AI-assisted autonomous manufacturing of (on-demand, tailored, tunable) drug-loaded nanoparticles by multi-step continuous-flow platform

Prof. Dong-Pyo Kim

CONTROLLED RELEASE SOCIETY
CRS 2024 Annual Meeting AND Exposition
JULY 8-12, 2024 • BOLOGNA, ITALY

INTEGRATING
Delivery Science
ACROSS DISCIPLINES

THE FOUR INDUSTRIAL REVOLUTIONS

INDUSTRY 1.0

Mechanization

Mechanization and the introduction of steam and water power

INDUSTRY 2.0

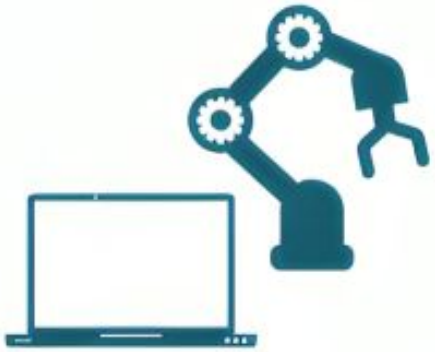
Electrification

Mass production assembly lines using electrical power

INDUSTRY 3.0

Automatization

Automated production, computers, IT-systems and robotics



INDUSTRY 4.0

Cyber-Physical Systems

The Smart Factory. Autonomous systems, IoT, machine learning

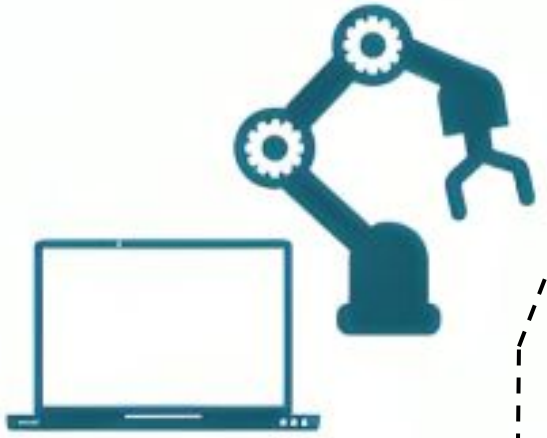
Emergence of AI-Based Autonomous Systems

NDUSTRY 4.0

r-Physical Systems

The Smart Factory.
nomous systems, IoT,
machine learning

Autonomous Platforms in Chemical Science

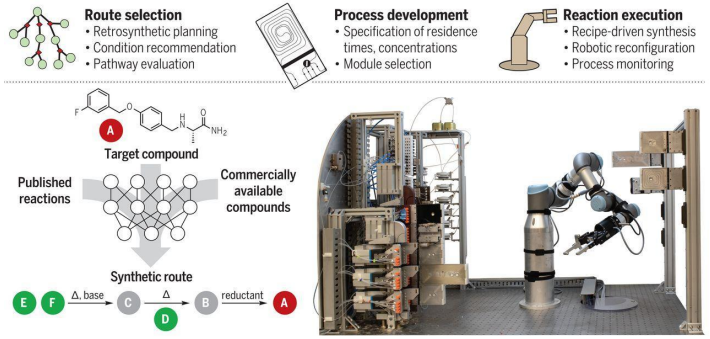


INDUSTRY 4.0

Cyber-Physical Systems

The Smart Factory.
Autonomous systems, IoT,
machine learning

Science

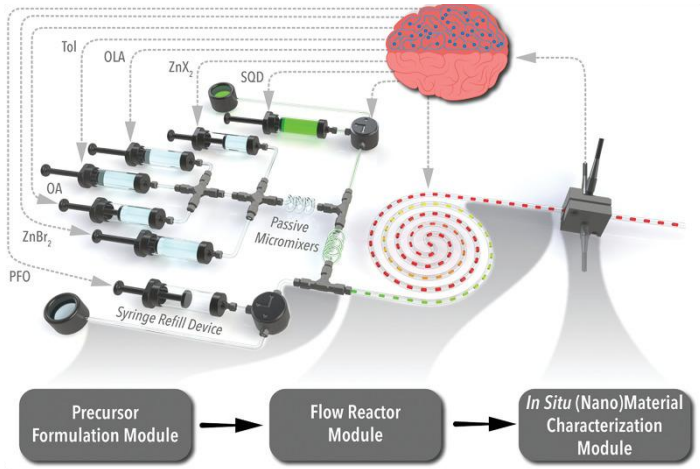


*Science***365**,eaax1566(2019)

nature

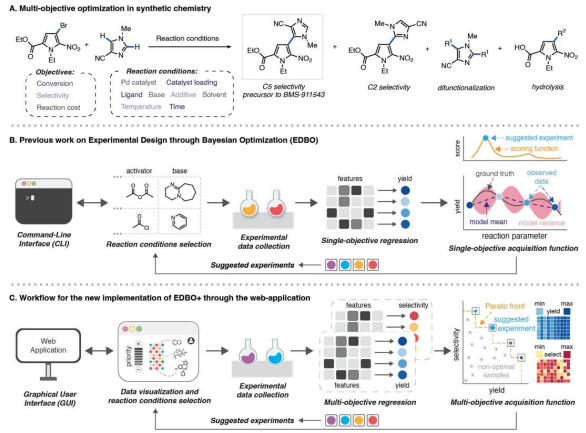
Nature **583**, 237–241 (2020)

ADVANCED MATERIALS



Adv. Mater. 2020, 32, 2001626

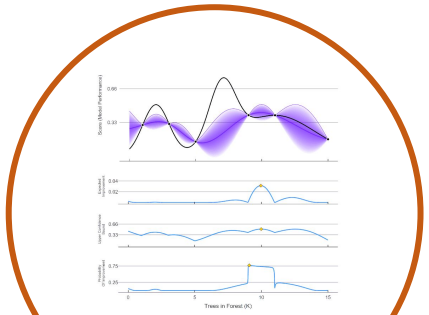
JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY



J. Am. Chem. Soc. 2022, 144, 43, 19999–20007

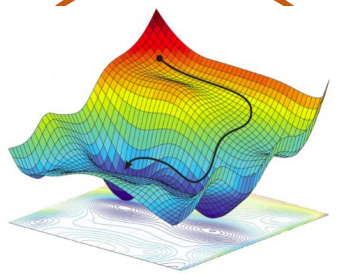
Introduction: Autonomous Platforms

How Does a Self-Driving Chemical Platform Work?



Bayesian Optimization

Optimization Algorithm



Multi-Objective Optimizer

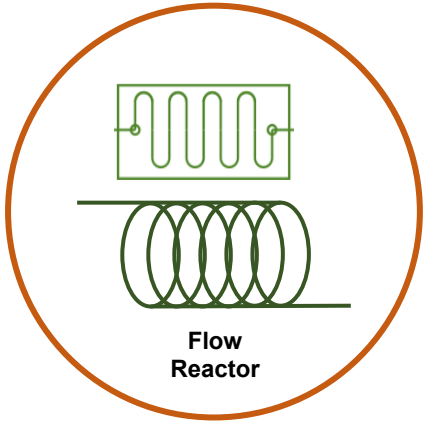
Chromatography

Spectroscopy

Automated Analysis

Closed-Loop

Automated Reactor

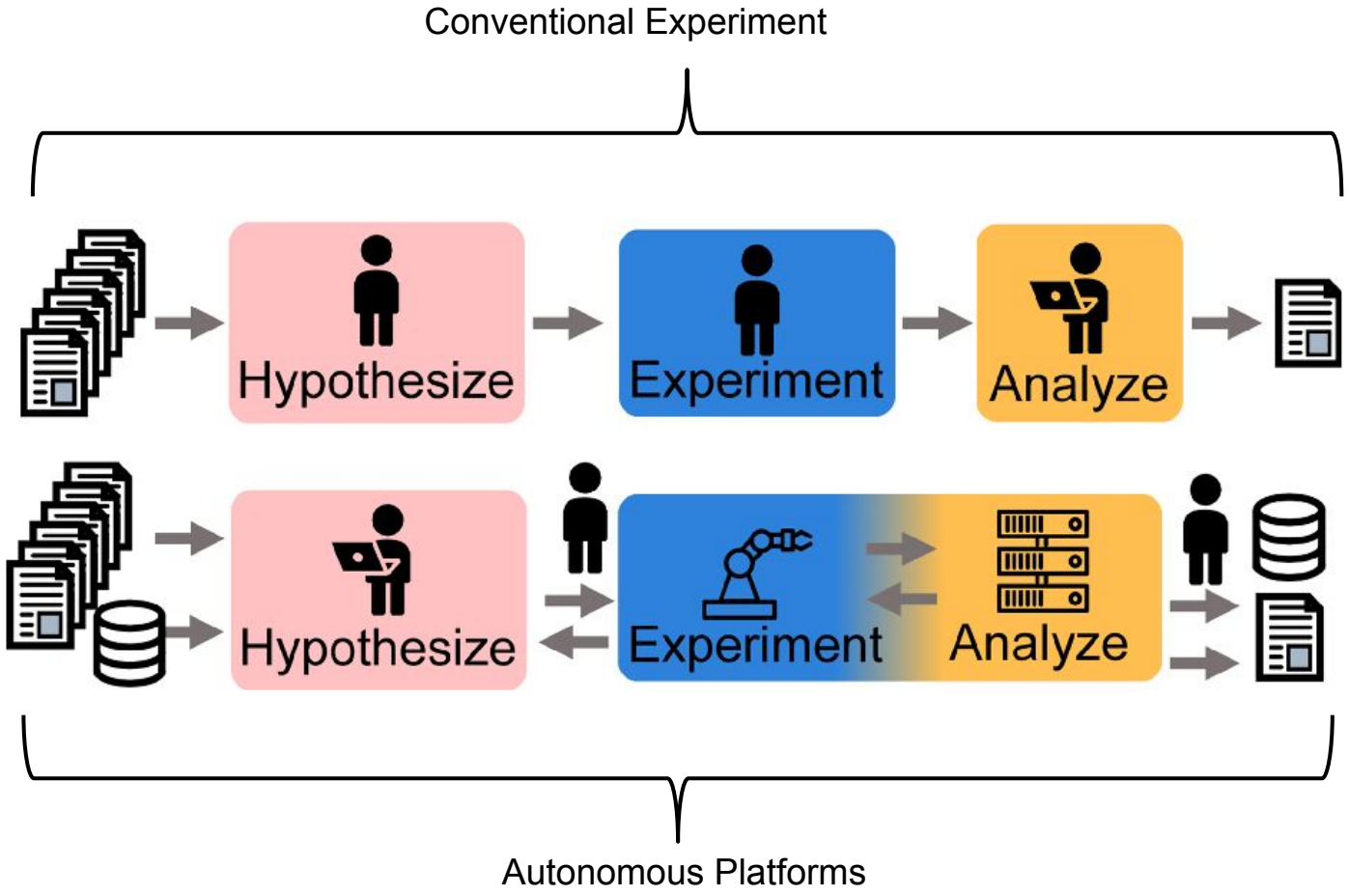


Flow Reactor

5

Introduction: Advantages of Autonomous Platforms

Why develop autonomous platforms?

