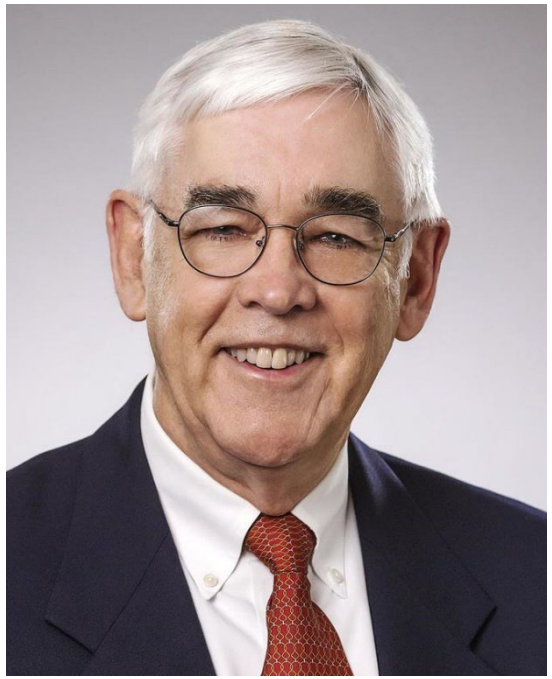
# An Integrated Platform for Continuous RNA Nanoparticle Formulation and Drying

Kurt Ristroph
Agricultural and Biological Engineering, Purdue University



Robert Prud’homme,
Princeton University

Kurt Ristroph,
Purdue University

Mark Kastantin,
Serán Bioscience

# Part 1: Flash NanoPrecipitation principles and use for lipid nanoparticle preparation

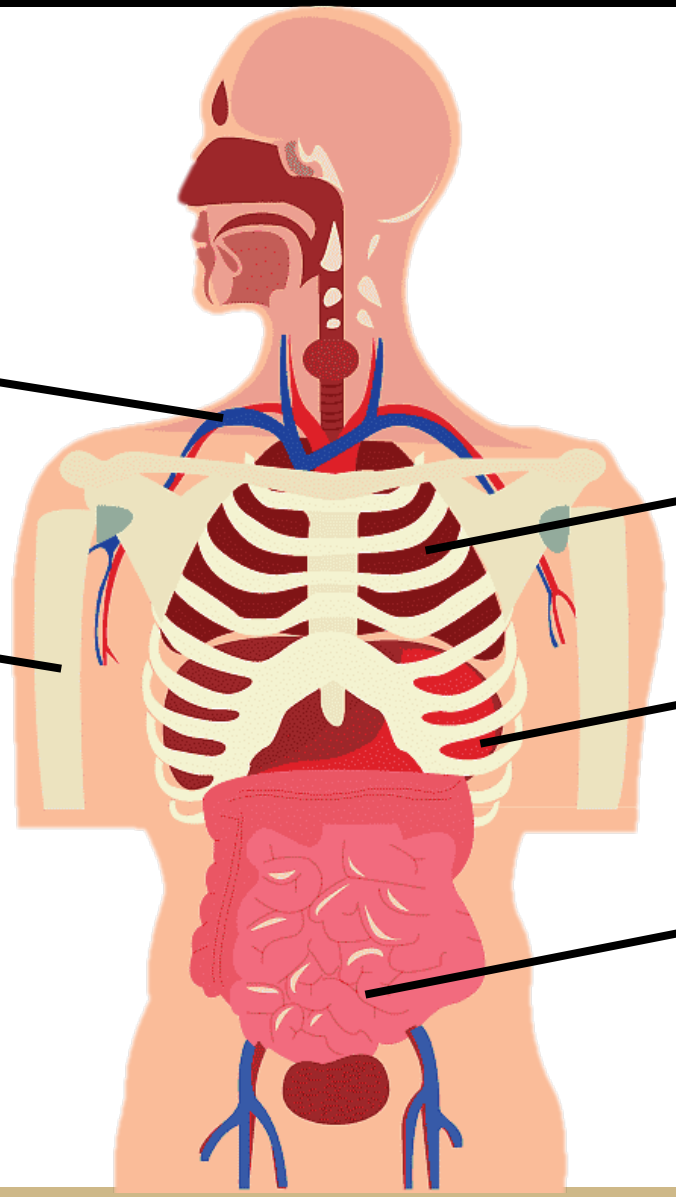
# Potential benefits of nanoencapsulation, at a glance

*Improved circulation time in blood, residence time in body (parenteral)*

*Targeting to macrophages, tumors, etc. (parenteral)*

*Co-delivery of multiple payloads for multimodal therapy and/or drug synergism (oral, parenteral)*

*<u>Combinations of the above</u>*



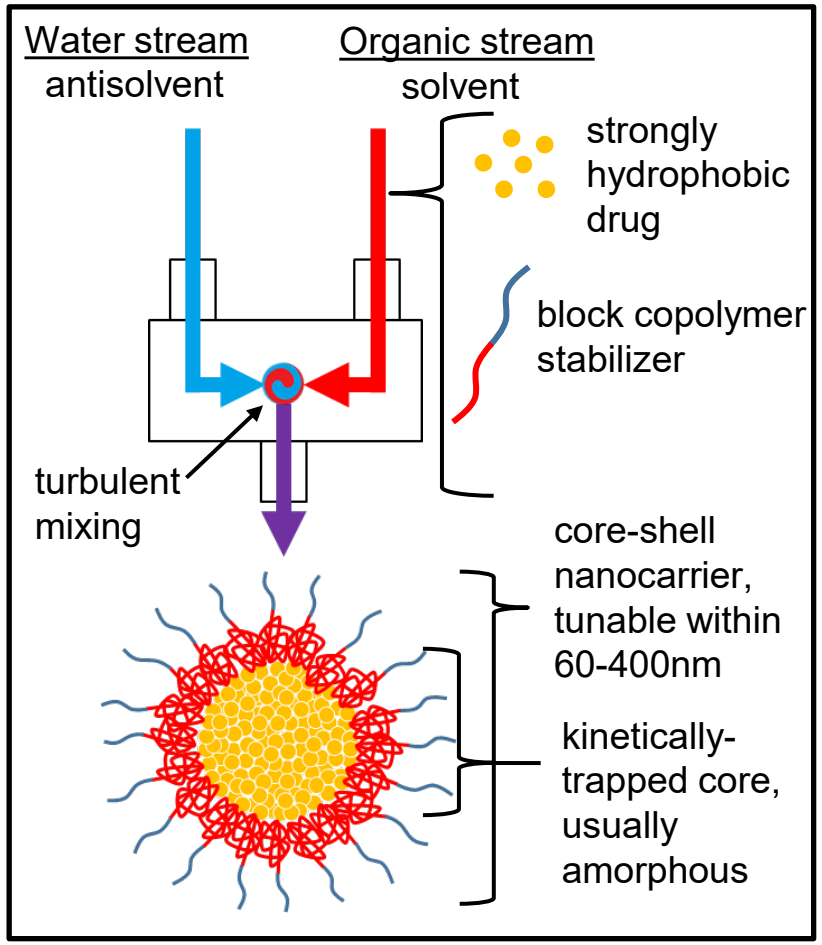
*Ability to penetrate physiological mucus (oral, parenteral)*

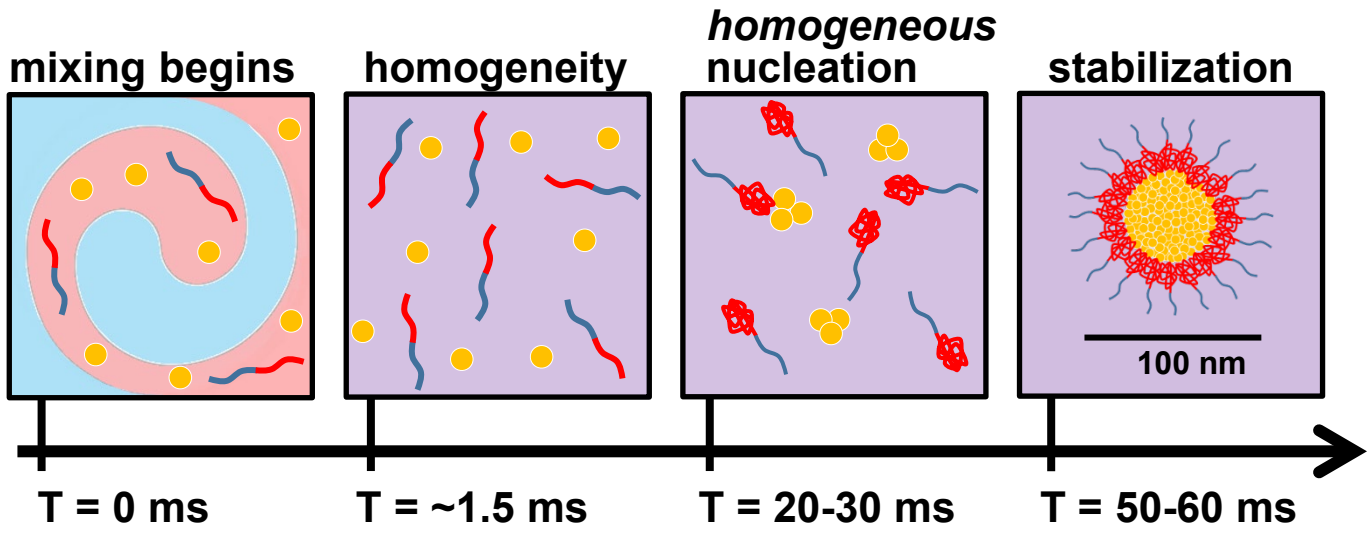
*Controlled or stimulus-responsive release (**hydrophilic API**; parenteral)*:

*Rapid dissolution in GI tract (**hydrophobic API**; oral)*

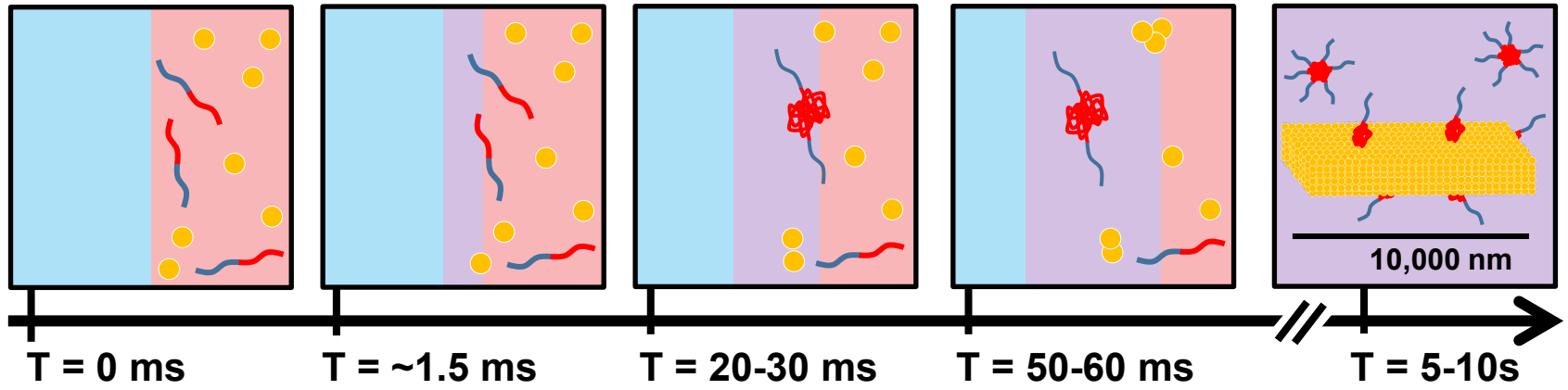
# Nanoparticle formulation technique: Flash NanoPrecipitation

