

Nanomedicine: from high tech to global health

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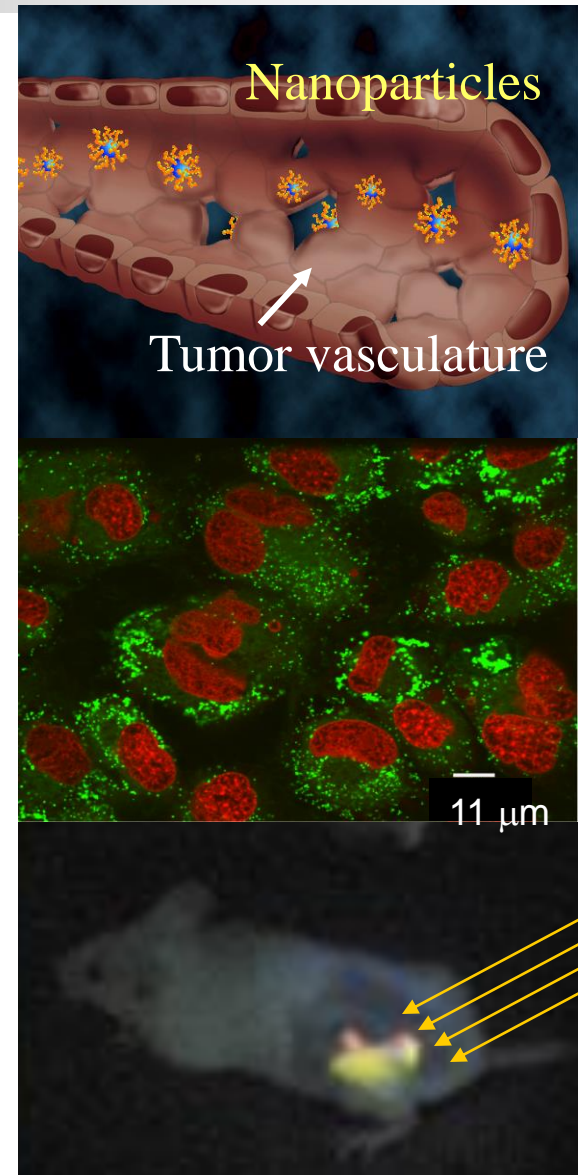
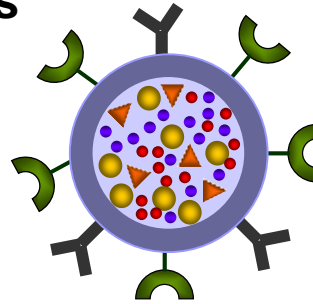
***Dept. Chemical Engineering
Princeton University***

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

Next Generation Nanoparticles (NPs)

Motivation

- Bioavailability: 40% of new drug compounds are hydrophobic
- EPR targeting of solid tumors
- Targeting toxic API
- Multiple drugs cocktails
- Imaging where toxic APIs go
- siRNA, mRNA, proteins and peptide delivery



More than just small

- Size
- Surface functionality
- Stoichiometric encapsulation of multiple species: cocktails
 - Imaging plus delivery
 - Targeting plus delivery
- Scalable and manufacturable

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.



Scott McNeil, the head of the National Cancer Institute's Nanotechnology Laboratory

*"Another big hurdle in developing nanomedicines is **scaling up** the synthesis of the particles**developing a synthesis that yields particles ... on a consistent basis. That is still a difficult process.**" (C&ENews. ACS.org, June 20, 2016, p. 19)*

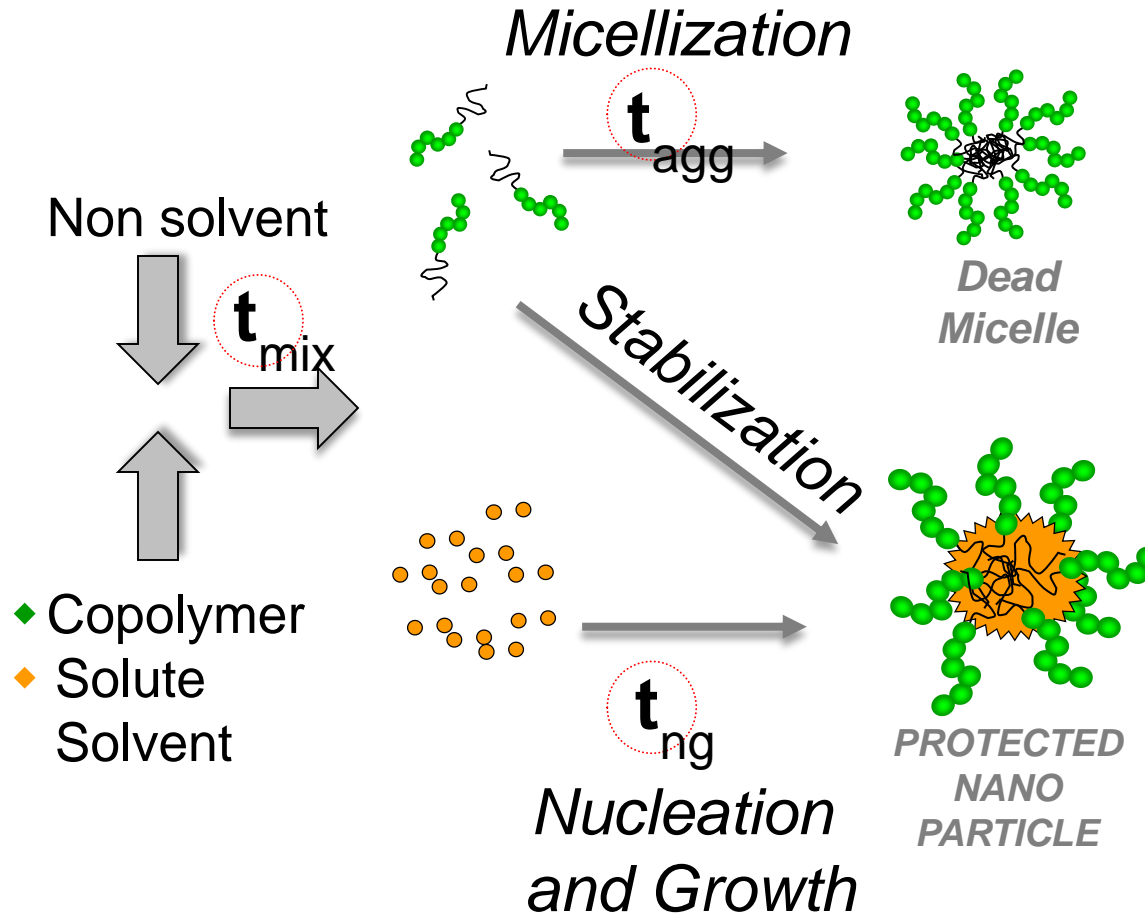


Outline

1. ***Nanoparticles by turbulent micromixing in confined impinging jet mixers (CIJ)***
 1. *Development of an idea: Flash NanoPrecipitation (FNP)*
2. ***Nanoparticle drug delivery***
 1. ***Oral: increased bioavailability (Gates funding)***
 2. ***Parenteral: Controlled release with conjugation***
 3. ***Parenteral: Ion pairing for hydrophilic drugs (LNPs RNA, peptides, proteins)***
3. ***Targeting***

Nanoparticle formation by Flash NanoPrecipitation

Block copolymer directed rapid precipitation

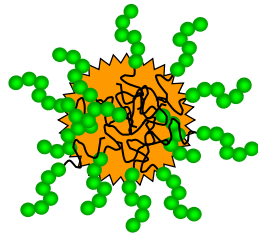


Johnson, Prud'homme AICHE J (2003), Liu, Prud'homme, Fox, Chem Engr. Sci. (2008)

Polymer Protected vs Unprotected Particle Growth

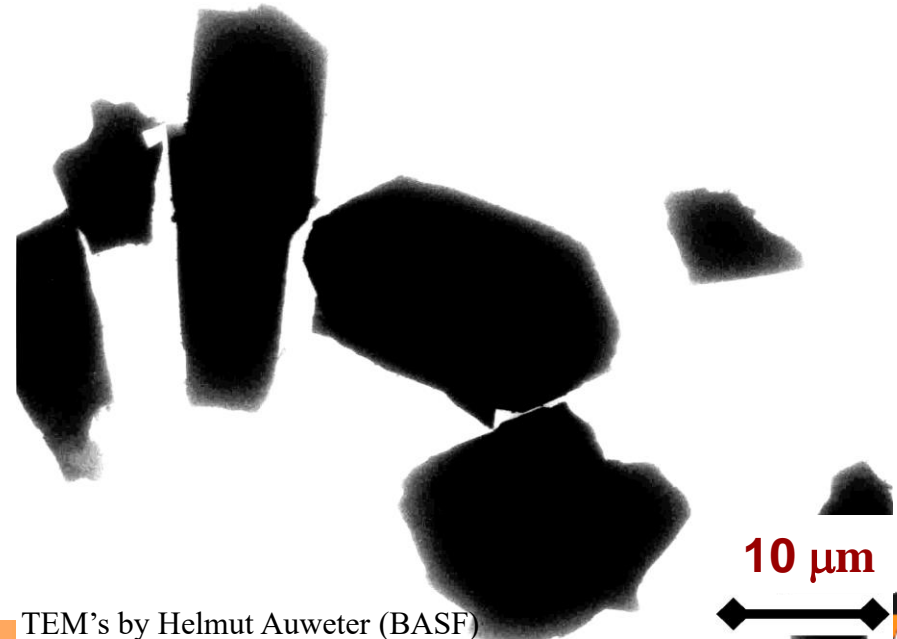
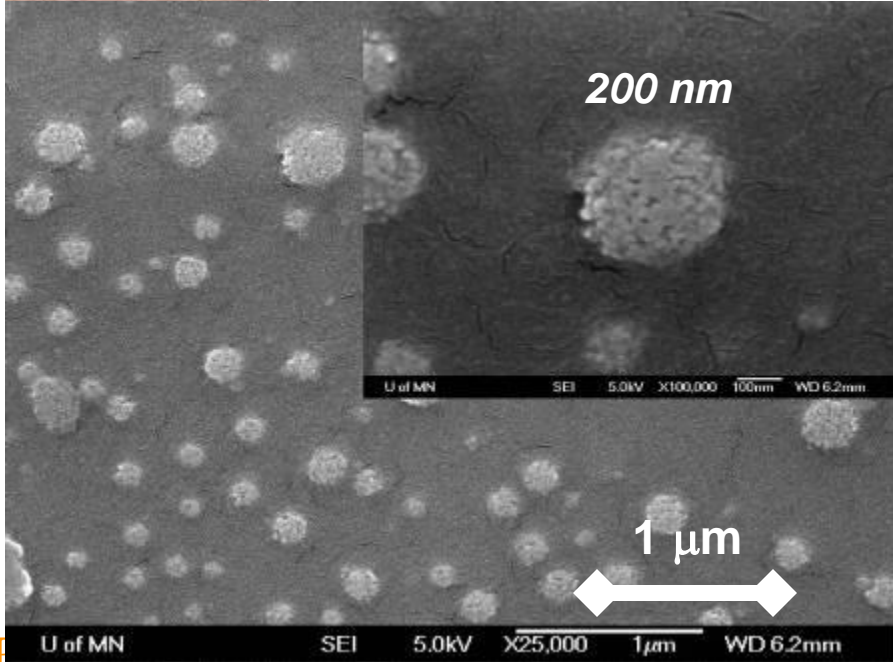
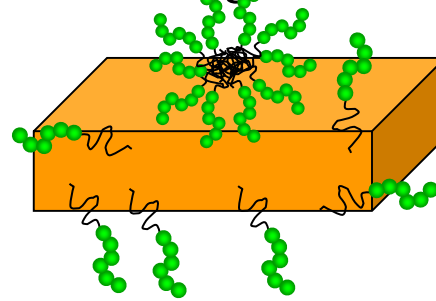
Flash Precipitation

Image analysis = 249 nm
Light diffraction = 370 nm
SSA = 22 m²/gram



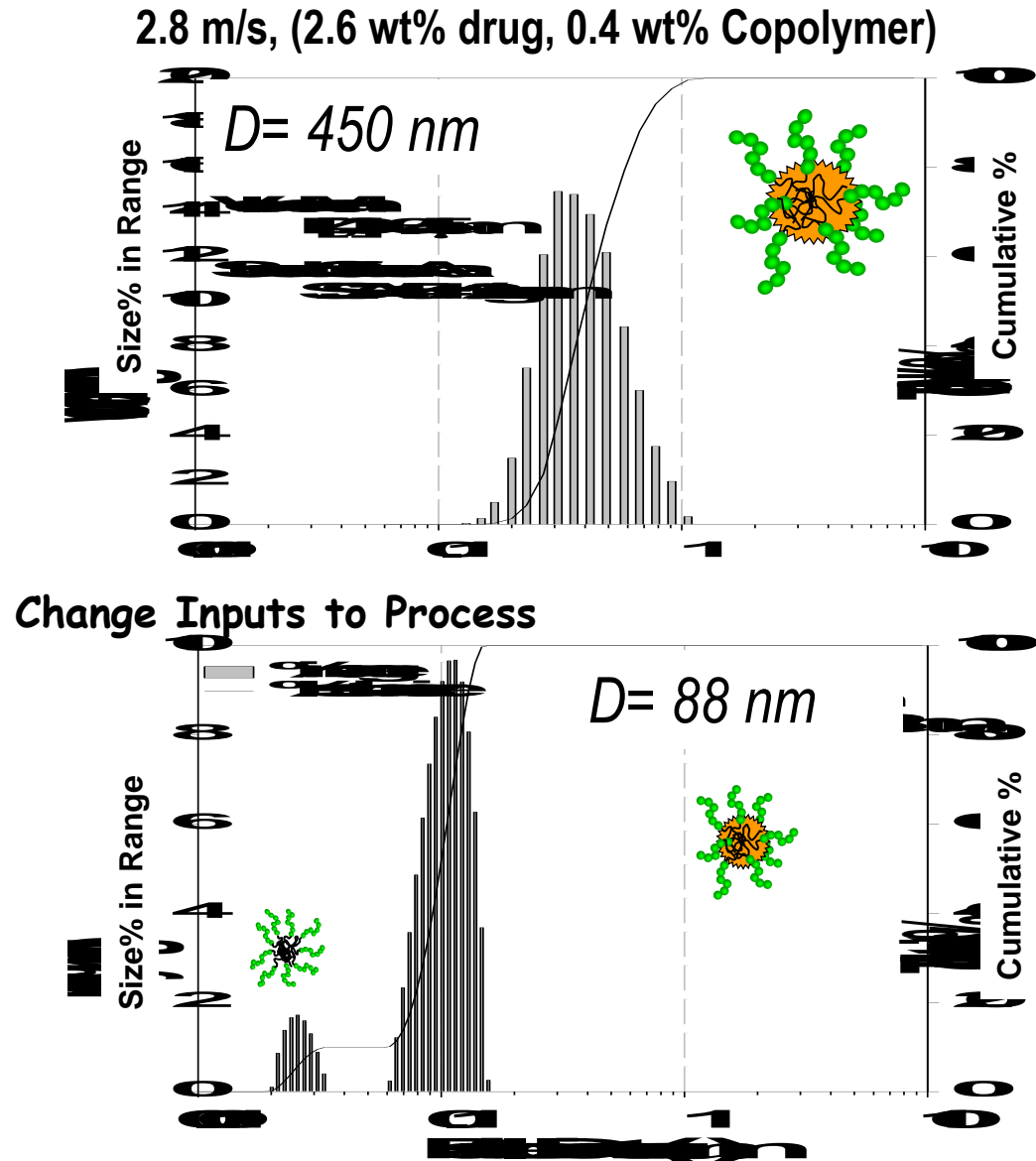
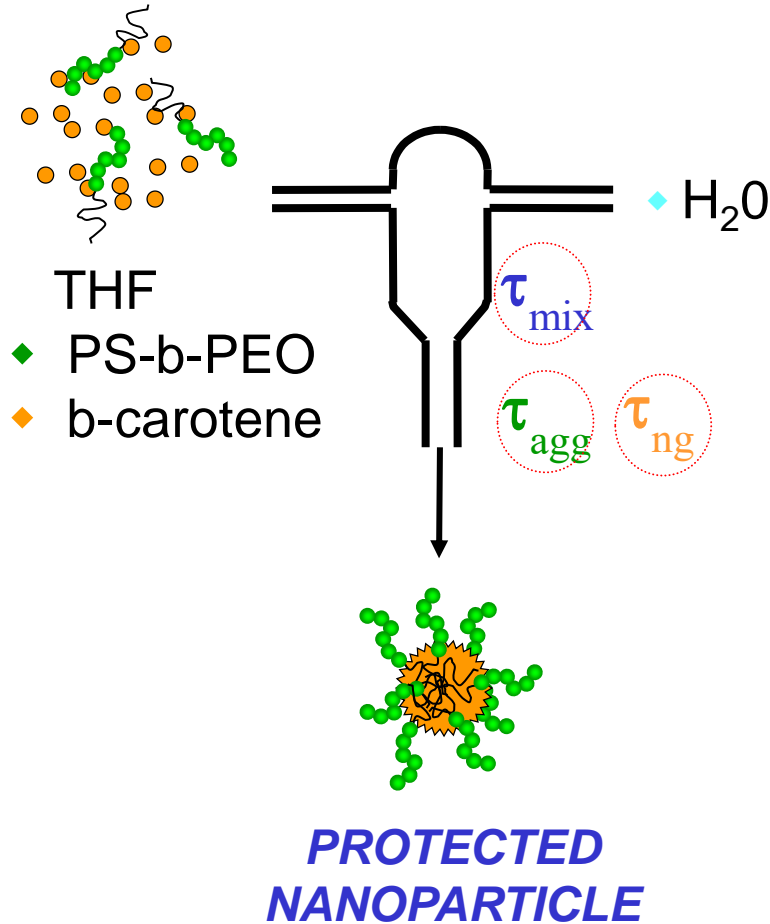
“Typical” Precipitation

