# Engineering Prodrug Therapies for Infectious Disease and Cancer Therapies

# Long-Acting PrEP for Africa

- Women in Sub-Saharan Africa have poor pill adherence
- Long-acting delivery systems are implants or injectable
- Although implants provide longer duration, some women prefer injectable DDS

# Current State of the Art in Long-Acting Pre-Exposure Prophylaxis (PrEP) for HIV Prevention


## Release Profile Good but…

Drug falls across wide range and tails

"The clinical significance of the long pharmacokinetic tail of cabotegravir observed in female participants compared with male participants, and those with higher BMI compared with a lower BMI, need to be addressed in future trials."

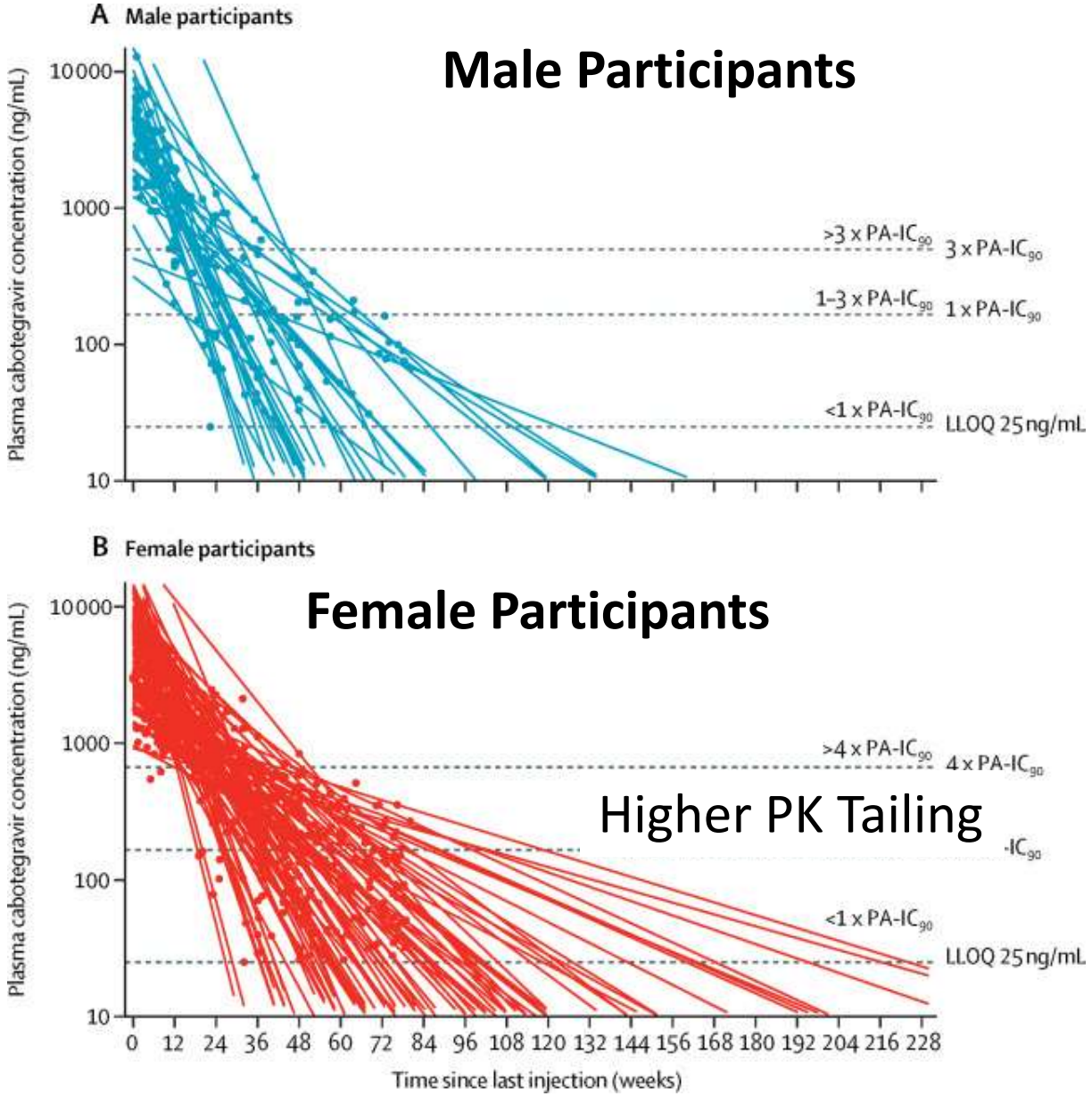
## Cabotegravir/Apretude Approved 2023

600-mg (3-mL) intramuscular (IM)

2month duration

injection in the muscle of the buttock by a health care professional

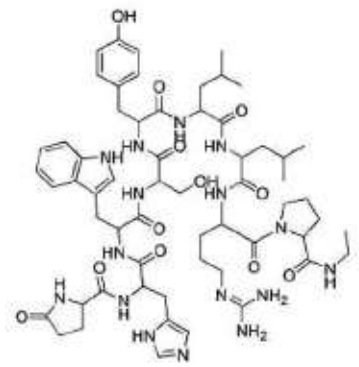
The Lancet
7:E42

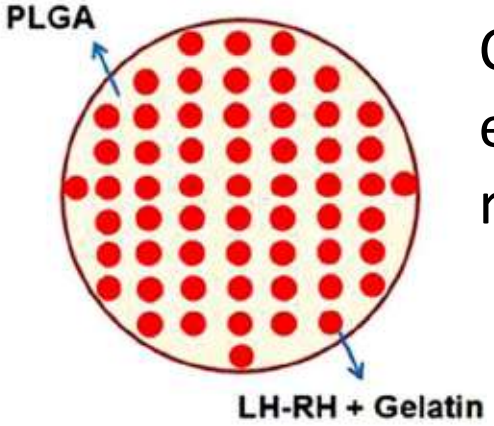


# The Bill and Melinda Gates Foundation Target Product Profile (TPP) For Women in Sub-Saharan Africa (and other resource poor settings)

- ***Longer-Acting/Better PK Injectables (>6months)***
- ***Lower injection volumes***
- ***Subcu less painful than IM***
- ***Lower Cost of Goods Sold***
- ***Limited cold chain***
- ***reduced clinic/healthcare worker requirements***
- ***Different duration products, i.e. 6mo, 12 mo, 1.5yr etc***

# Lupron Depot Example of Biodegradable Drug Delivery Systems

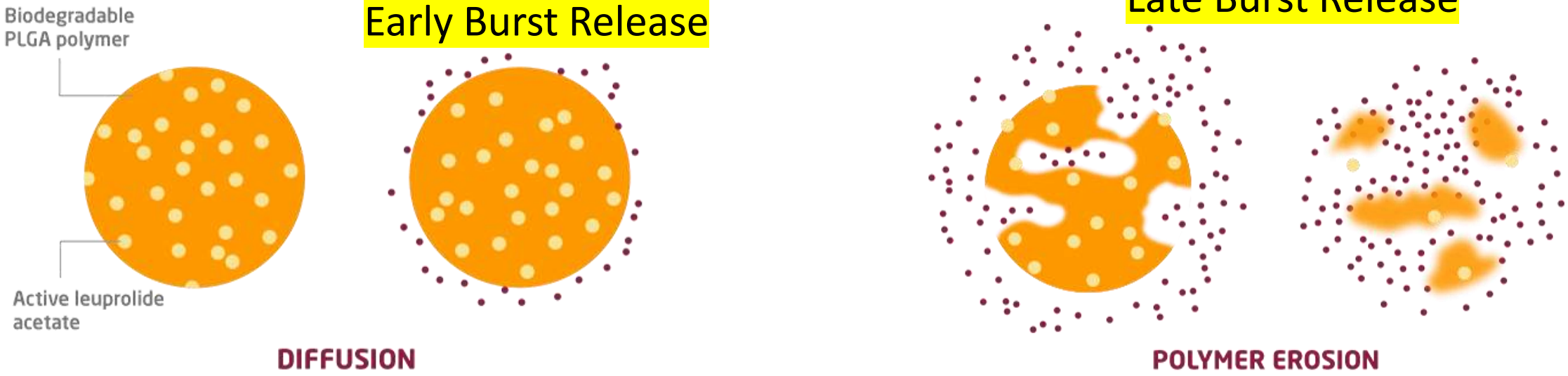





Current Depot Designs
e.g. PLGA depots erode to release encapsulated drug

Early Burst Release

Late Burst Release

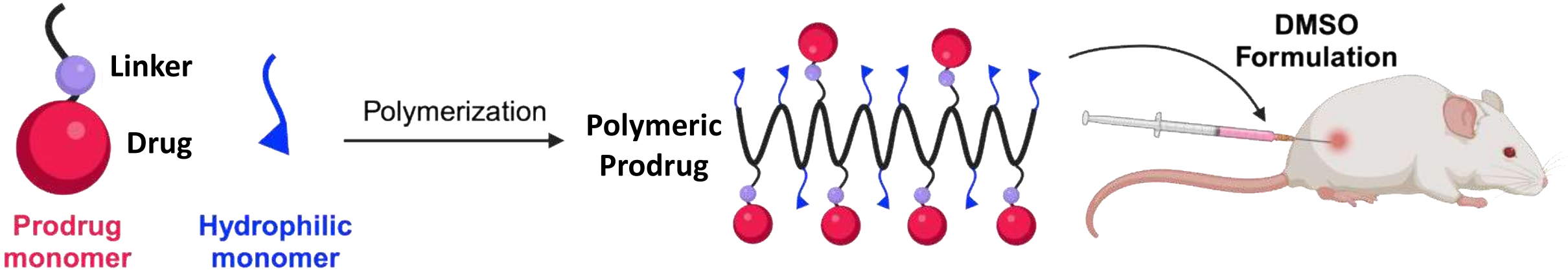



Much of the mass is the degradable polymer = low drug wt %

https://www.lupronprostatecancer.com/hcp/innovative-features

# Drugamer Depot is Polymeric Drug In Injection Solvent

## *Polymers Designed To Be Water Insoluble Initially at Injection*




**Schematic mechanism of drugamer depot**


Takuma Yoshikawa


Debashish Roy


Sreekanth Kokkonda


Sam Arnold

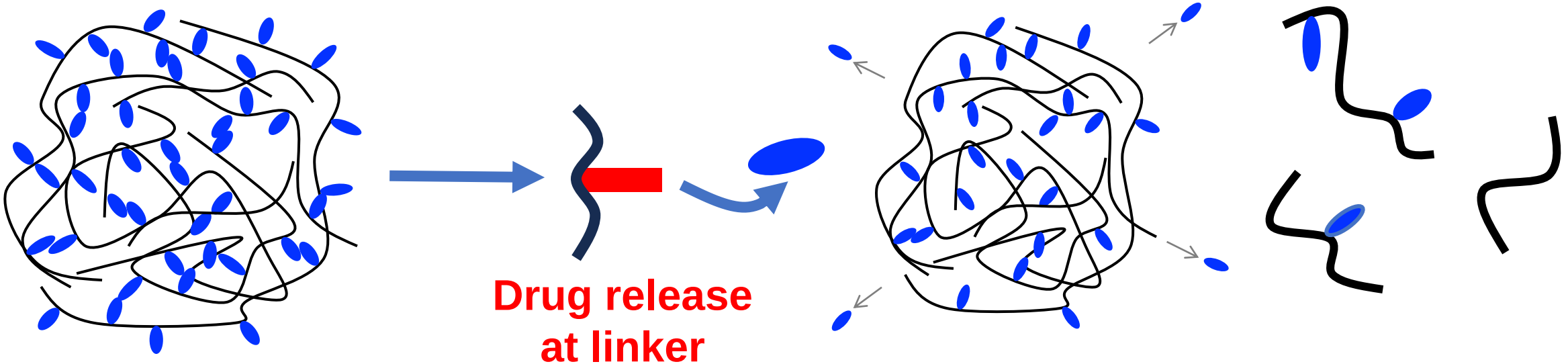

Cindy Zhang


John Chiefari


Almar Postma


Fei Huang

# Mechanism of Drugamer Depot Formation & Degradation/Clearance



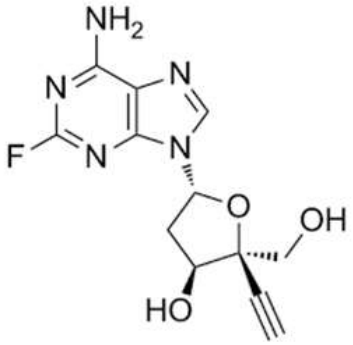

**Hydrophobic Effect & Other Interactions Drive Gel Formation**

**Drug Release Leaves Carboxylated Monomers & Chains**

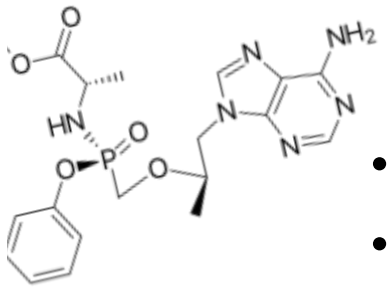
**De-gelling & Depot Dissolution is Connected to Drug Release**

# Drugamer Depots Have High Drug Loading for Long-Acting Formulations

***Synthetic manufacturing with lower "Cost of Goods"***




Merck
islatravir

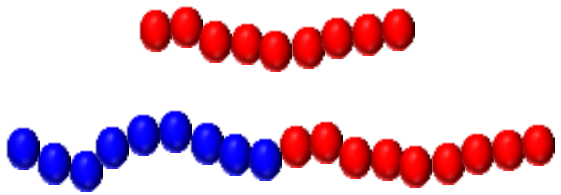



Gilead
TAF

## Drug Depot Goals

- Low Volume &Viscosity
- Narrow needle gauge
- Zero-order release, no early or late-stage burst release
- Low Cost of Goods Sold


sophisticated drug space possible

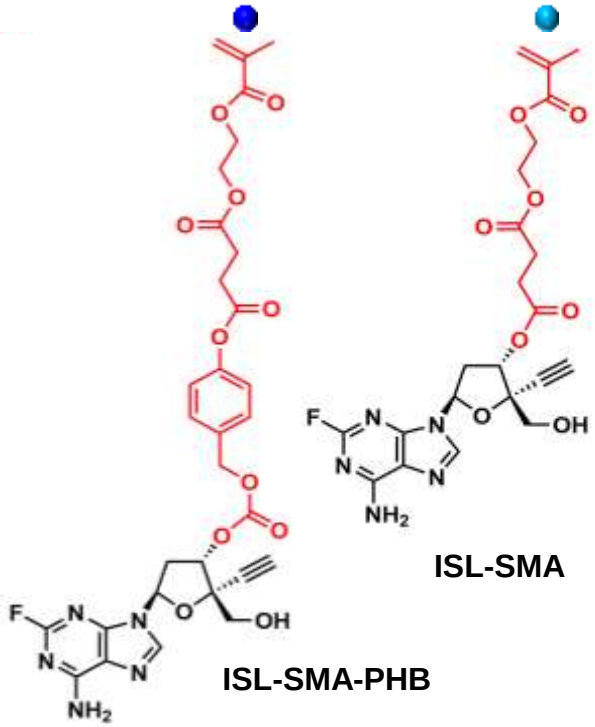


***Depot is polymer in injection solvent***

Ho et al., J. Controlled Release (2021) 329:257

# Synthetic Prodrugs Open New Design Space: Bulk Depot Release Engineered By Polymer Chemistry

## Linker Design



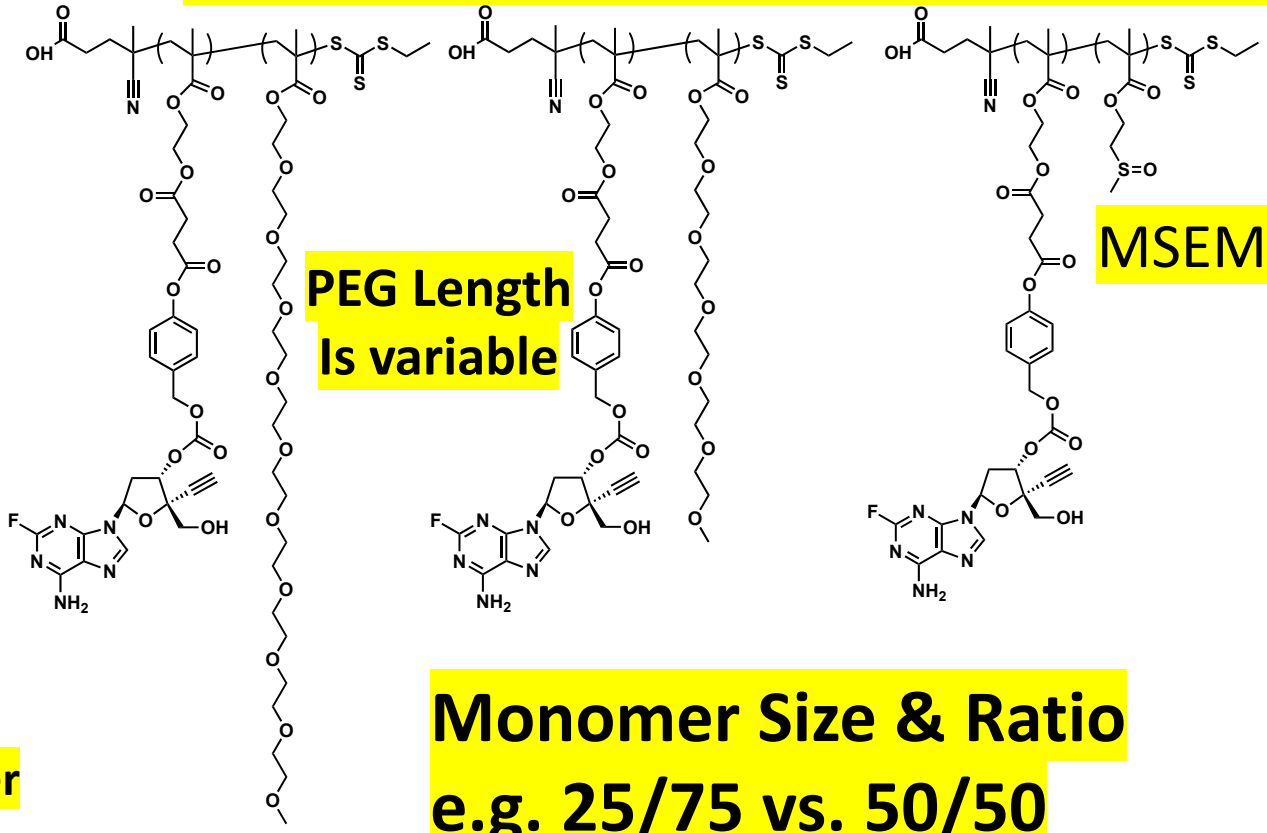
ISL-SMA

ISL-SMA-PHB

Slower Alkylester

Faster Benzylic Carbonate

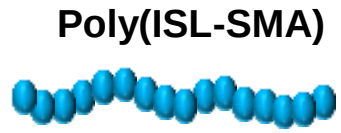
## Co-Monomer Sterics & Hydrophobicity



PEG Length Is variable

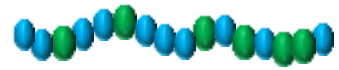
MSEMA

**Monomer Size & Ratio e.g. 25/75 vs. 50/50**



Poly(ISL-SMA)

Poly(ISL-SMA-PHB)



