

An Integrated Platform for Continuous RNA Nanoparticle Formulation and Drying

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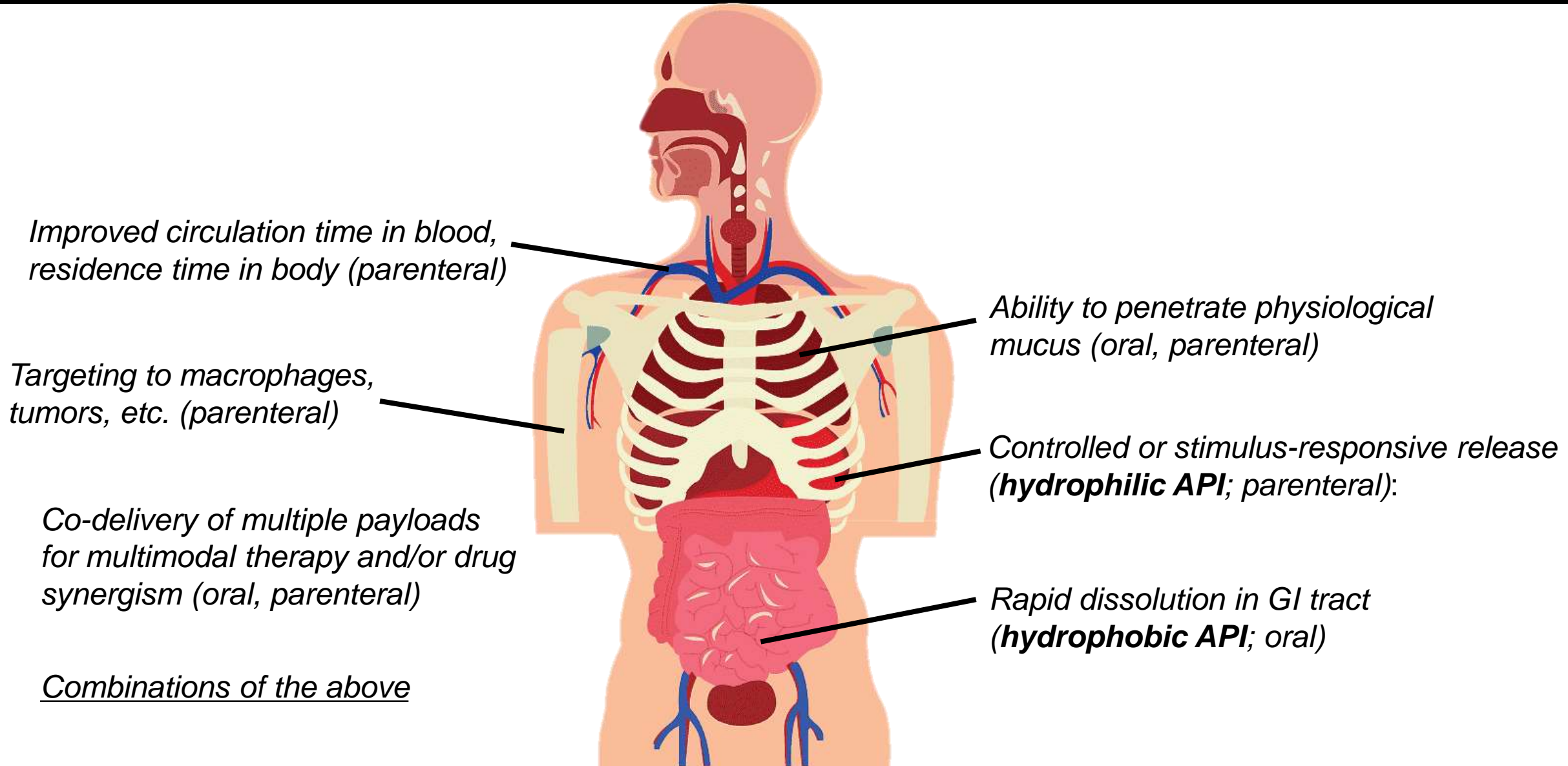
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Mark Kastantin,
Serán Bioscience

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

Potential benefits of nanoencapsulation, at a glance



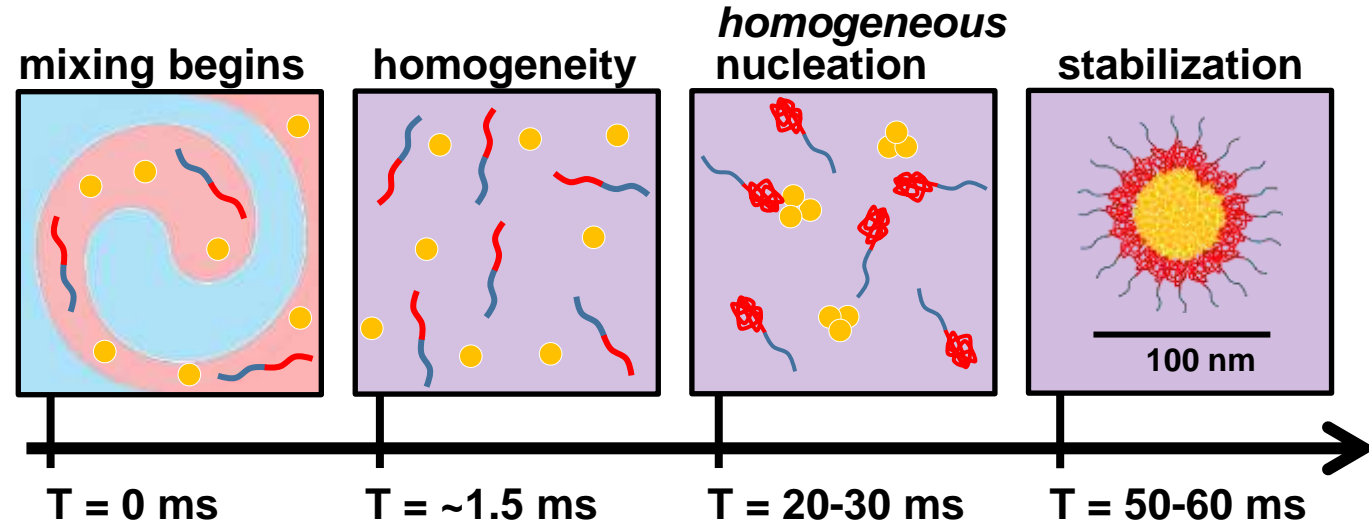
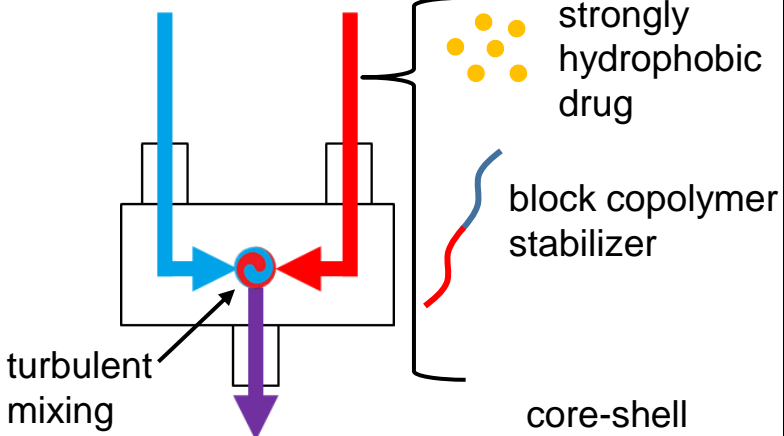
Nanoparticle formulation technique: Flash NanoPrecipitation

Flash NanoPrecipitation (FNP)

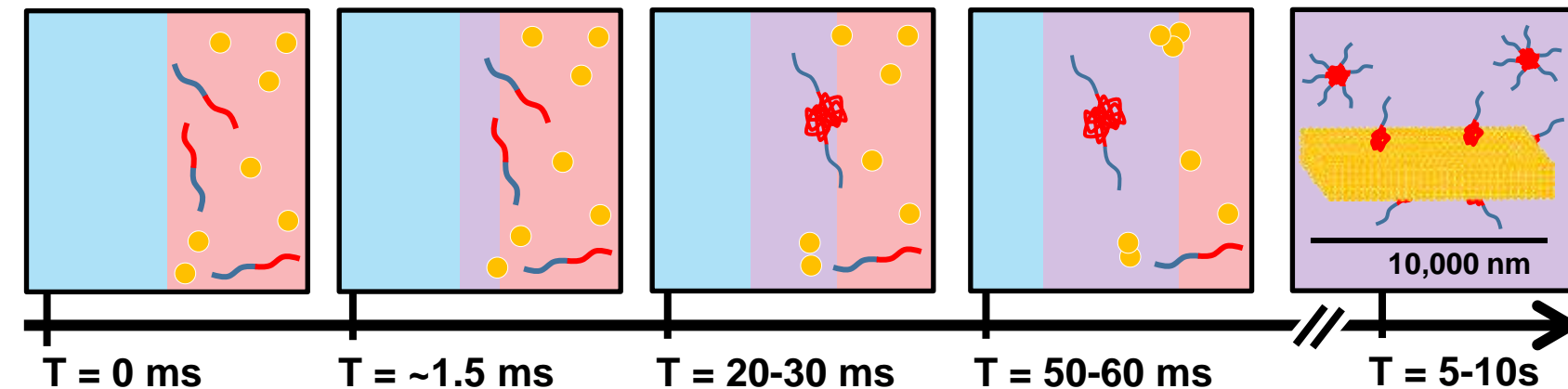
Hydrophobic molecule encapsulation

Water stream
antisolvent

Organic stream
solvent



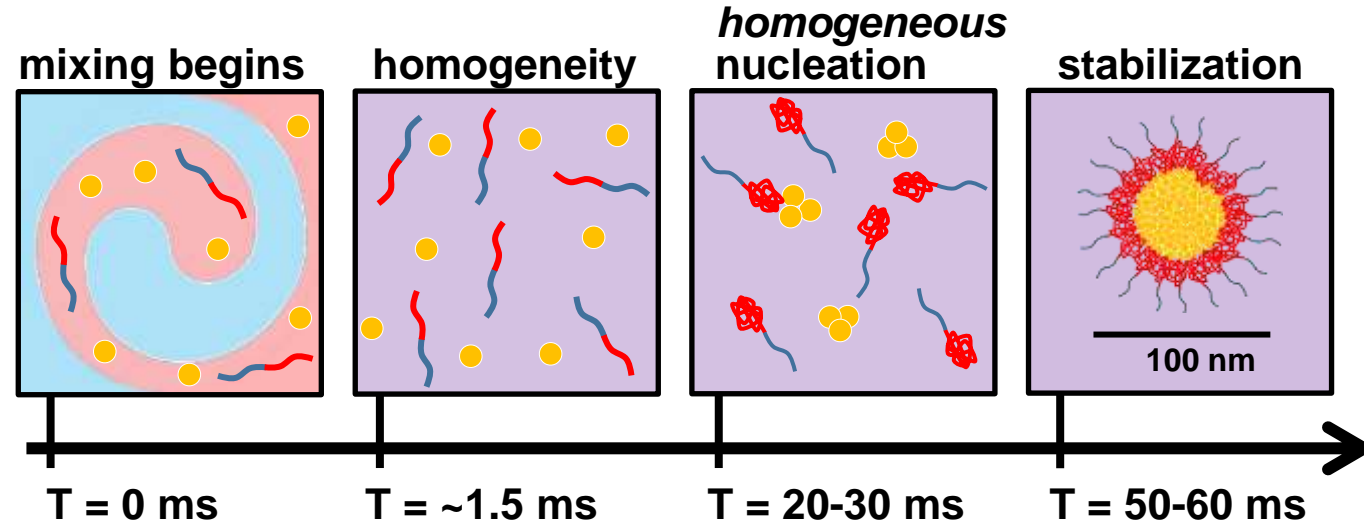
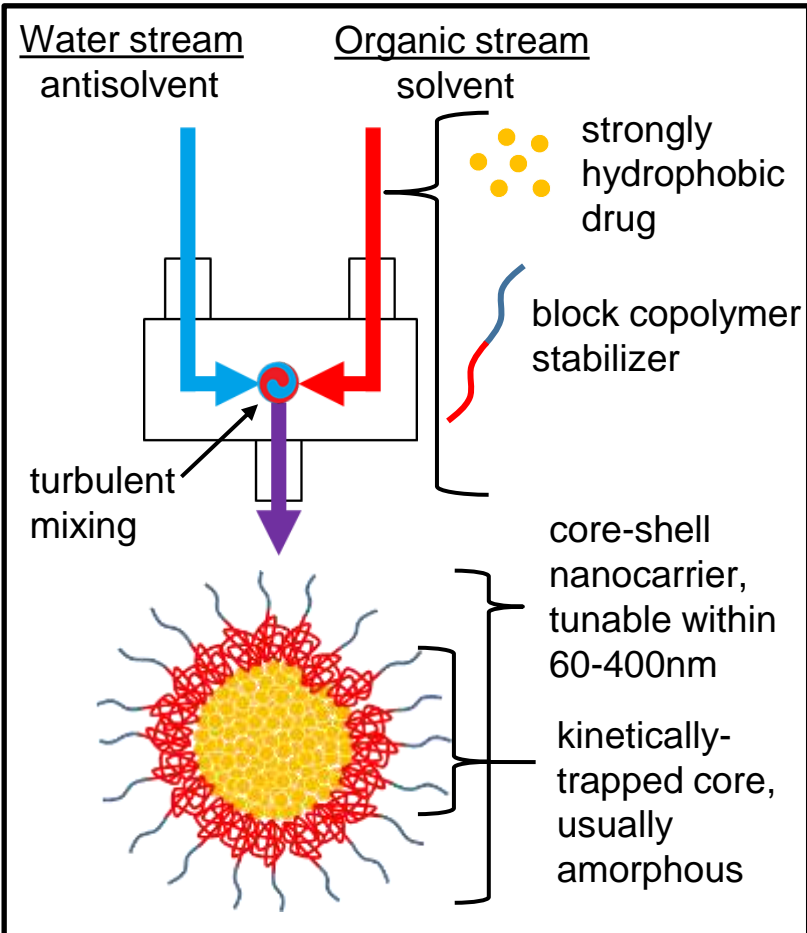
“Bulk nanoprecipitation” poor mixing:



Nanoparticle formulation technique: Flash NanoPrecipitation

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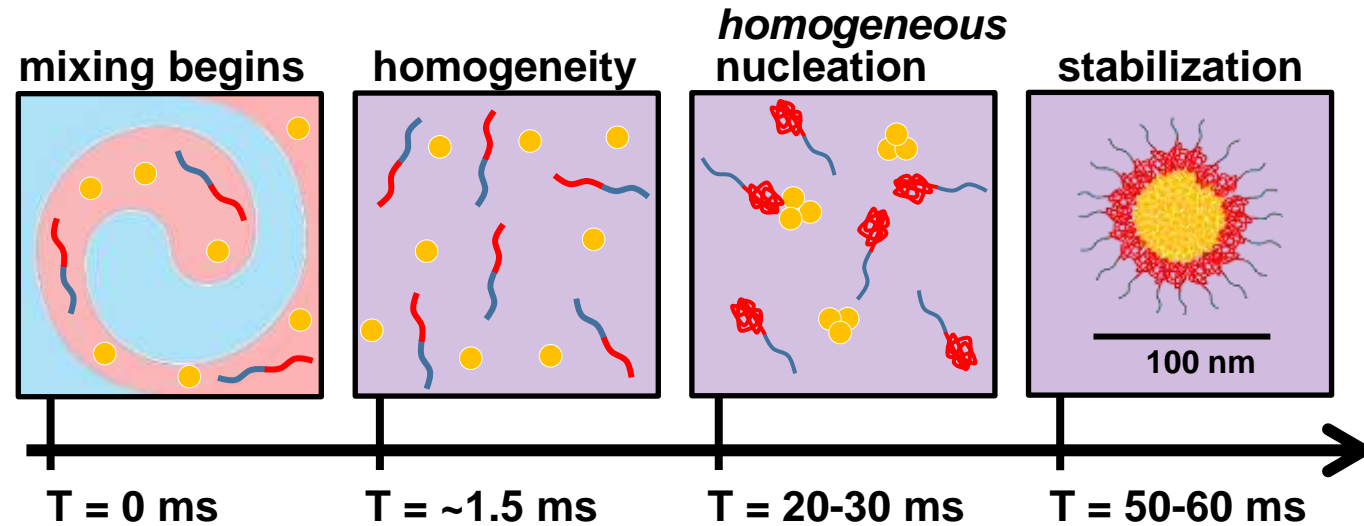
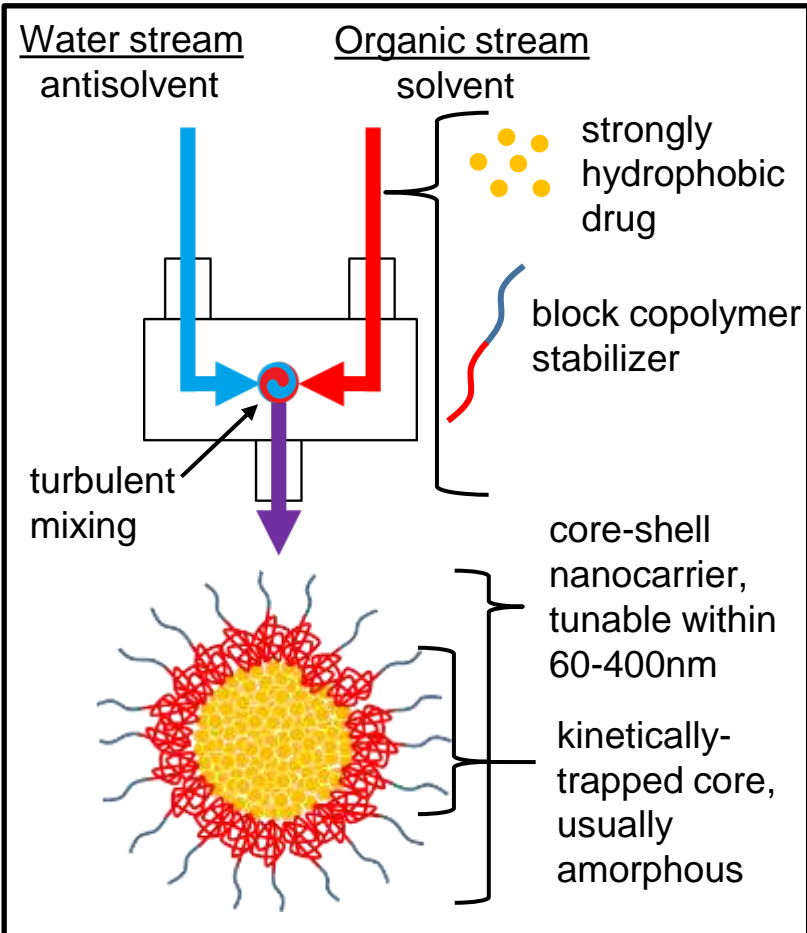
Key points for nanoprecipitation:

- 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



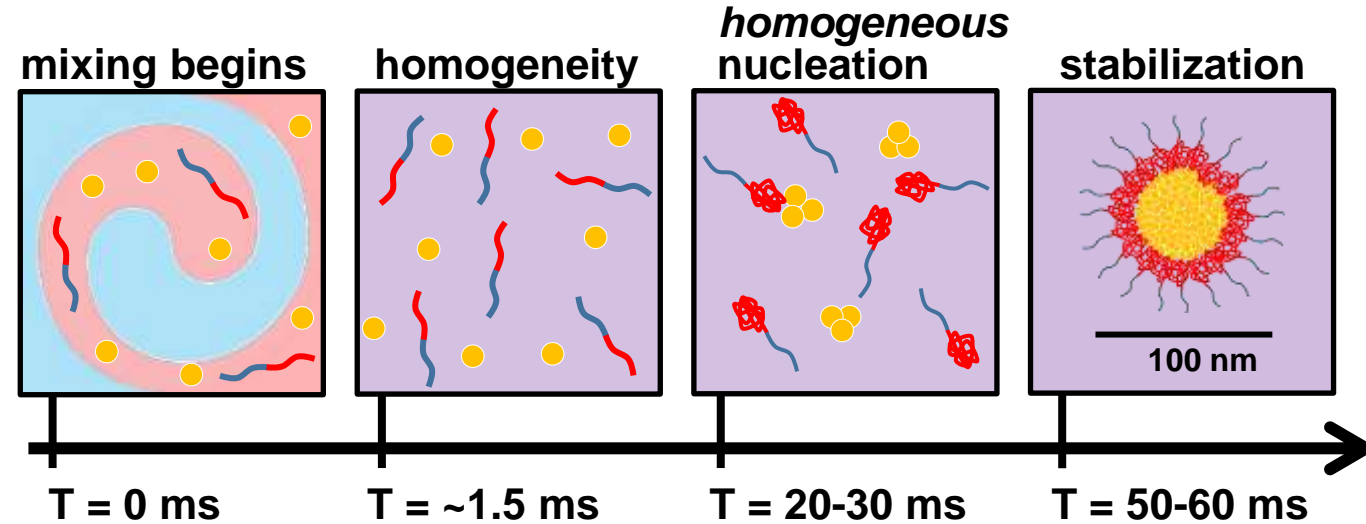
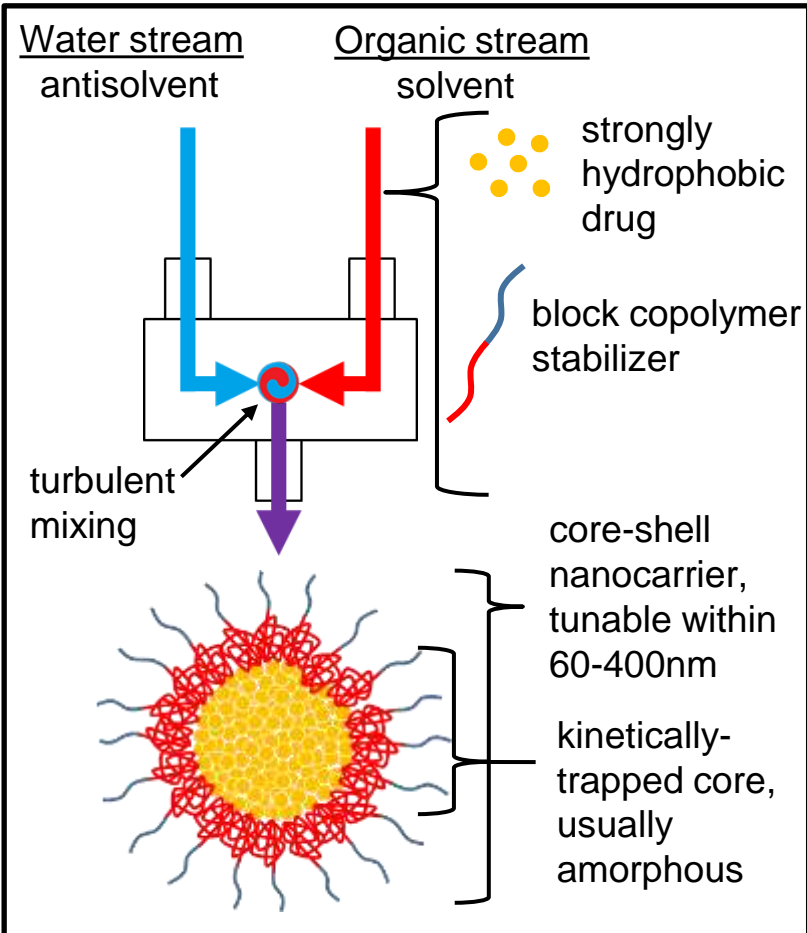
Nanoparticles made by FNP:

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

Nanoparticle formulation technique: Flash NanoPrecipitation

Flash NanoPrecipitation (FNP)

Hydrophobic molecule encapsulation



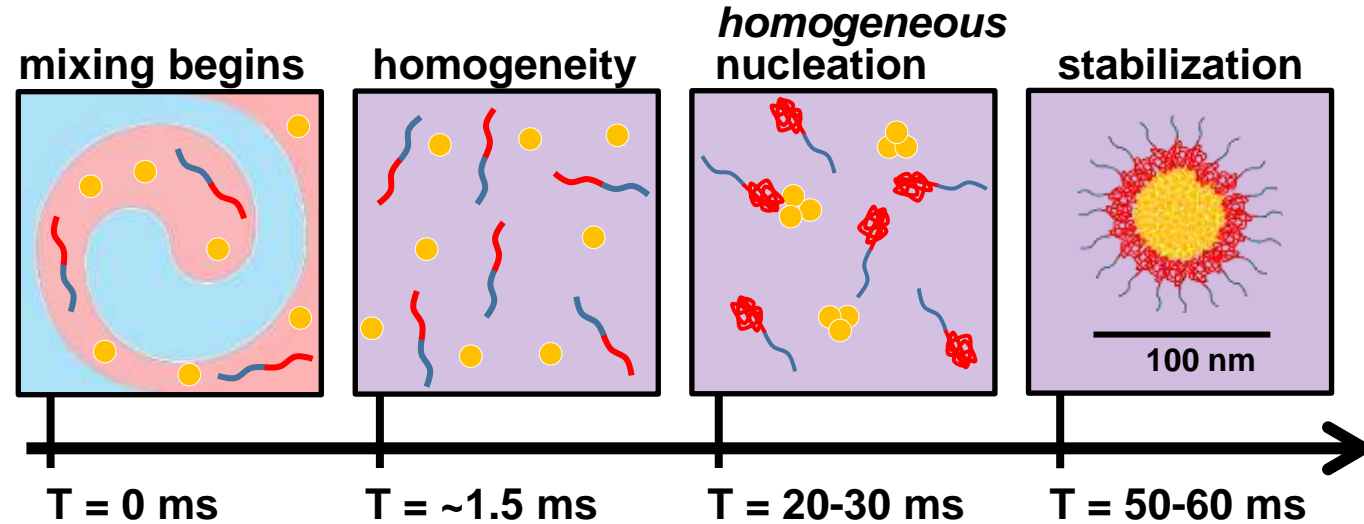
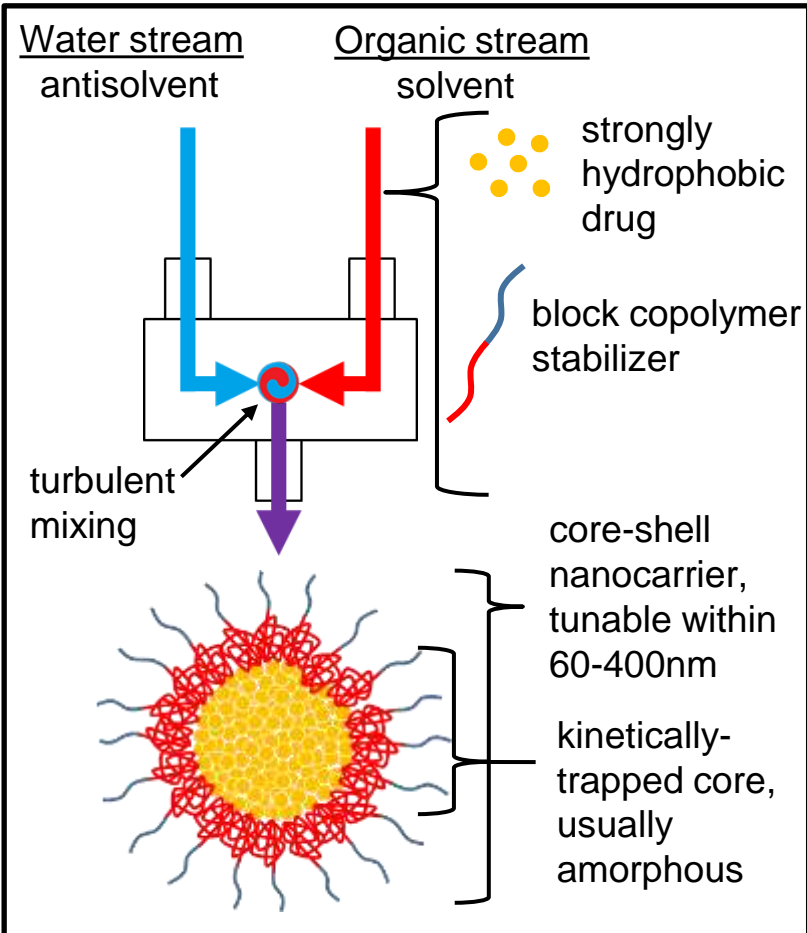
Key process advantages:

- scalability and reproducibility
- continuous operation

Nanoparticle formulation technique: Flash NanoPrecipitation

Flash NanoPrecipitation (FNP)

Hydrophobic molecule encapsulation



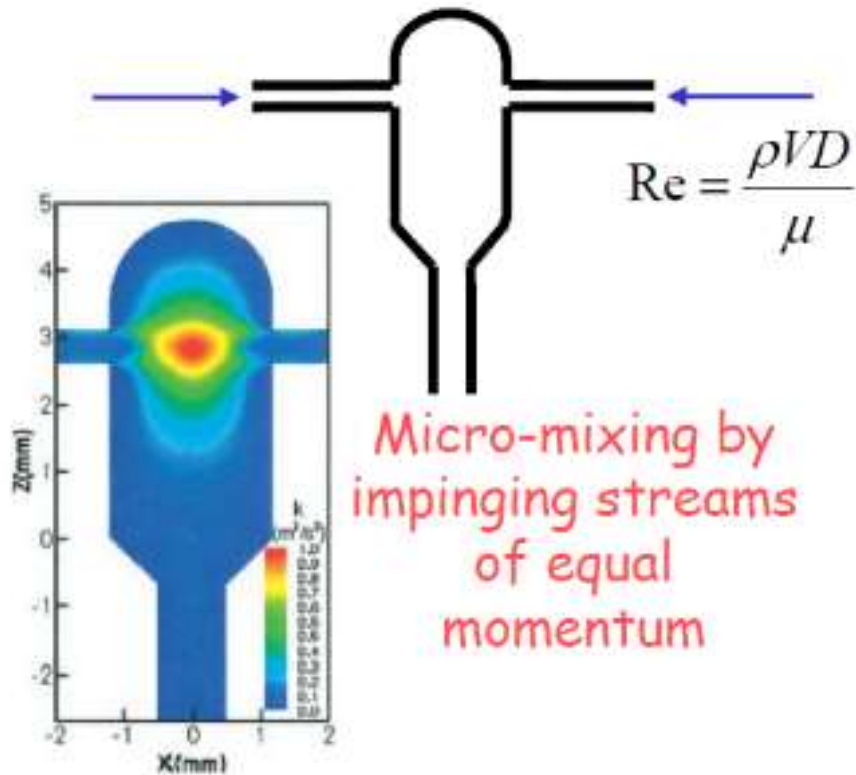
Key process advantages:

- scalability and reproducibility
- continuous operation

Key challenges:

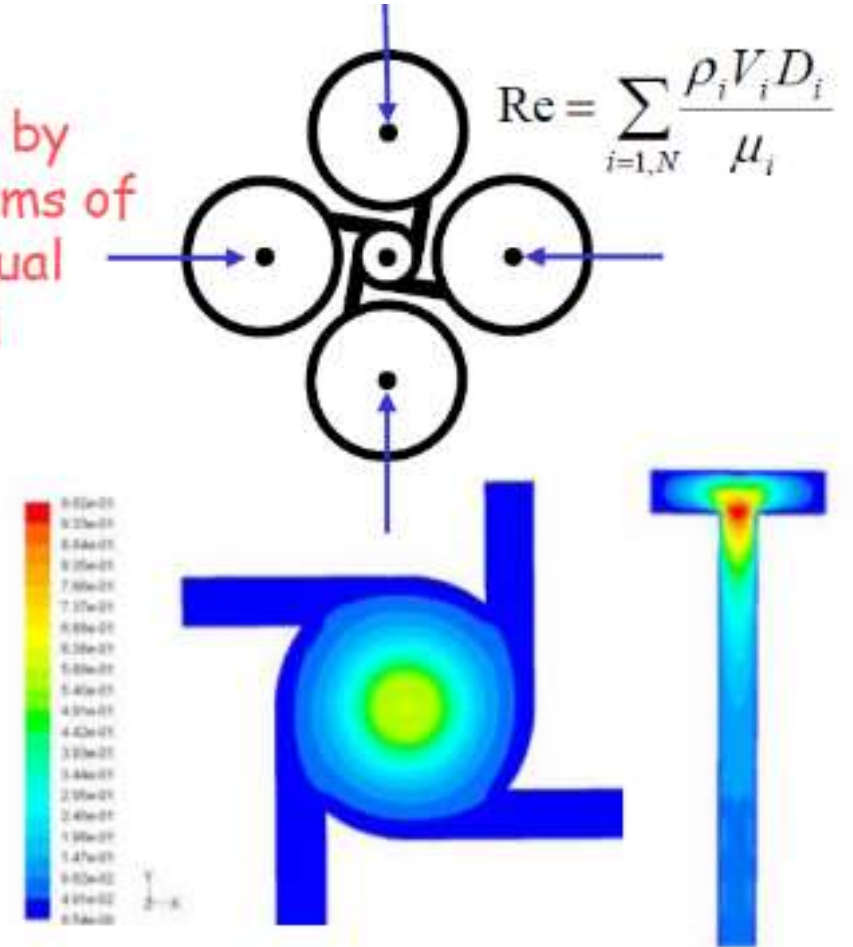
- APIs with low or intermediate hydrophobicity
- cost of excipients (global health, agchem applications)

Flash NanoPrecipitation scalability: two mixer geometries



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

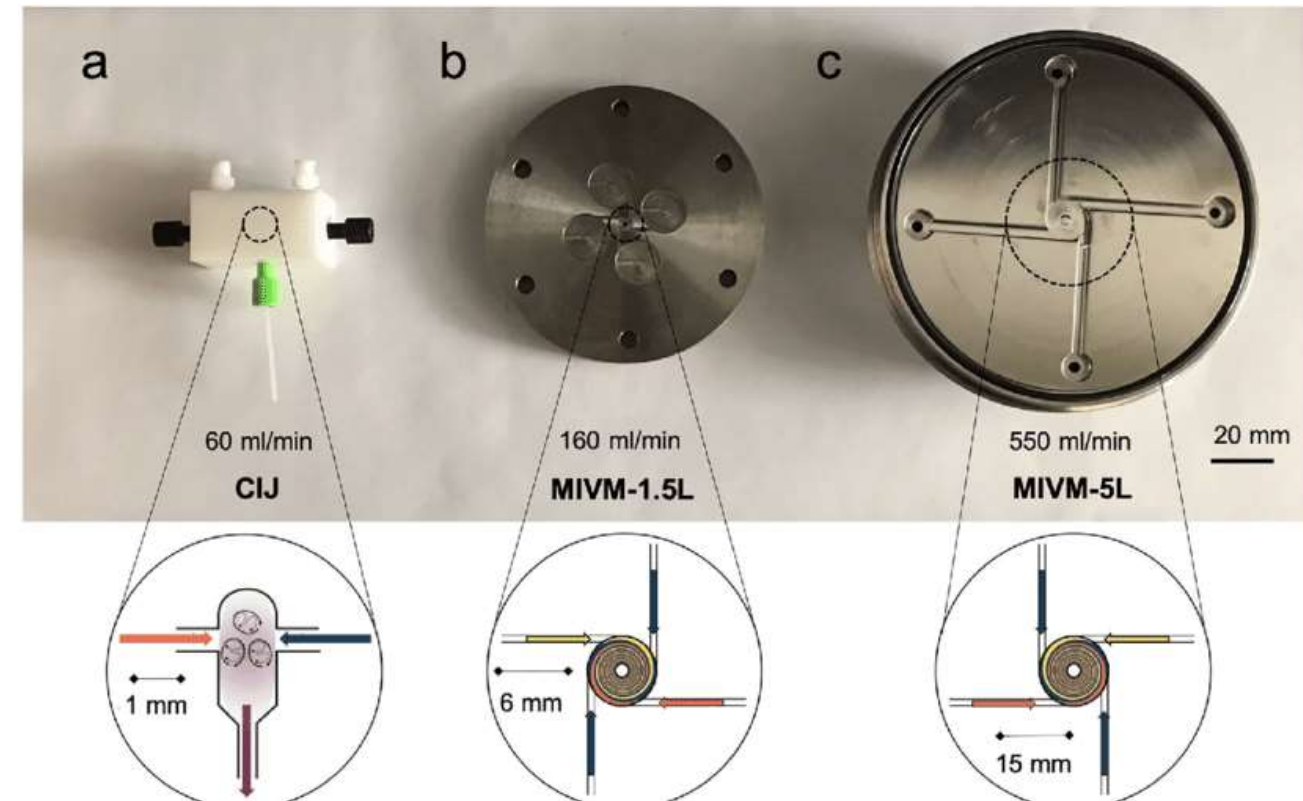
Re = Reynold's Number
 ρ = density
 V = velocity
 D = characteristic dimension
 μ = viscosity



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):

$$\frac{\rho \uparrow v \uparrow d \uparrow}{\mu} = Re = \frac{\rho \downarrow v \downarrow d \downarrow}{\mu}$$