Image analysis > 10 μm
Light diffraction > 10 μm
SSA > 0.6 m²/gram

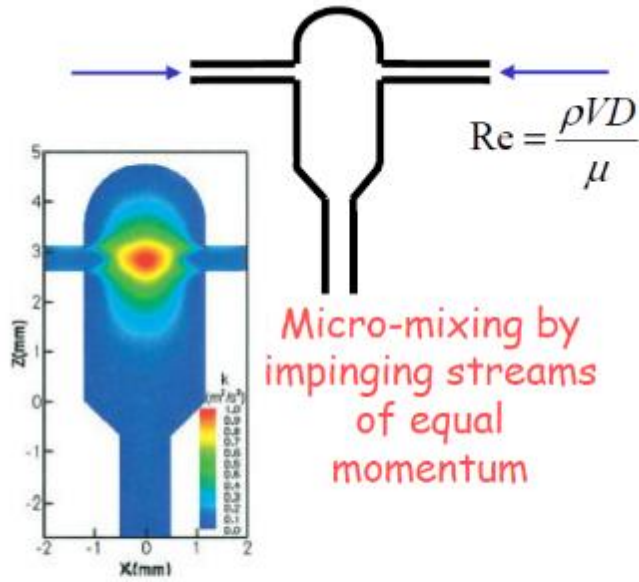


“FLASH” Nanoparticles Precipitation Size Control

- High throughput, > 2 wt% active
- High NP loading

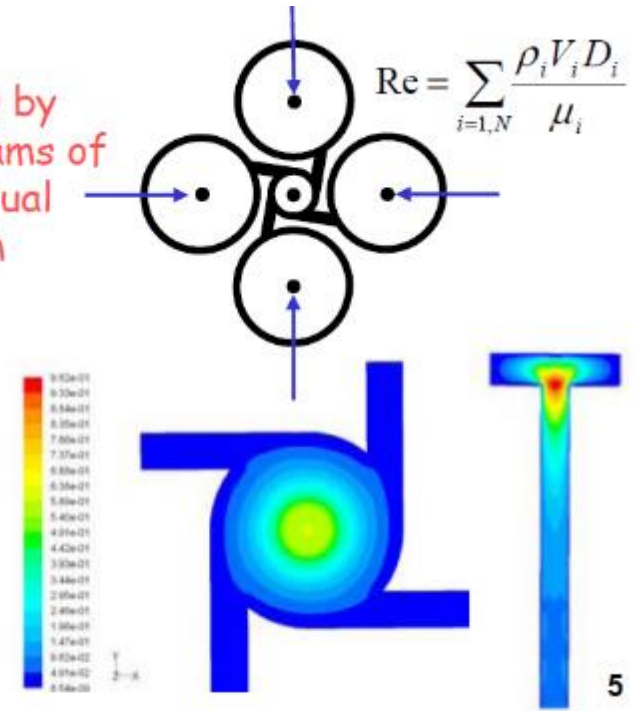


Confined impinging jet micro-reactors



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

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



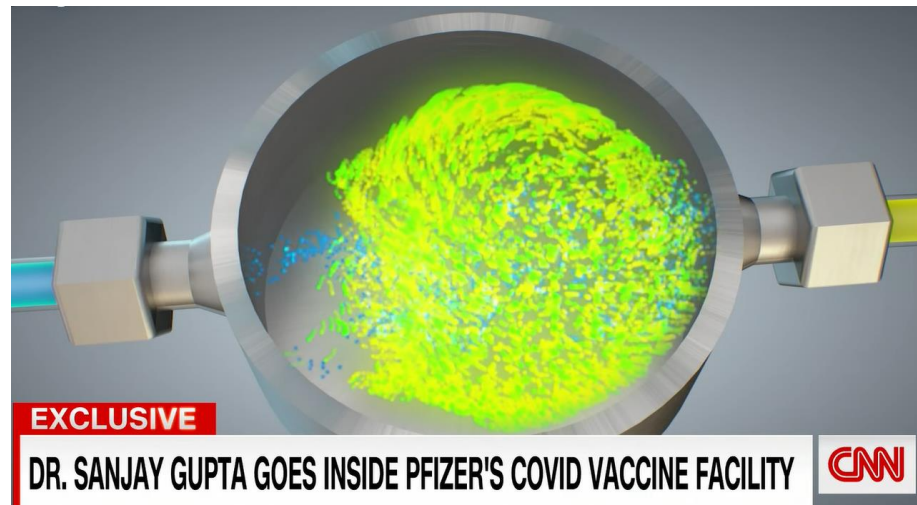
Y. Liu, R.K. Prud'homme *et al.*, *Chem. Eng. Sci.* 63 (2008) 2892-2842
 B.K. Johnson and R.K. Prud'homme, *AIChE J.* 49(2002) 2264-2282



Princeton CIJ mixers and COVID vaccines

*Pfizer COVID vaccine
3 billion doses*

*Mike McDermott, the
President of Pfizer Global
Supply, in the CNN
interview where they took
the press through the
Pfizer production
process: “**the
impingement jet mixer
makes it possible.**”*

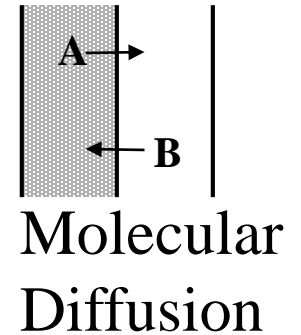
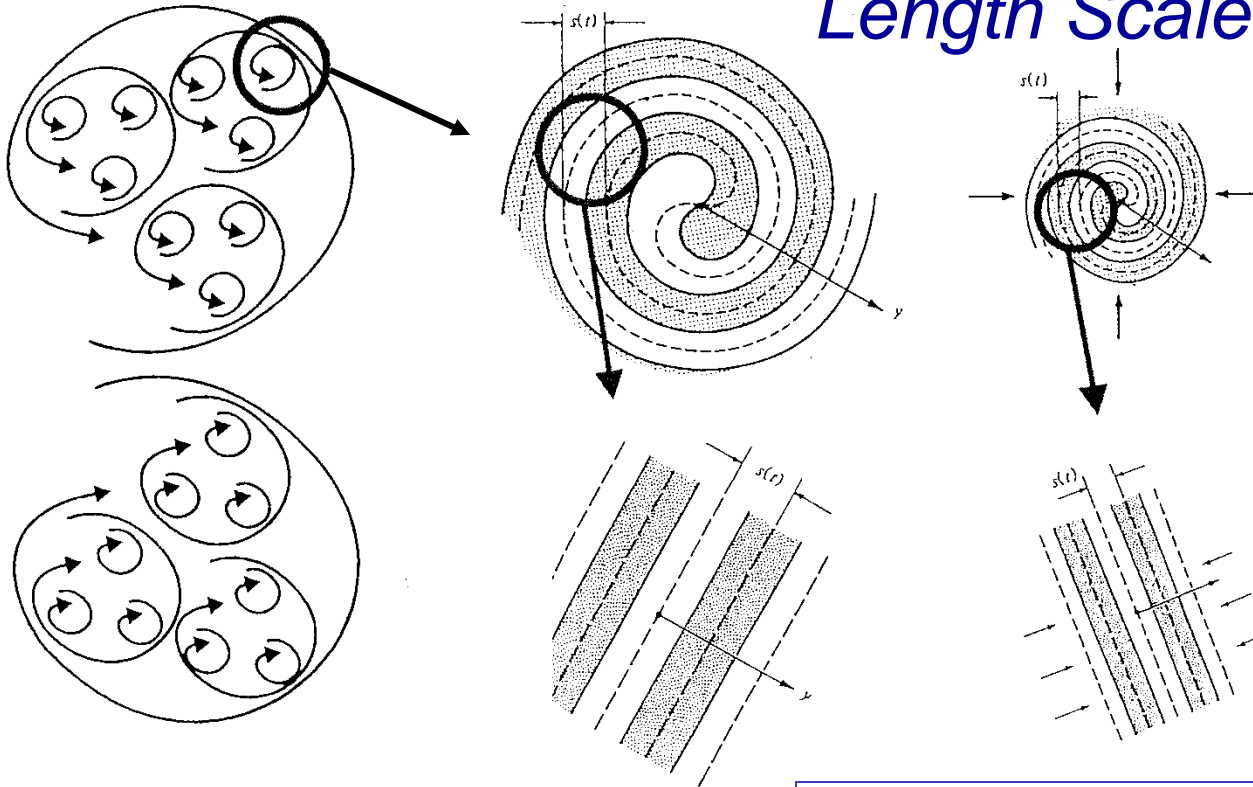


CNN. Manufacturing moonshot: How Pfizer makes its millions of Covid-19 vaccine doses <https://www.cnn.com/2021/03/31/health/pfizer-vaccine-manufacturing/index.html>.

TURBULENCE THEORY - Mixing Two Fluids

*Kolmogorov
Length Scale*

$$\lambda = \left[\frac{\nu^3}{\varepsilon} \right]^{1/4}$$



Mixing Time

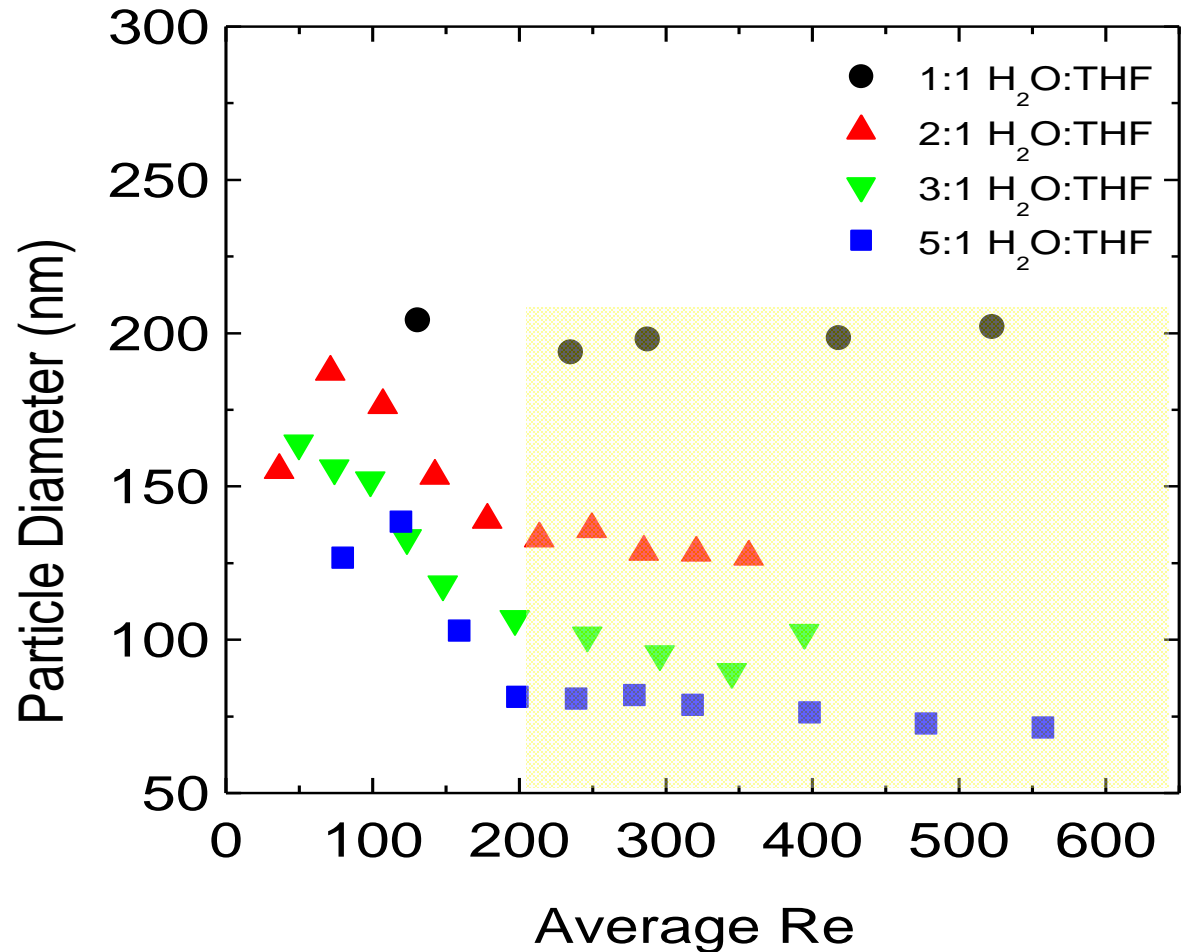
$$\tau_{mix} = \tau_{diffusion} = \frac{\lambda^2}{4D} \propto \mathbf{Re}^{-1.5}$$

Control of particle size

1. *Mixing intensity*

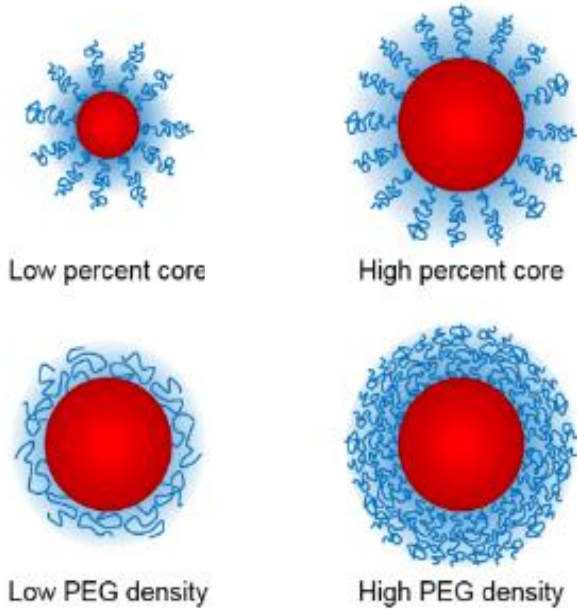
2. *Super-saturation*

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

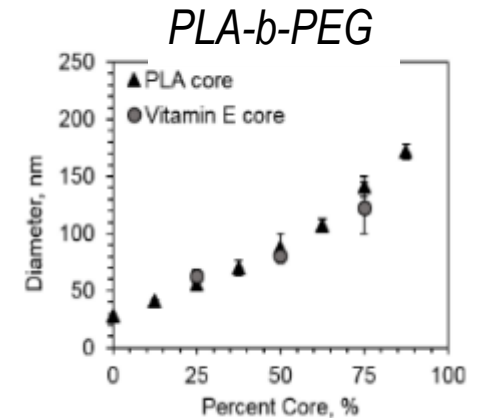
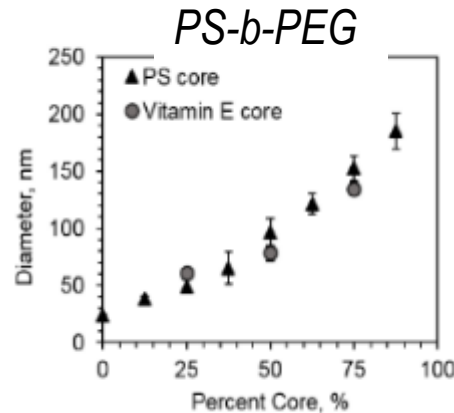


