



# Nanomedicine: from high tech to global health

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***Robert K. Prud'homme,***

***Brian Johnson, Walid Saad, Ying Liu, Marian Gindy, Stephanie Budijono,  
Margarita Herrera-Alonso, Varun Kumar, Suzanne D'Addio, Robby Pagels,  
Chet Markwalter, Brian Wilson***

*Dept. Chemical Engineering  
Princeton University*

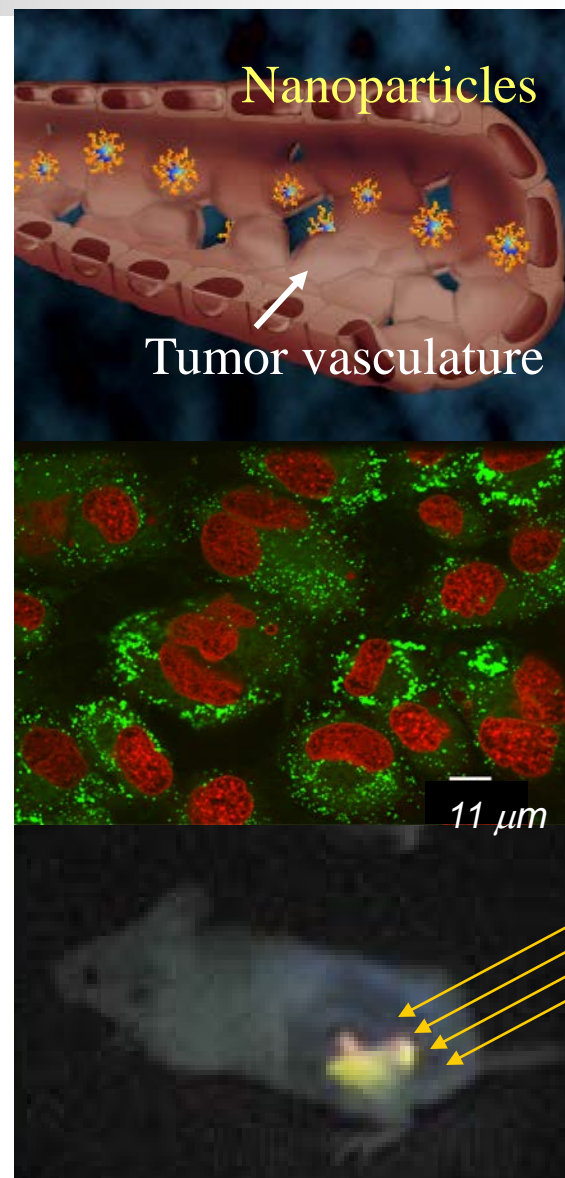
*Support: NSF, NIH, Gates Foundation, BASF, Evonik, Merck, J&J, GSK,  
Celator, Optimeos; Nanomedicine for BBB-crossing in CNS oncologic  
pathologies MAECI PROJECT 2019-2021*

# Next Generation Nanoparticles (NPs)

## Motivation

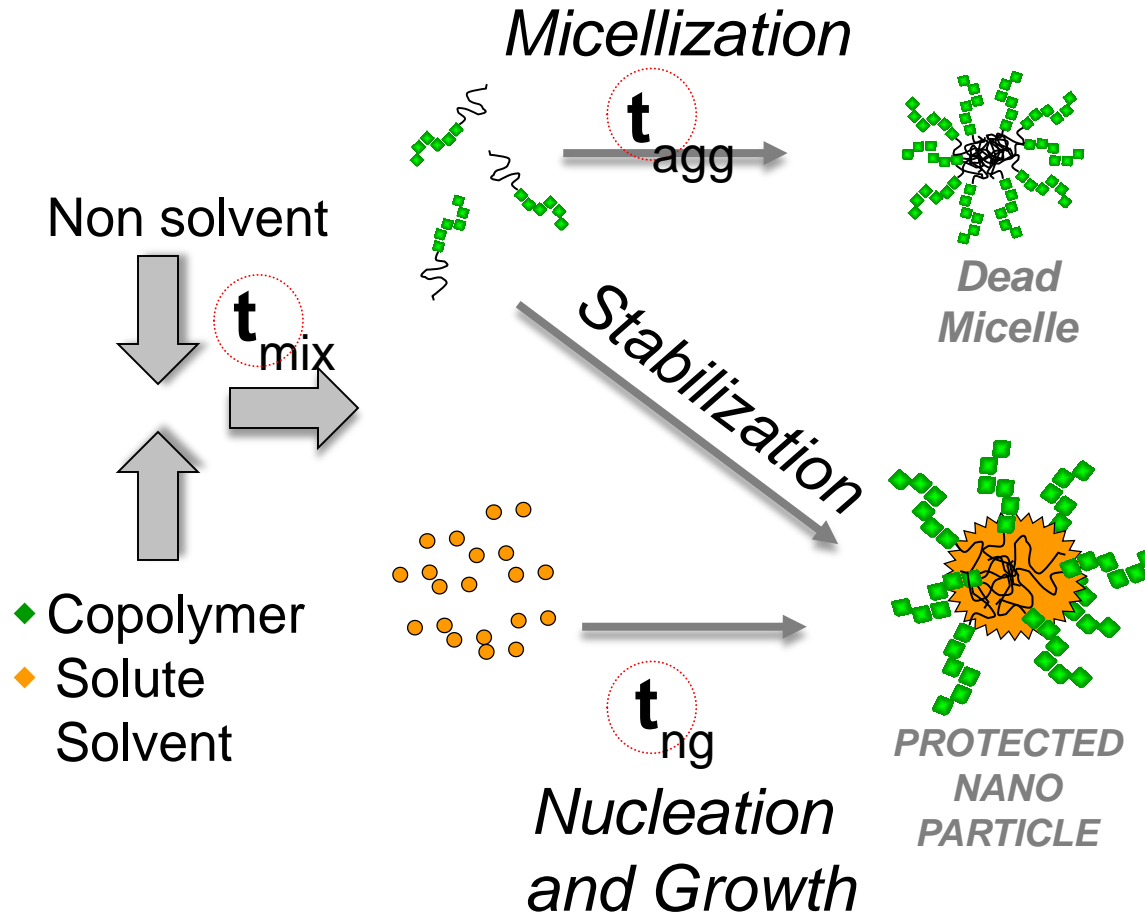
*“...over the next few years some of the complex theranostic strategies published rampantly in chemistry journals will fall out of contention...For me, something that’s too difficult to make or too complex to sustain in large-scale production is not what we are interested in.”*