High velocities
Large channels

Low velocities
Small channels

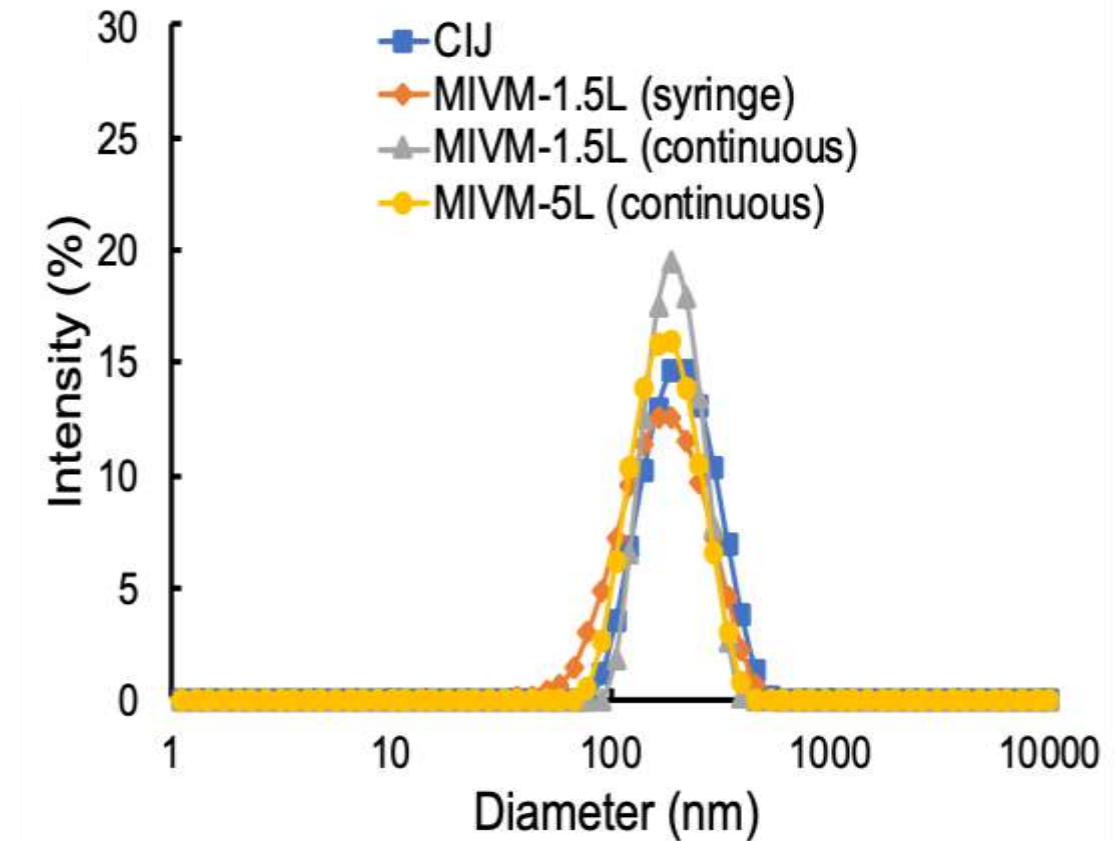
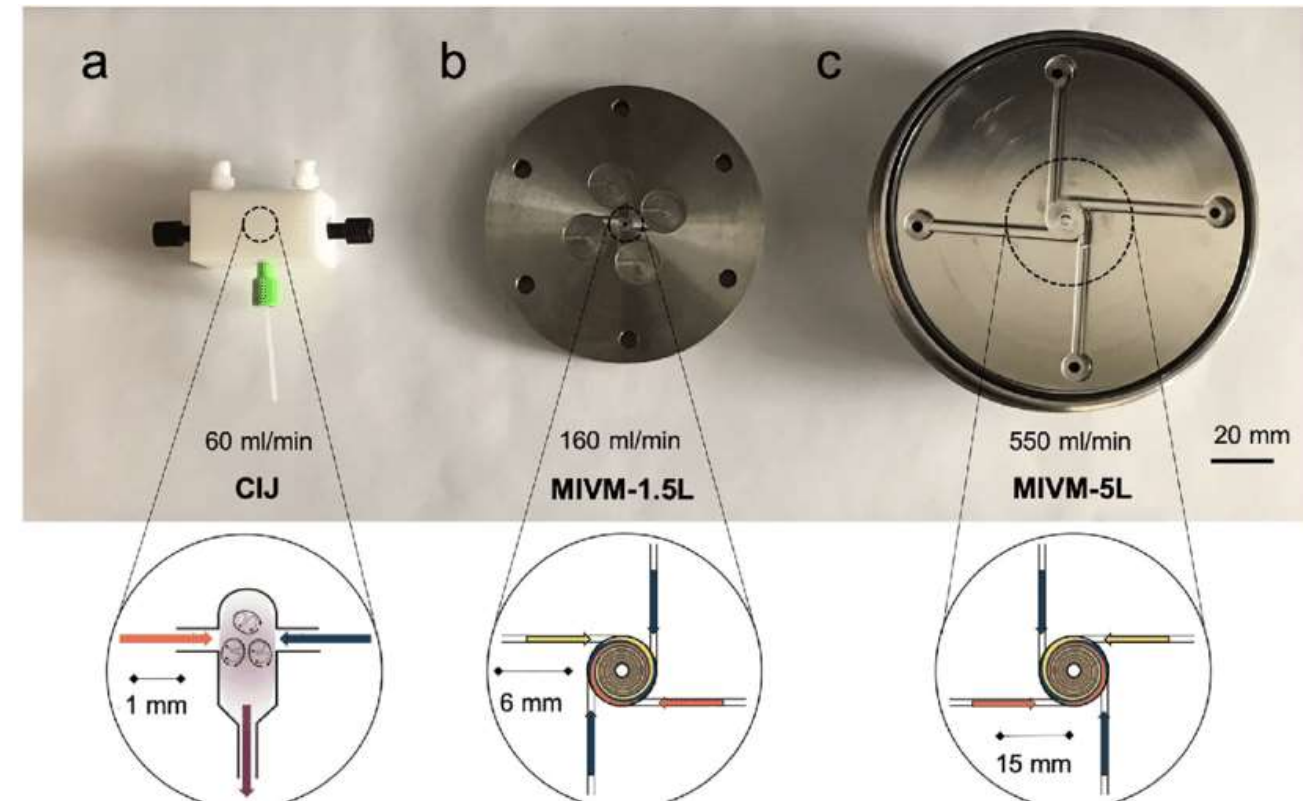
$$\frac{\pi \uparrow v \uparrow d^2 \uparrow}{4} = Q = \frac{\pi \downarrow v \downarrow d^2 \downarrow}{4}$$

High throughput
Fast mixing

Low throughput
Slower mixing

Flash NanoPrecipitation scalability

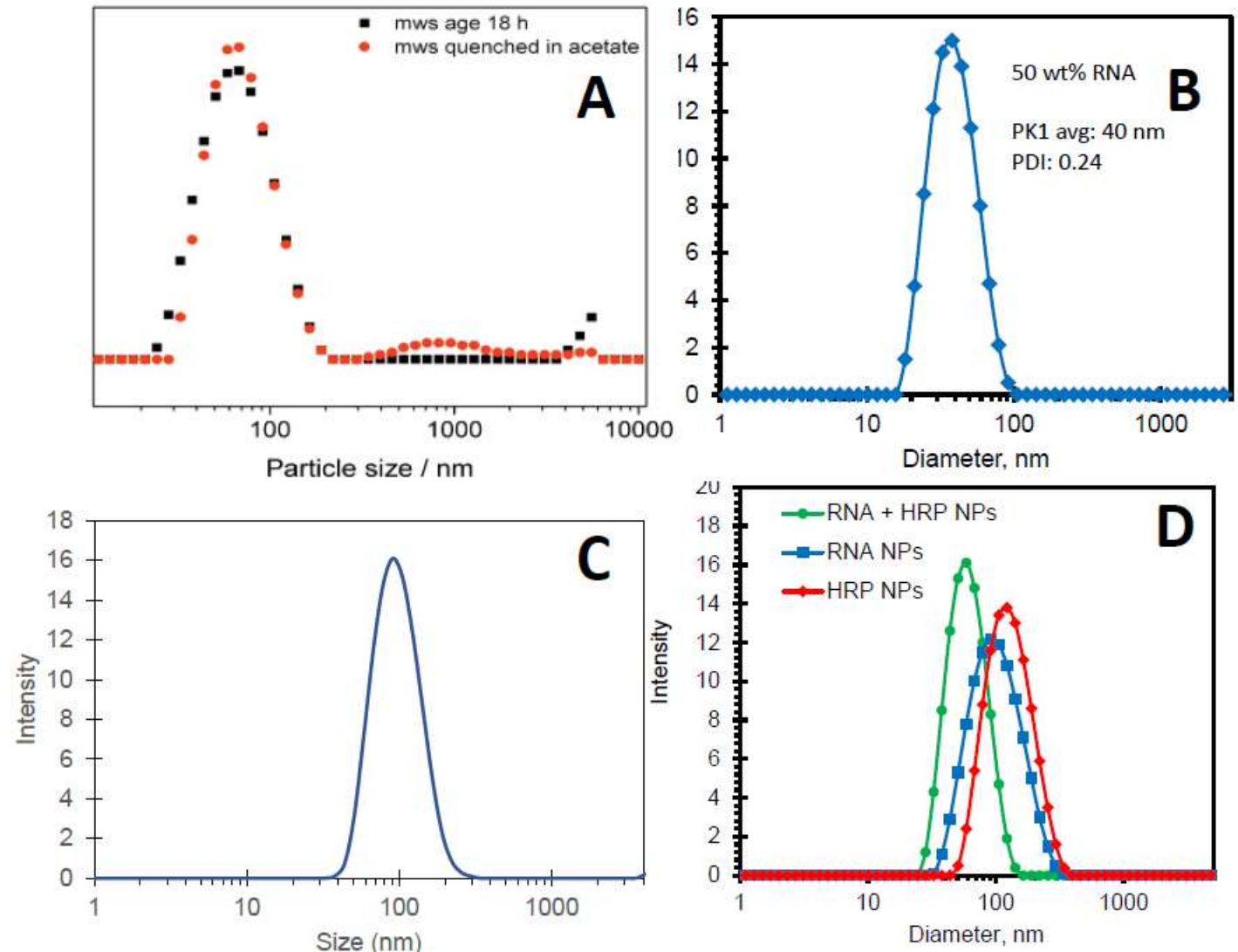
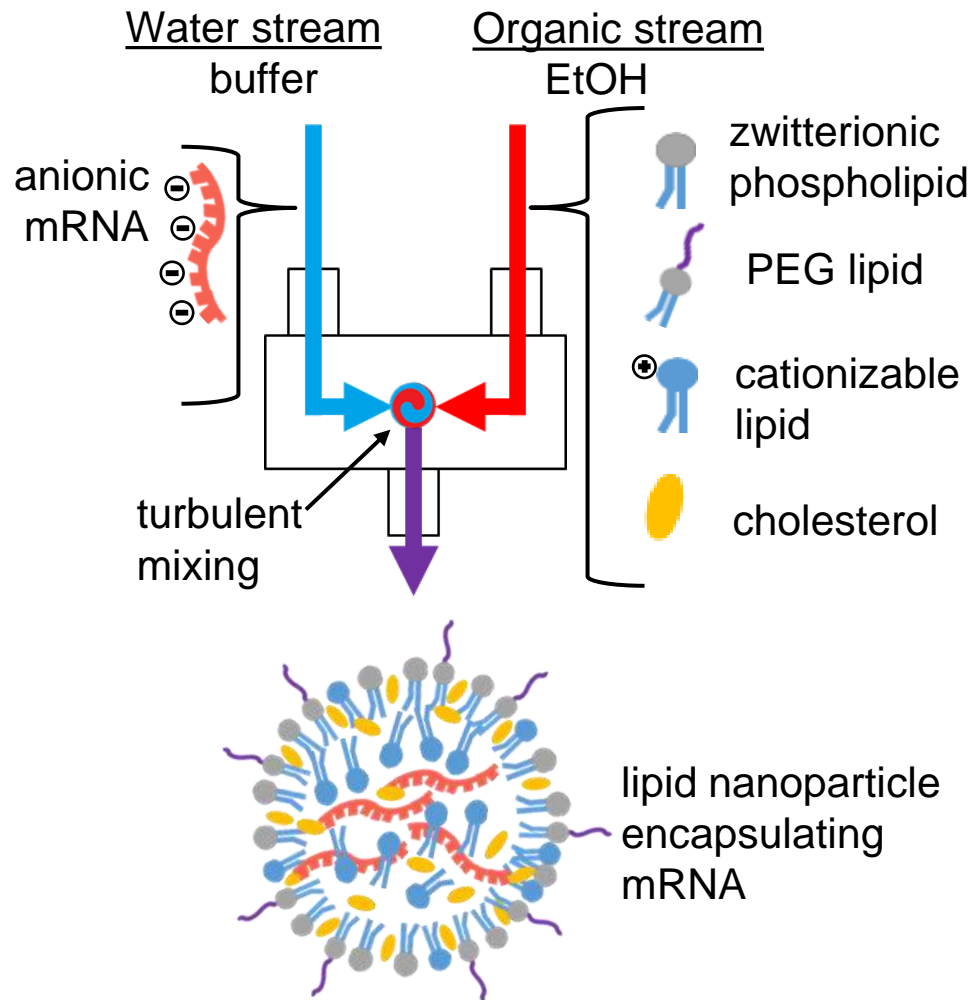
FNP mixers scaled up to 5L/min and down to 5mL batches



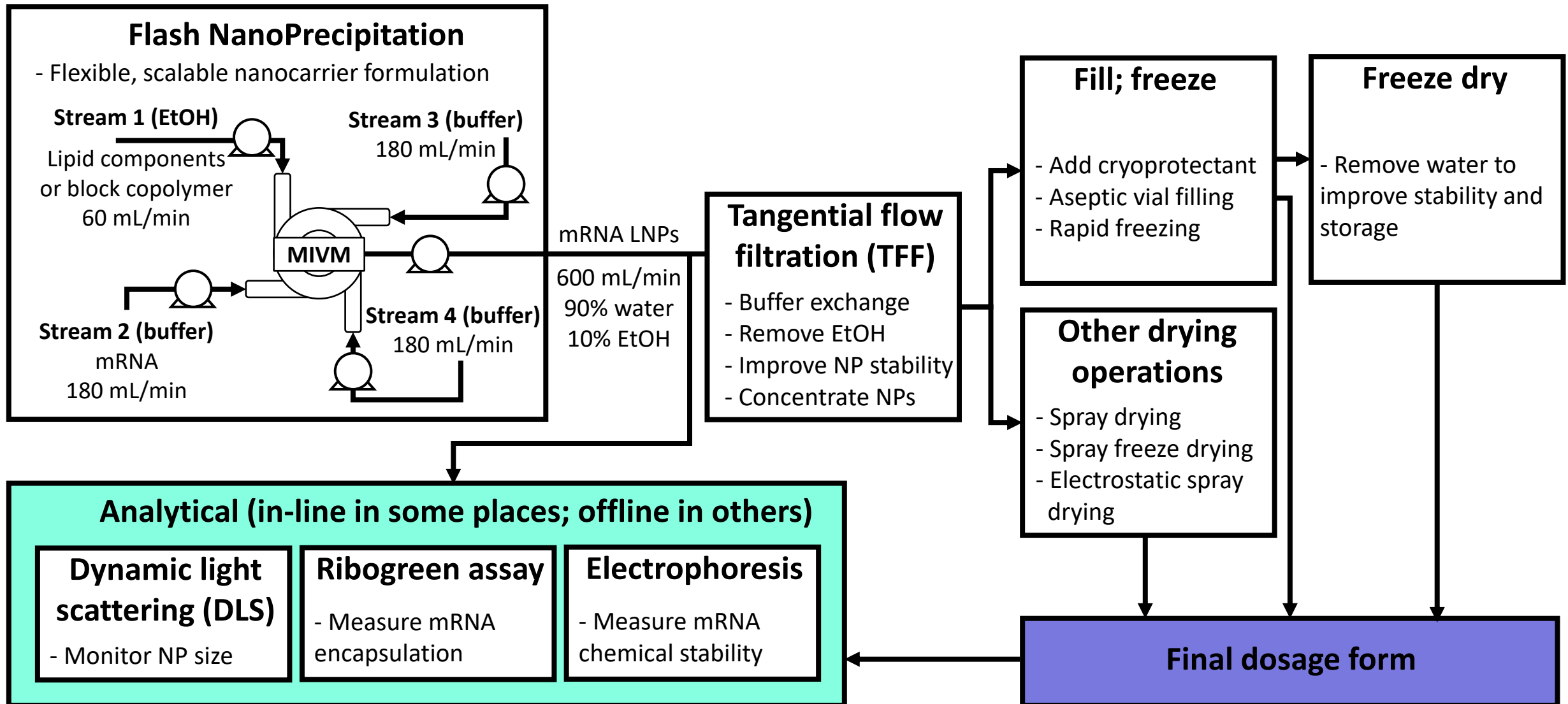
NP size and PDI are the same in 5mL batch, 30L batch, and 3000L batch.