NP Formation: PS to PLA NPs

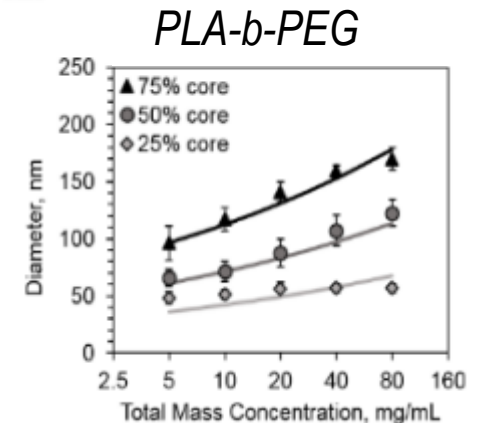
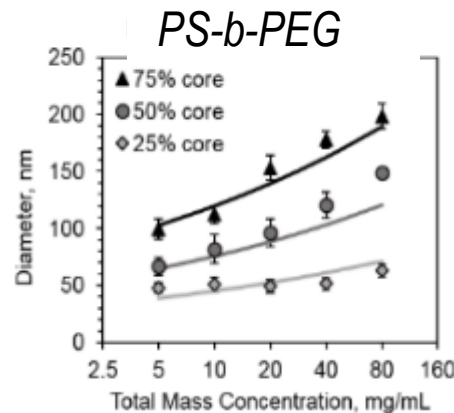
Want to shift from **model polystyrene (PS)** to **biocompatible polylactic acid (PLA)**. How does size depend on species in core?



b. Effect of Percent Core on NP Size



c. Effect of Total Mass Concentration on NP Size

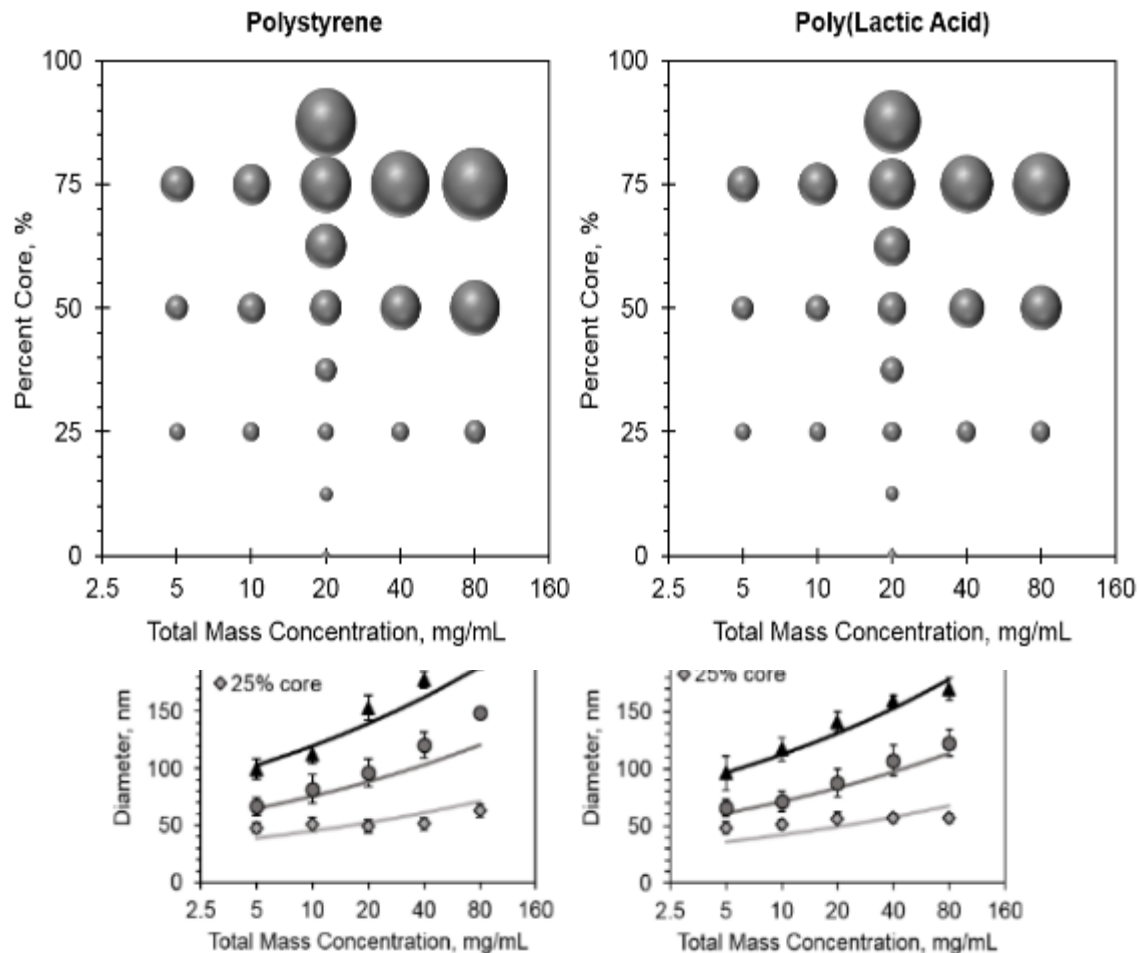
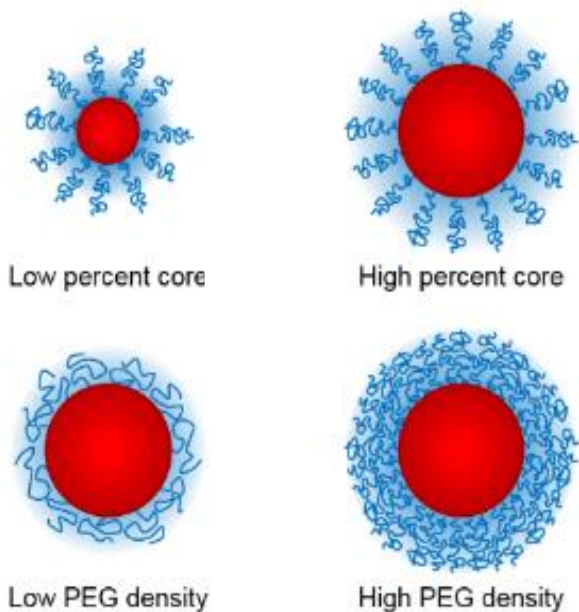


- What is in the core doesn't matter
- The ratio of core to BCP matters, C_{core}/C_{BCP}
- The total mass matters

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?



- *What is in the core doesn't matter*
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Pagels *Nano Letters* **18** 1139-1144 (2018)

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Conflicting Goals: Oral vs Parenteral Delivery

Schizophrenic communities

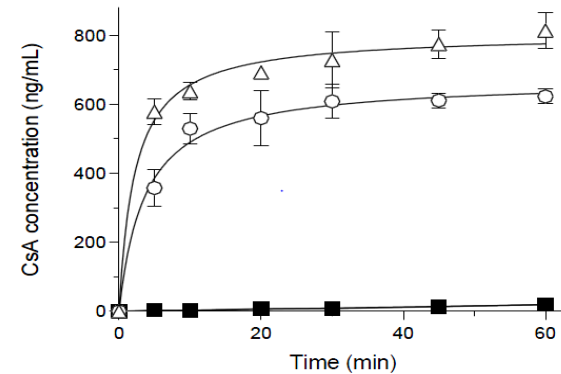
1. Increased bioavailability

1. **Oral: Gates global health drugs**
2. Milling and Solid Spray Dried Dispersions (Bend Res.): make things smaller, amorphous
3. Make things dissolve more rapidly

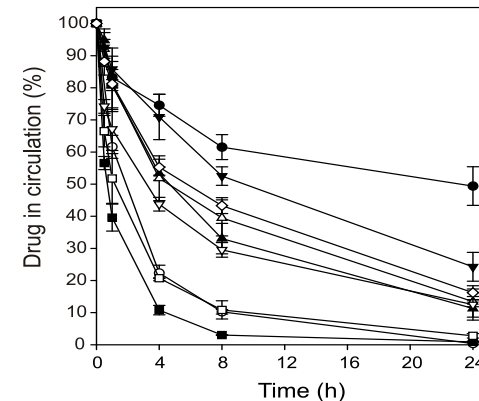
2. Targeted delivery to specific tissue (cancer, etc.)

1. **Parenteral : COVID vaccines**
2. Protected drug cargo (mRNA, peptides, proteins, anticancer API)
3. Keep things from dissolving/releasing until desired

- Cyclosporine A
- NPs ~200-300 nm
- Ppt with lecithin + mannitol and spray dried
- Supersaturations of 100
(Sato, Prud'homme *Intl.J.Pharm* (2017))

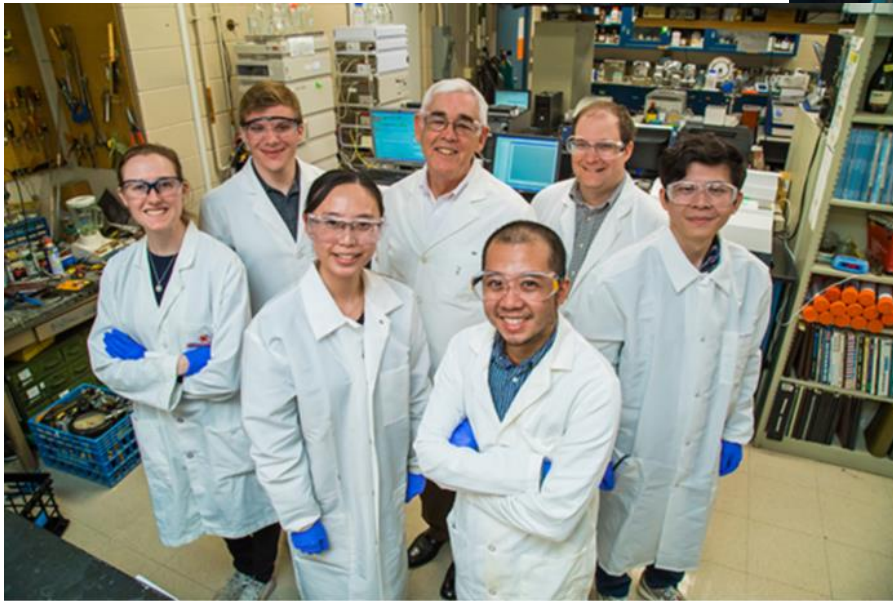


- Paclitaxel conjugate
- NPs 60 nm
- (Ansel, Prud'homme *Mol Pharm* (2102))



Gates Foundation and Global Health

- *Oral delivery*
- *Low Cost*
- *Translatable/Scalable*



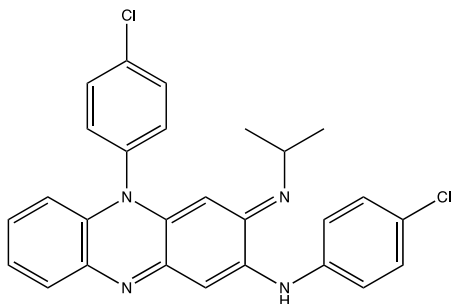
*Niya Bowers
(Gates)*



*Sunny Panmai
Wu Xi AppTec*

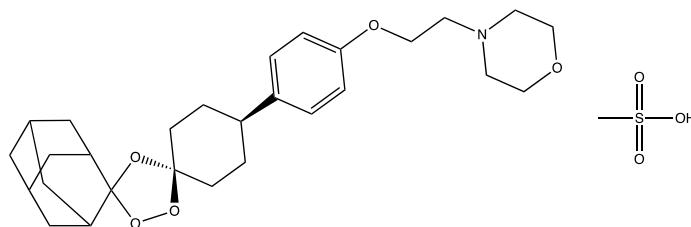
Drugs Considered

Clofazimine



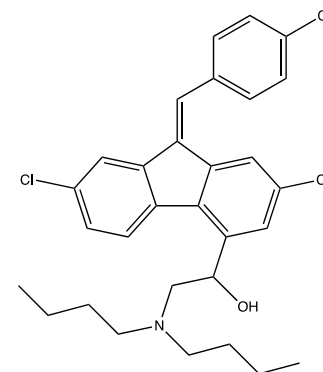
Ref: Design and Solidification of Fast-Releasing Clofazimine Nanoparticles for Treatment of Cryptosporidiosis". *Molecular Pharmaceutics*. **14**(10) 3480-3488 (2017); "Rapid Recovery of Clofazimine-loaded Nanoparticles with Long-term Storage Stability as Anti-Cryptosporidium Therapy". *ACS Applied Nano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

OZ439



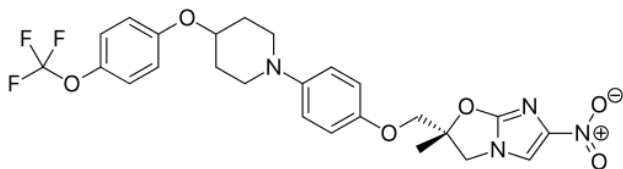
Ref: "Encapsulation of OZ439 into Nanoparticles for Supersaturated Drug Release in Oral Malaria Therapy". *ACS infectious diseases*. **4**(6) 970-979 (2018); "Spray drying OZ439 nanoparticles to form stable, water-dispersible powders for oral malaria therapy". *Journal of Translational Medicine* (2019),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

Lumefantrine



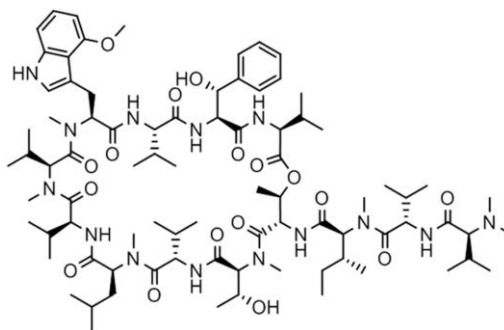
Ref: "Amorphous Nanoparticles by Self-assembly: Processing for Controlled Release of Hydrophobic Molecules". *Soft Matter*. **15** 2400-2410 (2019)

Delamanid



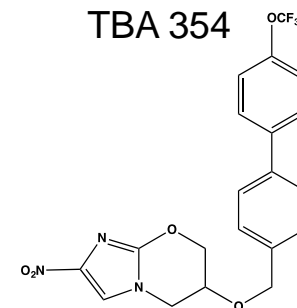
Ref:
<https://app.box.com/s/lvghy956h7pmzvdd1nlj9zz eotabijke>