*J.Janijic C&EN Sept 26,2011.*



# Nanoparticle formation by Flash NanoPrecipitation

## Block copolymer directed rapid precipitation



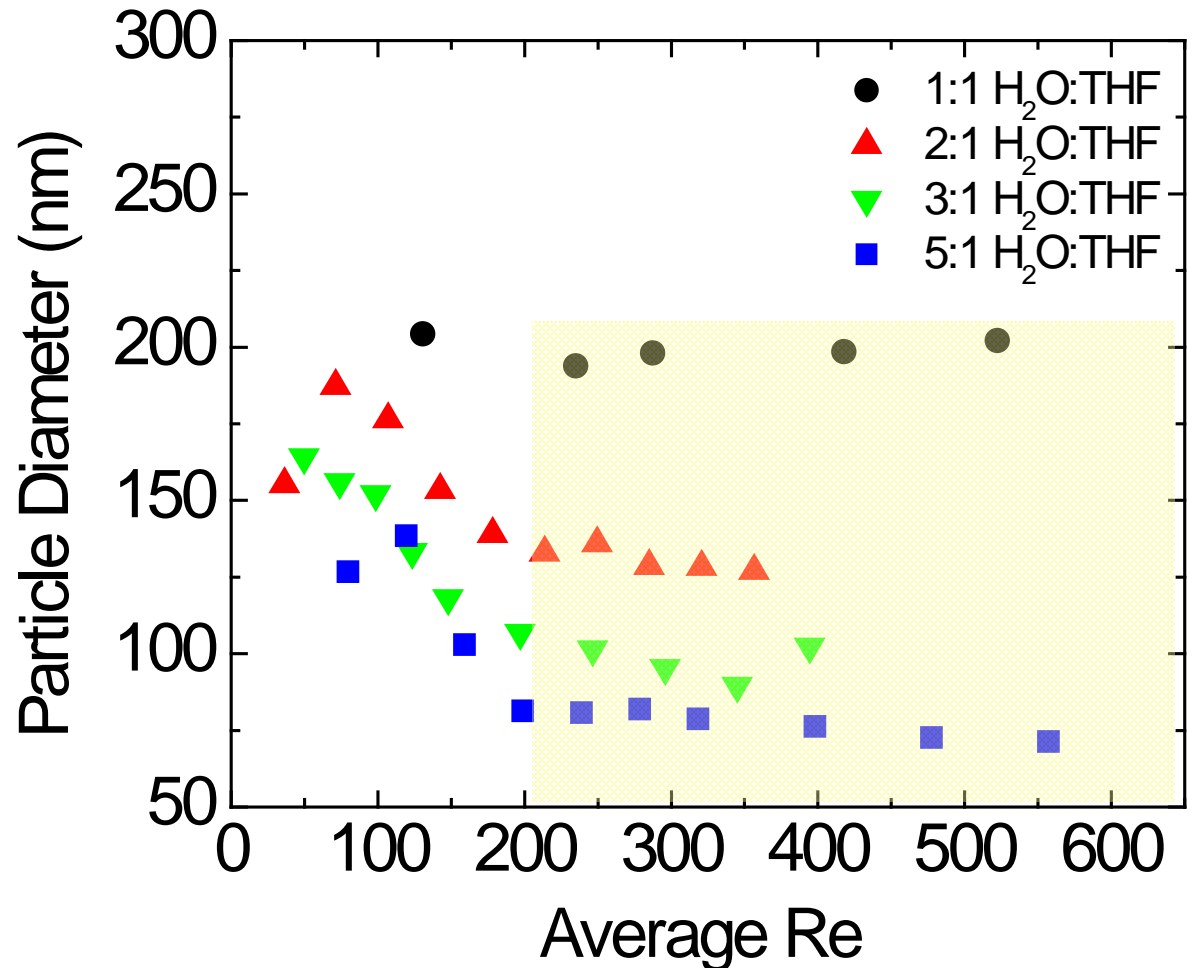
Johnson, Prud'homme AICHE J (2003), Liu, Prud'homme, Fox, Chem Engr. Sci. (2008)

# Control of particle size

## 1. *Mixing intensity*

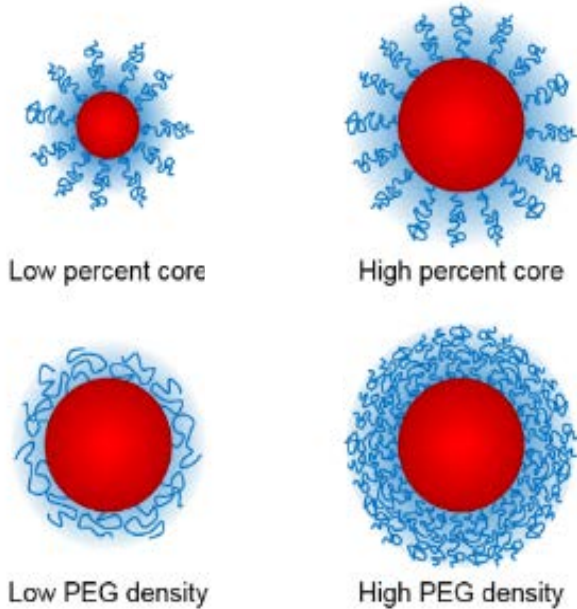
## 2. *Super-saturation*

- *Control super-saturation by changing solvent quality or solute concentration.*
- *Higher super saturation leads to smaller particles*

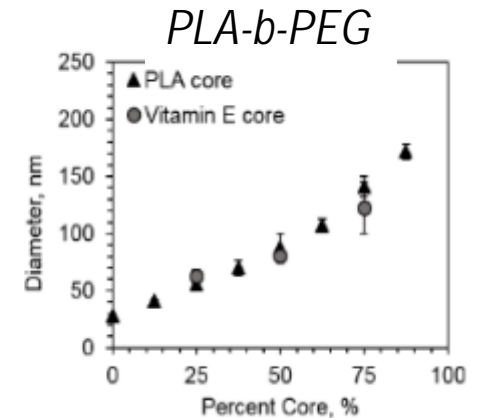
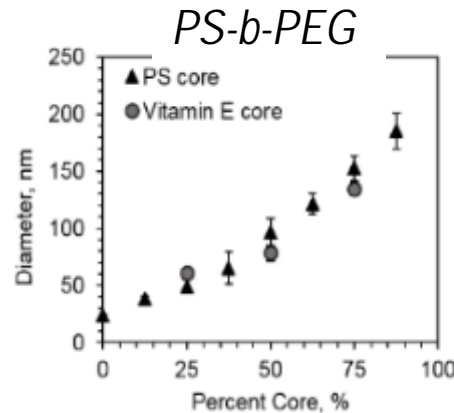


# NP Formation: PS to PLA NPs

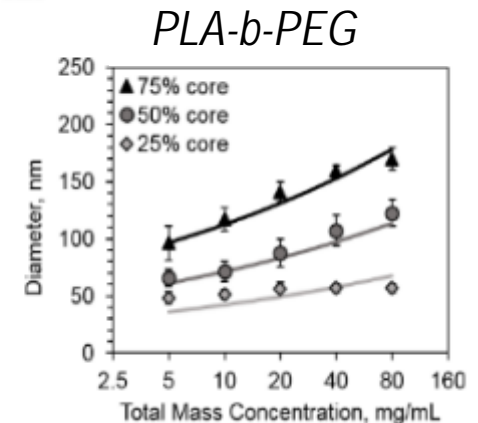
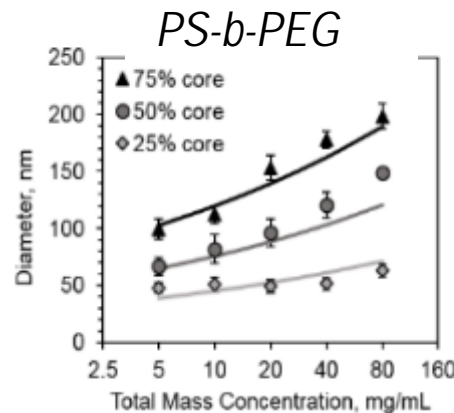
Want to shift from *model polystyrene (PS)* to *biocompatible polylactic acid (PLA)*. How does size depend on species in core?