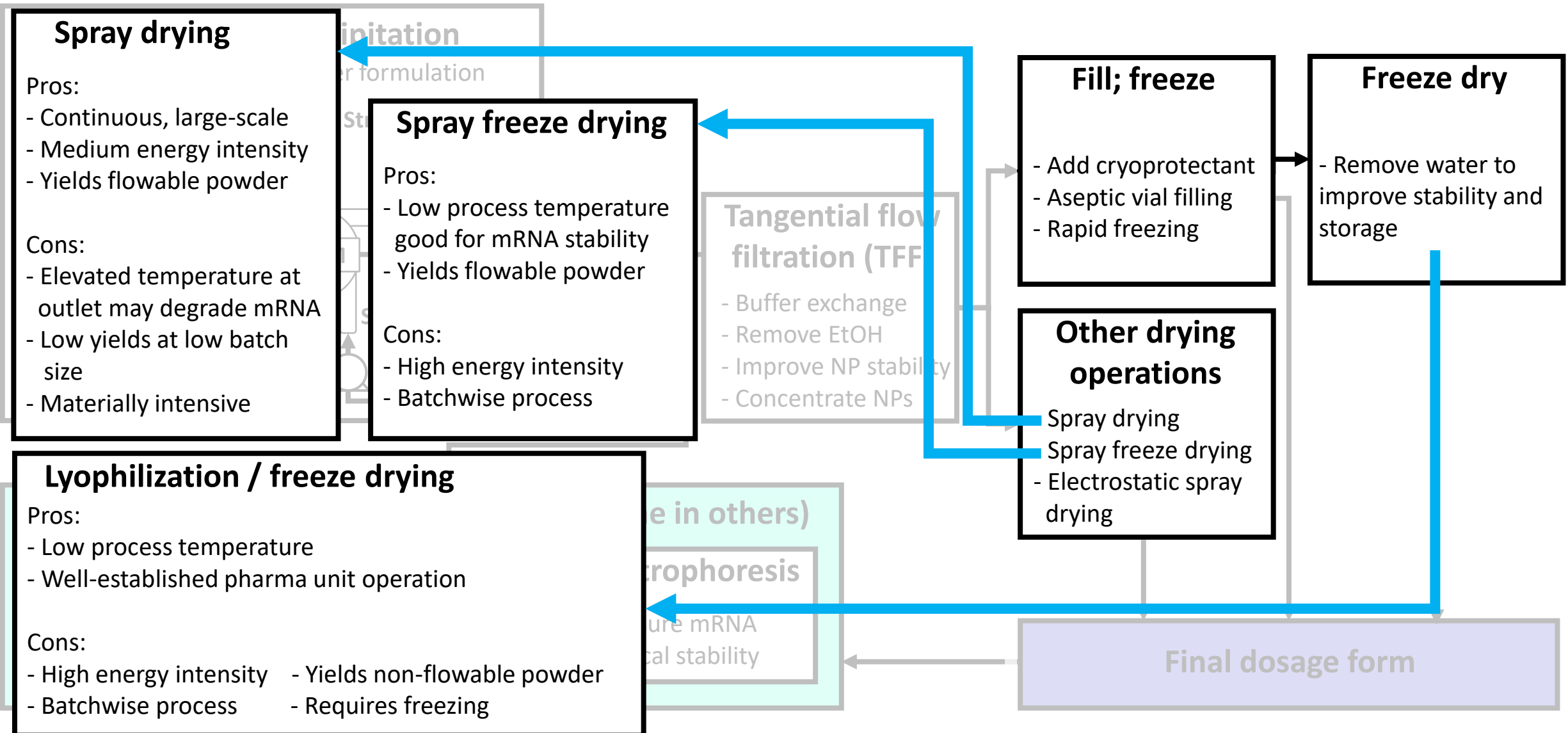
Armstrong *et al.* (2023) DOI: [10.1016/j.xphs.2023.04.003](https://doi.org/10.1016/j.xphs.2023.04.003)

Flash NanoPrecipitation for lipid nanoparticles



Overall process design for continuous mRNA LNP manufacture



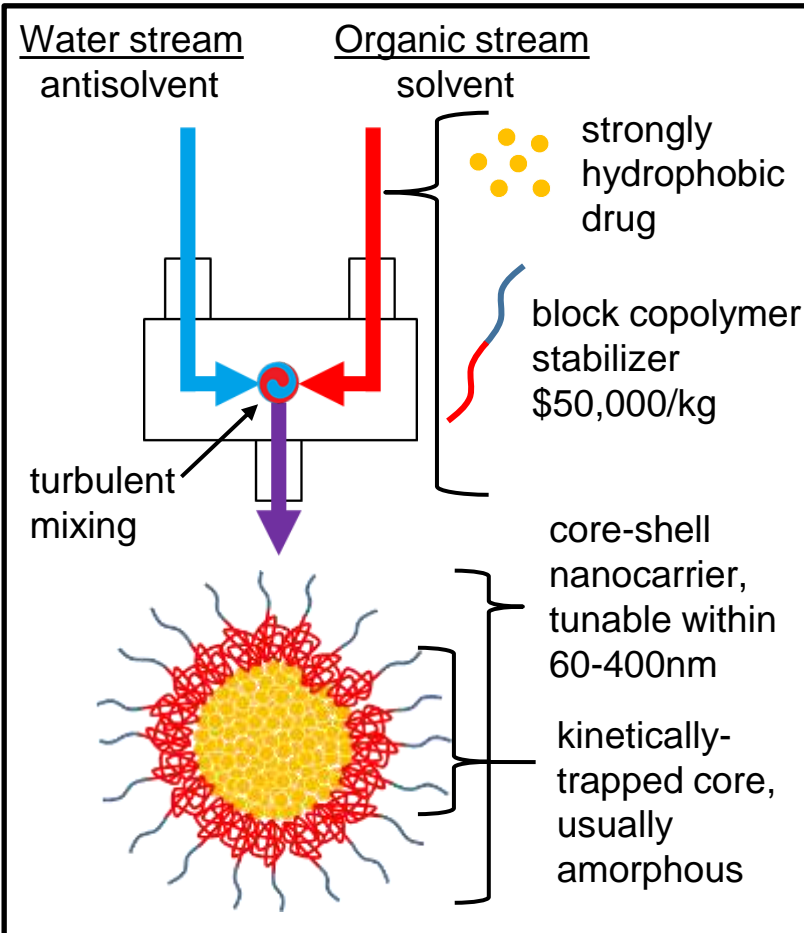


Part 2: Selected examples of Flash NanoPrecipitation to formulate small molecules and biopharmaceuticals

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

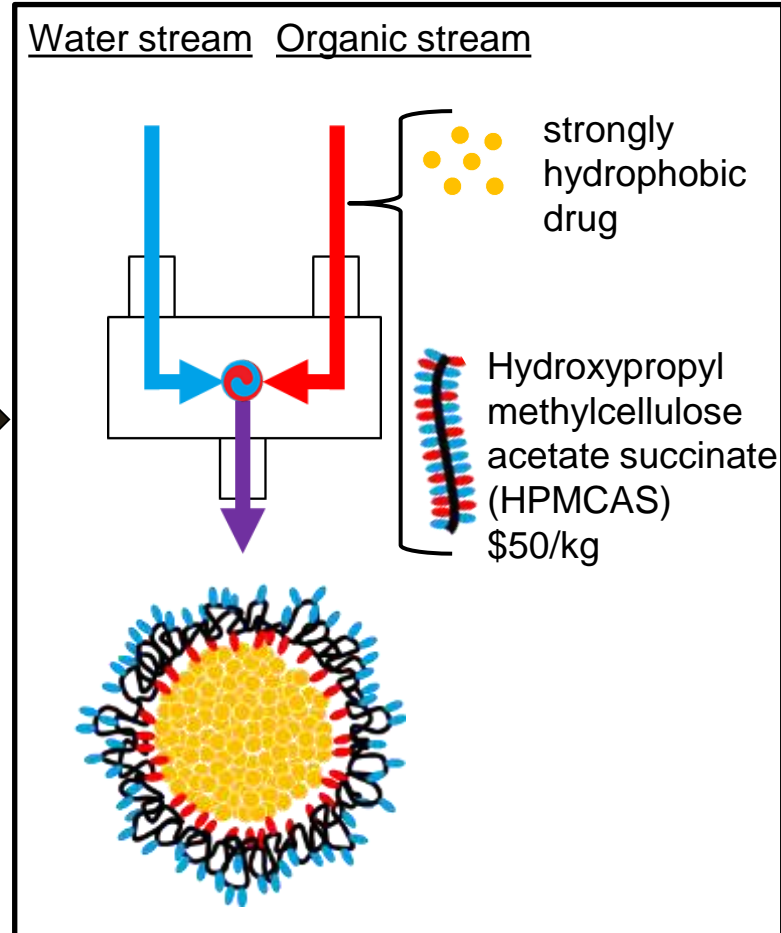
Flash NanoPrecipitation (FNP)

Hydrophobic molecule encapsulation



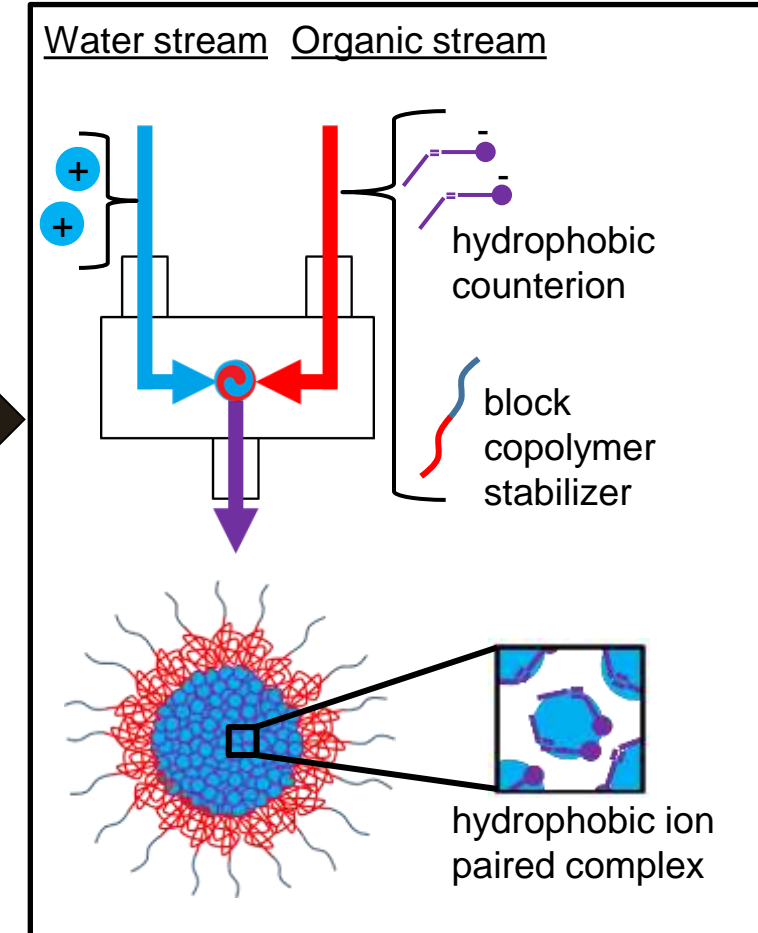
Low-cost stabilizer

Amphiphilic modified cellulosic



Hydrophobic ion pairing (HIP)

Hydrophilic molecule encapsulation



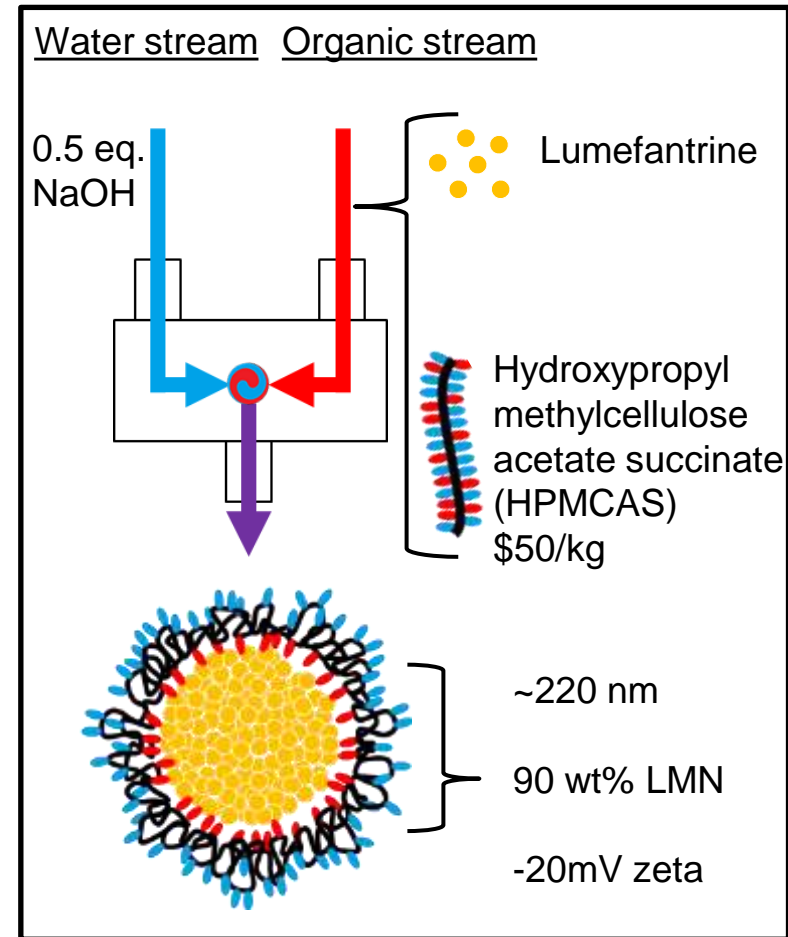
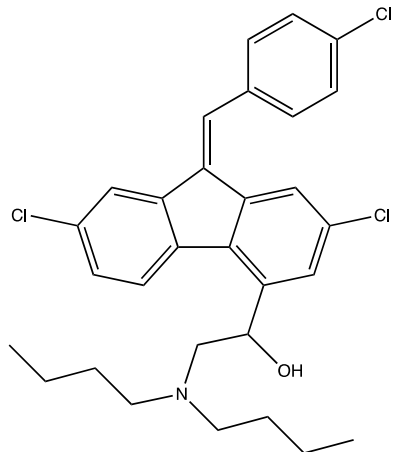
Example 1: improving bioavailability of hydrophobic antimalarial lumefantrine