Ecumicin



Ref:
<https://app.box.com/s/5lggnm4tv84kcb0cry0tscb y9oszzqzi>

TBA 354



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

Malaria is a global health pandemic

210 Million

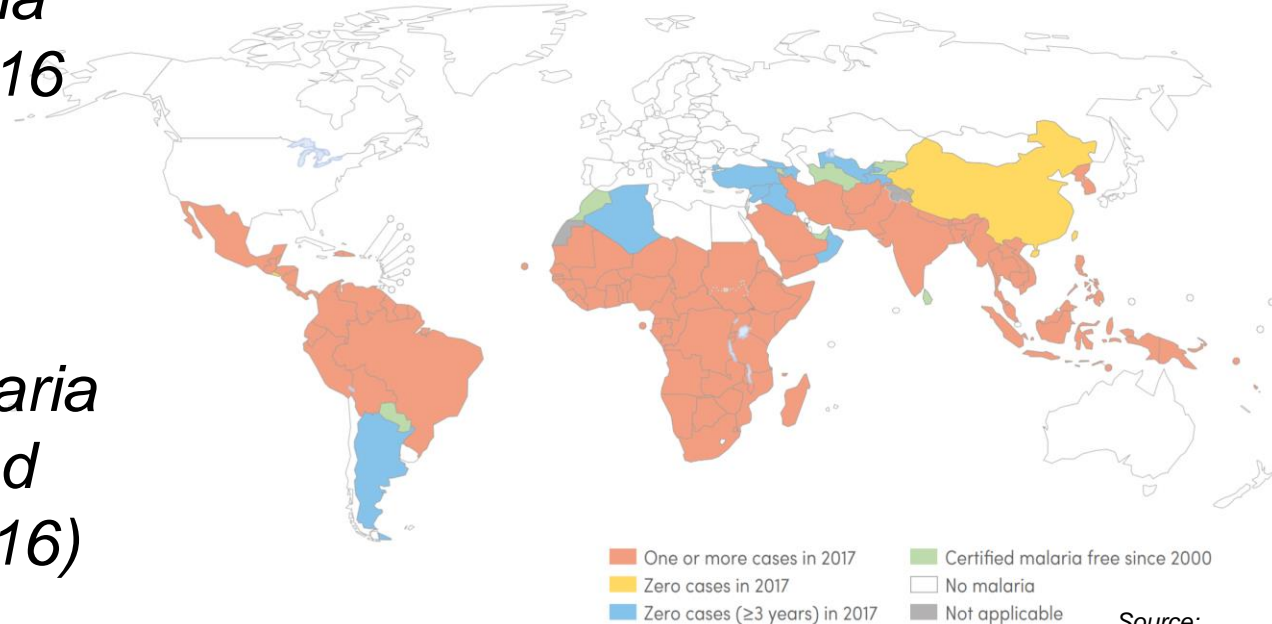
Cases of malaria worldwide in 2016 alone

429,000

Deaths due to malaria or malaria-related complications (2016)

>70%

Of deaths were in children under 5 years of age

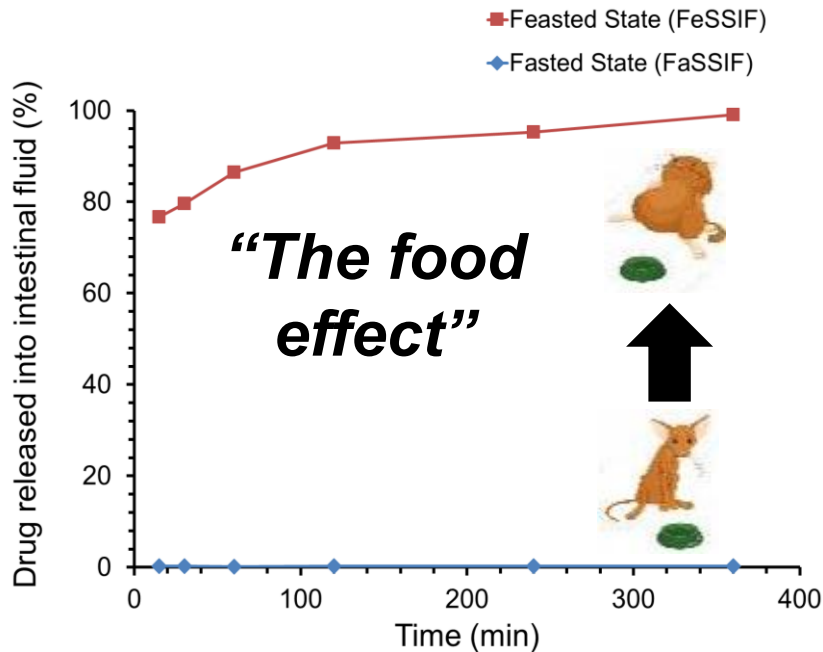


Source:
WHO

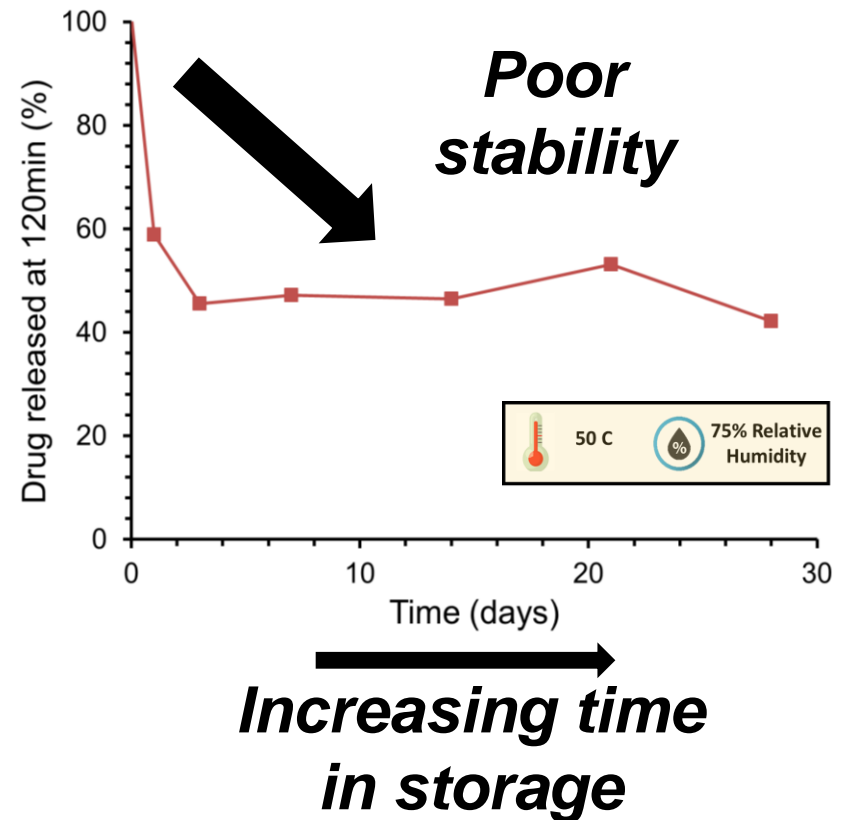
Lumefantrine: Two pharmaceutical challenges

Solid Spray Dried Dispersion

1 Bioavailability is dependent on food intake

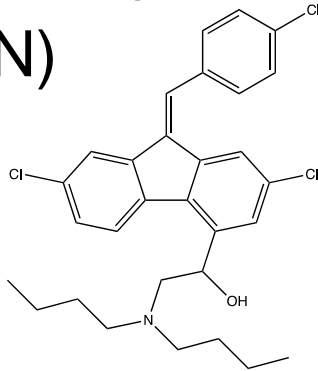


2 Crystallization over time reduces shelf-life



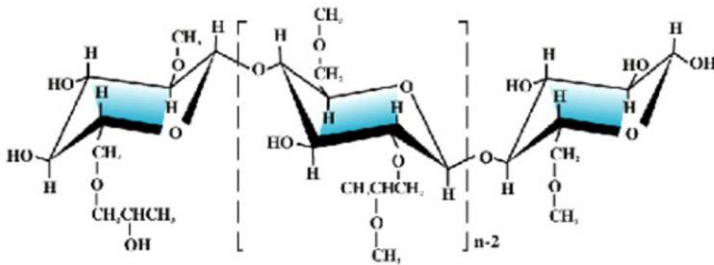
Lumefantrine Stabilizers

Lumefantrine (LMN)



• HPMCAS:

- NP size: ~ 100 nm
- HPMCAS126, 716, 912 all form NPs, but 126 most stable
- 90% drug loading (60% in previous work)

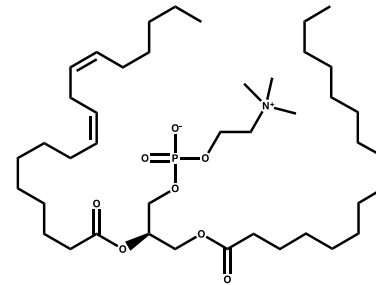


• Lecithin:

- NP size: ~ 400 nm
- 67% drug loading
- In 10% organics (THF), stable for ~ 4-6 h

• Zein/Casein

- NP size: ~ 200 nm
- 40% drug loading
- In 10% organics (EtOH& acetone), stable for ~ 6h



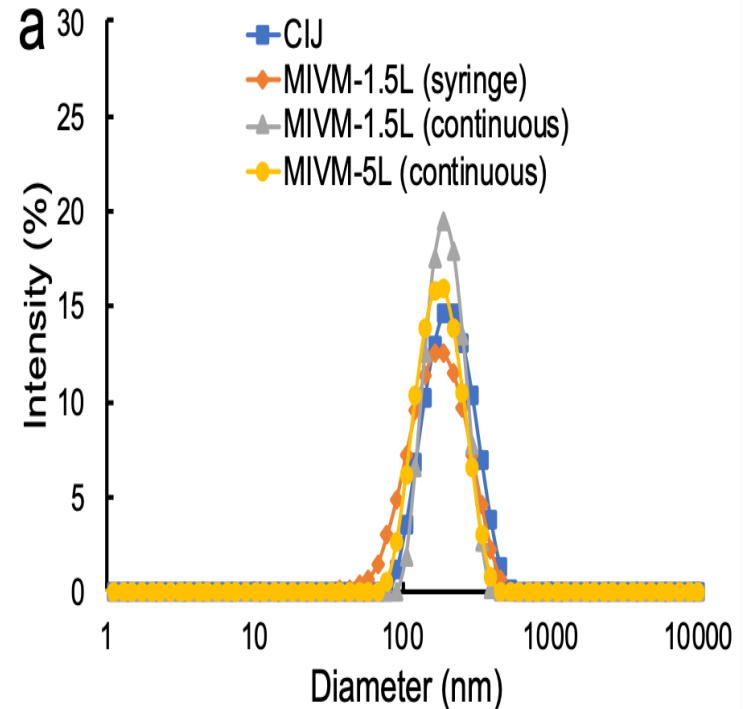
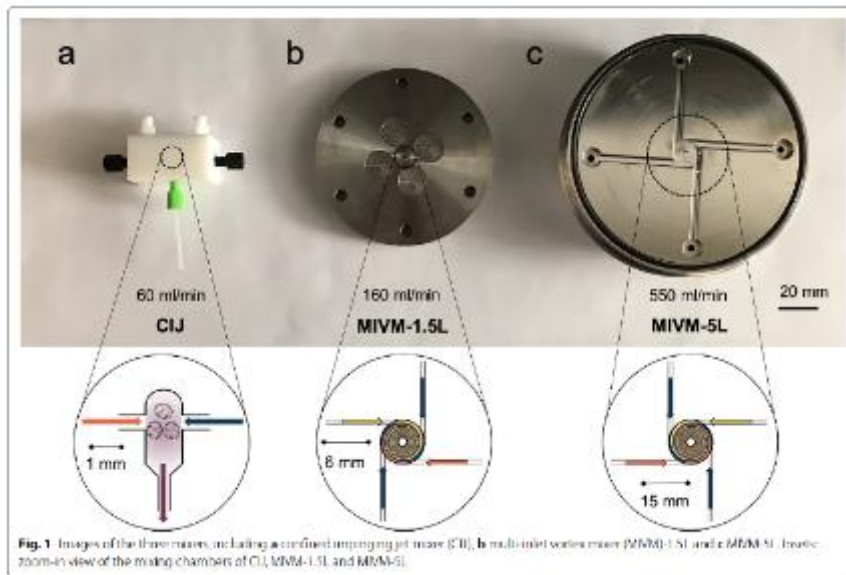
Lecithin



Zein

SCALEUP: Equivalent NPs from 1 ml to 5 L/min)

- *Identical NPs produced at sub-mg scale to 3000 L samples*
- *Funded by the Gates Foundation*
- *GMP line developed at WuXi Aptec for global health drugs*



Armstrong J. Pharm Sci (2023)

PRINCETON: Large scale NP process

*Flow
controller
large-scaled
FNP*



*TFF increases NP
Concentration*

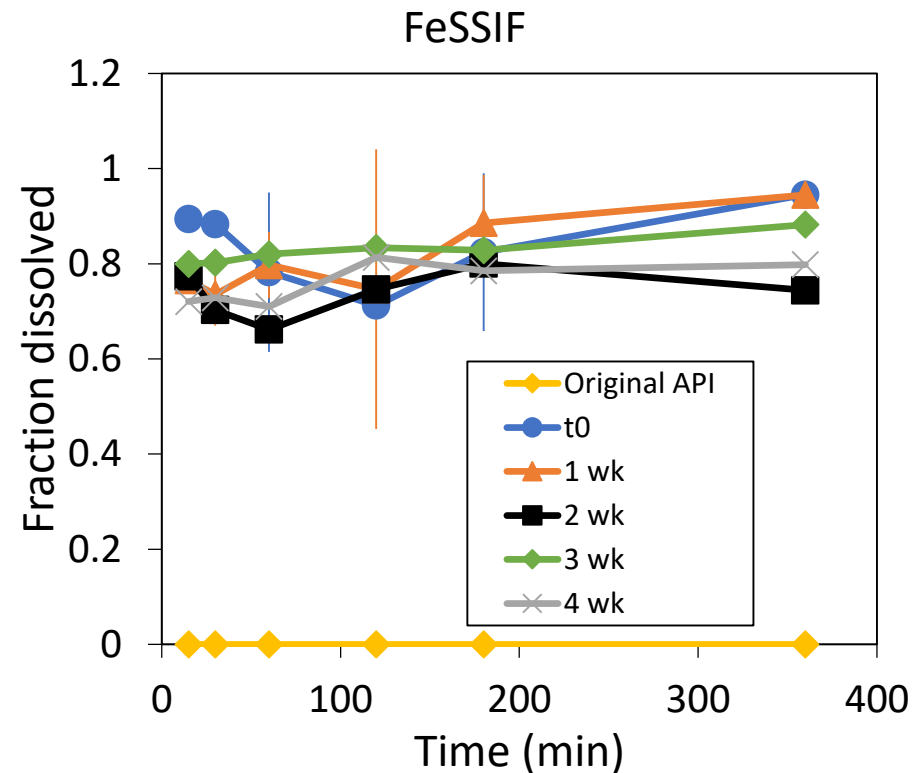
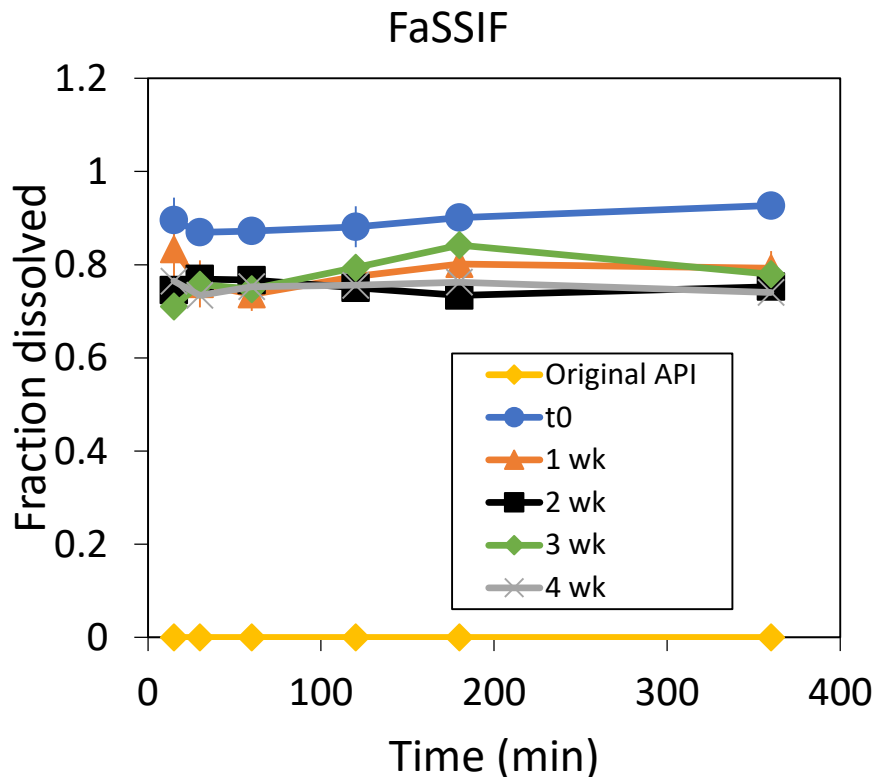