b. Effect of Percent Core on NP Size



c. Effect of Total Mass Concentration on NP Size

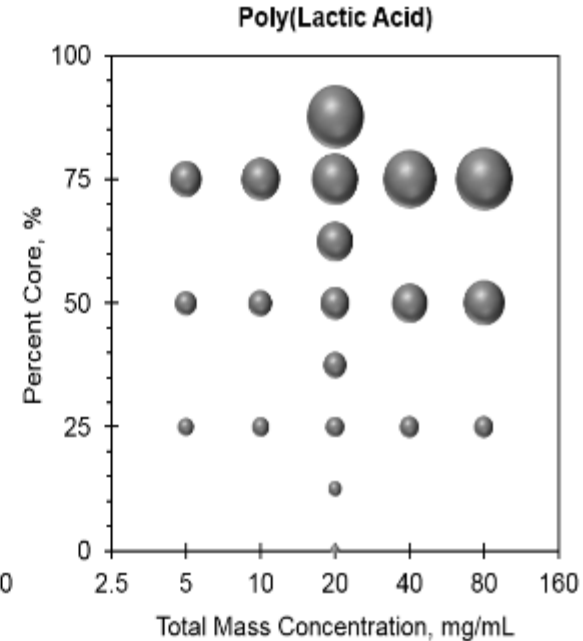
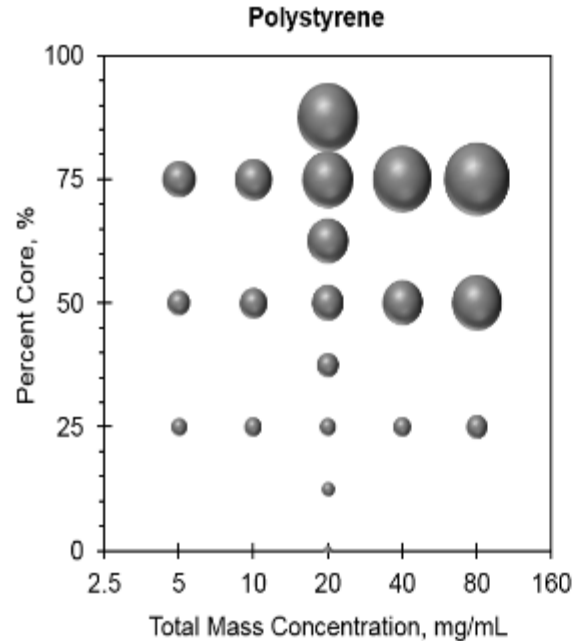
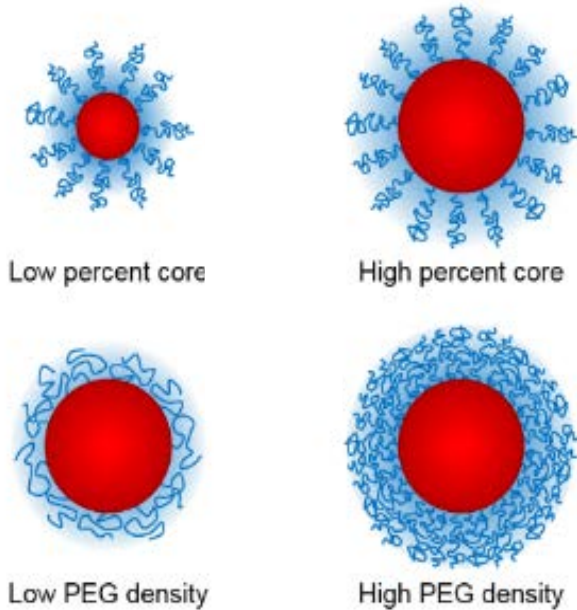


- What is in the core doesn't matter
- The ratio of core to BCP matters,  $C_{core}/C_{BCP}$
- The total mass matters

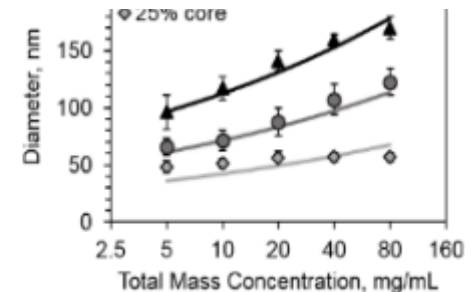
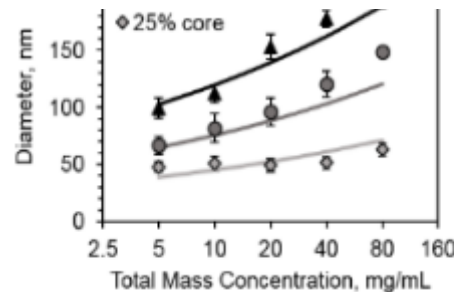
Pagels Nano Letters **18** 1139-1144 (2018)

# NP Formation: PS to PLA NPs

Want to shift from model polystyrene (PS) to biocompatible polylactic acid (PLA). How does size depend on species in core?



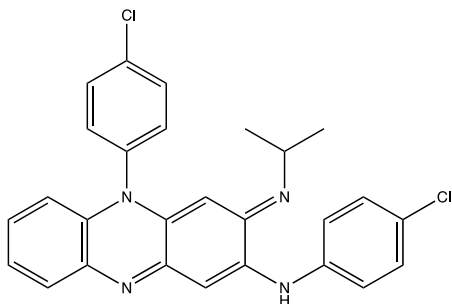
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Pagels Nano Letters 18 1139-1144 (2018)

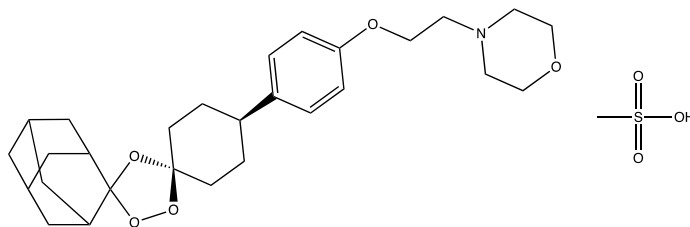
# Drugs Considered

## Clofazimine



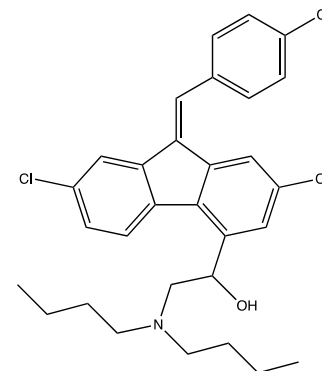
Ref: Design and Solidification of Fast-Releasing Clofazimine Nanoparticles for Treatment of Cryptosporidiosis". *Molecular Pharmaceutics*. **14**(10) 3480-3488 (2017); "Rapid Recovery of Clofazimine-loaded Nanoparticles with Long-term Storage Stability as Anti-Cryptosporidium Therapy". *ACS Applied Nano Materials*. **1**(5) 2184-2194 (2018)

## OZ439



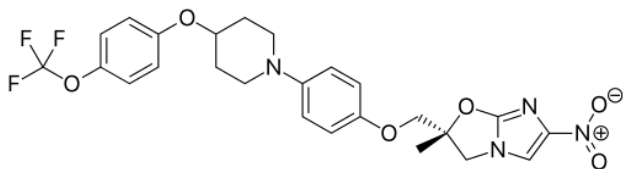
Ref: "Encapsulation of OZ439 into Nanoparticles for Supersaturated Drug Release in Oral Malaria Therapy". *ACS infectious diseases*. **4**(6) 970-979 (2018); "Spray drying OZ439 nanoparticles to form stable, water-dispersible powders for oral malaria therapy". *Journal of Translational Medicine* (2019), just accepted

## Lumefantrine



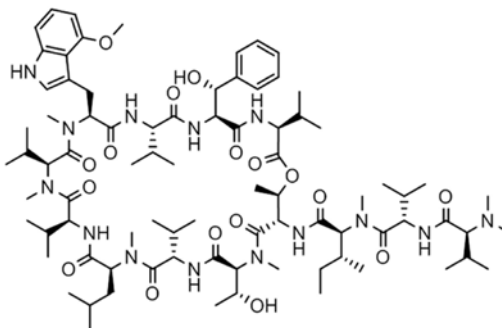
Ref: "Amorphous Nanoparticles by Self-assembly: Processing for Controlled Release of Hydrophobic Molecules". *Soft Matter*. **15** 2400-2410 (2019)

## Delamanid



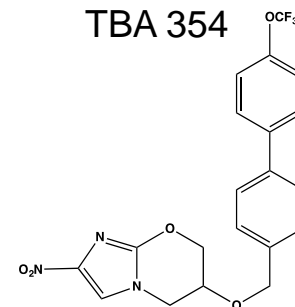
Ref:  
<https://app.box.com/s/lvghy956h7pmzvdd1nlj9zz eotabijke>

