Nanomedicine: from high tech to global health

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, Kurt Ristroph

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

Enchanced Bioavailability Using Scalable Flash NanoPrecipitation

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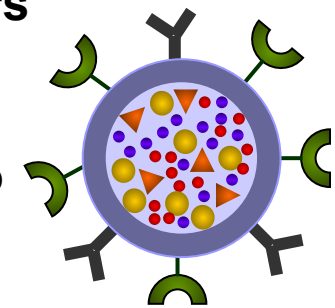
Outline

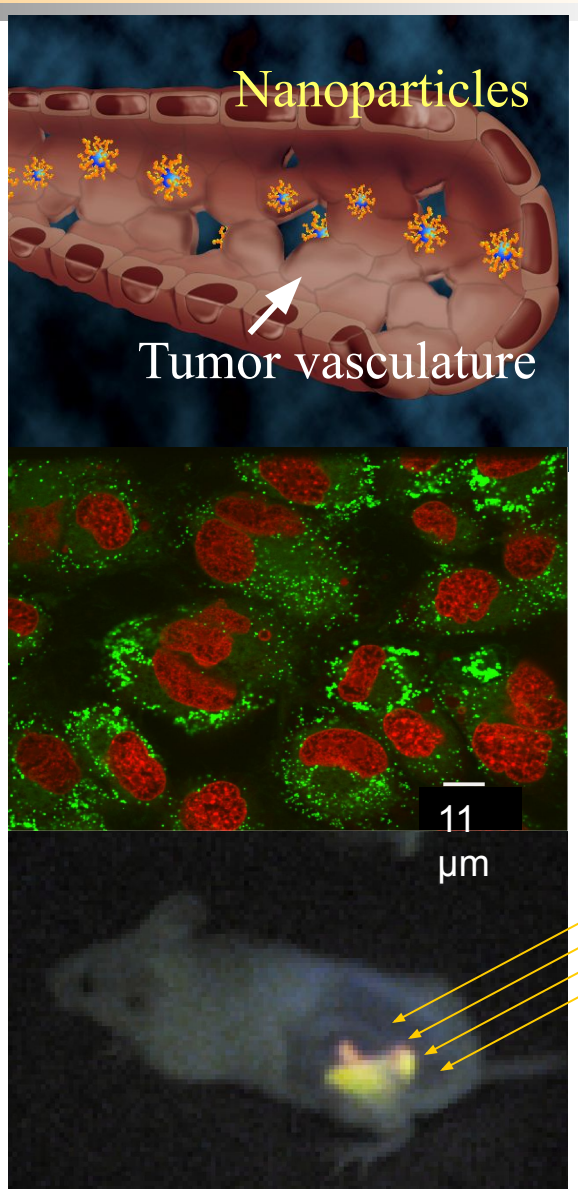
1. ***Nanoparticles by turbulent micromixing in confined impinging jet mixers (CIJ): FNP, MIVM***
    1. *Development of an idea: Flash NanoPrecipitation (FNP)*
2. ***Nanoparticle drug delivery***
    1. ***Oral**: increased bioavailabiity (Gates funding)*
    2. ***Parenteral**:Controlled release with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

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
- Scaleable 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)*

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Conflicting Goals: Oral vs Parenteral Delivery

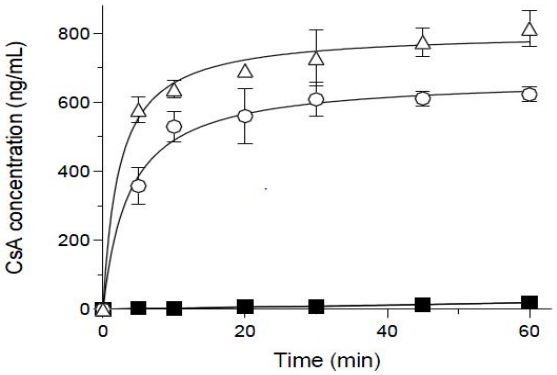
Schizophrenic communities

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*

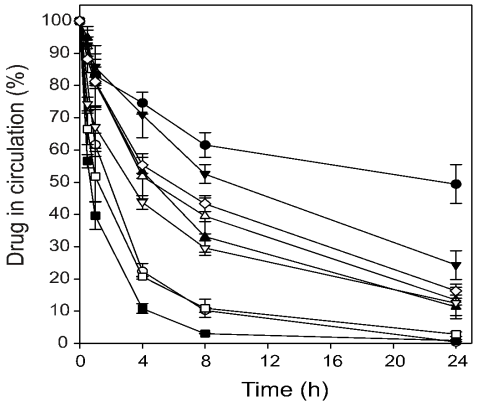
2. *Targeted delivery to specific tissue (cancer, etc.)*
   1. *Parenteral : COVID vaccines*
   2. *Protected drug cargo (mRNA, peptides, proteins, anticancer API)*
   3. *Keep things from dissolving/releasing until desired*

- *Cyclosporine A*
- *NPs ~200-300 nm*
- *Ppt with lecithin + mannitol and spray dried*
- *Supersaturations of 100*

(Sato,Prud'homme *Intl.J.Pharm (2017)*)

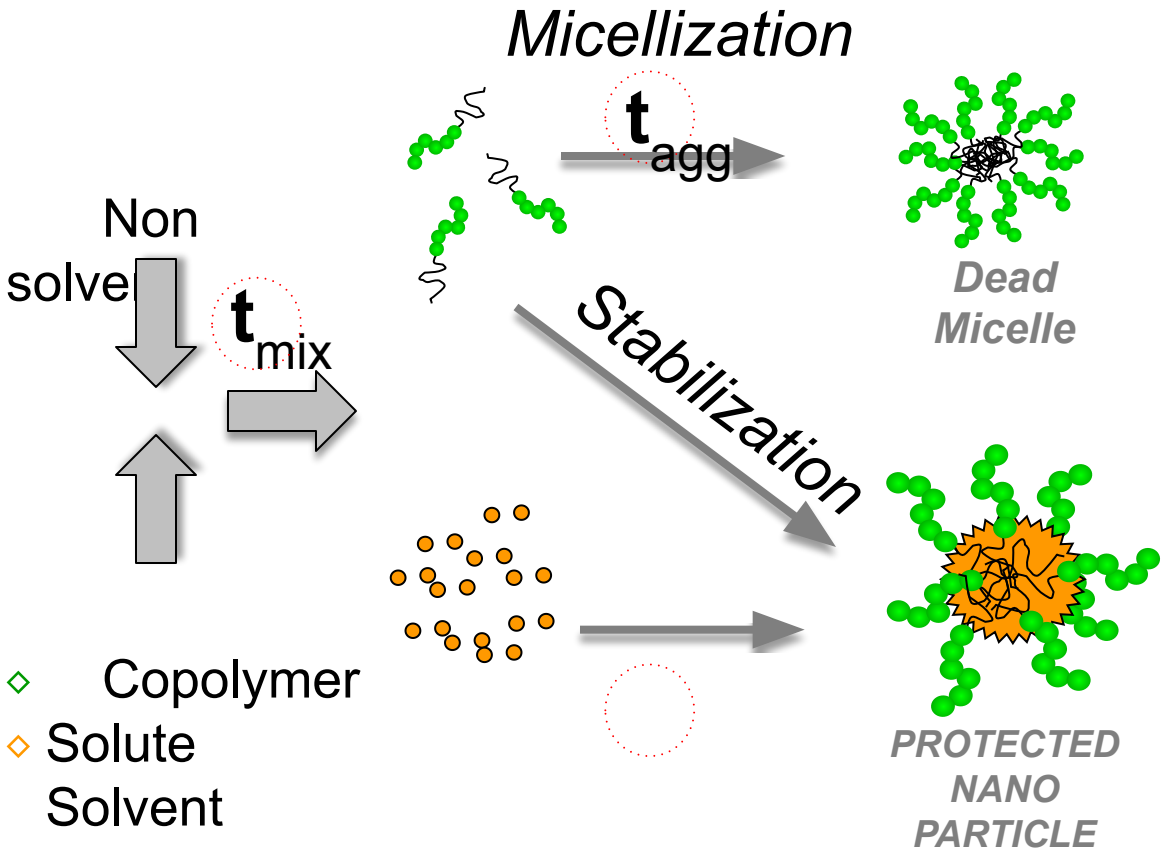


- *Paclitaxel conjugate*
- *NPs 60 nm*
- *(Ansel,Prud'homme Mol Pharm (2102)*



Nanoparticle formation by Flash NanoPrecipitation

Block copolymer directed rapid precipitation

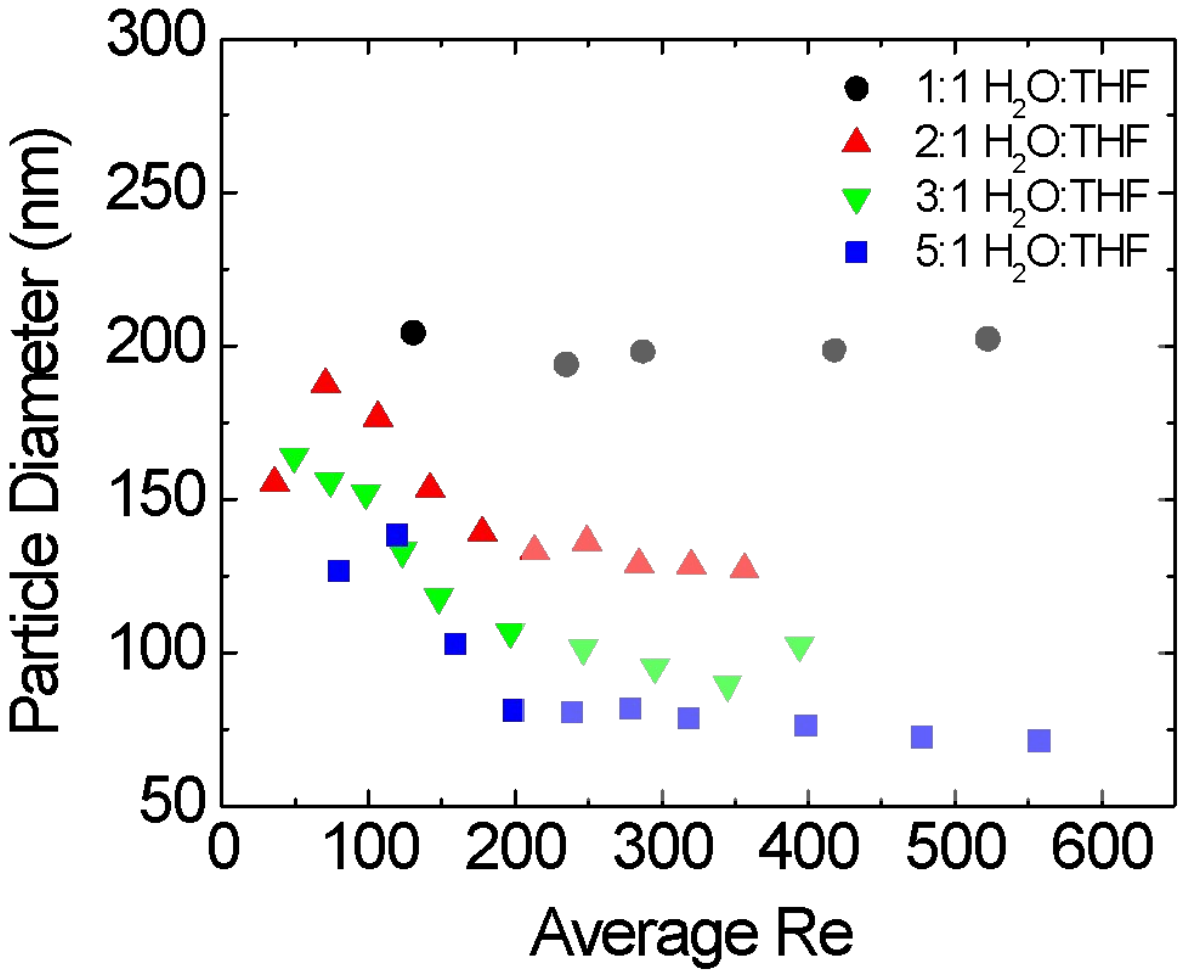


Johnson, Prud'homme AICHE J (2003), Liu, Prud'homme, Fox, Chem Engr. Sci. (2008)

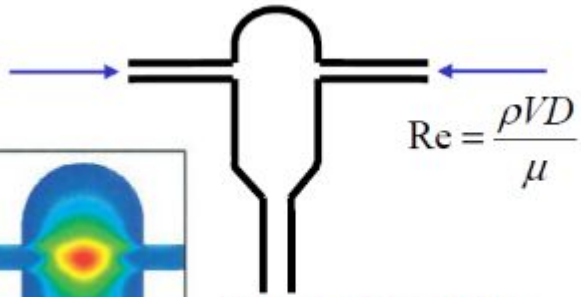
Control of particle size

1. ***Mixing intensity***
2. ***Super-saturation***

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

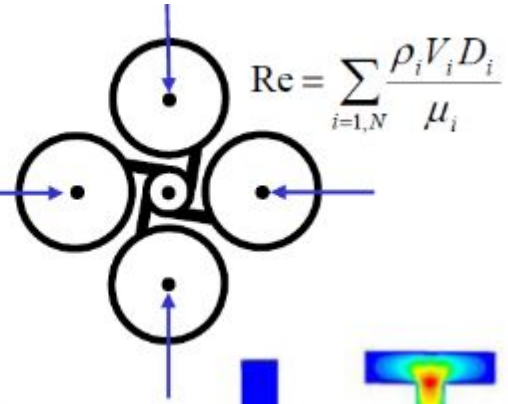


Confined impinging jet micro-reactors

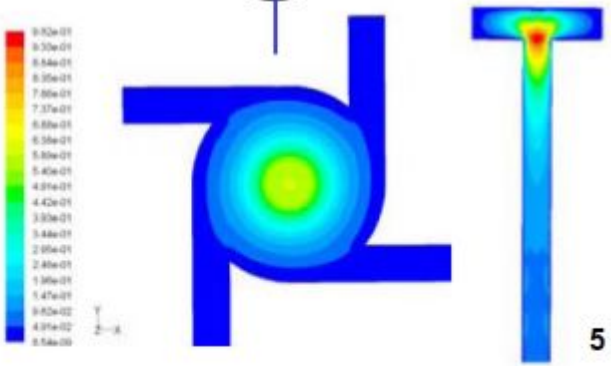


Micro-mixing by impinging streams of equal momentum

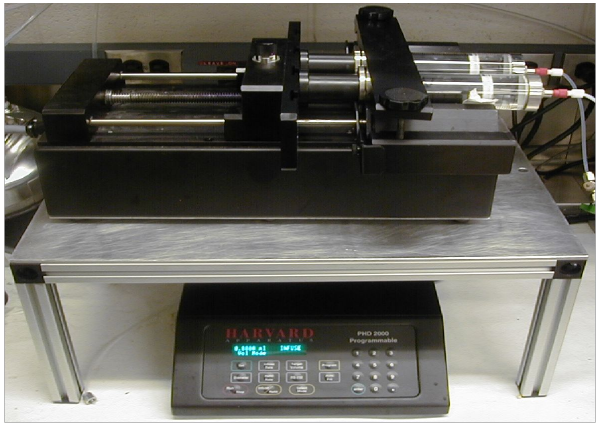
Micro-mixing by tangential streams of equal/non-equal momentum