*Spray-drier
Produces Dry
Powder*



Dissolution kinetics: fed/fasted state & stability

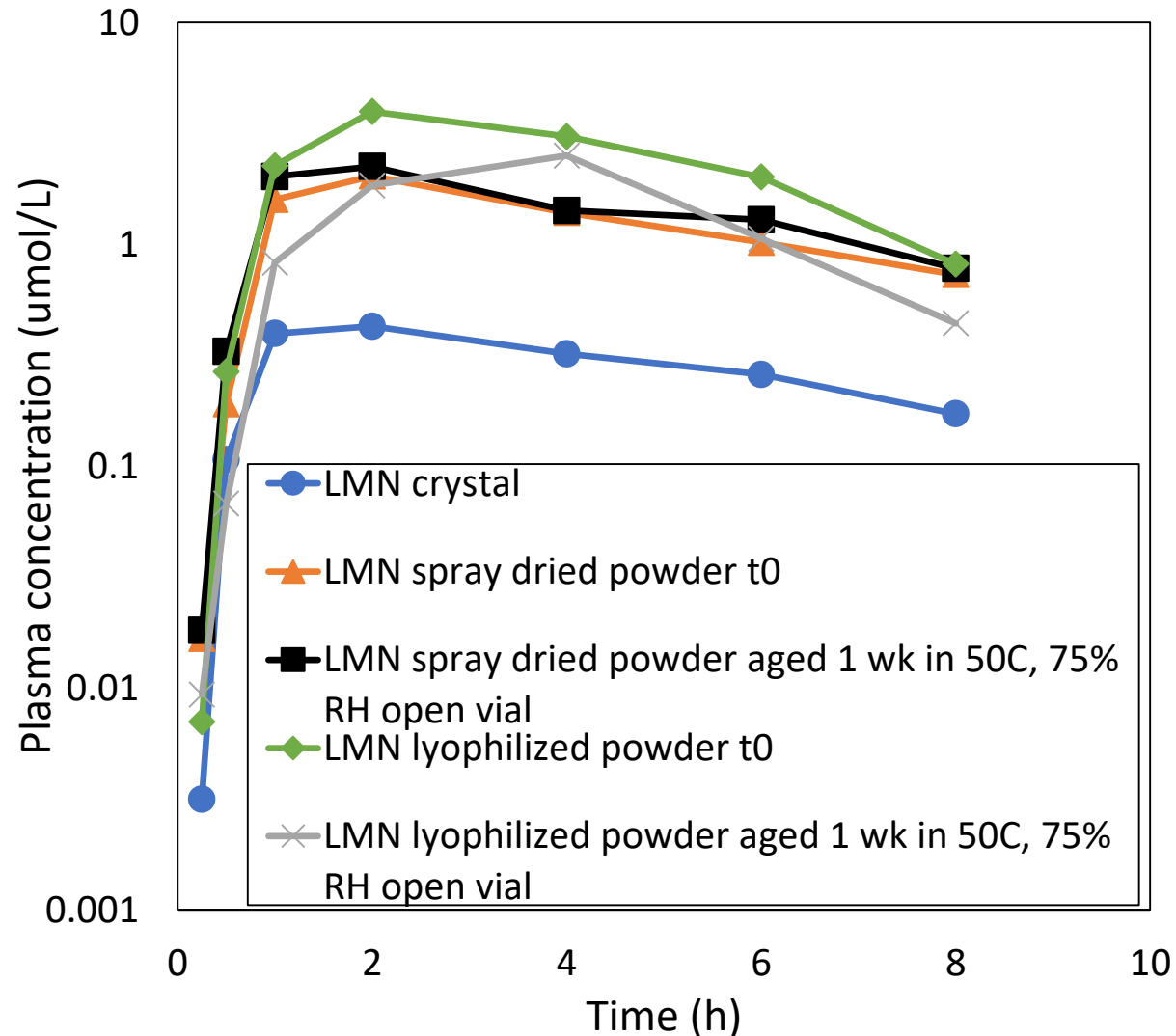
- *4 week stability under accelerated aging conditions (50C, 75% RH, open vial)*
- *Similar release kinetics in fasted or fed state media (no food effect)*



Enhanced bioavailability of LMN in animal study

LMN NP powder samples increased the bioavailability of the LMN 4.2X compared to the crystalline drug

Sample	AUC (hr*umol/L)
LMN crystal powder	2.29
LMN spray dried powder t=0	9.74
LMN spray dried powder 1 wk	11
LMN lyophilized powder t=0	18.3
LMN lyophilized powder 1 wk	10.6



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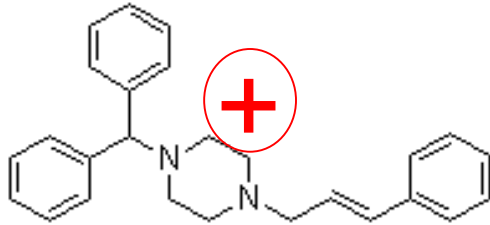
Stable NPs by ion pair complexation

Cinanzarizine

(antihistomine, weak base)

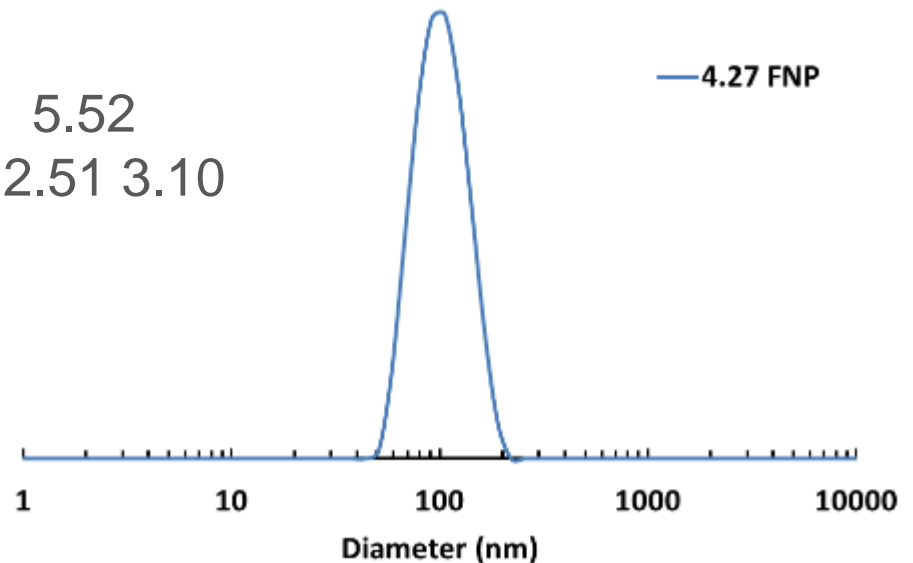
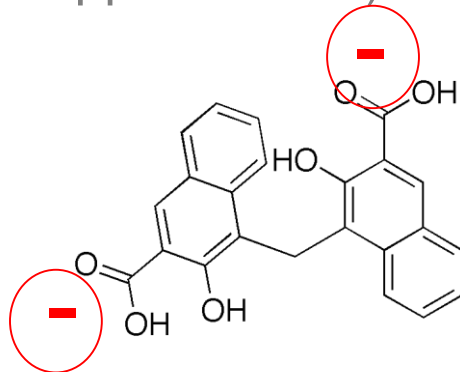
LogP 5.3
pK_b 7.5, 1.95

- Individual cnds can not form stable NPs
- Complex eliminates polarity of charge groups



Pamoic acid (FDA approved salt)

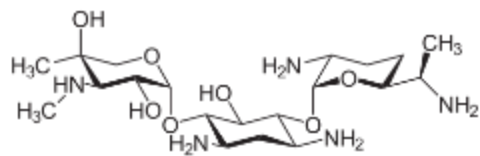
LogP 5.52
pK_a 2.51 3.10



Pinkerton, Grandeury[‡], Fisch[‡], Jörg Brozio[‡], Bernd U. Riebesehl et al., Mol Pharm (2012)

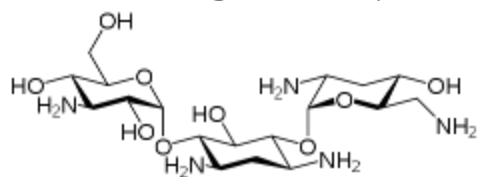
Ion Pairing P. Aeruginosa Antimicrobials

Cationic API



clogP = -6.3

gentamicin

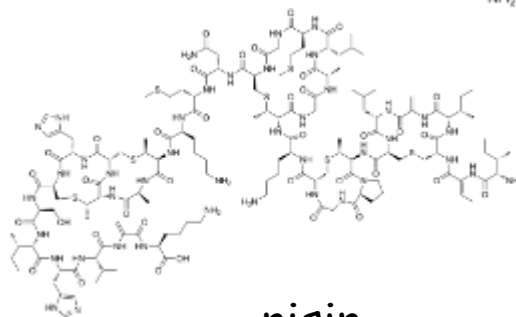


clogP = -7.2

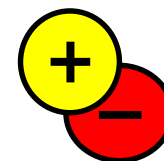
tobramycin



polymyxin

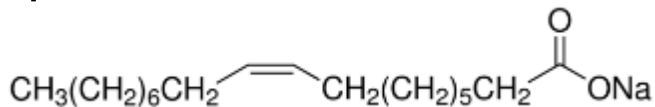


nisin



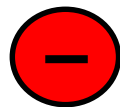
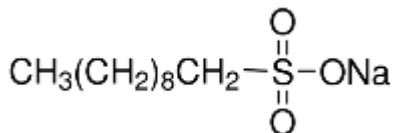
Hydrophobic salt-form of API

Hydrophobic + Anionic Ion Pair



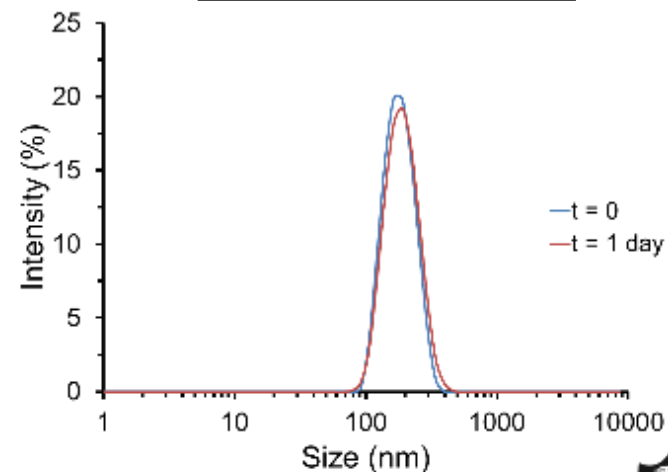
clogP = 4.9

pKa = 5.0

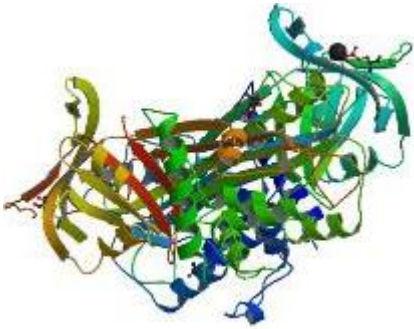


clogP = 0.8

pKa = -1.5



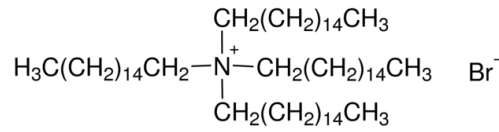
Ion Pairing of Proteins: Ovalbumin



Protein: chicken egg ovalbumin (OVA)

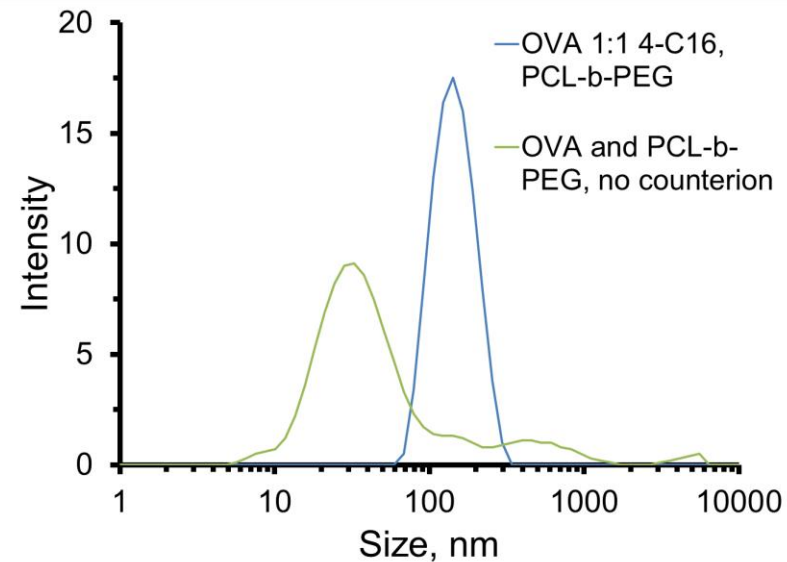
- 43,000 Da MW
- -48 charge at physiological pH

+



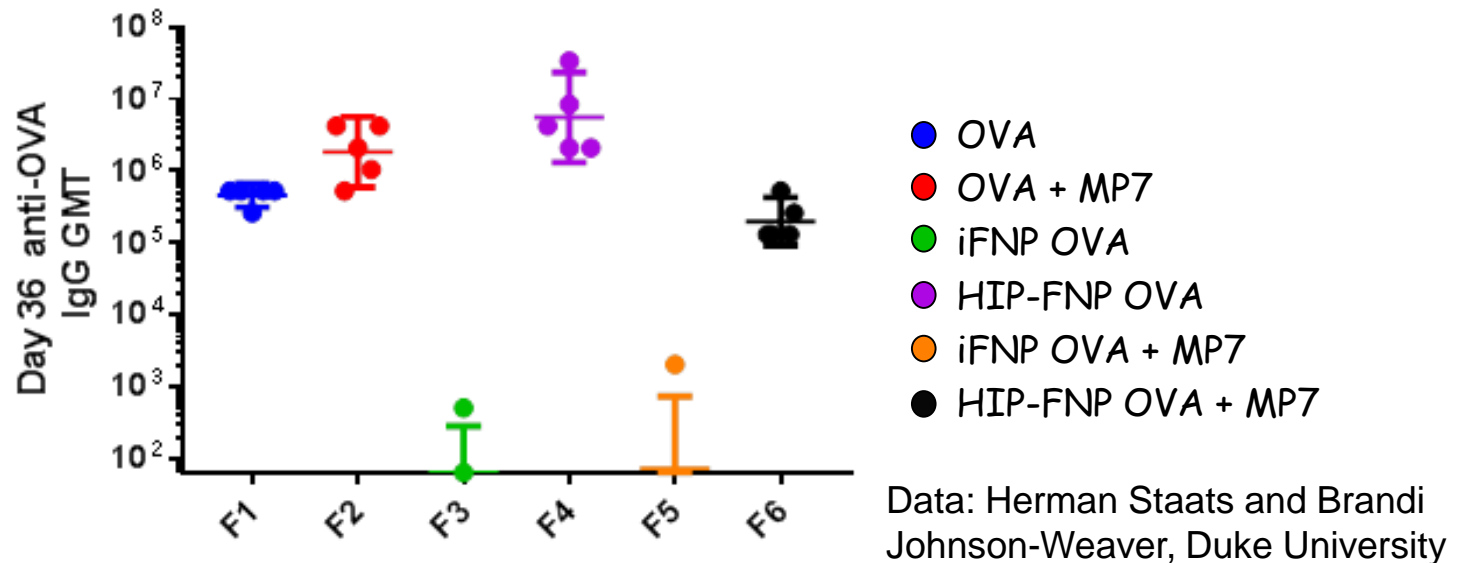
Counterion: quaternary amine surfactants