## Ecumicin



Ref:  
<https://app.box.com/s/5lggnm4tv84kcb0cry0tscb y9oszzqi>

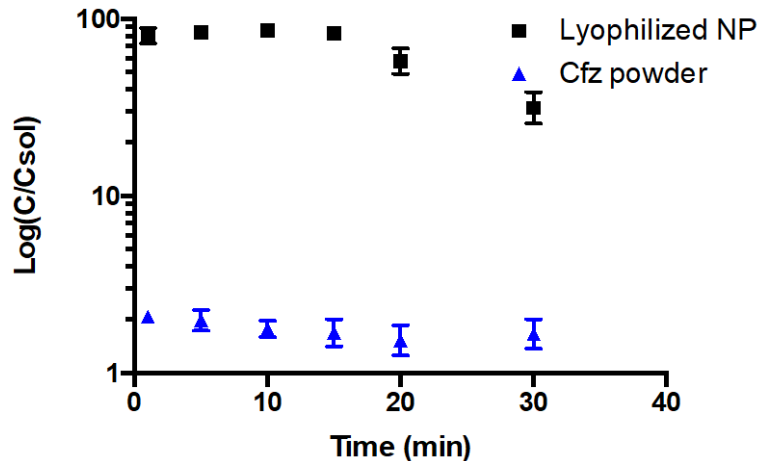
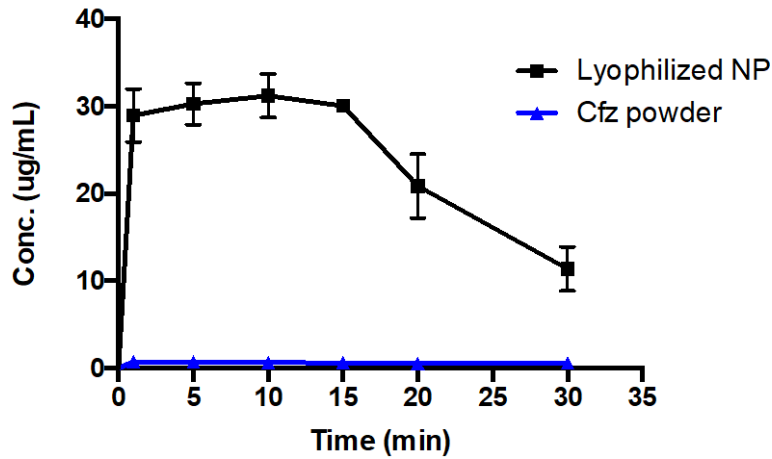
## TBA 354



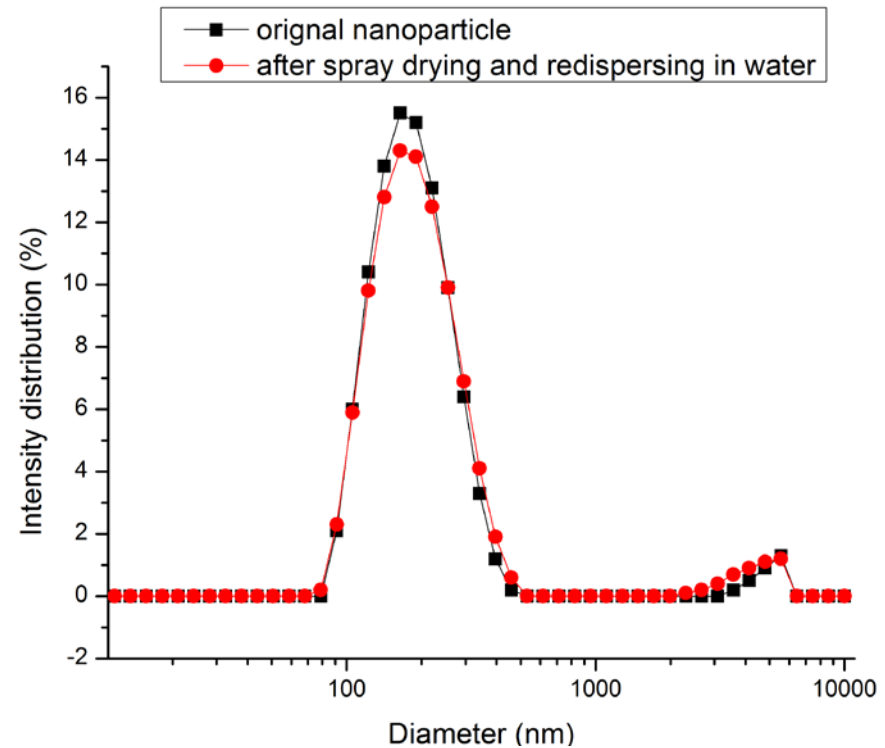
Ref:  
<https://app.box.com/s/57cromgxhg8uyqiz4zegtaf wqbcg3nk>

# Clofazimine – Zein/casein

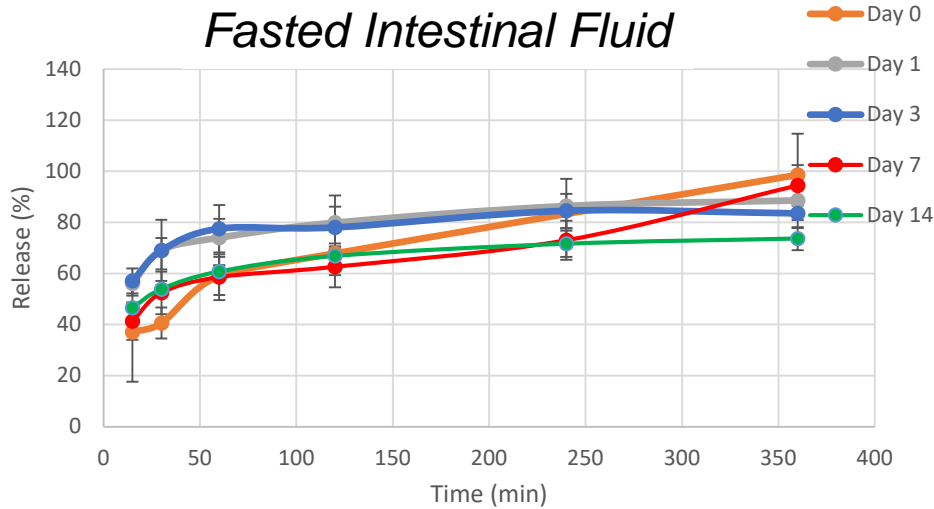
*40X higher dissolution in GF*



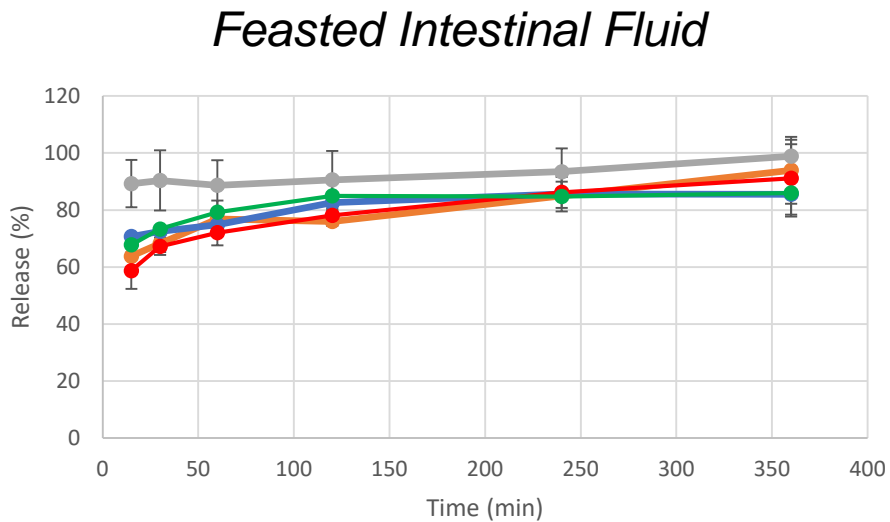
*Direct spray drying  
redisperses to NP*