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



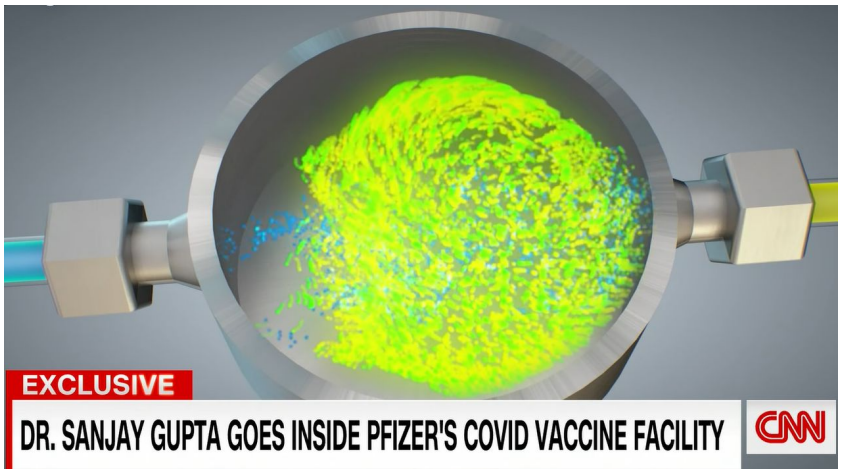
Y. Liu, R.K. Prud'homme *et al.*, *Chem. Eng. Sci.* 63 (2008) 2892-2842
B.K. Johnson and R.K. Prud'homme, *AIChE J.* 49(2002) 2264-2282



Princeton CIJ mixers and COVID vaccines

Pfizer BioNTechg COVID vaccine have produced 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.”**



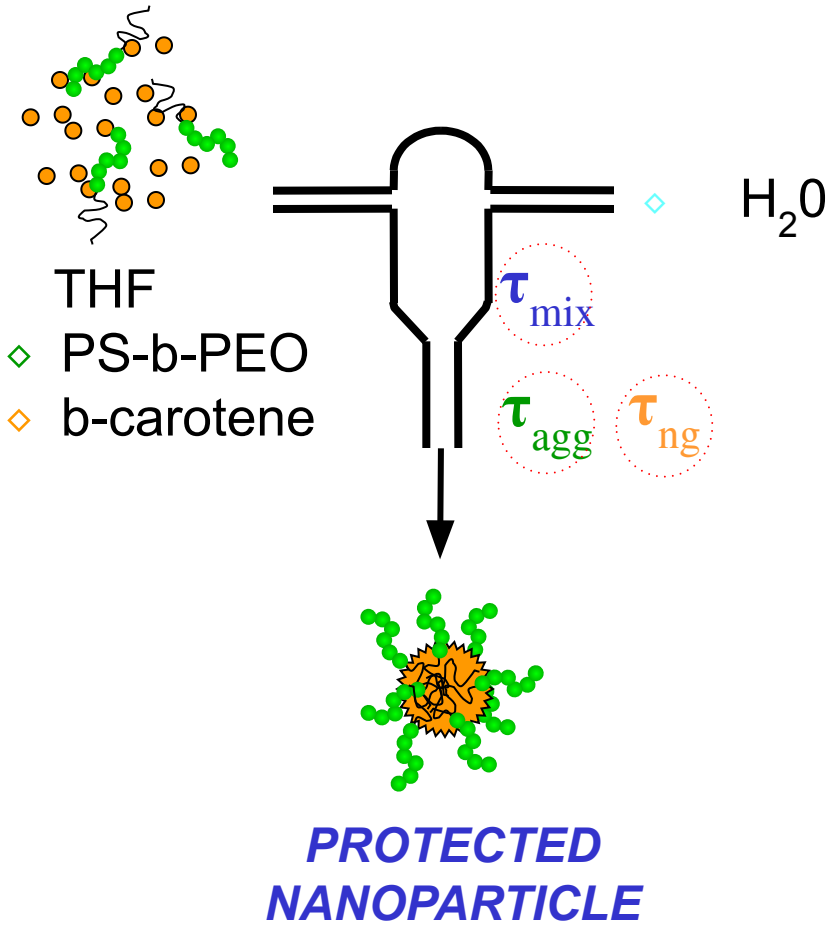
CNN. Manufacturing moonshot: How Pfizer makes its millions of Covid-19 vaccine doses https://www.cnn.com/2021/03/31/health/pfizer-vaccine-manufacturing/index.html.

https://fb.watch/5q7eNA3wnx

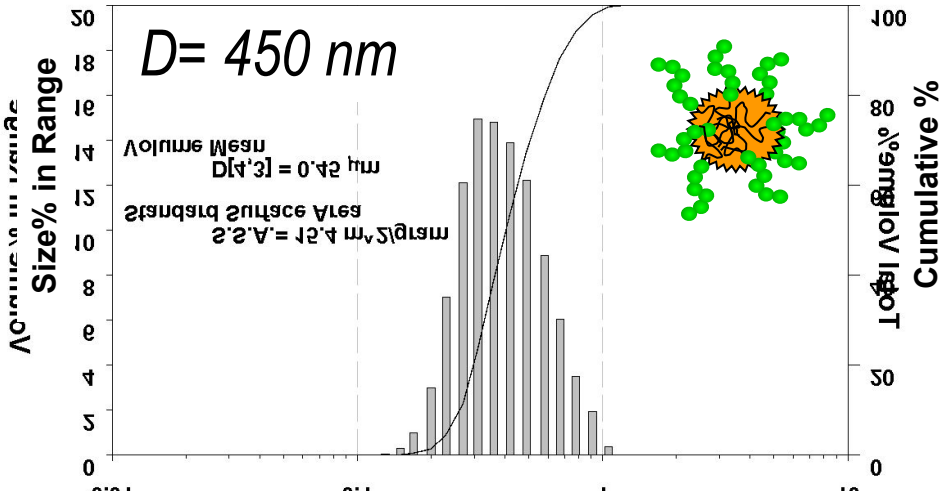
Johnson, Brian K., and Robert K. Prud'homme. "Chemical processing and micromixing in confined impinging jets." *AIChE Journal* 49.9 (2003): 2264-2282.
Johnson, Brian K., and Robert K. Prud'homme. "Flash nanoprecipitation of organic actives and block copolymers using a confined impinging jets mixer." *Australian Journal of Chemistry* 56.10 (2003): 1021-1024.

"FLASH" Nanoparticles Precipitation Size Control

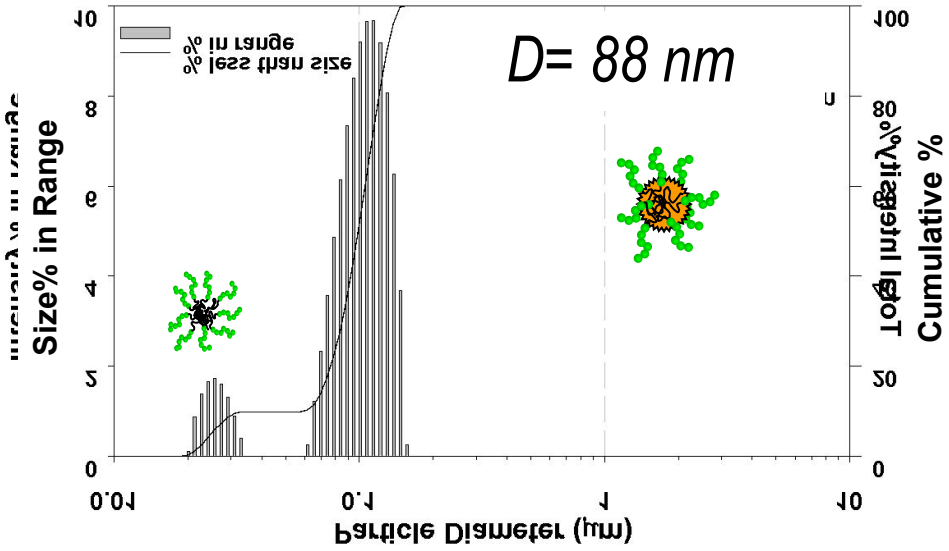
- *High throughput, > 2 wt% active*
- *High NP loading*



2.8 m/s, (2.6 wt% drug, 0.4 wt% Copolymer)

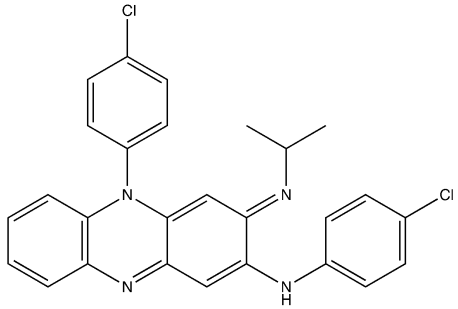


Change Inputs to Process



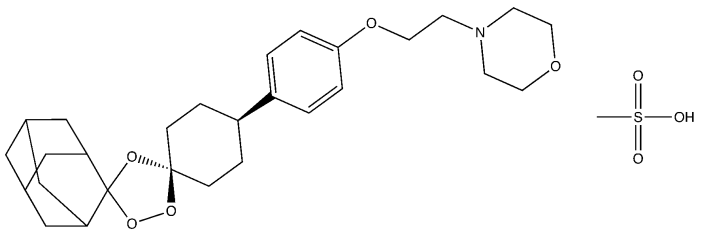
Drugs Considered

Clofazimine



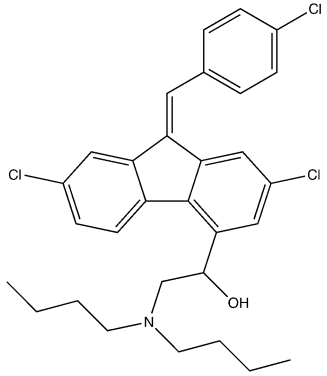
Ref: Design and Solidification of Fast-Releasing Clofazimine Nanoparticles for Treatment of Cryptosporidiosis". *Molecular Pharmaceutics*. **14**(10) 3480-3488 (2017); "Rapid Recovery of Clofazimine-loaded Nanoparticles with Long-term Storage Stability as Anti-Cryptosporidium Therapy". *ACS Applied Nano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

OZ439



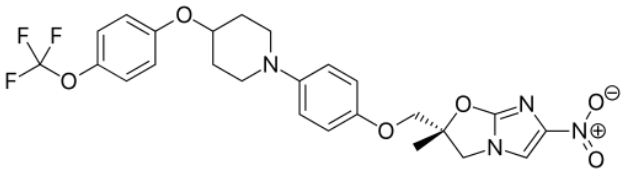
Ref: "Encapsulation of OZ439 into Nanoparticles for Supersaturated Drug Release in Oral Malaria Therapy". *ACS infectious diseases*. **4**(6) 970-979 (2018); "Spray drying OZ439 nanoparticles to form stable, water-dispersible powders for oral malaria therapy". *Journal of Translational Medicine* (2019),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

Lumefantrine



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

Delamanid



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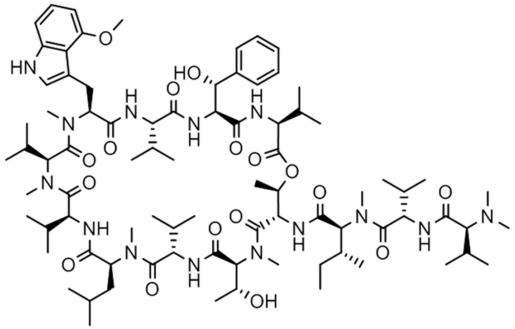
Ecumicin



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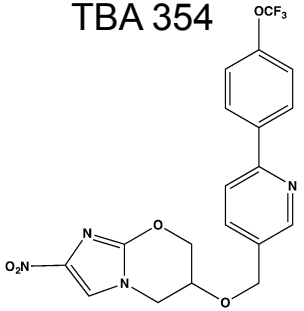