- Various carbon chain lengths and number of chains



- Successfully formed NPs
- Without hydrophobic counterion, OVA does not precipitate as desired
- Tuned size by altering counterion chemistry and charge ratio

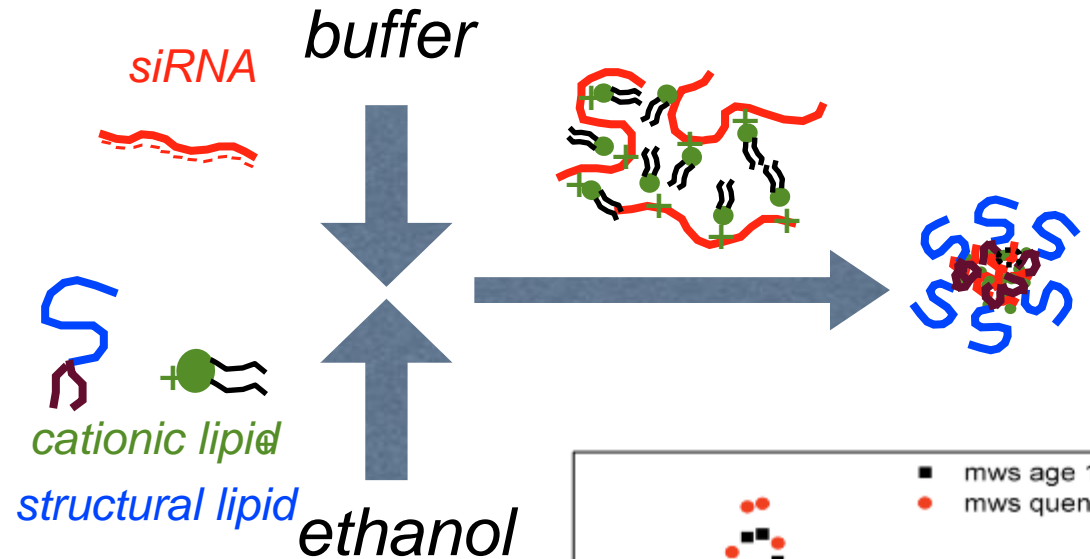
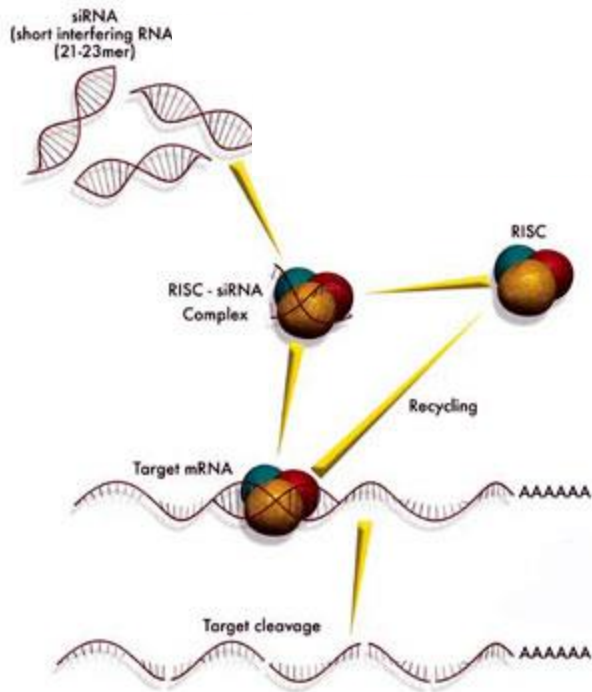
Protein encapsulation by ion pairing: ovalbumin



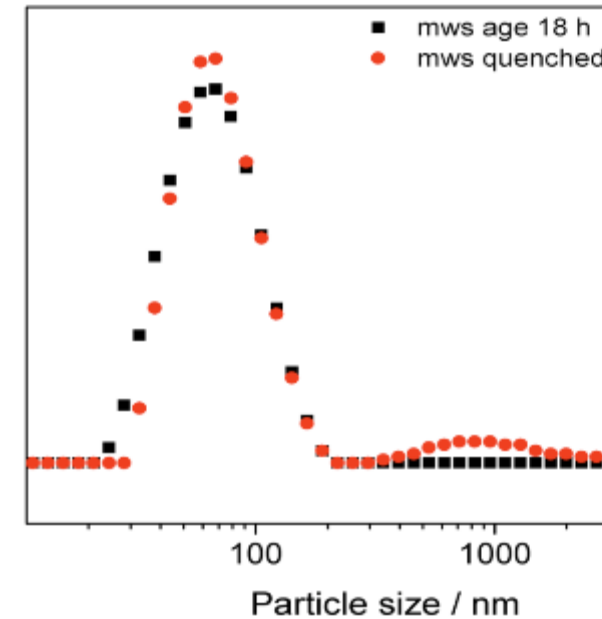
- Nasal vaccine collaboration with Herman Staats, Duke University
- OVA-containing NPs prepared by iFNP and HIP-FNP
- NPs and soluble OVA control delivered intranasally with and without immunomodulatory peptide MP7 in bulk
- Highest *in vivo* immune response seen from OVA-containing HIP-FNP particles (purple and black)

siRNA or mRNA Lipid Nanoparticles

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



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

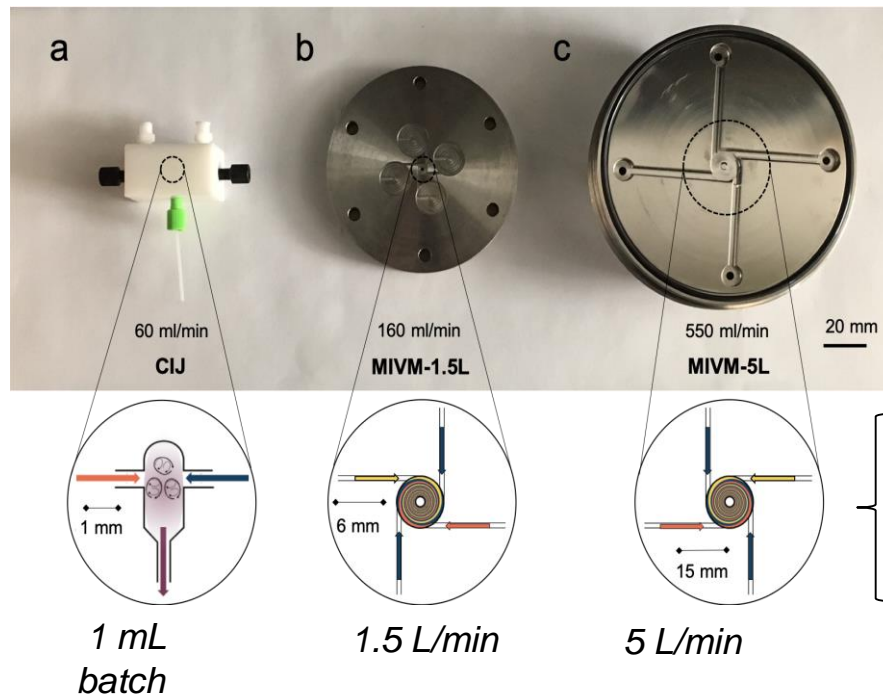


Four Scales of CIJ & MIVM

CIJ
Confined Impinging
Jet

MIVM
Multi Inlet
Vortex Mixer

Micro-MIVM
m-Multi Inlet
Vortex Mixer

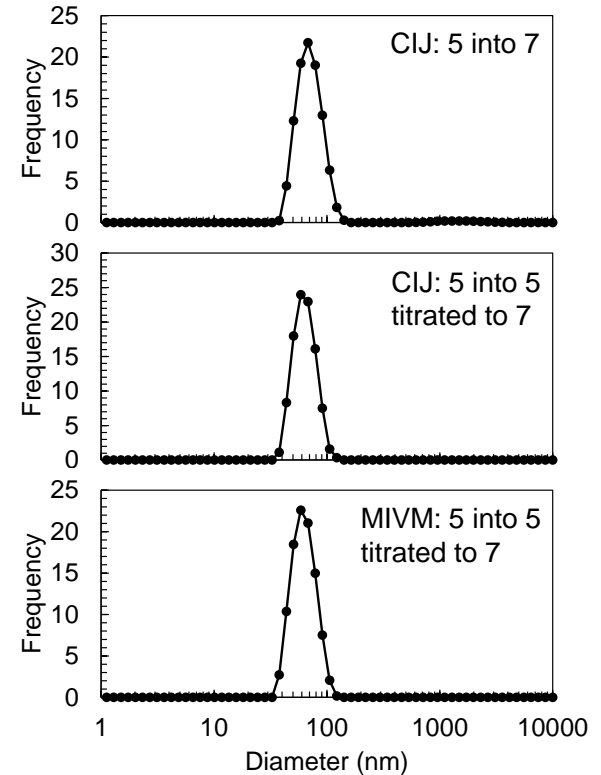
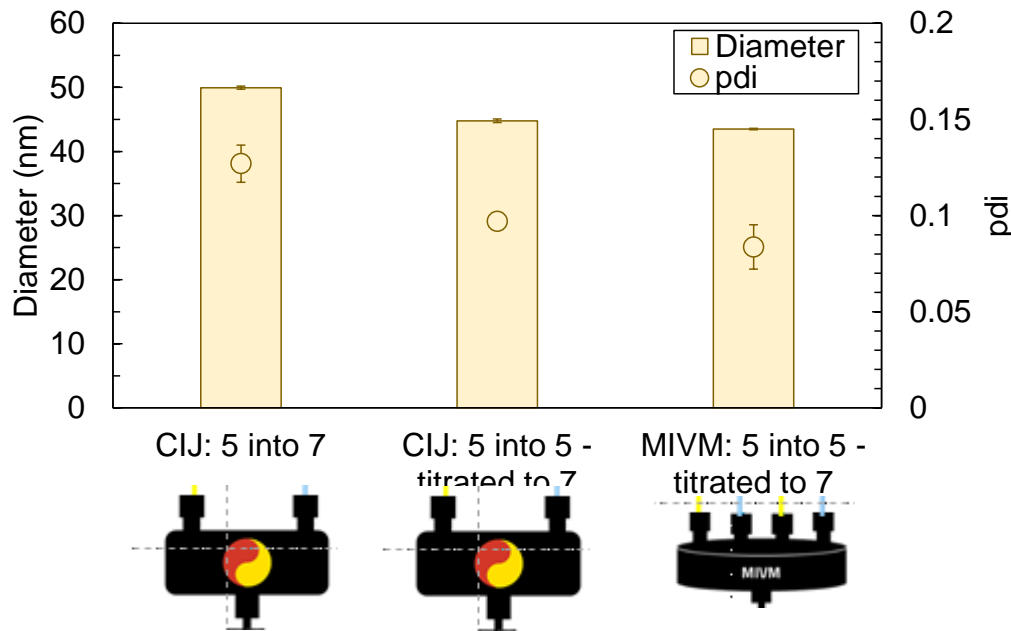


- 4 glass syringes: 2 ml
- 0.3-0.6 mg RNA (N/P=6)

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

Sub 100 nm LNPs are produced via CIJ and MIVM

No effect of mixer geometry



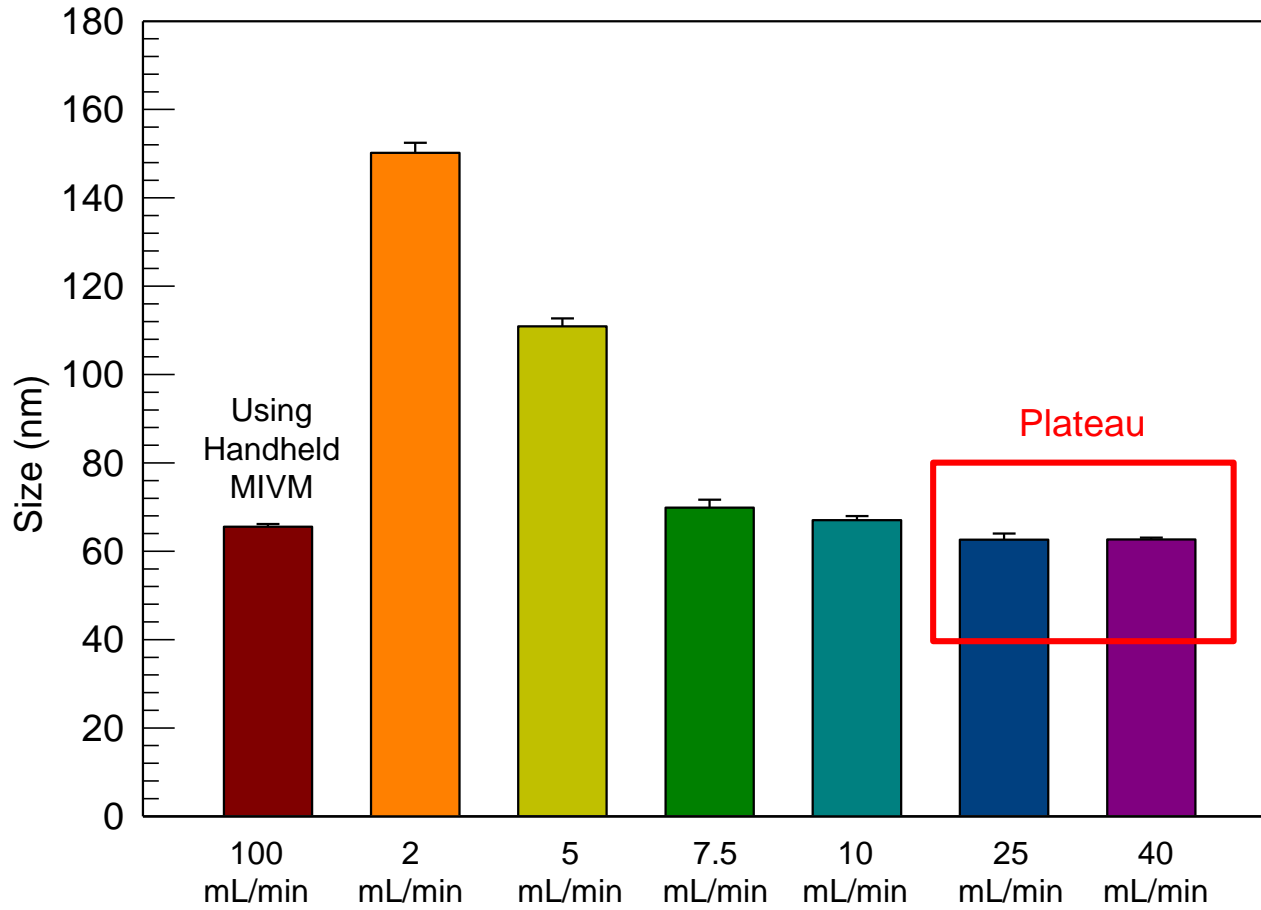
LNP size is independent of pH shifting process: pH= 5 → pH 7