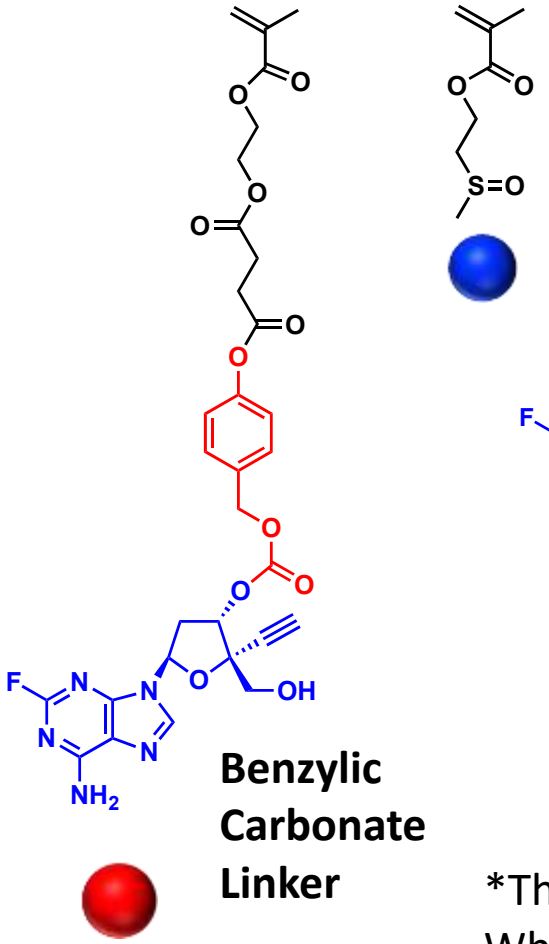
Poly(ISL-SMA-co-PEGMA)

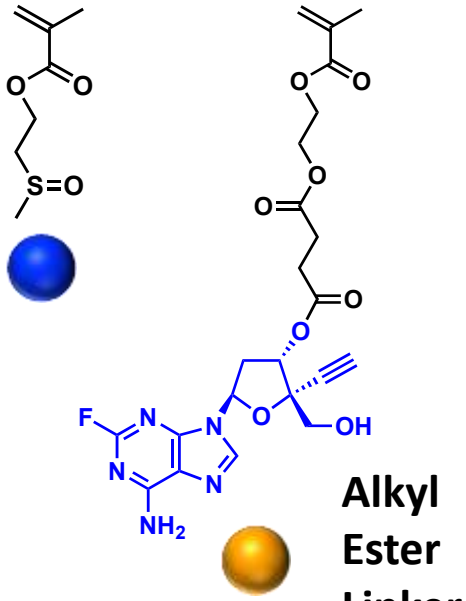
Poly(ISL-SMA-co-MSEMA)

Poly(ISL-SMA-PHB-co-MSEMA)

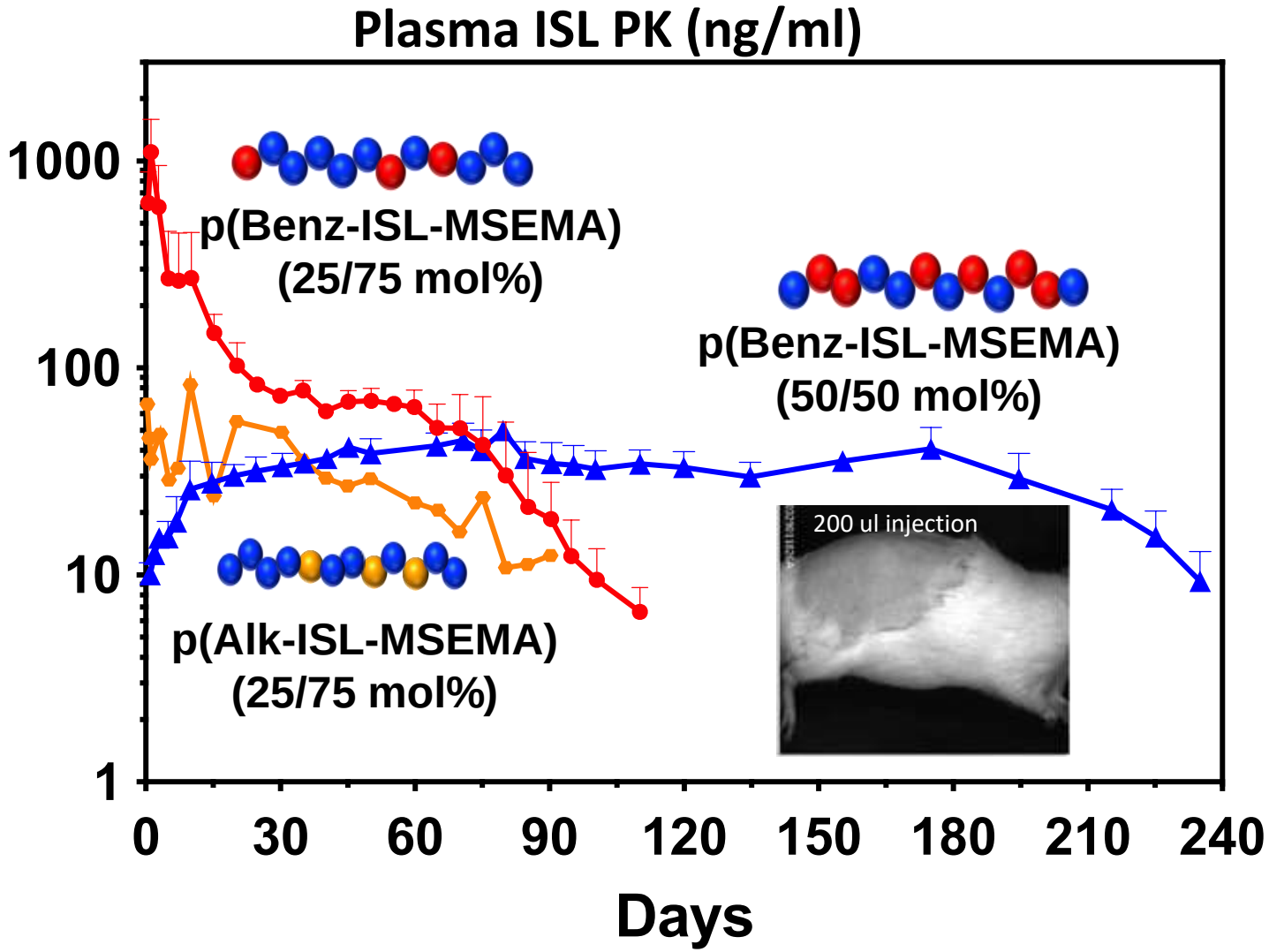
Project with Almar Postma, John Chiefari, Fei Huang, CSIRO Melbourne

# Depot Release Profiles Engineered By Linker & Copolymer Design



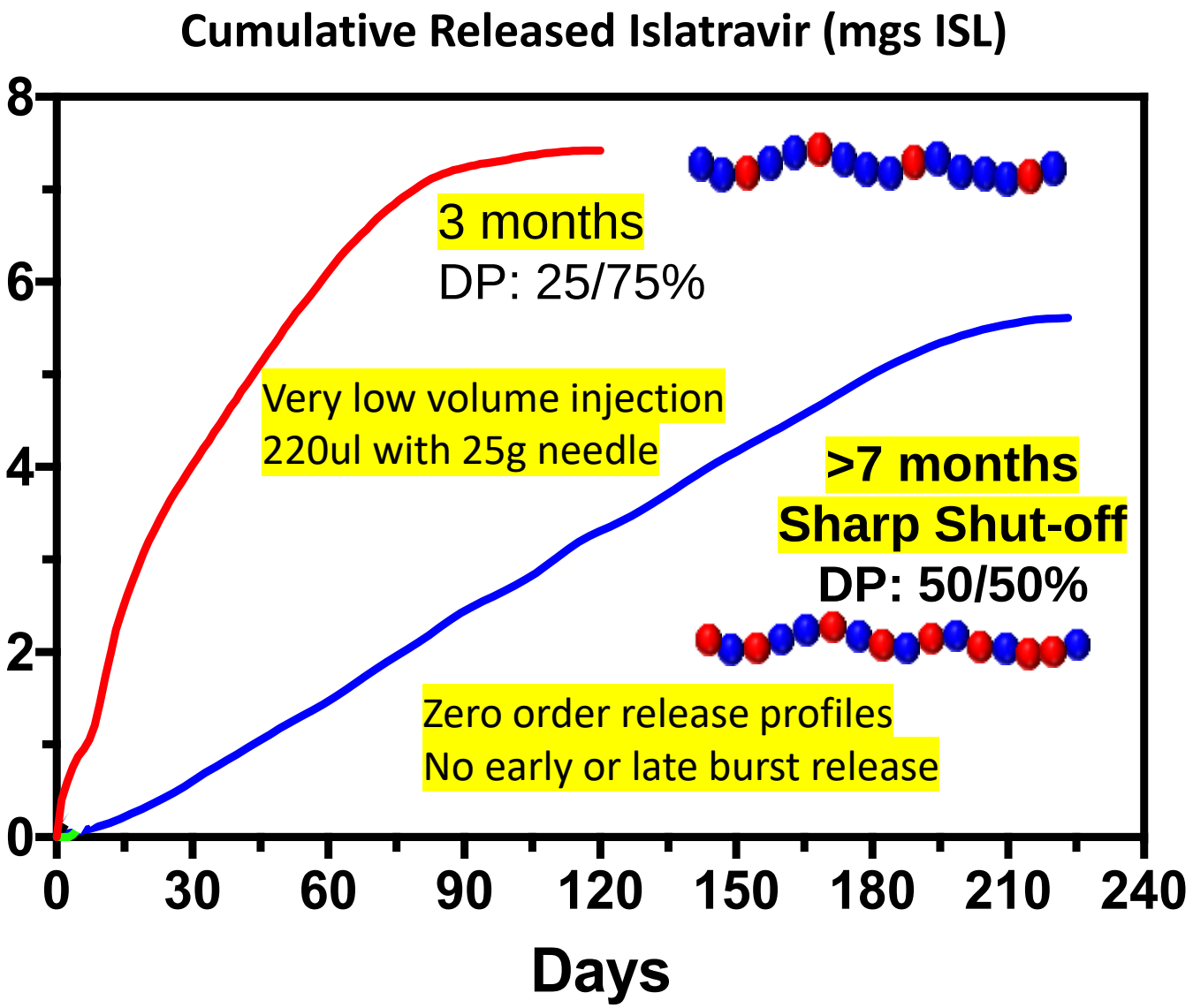




*Thanks to Andrew Whittaker suggesting MSEMA at APS 2019

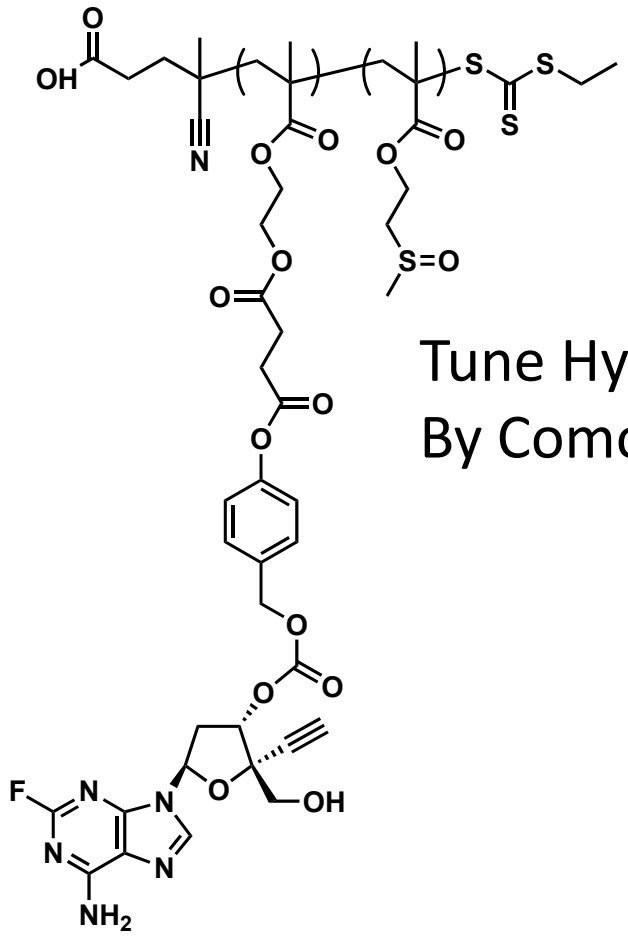



***Low Volume (220μl) Depots Deliver Human Doses 7 Months in Rat Model***

# Long-Acting Depots Achieve Human Daily Dosing Requirements




Copolymer With MSEMA Co-Monomer

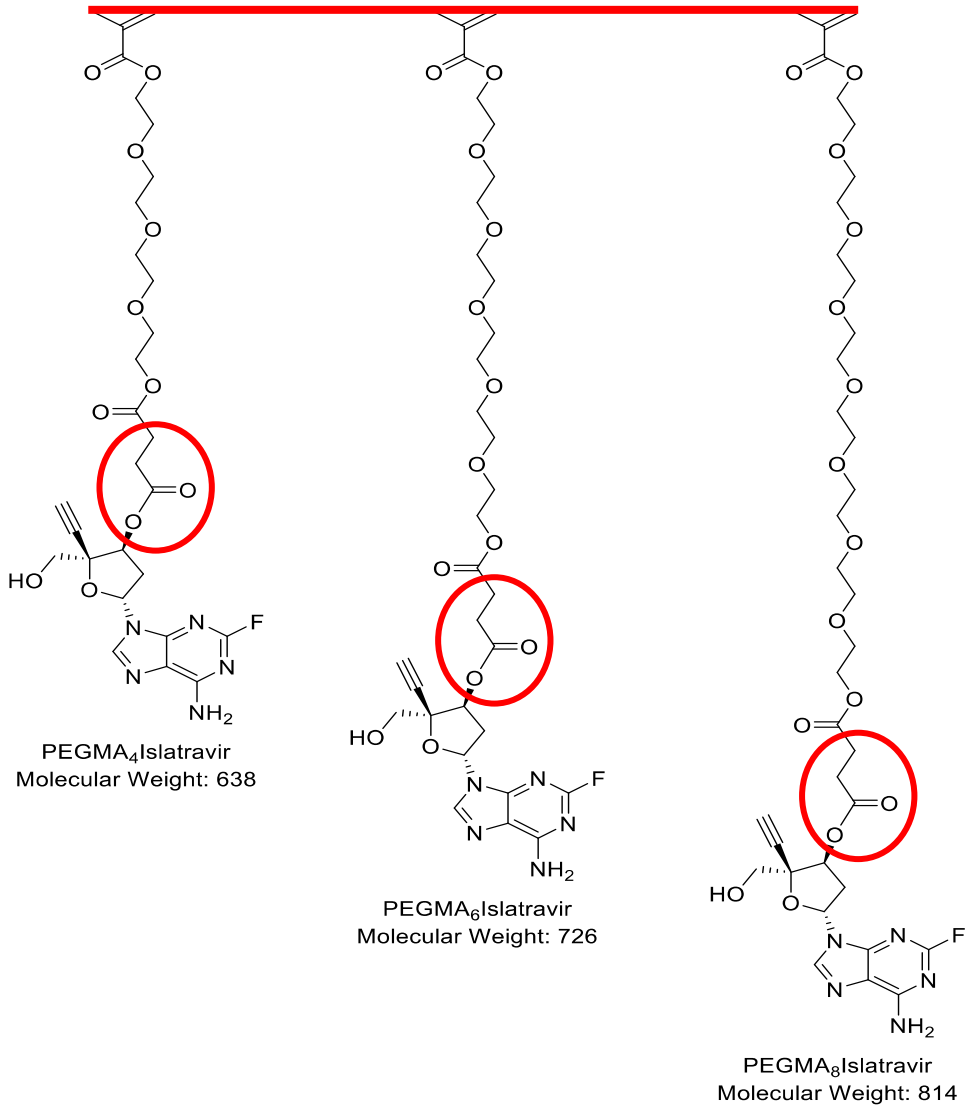


Tune Hydrophobicity
By Comonomer Ratio

# Spacer Design Enables Homopolymer Depots

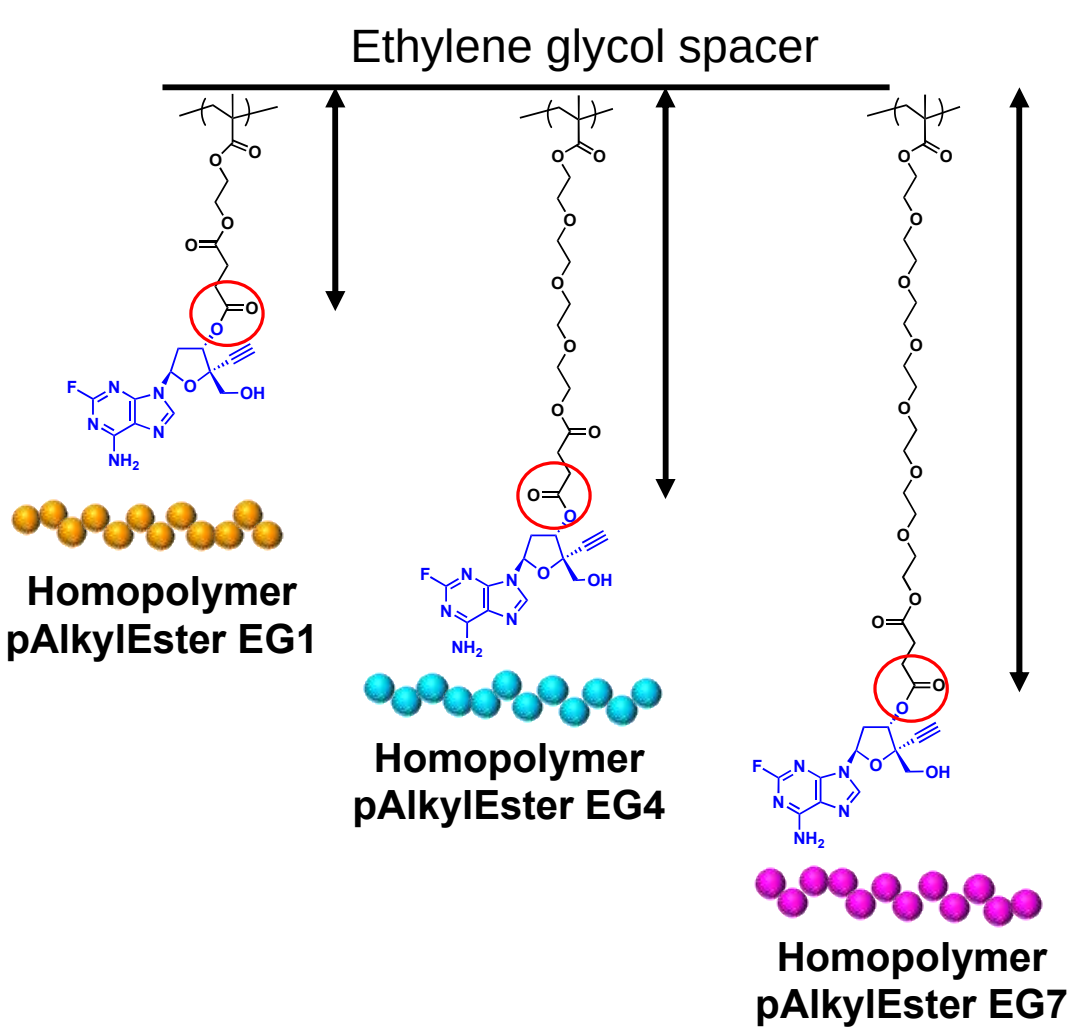
**EG Spacer Can Be Varied In Length**

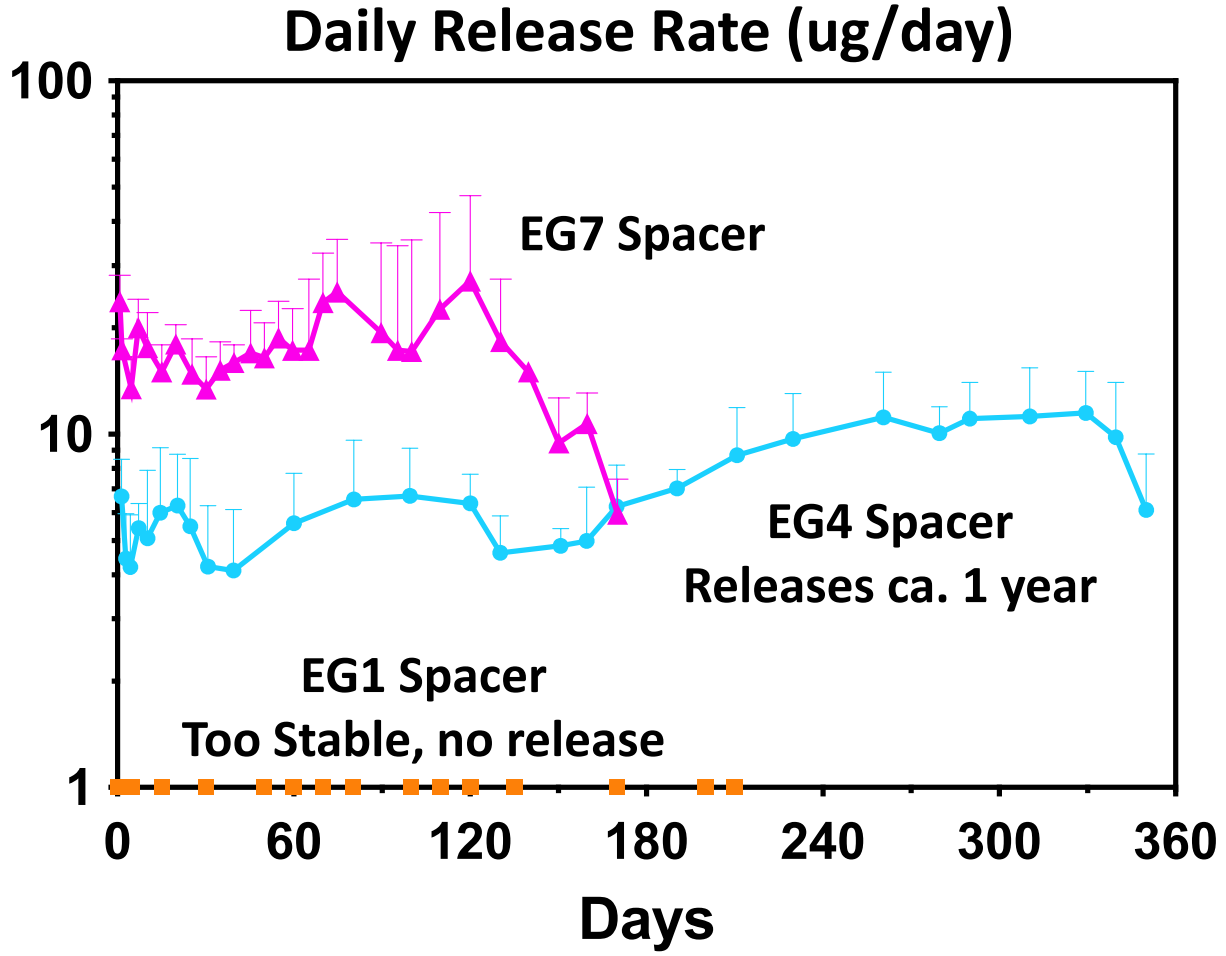
**Spacer Adds Hydrophilicity**
**Linker Is More Accessible**