# Long-term Zein Clofazimine NPs



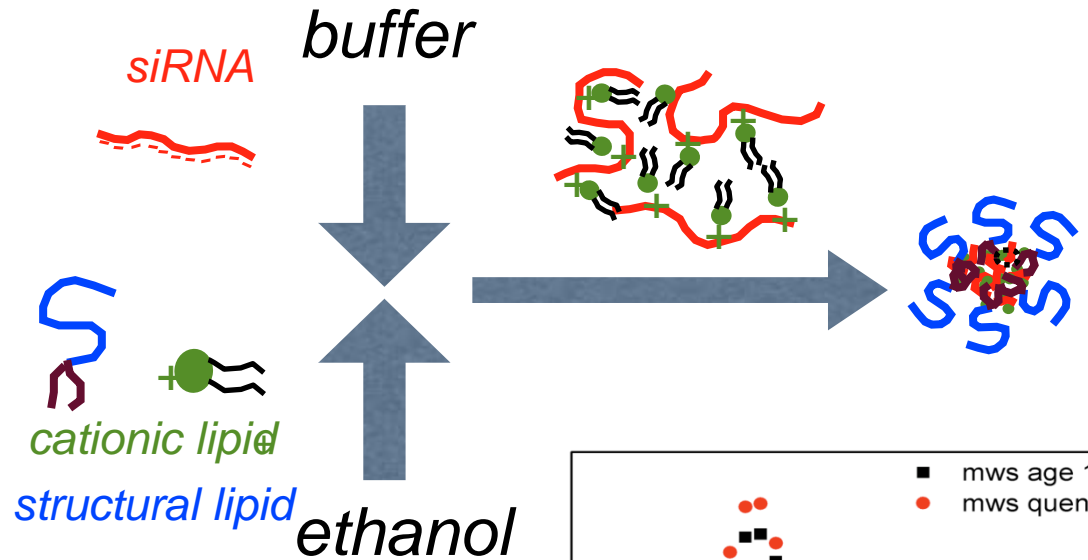
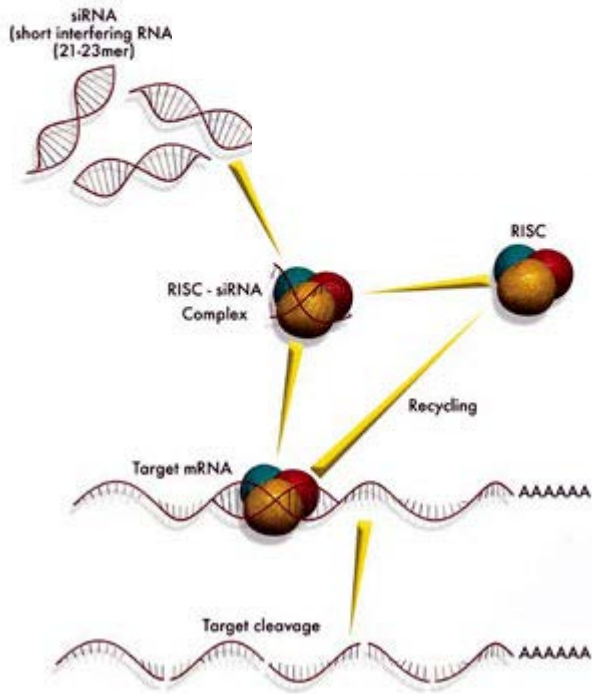
| Day | FaSSIF    | FeSSIF    |
|-----|-----------|-----------|
| 0   | 5.4 ug/mL | 5.4 ug/mL |
| 1   | 4.5 ug/mL | 4.8 ug/mL |
| 3   | 4.7 ug/mL | 5.9 ug/mL |
| 7   | 6.2 ug/mL | 5.6 ug/mL |
| 14  | 4.6 ug/mL | 5.1 ug/mL |



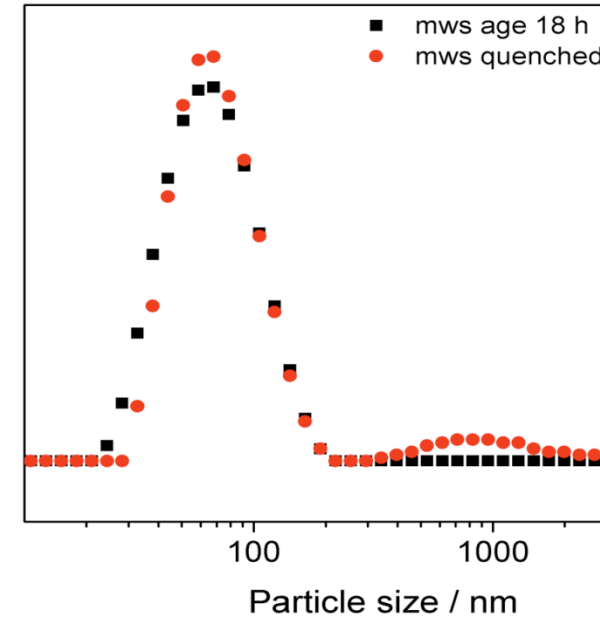
- *Open vial: 75 % RH, 50 C*
- *Have gone to 28 da +*
- *FeSSIF: influence was not significant*

# siRNA for gene silencing

- *siRNA complex cleaves mRNA and stops protein expression*
- *Problem is delivery*



- *Successful prep of 100 nm PEG protected siRNA*
- *Stable and transfect efficiently*



