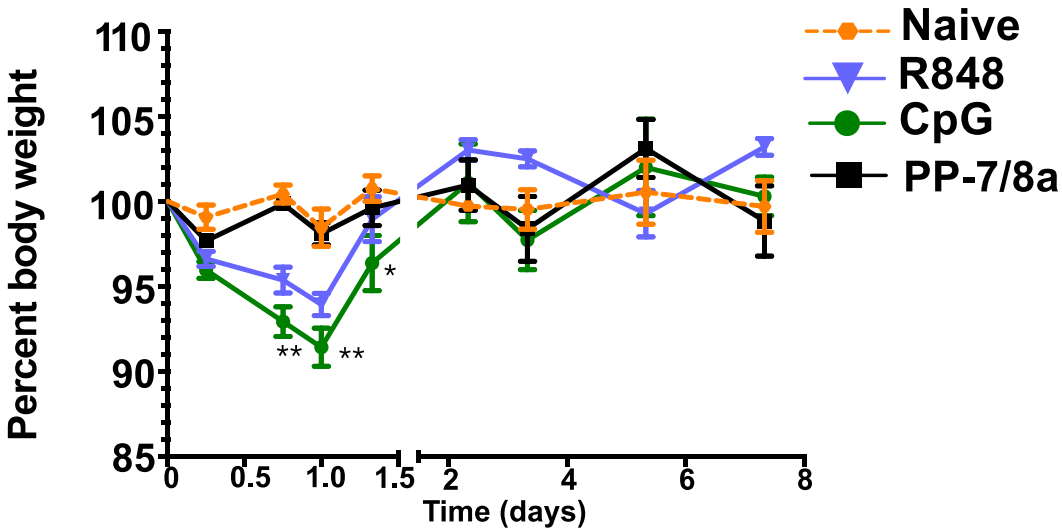
Polymer particle (PP)

Pharmacodynamics of PP-7/8a and CpG



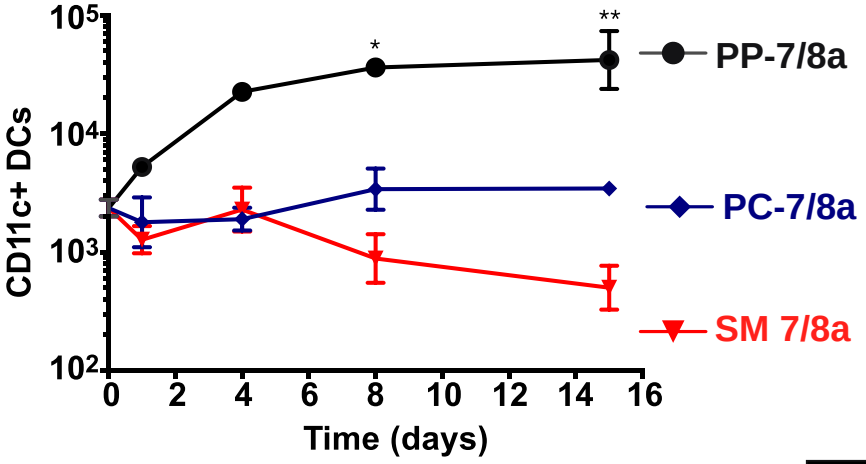


- R848 -> Transient systemic
- PP-7/8a -> Persistent local
- CpG -> Systemic and local

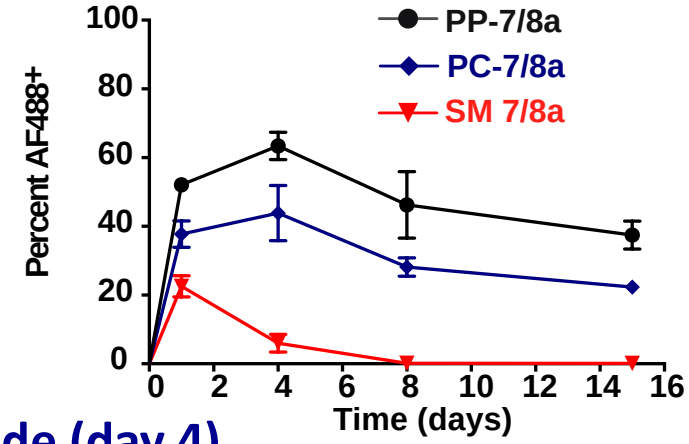


Particulate Poly-7/8a (PP-7/8a) Enhances Dendritic Cell Uptake and Trafficking to Draining Lymph Node