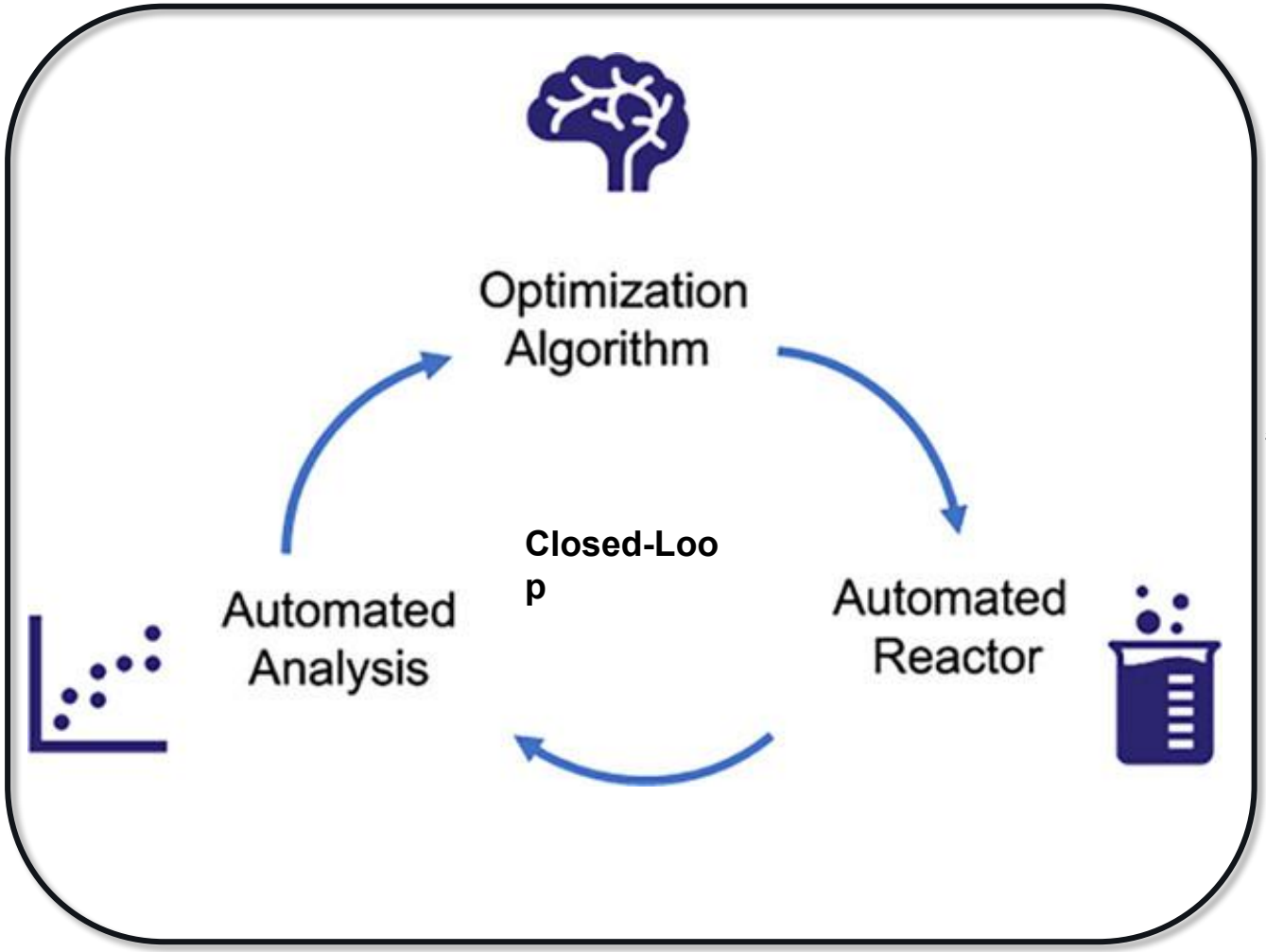


Benefits

- Cheaper, faster, greener
- Enhance the **capabilities** of the researcher
- Remove **labor-intensive** part
- Easier to solve **complex** problems with **AI-guided** algorithm
- Increase **reproducibility**
- **Discovery** of new compounds and formulations

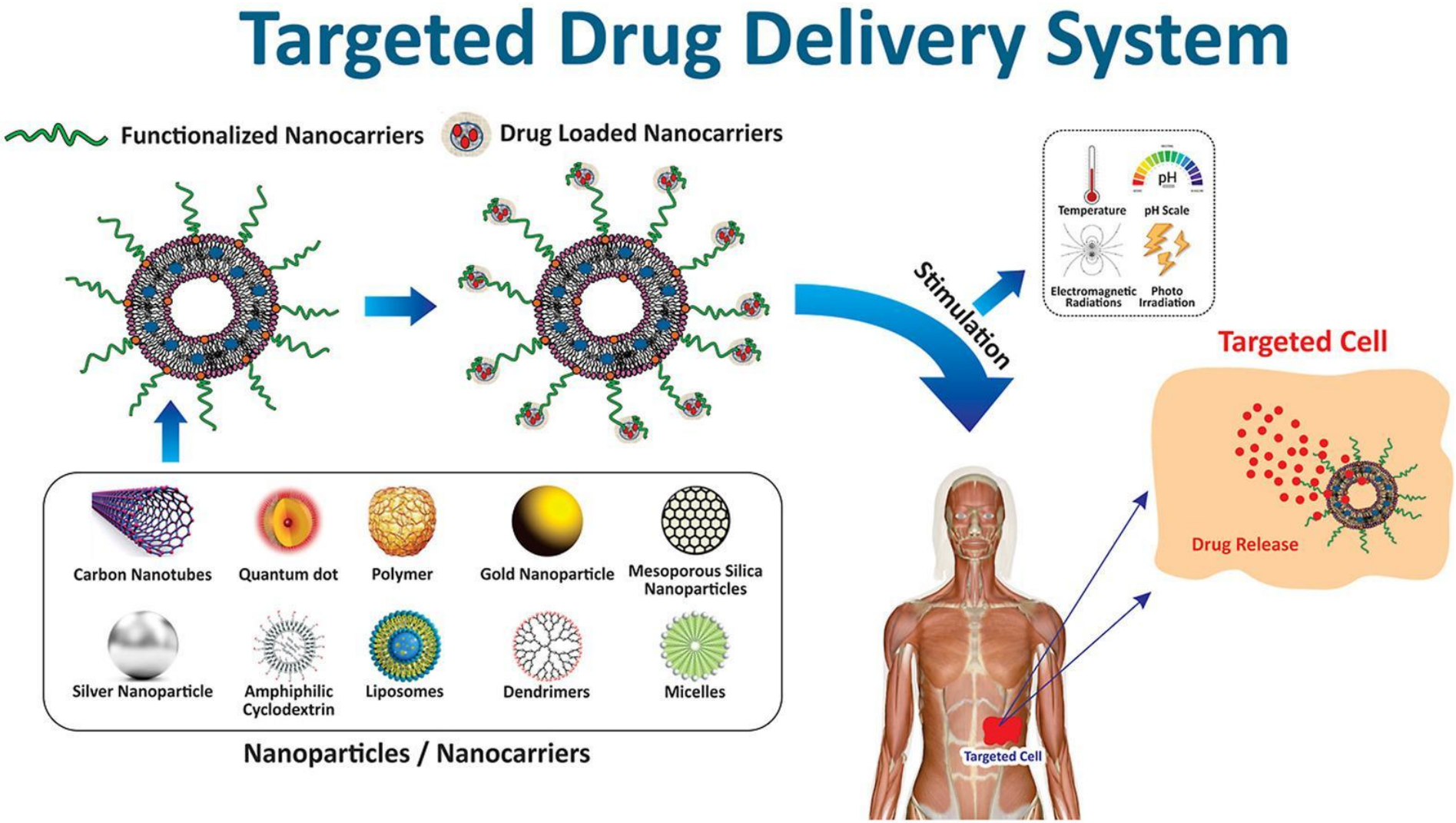
Introduction: Advantages of Autonomous Platforms

Which fields have autonomous platforms been used?



- **Chemical Synthesis**
  - Catalytic reaction, Photoredox reaction, electrochemical reaction, etc.
- **Materials Science**
  - Metal nanoparticle synthesis, quantum dots, etc.
- **Nanomedicines**
  - Nanoparticle Drug Delivery Systems
  - Lack of detailed study in nanomedicines

Introduction: Nanoparticle Drug-Delivery Systems

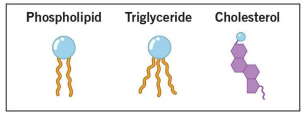


Introduction: Challenges in Conventional DDS (example of Liposome)

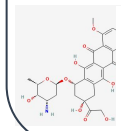
Challenges for development of Autonomous Platform in Nanoparticle DDS?

Formulation

Lipids



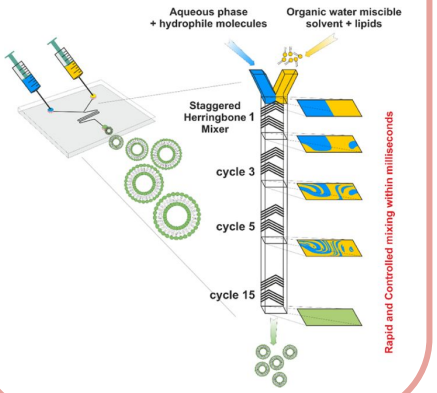
Drug



Solvent

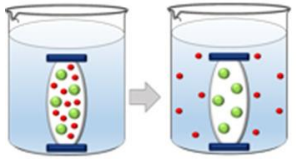
Flow Conditions

Nanoprecipitation

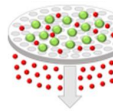


Purification

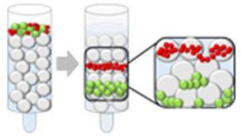
Dialysis



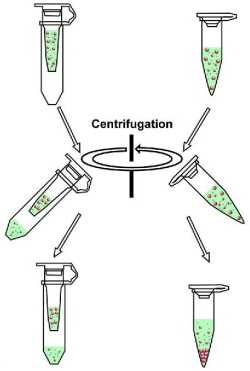
Filtration



Chromatography

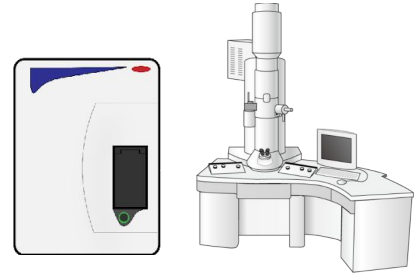


Ultrafiltration



Characterization

Size and Morphology



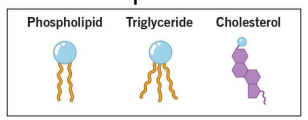
Drug Loading

Introduction: Challenges in Conventional DDS (example of Liposome)

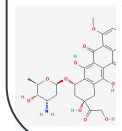
Challenges for development of Autonomous Platform in Nanoparticle DDS?

Formulation

Lipids



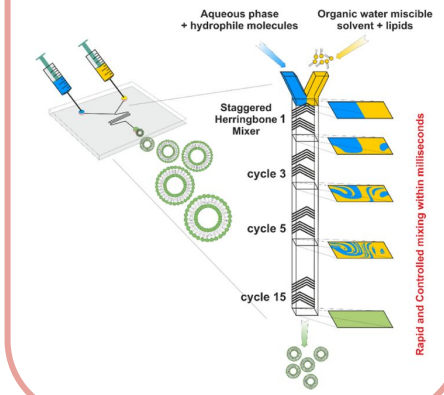
Drug



Solvent

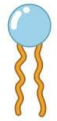
Flow Conditions

Nanoprecipitation

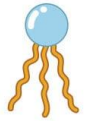


Recipe Development

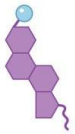
Phospholipid



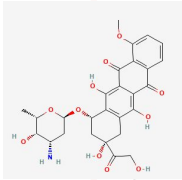
Triglyceride



Cholesterol

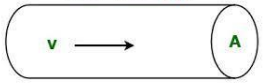


Drug



Solvent & Buffer

Flow Conditions



(flowrate, ratio)

40 Combinations

20 Concentrations

10 Combinations

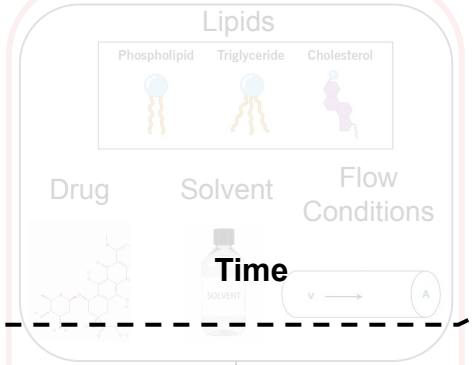
50 Combinations

Large Number of Combinations

Introduction: Challenges in Conventional DDS (example of Liposome)

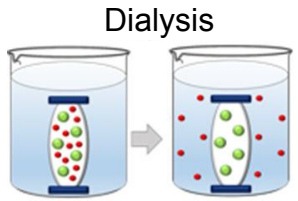
Challenges for development of Autonomous Platform in Nanoparticle DDS?