**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation



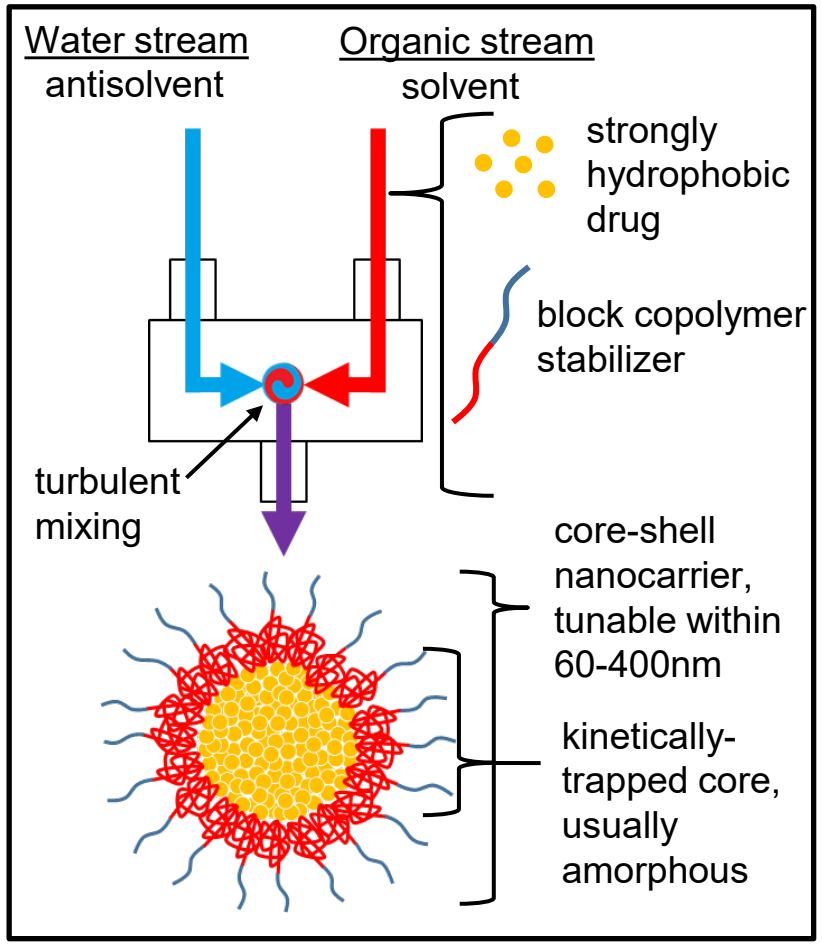



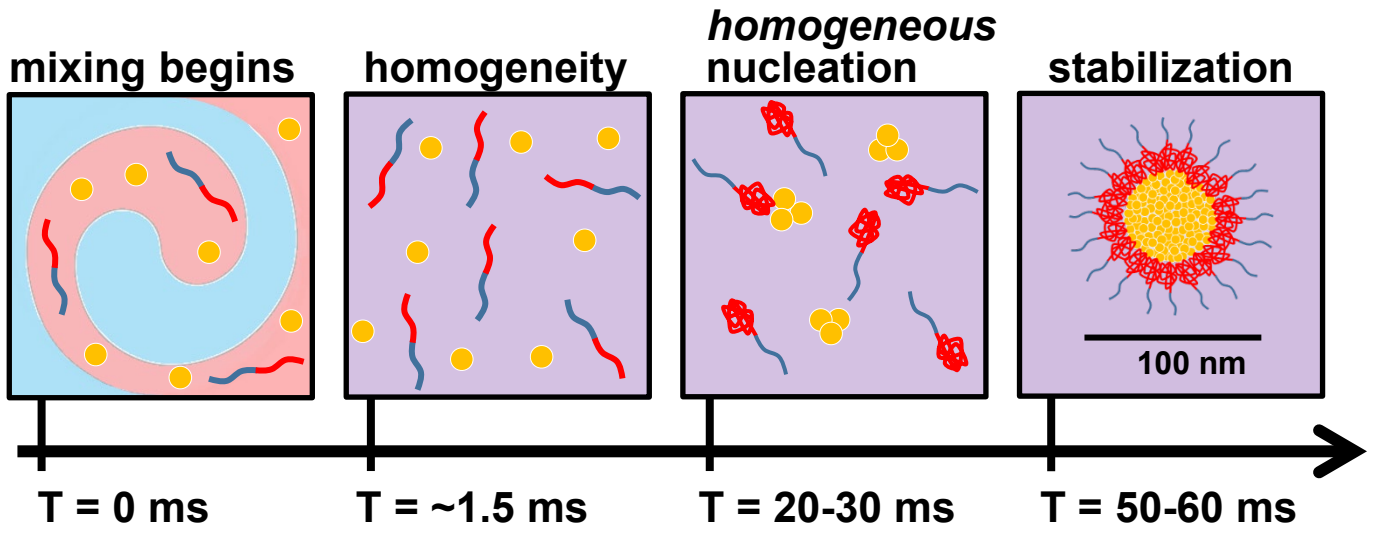

**"Bulk nanoprecipitation" poor mixing:**



# Nanoparticle formulation technique: Flash NanoPrecipitation

**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation



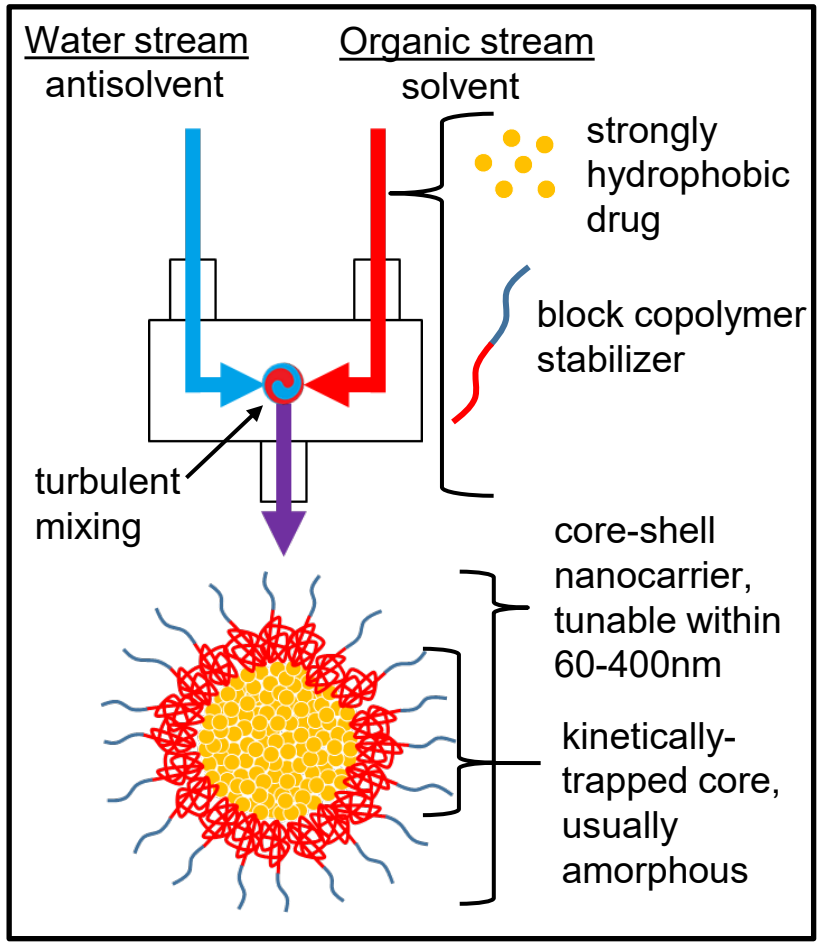



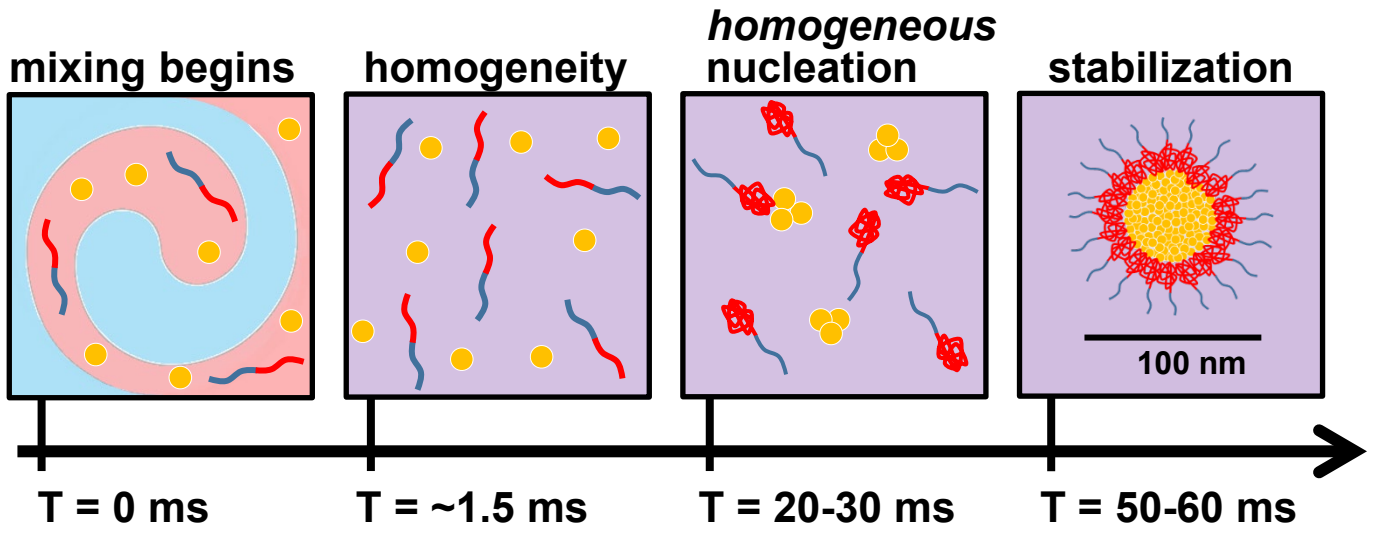

**Key points for nanoprecipitation:**

- Mixing must occur on a *shorter* time scale than particle assembly.
- All compounds must *precipitate* on a similar time scale (i.e. must be strongly hydrophobic or must be made strongly hydrophobic).

# Nanoparticle formulation technique: Flash NanoPrecipitation

**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation



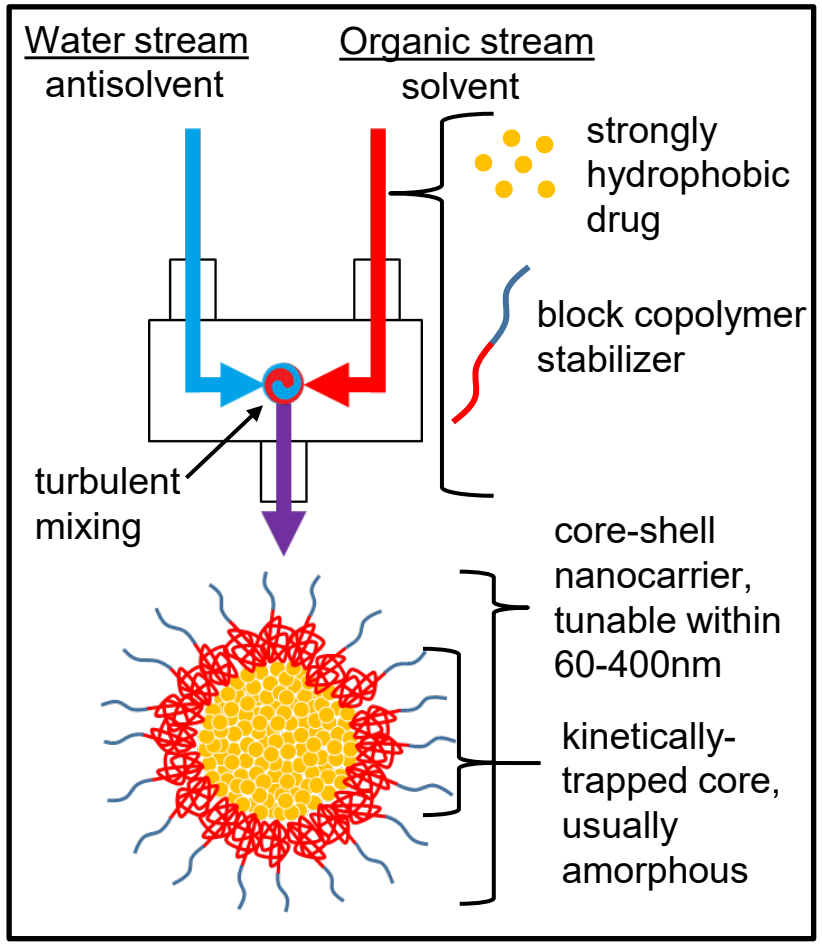



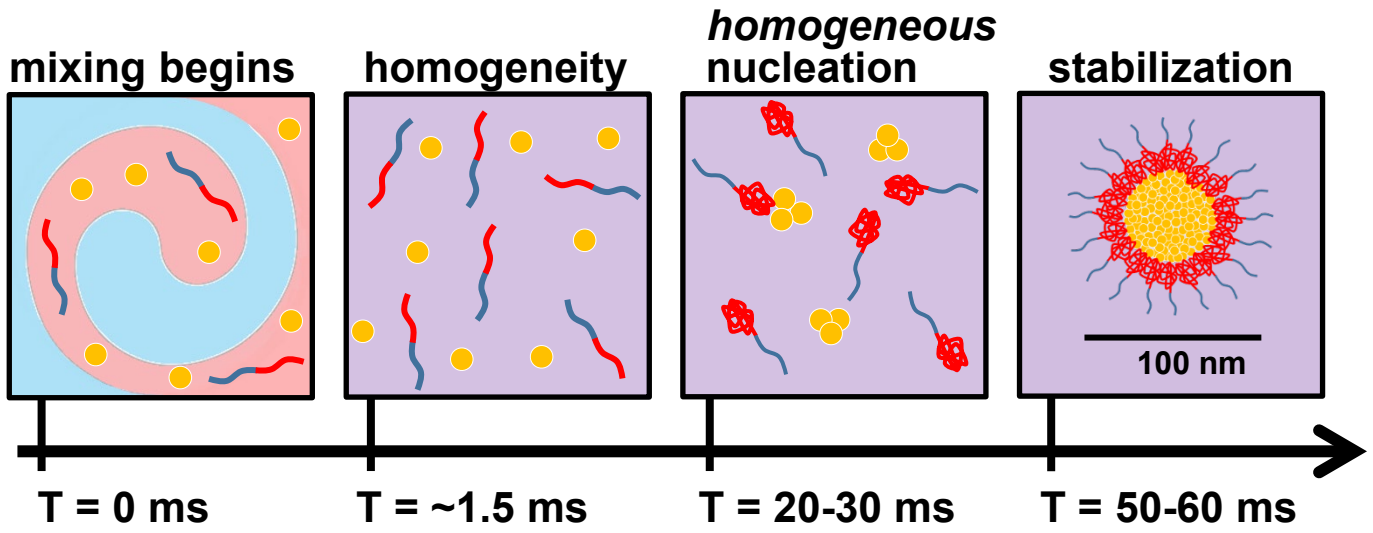

## Nanoparticles made by FNP:

- Dense polymer surface layer (~2 chains / $nm^2$); brush conformation
- Mixtures of surface polymers possible
- Core and shell chemistries are independent; modular formulations
- For hydrophobic cores with logP > ~4.5:
  - >95% encapsulation efficiency
  - 50-70% drug loading (recently up to 90%)