# Four Scales of CIJ & MIVM

## CIJ

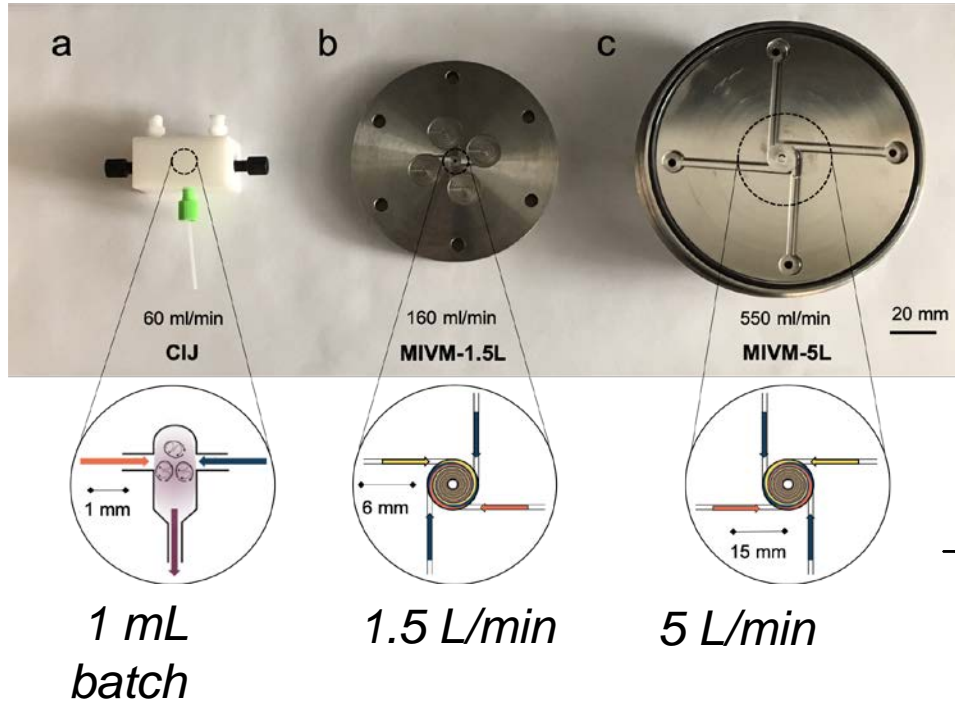
Confined Impinging Jet

## MIVM

Multi Inlet Vortex Mixer

## Micro-MIVM

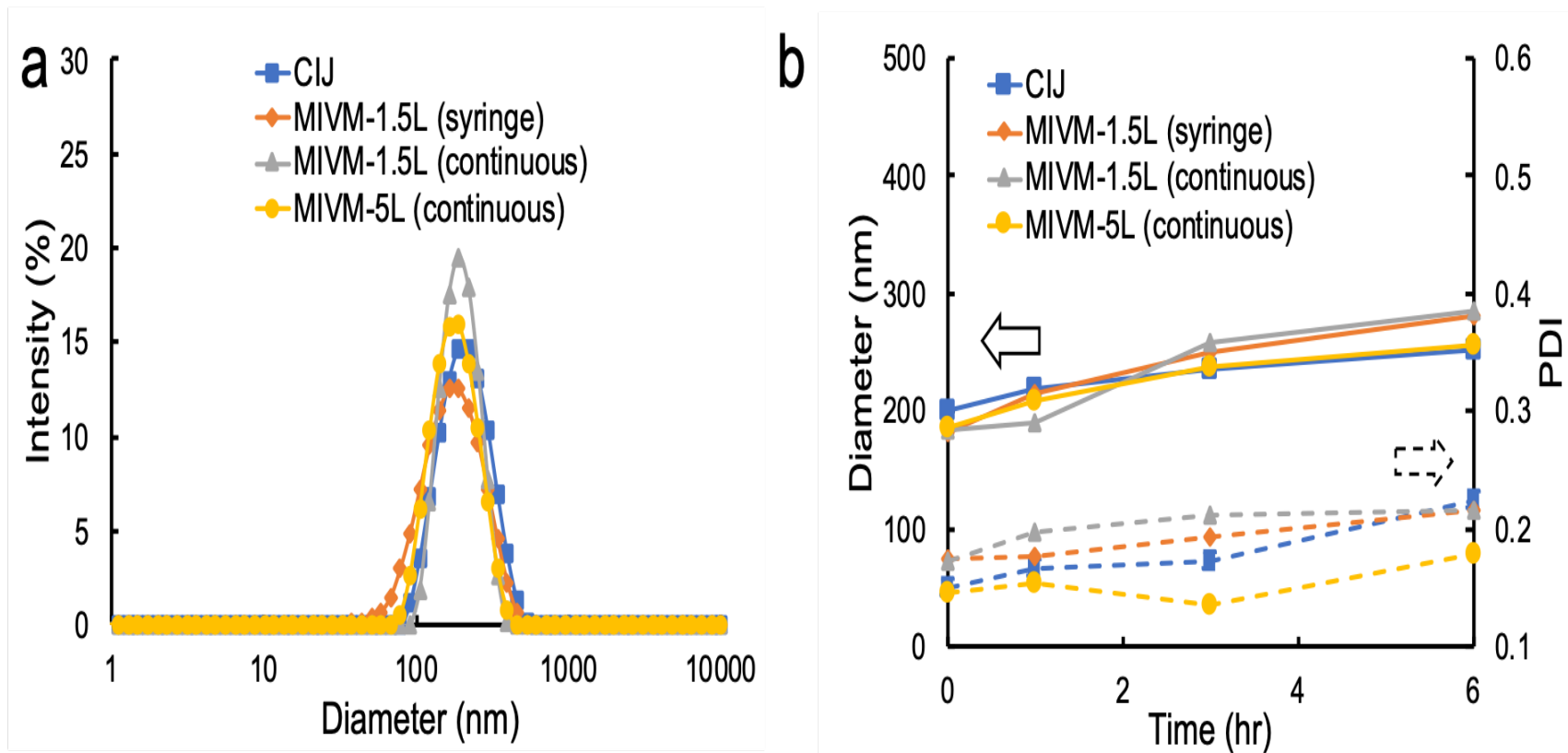
$\mu$ -Multi Inlet Vortex Mixer



Markwalter, et al *JOVE* **143** (2019), *J Pharm Sci* **107** (2018); Feng, *J Trans Med* (2019)

# Equivalent NP formation vs Reynolds No.

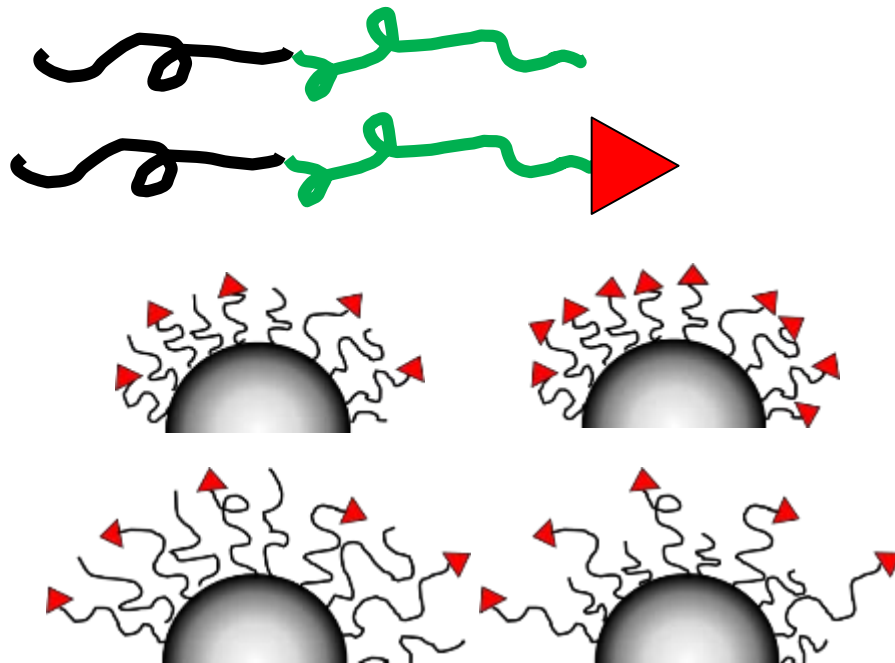
- **Similar NPs produce from batch (1 ml scale) to 5 L/min**



Feng, et al. *J. Translational Medicine* (2020)

# Active targeting

- Conjugate targeting group to block copolymer
- Do hard chemistry and characterization on conjugate
- Quantitatively assemble targeted NP with control on % targeting groups
- Conjugate chemistry: click chemistry, maleimide, carbodiimide, carbamate



Small molecule:  
(mannose, folate, LHRH)



V domain



V<sub>H</sub>

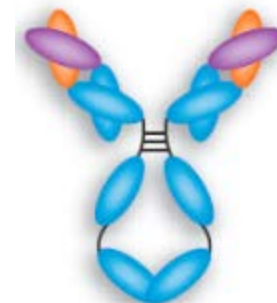


V<sub>K</sub>

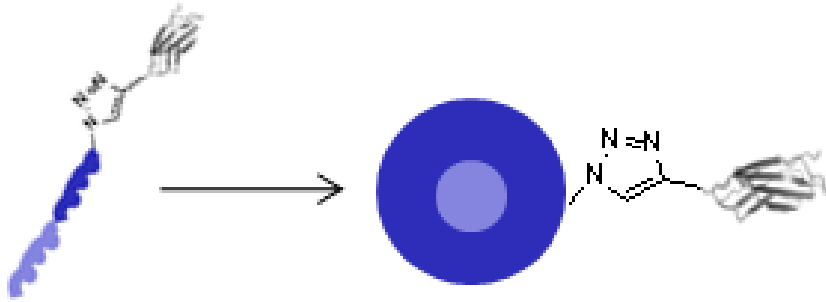
scFv



IgG

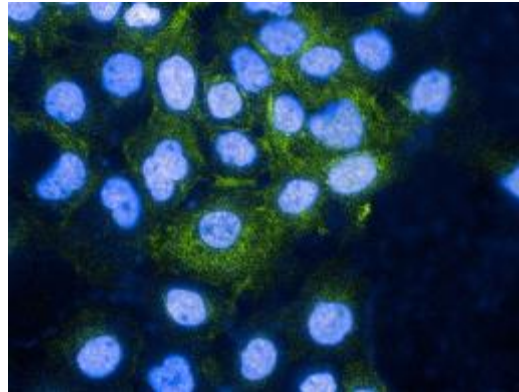


# Variable domain antibody targeting

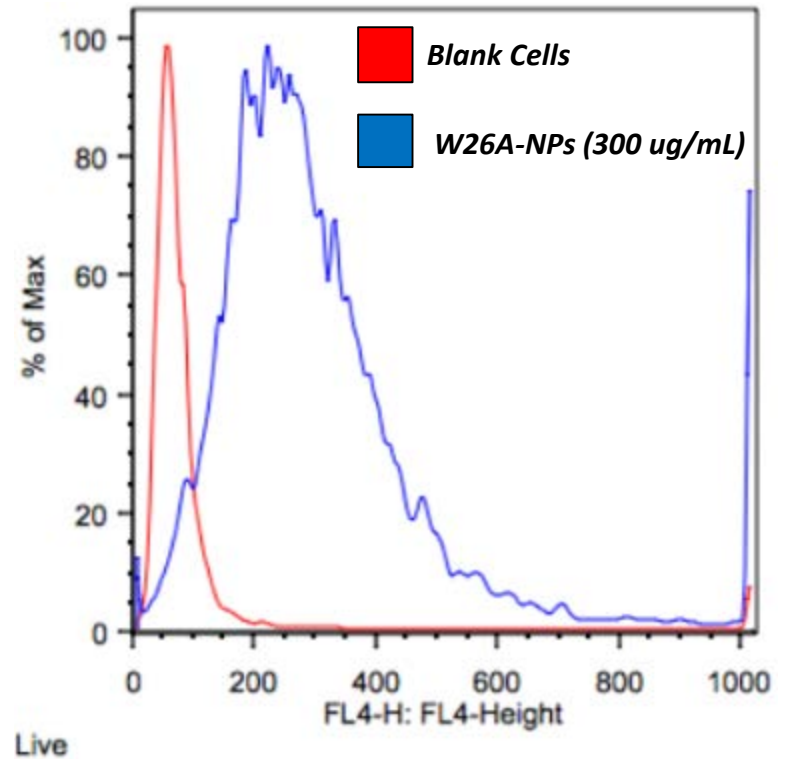
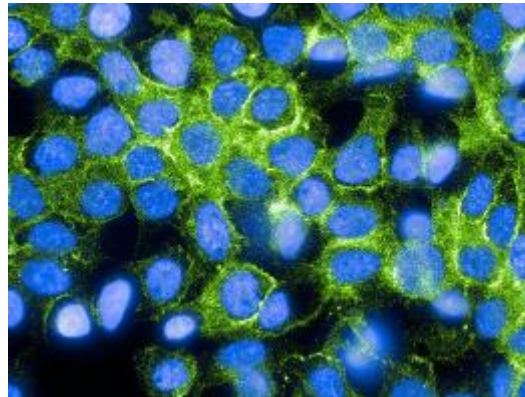