Ref: https://app.box.com/s/57cromgxhg8uyqiz4zegtafwqbclg3nk

Malaria is a global health pandemic

210 Million

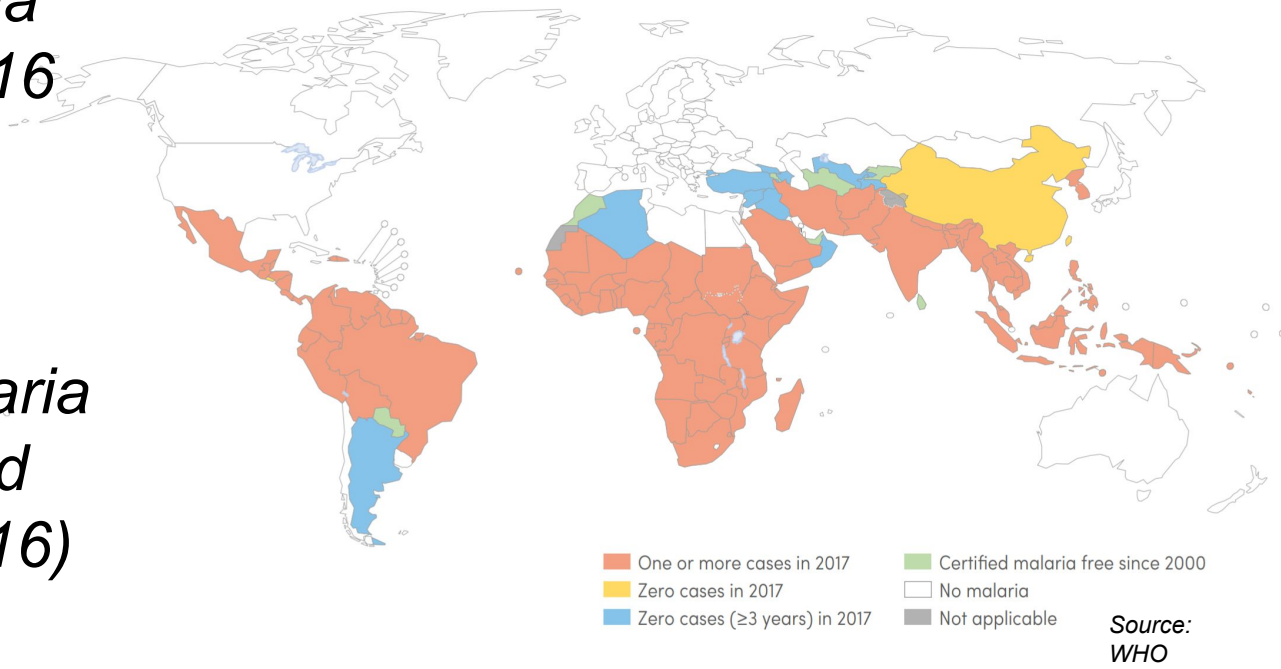
Cases of malaria worldwide in 2016 alone

429,000

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

>70%

Of deaths were in children under 5 years of age

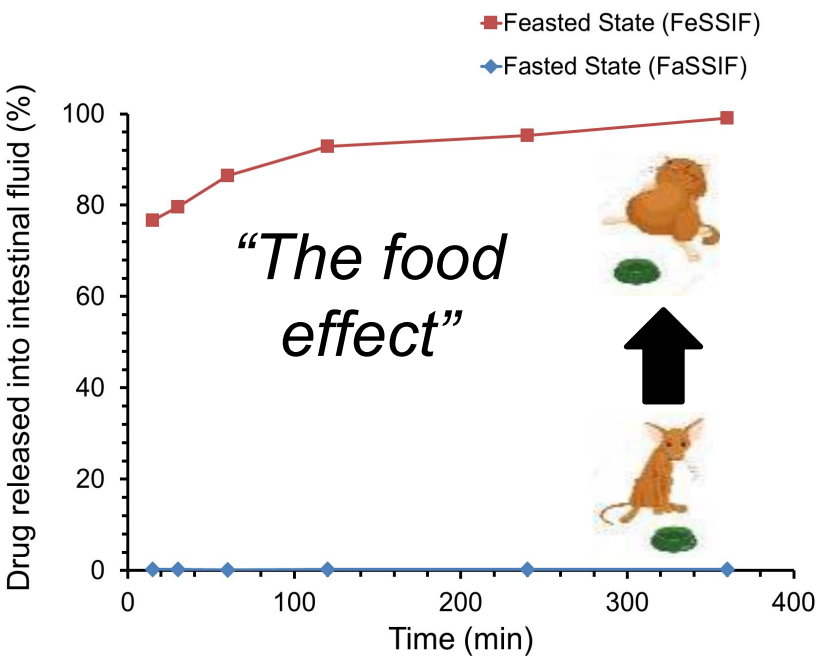


Source: WHO

Lumefantrine: Two pharmaceutical challenges

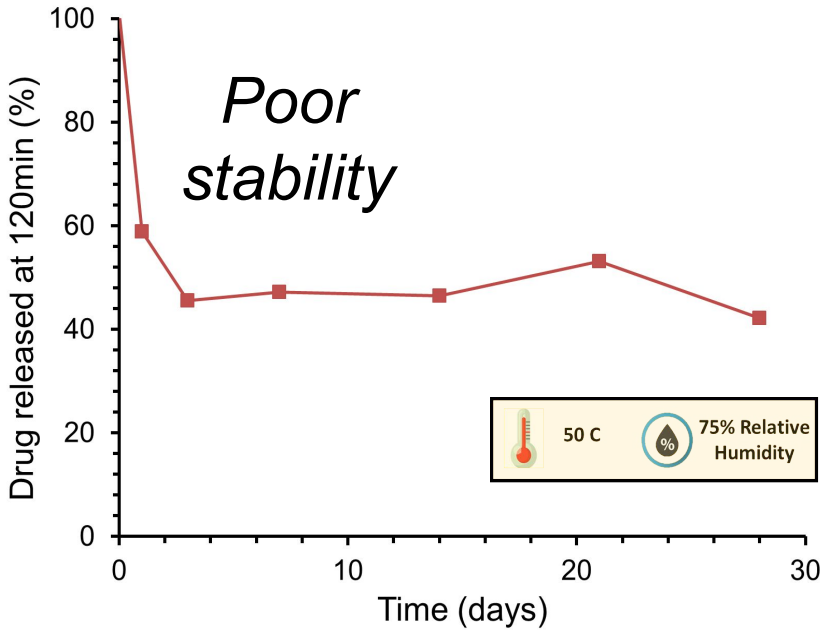
Solid Spray Dried Dispersion

1 ***Bioavailability is dependent on food intake***



Feng et al. J Transl Med (2019), Feng et al. Soft Matter (2019)

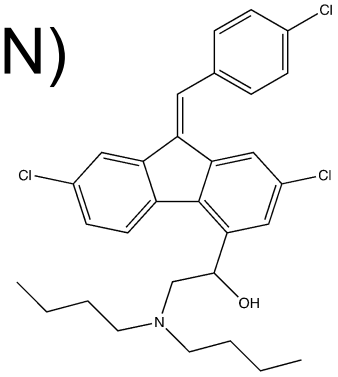
2 ***Crystallization over time reduces shelf-life***



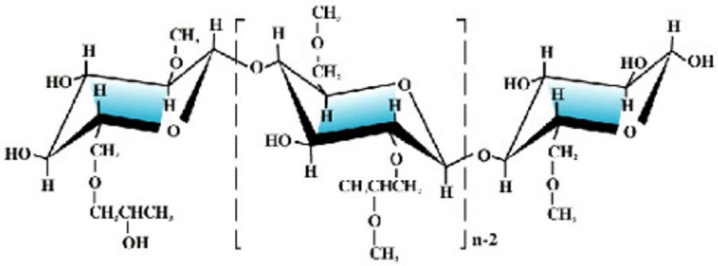
Increasing time in storage

Lumefantrine Stabilizers

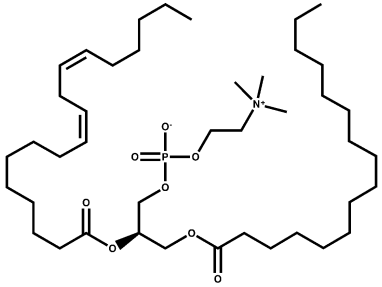
Lumefantrine (LMN)



- **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

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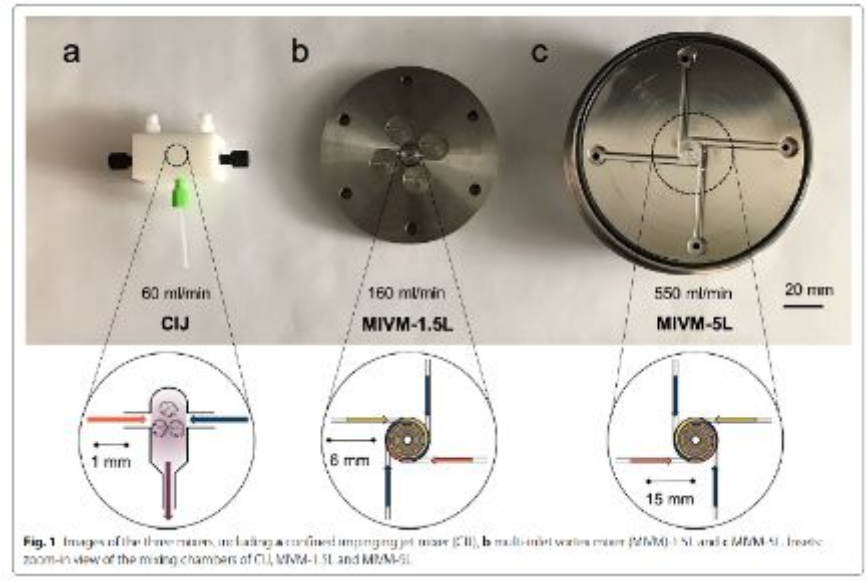
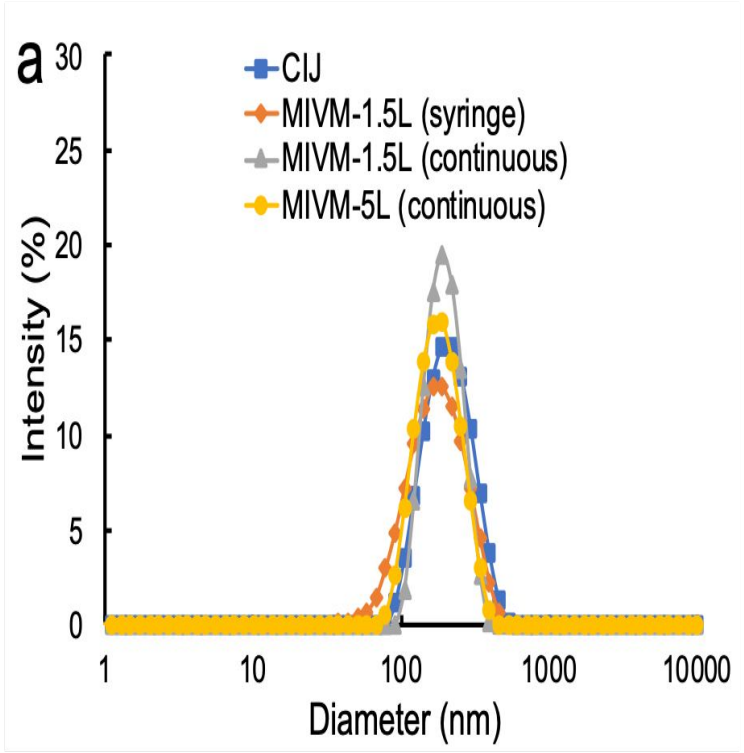
SCALEUP: Equivalent NPs from 1 ml to 5 L/min)