Pump-based MIVM vs handheld MIVM

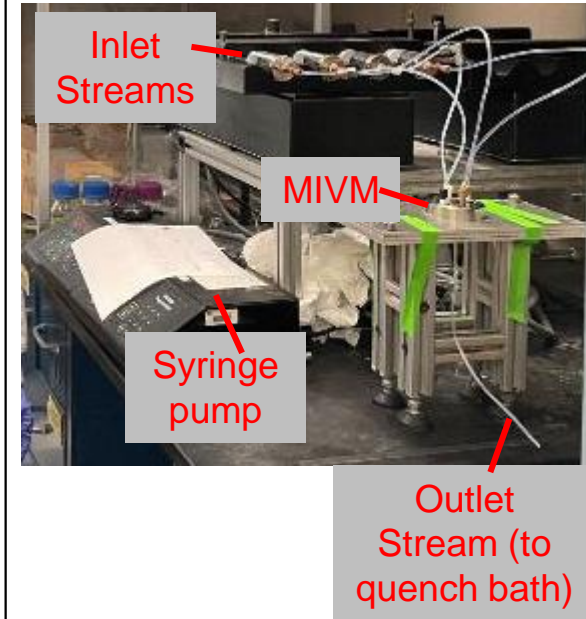
Before
concentration

DODMA lipid, mRNA (yeast) LNPs

N/P ratio: 6



MIVM setup

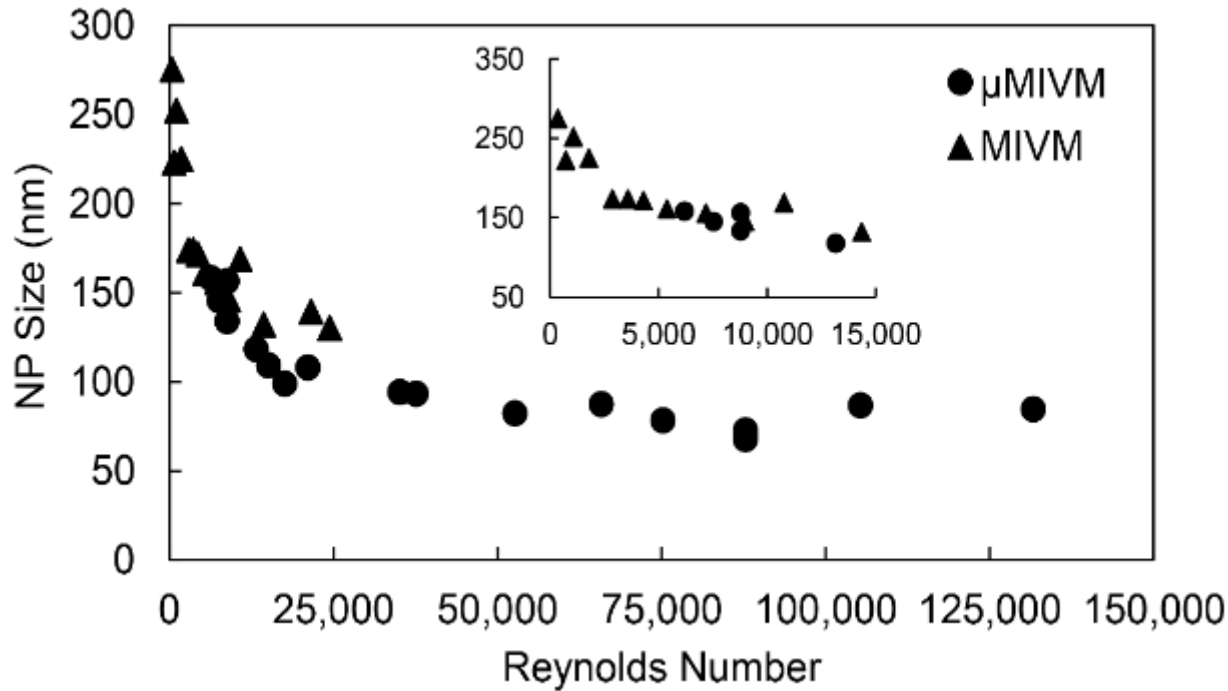


Pump-based MIVM vs handheld MIVM

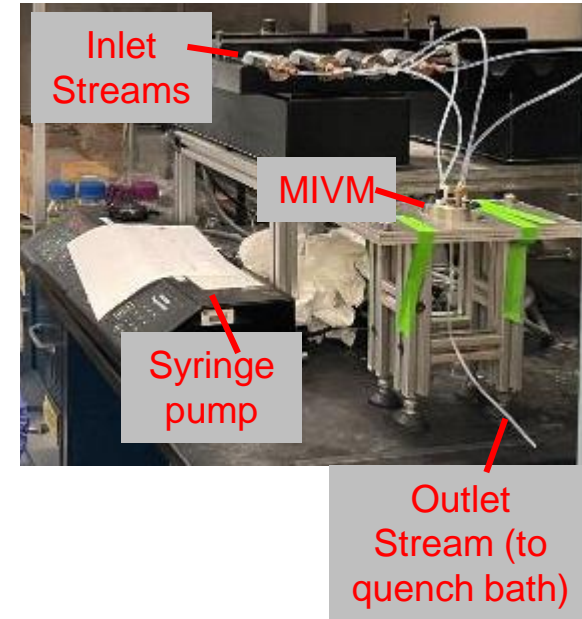
Before
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MIVM setup

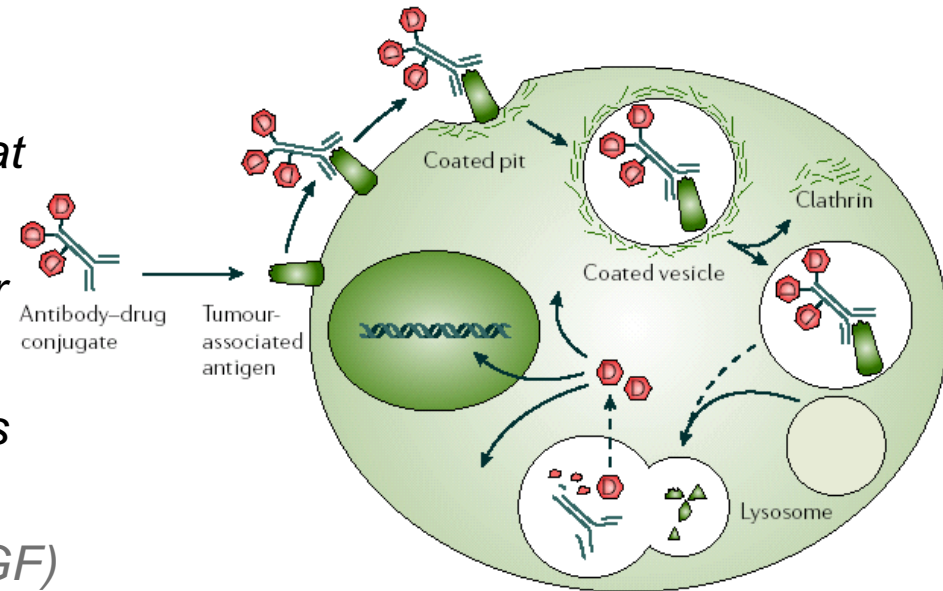


Outline

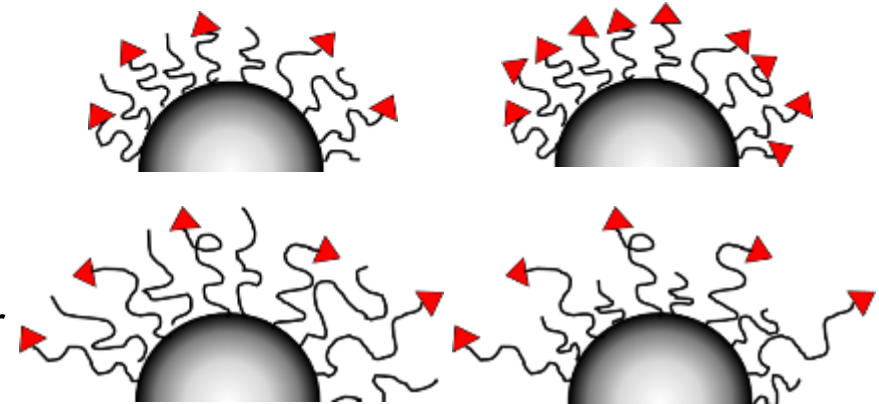
1. ***Nanoparticles by turbulent micromixing in confined impinging jet mixers (CIJ)***
 1. *Development of an idea: Flash NanoPrecipitation (FNP)*
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 3. ***Parenteral: Ion pairing for hydrophilic drugs (LNPs RNA, peptides, proteins)***
3. ***Targeting***

Active targeting

- *Over-expression of specific receptors at surfaces of diseased cells*
- *Allows for targeting of metastatic tumor cells, macrophages, inflammation, etc.*
- *NP surfaces functionalized with ligands*
 - *Small molecules (folate, mannose)*
 - *Peptides and proteins (LHRH, VEGF)*
 - *Antibodies (2C5)*
- *Key research questions*
 - *Density of ligand attachment*
 - *Preservation of ligand integrity (functionality)*
 - *PEG Mw*
 - *Presentation of PEG vs steric layer*



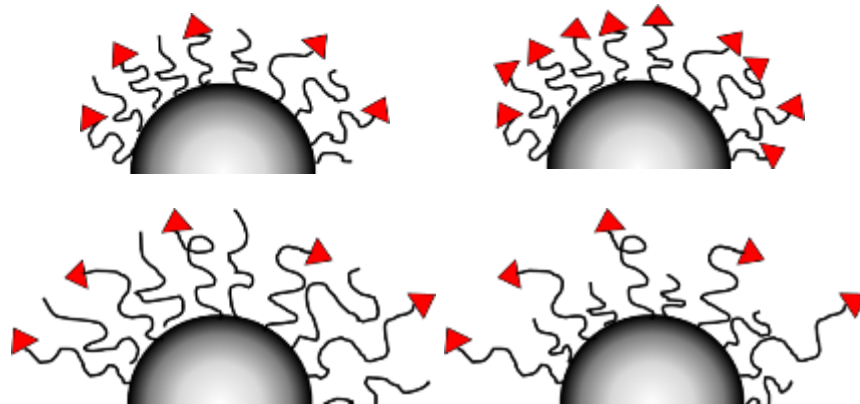
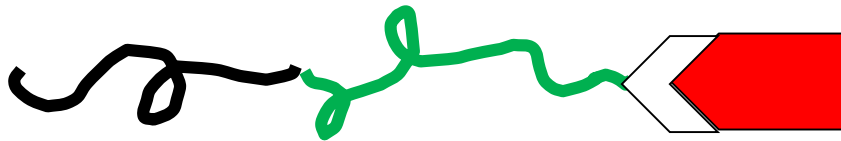
Nature Reviews 2006, 5, 147-159



Gindy, Biomaterials (2008)
Akbulut, Adv. Fn. Mat. (2009)

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_H

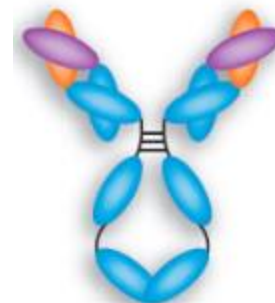


V_K

scFv

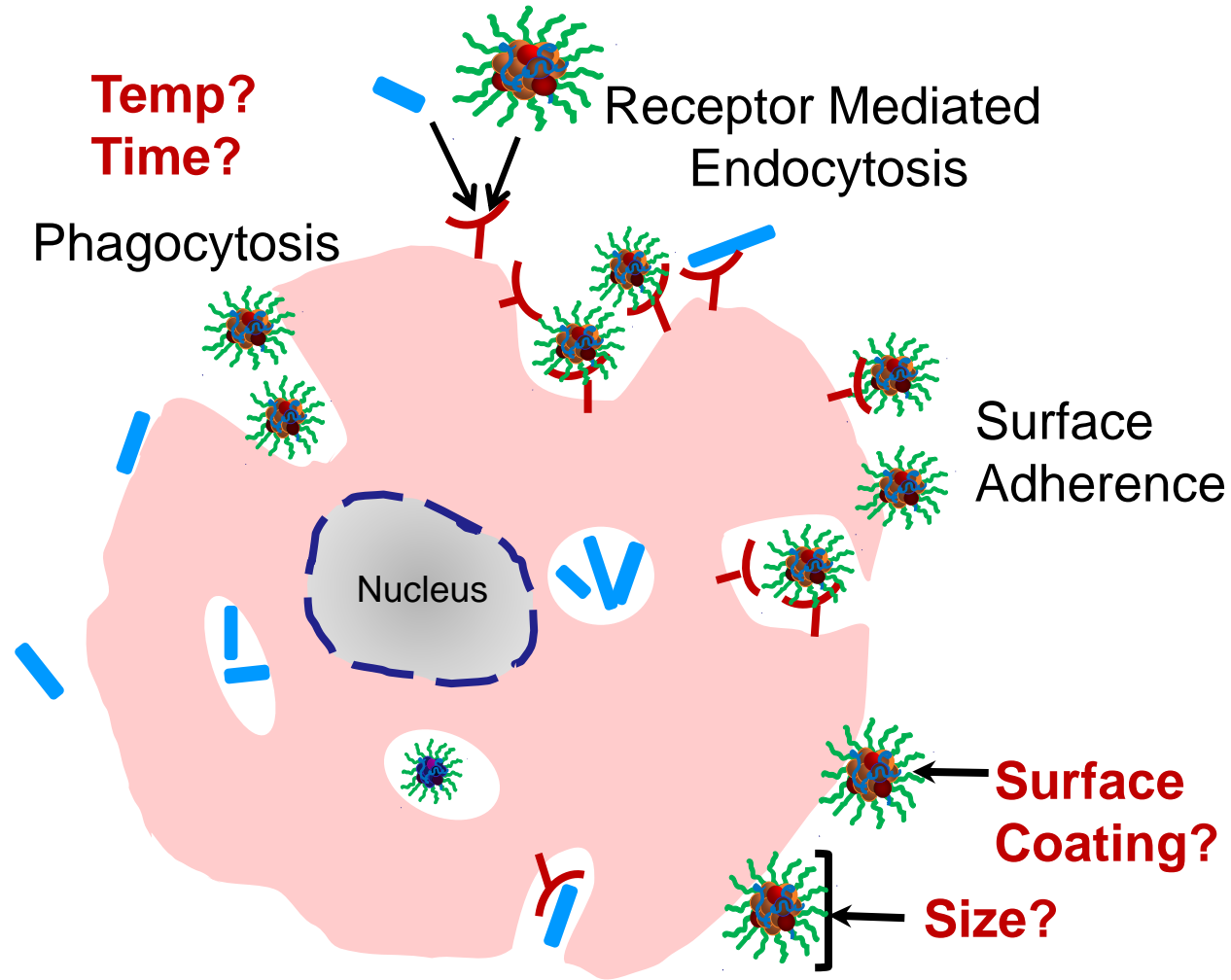


IgG



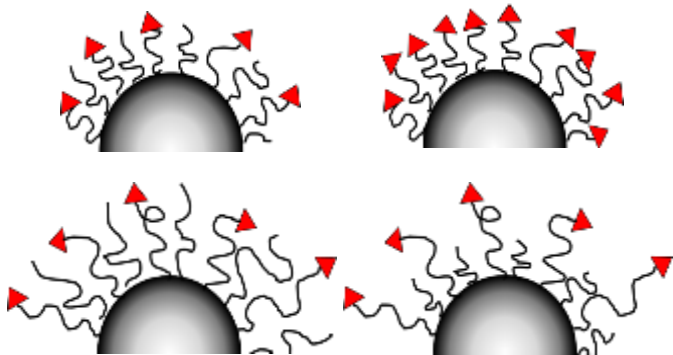
Targeting of Macrophages for TB

1. Engineer a range of different surface-coated and sized NPs
2. Explore the effects of temperature, incubation time, NP size, surface coating properties on uptake by macrophages
3. Coat NPs with ligands to enhance NP uptake by MACs