# Nanoparticle formulation technique: Flash NanoPrecipitation

**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation

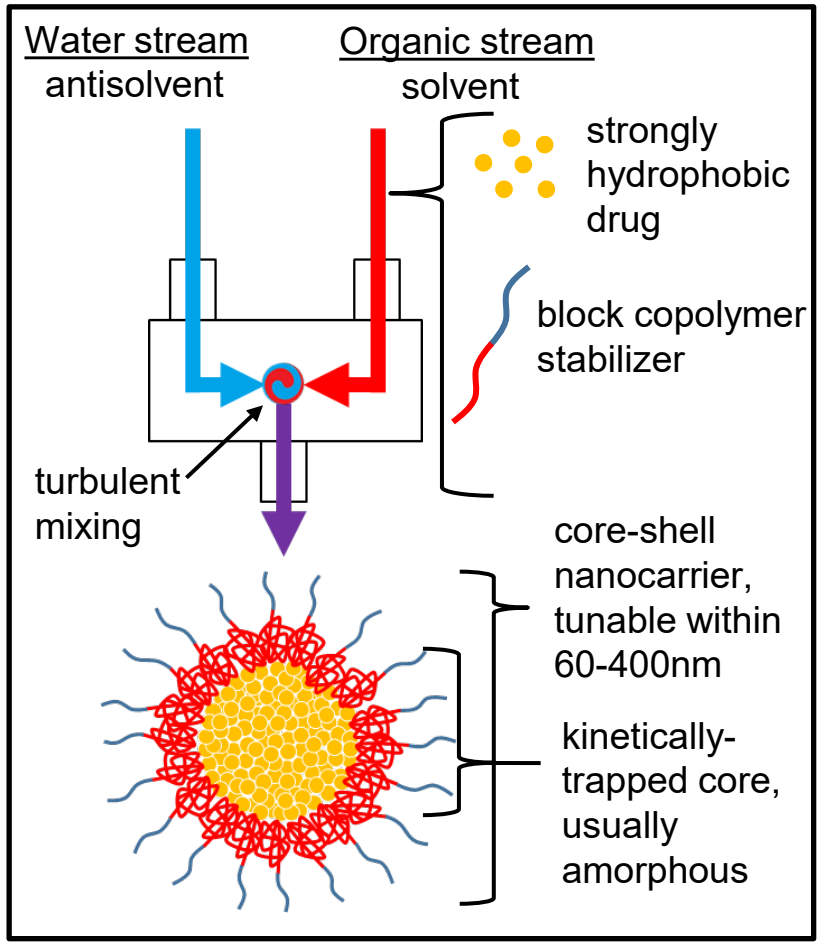


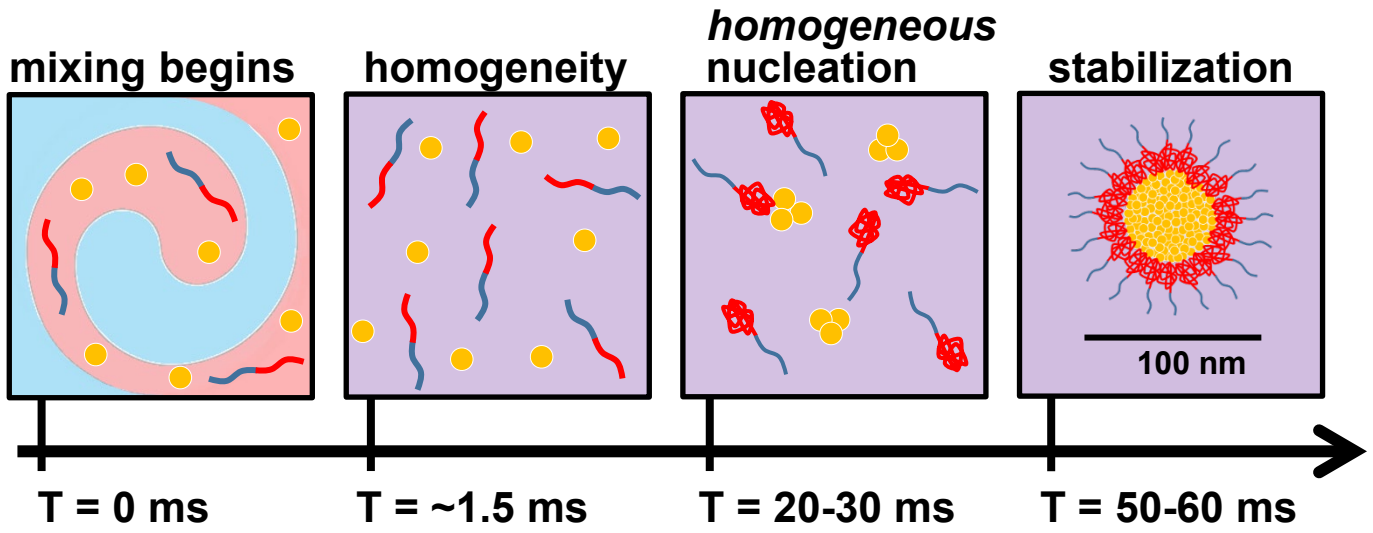




*Key process advantages:*
*- scalability and reproducibility*
*- continuous operation*

# Nanoparticle formulation technique: Flash NanoPrecipitation

**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation

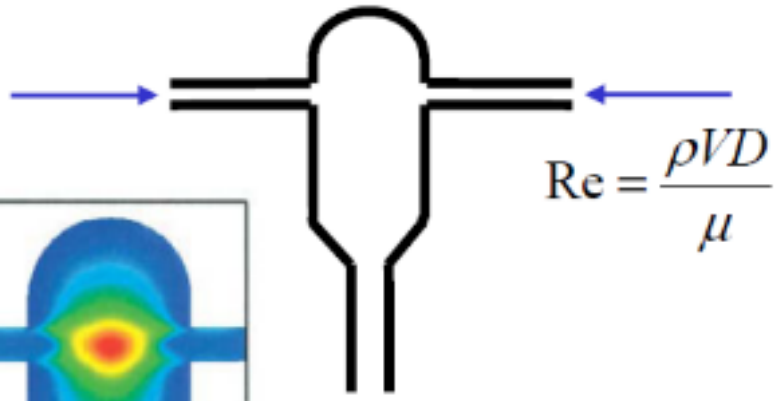



*Key process advantages:*
*- scalability and reproducibility*
*- continuous operation*

*Key challenges:*
*- APIs with low or intermediate hydrophobicity*
*- cost of excipients (global health, agchem applications)*

# Flash NanoPrecipitation scalability: two mixer geometries

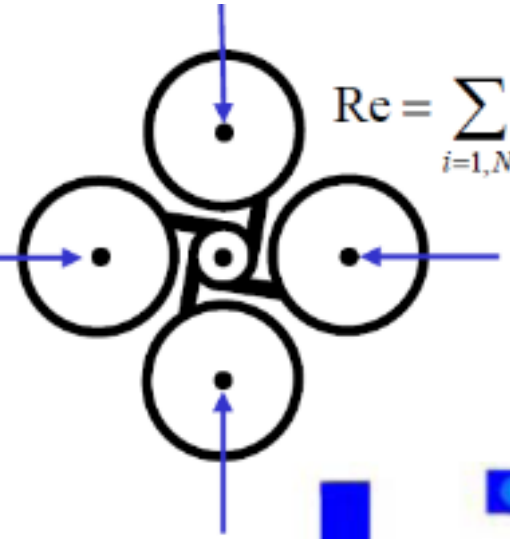


$$\mathrm{Re} = \frac{\rho V D}{\mu}$$

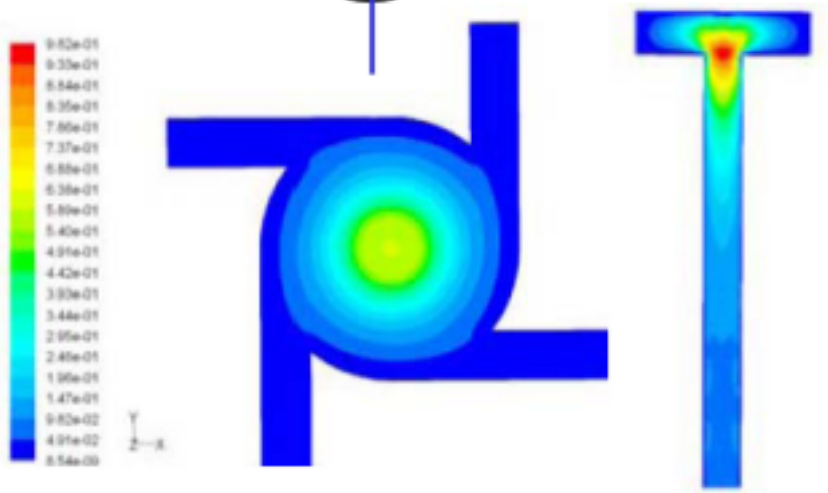
Micro-mixing by impinging streams of equal momentum

Micro-mixing by tangential streams of equal/non-equal momentum

$$\mathrm{Re} = \sum_{i=1,N} \frac{\rho_i V_i D_i}{\mu_i}$$



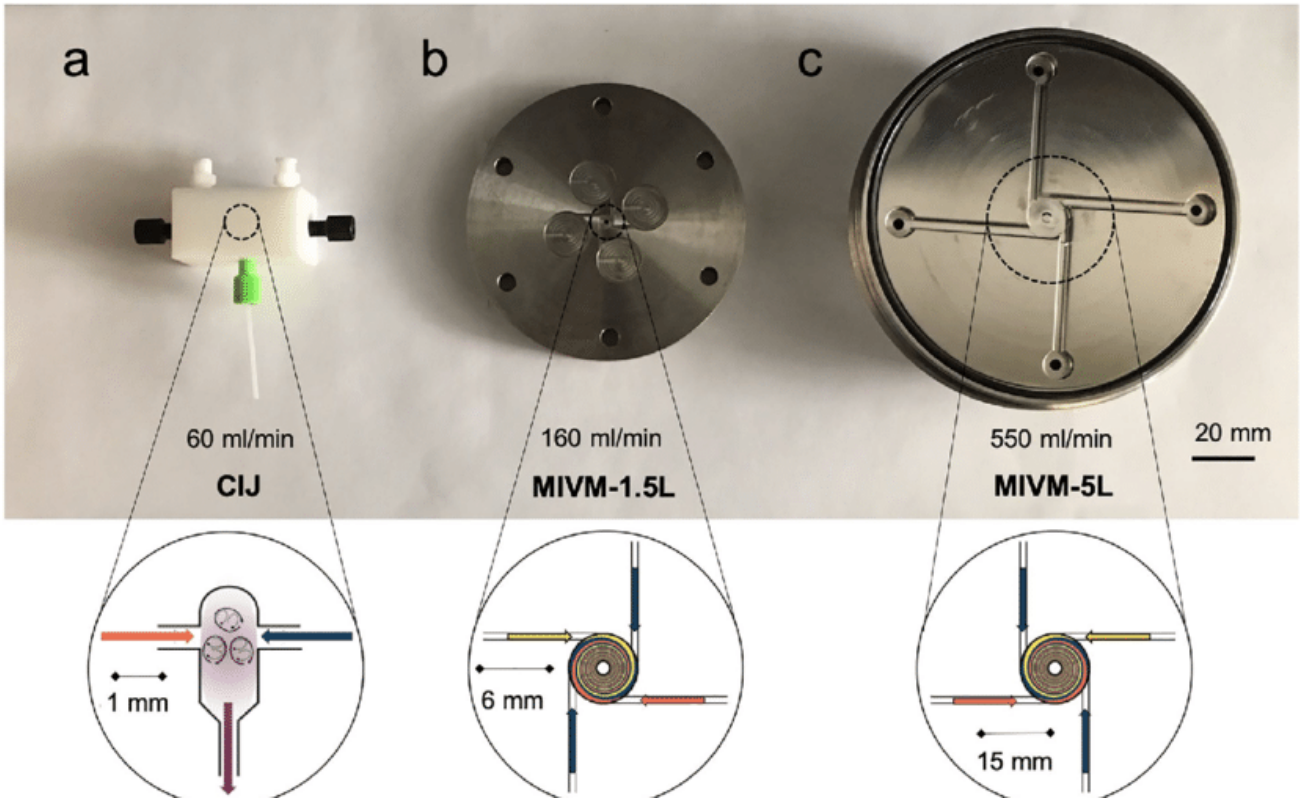
Re = Reynold's Number
ρ = density
V = velocity
D = characteristic dimension
μ = viscosity



Y. Liu, R.K. Prud'homme *et al.*, *Chem. Eng. Sci.* 63 (2008) 2892-2842
B.K. Johnson and R.K. Prud'homme, *AIChE J.* 49(2002) 2264-2282

# Flash NanoPrecipitation scalability

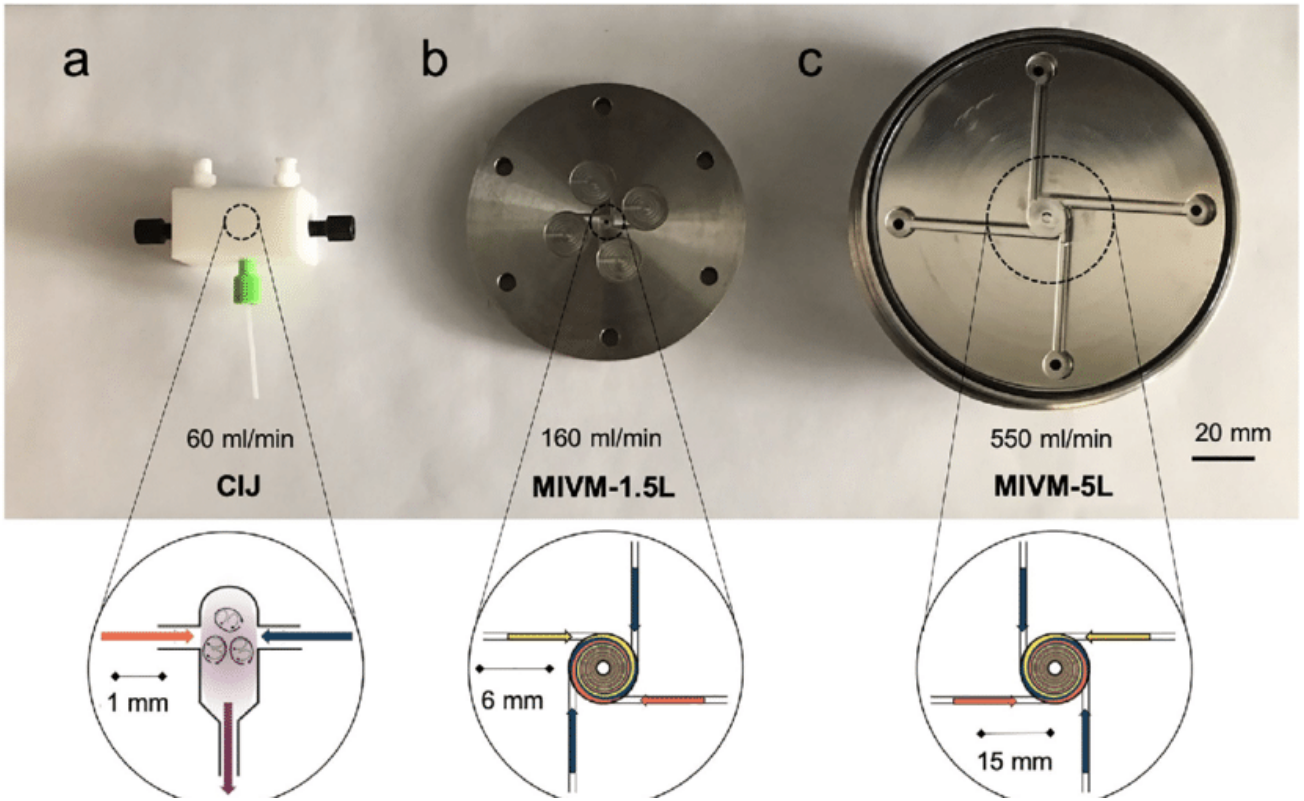
FNP mixers scaled up to 5L/min and down to 5mL batches