- *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*



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

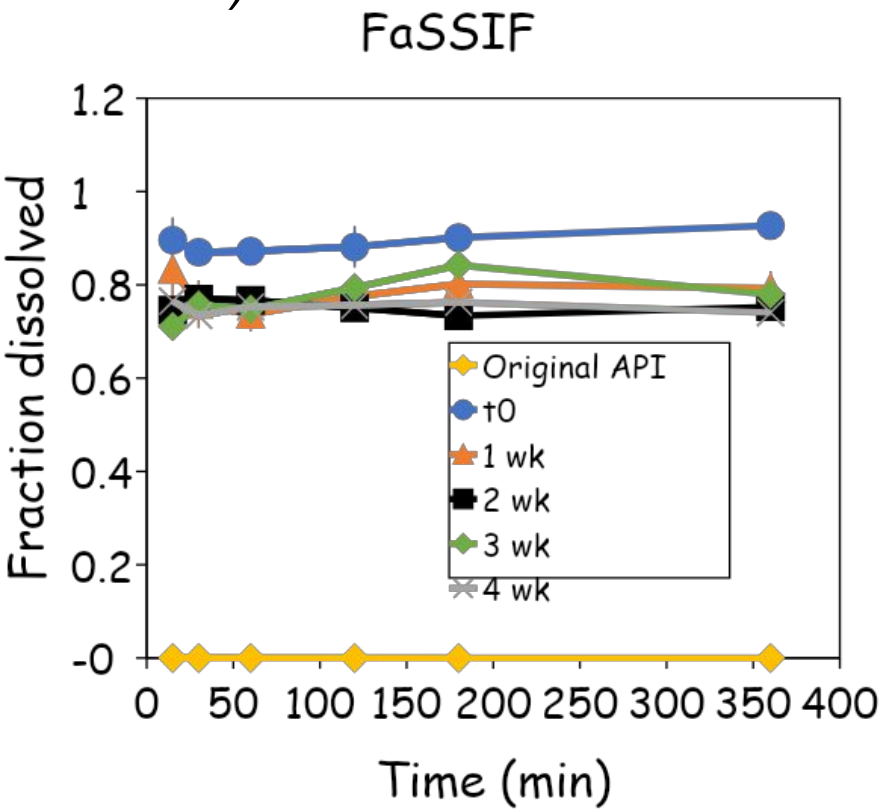


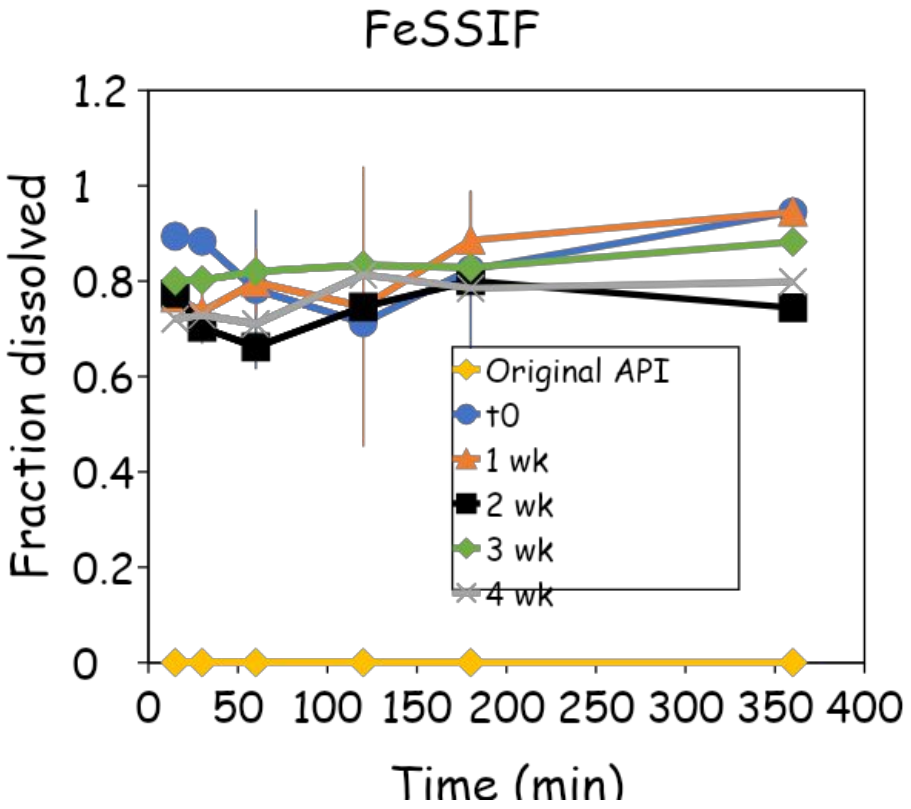
90% DRUG LOADING IN NP

Armstrong J. Pharm Sci (2023)

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)*

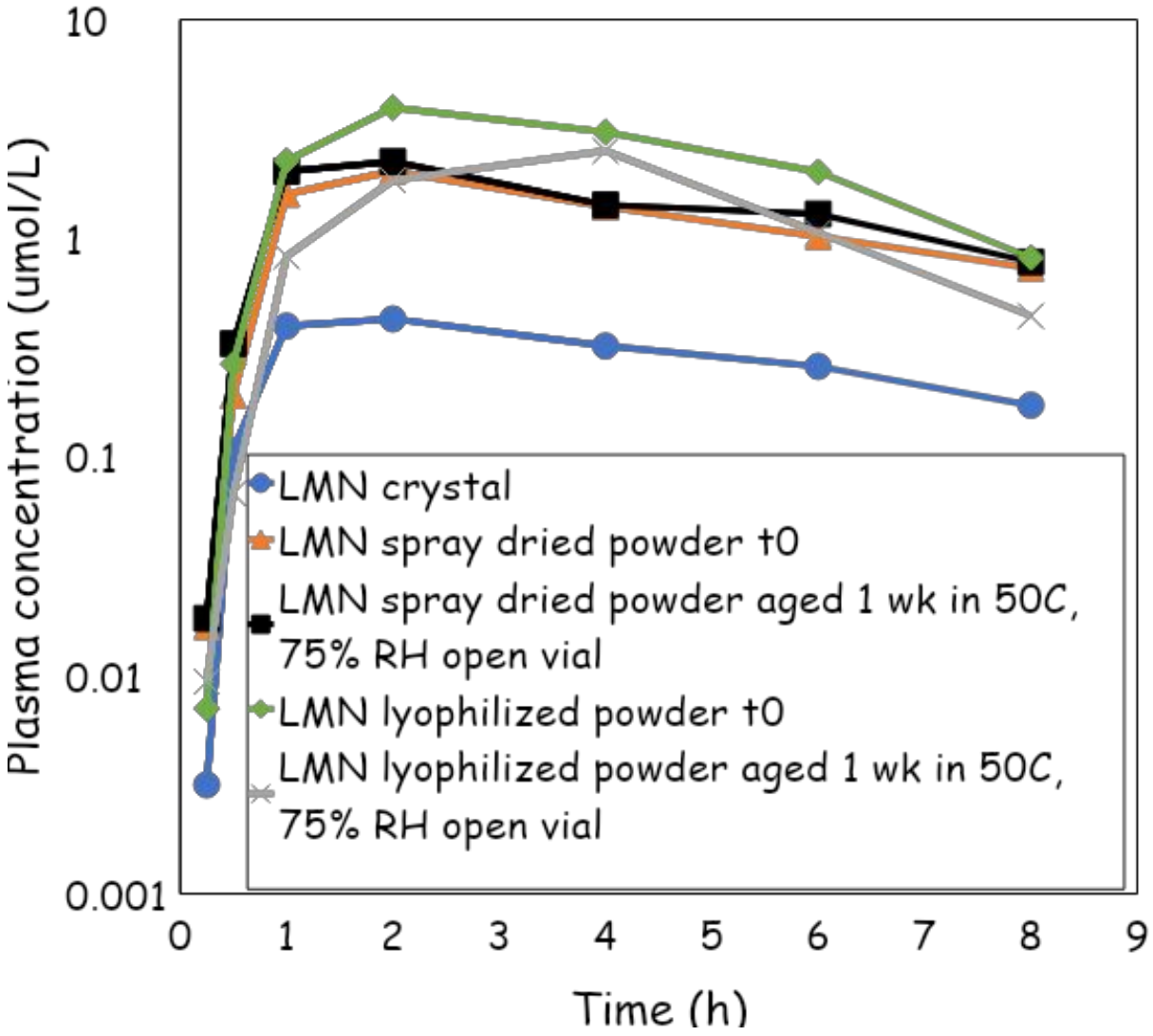




Enhanced bioavailability of LMN in animal study

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

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



Study conducted by Genentech

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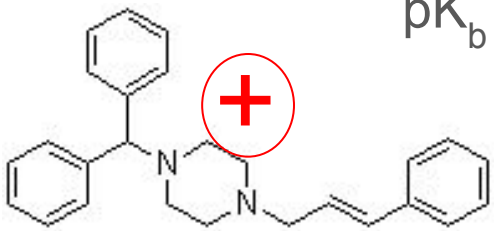
Encapsulation by ion pair complexation

Cinanarizine
(antihistomine, weak base)

LogP 5.3

pK_b 7.5, 1.95

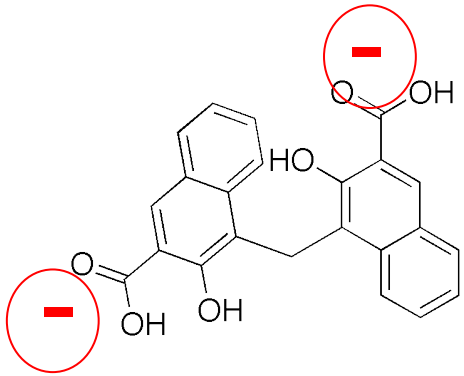


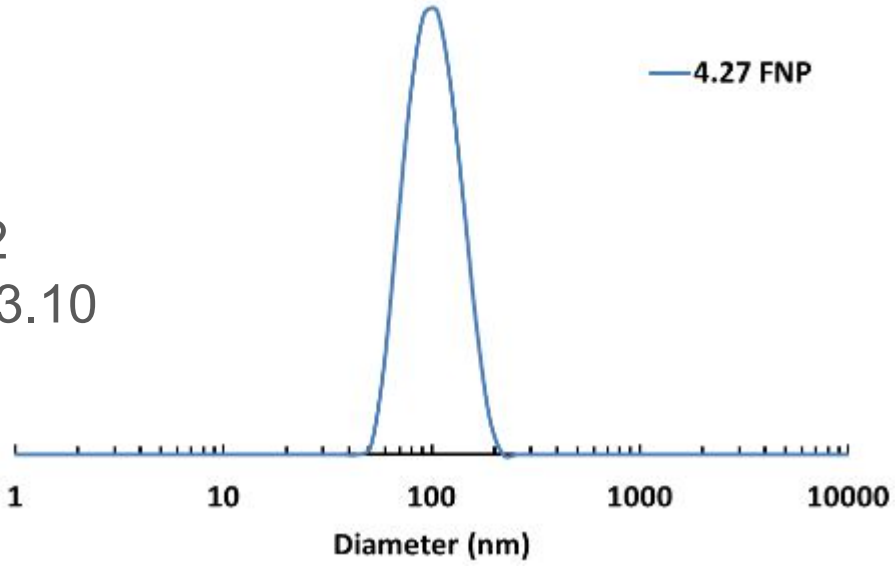
- *Individual cmds can not form stable NPs*
- *Complex eliminates polarity of charge groups*

Pamoic acid (FDA approved salt)

LogP 5.52

pK_a 2.51 3.10

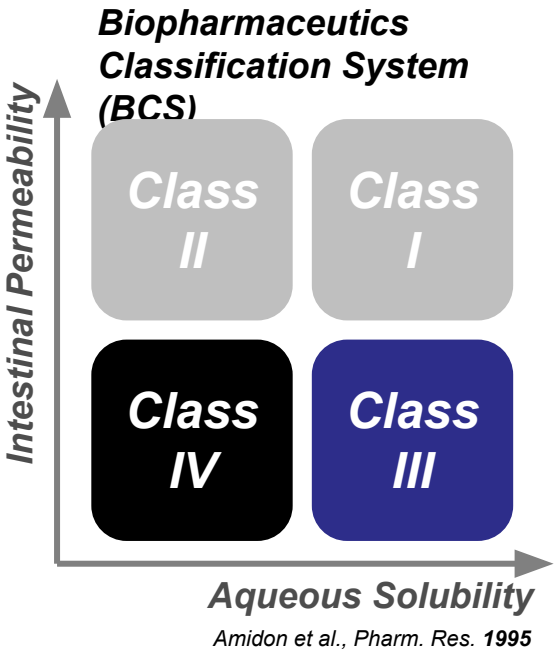




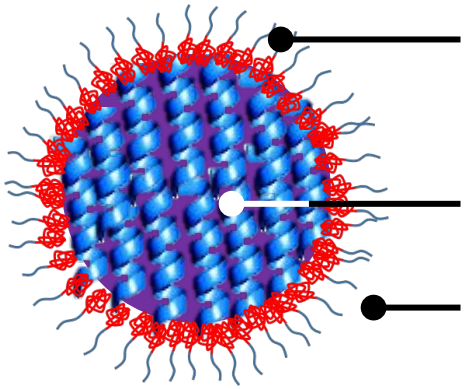
Pinkerton, Grandeury‡, Fisch‡, Jörg Brozio‡, Bernd U. Riebesehl et al., Mol Pharm (2012)

Peptide nanoparticles by ion pairing: BMS-PCI

Nanoencapsulation can help address solubility and permeability limitations to oral peptide delivery



Solubility and GI membrane permeability can vary widely with peptide architecture, charge, and functional modification



-PEG brush facilitates muco-diffusion in GI tract

-Targeting ligands

Disordered core can enhance solubility

Tune ion pairing and stabilizer shell to modulate release kinetics

<u>0.1% bioavailability</u>

- *insolubility*
- *too polar (soluble), membrane impermeable*

Peptide nanoparticles by ion pairing: BMS-PCI

Cyclic peptides synthesized by BMS

Bristol Myers Squibb

1 x6(HOOC) ⊙Ion Pair $NH_3^{\oplus}$ x4

2 x6(HOOC) x1(HO_3S) ⊙Ion Pair $NH_3^{\oplus}$ x4

3 x8(HOOC) ⊙Ion Pair $NH_3^{\oplus}$ x4

4 x6(HOOC) ⊙Ion Pair $NH_3^{\oplus}$ x5

MW 2500-3500 Da

Across four peptides variations in:

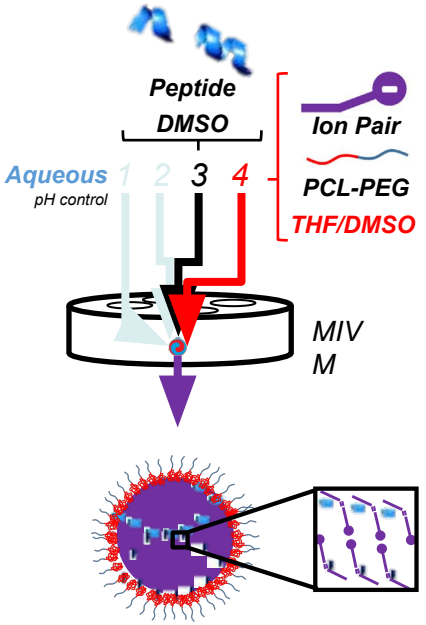
- *Charge*
- *Alkylation*
- *PEGylation*
- *Molecular weight*

Parameters explored in FNP formulation:

- *Ion pairing agent*
- *Polymer stabilizer*
- *Charge ratio*
- *Cryoprotectant (lyophilization)*
- *Co-core*