# Spacer Length Also Tunes Release in Hompolymer Design

*Monomer design translates directly into bulk depot drug release PK*

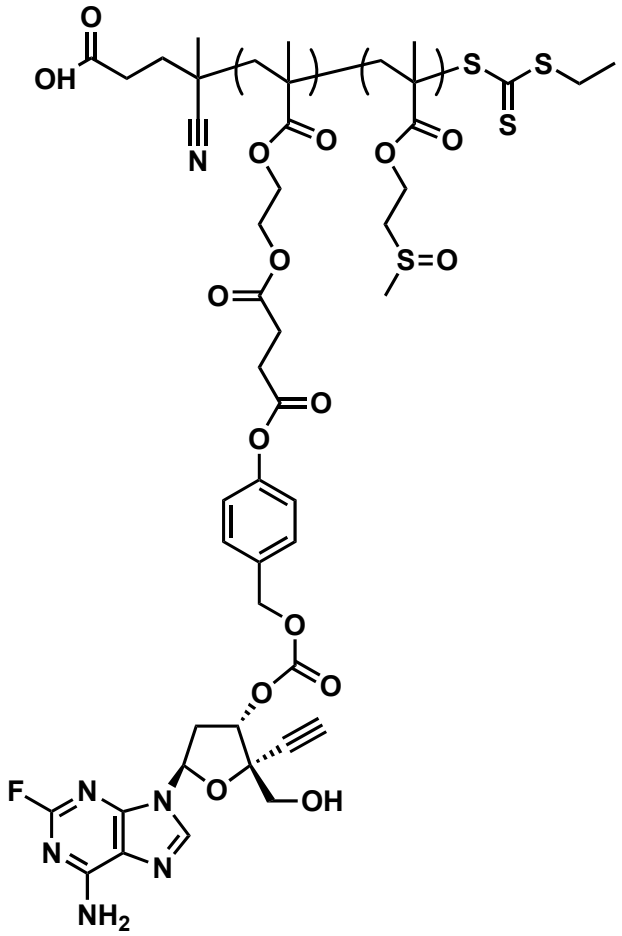



*With Almar Postma, John Chiefari, Fei Huang, CSIRO Melbourne

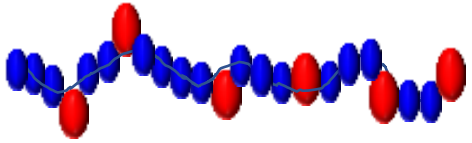
# Knobs For Tuning Release Profiles

## Copolymer Design

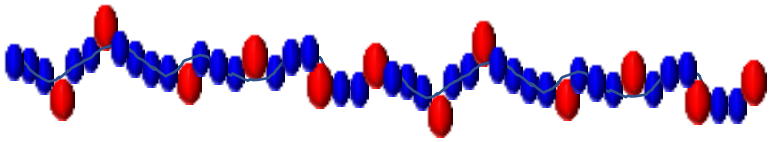


## Mw Series Design

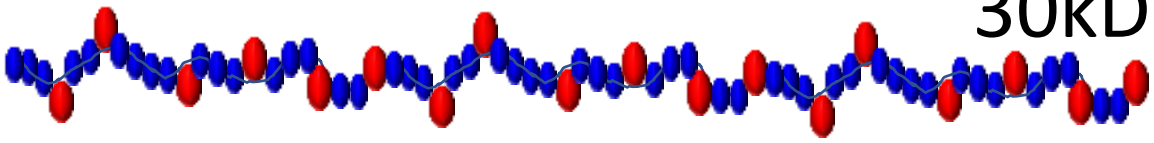
*25% ISL – co – 75% MSEMA Kept Same*



8kD Mw



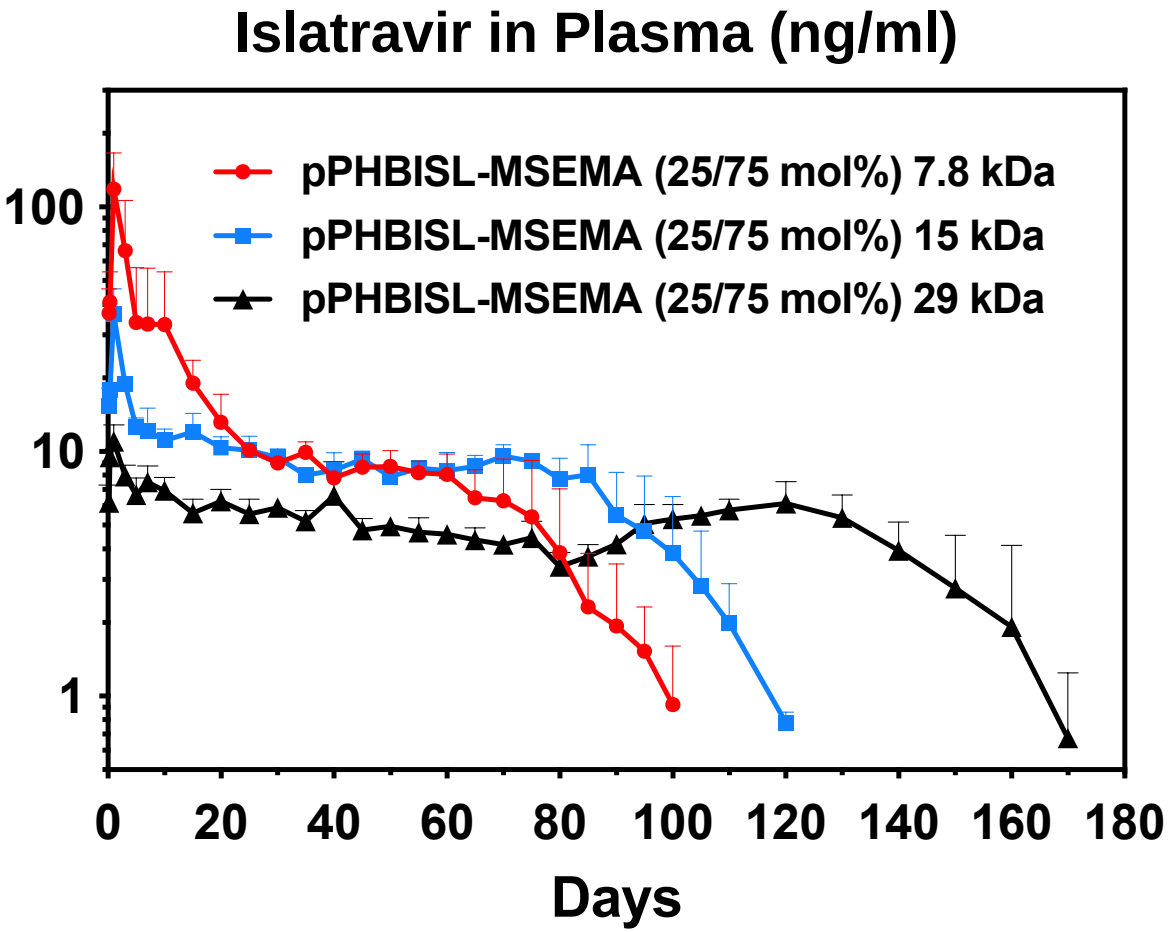
16kD Mw

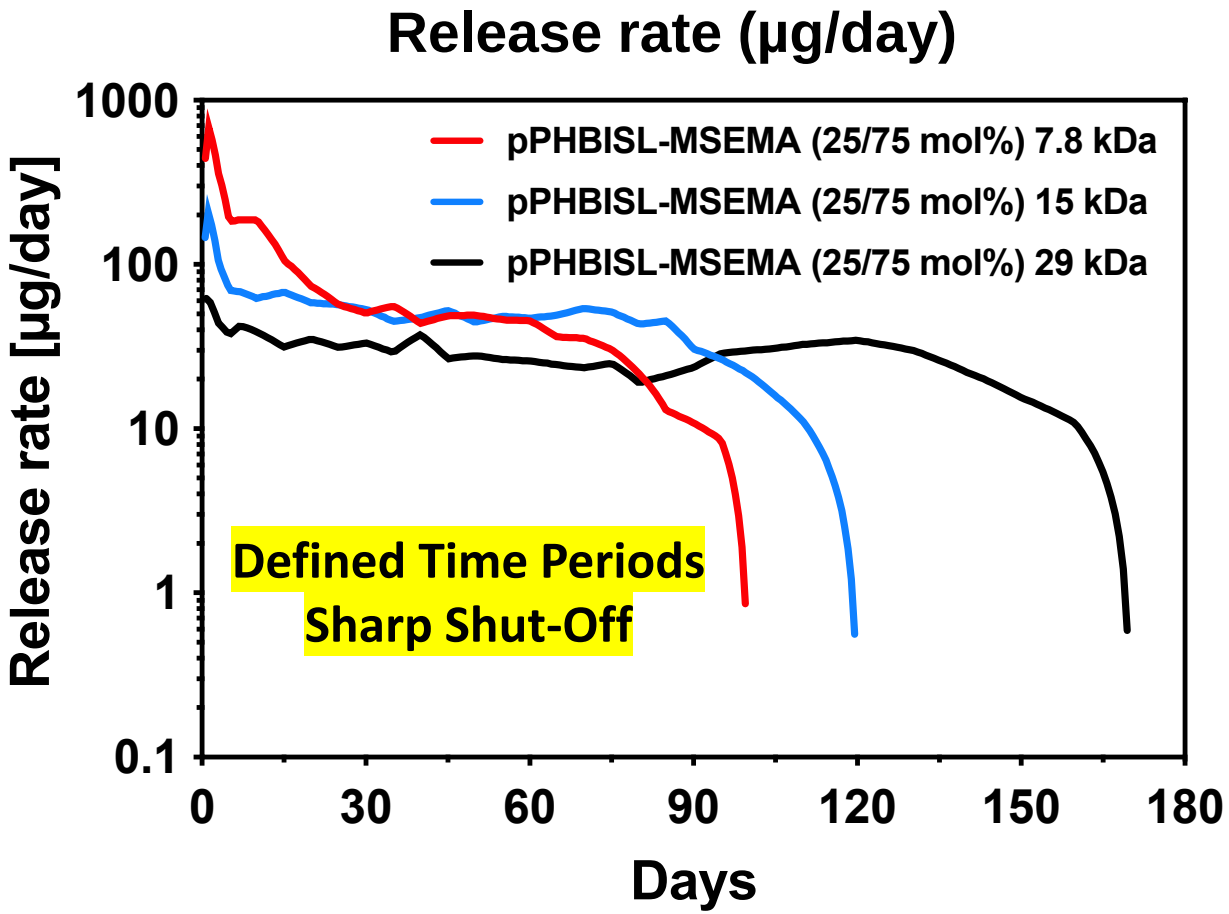


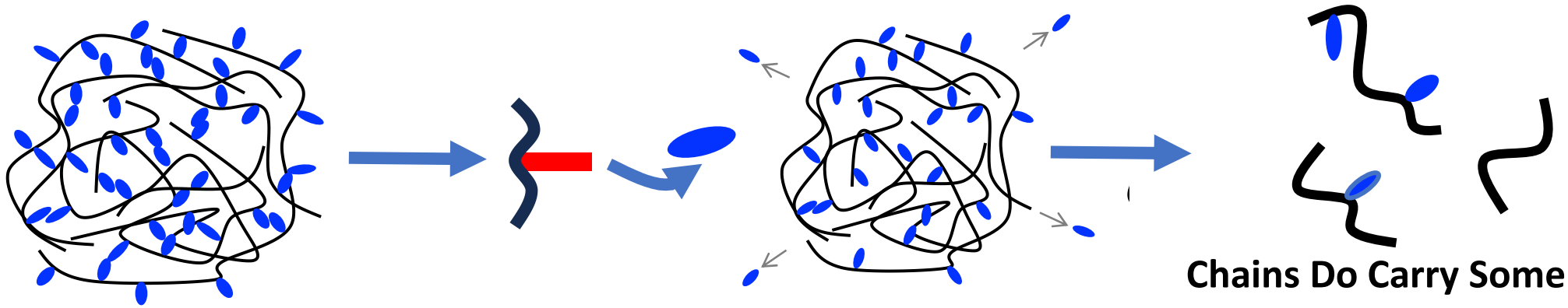
30kD Mw

# Molecular Weight Tunes Longer Depot Release

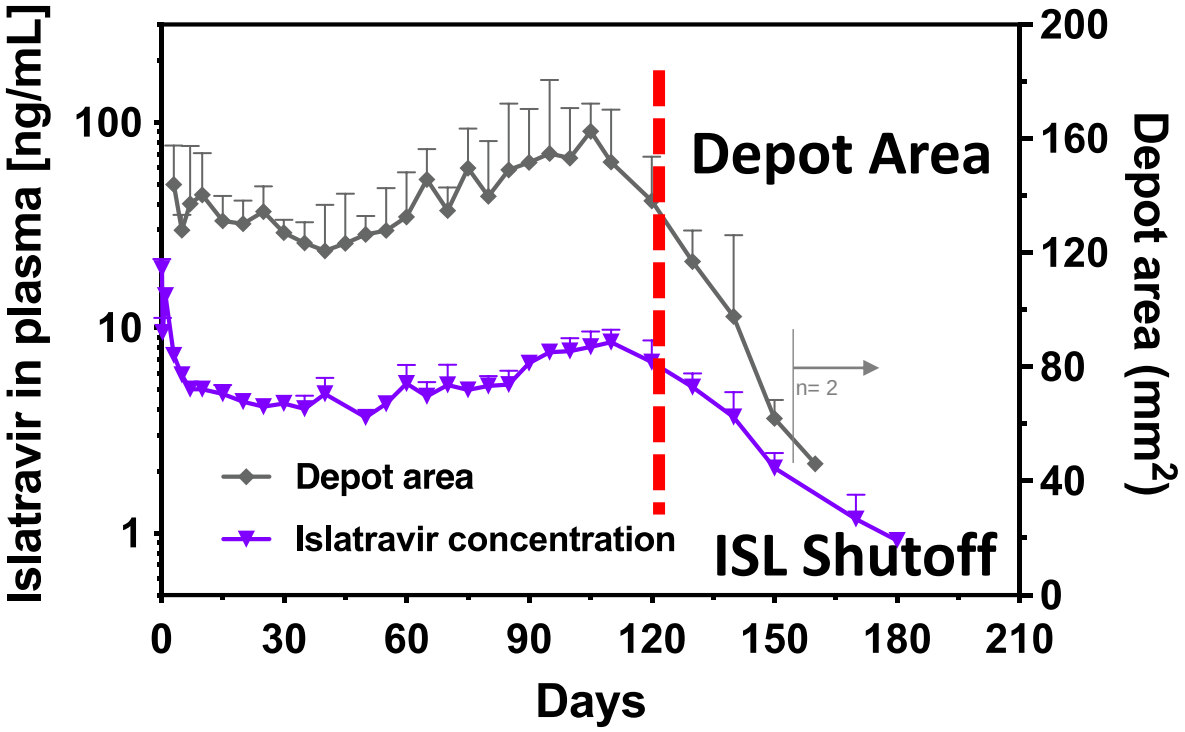
***Copolymer Composition Kept Constant While Mw Varies***