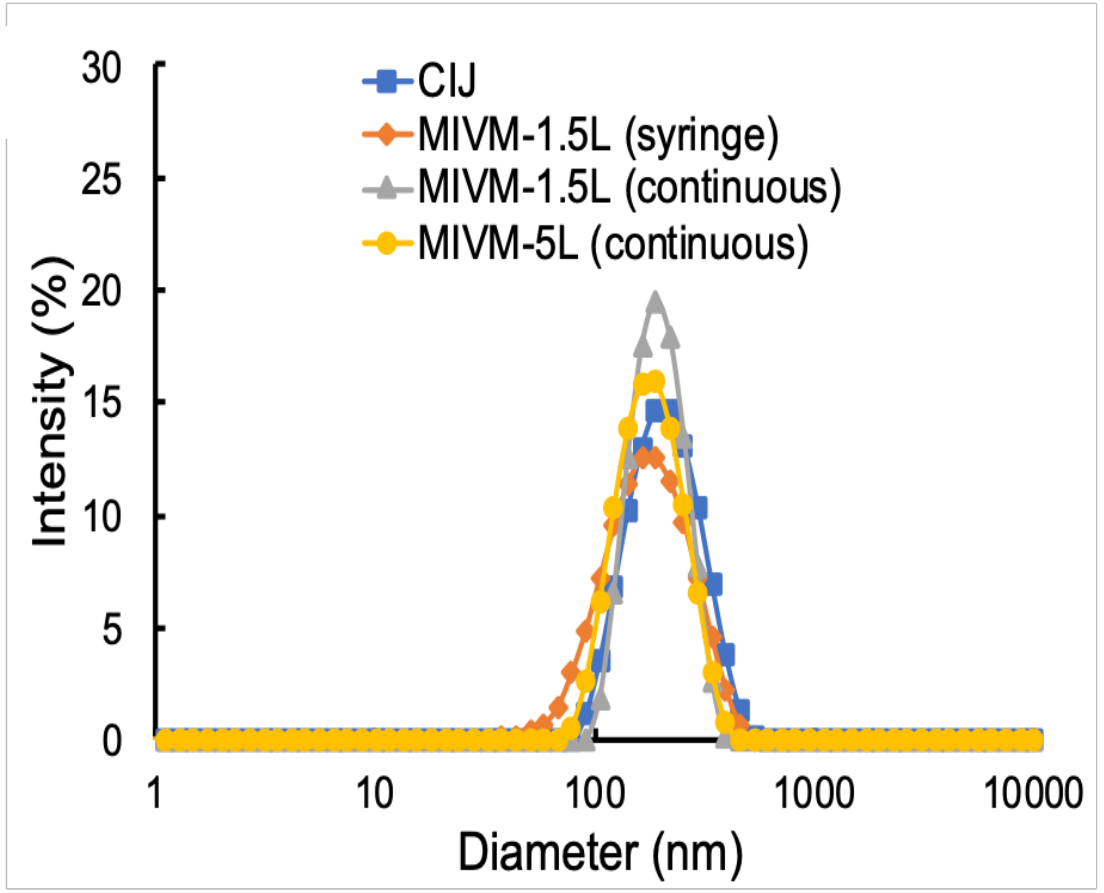



| ***Turbulent* flow mixing (FNP):** | ***Laminar* flow mixing (microfluidics):** |
|---|---|
| $\frac{\rho\, v\uparrow d\uparrow}{\mu} = Re = \frac{\rho\, v\downarrow d\downarrow}{\mu}$ | |
| *High velocities* | *Low velocities* |
| *Large channels* | *Small channels* |
| $\frac{\pi\, v\uparrow d^2\uparrow}{4} = Q = \frac{\pi\, v\downarrow d^2\downarrow}{4}$ | |
| *High throughput* | *Low throughput* |
| *Fast mixing* | *Slower mixing* |

# Flash NanoPrecipitation scalability

FNP mixers scaled up to 5L/min and down to 5mL batches

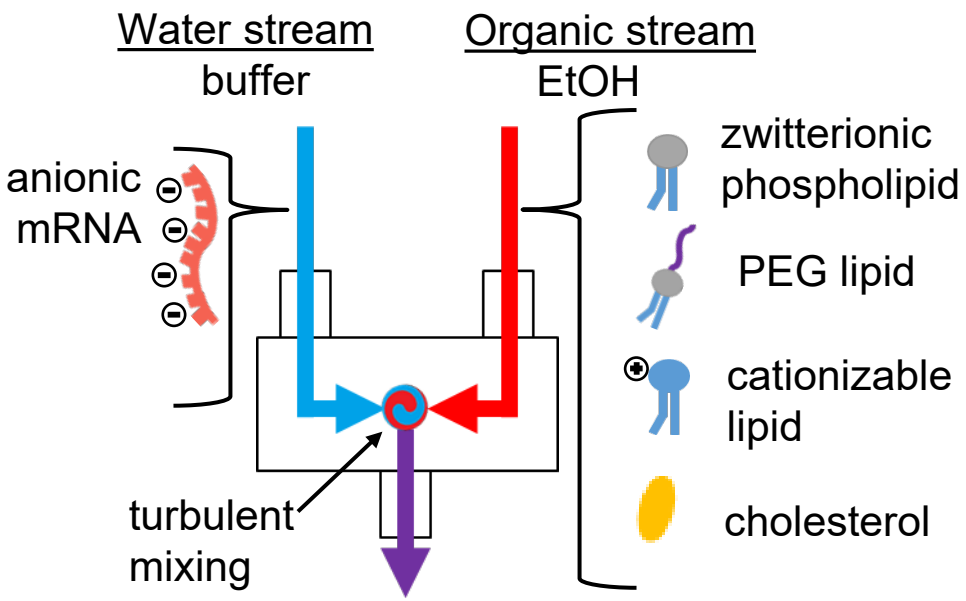


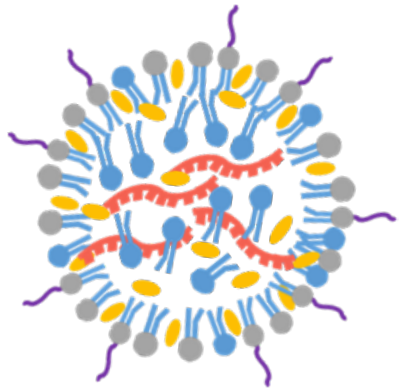




***NP size and PDI are the same in 5mL batch, 30L batch, and 3000L batch.***

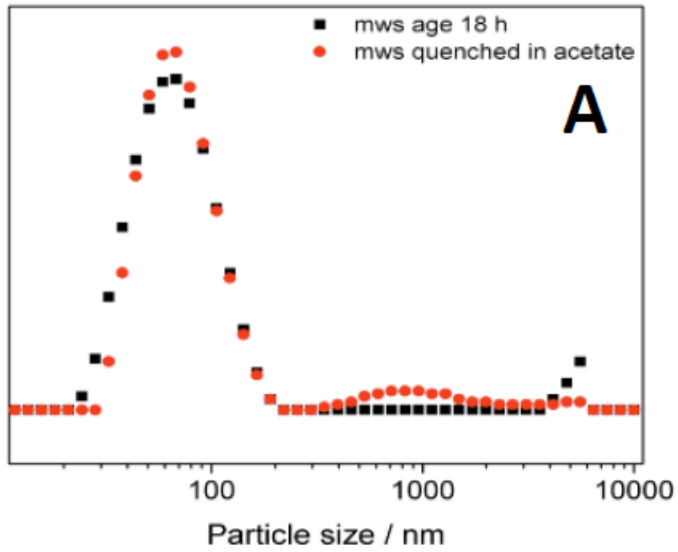
Armstrong *et al.* (2023) DOI: 10.1016/j.xphs.2023.04.003

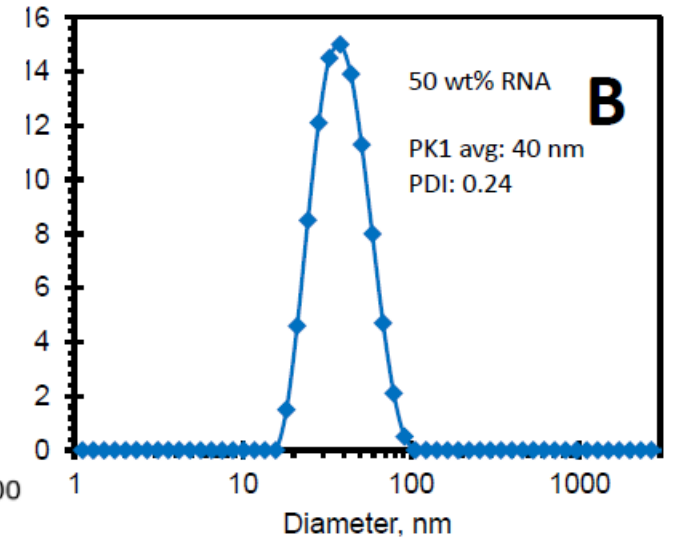
# Flash NanoPrecipitation for lipid nanoparticles