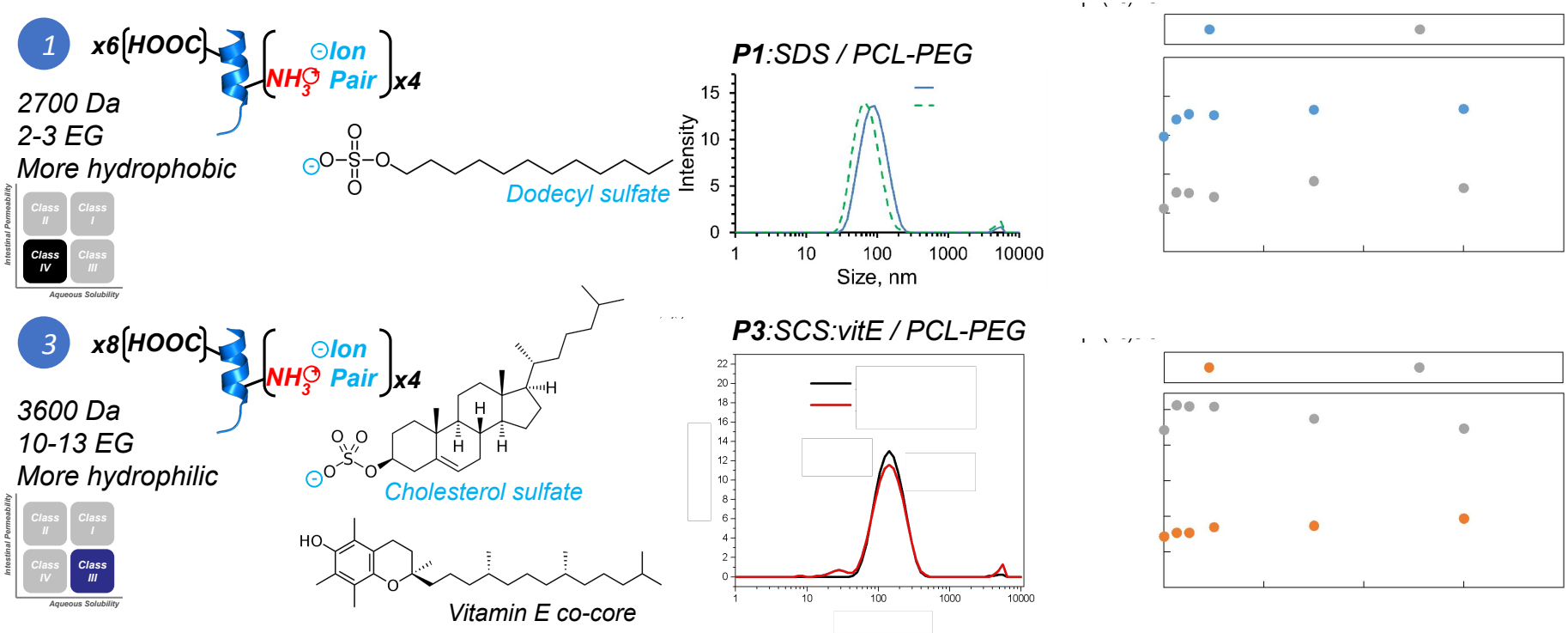
Multi-Inlet Vortex Mixer (MIVM)

Increased compositional and flowrate flexibility



Peptide nanoparticles by ion pairing: BMS-PCI

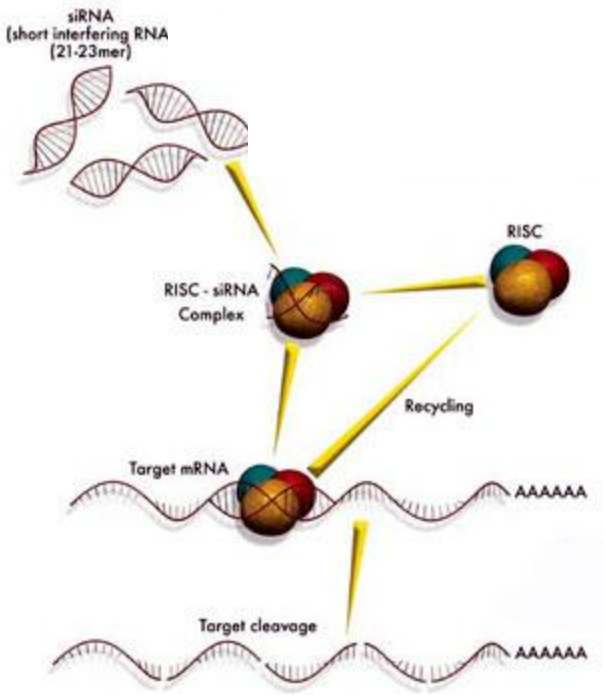
Different formulation strategies required for more hydrophobic vs more hydrophilic peptides

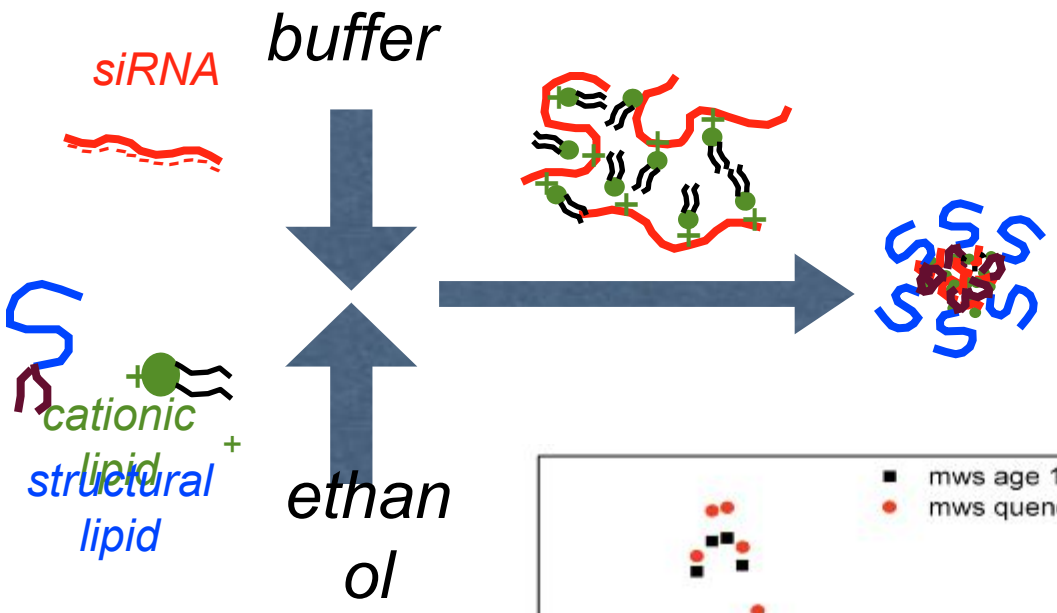


- *More hydrophobic peptide is directly ion paired with SLS*
- *More hydrophilic requires capture in a hydrophobic matrix*

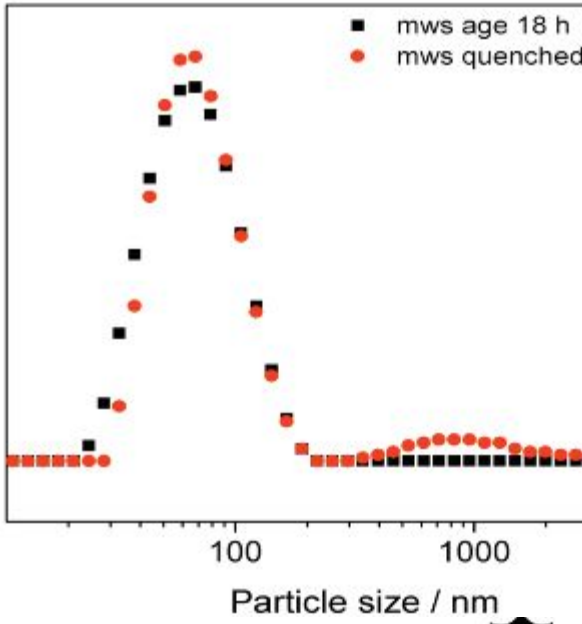
siRNA or mRNA Lipid Nanoparticles (Merck)

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





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



RNA Encapsulation

•*siRNA complex cleaves*

Marian Gindy
Merck

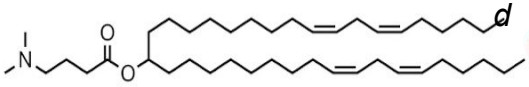
Paul Burke
Merck

Varun Kumar

mRNA and DNA Lipid nanoparticles (LNPs)

LNP Formulation

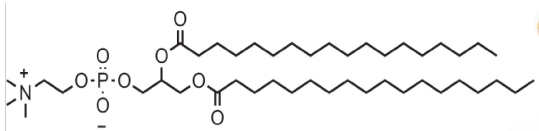
Ionizable lipid (Dlin-MC3-DMA)



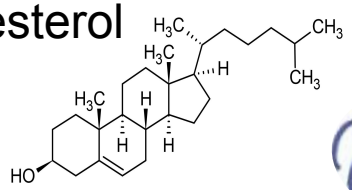
Uncharged

Charged

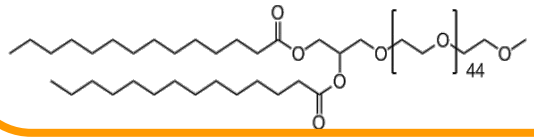
Zwitterion lipid (DSPC)

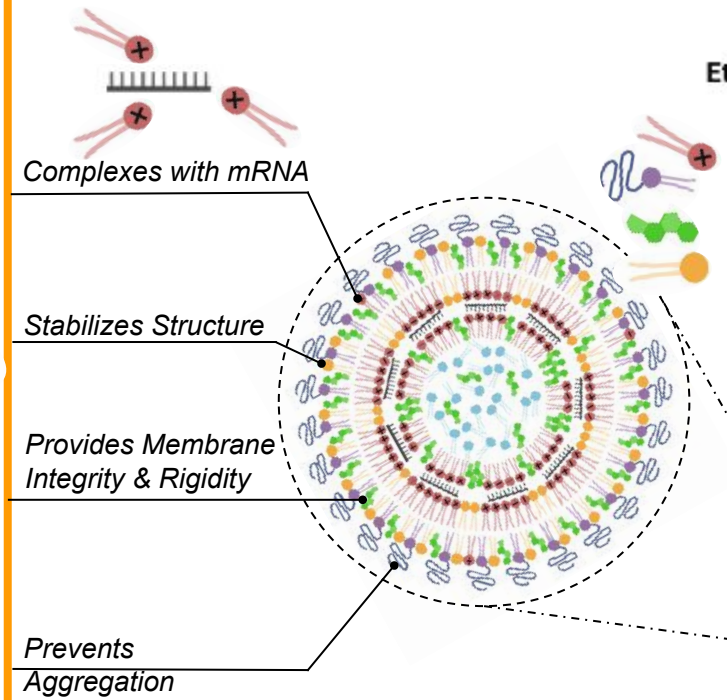


Cholesterol

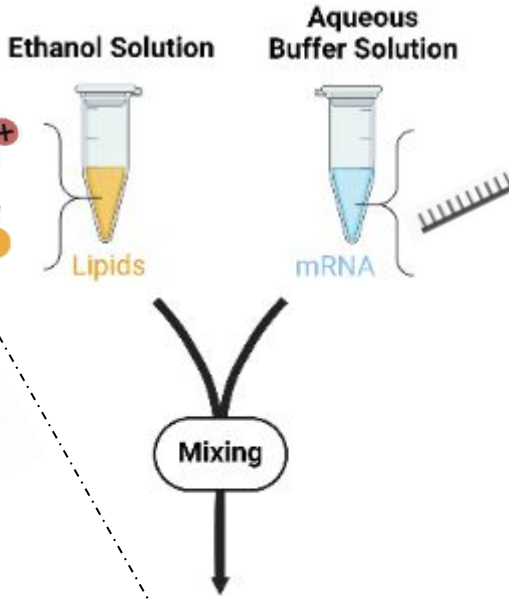


Stabilizing lipid (DMG-PEG)





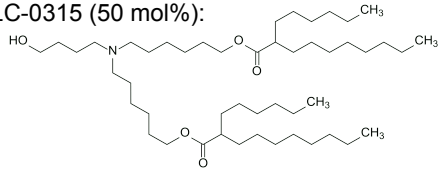
Co-loading



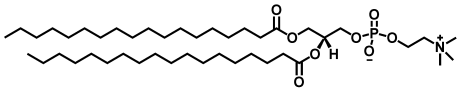
Formulation and mRNA/DNA LNP production

Formulation

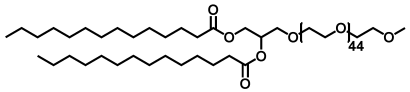
ALC-0315 (50 mol%):



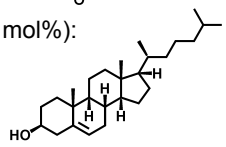
DSPC (10 mol%):



DMG-PEG$_{2000}$ (1.5 mol%):



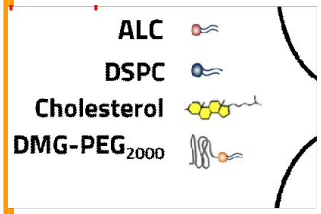
Cholesterol (38.5 mol%):