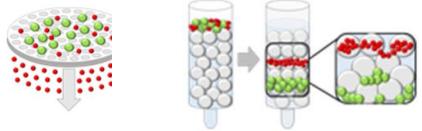
Formulation



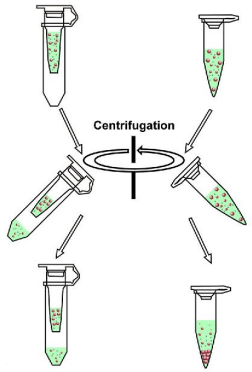
- **Long processes**
- **Multiple steps → hours scale**

Purification

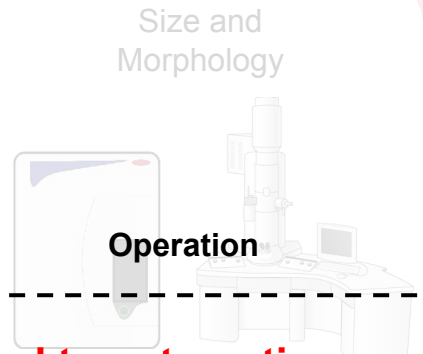




Ultrafiltration



Characterization



Operation

- **Hard to automatize**
- **Challenging to integrate**

Introduction: Challenges in Conventional DDS (example of Liposome)

Challenges for development of Autonomous Platform in Nanoparticle DDS?

Formulation

Lipids

Phospholipid Triglyceride Cholesterol

Drug Solvent Flow Conditions

Nanoprecipitation

Aqueous phase + hydrophile molecules

Organic water miscible solvent + lipids

Staggered Herringbone 1 Mixer

cycle 3

cycle 5

cycle 15

Rapid and Controlled mixing within milliseconds

Purification

Dialysis

Filtration Chromatography

Ultrafiltration

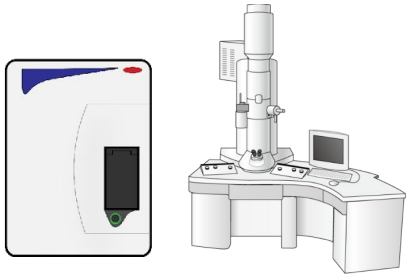
Centrifugation

Data Acquisition

Generally off-line analysis instruments → not suitable for automation

Characterization

Size and Morphology



Drug Loading

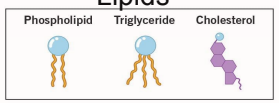
Introduction: Challenges in Conventional DDS (example of Liposome)

How to address the challenges for construction of a self-driving platform?

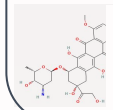
Optimization and Discovery

Formulation

Lipids



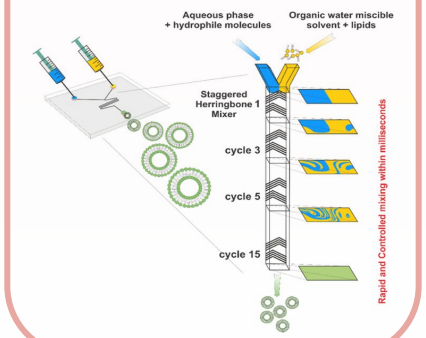
Drug



Solvent

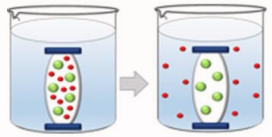
Flow Conditions

Nanoprecipitation

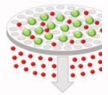


Purification

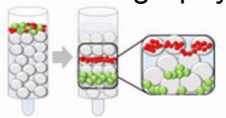
Dialysis



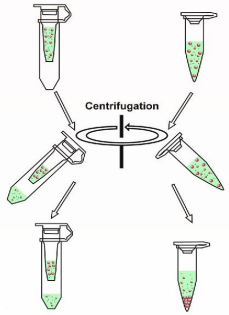
Filtration



Chromatography

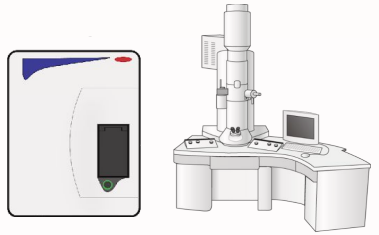


Ultrafiltration



Characterization

Size and Morphology



Drug Loading

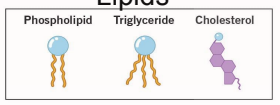
Introduction: Autonomous Integrated Platform

How to address the challenges for construction of a self-driving platform?

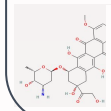
Optimization and Discovery

Formulation

Lipids



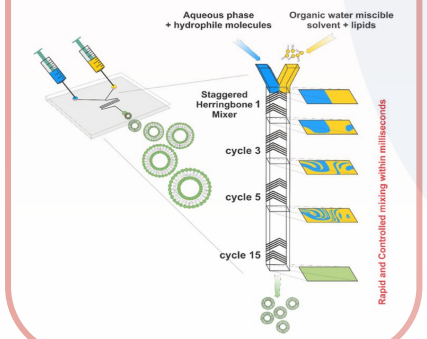
Drug



Solvent

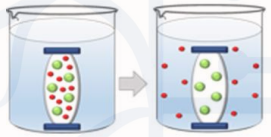
Flow Conditions

Nanoprecipitation

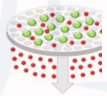


Purification

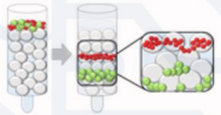
Dialysis



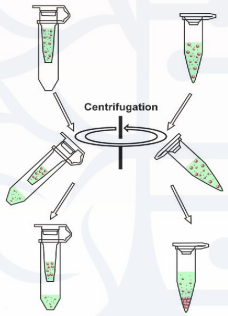
Filtration



Chromatography

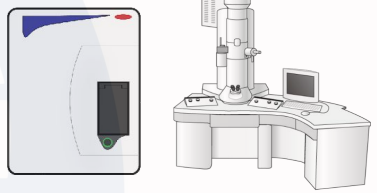


Ultrafiltration



Characterization

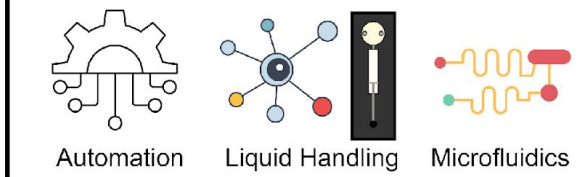
Size and Morphology

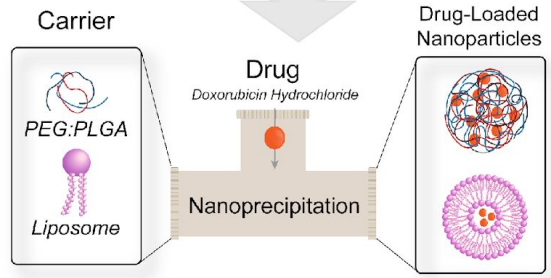


Drug Loading

Research Overview

1 Automated Nanoparticle Synthesizer





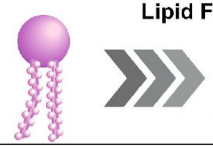
Synthesis Parameters

Flow Conditions

Total Flowrate (mL/min) *Dox Conc. (mg/mL)*

Flowrate Ratio (mL/min) *Polymer Conc. (mg/mL)*

Lipid Formulation

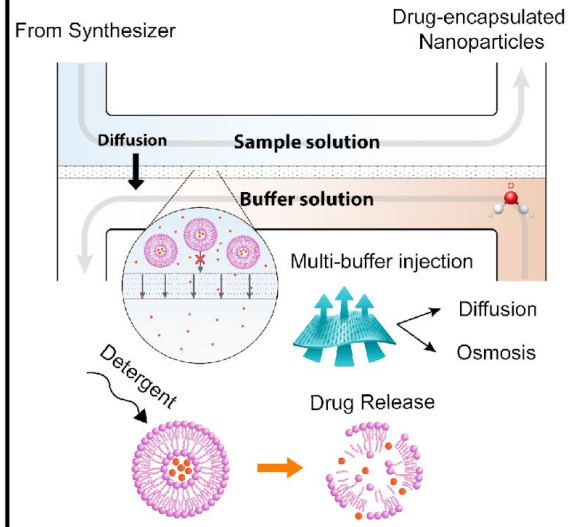


POPG (mol%)

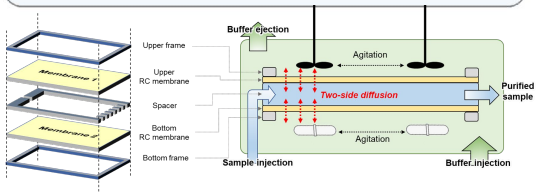
POPC/ Cholestrol (P/C)