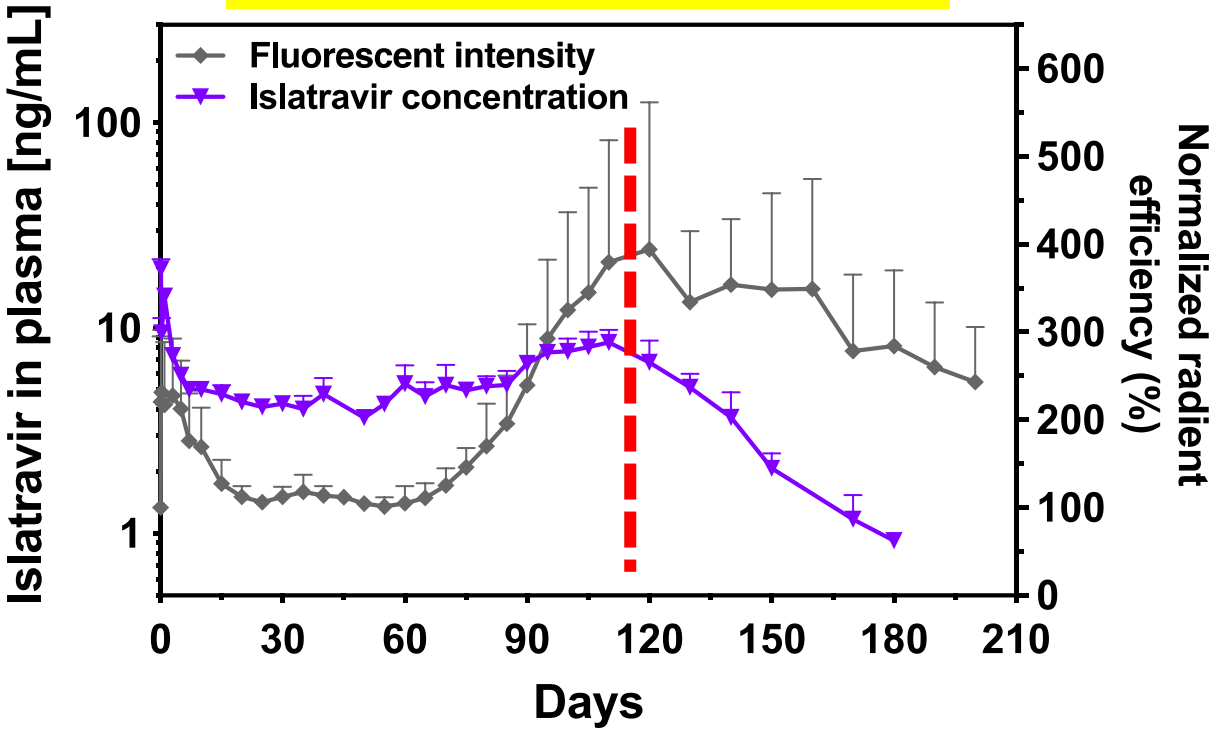





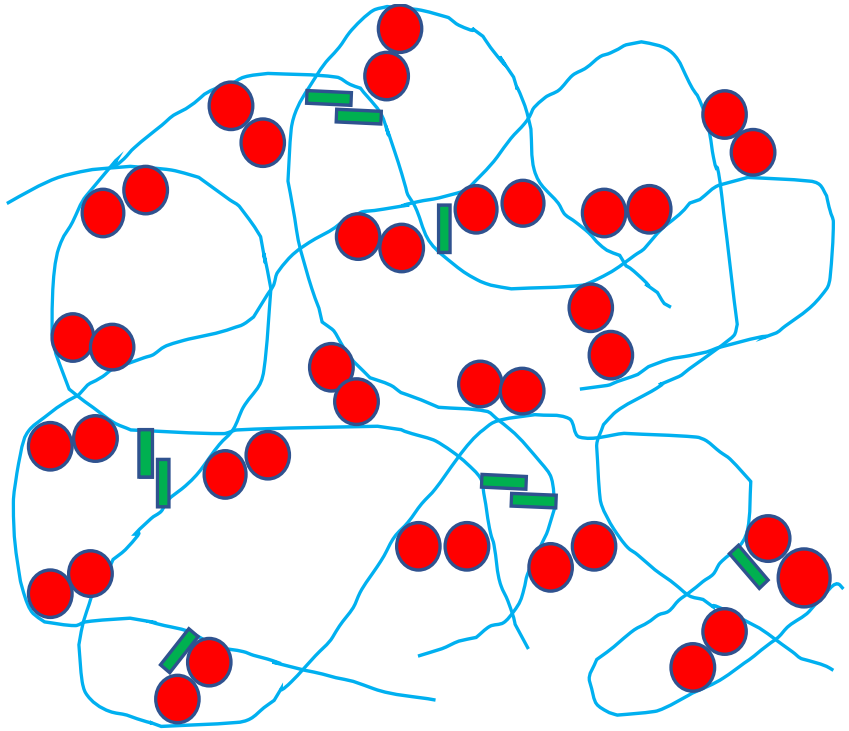

## Depot Shutoff Correlates with De-Gelling



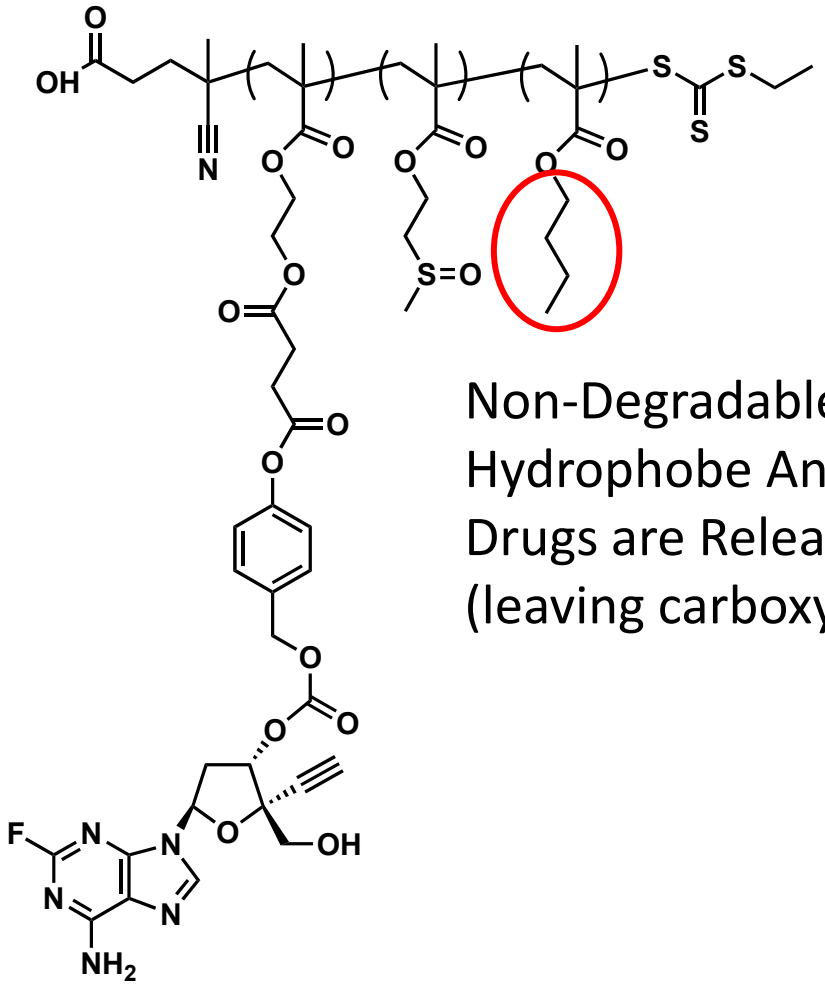

## IVIS Fluorescence Transition



# Knobs For Tuning Release Profiles

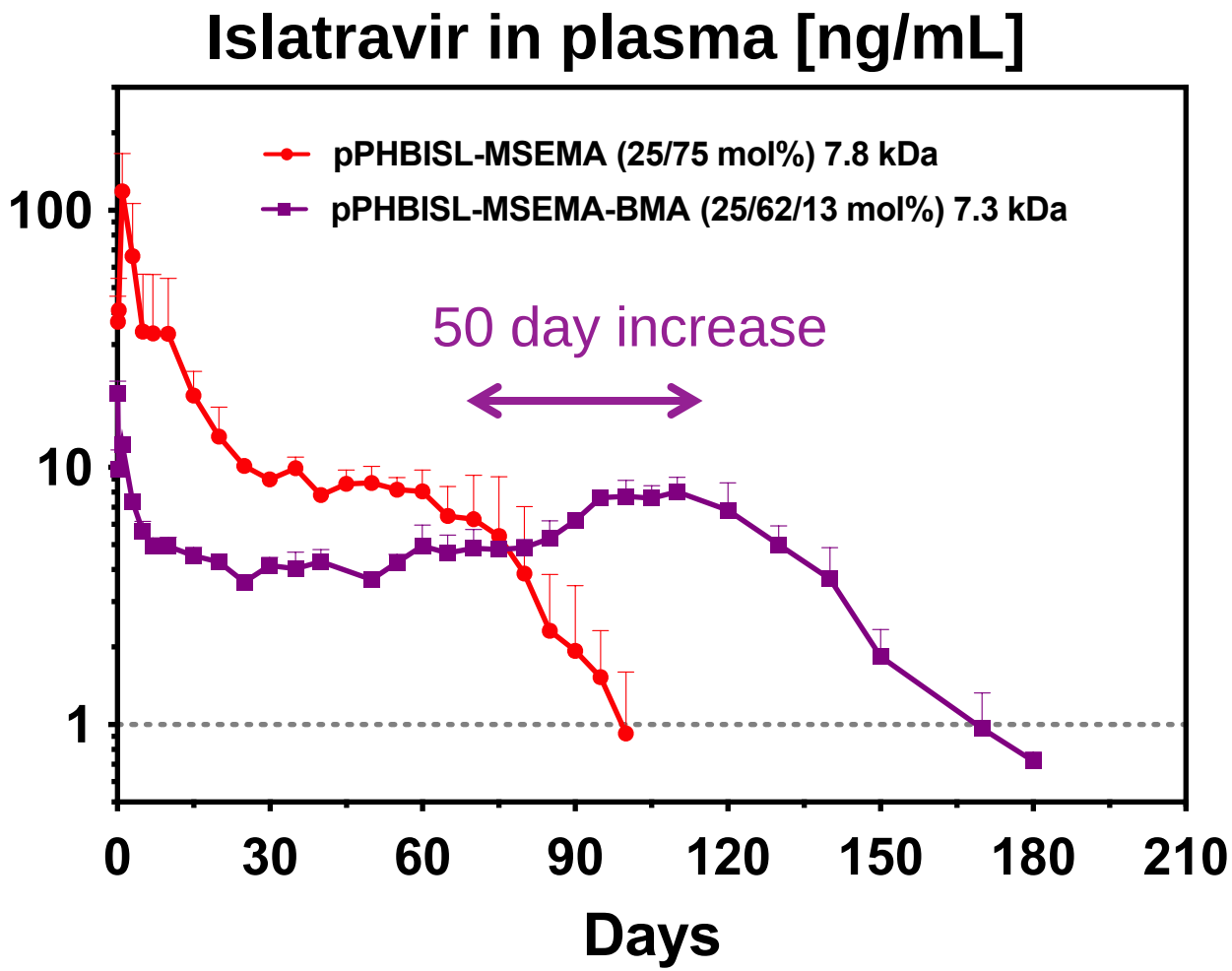


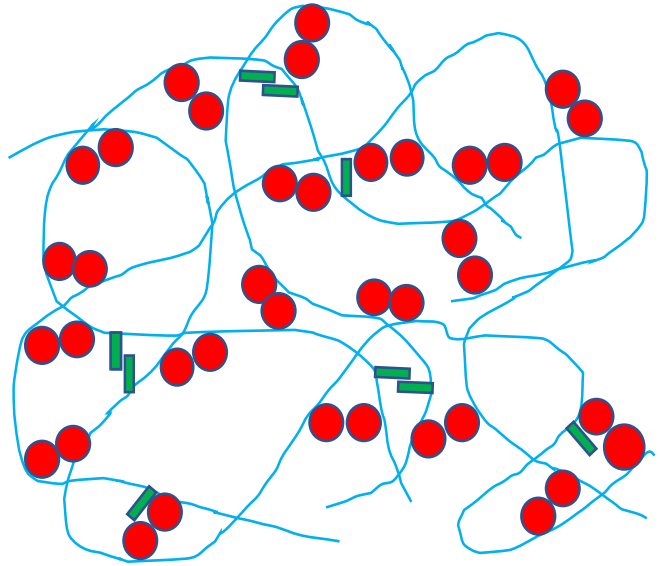
## Gel Anchoring Designs




Non-Degradable Butyl Hydrophobe Anchors Gel As Drugs are Released (leaving carboxylate group)

# Hydrophobic Anchoring Design Extends Release

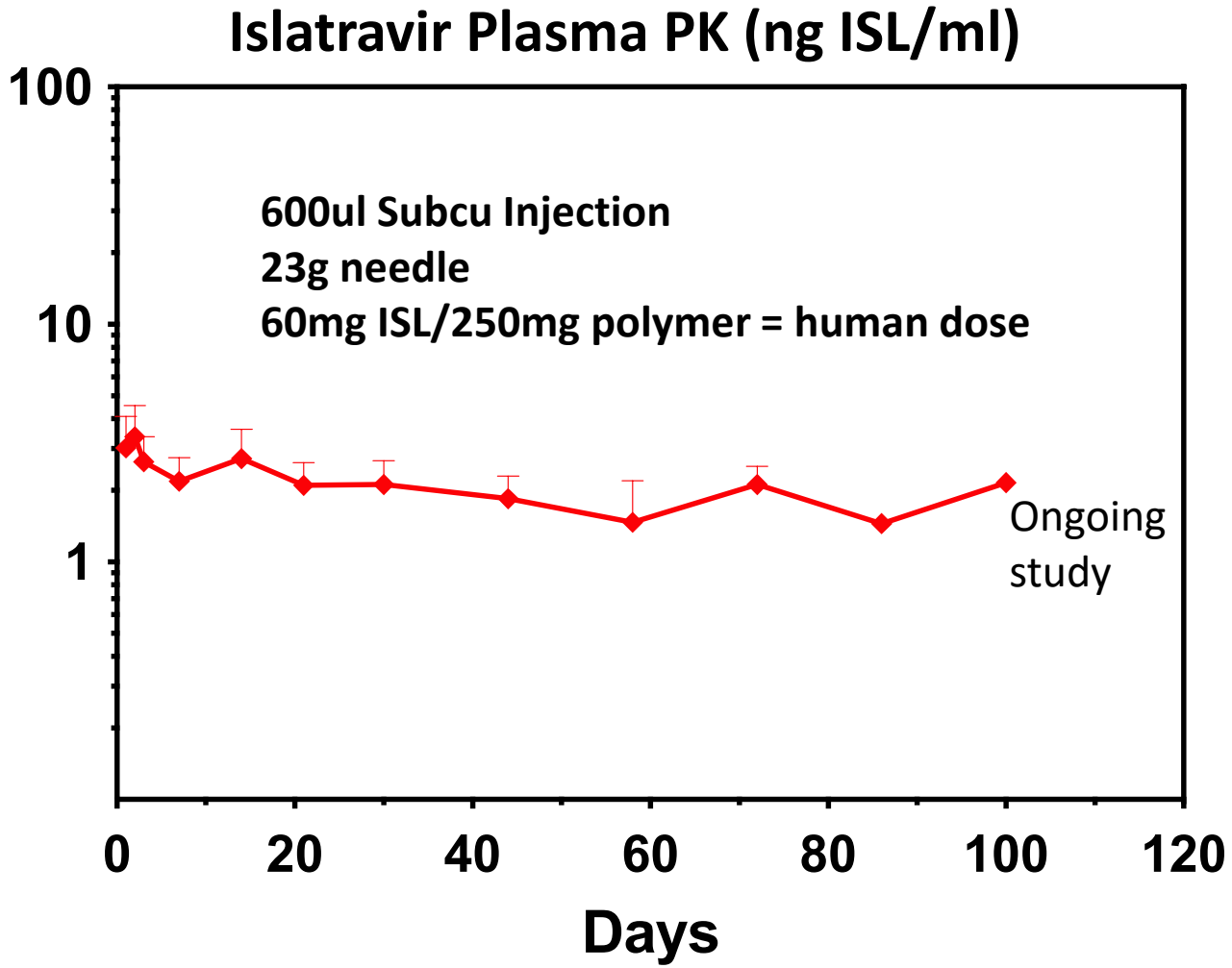


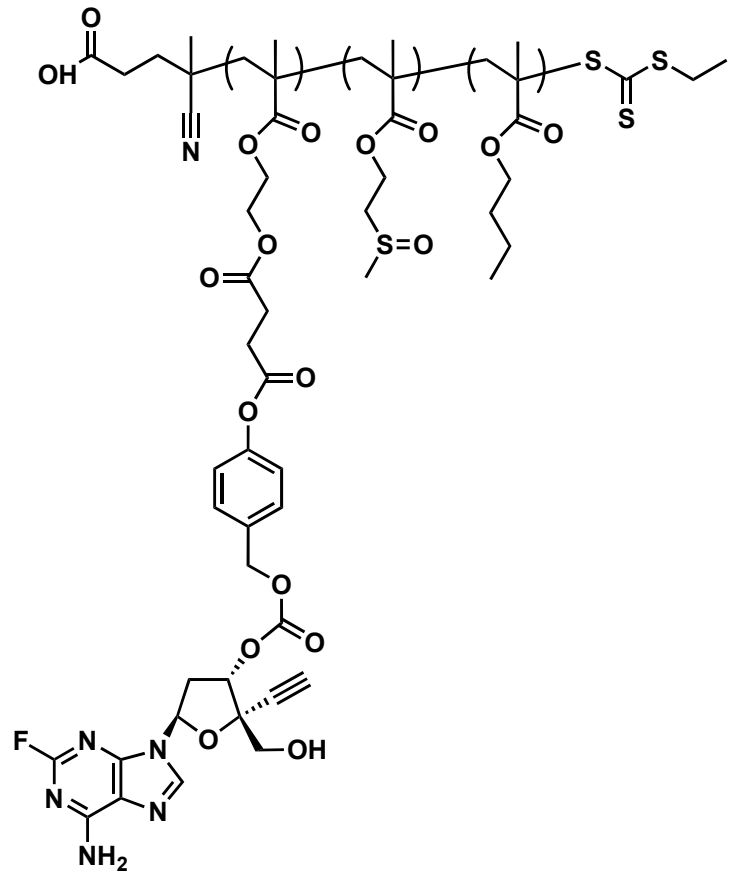



Decouple gel anchoring from drug release to slow de-gelling

Adding just 3 Butyl segment anchors increased working duration by ca. 50 days, eliminated early release

# Non-Human Primate Pilot PK Study



**p(PHBISL-*co*-MSEMA-*co*-BMA)**
**(DP 10/24/3, 12 kDa)**

SD= independent measurements of same
n=1 monkey plasma sample

# Manufacturability and Cost of Goods Sold Analysis Conclusions

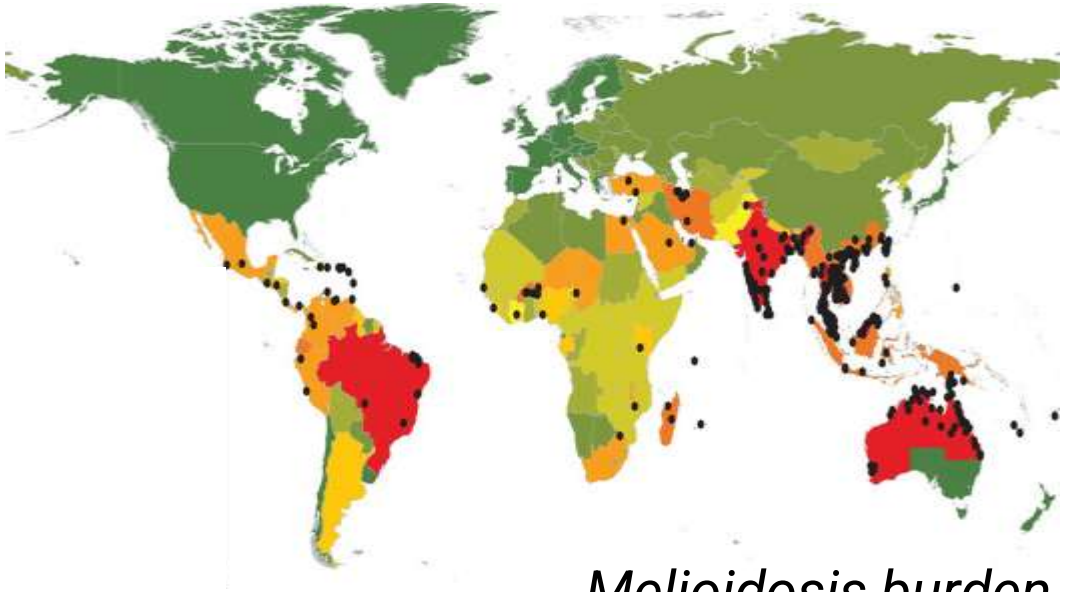
- The process was concluded to be commercially manufacturable up to 50 metric ton scale, with available raw materials, reasonable process safety and environmental issues, and existing synthetic reactor scale
- Recommended RAFT CTA & monomers non-GMP, then final polymeric prodrug synthesized with GMP practice
- Cost of Goods Sold (COGS) was estimated to be $0.74/1 g at the 50 metric ton scale for more complex malaria polymeric prodrug, $1.40 at 5MM, and $2.64 at 1MM scale
- For injectable drugs such as contraceptives that are potent at much lower doses, long-acting depots could require only ca. 100-200 mgs/dose and so it is likely < $0.10 dose equivalent at higher global scales
- Product could be lyophilized polymer that may not require cold-chain, or liquid vialed also may not require cold-chain

*George Tyson – UC Berkeley

# Pulmonary Bacterial Infections Remain an Important Unmet Need & Neglected Health Equity Issue

## Global Pathogens

- *Mycobacterium, tuberculosis*
- *Anthrax*
- *Burkholderia, melioidosis*
- *Francisella, tularemia*



*Melioidosis burden*
*Nature Microbiology* **1**,
Article number: 15008 (2016)

## Morbidity

- Leading global killers:

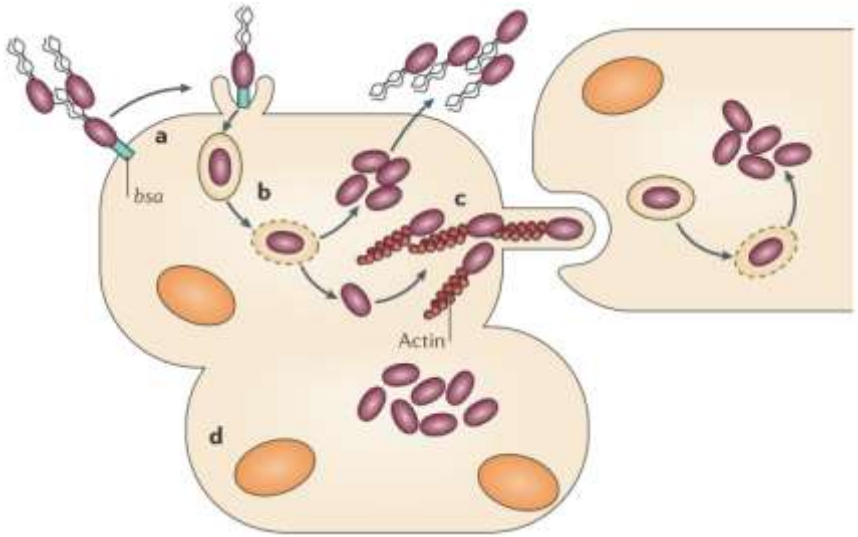
***TB 1.7 billion infected/yr and 1.8 million deaths***

***Melioidosis 430K cases/250 K deaths***

- Weaponized Tier 1 biological agents
- W.H.O predicts mass airborne release in a city of 5 mil. would produce *250,000* cases with *3,500* case-fatalities

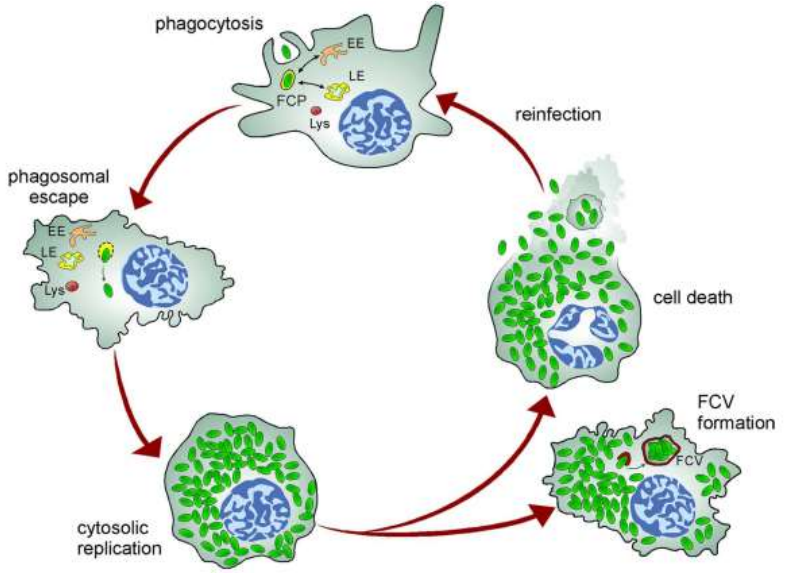
# Tier 1 Biodefense Agents & Global Health Threats: Tularemia and Melioidosis

- CDC Tier 1 select agents presenting greatest risk of deliberate misuse and most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence.
- The intracellular compartmentalization of these pathogenic organisms in alveolar macrophages is a significant barrier to bacterial clearance




*Francisella tularensis*

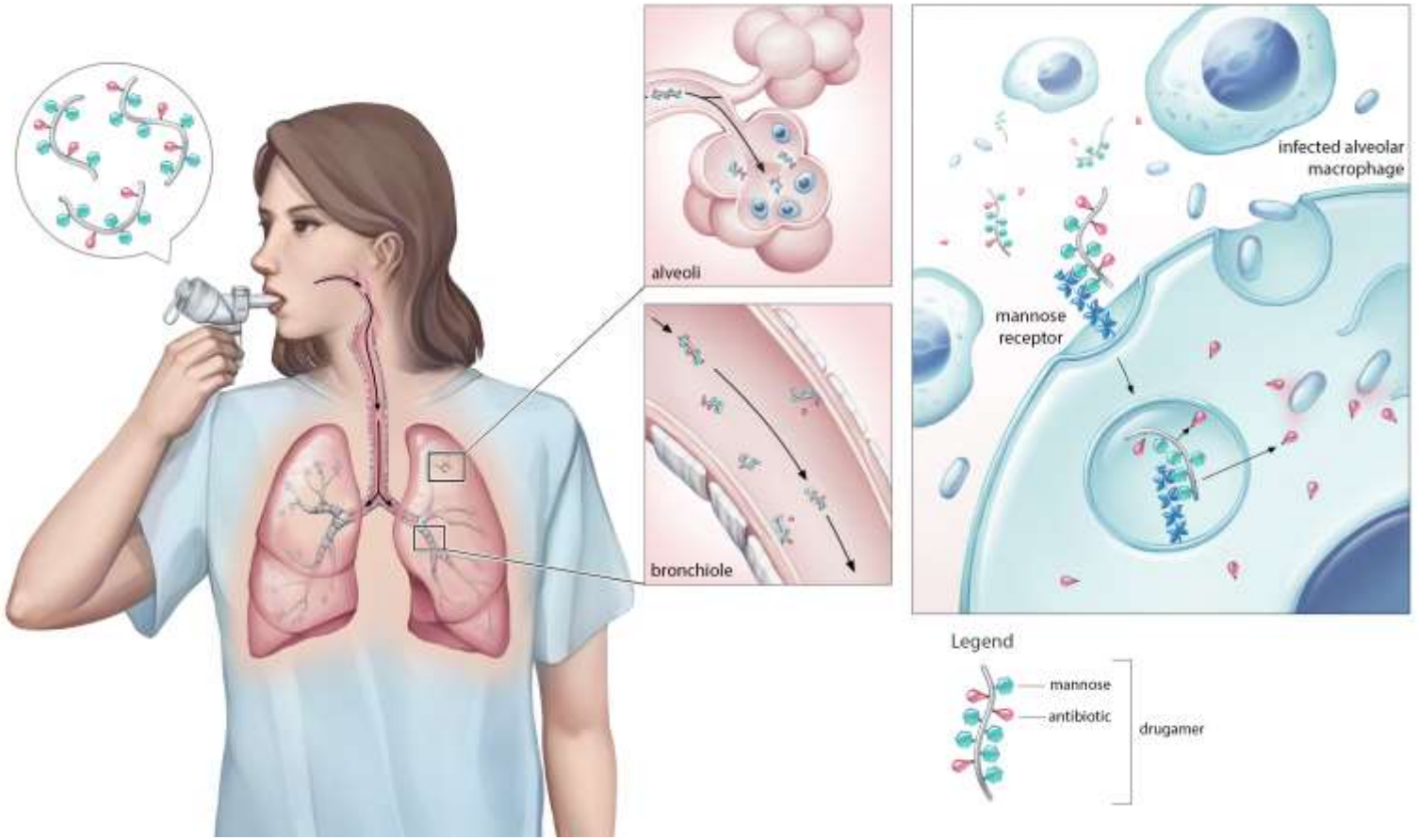
*Nat Rev Microbiol. 4(2006):272-82.*



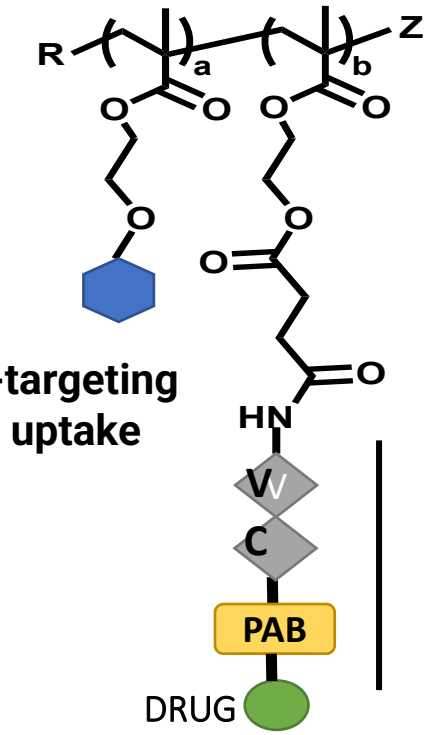

*Burkholderia pseudomallei*

*Microbiol. 1(2010):1-12.*

# Polymeric Prodrugs Exploiting The Lung Macrophage to Extend and Focus Drug PK




**Pulmonary Infection Therapy**
**Melioidoisis, TB, Covid**




**Mannose-targeting optimizes uptake**

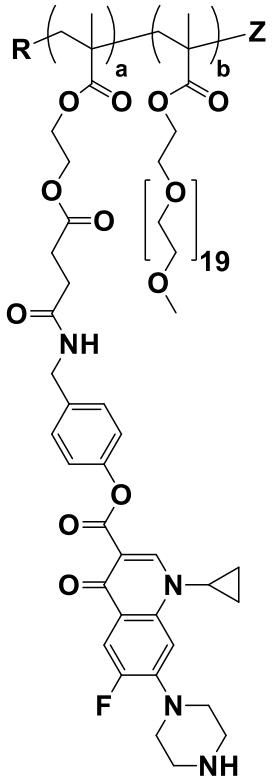
ADC linker increases extracellular stability & rapid drug release inside macrophages

J Control Release. 2021 330:284-292
J Control Release. 2018 287:1-11

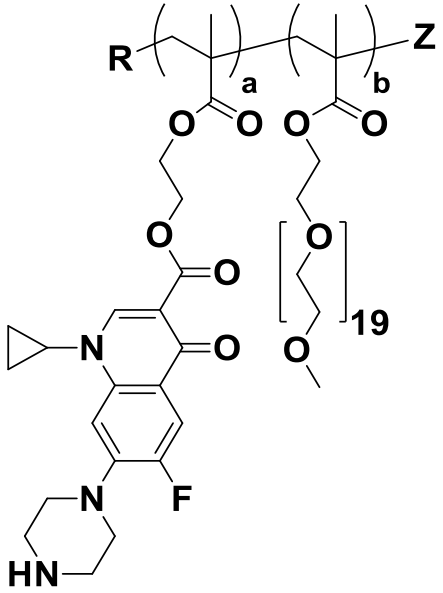
# Macromolecular prodrugs with controlled release kinetics

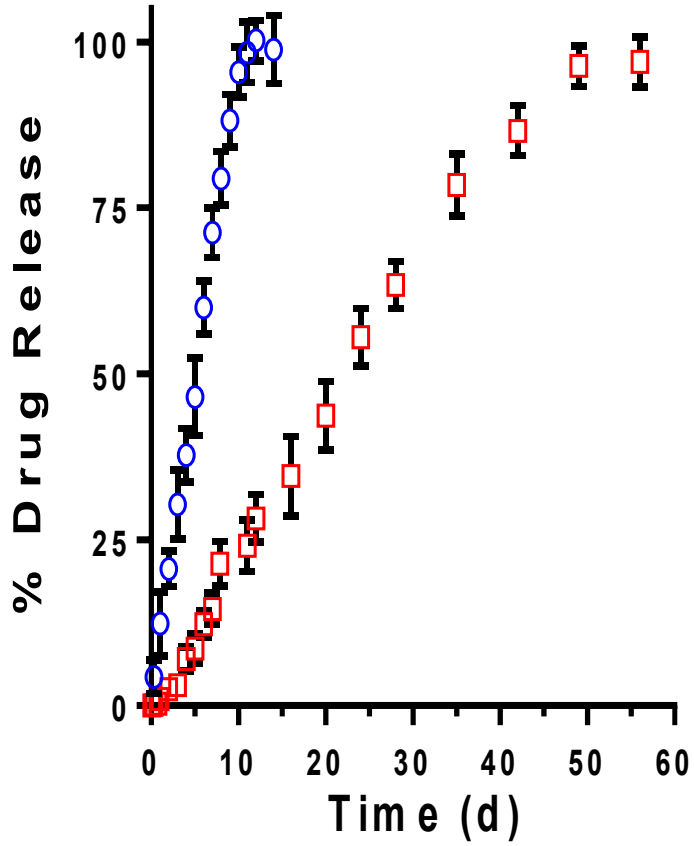
*Drugamers engineered with tunable timeframes matching disease biology settings*

**Faster Linker**




**Slower Linker**




**Drug release is zero order without burst release**

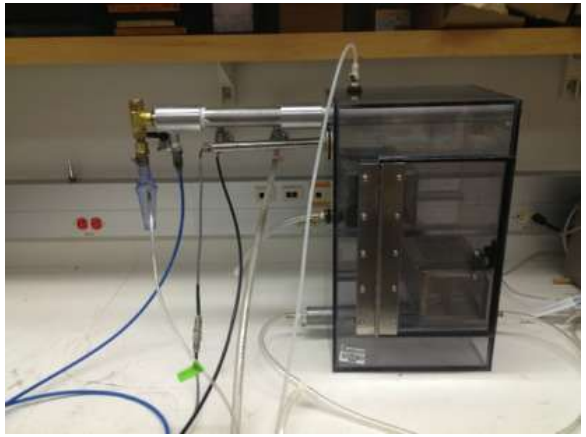
Das et al., *Polymer Chemistry* 7(4) (2016) 826-837

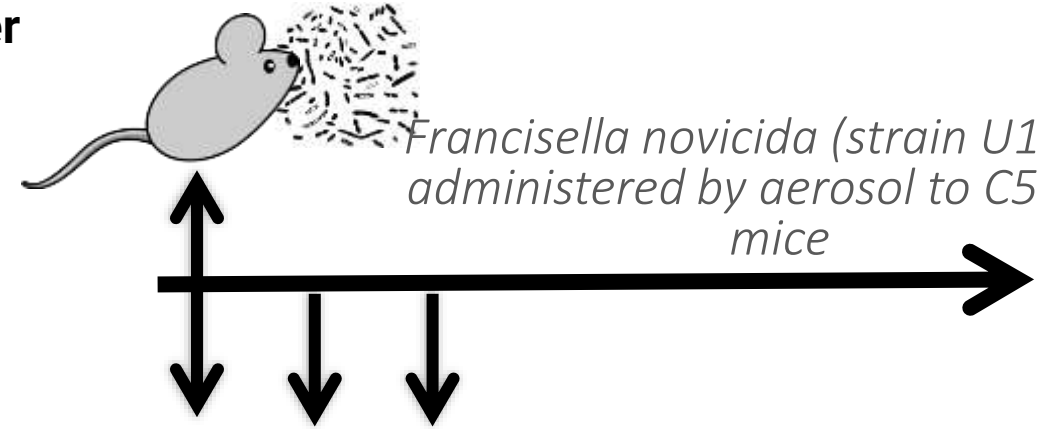
# Therapeutic Cipro Activity in Tularemia model

**Prof. Shawn Skerrett, MD**
Pulmonary and Critical Care Medicine
University of Washington

**Prof. T. Eoin West, MD, MPH, FCCP**
Pulmonary and Critical Care Medicine
Adjunct Assistant Professor of Global Health
University of Washington

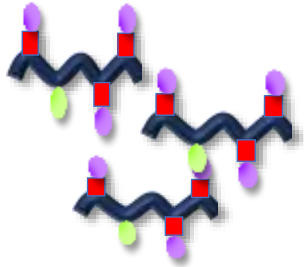
**Aerosolized Infection Chamber**





*Francisella novicida (strain U112) is administered by aerosol to C57Bl/6 mice*

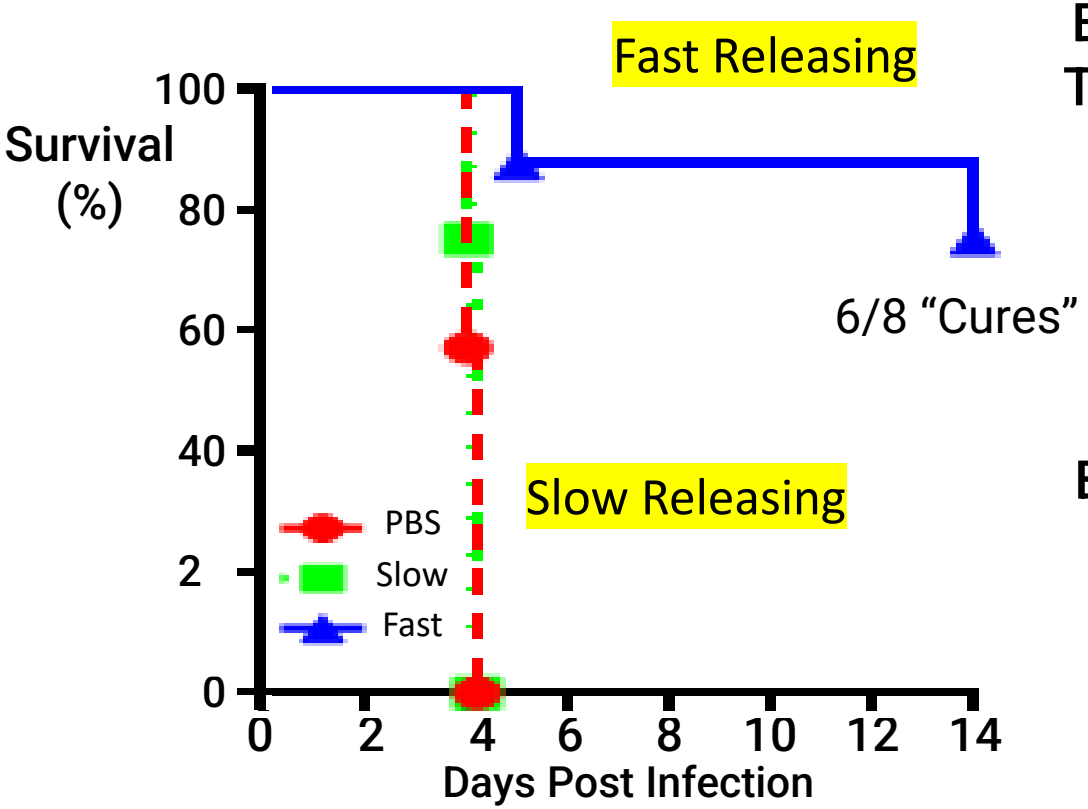
Intra-Tracheal Sprayer

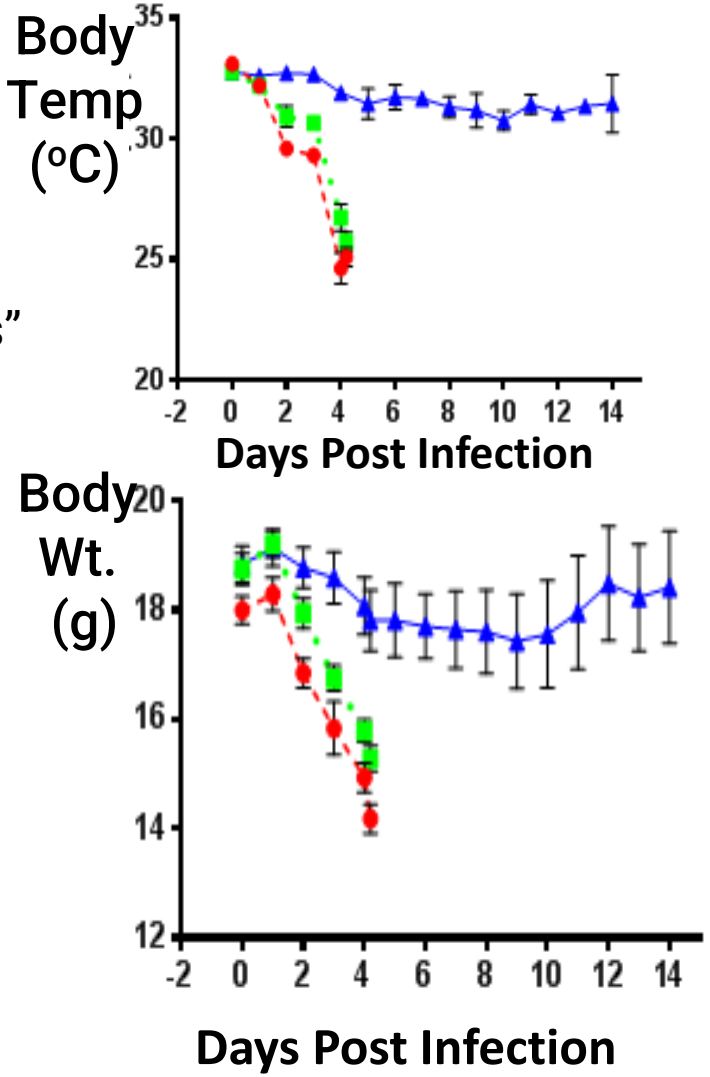


Polymer prodrug

40mg/kg ciprofloxacin

# Drugamers Active in Highly Lethal *Francisella* Model

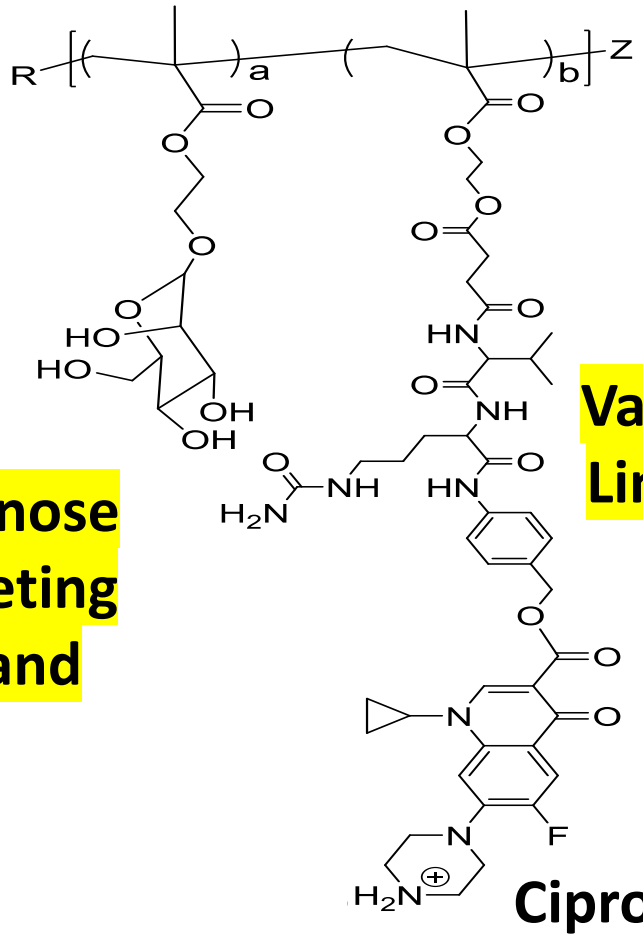



- *Francisella novicida* aerosolized at day 0
- Cipro dosed day 0, +1, +2 at 40 mg/kg
- N=8 mice for each cohort