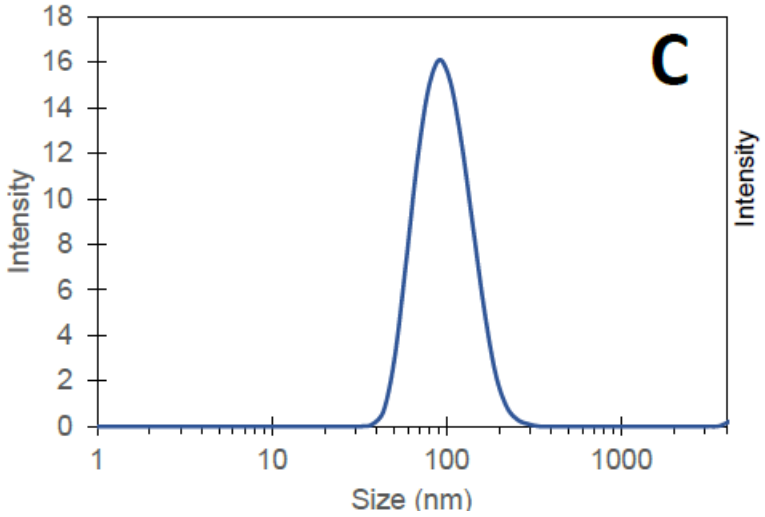


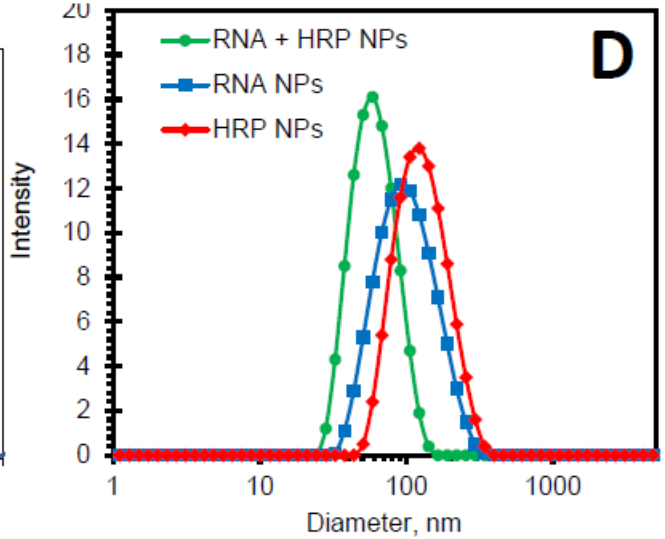



lipid nanoparticle encapsulating mRNA

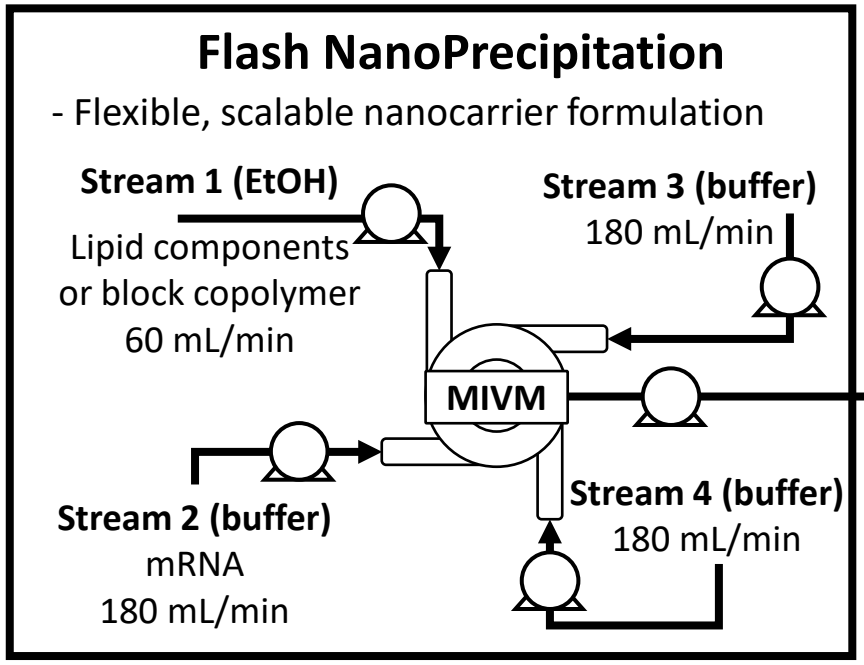


# Overall process design for continuous mRNA LNP manufacture




mRNA LNPs
600 mL/min
90% water
10% EtOH

**Tangential flow filtration (TFF)**
- Buffer exchange
- Remove EtOH
- Improve NP stability
- Concentrate NPs

**Fill; freeze**
- Add cryoprotectant
- Aseptic vial filling
- Rapid freezing

**Freeze dry**
- Remove water to improve stability and storage

**Other drying operations**
- Spray drying
- Spray freeze drying
- Electrostatic spray drying

**Analytical (in-line in some places; offline in others)**

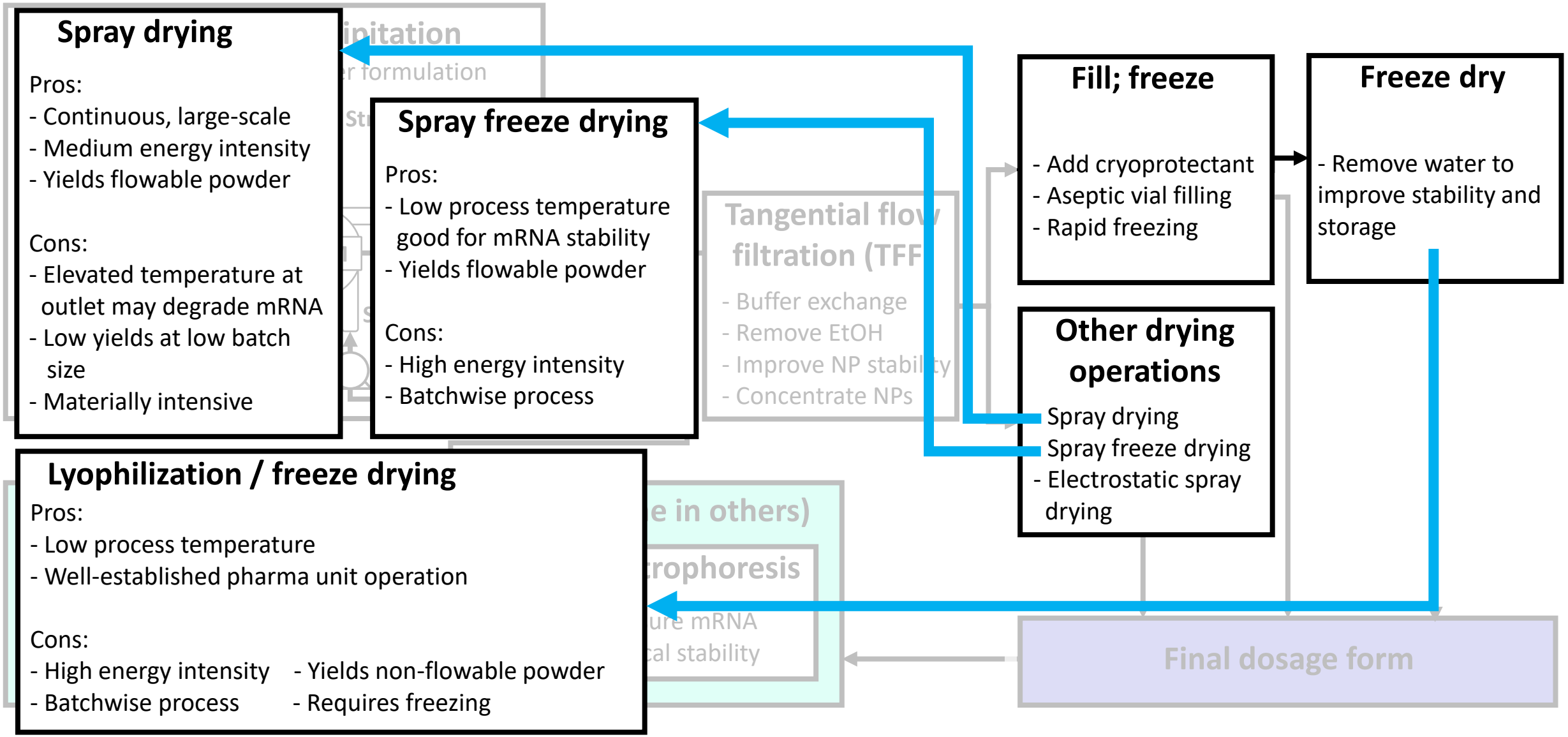
**Dynamic light scattering (DLS)**
- Monitor NP size

**Ribogreen assay**
- Measure mRNA encapsulation

**Electrophoresis**
- Measure mRNA chemical stability

**Final dosage form**

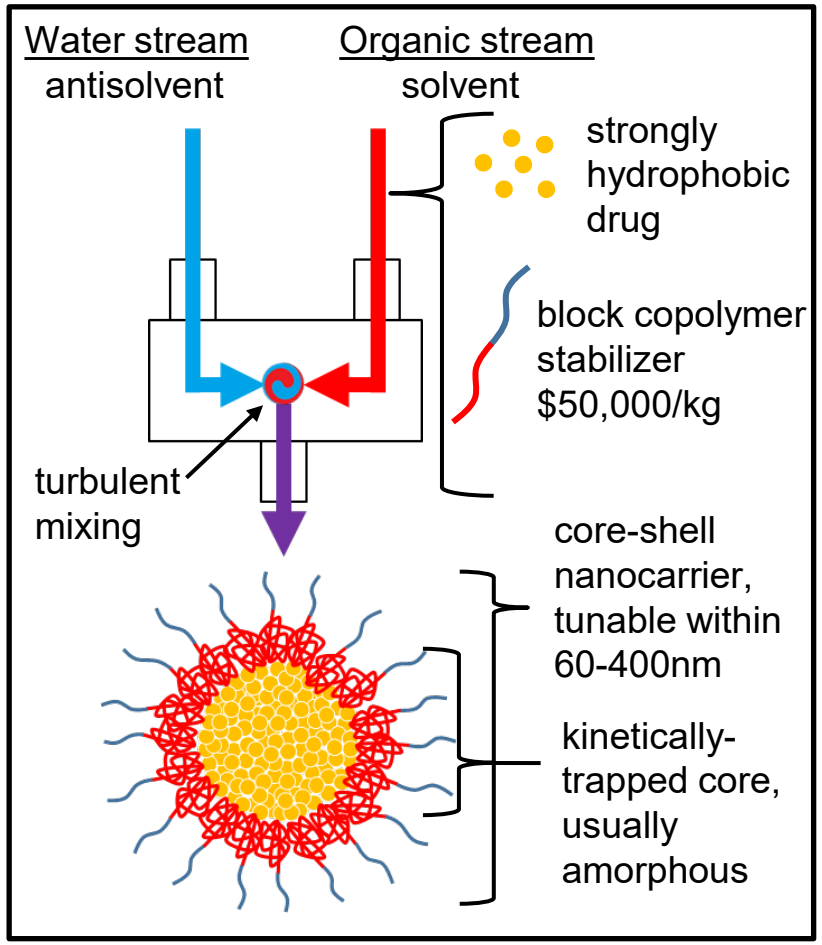
# Pros and cons of different downstream processing operations to explore



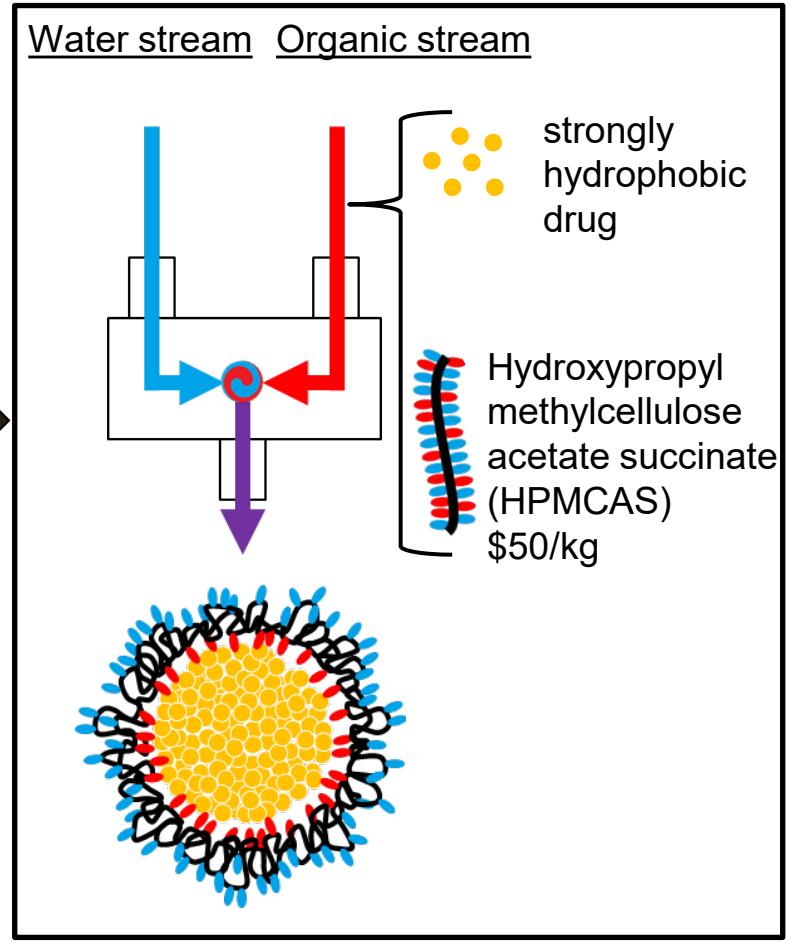
# Part 2: Selected examples of Flash NanoPrecipitation to formulate small molecules and biopharmaceuticals

# Adaptations extend traditional FNP platform to low-cost stabilizers, hydrophilic payloads

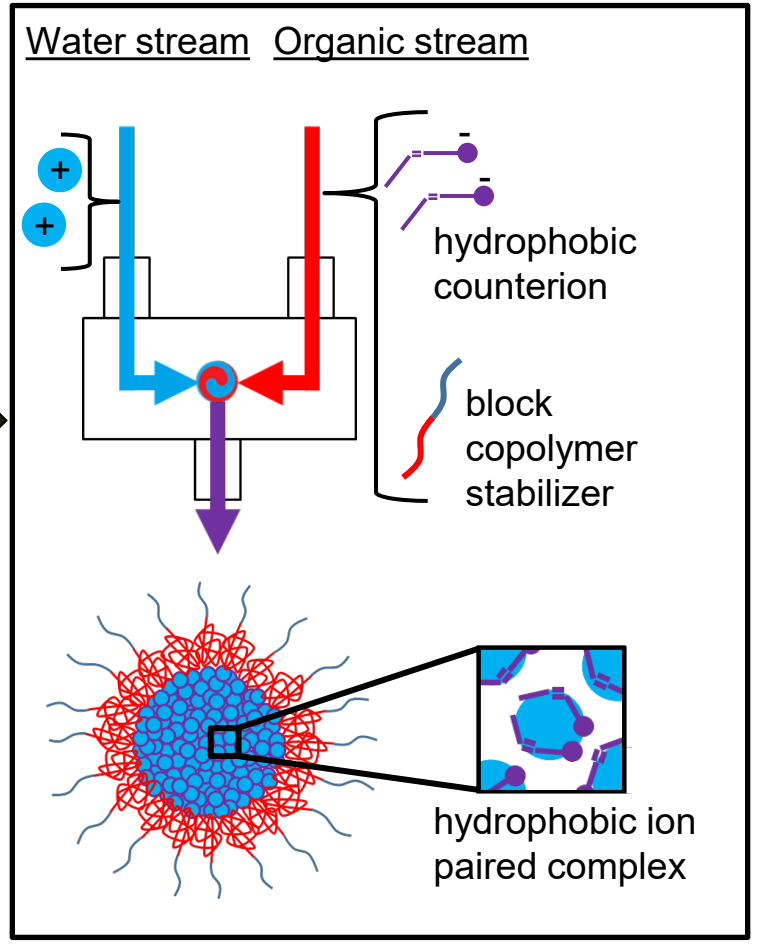
**Flash NanoPrecipitation (FNP)**
Hydrophobic molecule encapsulation




**Low-cost stabilizer**
Amphiphilic modified cellulosic

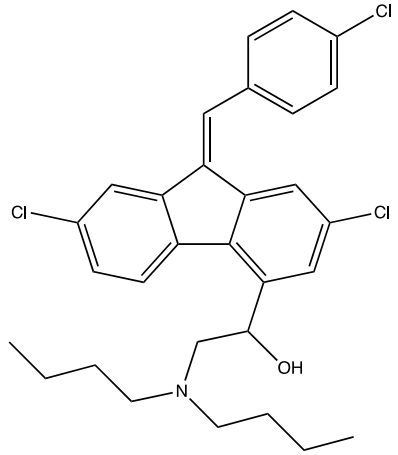



**Hydrophobic ion pairing (HIP)**
Hydrophilic molecule encapsulation



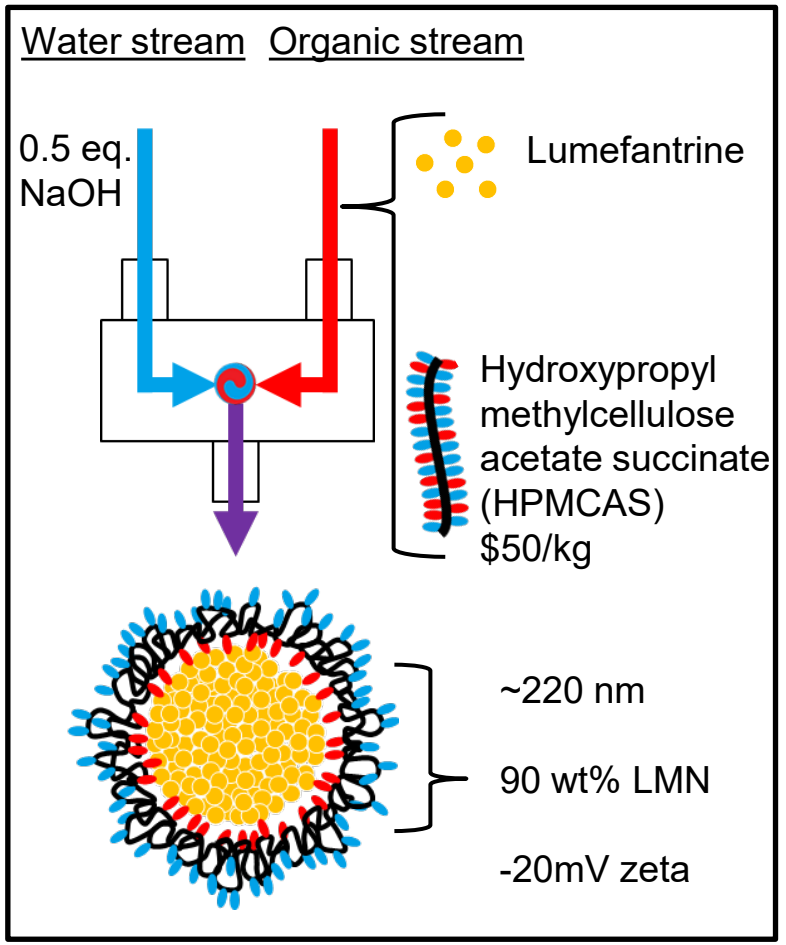
# Example 1: improving bioavailability of hydrophobic antimalarial lumefantrine