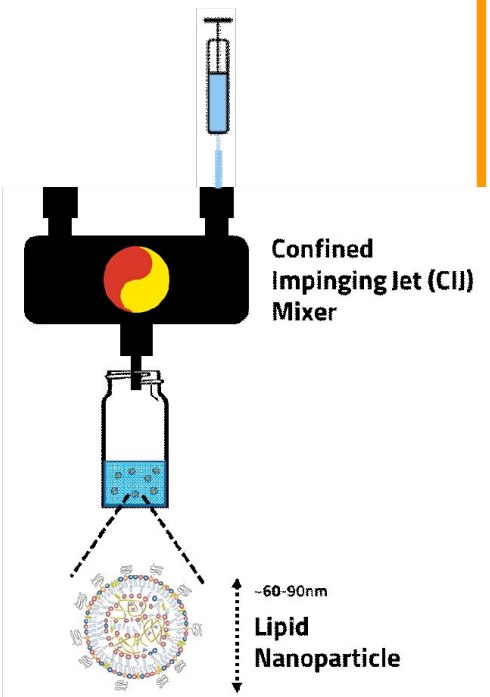


N/P 6 yeast RNA

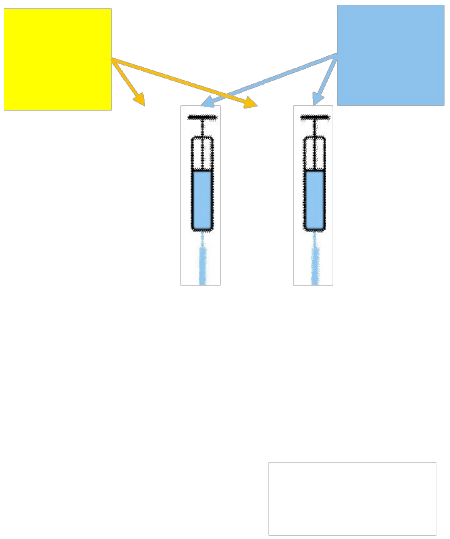
10% EtOH

CIJ setup





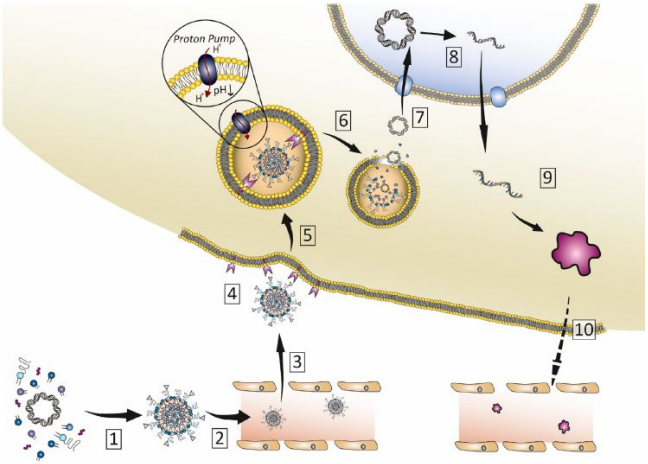
MIVM setup



DNA LNPs: Challenges and Opportunities

Challenges:

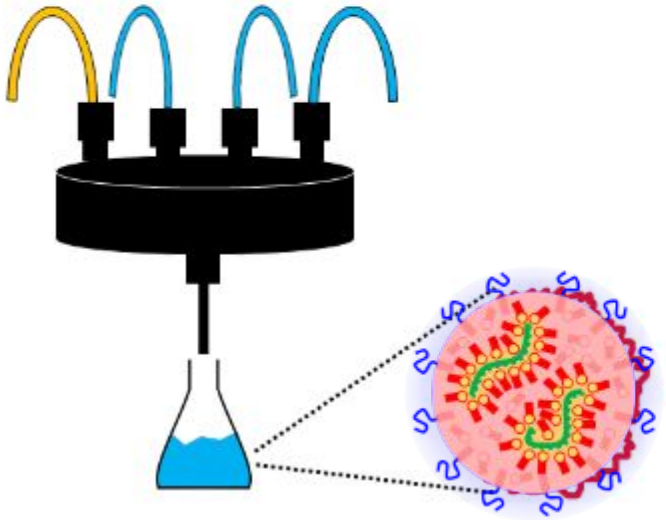
- DNA stiffer than mRNA, more difficult to collapse
- Requires delivery to the nucleus



Endosomal escape: Genentech - PCI

Solvent stream (Ethanol)
DOPE
Dlin-KC2-DMA
Stabilizer: PEG-DMG vs PEG-PCL

Anti-solvent stream (H_2O)
Sodium Acetate
pDNA or mRNA



Opportunities:

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

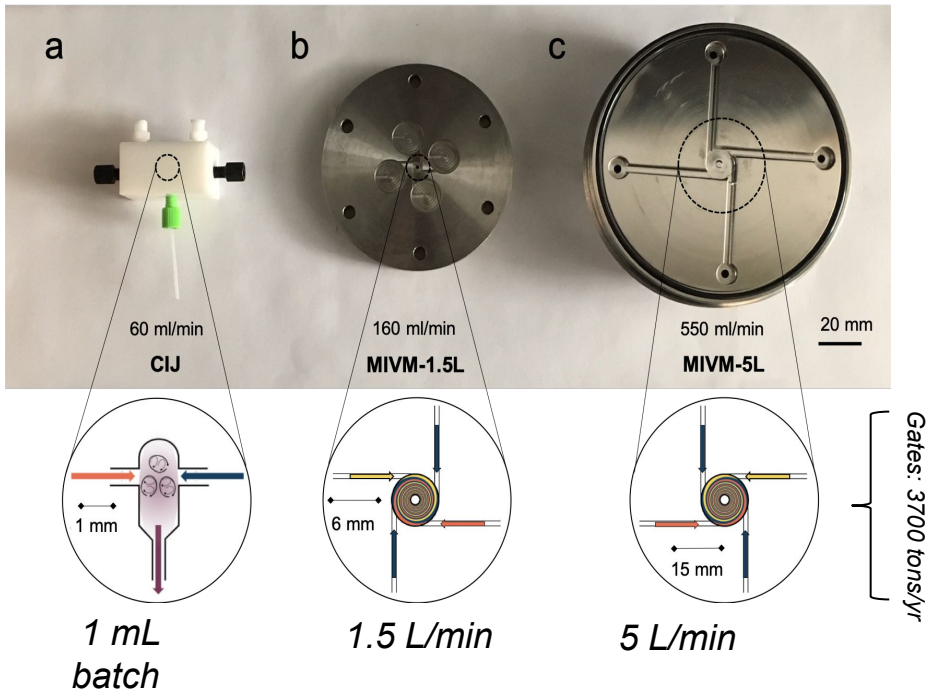
Buck, et al., *ACS Nano* 13, no. 4 (2019): 3754-3782.

Four Scales of CIJ & MIVM

CIJ
Confined Impinging Jet

MIVM
Multi Inlet Vortex Mixer

Micro-MIVM
m-Multi Inlet Vortex Mixer



1 mL batch

1.5 L/min

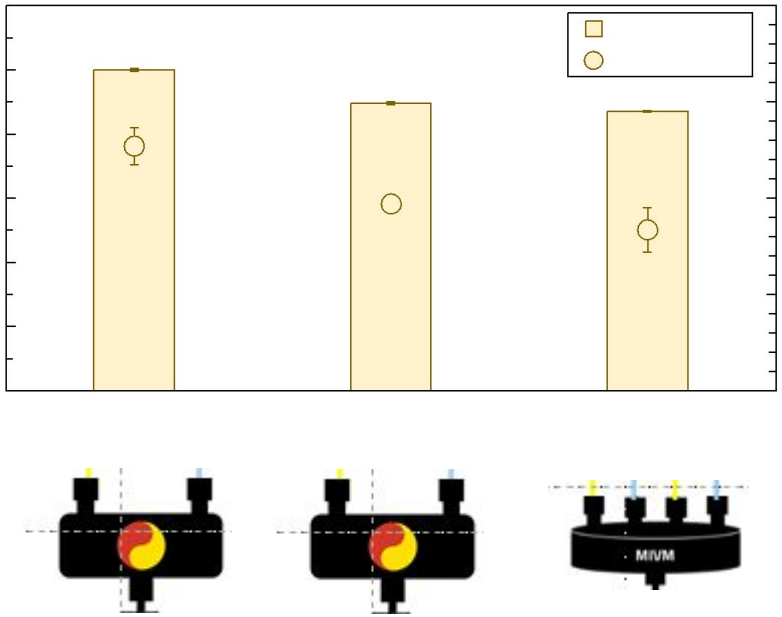
5 L/min

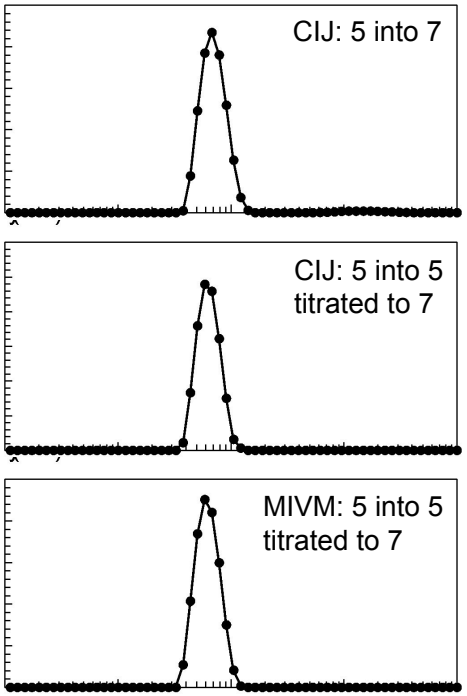
- *4 glass syringes: 2 ml*
- *0.3-0.6 mg RNA (N/P=6)*

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

50 nm DNA LNPs are produced via CIJ and MIVM

No effect of mixer geometry





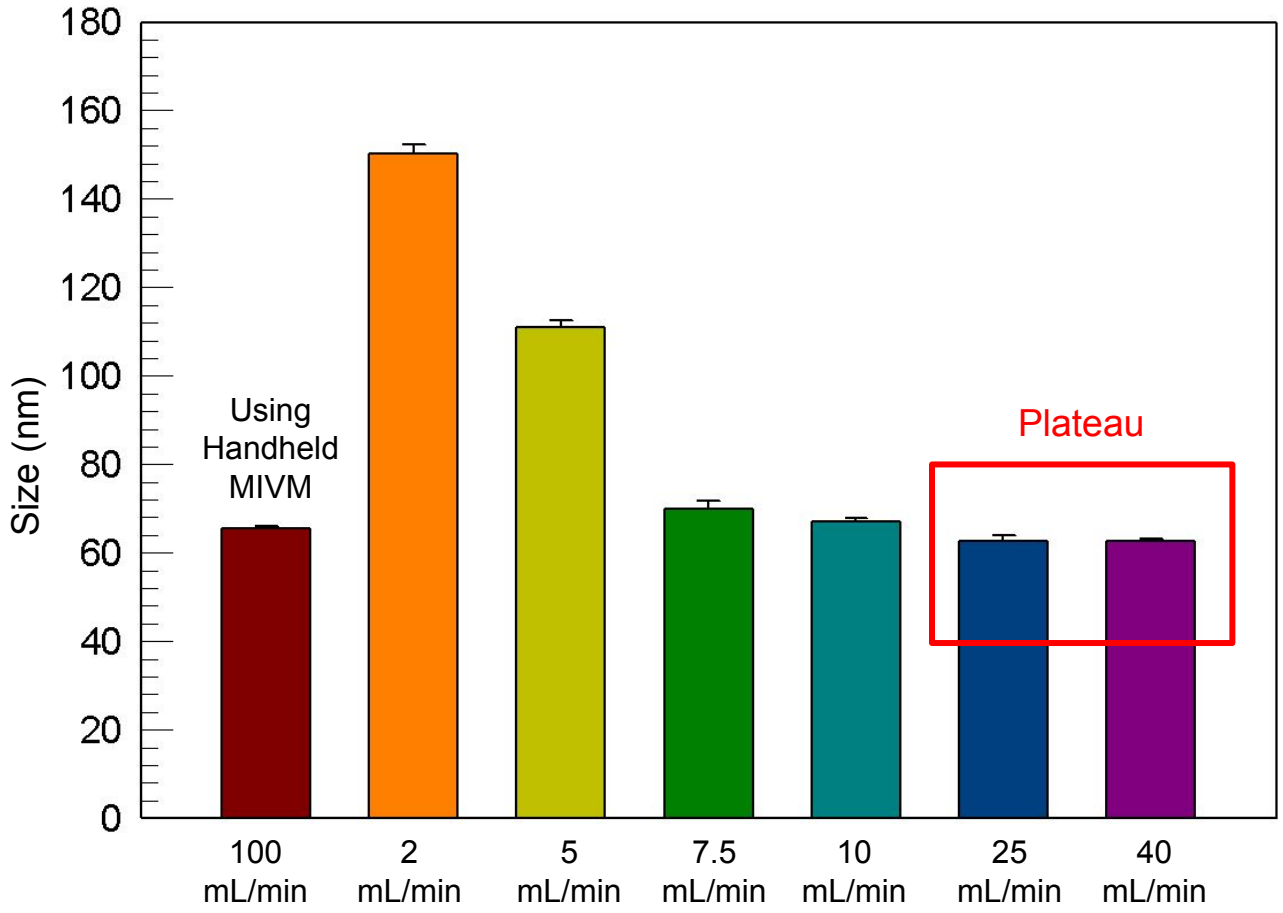
LNP size is independent of pH shifting process: pH= 5 → pH 7