- 14K fibrinogen peptide scaffold (Centryin)
- Pre functionalize BCP
- Assemble NP

Control  
(PEG NP)



5%  
ligand  
targeted

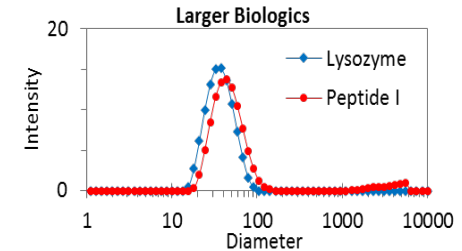
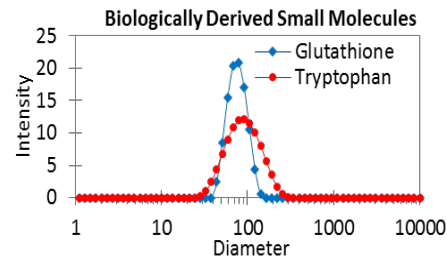
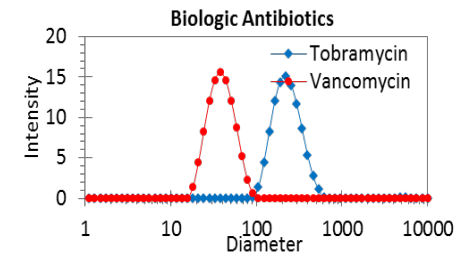
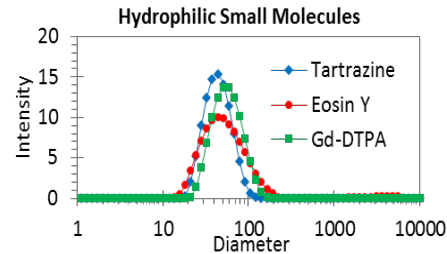
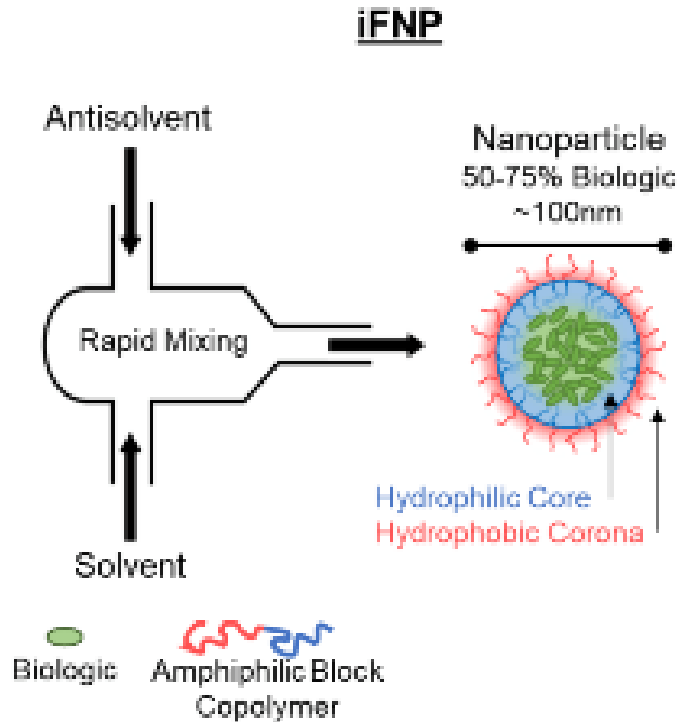


# Inverse Flash NanoPrecipitation (iFNP)

## *Precipitation process for the encapsulation of hydrophilic therapeutics*

*iFNP uses a non-polar antisolvent to produce NPs with a water-soluble core:*

*iFNP has been demonstrated on a variety of water-soluble molecules:*



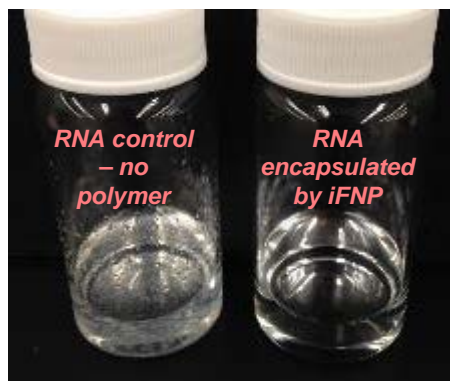
- All  $\geq 50\%$  loading
- Other encapsulated molecules include: polysaccharides, insulin, ovalbumin, peroxidase, Trp cage, mRNA

Pagels & Prud'homme, *J. Cont. Release* (2015)  
Markwalter, et al. *AAPS* (2020)

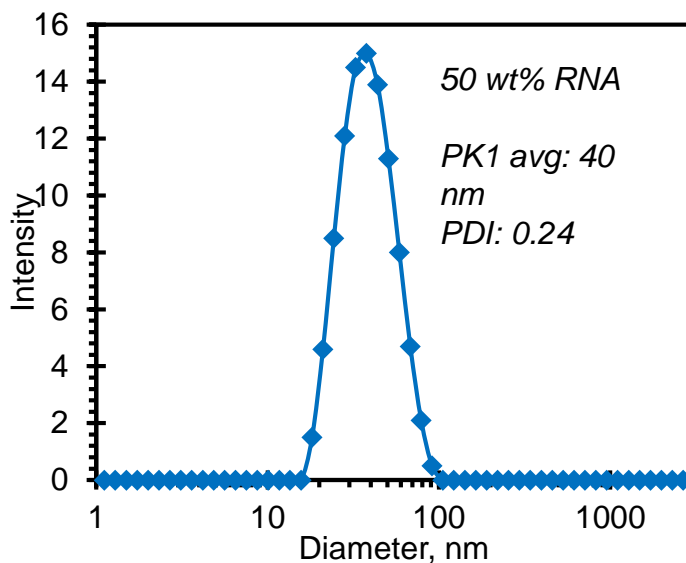
# Unique Technology Capability: RNA Encapsulation

*mRNA has been encapsulated (50% loading) into inverted nanoparticles by iFNP*

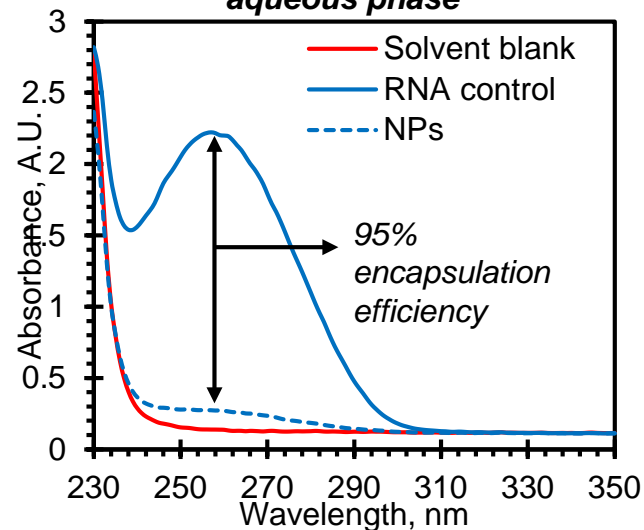
*RNA precipitate (left) and nanoparticles (right) in  $\text{CHCl}_3$*