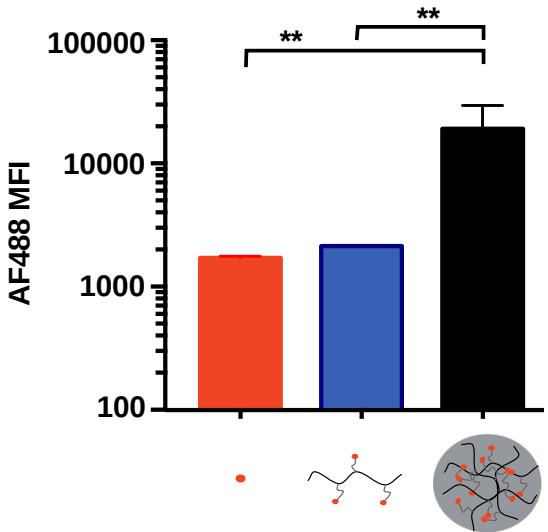
DC Trafficking (lymph node)



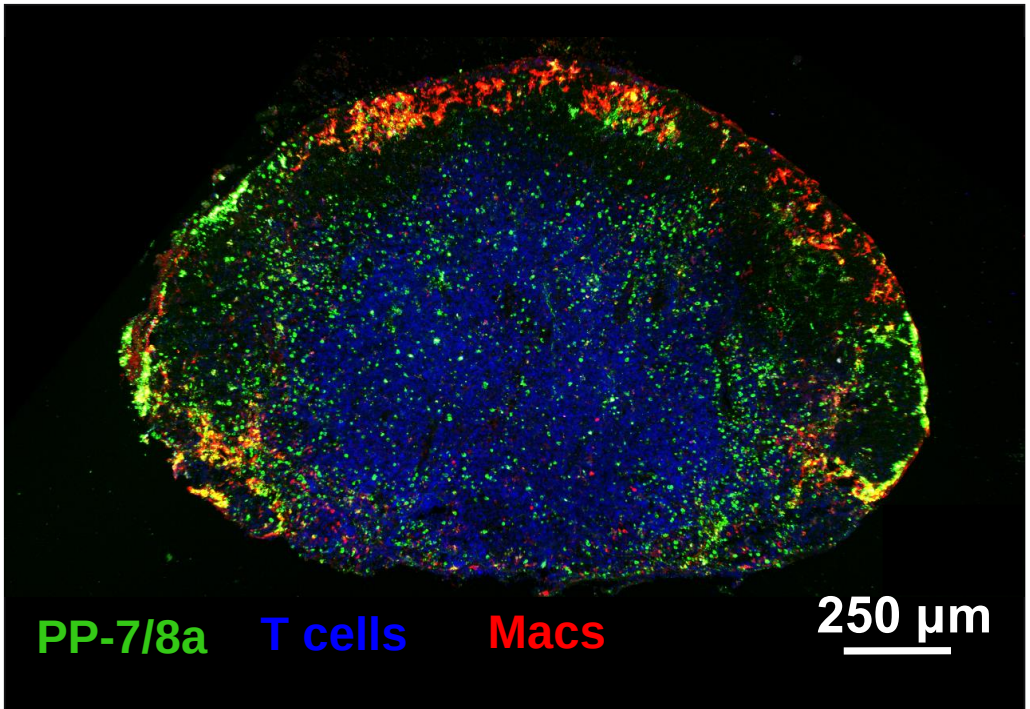
% DC Uptake



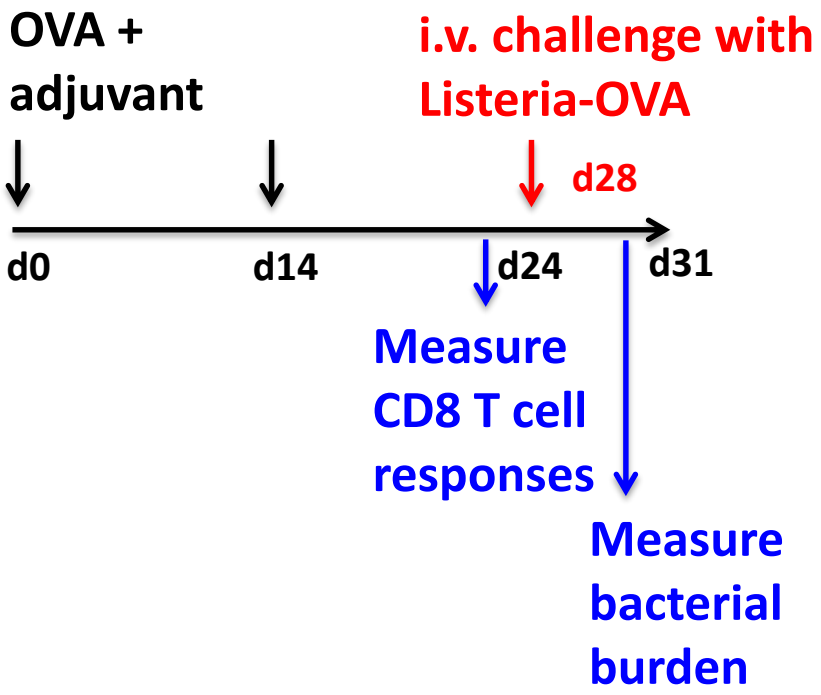
Uptake per cell (24h)