Pump-based MIVM vs handheld MIVM

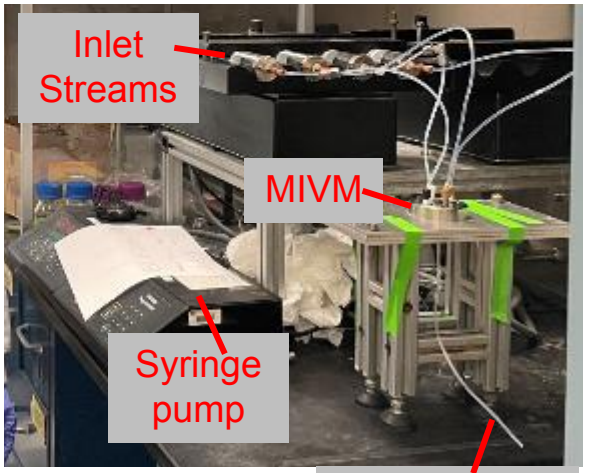
Before concentration

DODMA llipid, mRNA (yeast) LNPs

N/P ratio: 6



MIVM setup



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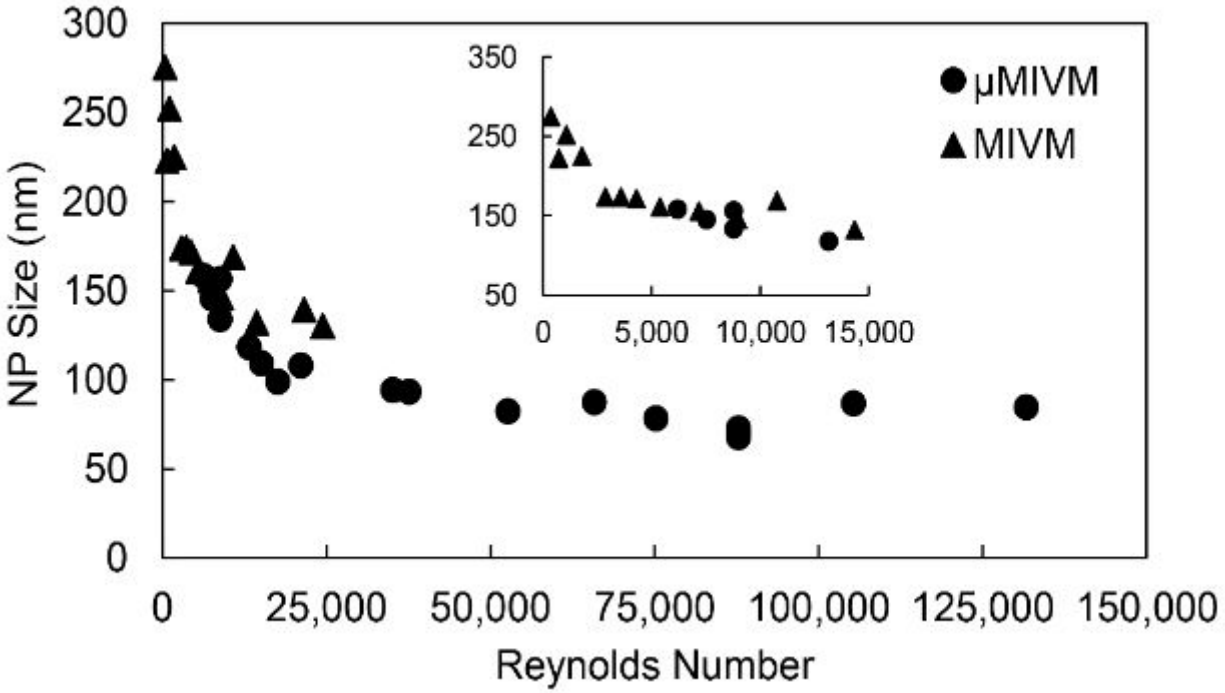
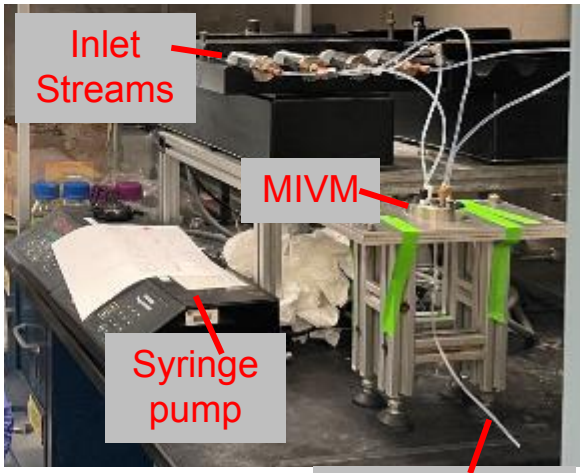


MIVM setup

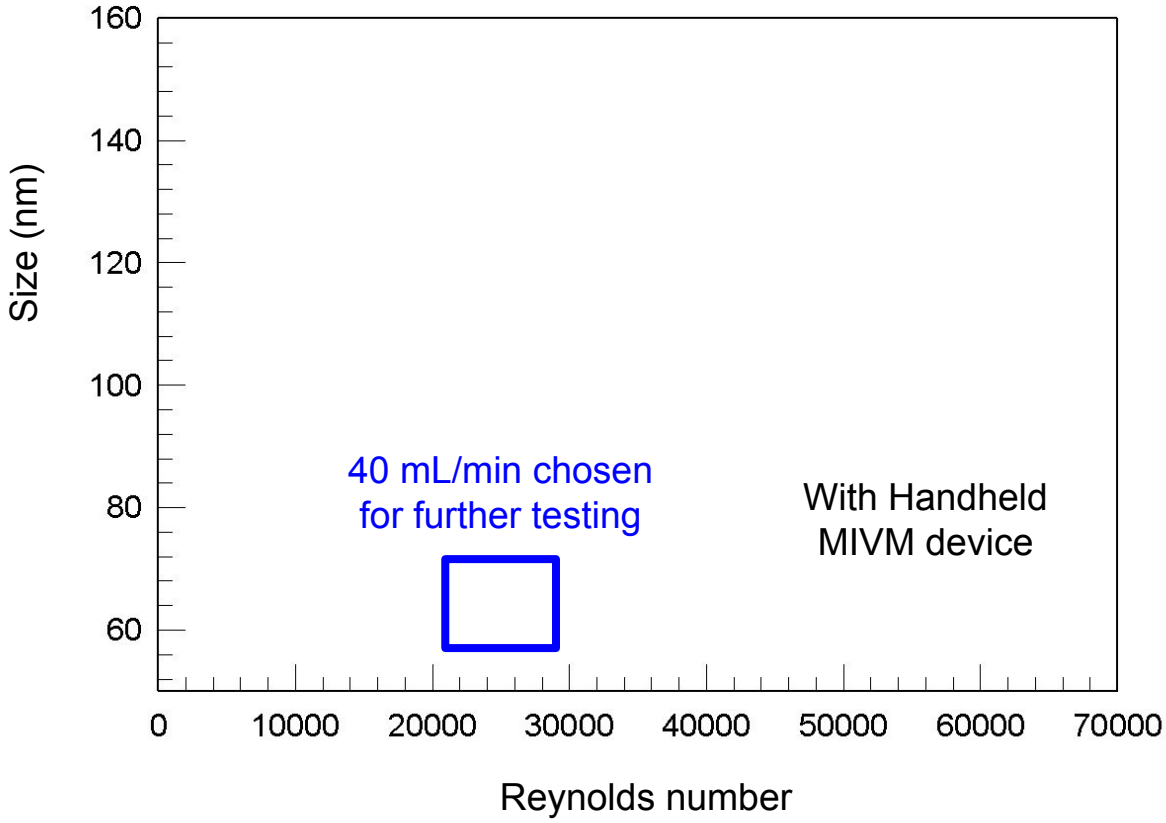


Nikhil Subraveti
Postdoctoral Research Associate
Project focus: Functionalized NPs

LNP RNA results

Understanding in terms of Size vs Reynolds number

'DODMA lipid, yeast RNA



$$Re = \sum_{i=1,N} \frac{V_i}{\nu_i} D$$

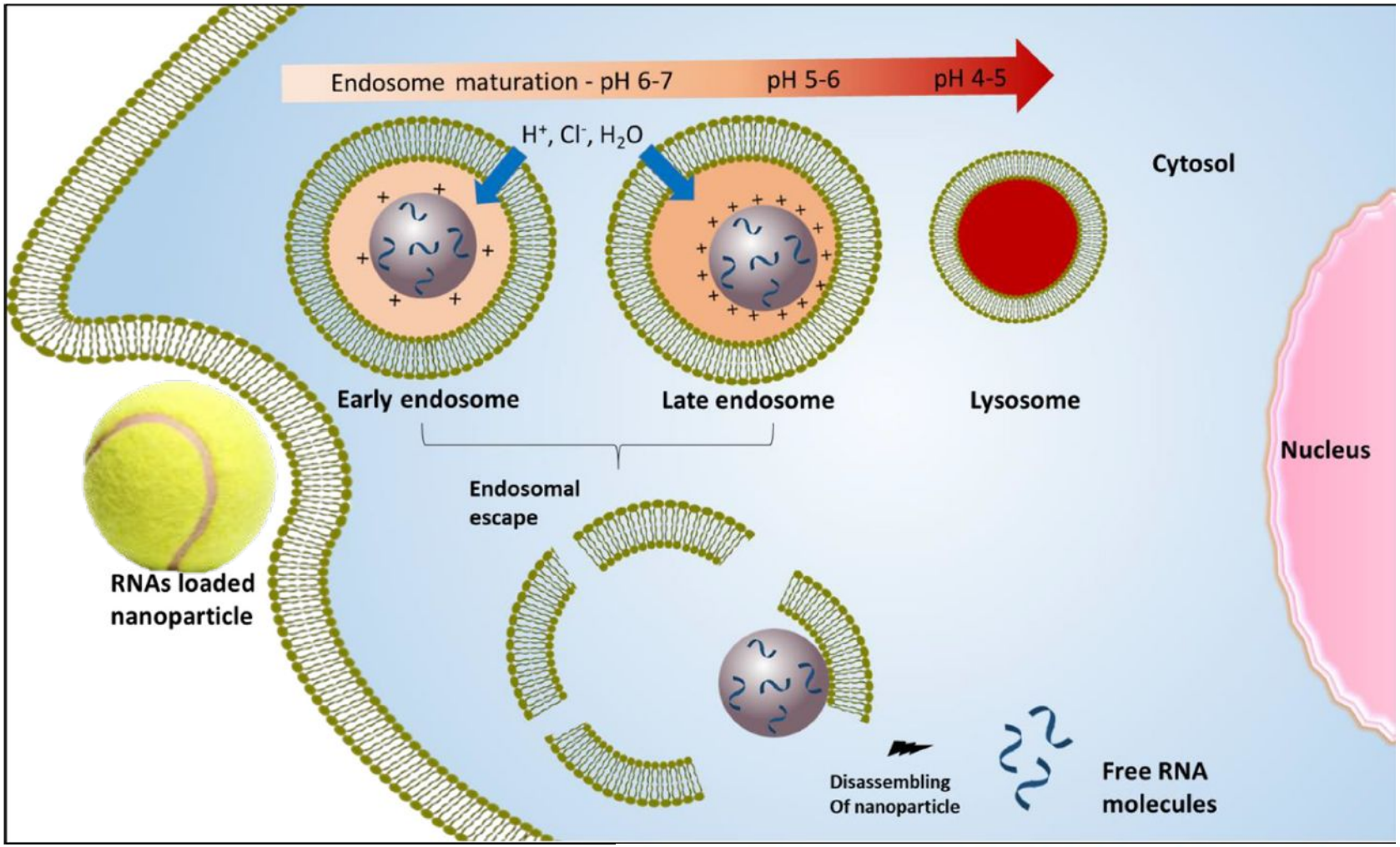
= Average inlet velocity of the ith stream

= Kinematic viscosity

= Chamber diameter

Size of LNPs plateau at a critical *Re* of 15000!

dosomal escape of COVID LNPs

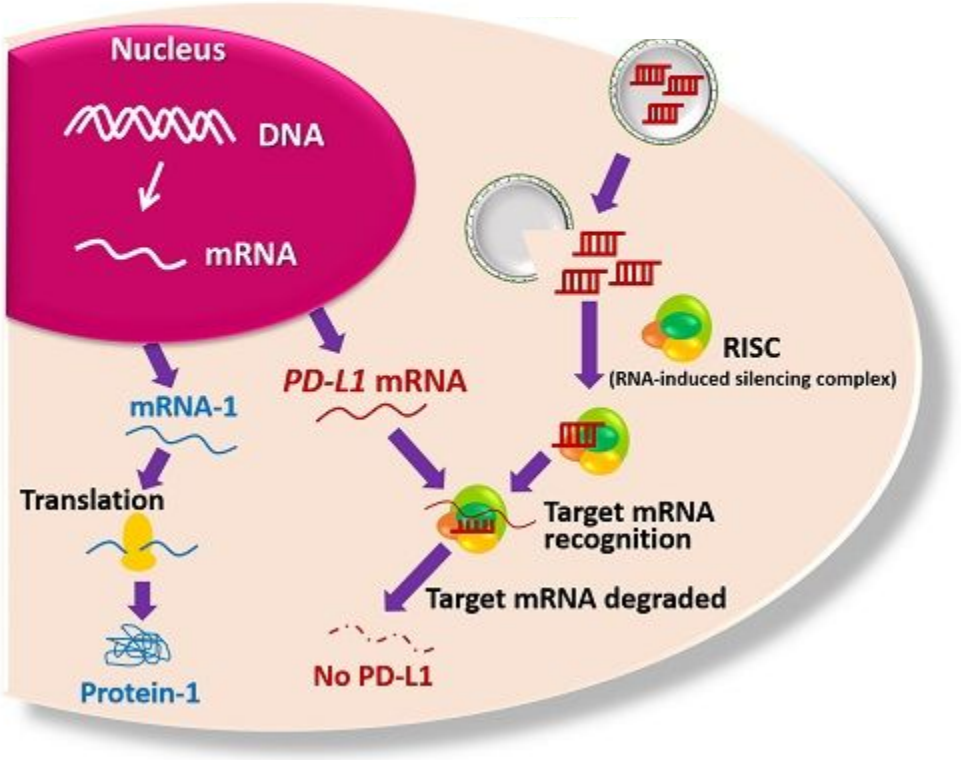


https://doi.org/10.1016/j.tips.2021.03.002

Oligonucleotides

- ***DNA*** *(deoxyribose nucleic acid): codes genetic information*
- ***siRNA*** *(silencing ribonucleic acid): stops mRNA synthesis*
- ***mRNA*** *(messenger RNA): synthesizes proteins*