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)

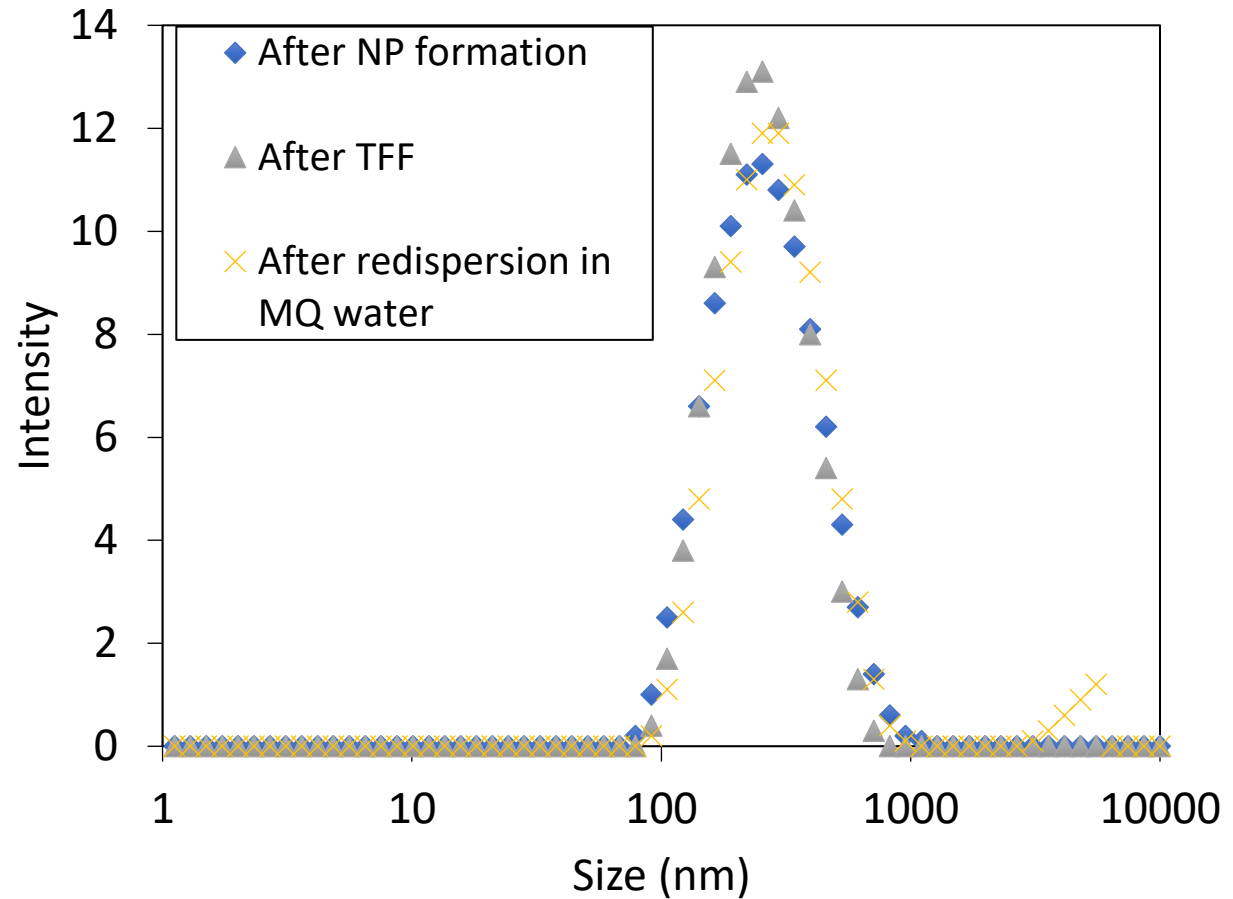
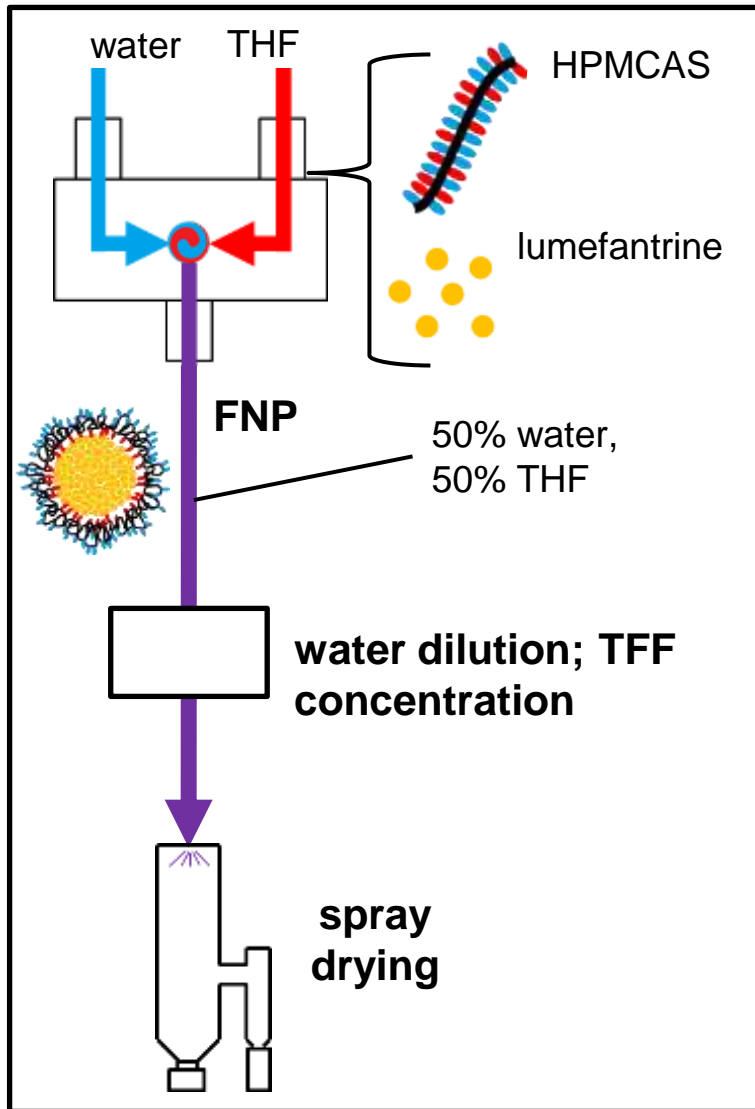


Formed NPs encapsulating LMN using HPMCAS as stabilizer

Feng *et al.* (2019) DOI: [10.1039/c8sm02418a](https://doi.org/10.1039/c8sm02418a)

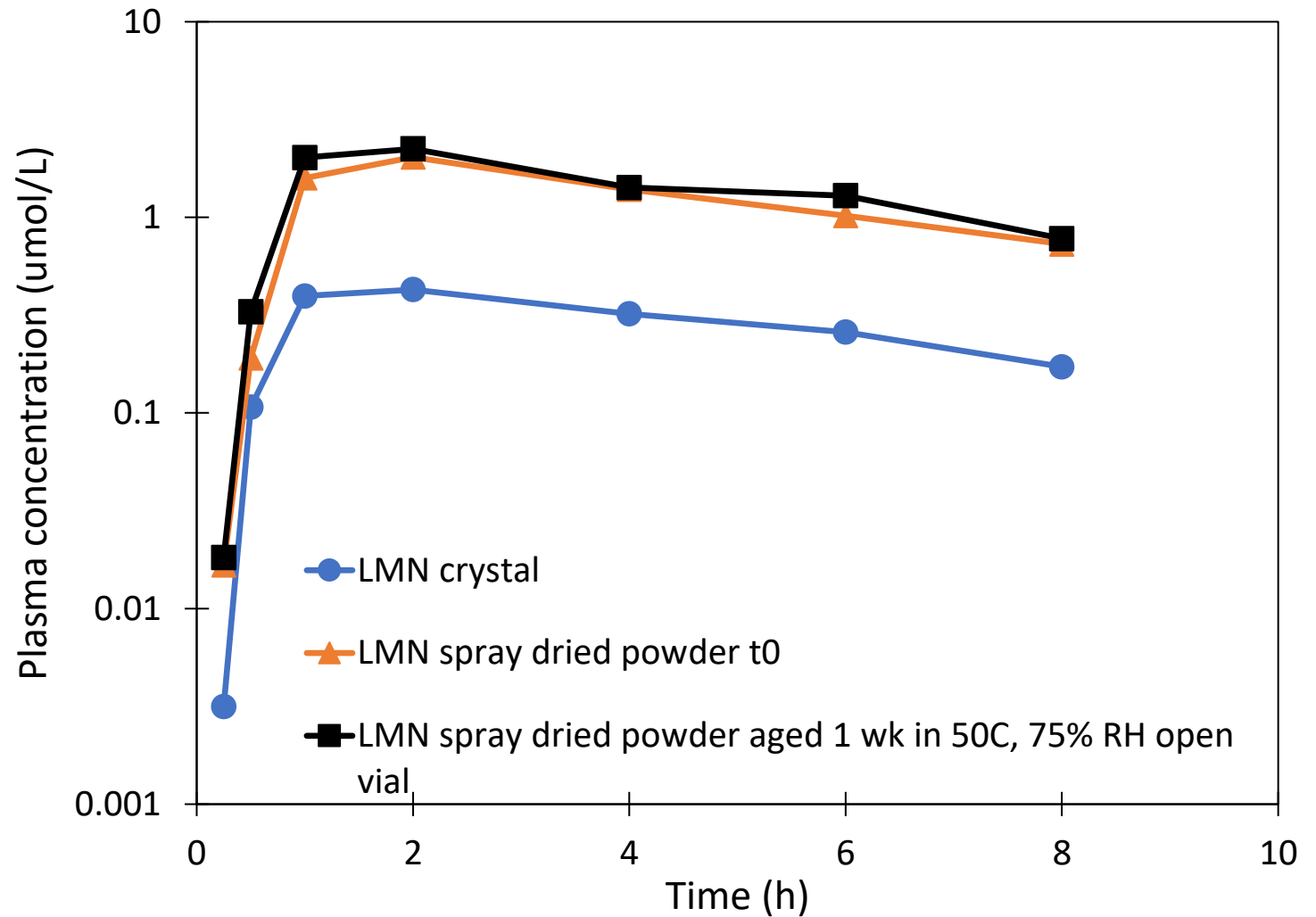
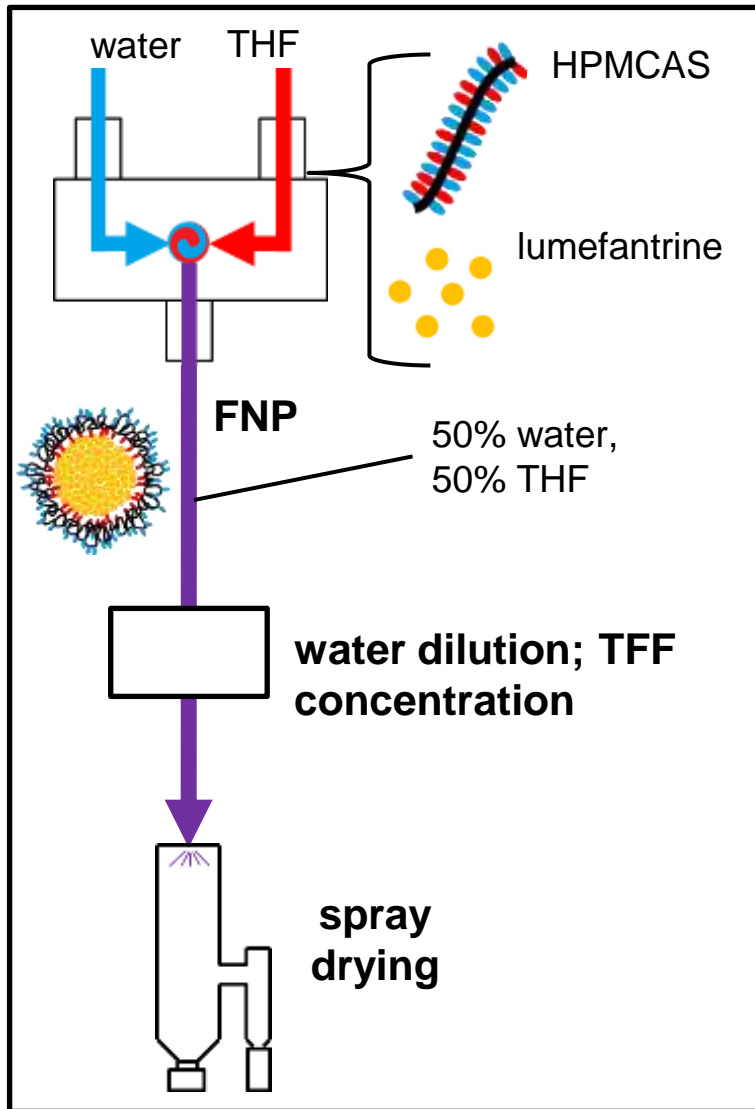
Ristroph *et al.* (2019) DOI: [10.3791/58757](https://doi.org/10.3791/58757)