2 Purification

Small-scale Flow Dialysis Module



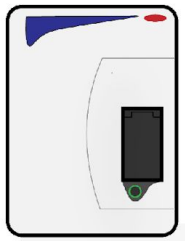
Scaled-up Flow Dialysis Module



3 Characterization & Optimization

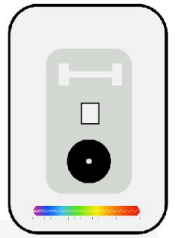
Real-time Analysis

Dynamic Light Scattering



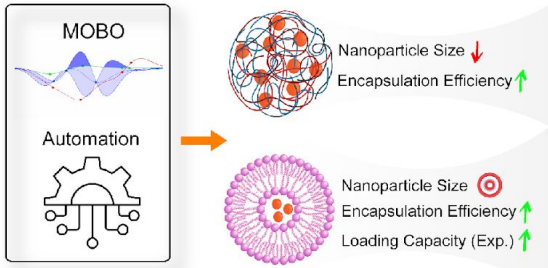
Size Distribution Uniformity

UV-Vis Spectroscopy

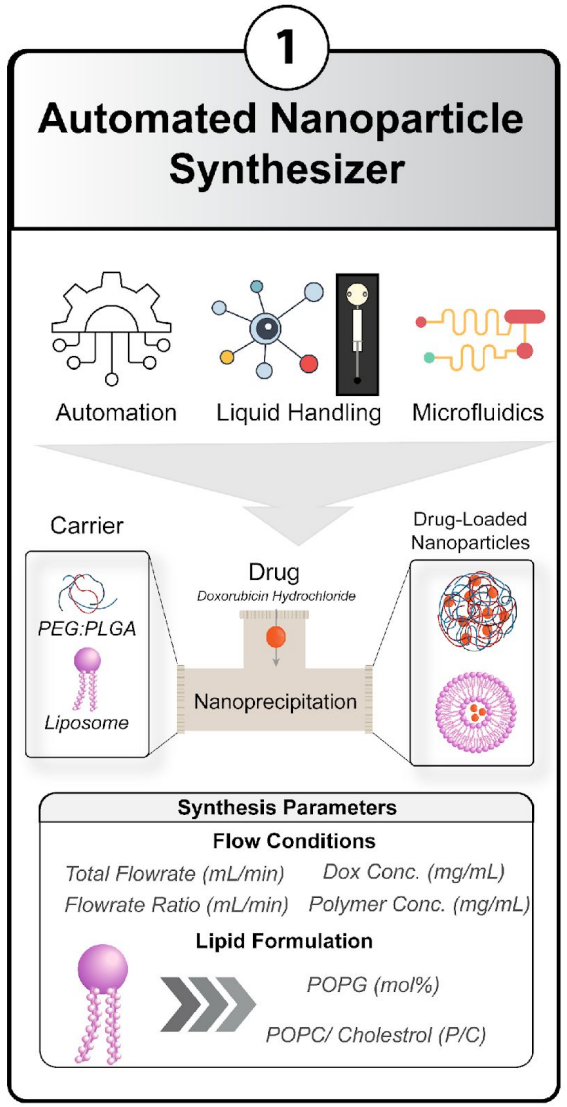


Drug Loading *at 485 nm for Dox*

Multi-Objectie Bayesian Optimization

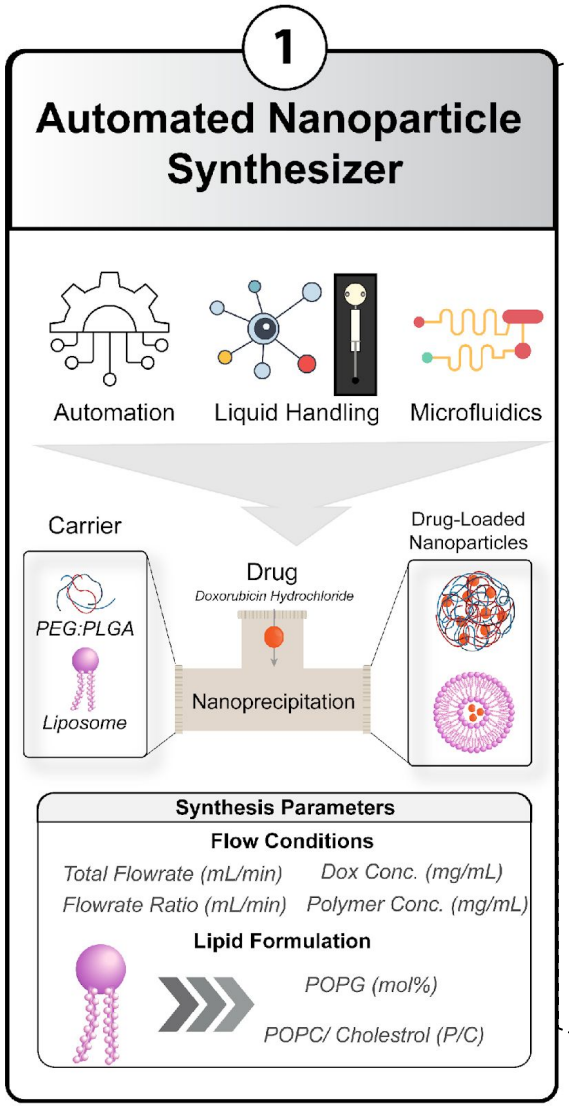


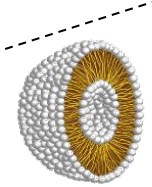
Module 1: Automated Nanoparticle Synthesizer



Module 1: Automated Nanoparticle Synthesizer

Without Drug Encapsulation





Liposome Nanoparticles

Process Control & Decision Making

Nanoparticle Size PDI

Objectives

Closed Loop

Ethanol

Dilution

Selector Valve Lipid Reservoir

PEG (Mol%)

0 0.5 1 1.5

Nanoparticle Synthesizer

Dynamic Light Scattering (DLS)

Intensity

Time

Selector Valve Antisolvent Reservoir

PBS Tris NaCl Citr.

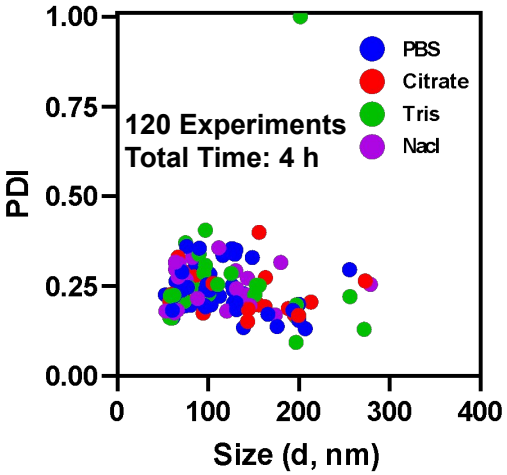
Liposome

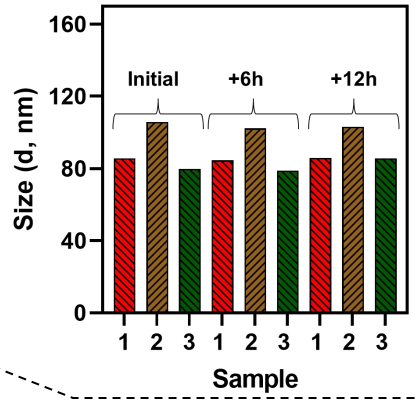
- POPC:Chlostrol = 55:45
- Anionic Lipid: 10 Mol%
- PEG: [0,1.5] Mol%

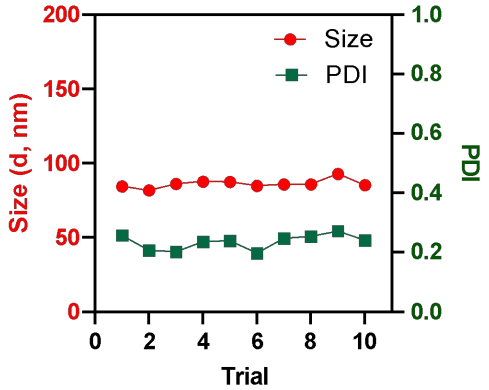
Total Flowrate
Flow rate Ratio
Lipid Concent.
PEG Mol%
Antisolvent

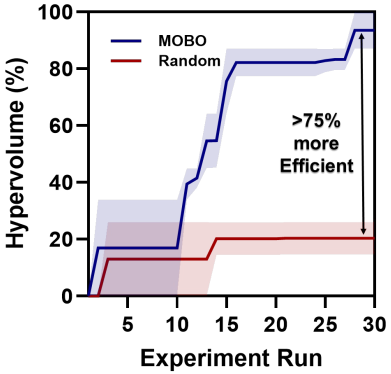
Parameters

Overall Data Obtained







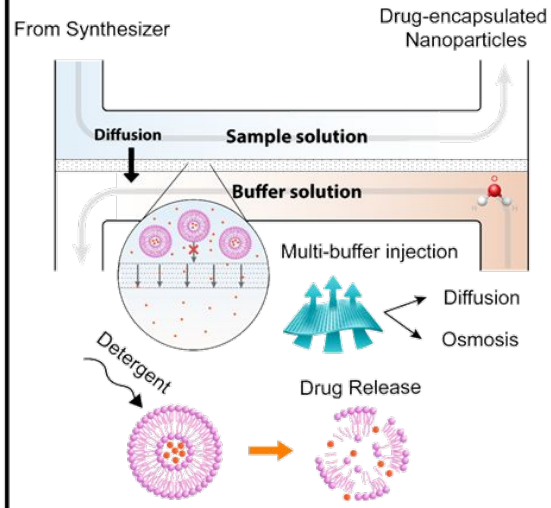


Module 2: Purification Module

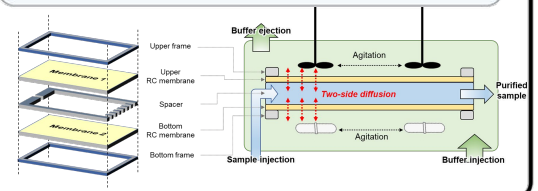
2

Purification

Small-scale Flow Dialysis Module



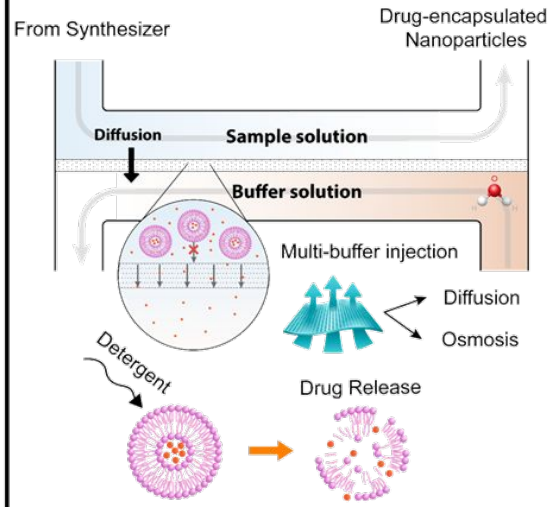
Scaled-up Flow Dialysis Module



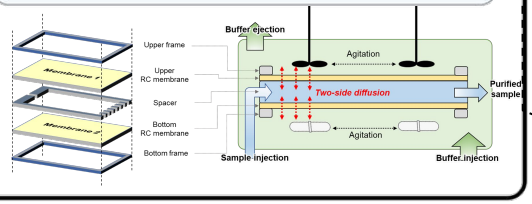
Module 2: Purification Module

2 Purification

Small-scale Flow Dialysis Module

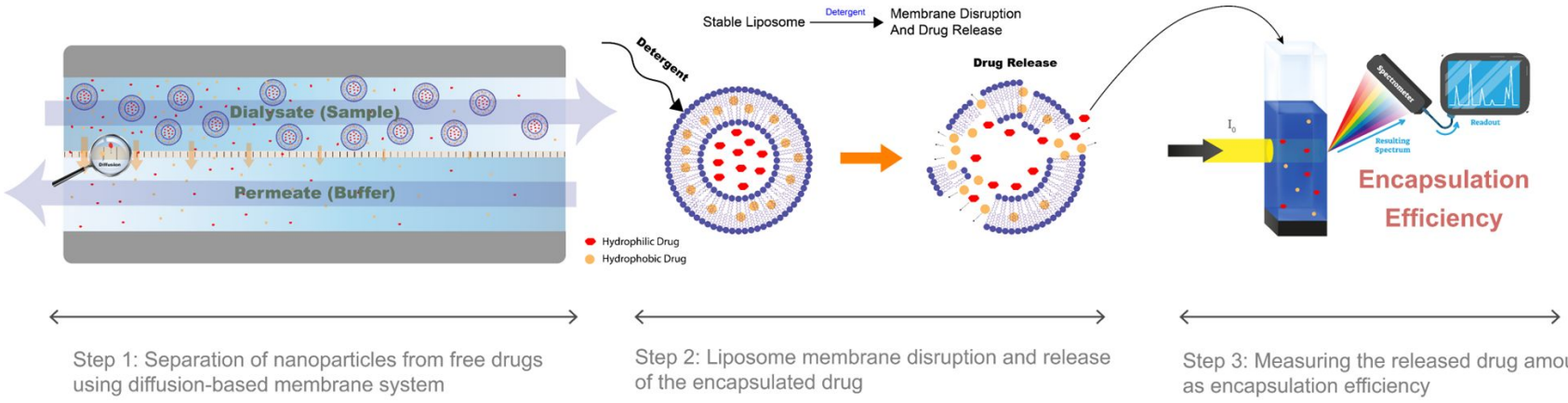


Scaled-up Flow Dialysis Module



1 Small Scale Flow Dialysis Module For Characterization

Multi-Buffer Injector (MBI)