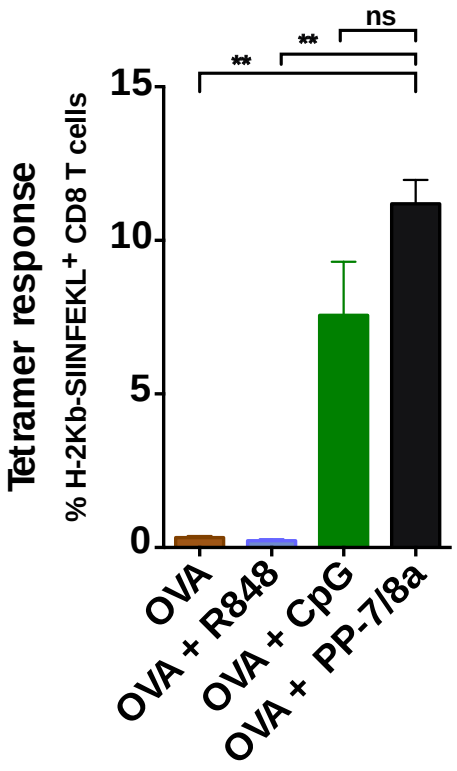


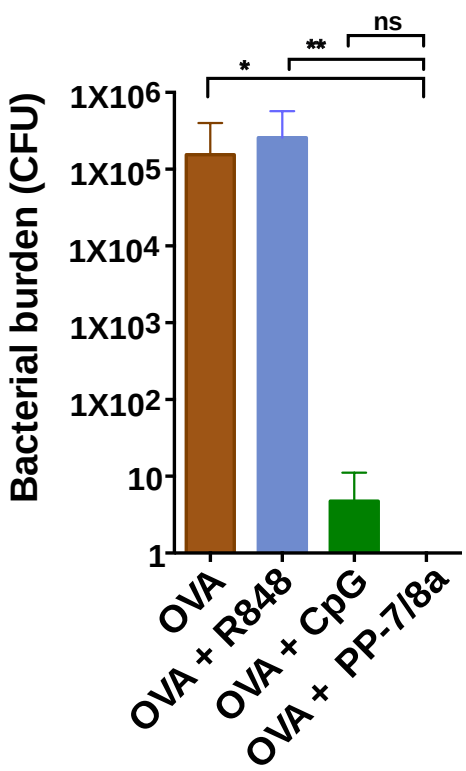
Lymph node (day 4)



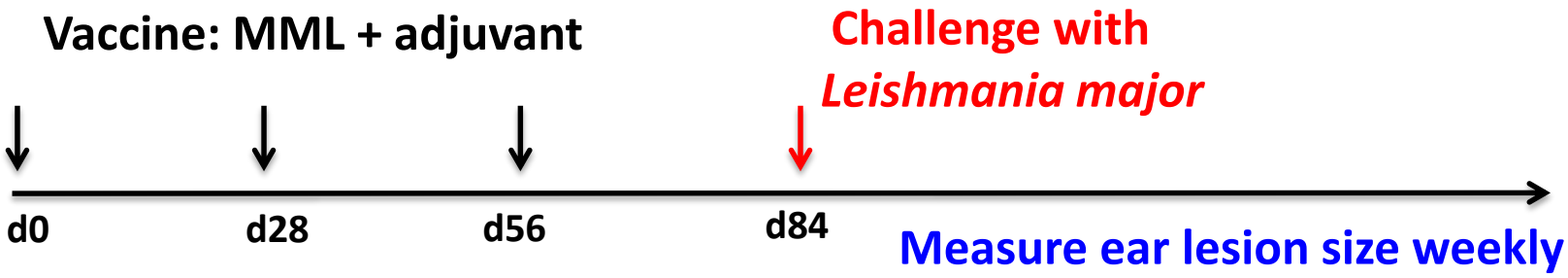
PP-7/8a Induces Protective CD8 T Cell Responses Against *Listeria*-OVA Challenge