**Lumefantrine (LMN)**
- Indication: malaria
- LogP: 8.7



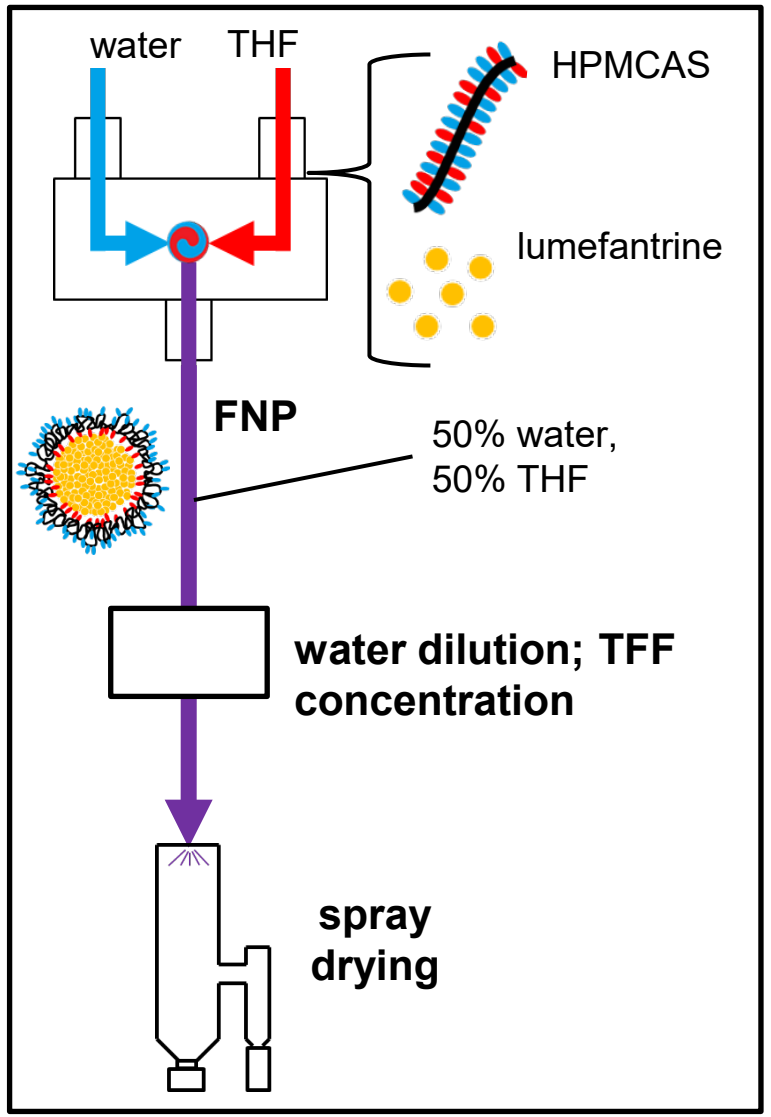

**Project objective**
- Develop process to prepare bioavailable solid oral dosage form
- Formulation process must:
  - Be continuous
  - Be scalable to ~4000 kg API/yr
  - Add no more than $0.60 per dose (material + processing)
  - Yield a dry, water-dispersible powder, stable in hot/humid climates without protective packaging (powder sachet only)

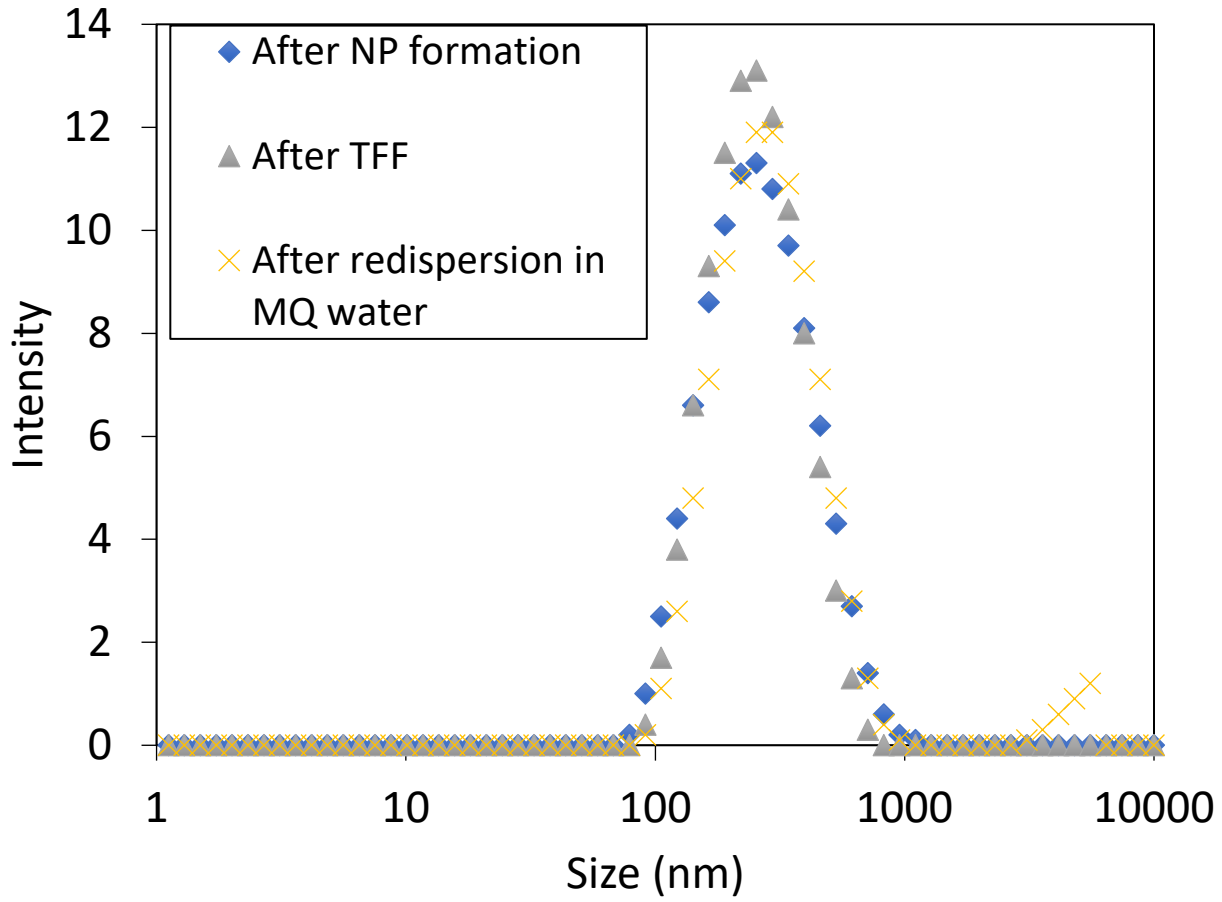
**Formed NPs encapsulating LMN using HPMCAS as stabilizer**




Feng *et al.* (2019) DOI: 10.1039/c8sm02418a
Ristroph *et al.* (2019) DOI: 10.3791/58757

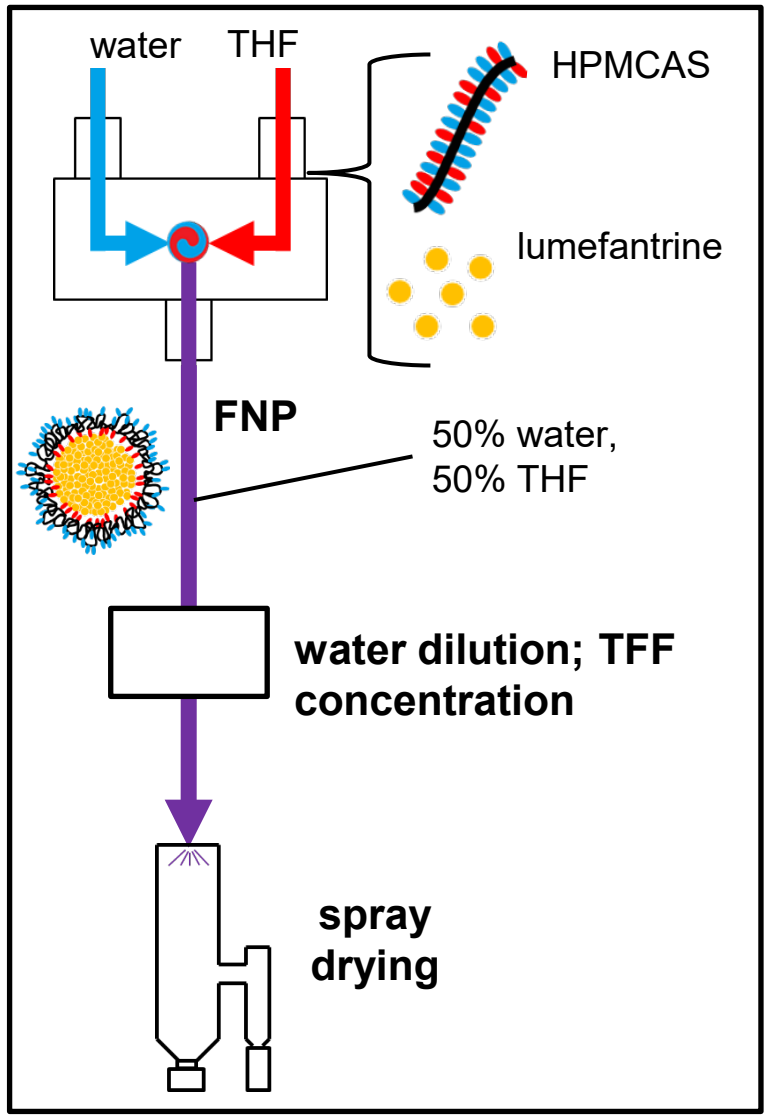
# Downstream unit operations concentrate and dry NPs; size stable throughout