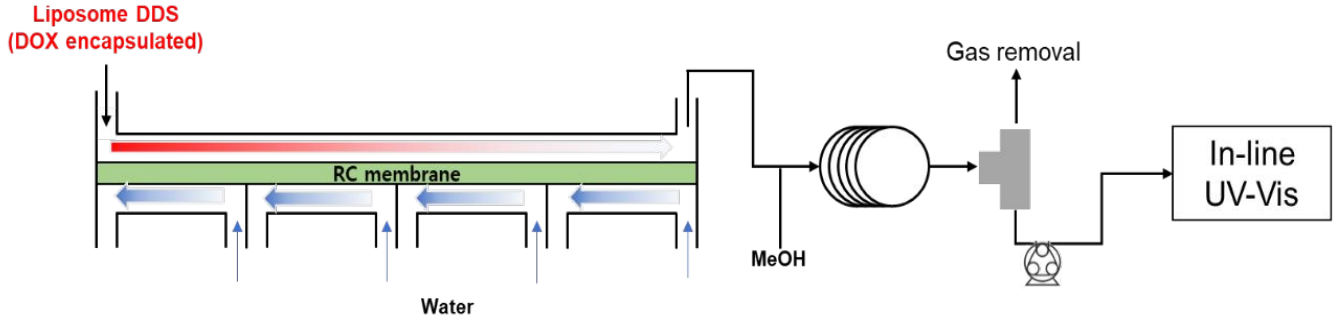


Step 1: Separation of nanoparticles from free drugs using diffusion-based membrane system

Step 2: Liposome membrane disruption and release of the encapsulated drug

Step 3: Measuring the released drug amount as encapsulation efficiency

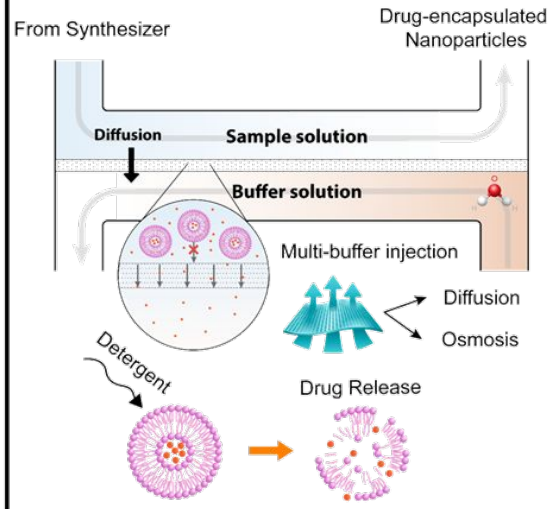
- Separation of the drug-loaded nanoparticles from free "unencapsulated" drugs
- Use of a detergent for disrupting nanoparticles (MeOH for Liposome case)
- Flowrate of 75 µL/min (retention time of 10 mins)
- RC Membrane (12 KDa), DI water instead of PBS buffer for rapid removal



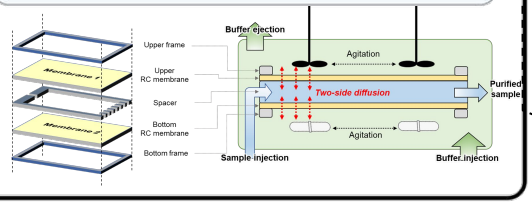
Module 2: Purification Module

2 Purification

Small-scale Flow Dialysis Module

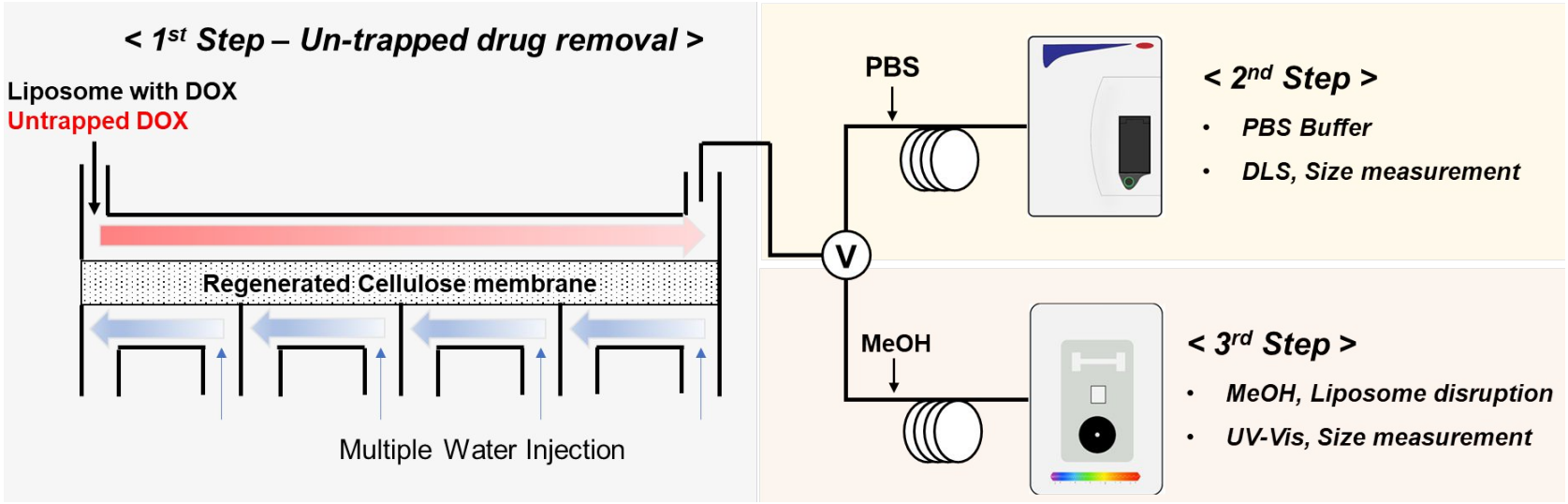


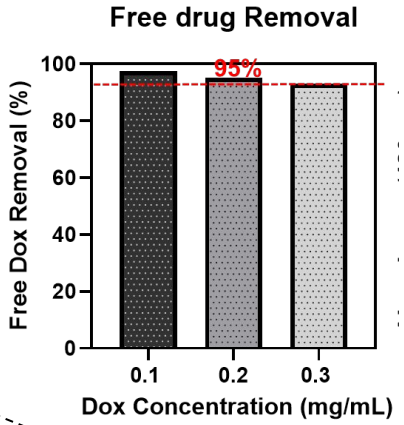
Scaled-up Flow Dialysis Module



1 Small Scale Flow Dialysis Module For Characterization

Multi-Buffer Injector (MBI)



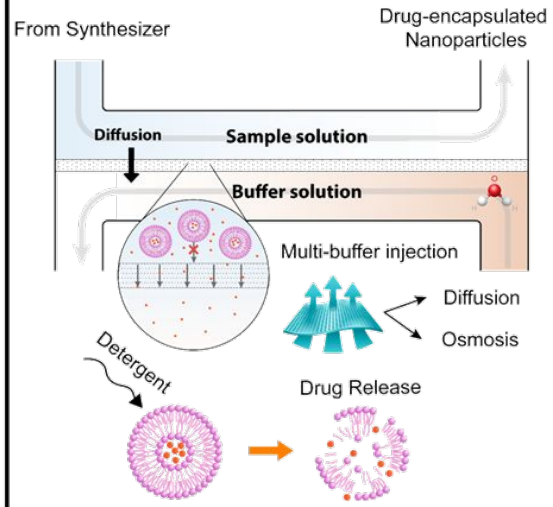


- The most important factor for the performance of MBI removal of the free Drug (DOX)
- Injection of DOX with different concentrations
- In-line UV-Vis at the end of the line checking the removal rate for each case (>95% acceptable)

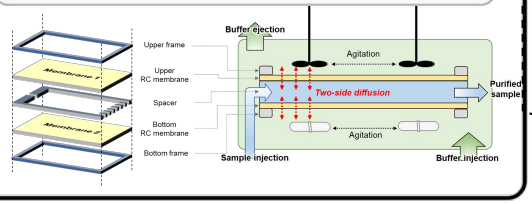
Module 2: Purification Module

2 Purification

Small-scale Flow Dialysis Module

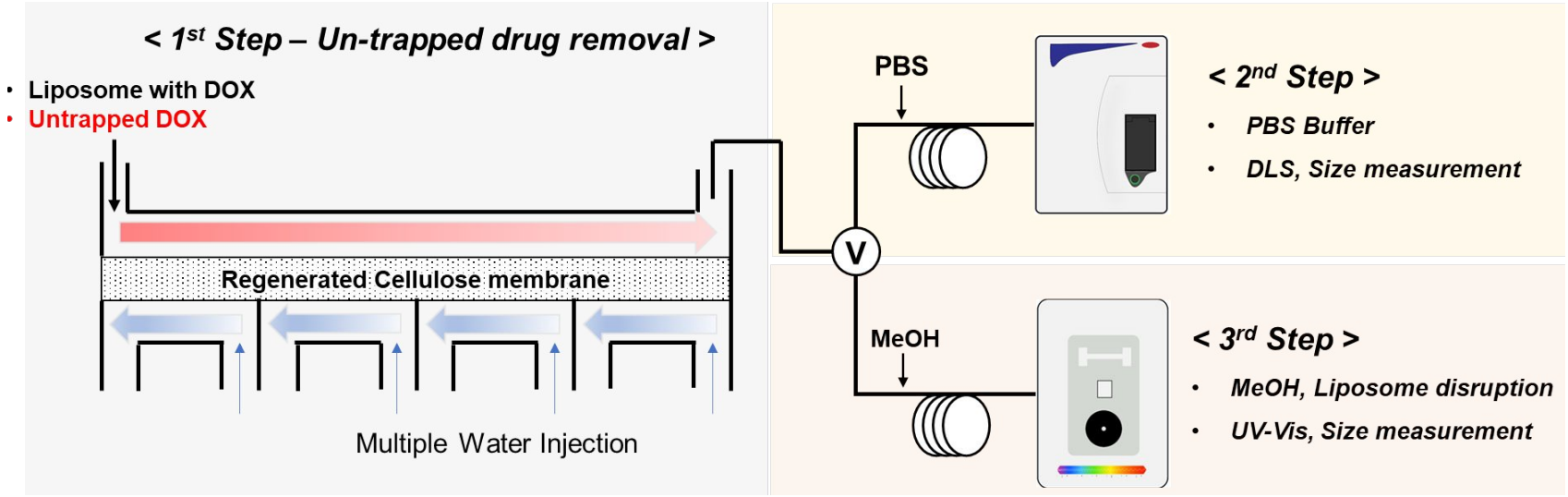


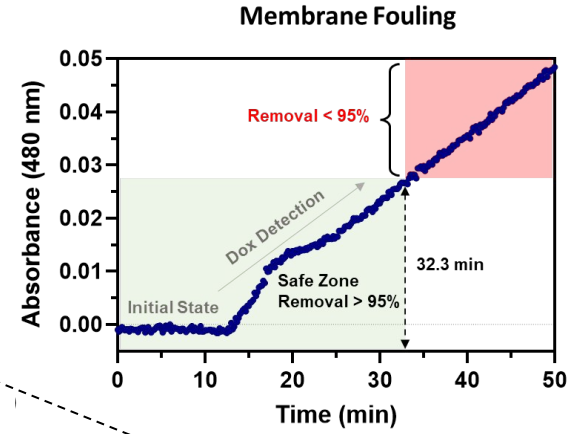
Scaled-up Flow Dialysis Module



1 Small Scale Flow Dialysis Module For Characterization

Multi-Buffer Injector (MBI)