**D. Das et al., 2017 *ACS Mol. Pharm.* 14:1988**

# CD206/Mannose Targeting and Enzyme Cleavable Linker Design

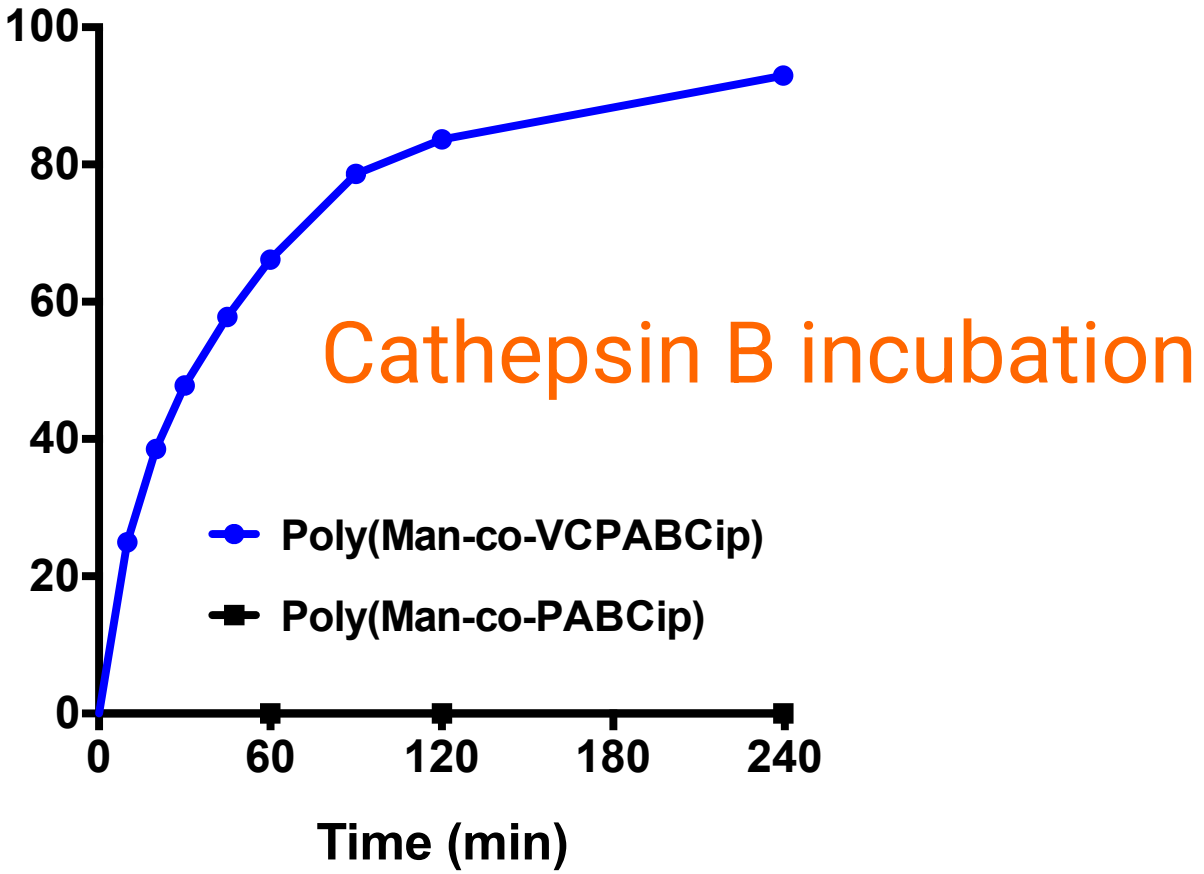



**Mannose Targeting Ligand**

**Val-Cit Linker**

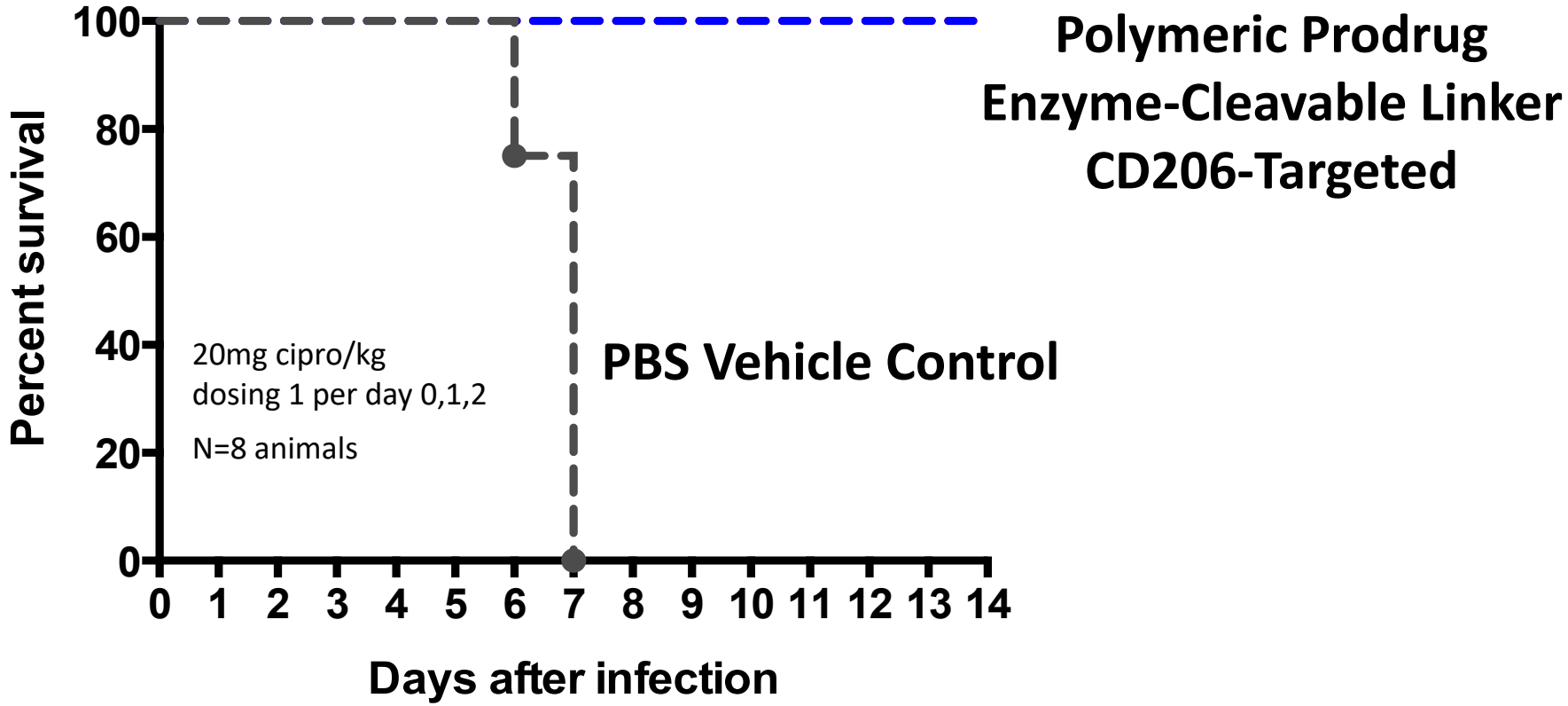
**Ciprofloxacin Drug**

**Rapid release in alveolar macs provides good Cmax/MIC**



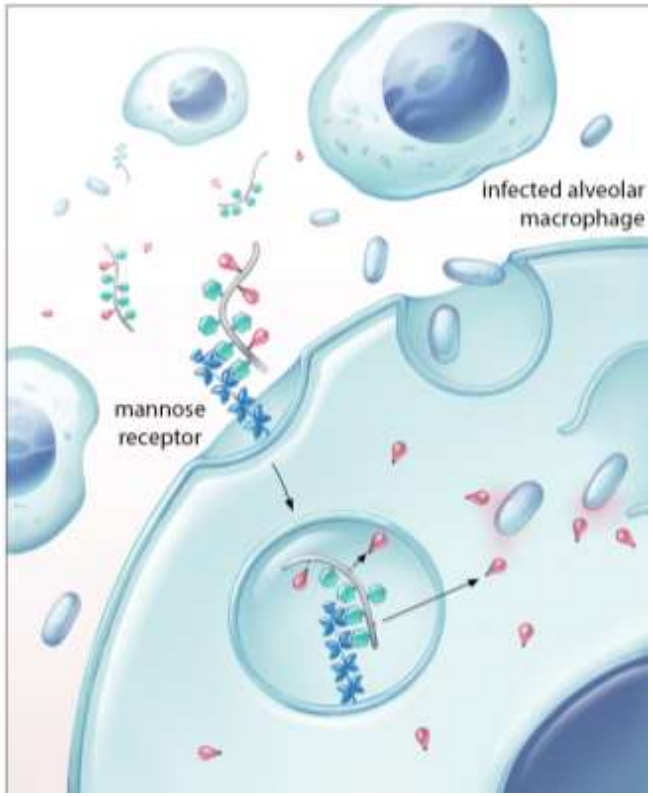
# Full Tularemia Survival Achieved

## Aerosolized *F.t. novicida*

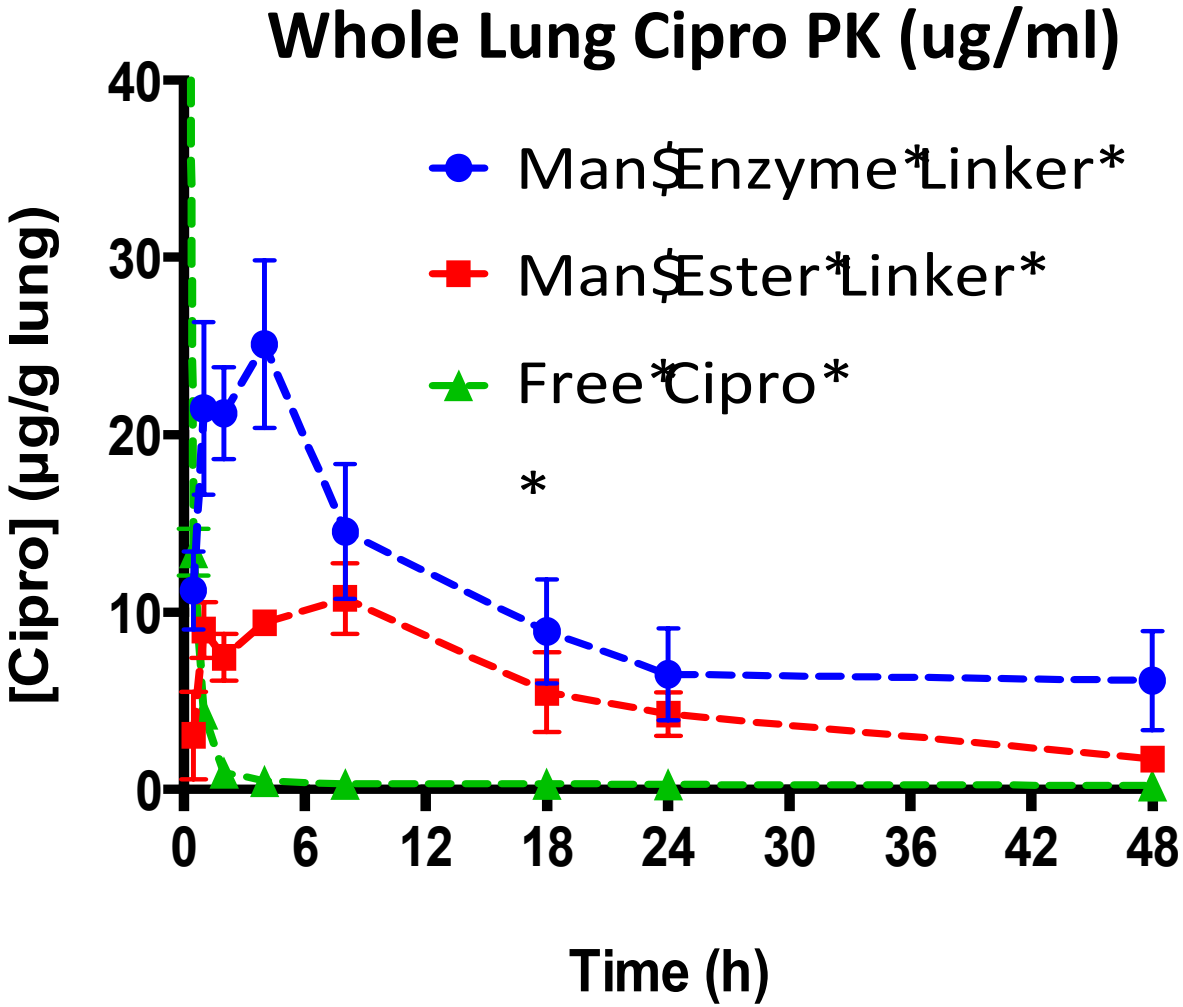



***Extended duration dosing + mac targeting = high efficacy***

# The macrophage can be used as a drug reservoir to extend lung PK

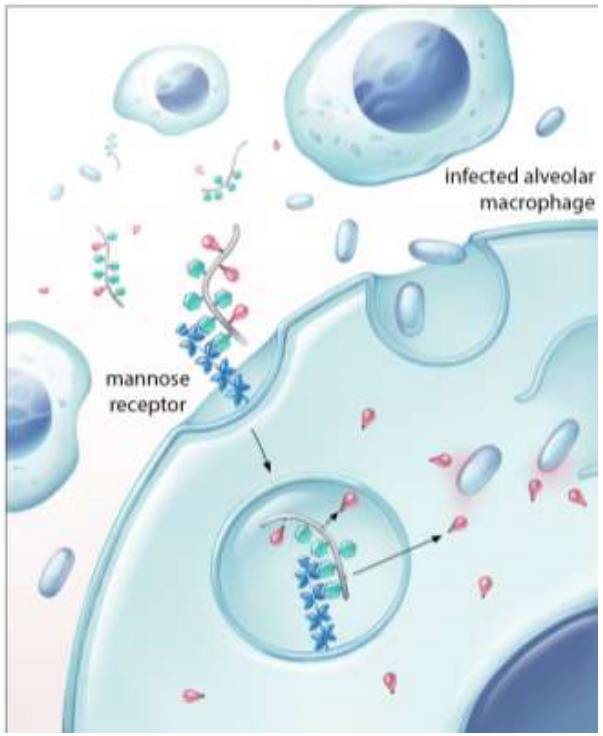



**Drugs Released in Lung Macs Can Disperse Back Out in to Whole Lung**

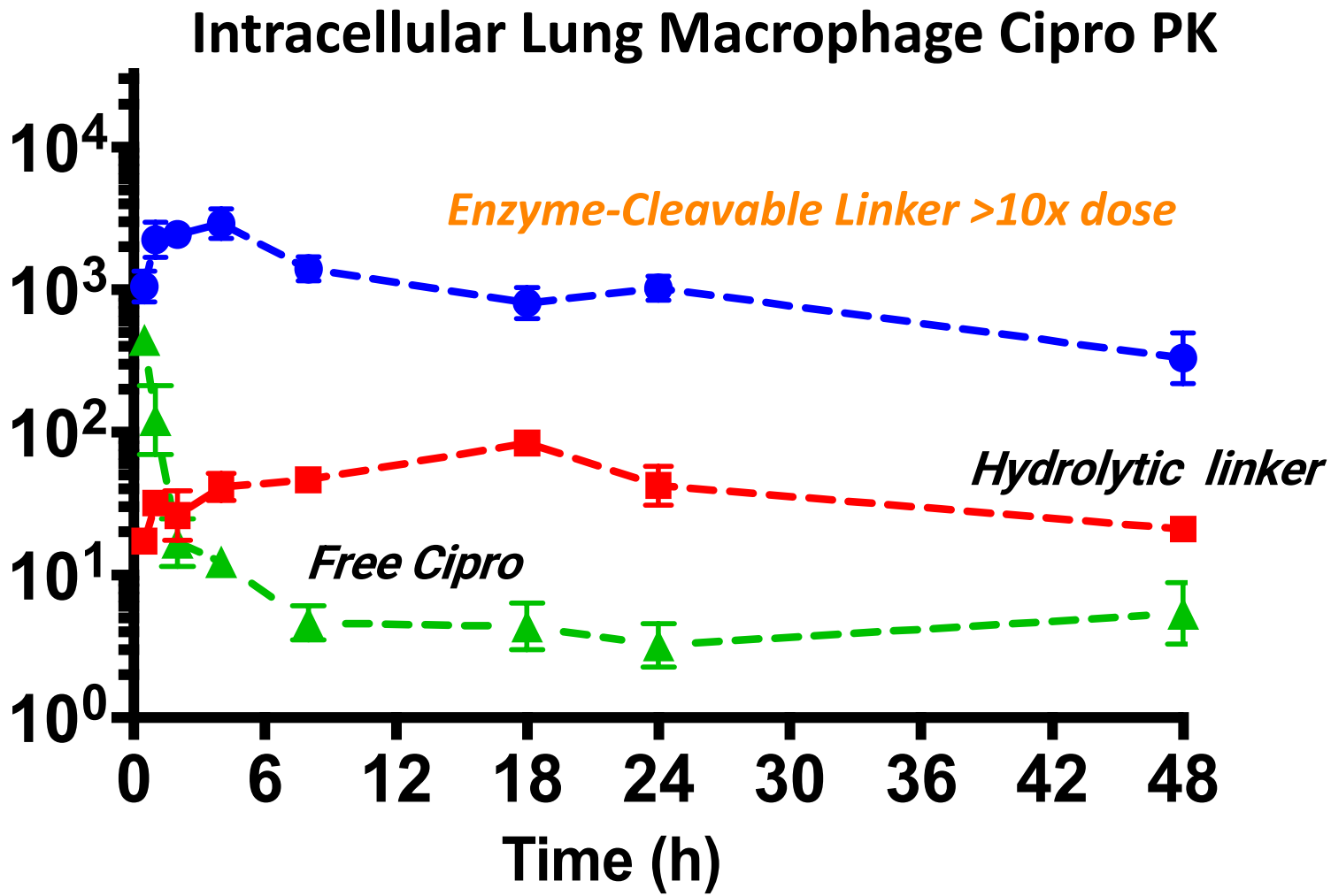



Su et al., J Control Release. (2018) 287:1-11
Chavas et al., J Control Release. (2021) 330:284-292