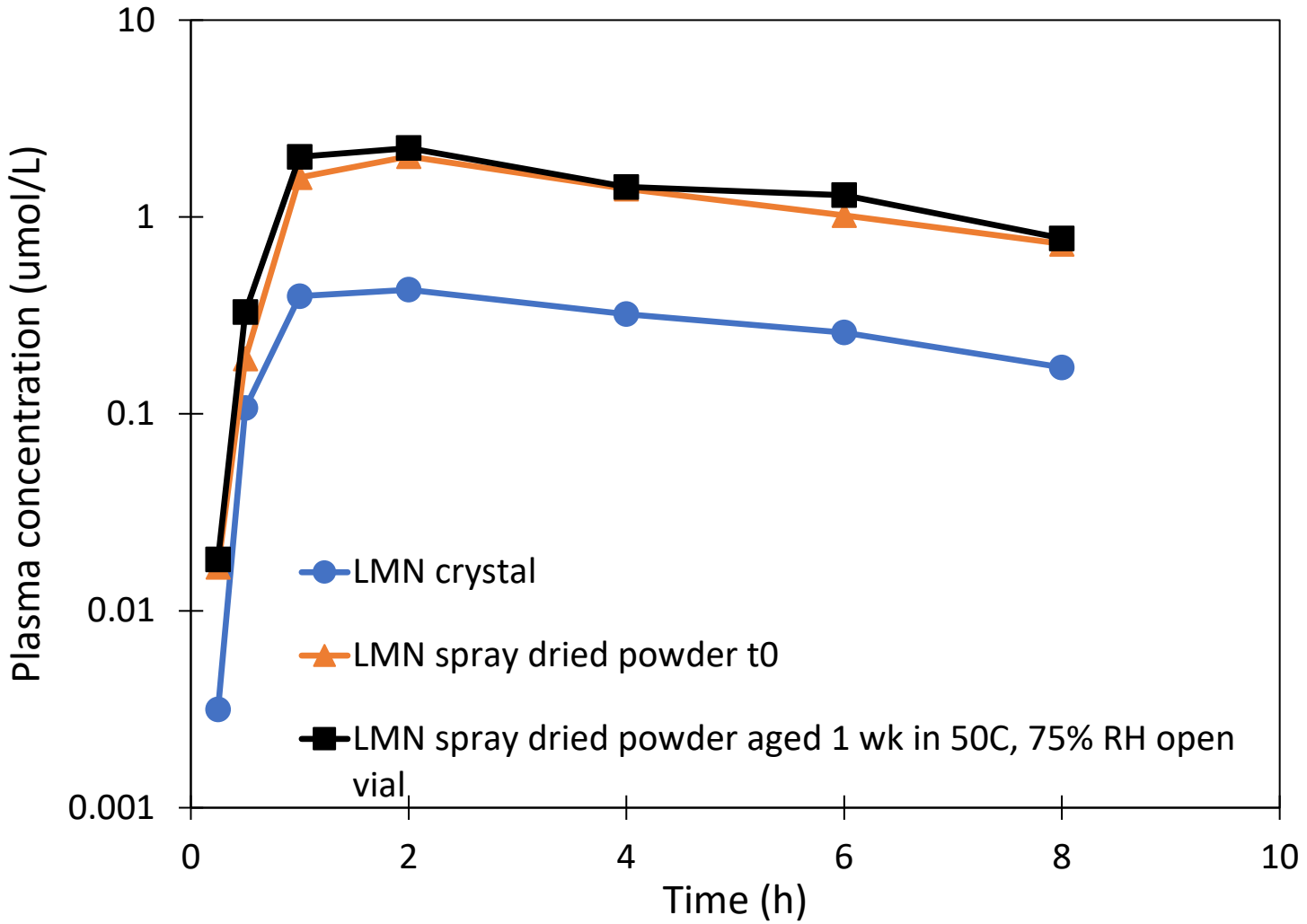



Armstrong *et al.* (2023) DOI: 10.1016/j.xphs.2023.04.003

# LMF bioavailability improved 4.2x compared to crystalline API, through aging




Armstrong *et al.* (2023) DOI: 10.1016/j.xphs.2023.04.003

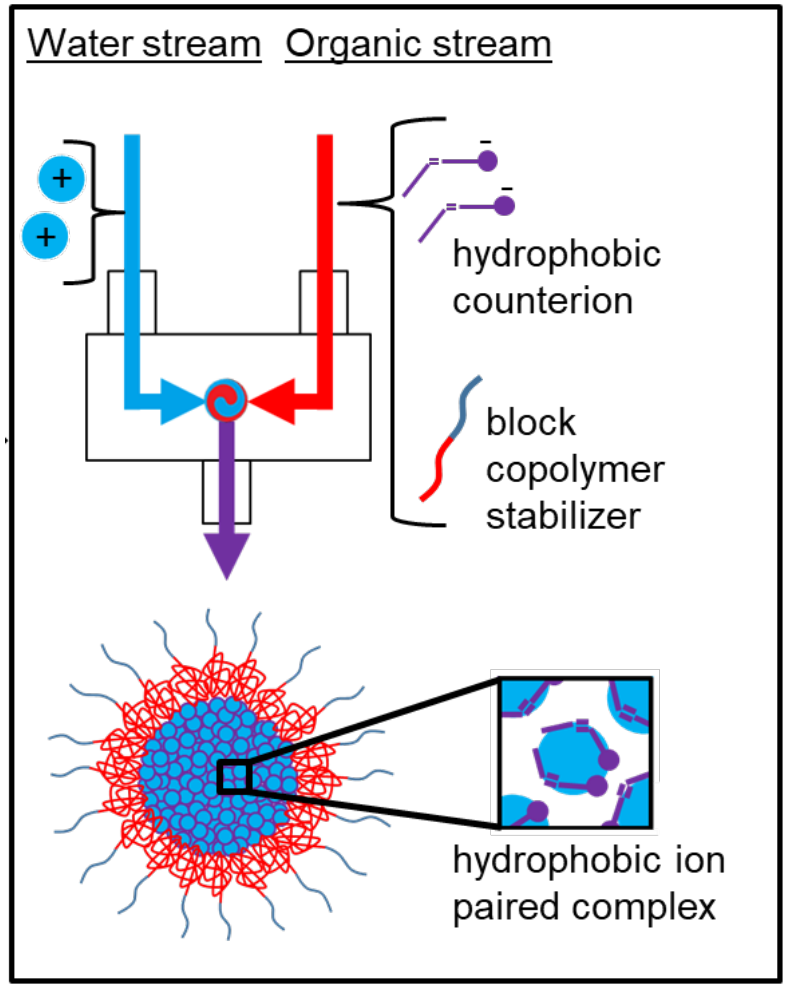
# Example 2: FNP with hydrophobic ion pairing to encapsulate biologics

- **Polymyxin B (PMB)**
  - Model hydrophilic antibiotic peptide with structural complexity & therapeutic relevance

- **Objective**
  - Increase encapsulation efficiency (EE) and loading of PMB NCs in FNP by incorporating hydrophobic ion pairing (HIP)

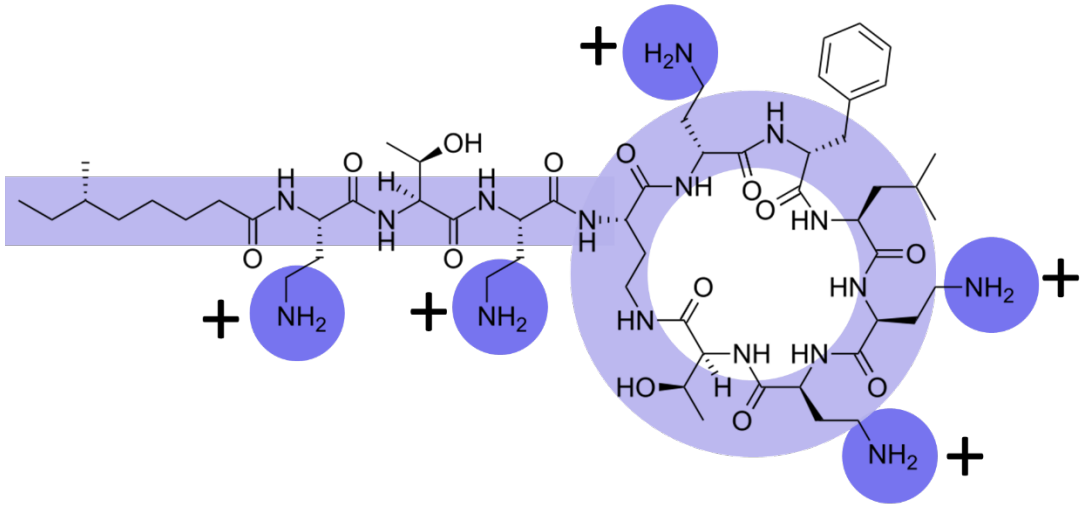
- **Specific goals**
  - Demonstrate feasibility of FNP with HIP for efficient biologics encapsulation
  - Identify major variables governing drug release; develop controlled-release formulations
  - Develop mechanistic understanding for controlling biologic release from NCs

**Hydrophobic ion pairing (HIP)**
Hydrophilic molecule encapsulation

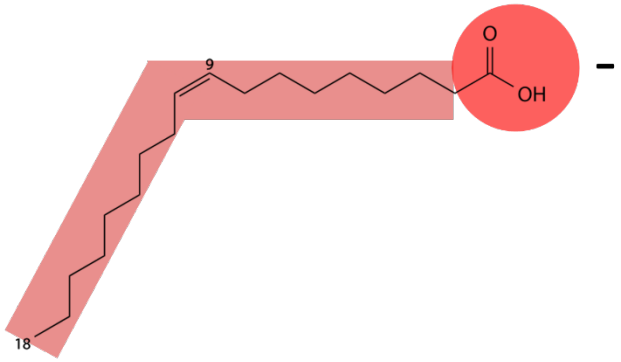


# Preparing NCs of antibacterial peptide polymyxin B

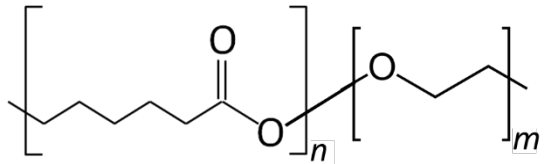
Model biologic: **polymyxin B (PMB)**



Counterion: **oleate (OL)**



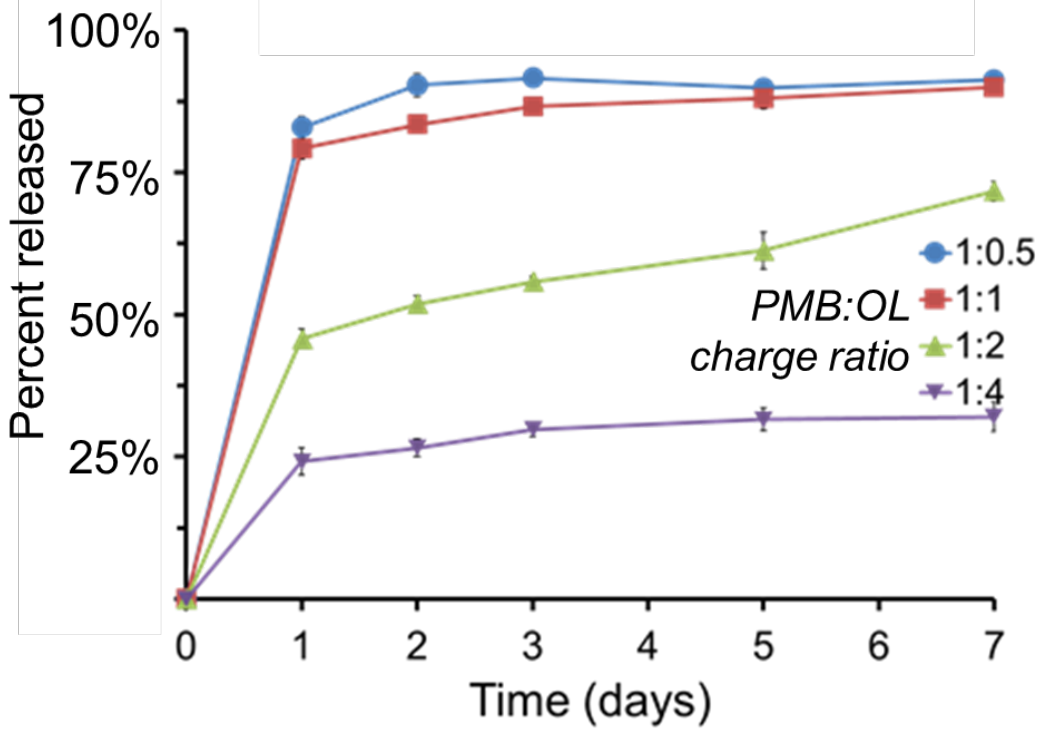
Stabilizer: **PCL-b-PEG**



**Results:**

- NCs formed with 120nm in diameter (tunable) at 90-100% EE and 30-40% PMB loading

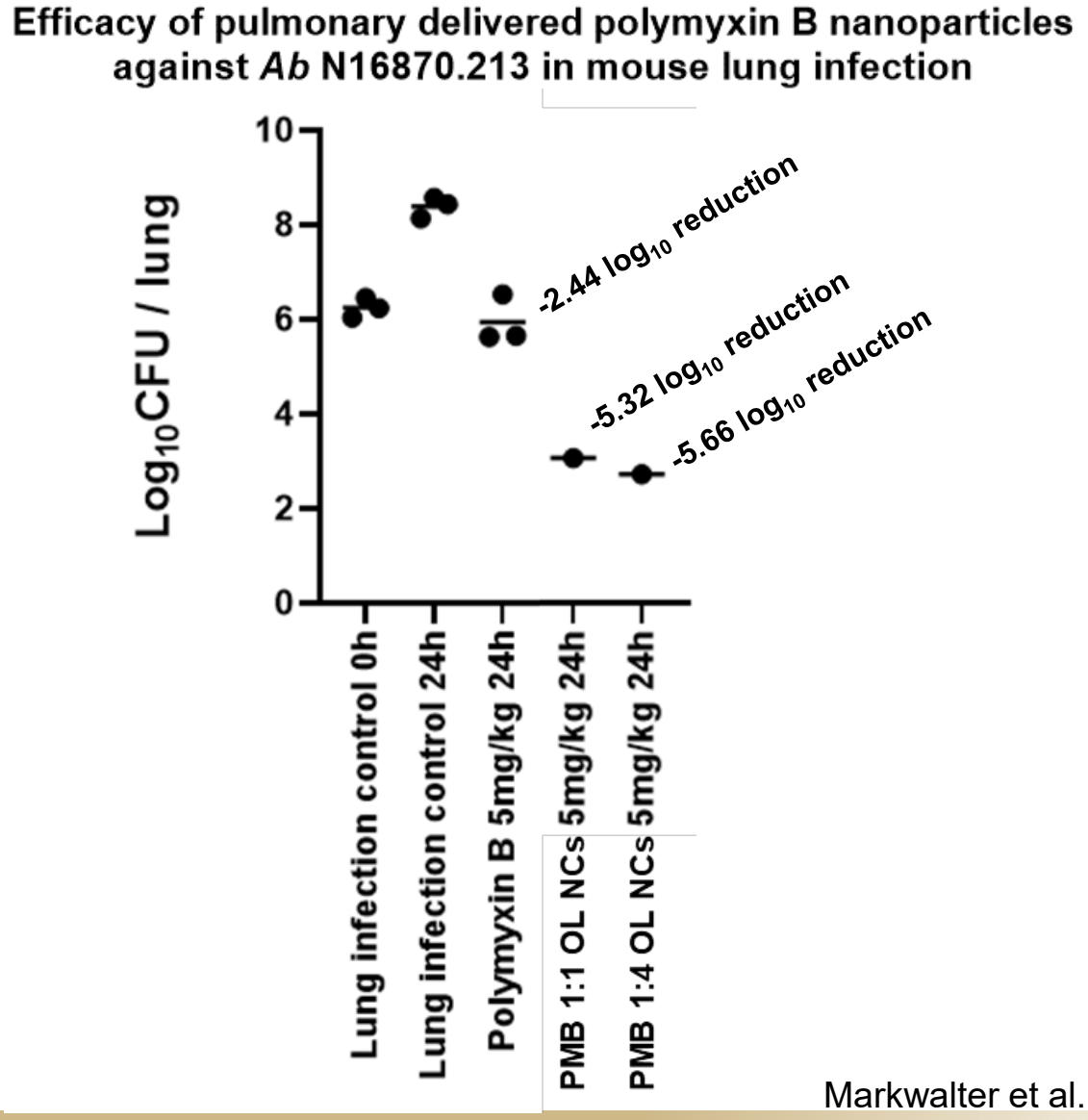
**PMB release in buffer varies with charge ratio**




Lu et al., *Mol. Pharm.* 2018

# *In vivo* efficacy of slow-releasing PMB NC formulations (with Jian Li, Monash)

- Bacterial isolate: *A. baumannii* N16870.213
- $1x10^5$ CFU in 25uL per lung (IT)
- Drug dosing route: intratracheal delivery, 25uL per mouse
- PMB NC formulations reduce *Ab.* CFU by more than 3 $log_{10}$ units compared to aqueous PMB after 24h.