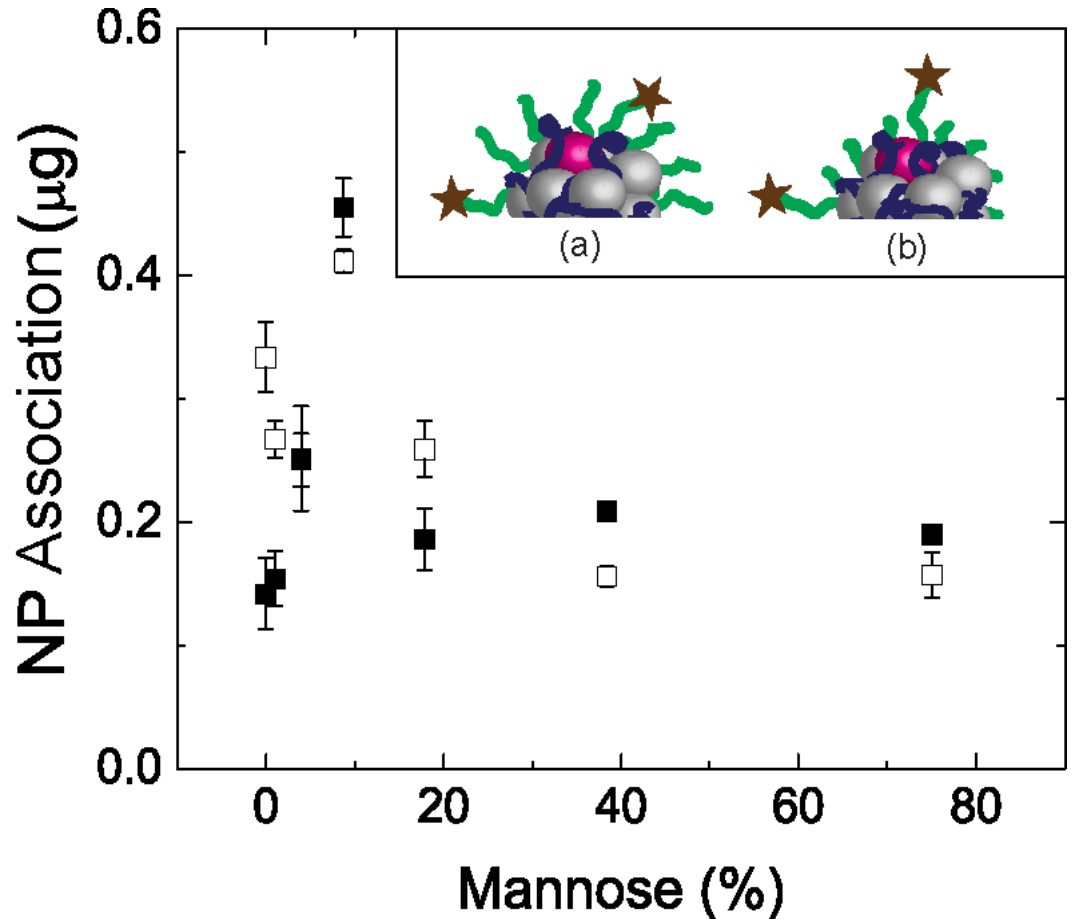
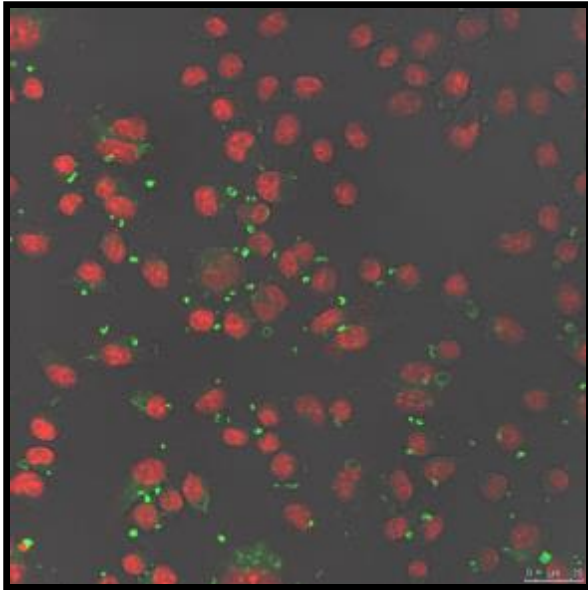


Note: also doing folate targeting (U Mich, click chemistry), VEGF (Sibtech, maleimide), antibody (V. Torchilin, pNP), LHRH

Mannose Receptor (MR) Targeting

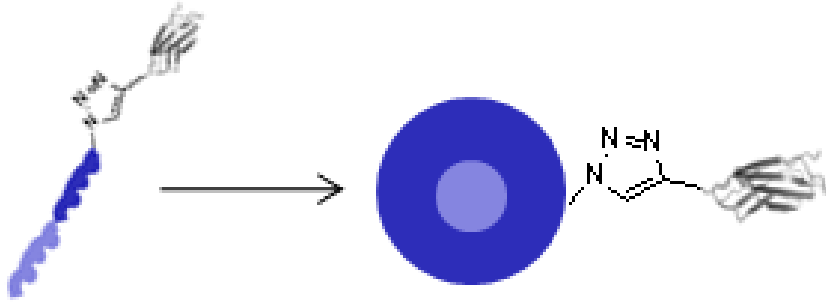


Gindy, Biomaterials (2008)
Akbulut, Adv. Fn. Mat. (2009)



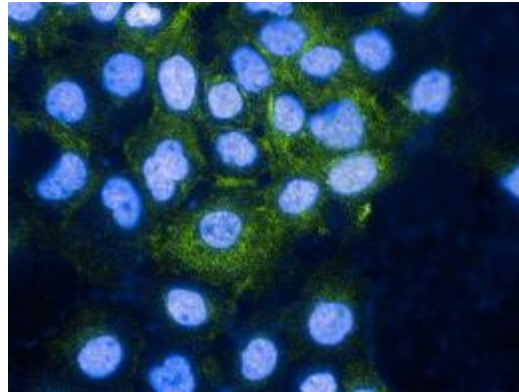
Binding and uptake of NPs is enhanced 3-fold when the corona is composed of 9% Mannose terminated ends.

Variable domain antibody targeting

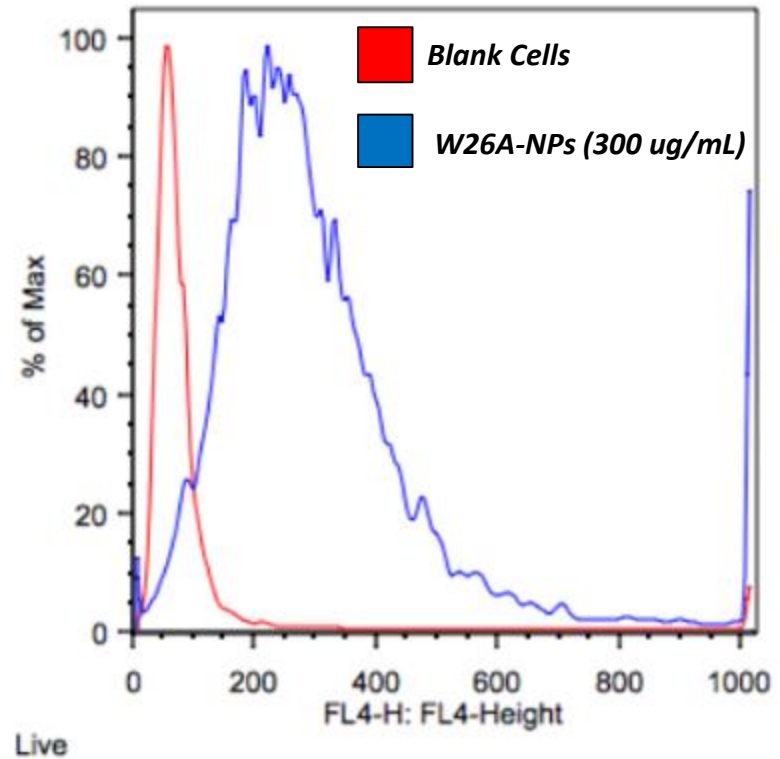
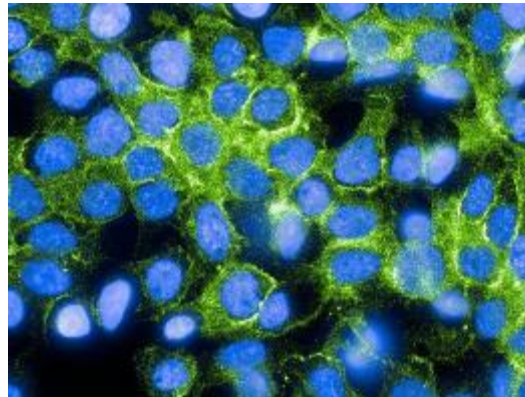


- 14K fibrinogen peptide scaffold (Centryin)
- Pre functionalize BCP
- Assemble NP

Control
(PEG NP)



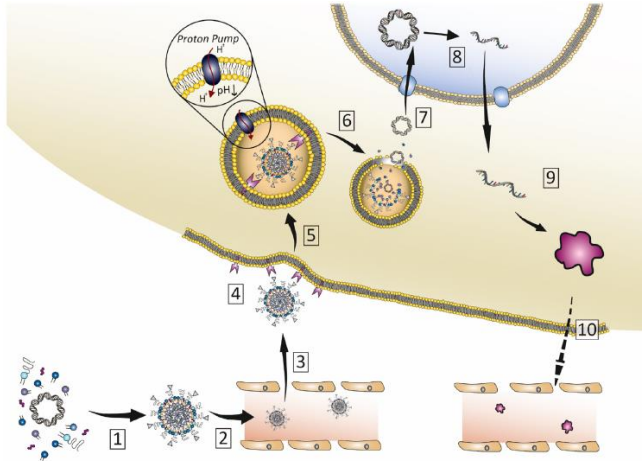
5%
ligand
targeted



DNA LNPs: Challenges and Opportunities

Challenges:

- DNA stiffer than mRNA, more difficult to collapse
- Requires delivery to the nucleus



■ Solvent stream (Ethanol)

DOPE

Dlin-KC2-DMA

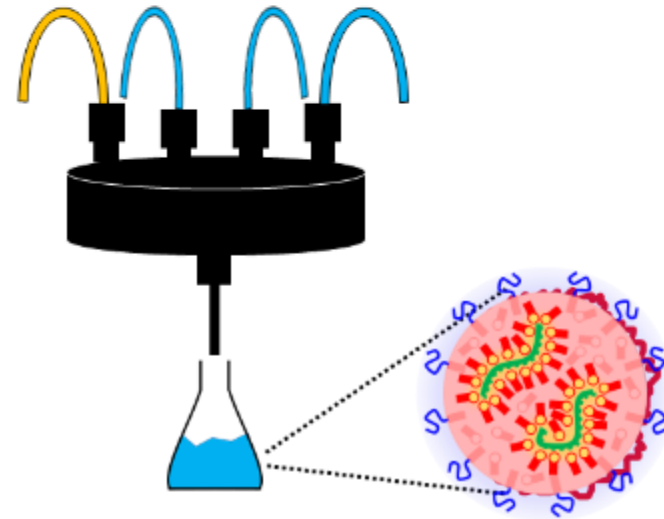
Cholesterol

Stabilizer: PEG-DMG vs PEG-PCL

■ Anti-solvent stream (H₂O)

Sodium Acetate

pDNA or mRNA



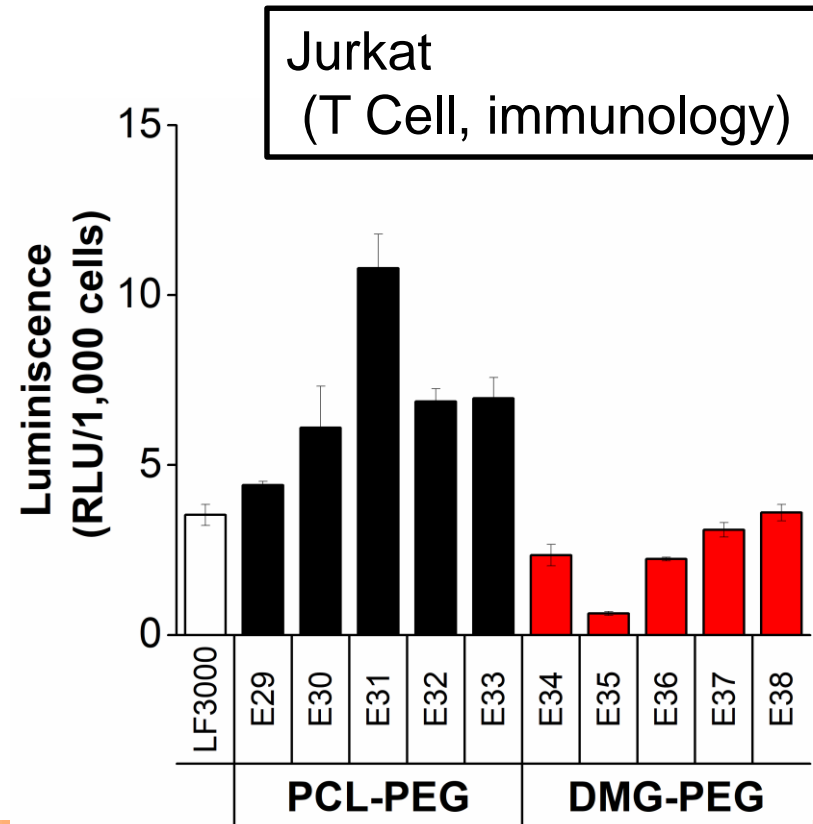
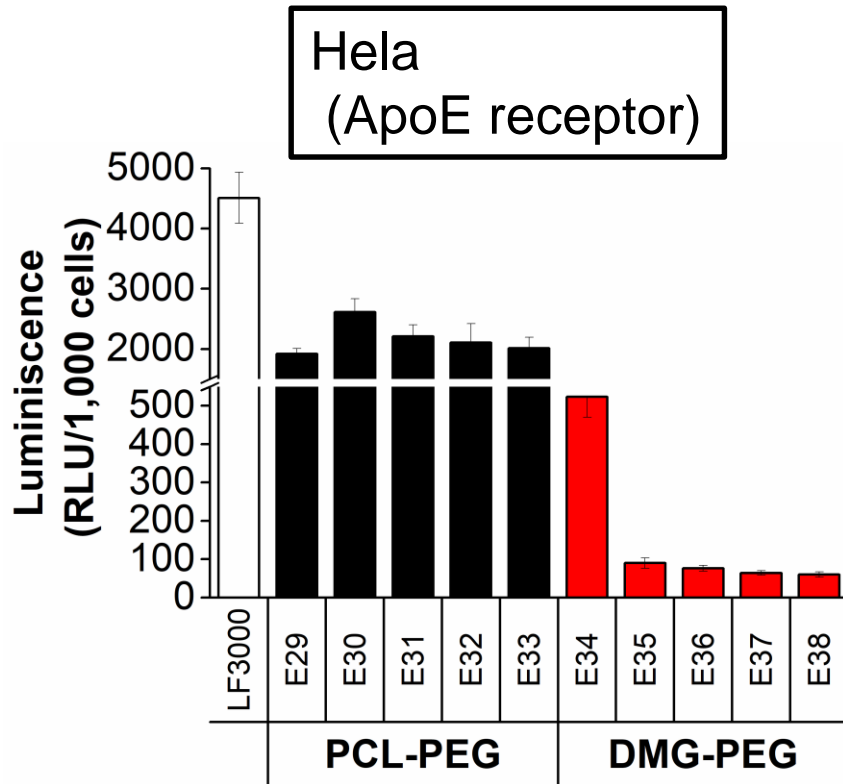
Opportunities:

- FNP, iFNP targeting
- Can encapsulate larger DNA than AAVs

Buck, et al., *ACS Nano* 13, no. 4 (2019): 3754-3782.

DNA LNPs by FNP: lipid vs PCL polymer anchoring

- PEG-lipids are designed to partition off the LNP
- PEG-PCL (block copolymers) stay anchored, provide a basis for targeting
- **Does anchored PEG prevent endosomal escape or transfection?**
 - **No! PCL-PEG DNA transfects better than lipid-PEGs**
 - **Similarly to Lipofectamine 3000**



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“It takes a village”