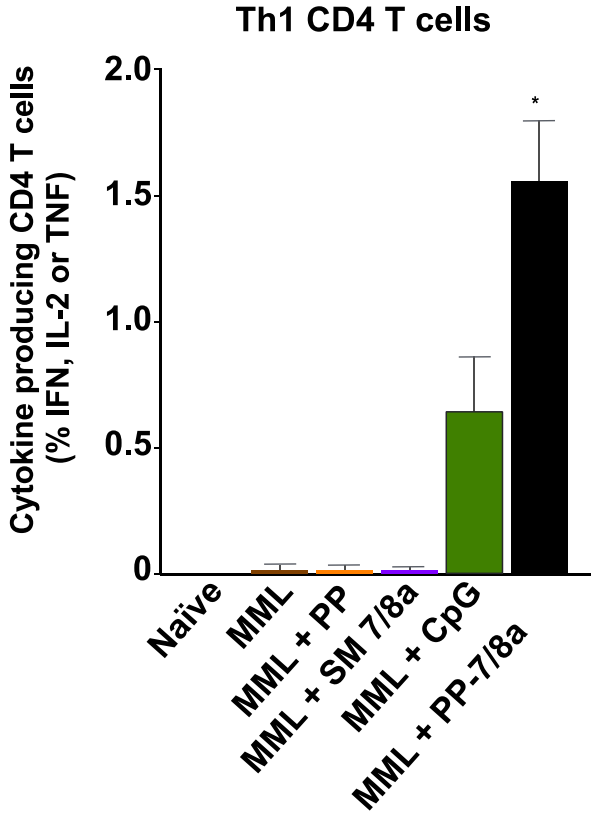


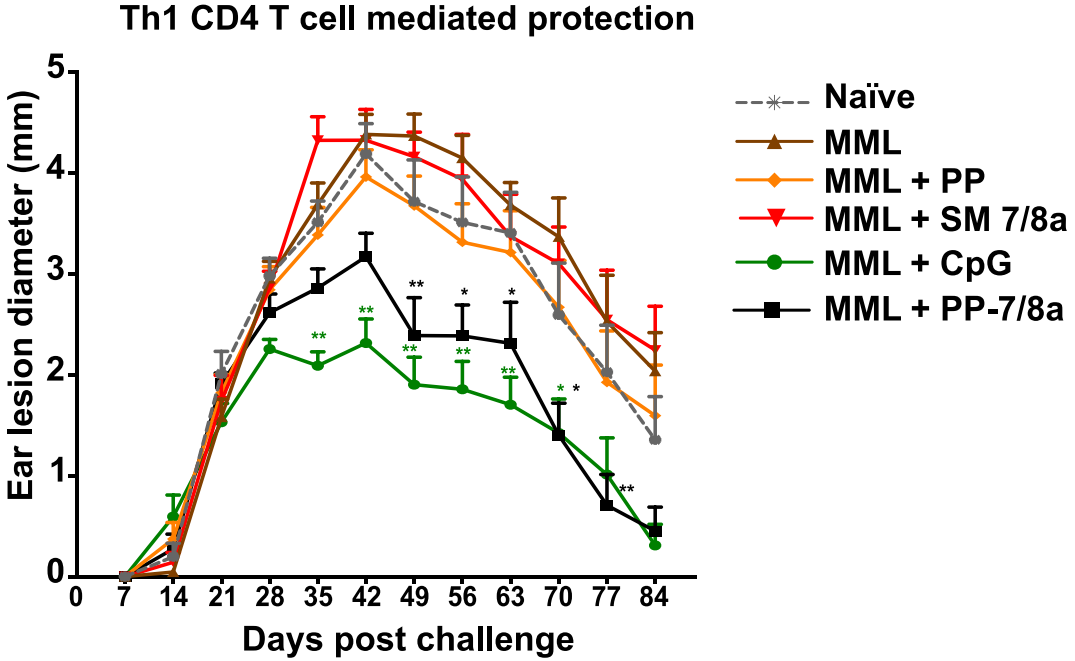




PP-7/8a Induces Protective CD4/Th1 Responses Against *Leishmania major* Challenge







Summary of Polymer Adjuvant Platform

- **Generalizable approach: modular polymer platform allows systematic evaluation of different physicochemical properties of multiple TLR agonists (TLR-7/8a, TLR-2/6a, TLR-4a)**
- **Mechanism: particle formation critical for enhancing persistent local innate immune activation**
- **Application: Vaccines requiring antibody and T cell immunity**

Extend platform to co-delivery of protein and adjuvant:

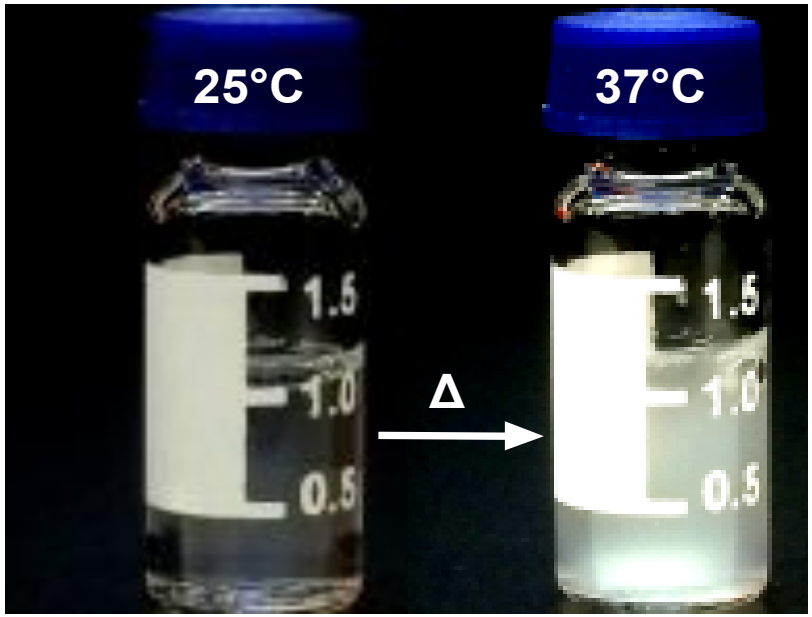
1. **Site-specific attachment**
2. **Controlled array of immunogen on particle**

Improving Formulation Stability

-Single vials of water soluble vaccines are stored at 4-20^{o}C are ideal for use, stability and cost

-Particles are more immunogenic than soluble molecules

Solution: Form immunogenic particles *in vivo*

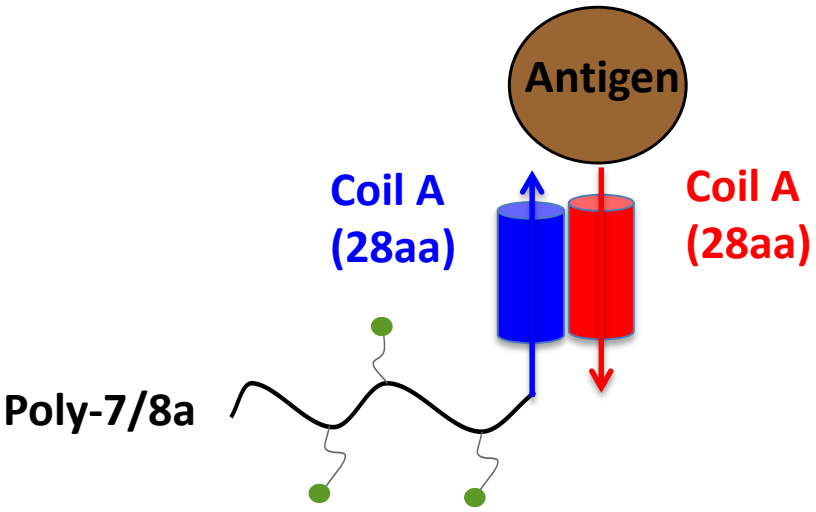


Coiled-Coil Interactions Co-Deliver Antigen and Adjuvant on Temperature Responsive Particles (TRP)

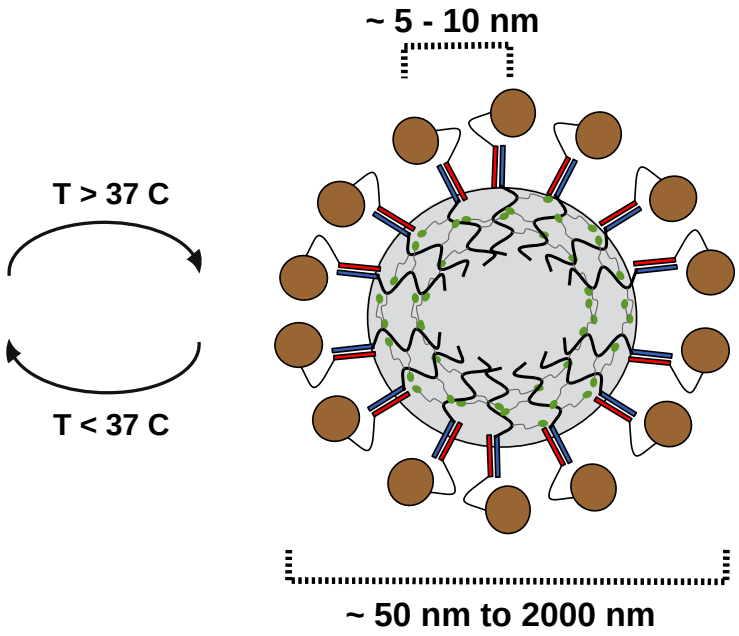
- Non-specific chemical cross-linkers can mask neutralizing epitopes

