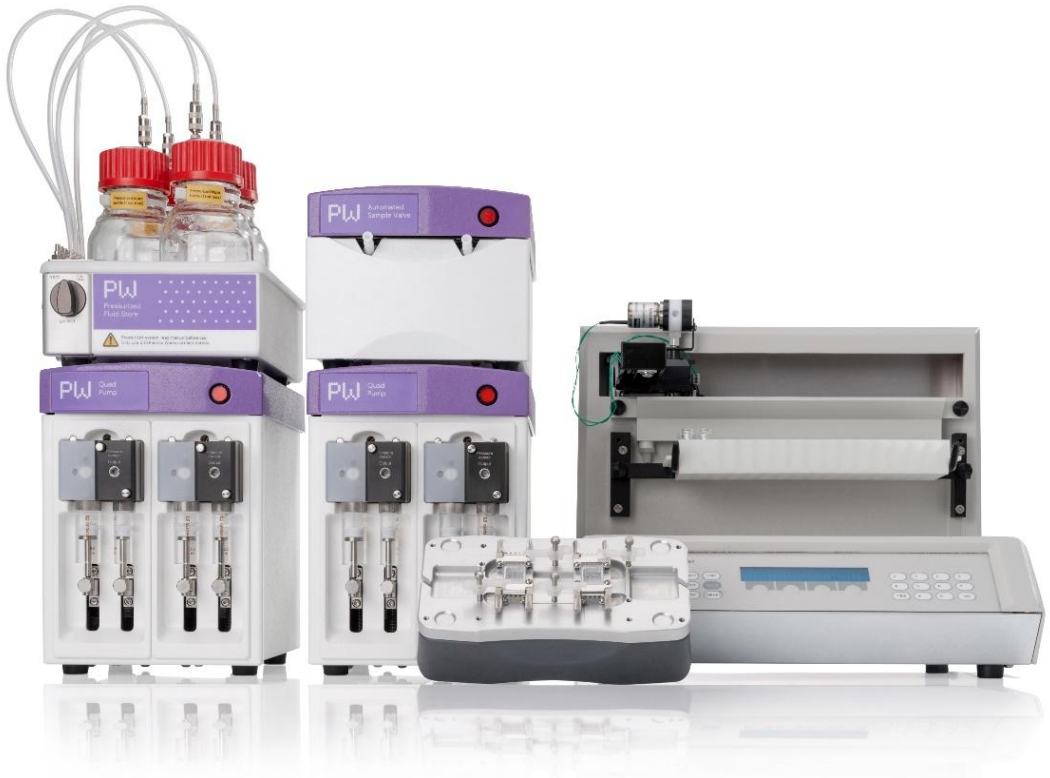


Particle Works

Microfluidics for Automated LNP screening and Scale Up

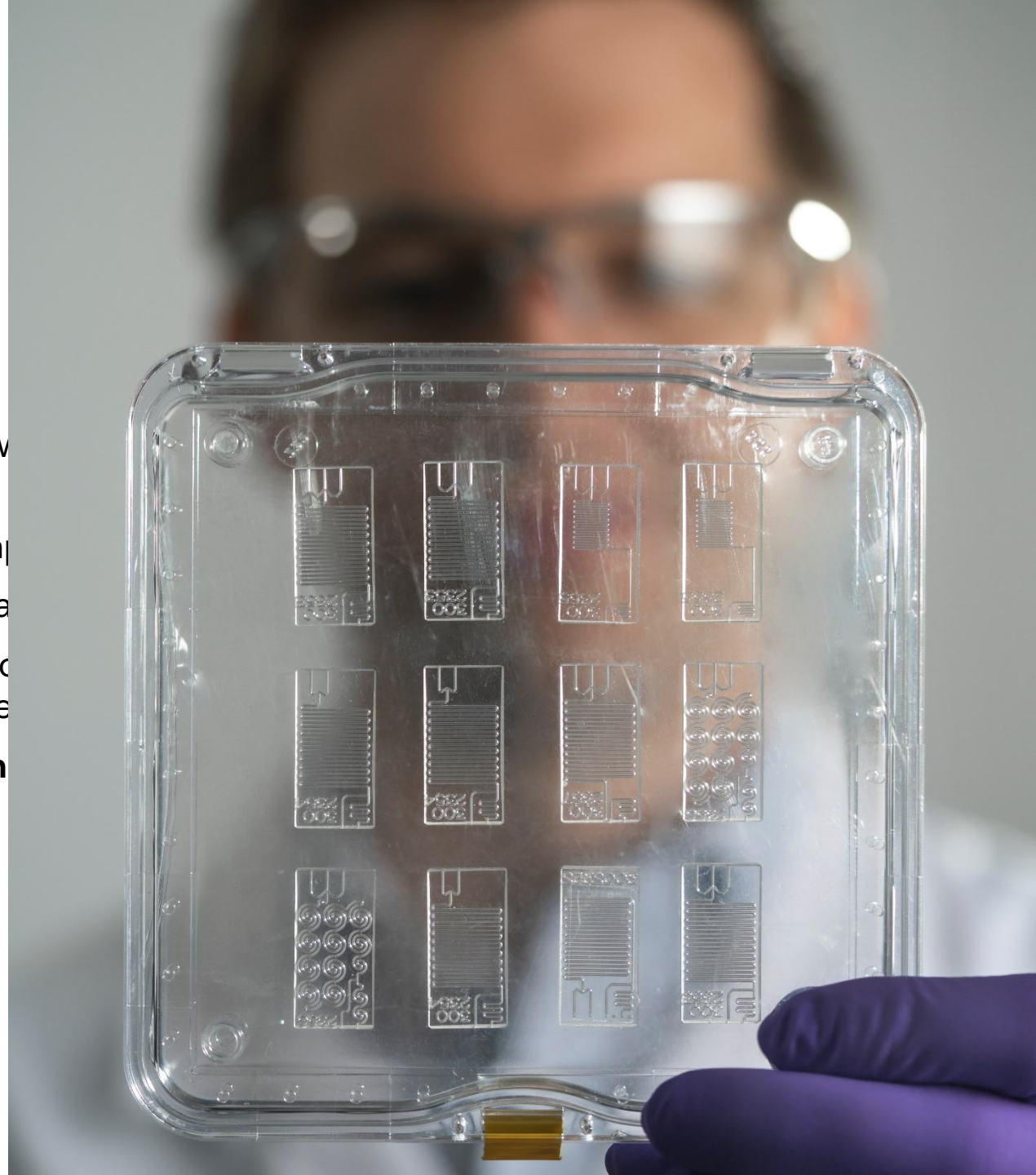
CRS Annual Meeting & Expo Montreal July 11 – 15, 2022 • Presenter
Tech Session 4: New Developments in Characterization and Process Scale-up (C&DP 2)

Richard Gray
Business Development Director, Blacktrace/ Particle Works

Particle Works

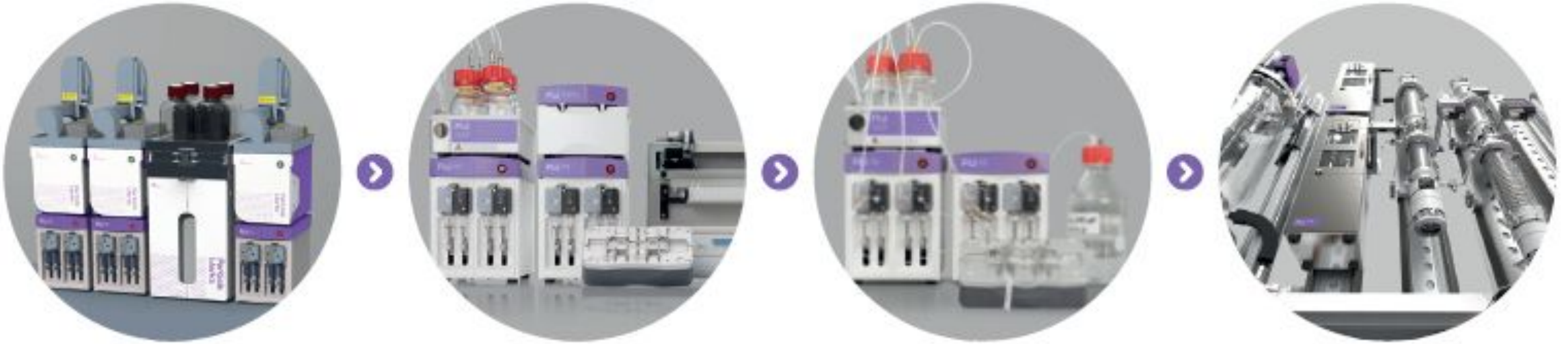
a new brand rooted in Blacktrace Microfluidic Technology

- Dolomite has a strong heritage in particle engineering, w
  innovative microfluidic devices and chip fabrication.
- We design and fabricate all our microfluidic chips, pump
- All our chips are made from glass for reusability, compa
- Our chips, combined with our innovative platforms, revo
  lipid nanoparticles (LNPs), polymer-based nanoparticle
- **We've created Particle Works to focus our efforts on**
  up