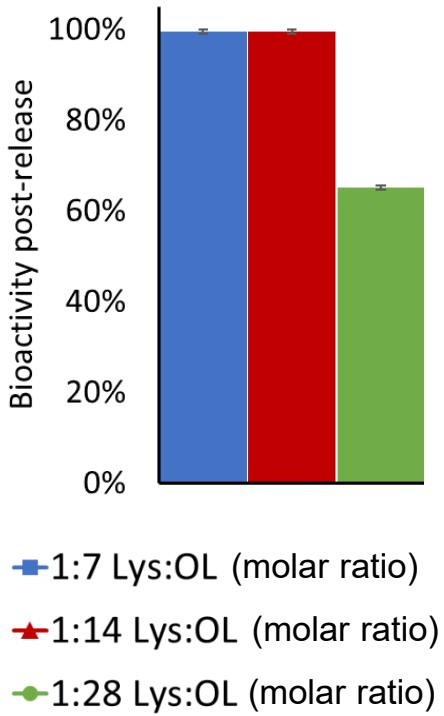



Markwalter et al., *J. Con. Rel.* 2021

# Example 3: protein encapsulation by FNP with HIP

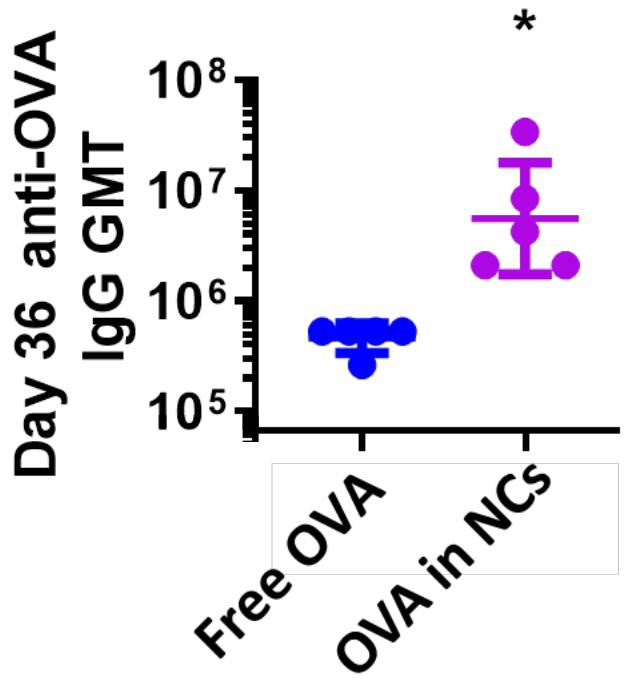
**Lysozyme** (Lys): 14.4 kDa, pI = 11.4

- EE: 99%  Lys loading: 39-47%
- Release tunable with charge ratio
- Up to 100% enzymatic activity post-release



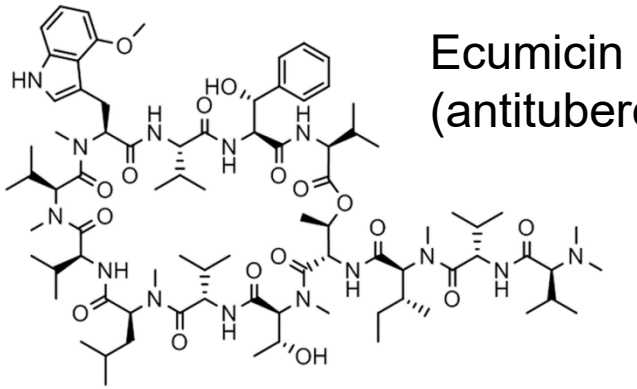

**Ovalbumin** (OVA): 43 kDa, pI = 5.2

- EE: 88%  OVA loading: 29%
- NC formulation improved immunogenicity *in vivo* in a nasal vaccine mouse model



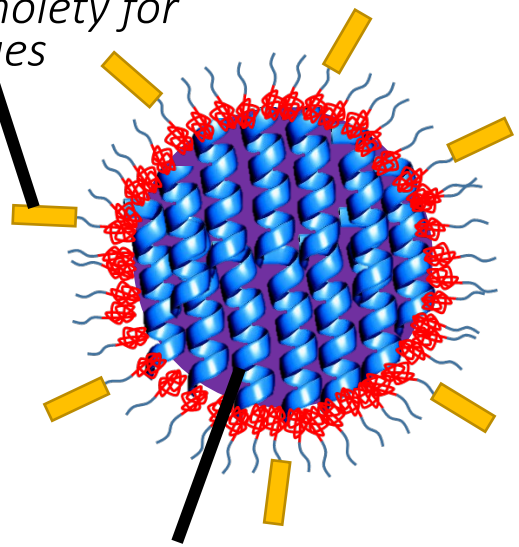

Ristroph et al., *Int. J. Pharm.* 2021

# Example 4: macrophage-targeted antitubercular NCs

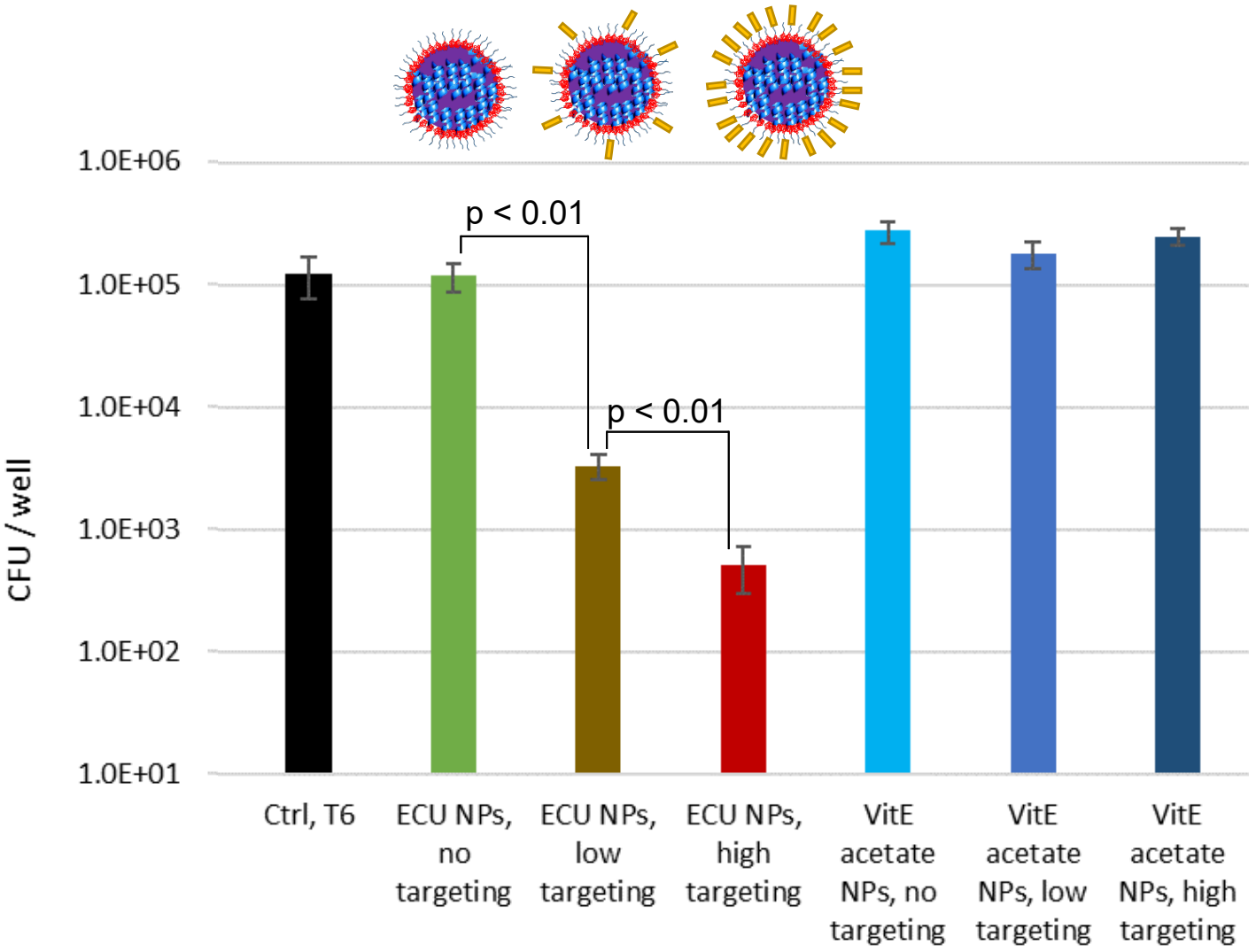


Ecumicin
(antitubercular peptide)

*Hexamannose targeting moiety for macrophages*



*Ecumicin core*




Ristroph *et al.* (2022) DOI: 10.1002/admt.202101748

# Active research areas

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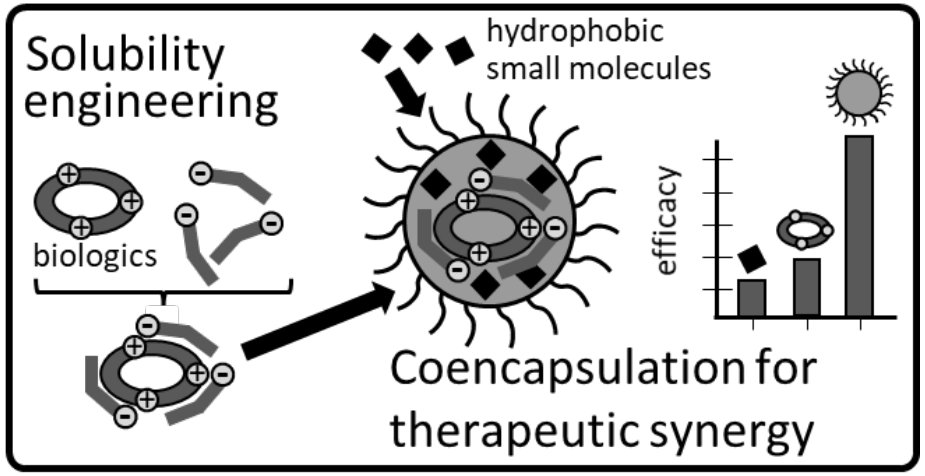
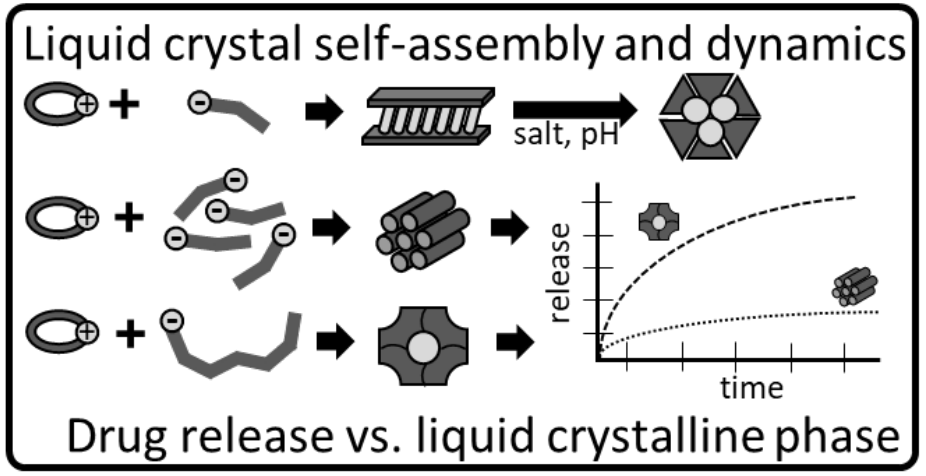
## I. Combined biologic/small molecule delivery

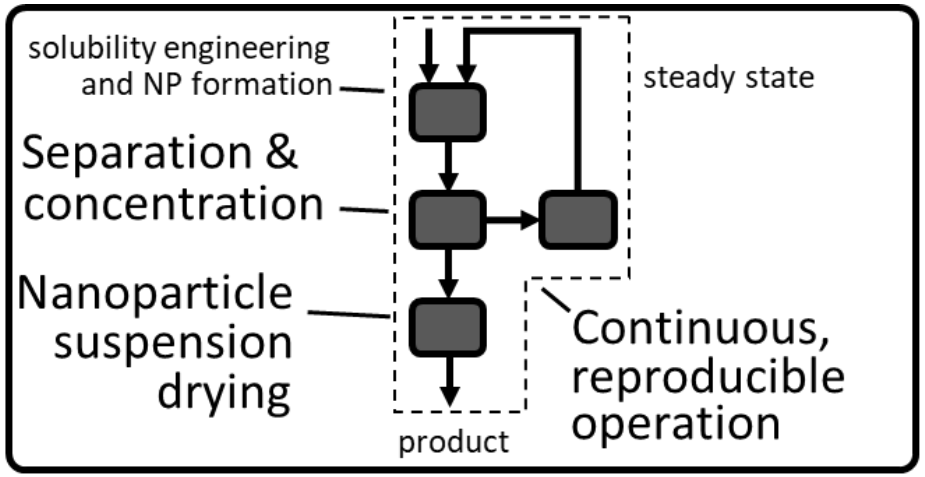



## II. Evolution of API liquid crystal phases

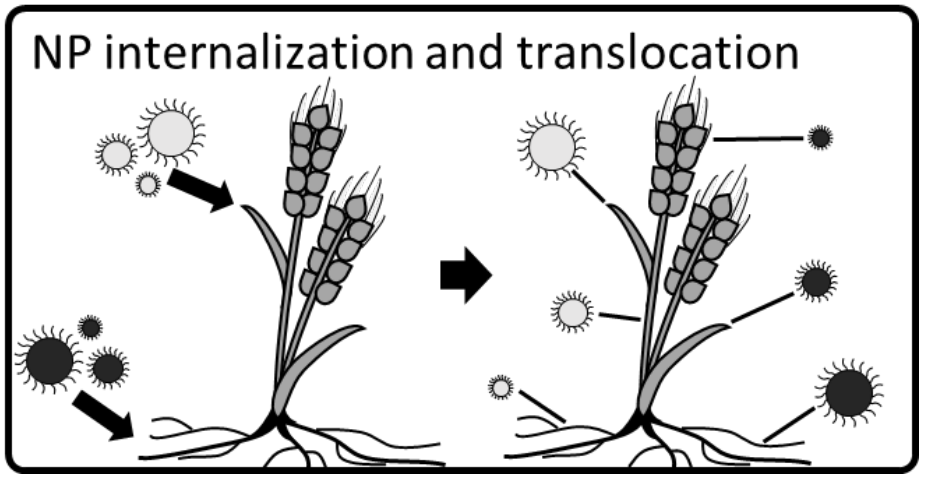



*Currently looking to hire 2 postdocs and 2-3 graduate students*

## III. Rapid unit operations for NPs at scale




## IV. Nanoparticle delivery to plants



# Acknowledgements

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Purdue
- Sophia Dasaro
- Luiza Oliveira
- Mojhdeh Baghbanbashi
- Luke Johnson
- Gabriel Harris
- Rachel Zheng

Princeton
- Robert Prud'homme
- Nick Caggiano
- Maddie Armstrong

BMGF
- Niya Bowers
- Chris Moreton
- Pius Tse
- Ellen Harrington







PRINCETON School of Engineering and Applied Science

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WuXi AppTec

MMV Medicines for Malaria Venture

MONASH University Institute of Pharmaceutical Sciences

Australian Synchrotron

USDA FDA NIH NSF

serán sparc BASF We create chemistry

Duke University School of Medicine

Carnegie Mellon University Civil and Environmental Engineering

# Purdue Agricultural & Biological Engineering


- Undergraduate and graduate programs ranked #1 or #2 nationally for 13 years
- State-of-the art building completed in 2021
- College of Agriculture: #3 nationally in 2024
- College of Engineering : #4 nationally in 2024 for graduate engineering
- engineering.purdue.edu/ABE