Downstream unit operations concentrate and dry NPs; size stable throughout



Armstrong *et al.* (2023) DOI: [10.1016/j.xphs.2023.04.003](https://doi.org/10.1016/j.xphs.2023.04.003)

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

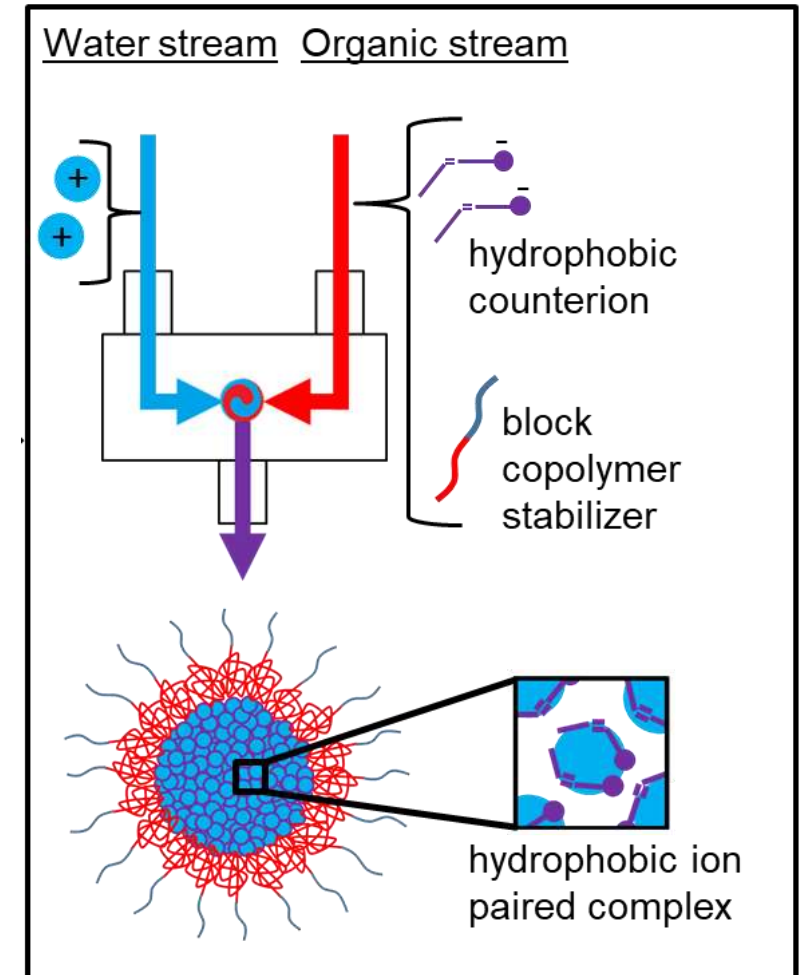


Armstrong *et al.* (2023) DOI: [10.1016/j.xphs.2023.04.003](https://doi.org/10.1016/j.xphs.2023.04.003)

Example 2: FNP with hydrophobic ion pairing to encapsulate biologics

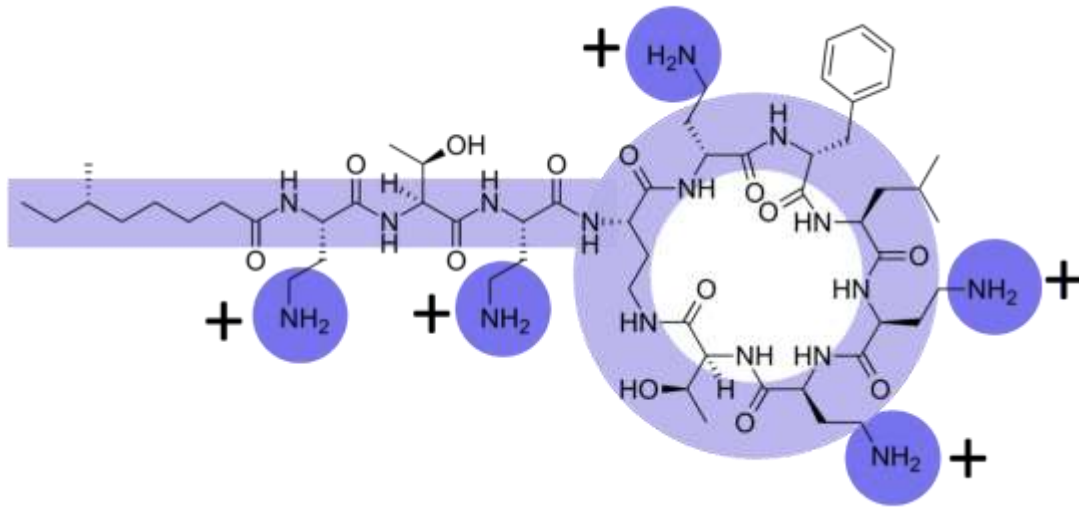
- **Polymyxin B (PMB)**
 - Model hydrophilic antibiotic peptide with structural complexity & therapeutic relevance
- **Objective**
 - Increase encapsulation efficiency (EE) and loading of PMB NCs in FNP by incorporating hydrophobic ion pairing (HIP)
- **Specific goals**
 - Demonstrate feasibility of 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

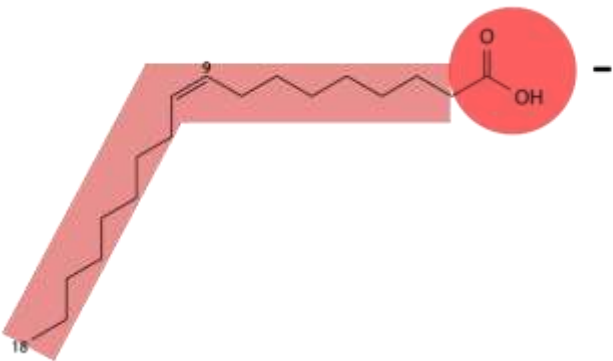


Preparing NCs of antibacterial peptide polymyxin B

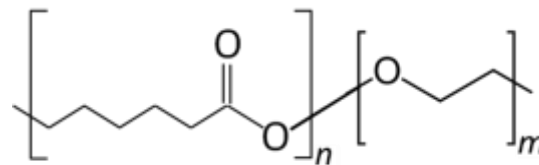
Model biologic: **polymyxin B (PMB)**



Counterion: **oleate (OL)**



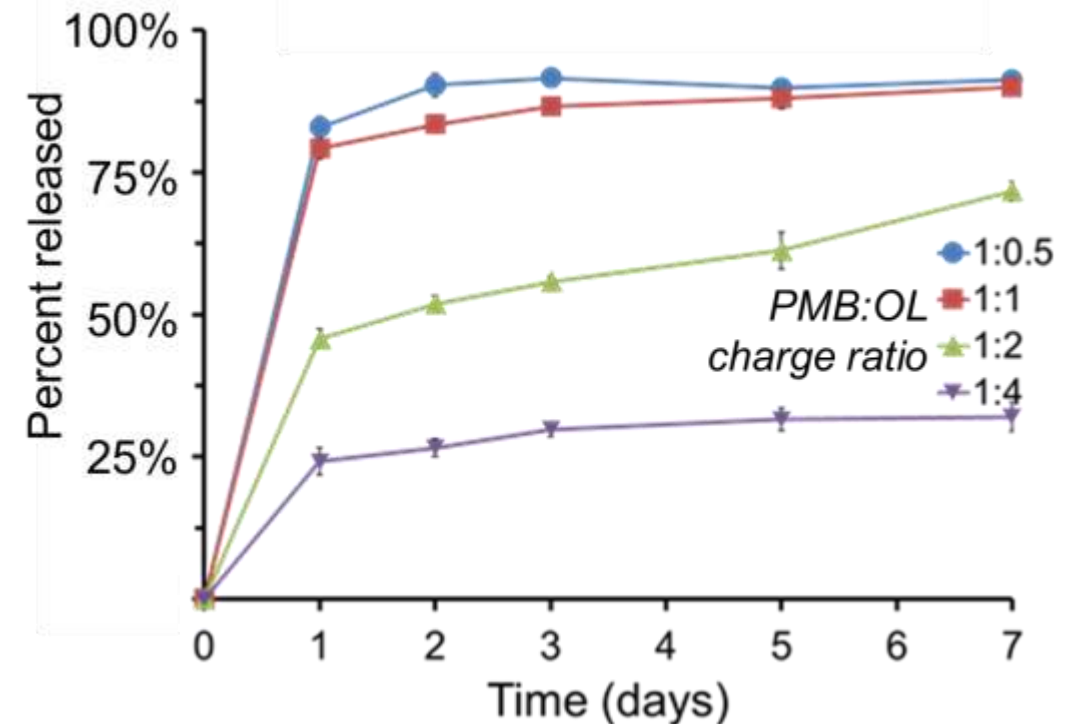
Stabilizer: **PCL-b-PEG**



Results:

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

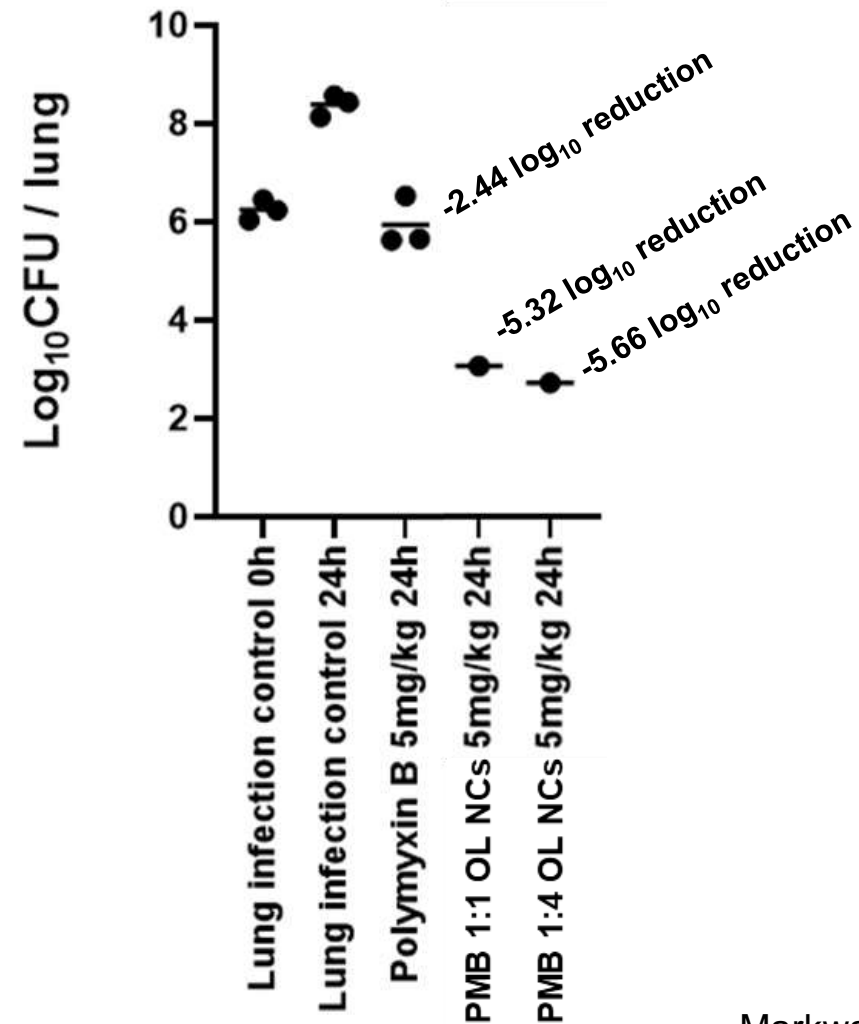
PMB release in buffer varies with charge ratio



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

- Bacterial isolate: *A. baumannii* N16870.213
- 1×10^5 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.