Use site-selective linkers (28 non-natural aa)

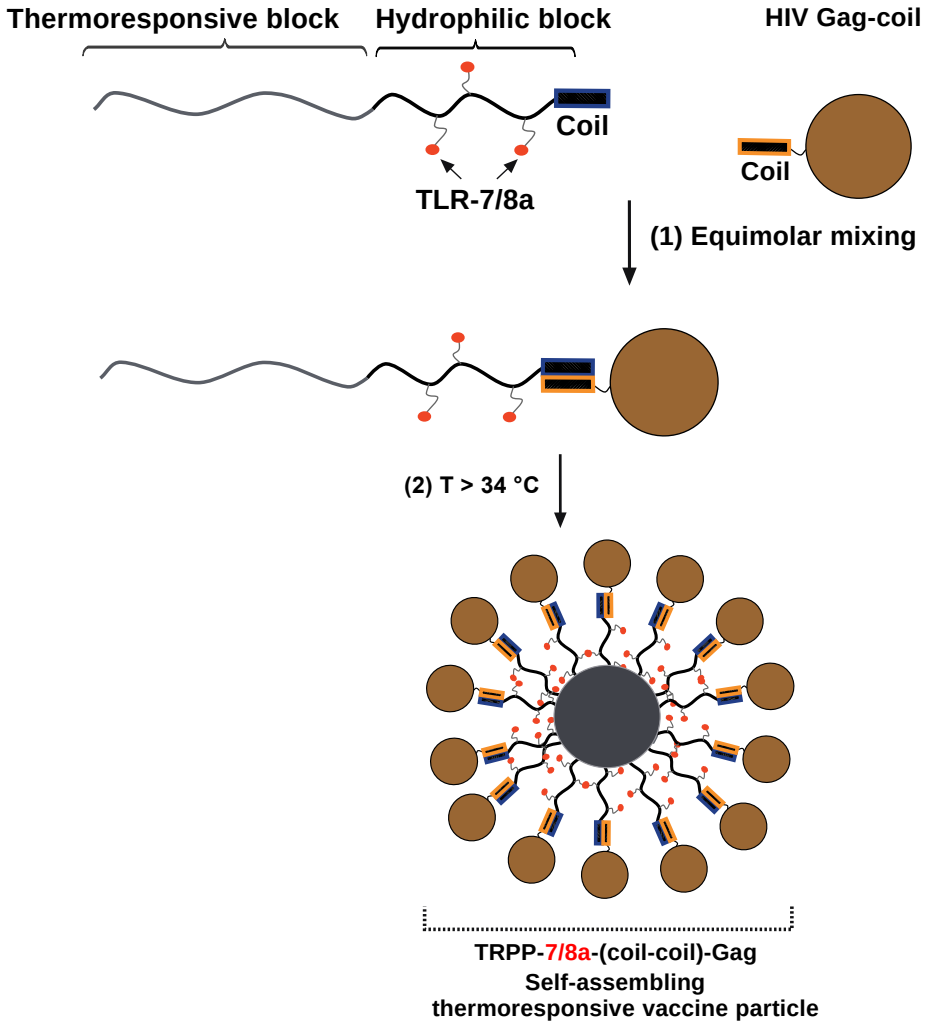
Co-delivery using coiled-coil interactions (hydrophobic/hydrophilic)