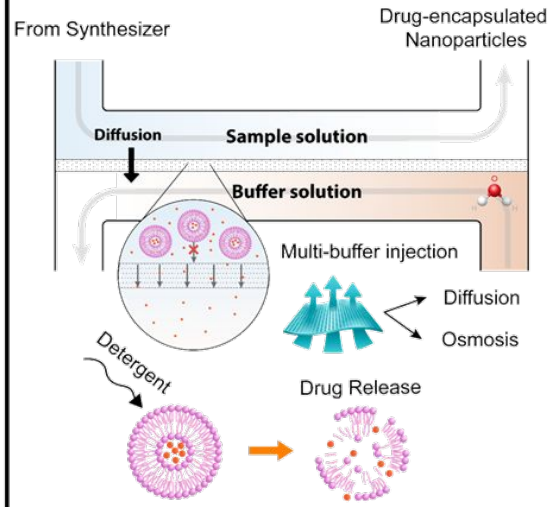


- Evaluation of the membrane Fouling by continues DOX injection (C_{DOX} = 0.3 mg/mL)
- Efficient continues performance of the module for more than 30 mins (Removal > 95%)

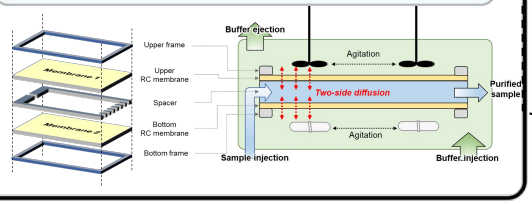
Module 2: Purification Module

2 Purification

Small-scale Flow Dialysis Module



Scaled-up Flow Dialysis Module

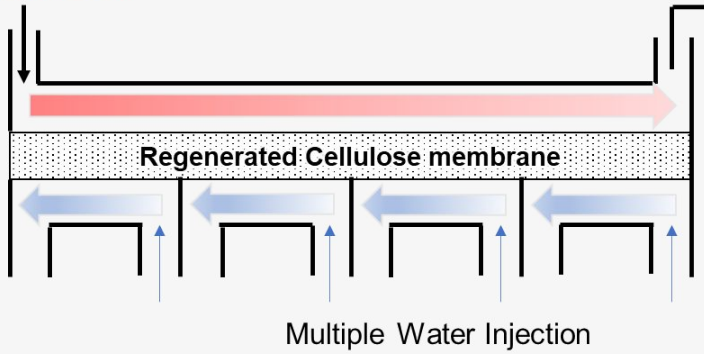


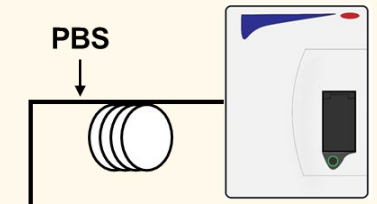
1 Small Scale Flow Dialysis Module For Characterization

Multi-Buffer Injector (MBI)

< 1st Step – Un-trapped drug removal >

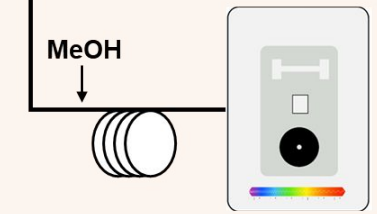
- Liposome with DOX
- Untrapped DOX





< 2nd Step >

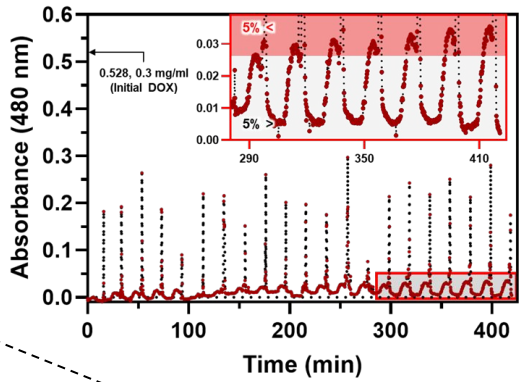
- *PBS Buffer*
- *DLS, Size measurement*



< 3rd Step >

- *MeOH, Liposome disruption*
- *UV-Vis, Size measurement*

Washing cycle

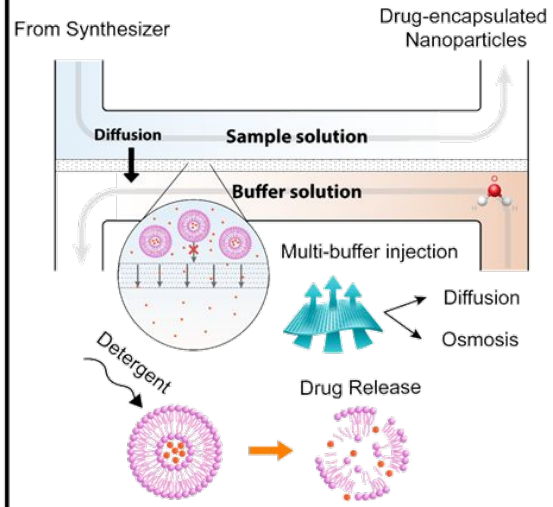


- Washing the membrane sequentially after injection of the sample (wash solvent: Ethanol) for around 400 mins
- In-line UV-Vis to measure the state of the washing [Sample → Wash → Sample → Wash → ….]

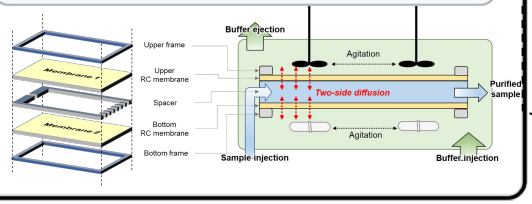
Module 2: Purification Module

2 Purification

Small-scale Flow Dialysis Module

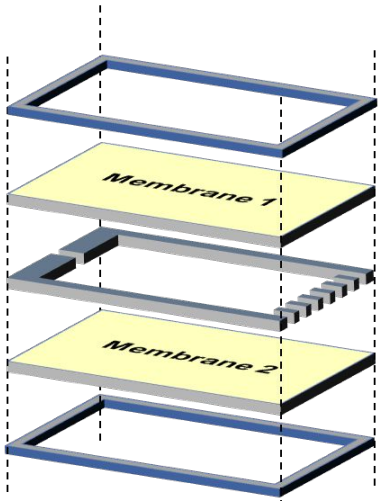


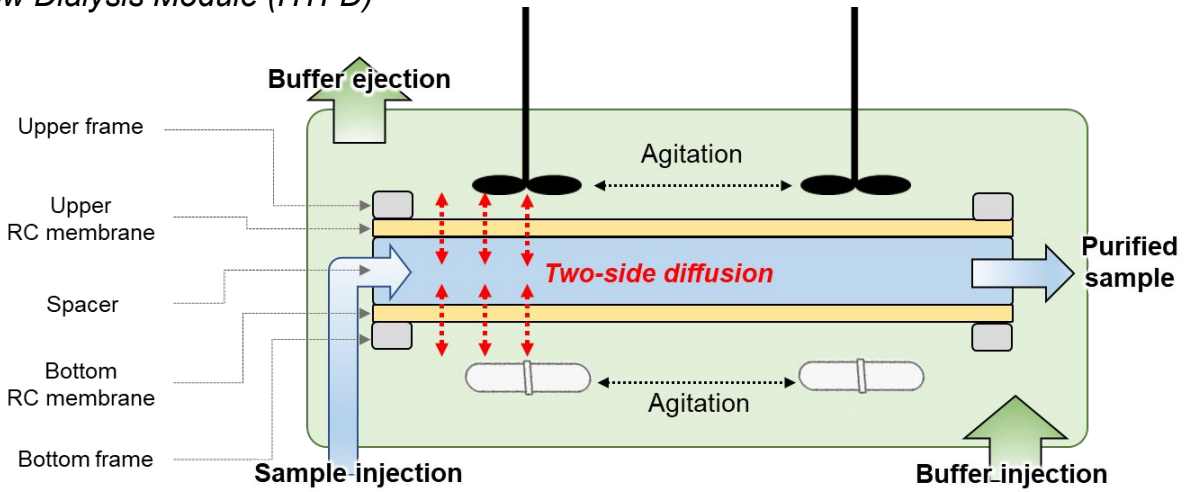
Scaled-up Flow Dialysis Module



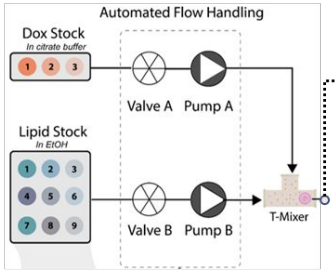
2 Large Scale Flow Dialysis Module For Large Scale Production

High-Throughput Flow Dialysis Module (HTFD)





DOX in citrate buffer Lipid in EtOH

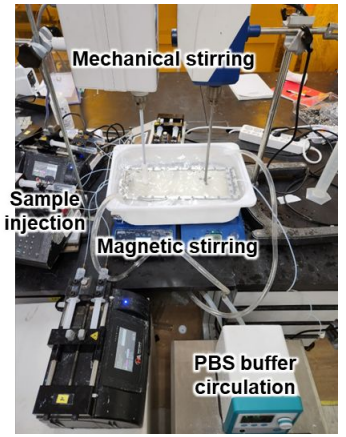


1:1 **EtOH : Buffer** 1:20

EtOH ↑ ***Buffer*** ↑

- pH ~ 7.4
- EtOH < 5000 ppm (0.5 %)

The purification condition (residence time) vary depending on the flow rate ratio



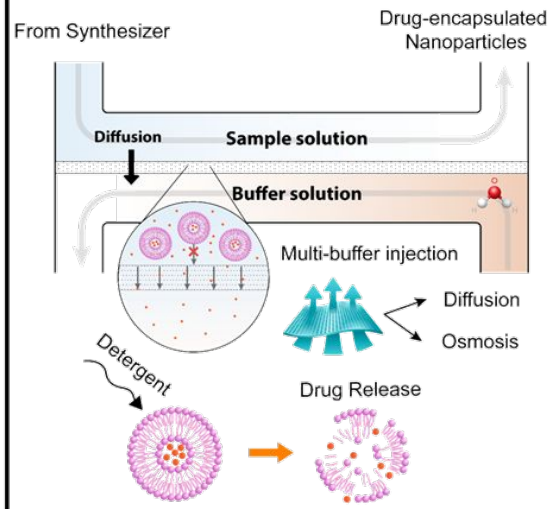
- Sample RT : 10 ~ 25 min
- PBS stirring RPM : 300
- **PBS circulation : 2.5 L/min**