# Using the macrophage compartment to target drug & extend PK

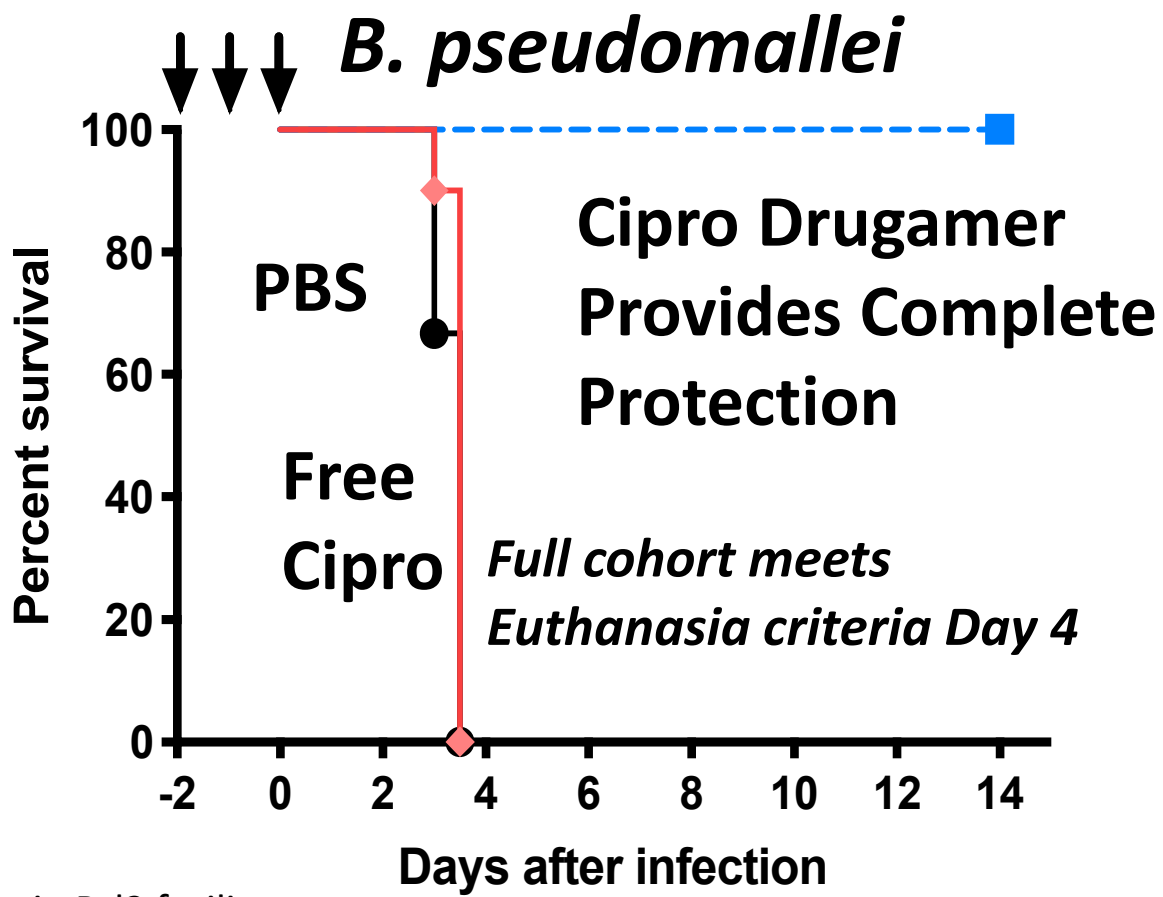



Polymer dosed inhalation
Lung lavaged/ Macs collected




Su et al., J Control Release. (2018) 287:1-11
Chavas et al., J Control Release. (2021) 330:284-292

# Extended PK of Drugamer Provides Prophylactic Protection in ABSL/3 Human Pathogen *Burkholderia pseudomallei* Model



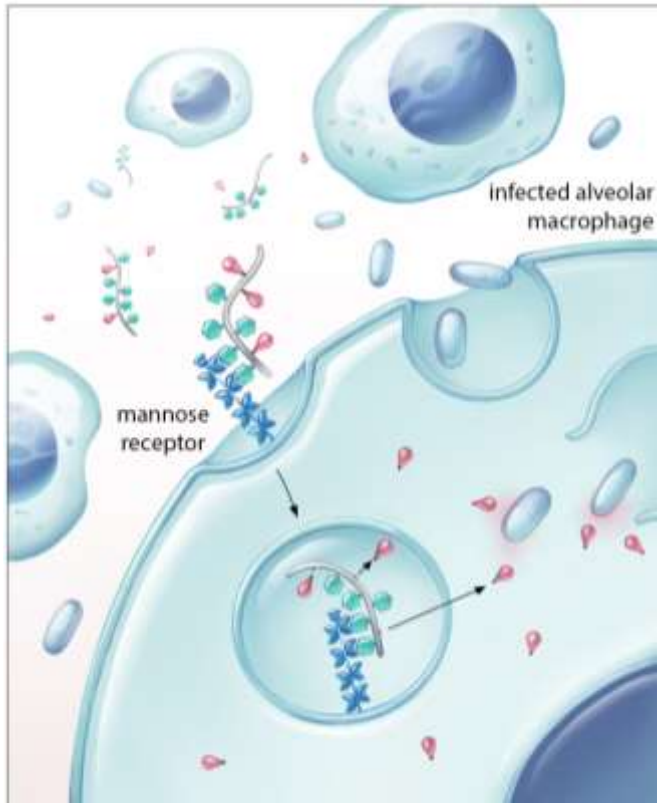

- N=10 mice in Bsl3 facility
- 20 mpk dose once per day for 3 days at day -2, -1, and 2h prior to Bp110 aerosol administration

*B.p.* > 100,000cases/yr
SouthEast Asia especially

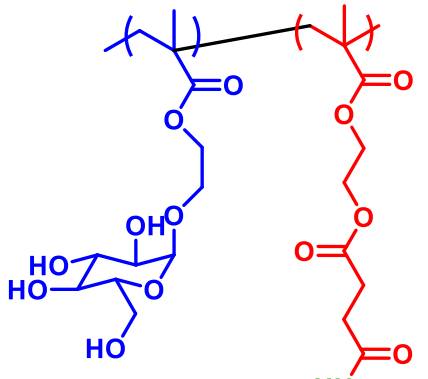
**Portable Pulmonary Therapeutics Covid-19 Modality (e.g. remdesivir)**

J Control Release. 2021 330:284-292

# Immune Therapy For Antibacterial and Antiviral Pulmonary Therapy

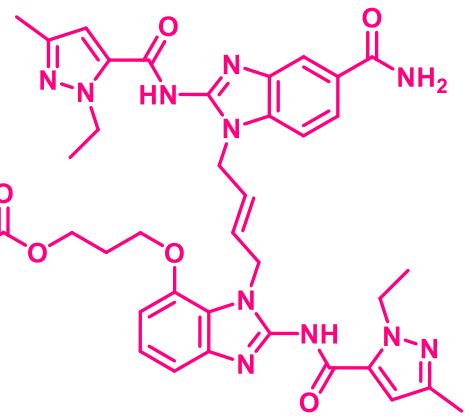



**Polymer dosed by inhalation**
**Activate Lung Macrophage**
**Against bacteria + virus**

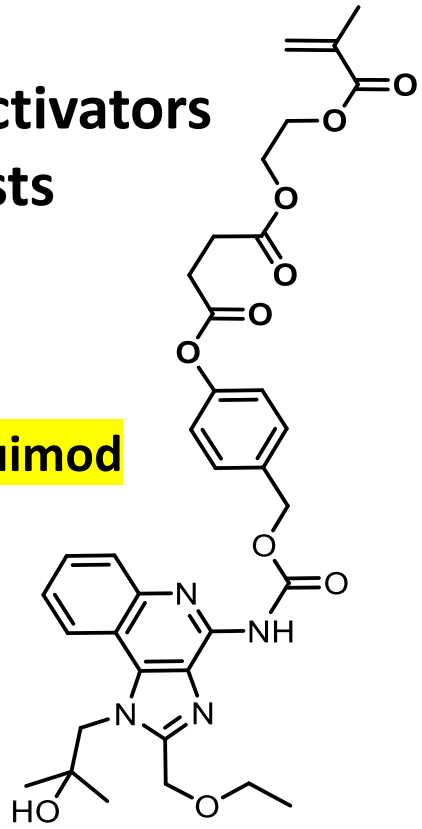


## CD206-Targeted Immune Activators
## STING and TLR Agonists

**DiABzi STING**



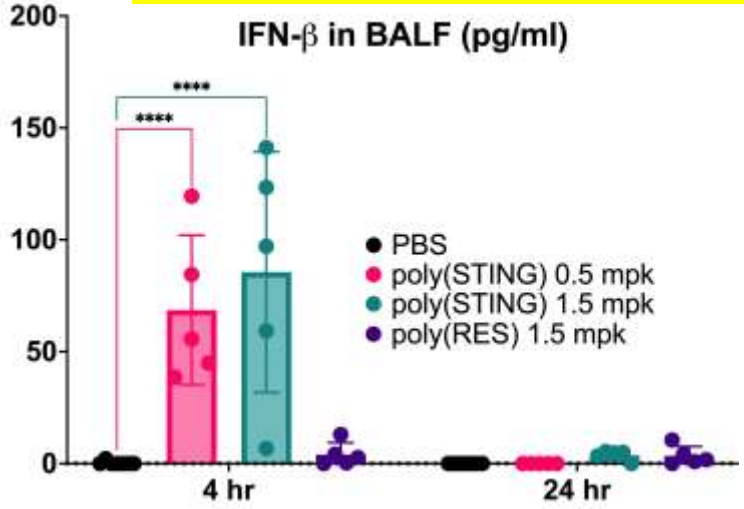
**Resiquimod**

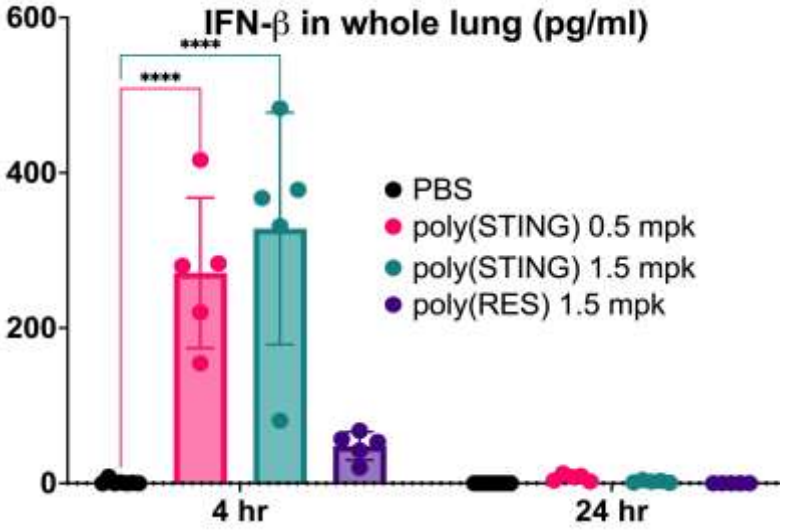


**Designed for sophisticated drug yet global health cost constraints**
**Fully Synthetic Manufacturing Gives Low COGS, ca. <$2/dose**

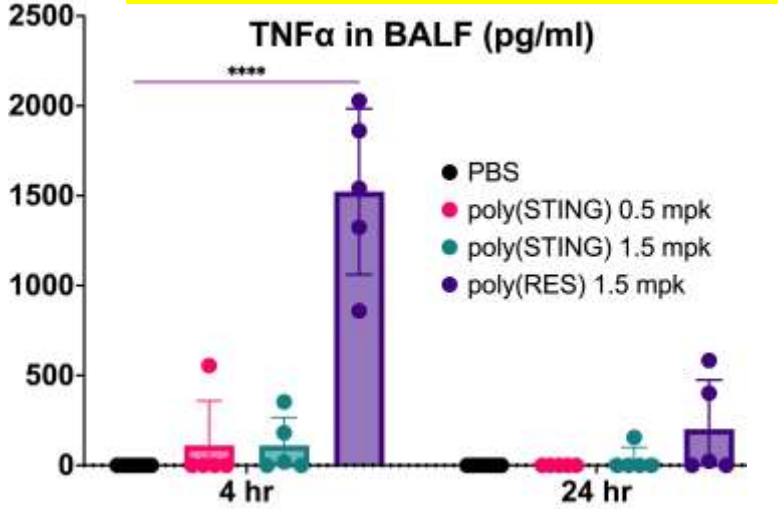
# Macrophage-Targeted Polymeric Prodrugs of STING and TLR Agonists Show High Immune-Signaling Specificity

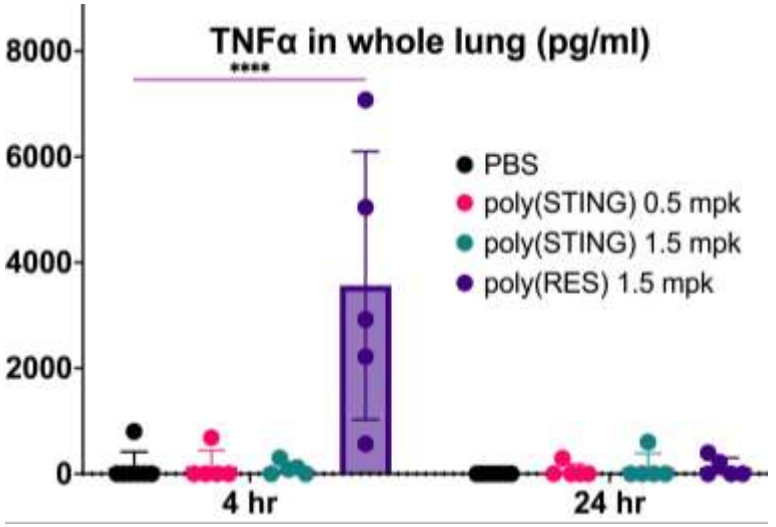
## STING Agonist Signature



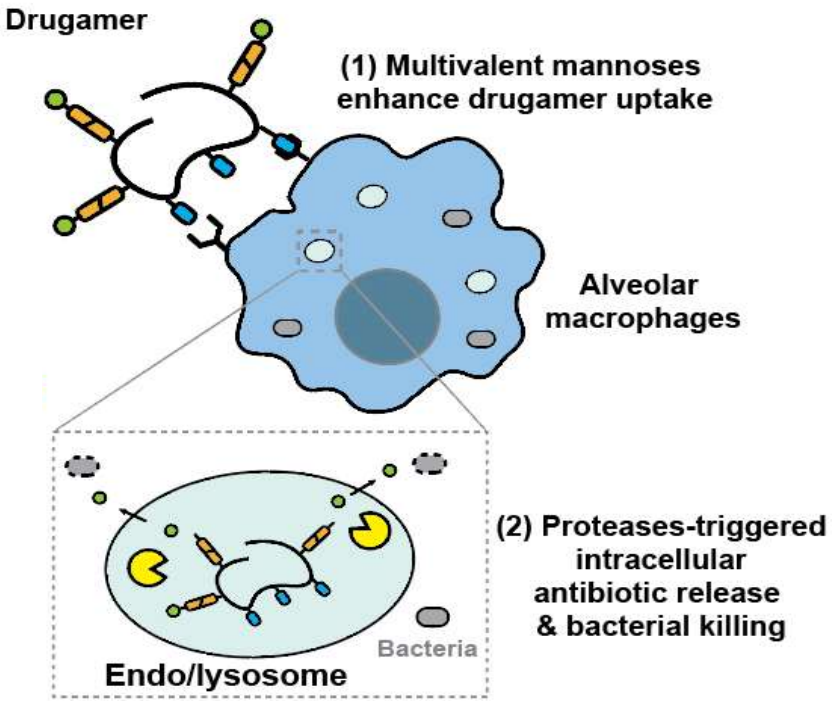

## TLR7/8 Agonist Signature



# polySTING Strongly Activates Host Macrophage Response

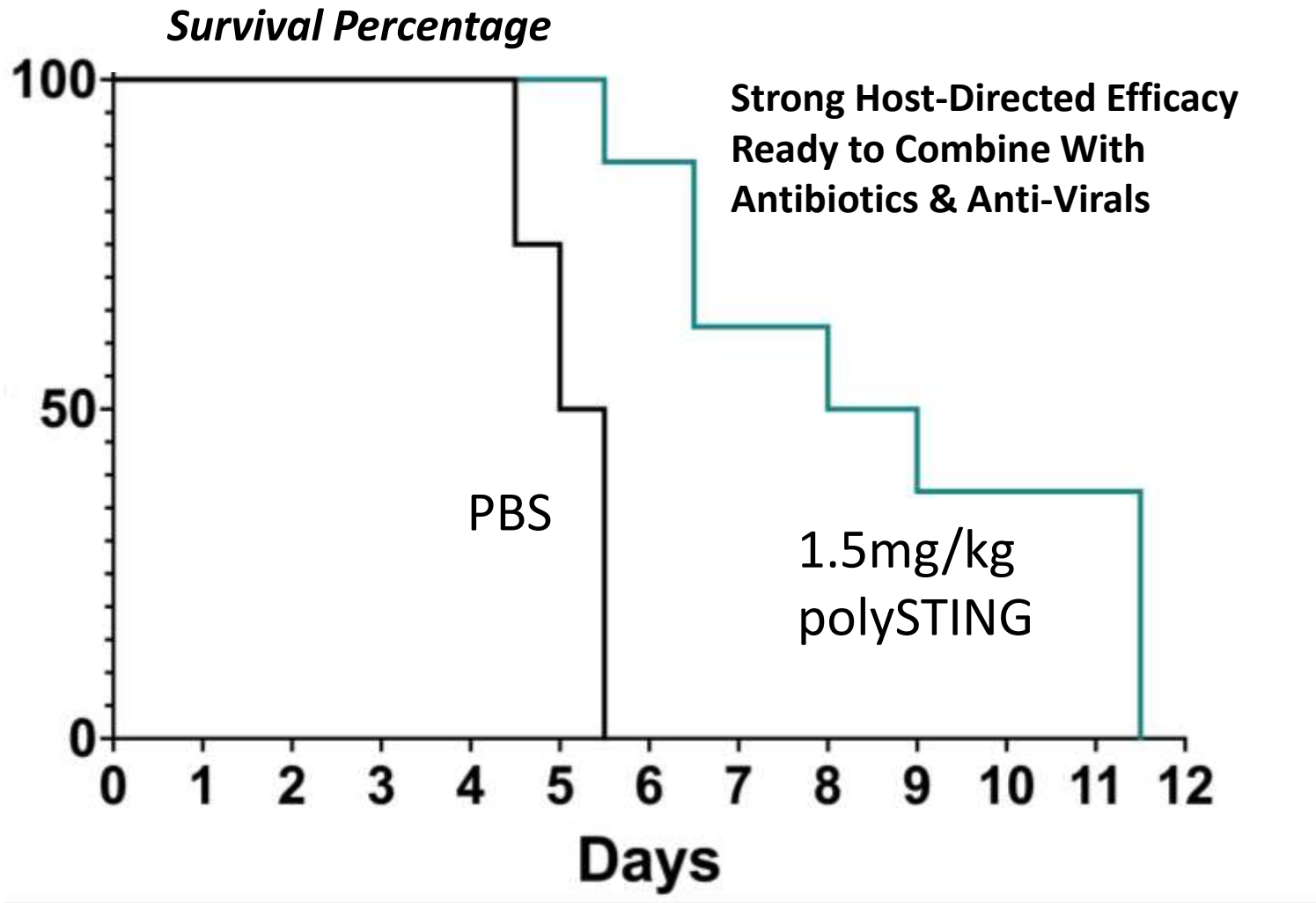
***Francisella novicida* down-regulates our macrophage immune response**