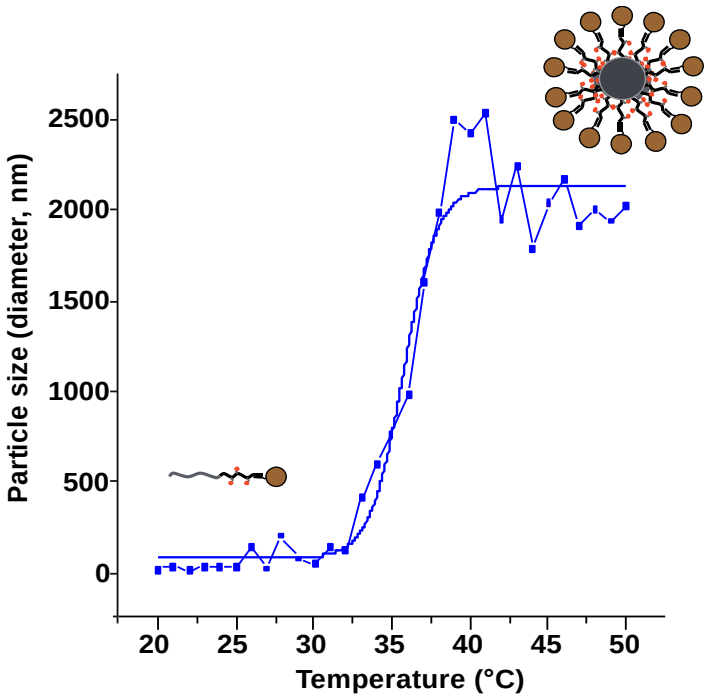


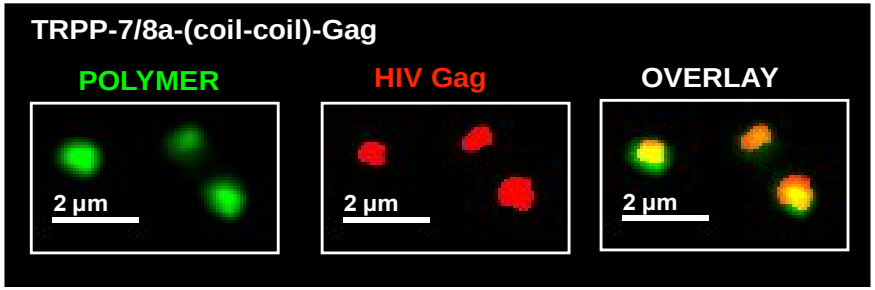
Self-assembly using stimuli-responsive polymers



Site-Selective Attachment of Protein/Peptide Antigen Using Coiled-Coil Interactions

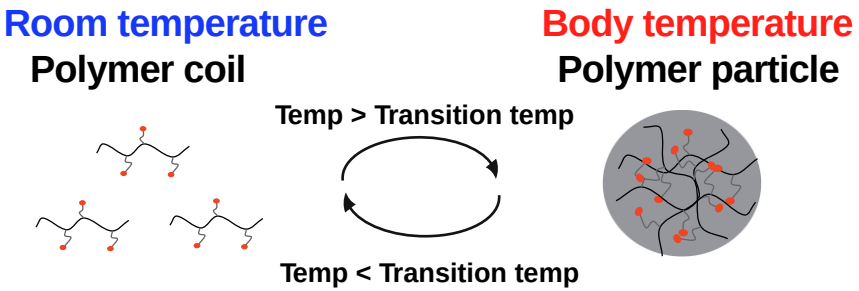


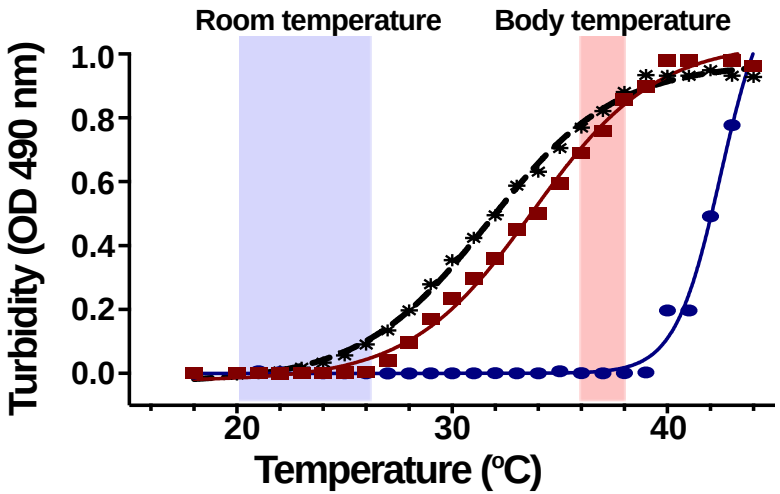




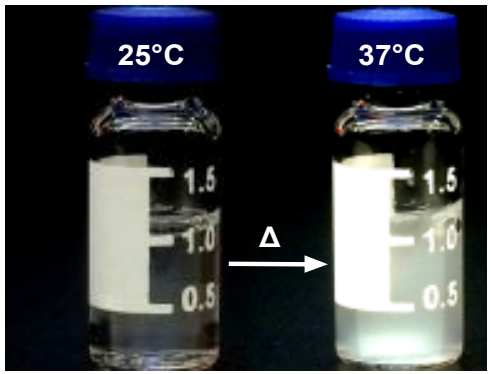
Pechar, M.; et al. Biomacromolecules. 12, 3645-3655 **(2011)** (IMC, Prague)