Module 2: Purification Module

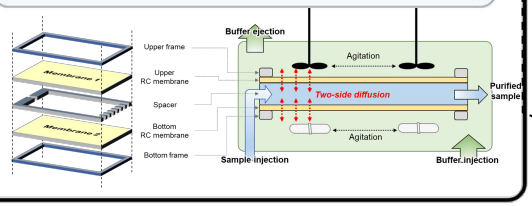
2

Purification

Small-scale Flow Dialysis Module

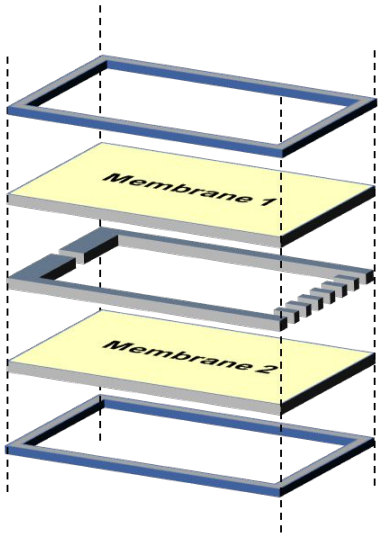


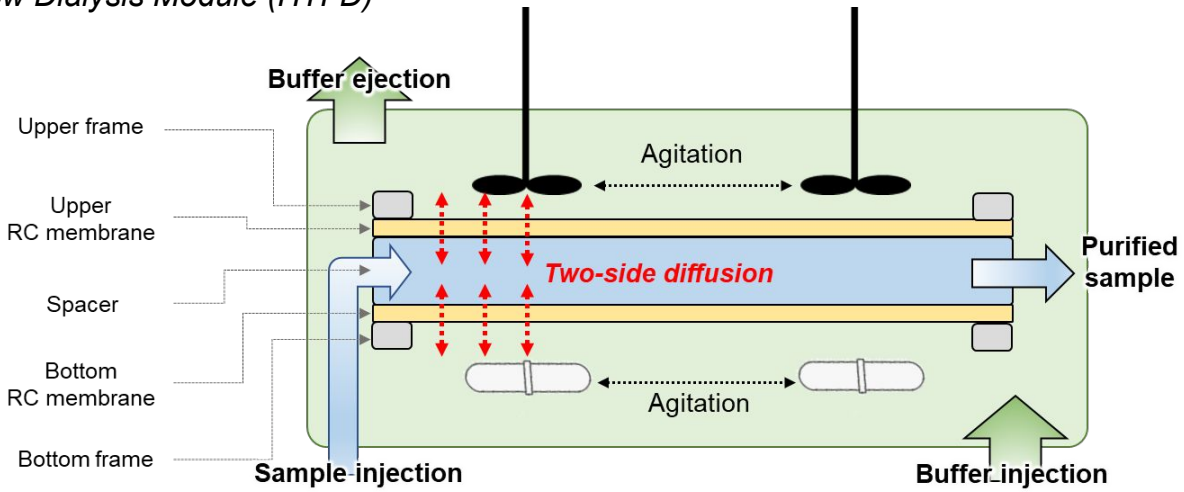
Scaled-up Flow Dialysis Module



2 Large Scale Flow Dialysis Module For Large Scale Production

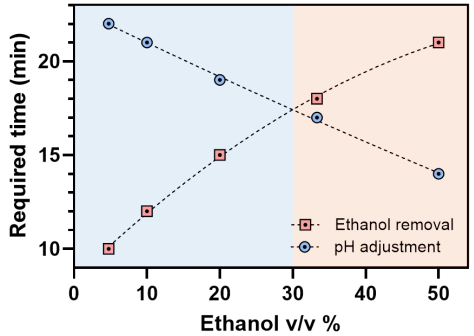
High-Throughput Flow Dialysis Module (HTFD)





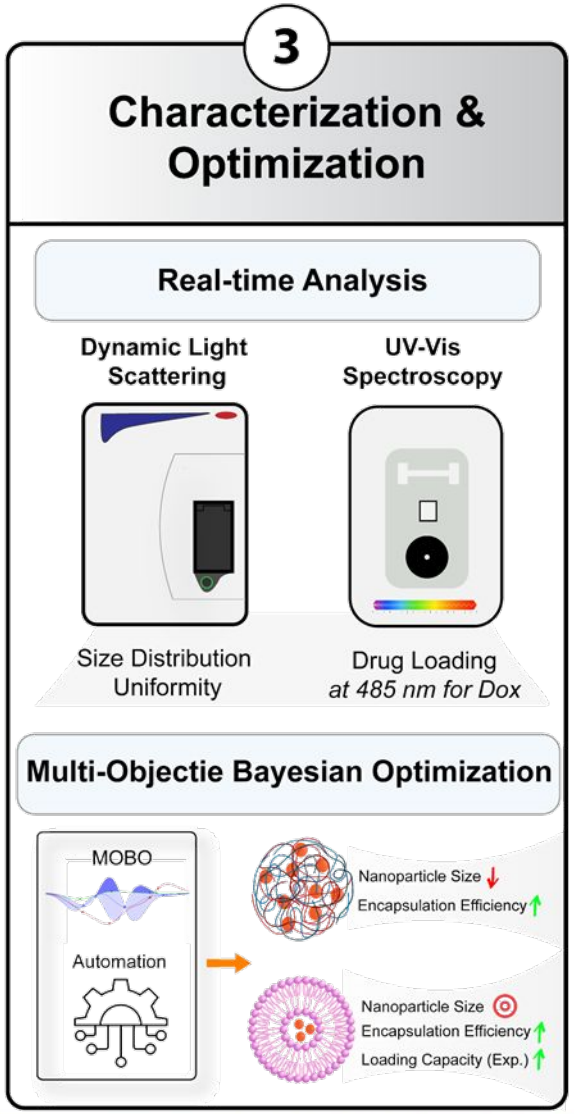
Sample With Specific Volume %
Of Ethanol (Antisolvent)

Calibration Curve for Automatic Retention Time Selection



Retention Time Required for Complete removal of the Ethanol from Sample

Module 3: Characterization and Optimization Module

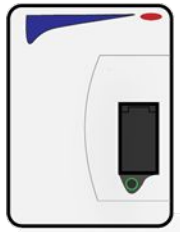


Module 3: Characterization and Optimization Module

3 Characterization & Optimization

Real-time Analysis

Dynamic Light Scattering

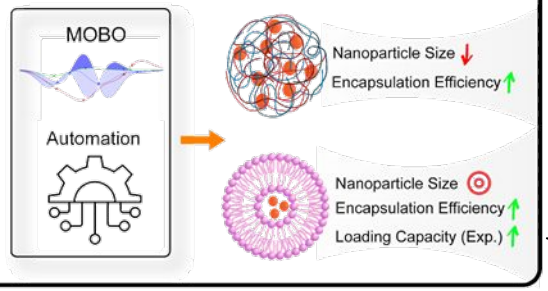


Size Distribution Uniformity

UV-Vis Spectroscopy

Drug Loading *at 485 nm for Dox*

Multi-Objectie Bayesian Optimization



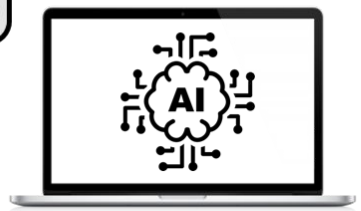
Global Black-Box Optimization Algorithm

Objectives

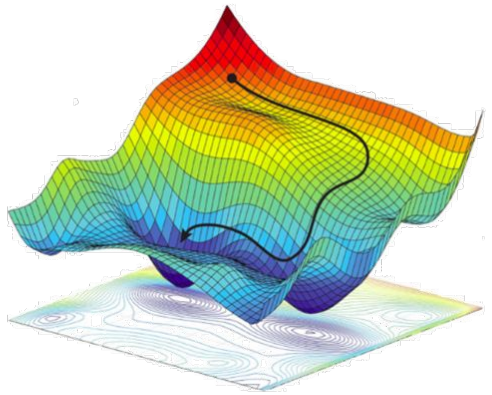
Size (Targeting)
Encapsulation Efficiency
Loading Capacity (Approx.)

Parameters

Total Flowrate
Flowrate Ratio
C_{dox}, An. Lipid %, P/C



Multi-Objective Bayesian Optimization



Decision making and controlling unit

- Using scripted sequences for repeating the same process and controlling the hardware
- Use of multi-objective optimization for optimizing the whole process