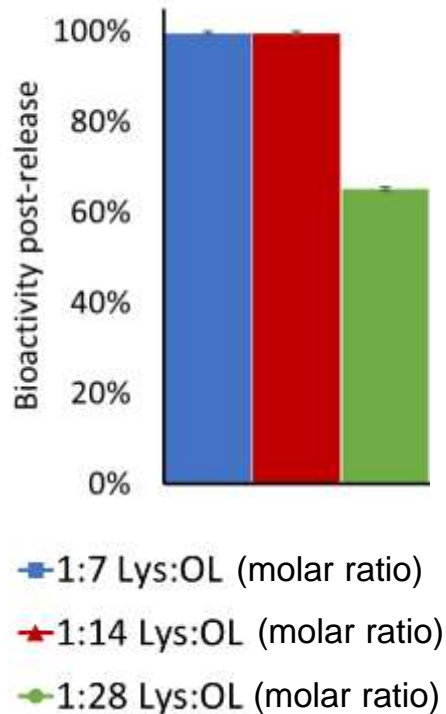
Efficacy of pulmonary delivered polymyxin B nanoparticles against *Ab* N16870.213 in mouse lung infection



Example 3: protein encapsulation by FNP with HIP

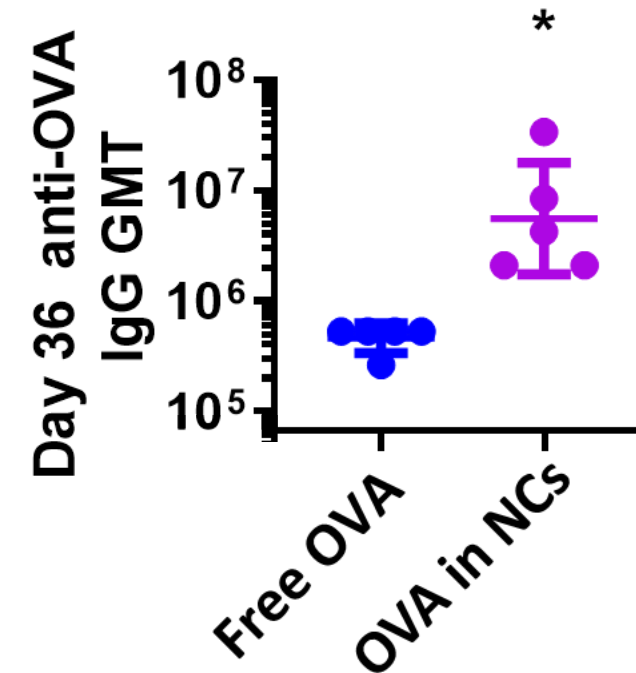
Lysozyme (Lys): 14.4 kDa, pI = 11.4

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

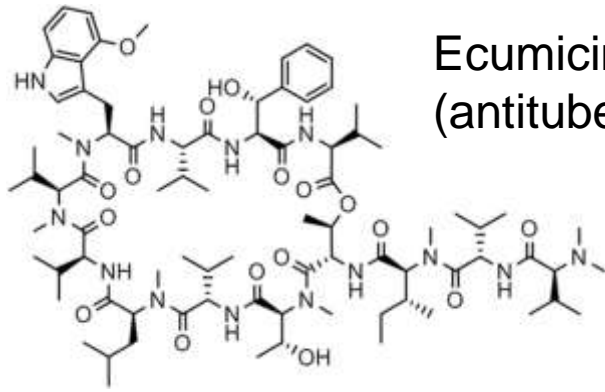


Ovalbumin (OVA): 43 kDa, pI = 5.2

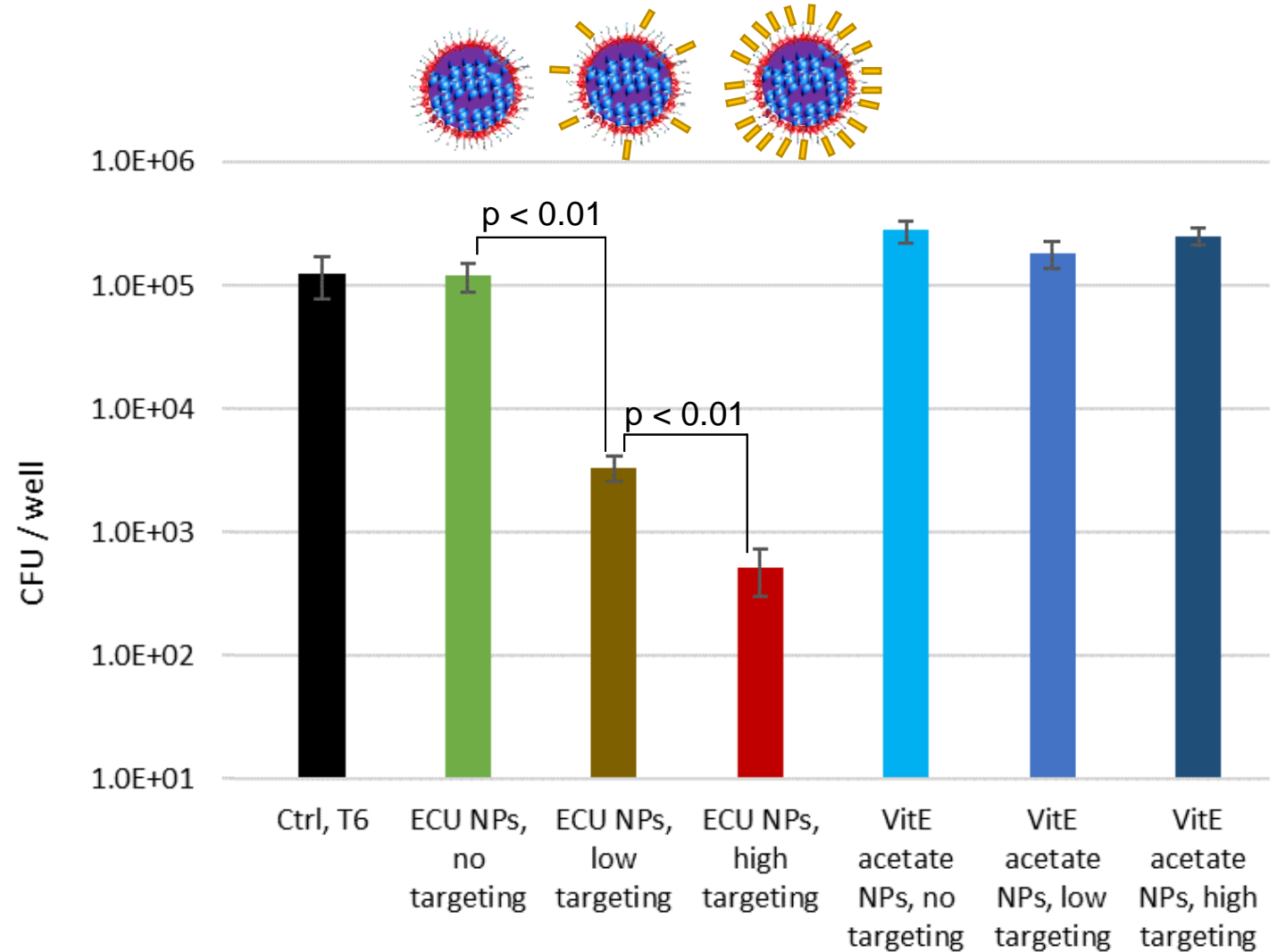
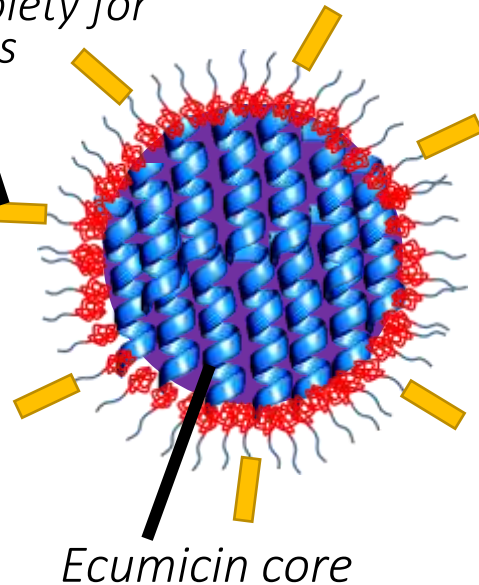
- EE: 88% OVA loading: 29%
- NC formulation improved immunogenicity *in vivo* in a nasal vaccine mouse model



Example 4: macrophage-targeted antitubercular NCs

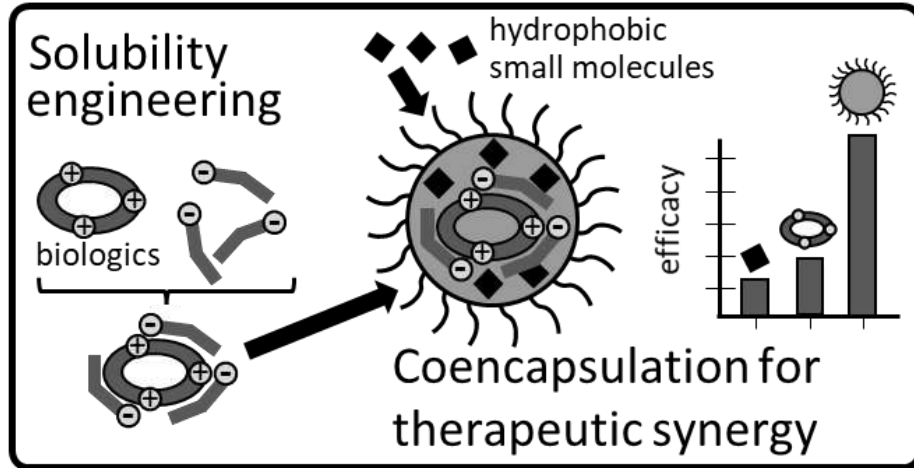


Hexamannose
targeting moiety for
macrophages

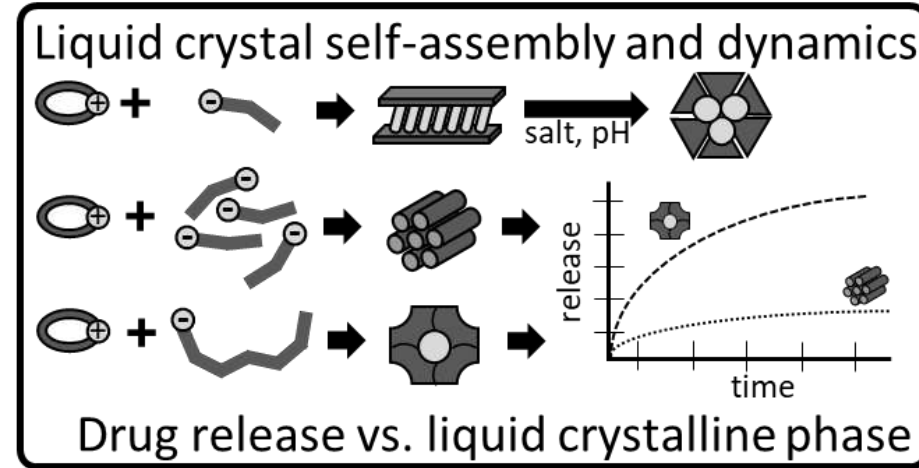


Ristroph *et al.* (2022) DOI: [10.1002/admt.202101748](https://doi.org/10.1002/admt.202101748)

I. Combined biologic/small molecule delivery

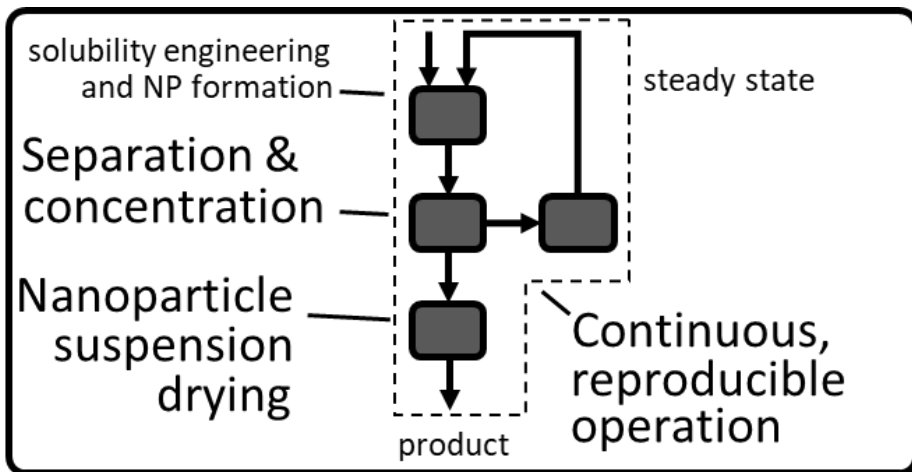


II. Evolution of API liquid crystal phases

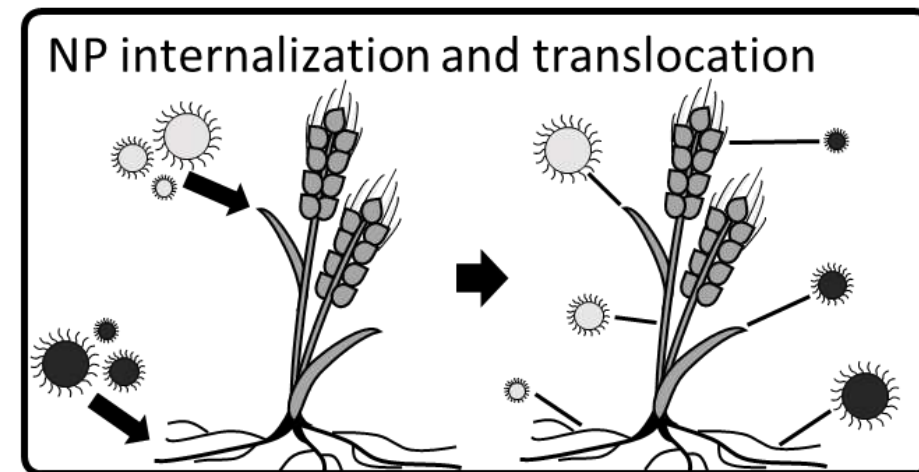


Currently looking to hire 2 postdocs and 2-3 graduate students

III. Rapid unit operations for NPs at scale



IV. Nanoparticle delivery to plants



Acknowledgements

ristrophlab.com

Purdue

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



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- Nick Caggiano
- Maddie Armstrong



BMGF

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



PRINCETON
School of Engineering and Applied Science

BILL & MELINDA
GATES foundation



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