*Particle size distribution*



*Encapsulated RNA is protected from extraction into an aqueous phase*

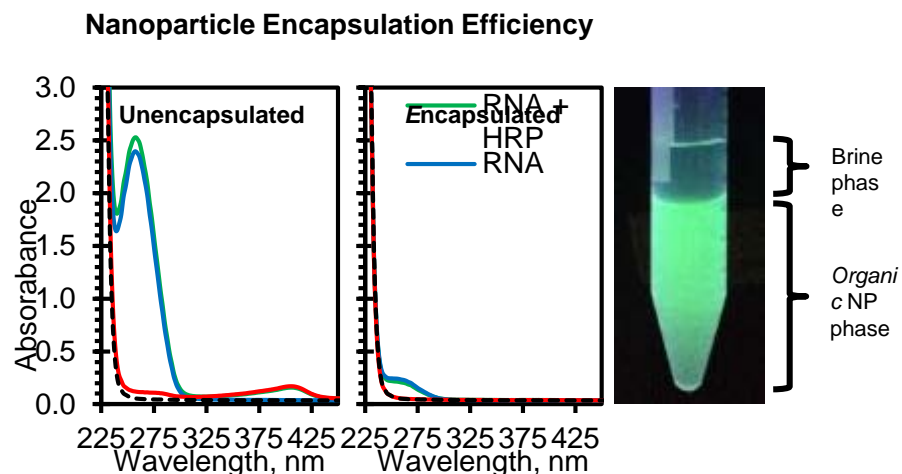
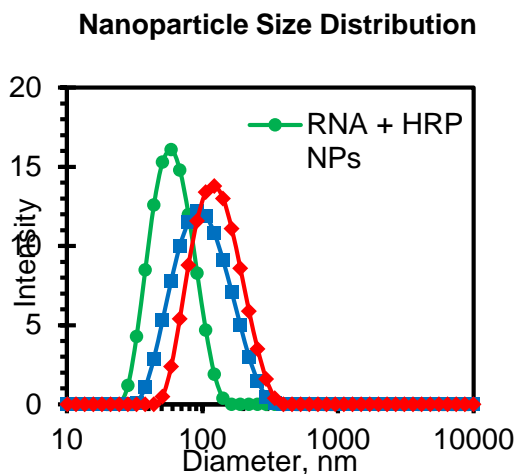
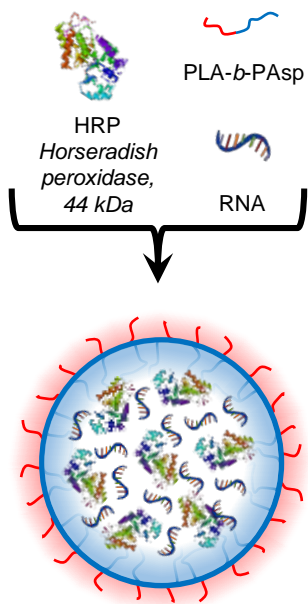


*Cationic lipids are not required for encapsulation of RNA – different from currently available technologies*

*Model RNA used: RNA from *Torula utilis*,  $M_r$  5,000-8,000 Da (15-25 nucleotides)*

# Unique Technology Capability Co-Encapsulation

**RNA (33 wt% loading) and a model protein (33 wt% loading)**



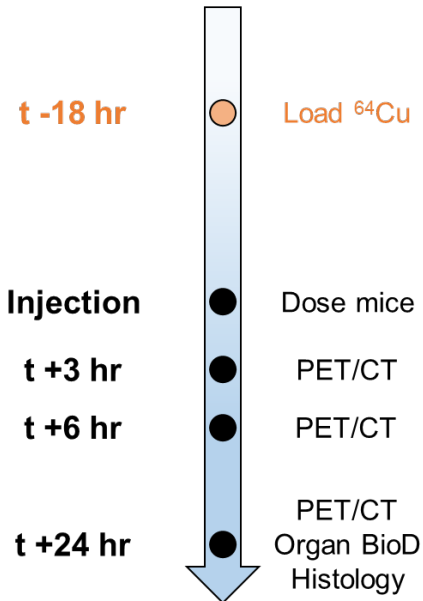
|               | Nanoparticle Composition, wt% |       |         | Encapsulation Efficiency |     |
|---------------|-------------------------------|-------|---------|--------------------------|-----|
|               | RNA                           | HRP   | Polymer | RNA                      | HRP |
| RNA NPs       | 50%                           | --    | 50%     | 92%                      | --  |
| HRP NPs       | --                            | 50%   | 50%     | --                       | 99% |
| RNA + HRP NPs | 33.3%                         | 33.3% | 33.3%   | 93%                      | 99% |

# 23 Cu-64 PET/CT imaging allows tracking of biodistribution *in vivo*

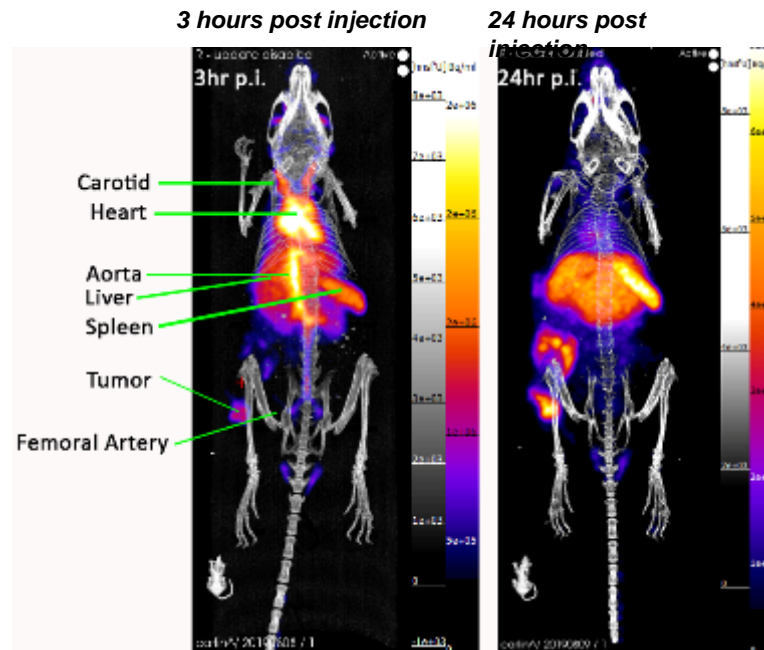
For Discussion with Spark  
Therapeutics Only  
Please Do Not Distribute



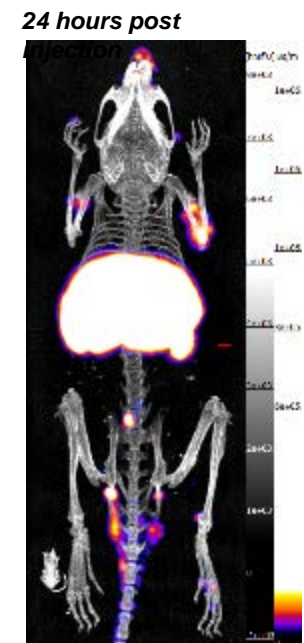
## Experiment Timeline



Neutral PEG coating leads to tumor accumulation



Negative coating promotes immune cell interactions



# Summary (prudhomm@Princeton.edu)

- *Flash NanoPrecipitation (FNP)*
  - Polymer-directed, rapid precipitation process
  - Hydrophobic drugs, and drugs made hydrophobic by ion pairing
  - Scaleable: mg to 1000 kg
  - Enables targeting incorporation and imaging (PET, fluorescence, MRI)
  
- *Inverse Flash NanoPrecipitation (iFNP)*
  - Polymer-directed, two step rapid precipitation process
  - Hydrophilic peptides, proteins, mRNA, DNA
  - High loading (20-50%), high encapsulation efficiency (80-99%)
  - Makes nanoparticles, or microparticles for depot delivery (2 mo release)