Highly negatively charged, highly water soluble

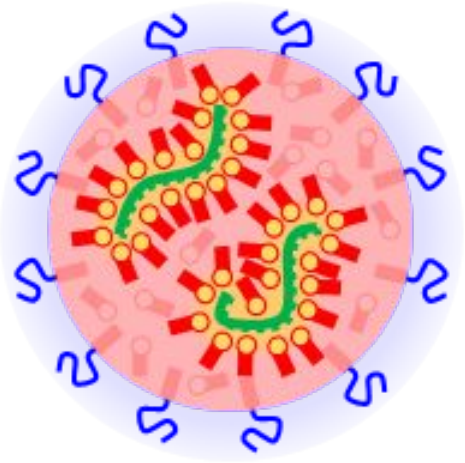


https://www.mdpi.com/2073-4409/9/9/2043#

Questions/Problems to Address: Targeting

Targeting with more complex ligands: antibodies, nanobodies

- *Lipids are poorly anchored and large targeting ligands will pull lipids from LNPs*
- *Larger ligand-PEG-lipid can not be incorporated in LNPs by microfluidics*



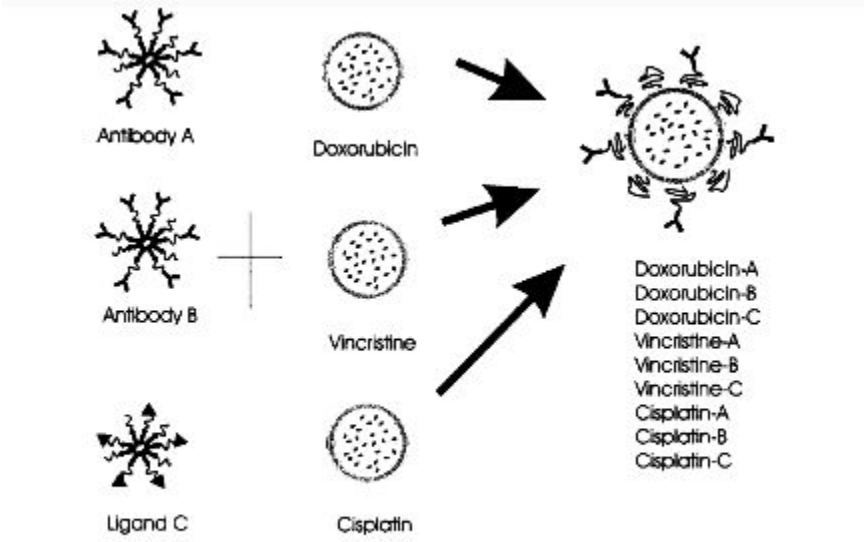


Fig. 1. Cartoon depicting the combinatorial approach to the formation of ligand-targeted liposomal anticancer drugs.

Ishida, T., D.L. Iden, and T.M. Allen, *A combinatorial approach to producing sterically stabilized (Stealth) immunoliposomal drugs.* FEBS letters, 1999. **460**(1): p. 129-133.

Parhiz, Hamideh, et al. "PECAM-1 directed re-targeting of exogenous mRNA providing two orders of magnitude enhancement of vascular delivery and expression in lungs independent of apolipoprotein E-mediated uptake." *Journal of controlled release* 291 (2018): 106-115.

Questions/Problems to Address: 4)Targeting

Targeting Summary:

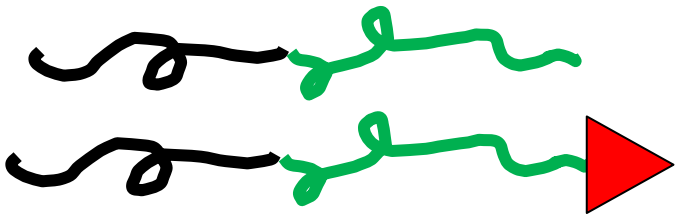
1) Targeting by changing LNP surface charge can bias deposition in the liver/lungs/spleen

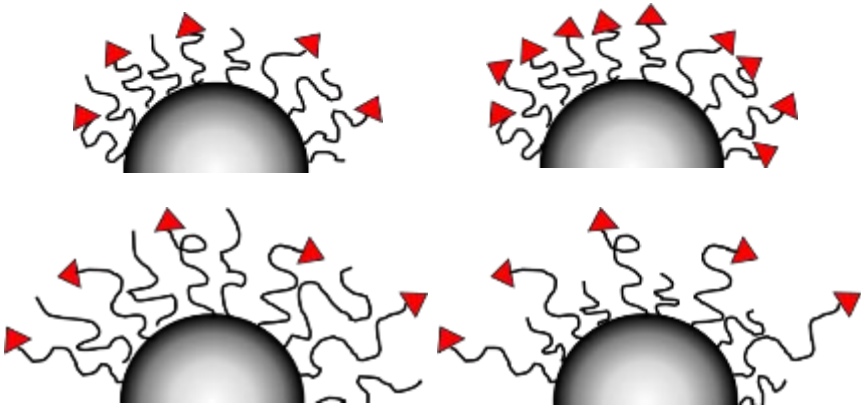
2) Adding cationic lipids to alter charge raises immunogenic problems

3) Targeting using "high specificity ligands" (e.g. antibodies) wil require a different platform than current LNP processes

Active targeting

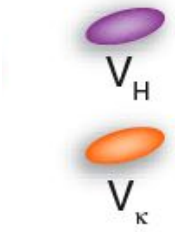
- *Conjugate targeting group to block copolymer*
- *Do hard chemistry and characterization on conjugate*
- *Quantitatively assemble targeted NP with control on % targeting groups*
- *Conjugate chemistry: click chemistry, maleimide, carbodiimide, carbamate*





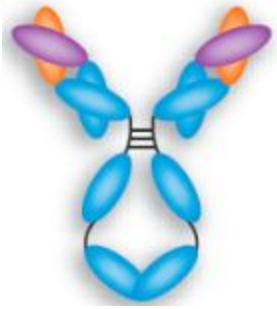
Small molecule:
(mannose, folate, LHRH)

V domain



scFv

IgG



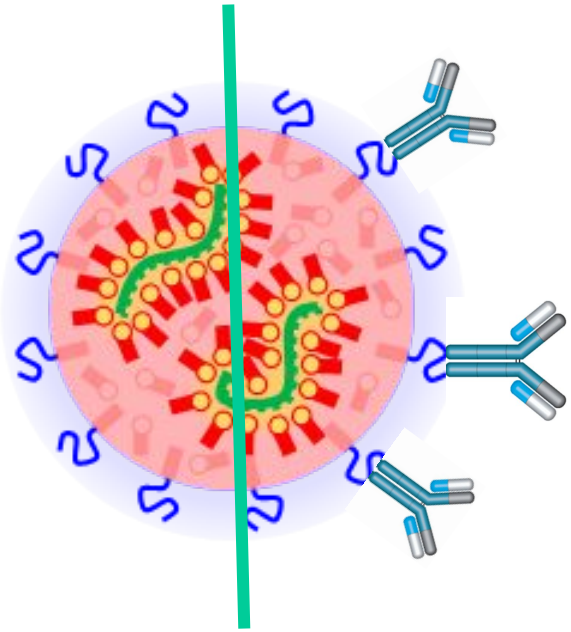
Cindy, Biomaterials (2008)

Akbulut, Adv. Fn. Mat. (2009)

Targeting mRNA and DNA LNPs

Current LNPs

- *Anchored by lipid (PEG-lipids)*
- *Meant to partition off in 30 min*



Princeton NPs

- *Anchored by block copolymer: PEG-PCL*
- *Irreversibly anchored*
- *Targeting antibody conjugated to PEG*
- ***Question**: does PEG layer prevent endosomal escape?*

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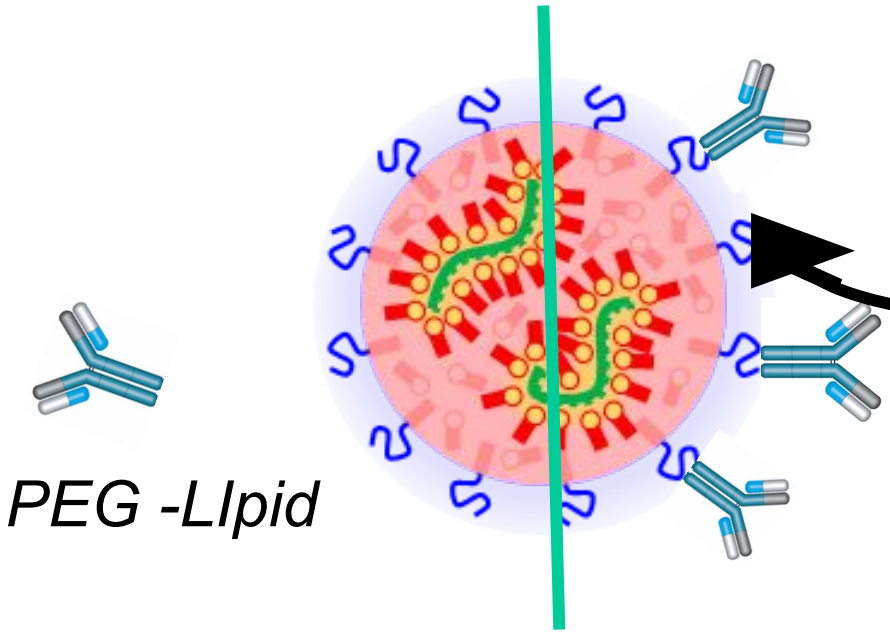
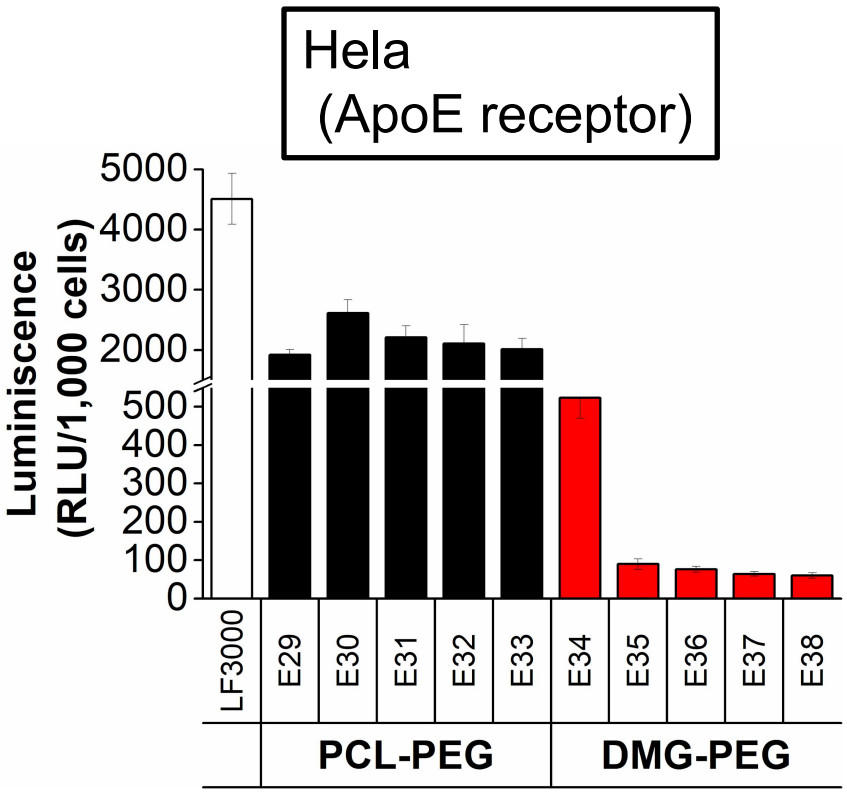
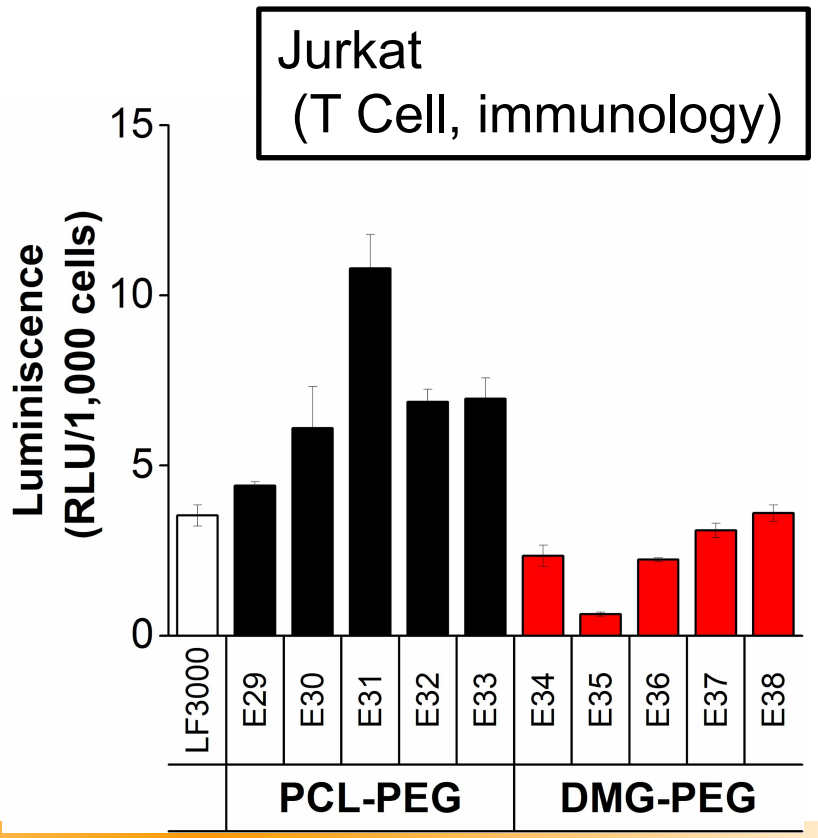


DNA LNPs by FNP: lipid vs PCL polymer anchoring

- *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***





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 bioavailabiity (Gates funding)*
    2. ***Parenteral**:Controlled release with conjugation*
    3. ***Parenteral**:Ion pairing for hydrophilic drugs (LNPs RNA, peptides, proteins)*
3. ***mRNA and DNA Lipid Nanoparticles:Targeting***

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3. ***Targeting***
4. ***Inverse FNP of biologics: NPs and Depot MCs***

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3. ***Targeting***

“It takes a village”

2005

2011

2014

2018