Sarah Snyder

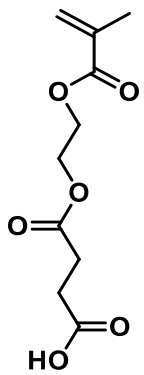
Debashish Roy

Simba Jokonya



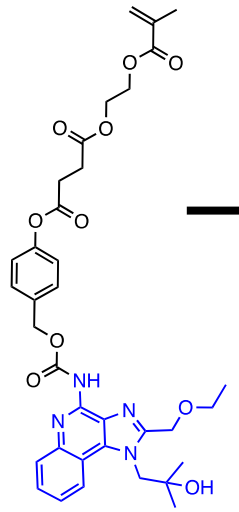

**Dosing at -4h, +24h, +48h**

# “Caging” Drugamers For Immune Vaccines



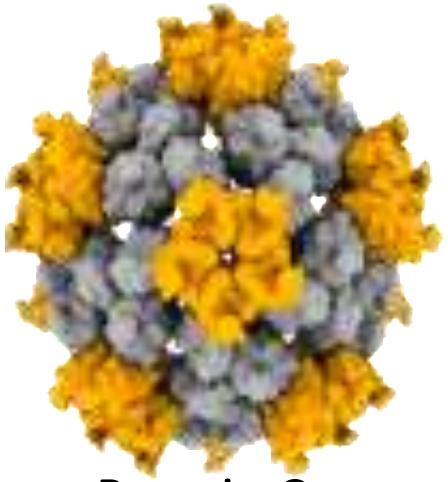
SMA

Negative Charge in
Arms of Polymer

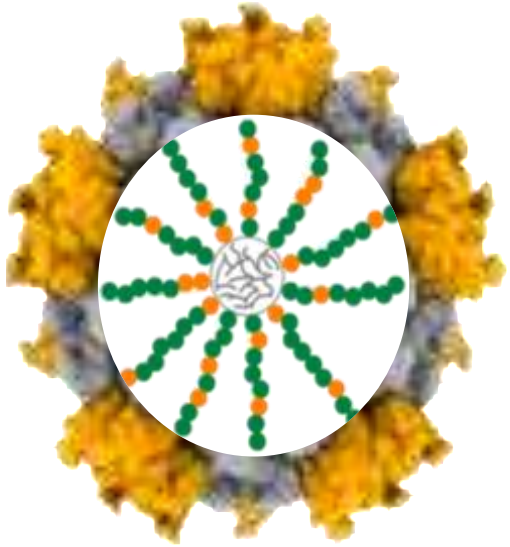


Resiquimod TLR 7/8Prodrug
Adjuvant Synthesized into Arms

Radiant Star
Nanoparticles
Negative charge



Protein Cages
Positive Charge Protein Cage
& Antigen Displayed



Encapsulated
Radiant Star in Cage

Concept: Encapsulate pro-adjuvants with controlled release from computationally designed protein cages for vaccines

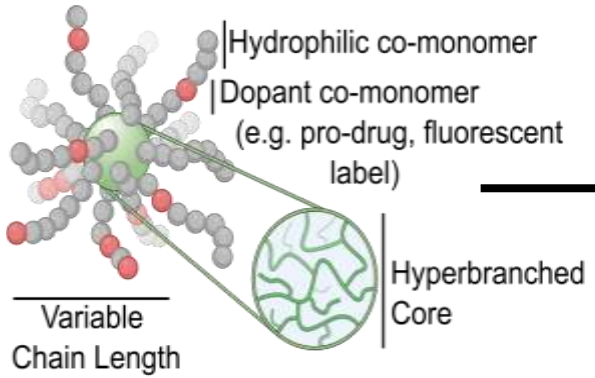
The 3D shape and exact size had to be engineered just right to assemble and stay in cage: DP<50 best

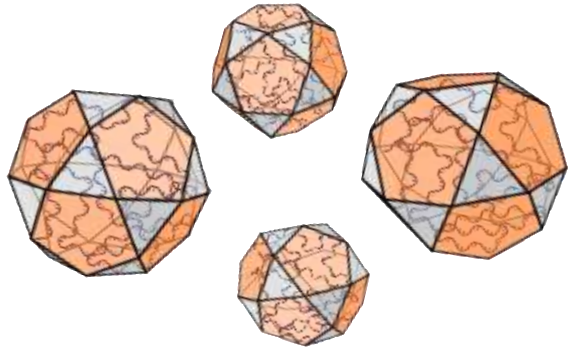
Computationally Designed Protein Cages
With Neil King & David Baker

King, N. P. et al. Science 336, 1171–1174 (2012)
King, N. P. et al. Nature 510, 103–108 (2014)

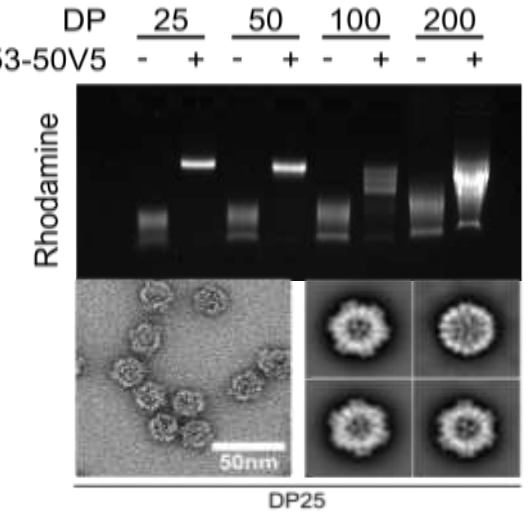
# Caged Resiquimod Drugamer Gives 20-fold Better Immune Response with Lowered Toxicity Compared Free Resiquimod

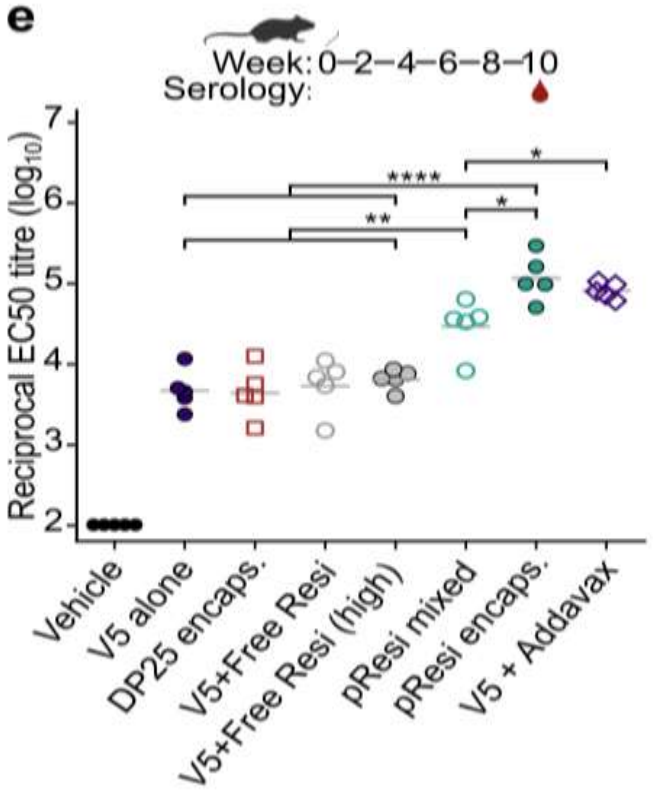
*Herpoldt et al.*
*Adv. Healthcare Mat.*
*2024, in press*

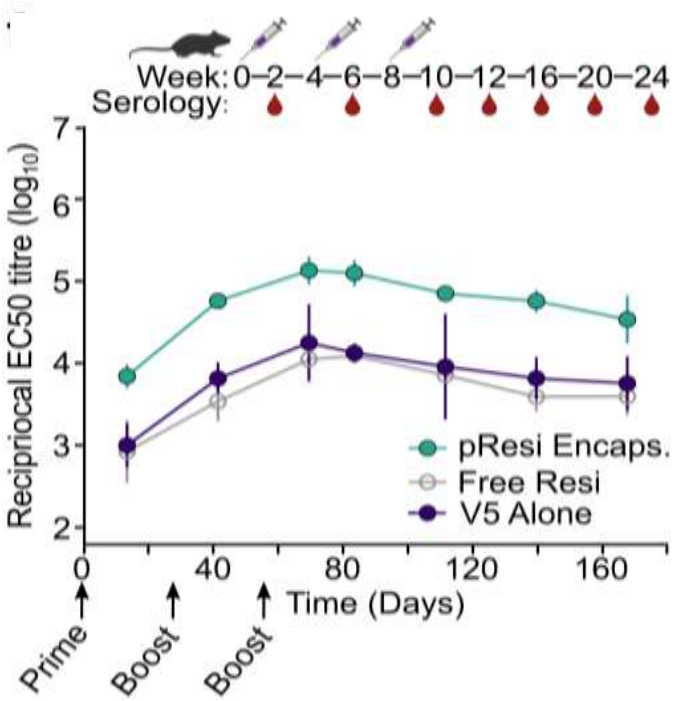


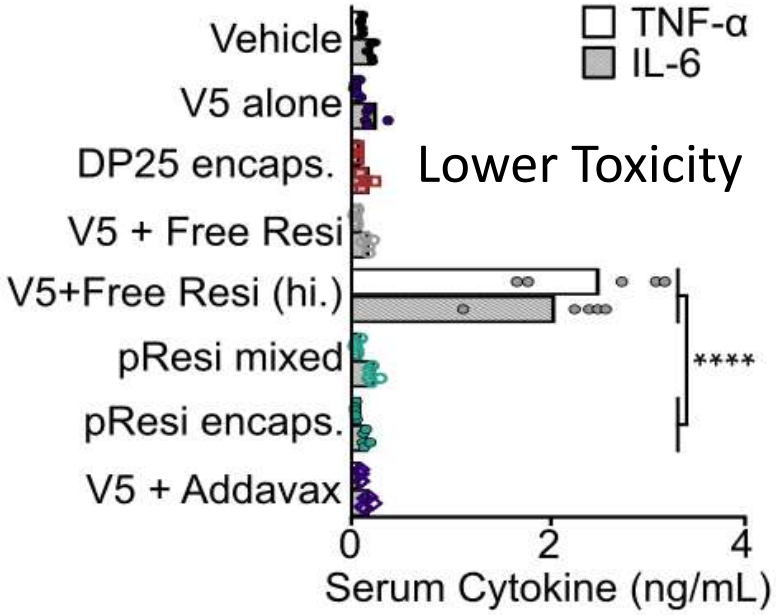



Single Polymer Nanoparticle Encapsulated in Protein Cage



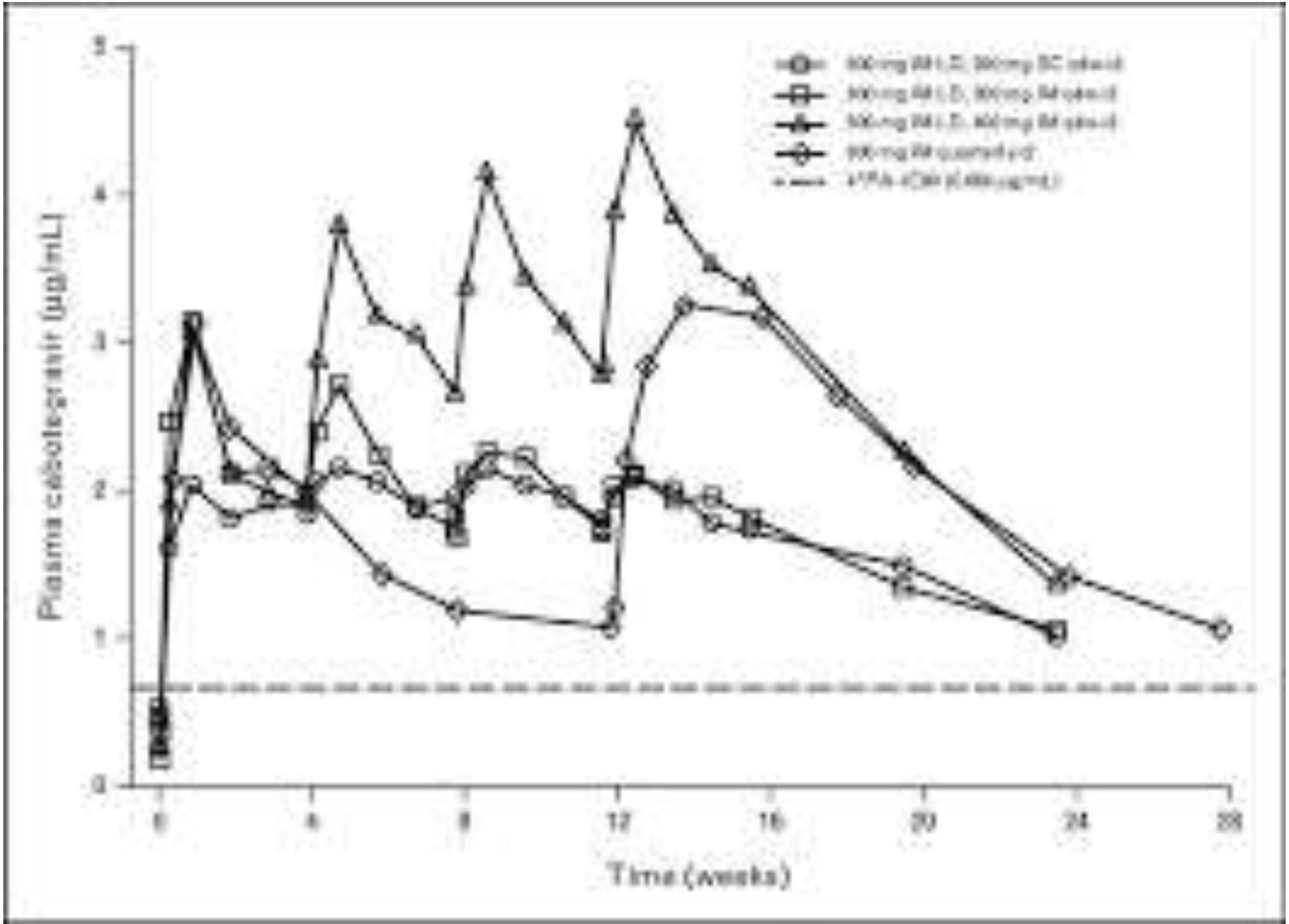



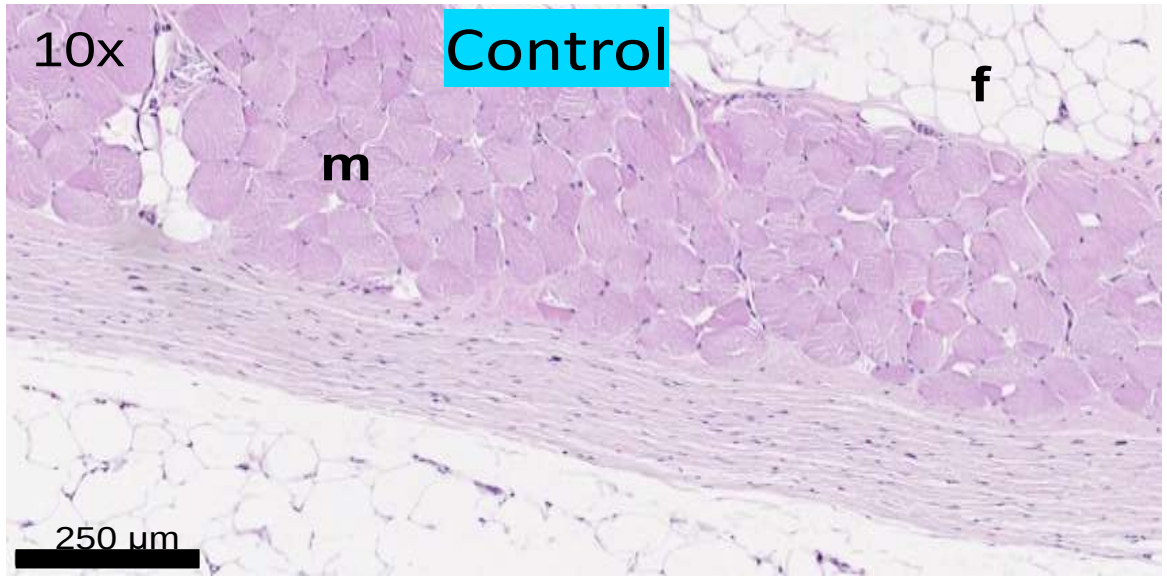



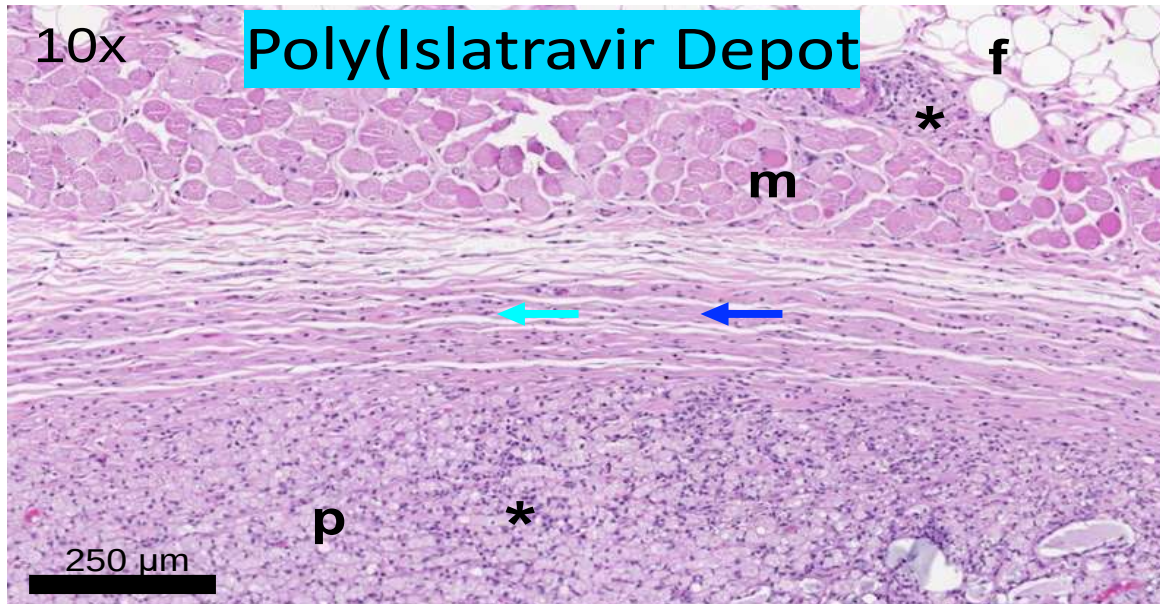
**Thank you to our collaborators at UW, Melbourne CSIRO, Seattle Children's Research Institute, Dana Farber Cancer Institute, Houston Methodist, Fred Hutch CRC**

**Work funded by DTRA, NIH, Bill & Melinda Gates Foundation, Cystic Fibrosis Foundation**


Plasma cabotegravir (µg/mL)
0
1
2
3
4
5
0
4
8
12
16
20
24
28
Time (weeks)

# Pathology Assessment of Subcutaneous Injection Site




Initial pathology analysis of the poly(islatravir) depot (7 month timepoint), conducted by Dr. Jessica Snyder, Asst. Professor in the Pathology Dept, UW. She found a minimal to mild foreign body reaction. The asterisk (*) denotes infiltrating inflammatory cells, d is dermis layer, m is muscle layer, f is fat layer, p is the polymer depot site, and the light blue and dark blue arrows fibroblasts and collagen, respectively.

Fixed in 10% Formalin, H&E stain, Entire cross section of tissue from dermis to subcutaneous