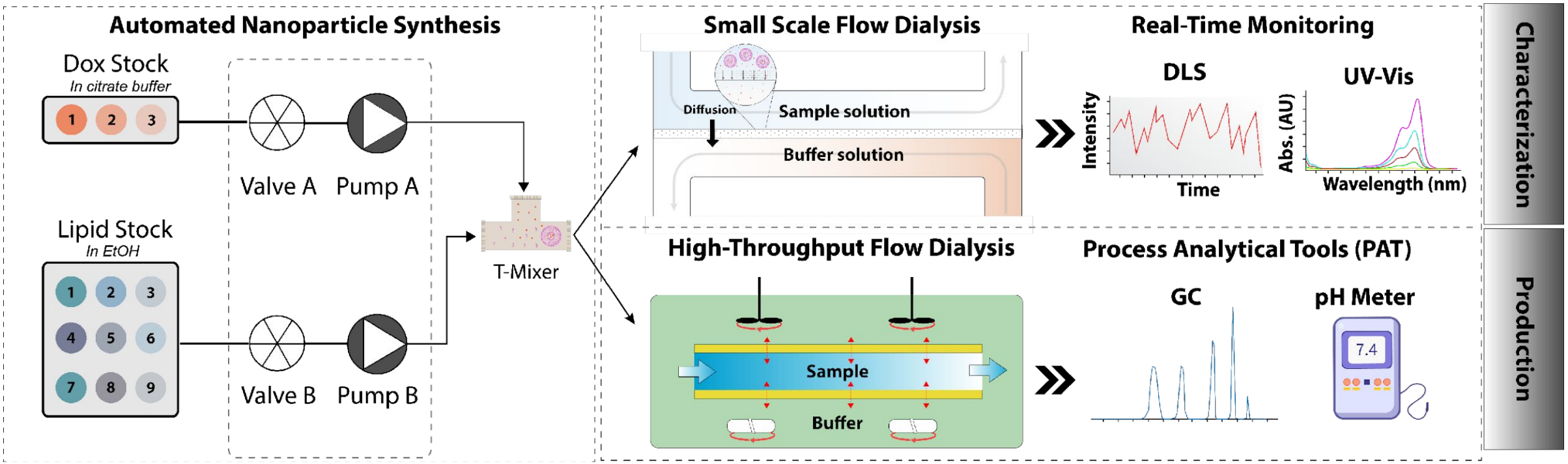
Objectives

1 **Size Targeting**
$min(|Size - target|)$
70, 140, 350 nm

2 **Encaps. Effic.**
$max(EE)$

3 **E. Loading Capacity**
ELC= $\frac{loaded\ drug}{Consumed\ Lipid}$

Integrated Platform



Automated Nanoparticle Synthesis Module

Total Flowrate | POPG | C_{Dox}
Flowrate Ratio | POPC/Cholestrol

Parameters

Purification Module

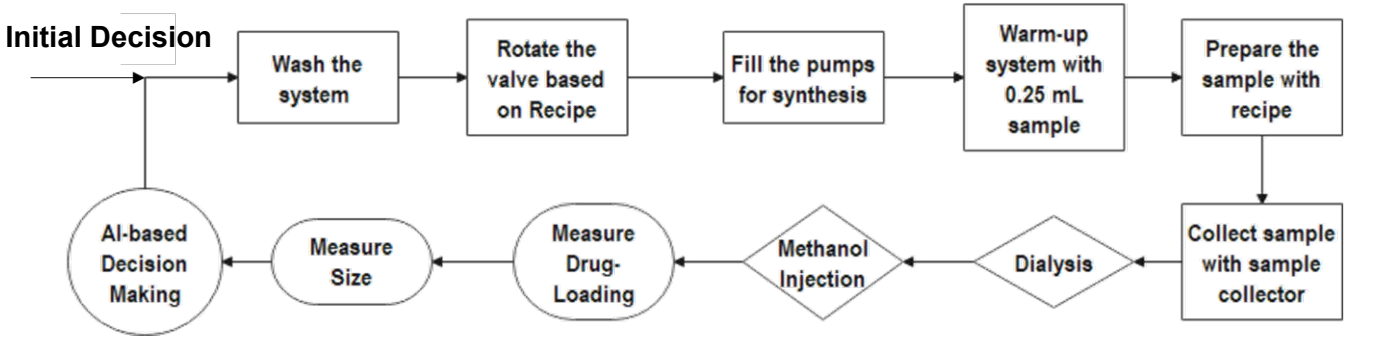
AI-Based Decision Policy

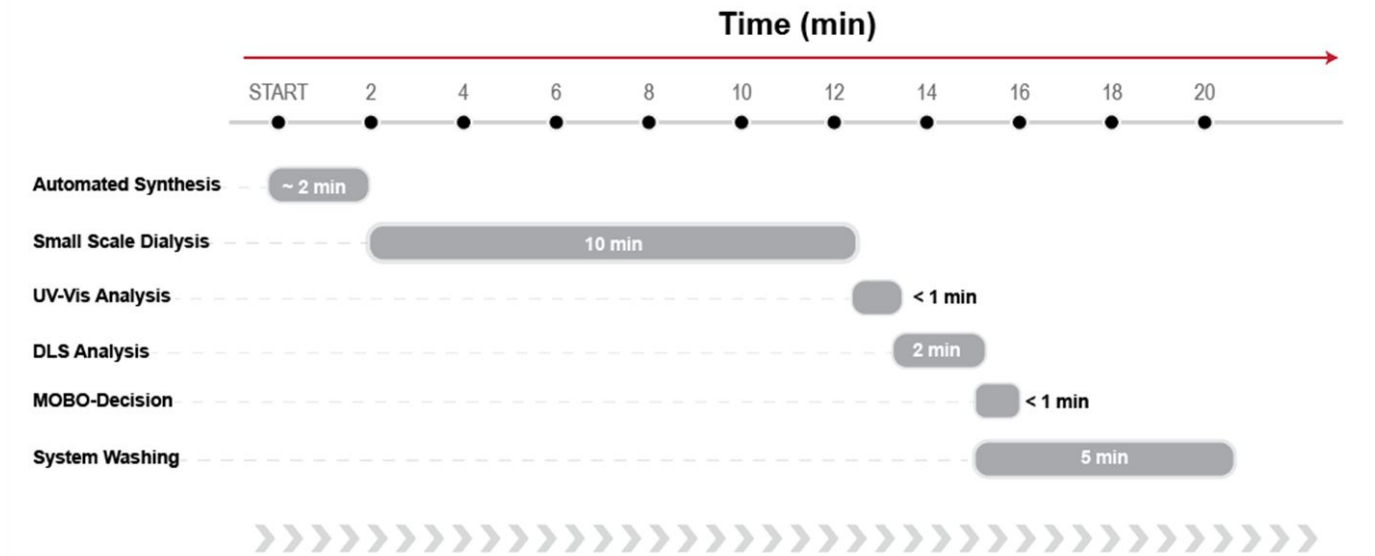
Characterization Module

Nanoparticle Size | Drug Loading
Ethanol Removal | pH Adjustment

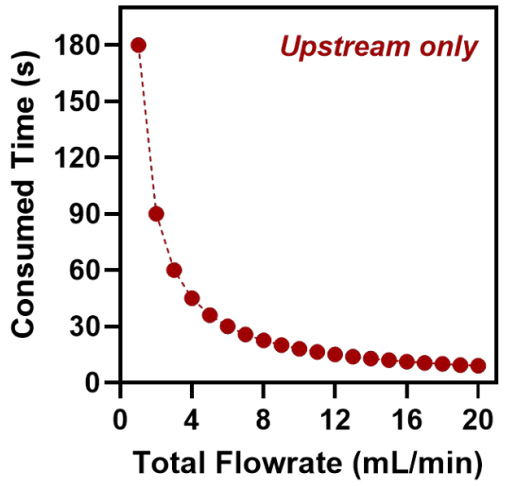
Objectives

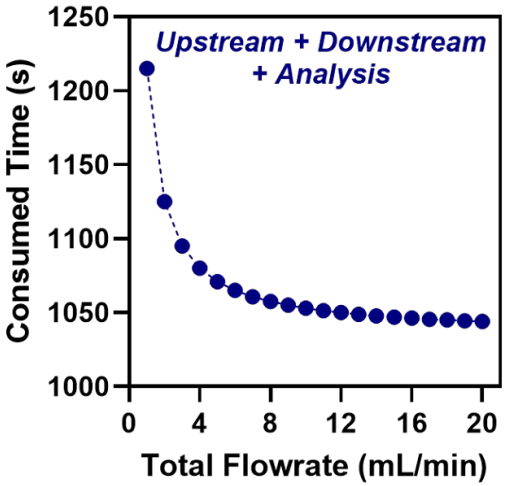
Integrated Platform: Time and Sample Efficiency



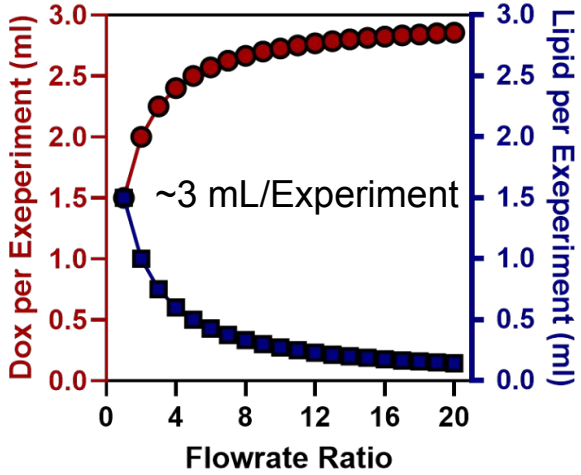


Time Consumption for Upstream and Overall System





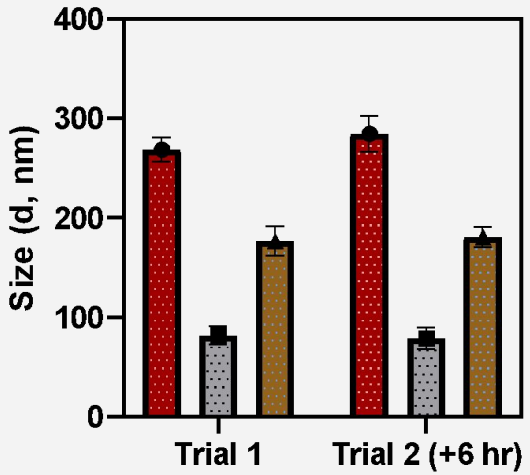
Sample Efficiency

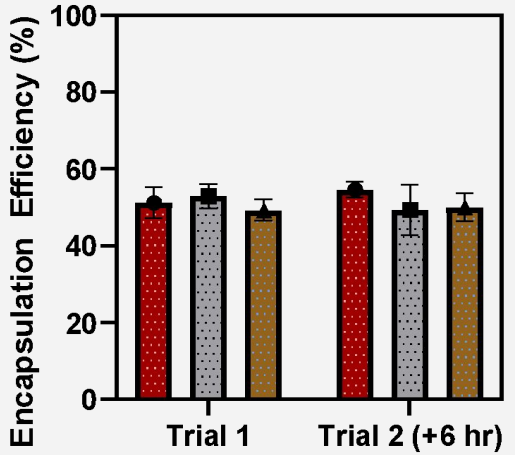


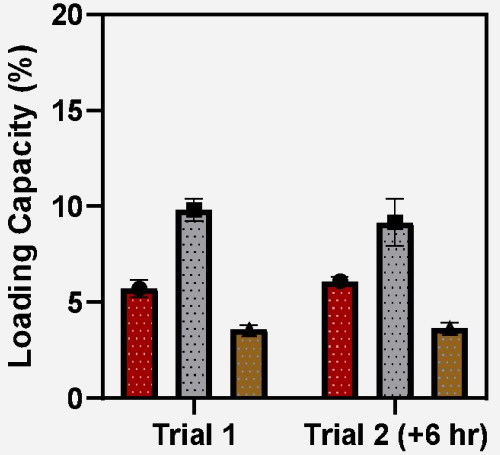
Performance Evaluation: Liposome

Robustness

- **Trial 1** and **Trial 2** with three different samples and **6 hr** of time gap
- **Size, EE, and ELC** measured for each sample in Trial 1 and Trial 2
- Evaluation of how **robust** system can perform over a long system **pause**

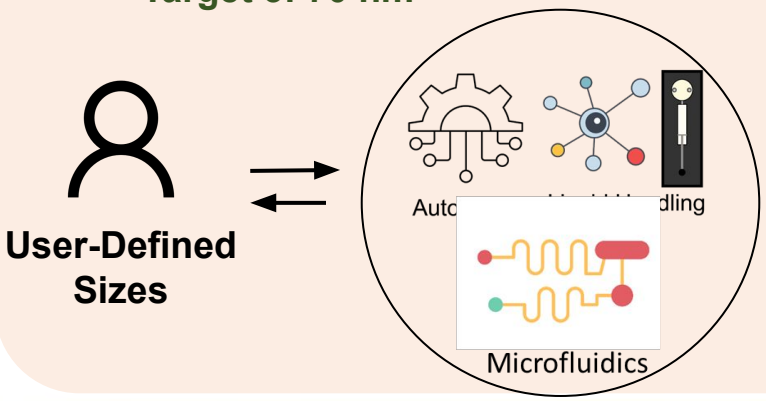


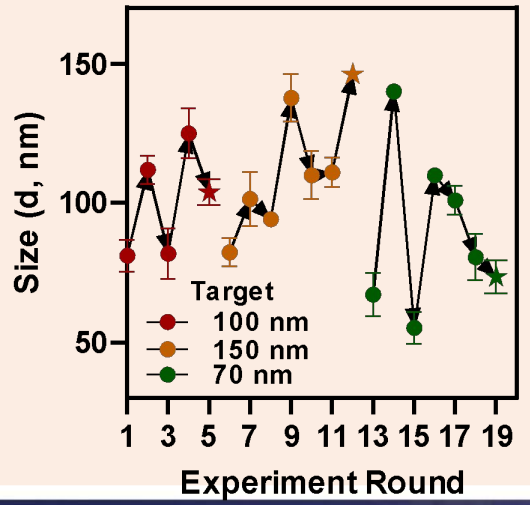


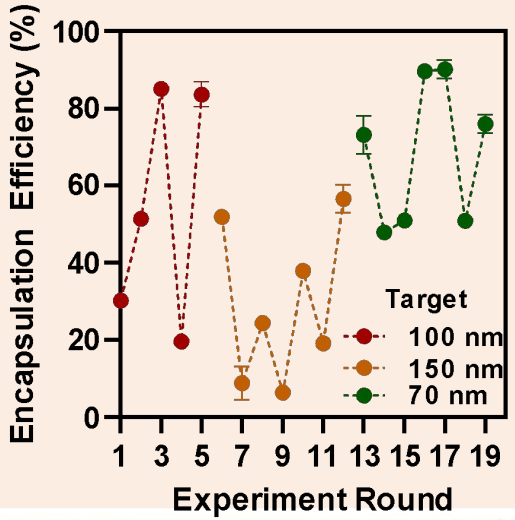


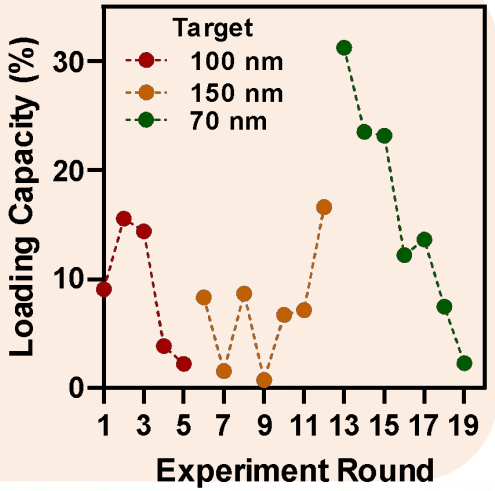
AI-Base Autonomous Experimentation: Size Targeting

- **Target 1: 100 nm**, **Target 2: 150 nm**
  Target 3: 70 nm









Performance Evaluation: Polymeric Nanoparticles (PEG:PLGA)