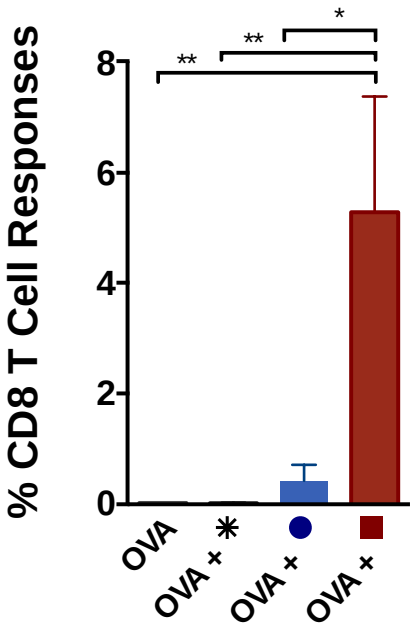
Thermo-Responsive Polymers (TRP)

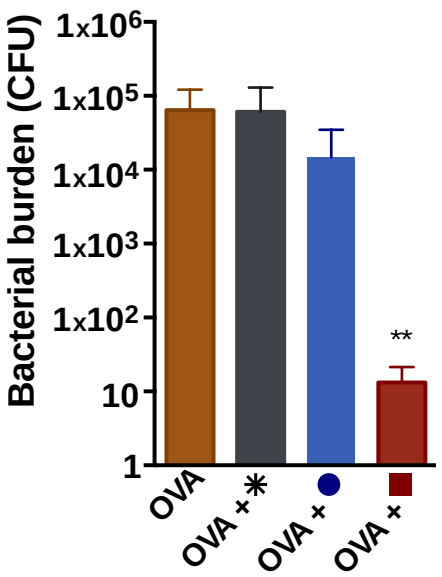




Adjuvant	TLR-7/8a	TT (°C)
✱ TRPP	-	32
● TRPC-7/8a	+	43
■ TRPP-7/8a	+	34





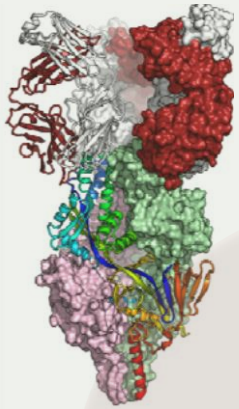


In vivo **particle formation leads to enhanced immunogenicity**

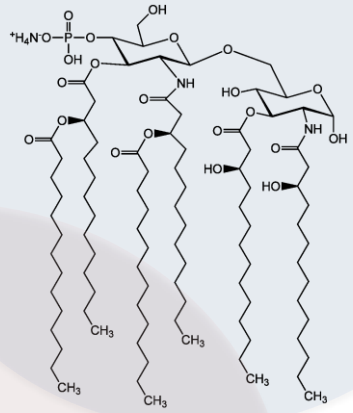
Optimizing Subunit Vaccine Immunogenicity

Structure-based immunogen design

Antigen



Immunostimulant



Delivery Platform

CONTROL

Antigen delivery

- Multi-epitope array to optimally engage BCRs
- Defined orientation to conserve antigenicity
- Use to deliver peptides
- Improve quality of Ab

Immunostimulant

- Spatially restrict activity to prevent acute toxicity
- Target specific APCs to optimize T cell immunity
- Control innate activation

Acknowledgements

Seder Lab
Geoff Lynn
Tricia Darrah
Joe Francica
Alexandra Balaci
Andrew Ishizuka
Azza Idris
Barbara Flynn
Kailan Sierra-Davidson
Mercy Ude
Neeha Zaidi
Neville Kisalu

Graham Lab
Barney Graham
Man Chen

Kwong Lab
Peter Kwong
Gordon Joyce
Ivelin Georgiev
Guillaume Stewart-Jones
Baoshan Zhang
Tongqing Zhou

Animal facility
Carmelo Chiedi
Marlon Dillon
Wuddi Kefale
Alida Taylor
Hana Bao

National Institute of Allergy and Infectious Diseases

Margery Smelkinson
Michael Gerner
Brenda Klaunberg

Seymour Lab
Len Seymour
Kerry Fisher
Ryan Cawood
Tom Hills

IMC (Prague)
Karel Ulbrich
Richard Laga
Michal Pechar
Robert Pola
Karel Ulbrich

NIH National Heart, Lung, and Blood Institute

JOHNS HOPKINS MEDICINE

IPDC
Rolf Swenson
Olga Vasalatiy
Andres Dulcey
Changhui Li

Green Lab
Jordan Green
Joel Sunshine