Providing an End-to-End Solution

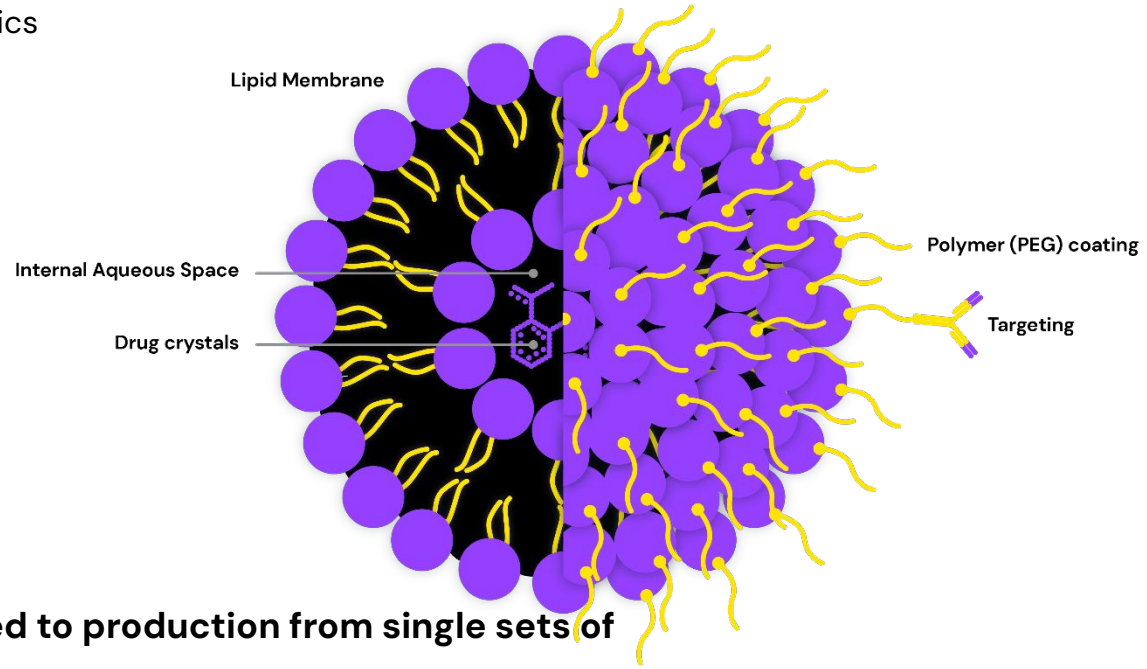
- The Particle Works range of systems provide a complete solution from screening to large scale production:



Background

- **Nanoparticles are an excellent delivery method for vaccines, drugs, gene therapies and other APIs**
  - Liposomes and Lipid nanoparticles (LNPs) are cell-like structures which give good bioavailability
  - Can encapsulate both hydrophobic and hydrophilic APIs or markers
  - Polymer-based nanpparticles offer excellent controlled release characteristics

- **Current LNP production methods have significant drawbacks**
  - Particle size is not consistent
  - Encapsulation efficiency can be poor
  - Minimum sample size is typically large
  - Poor scalability and reproducibility



- **Microfluidics offers an excellent way to generate LNP**
  - Excellent particle size monodispersity and encapsulation efficiency
  - Highly scalable via numbering up
  - Preserve particle integrity and avoid damage caused my mechanical forces

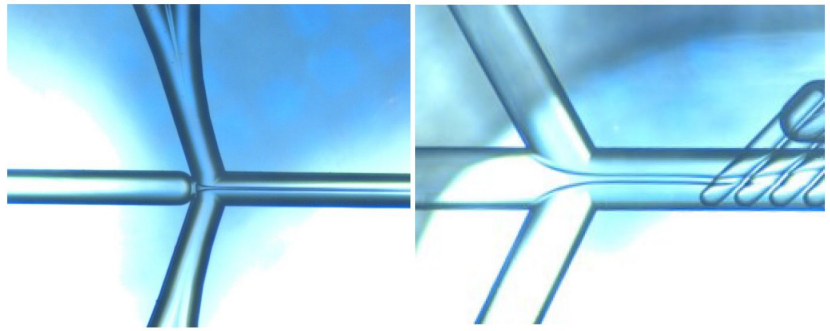
- **BUT current traditional and microfluidic approaches are best suited to production from single sets of reagents**
  - Hard to vary production protocol quickly and automatically (flow rates, temperatures, etc)
  - Hard to generate test samples from a range of APIs and/or Lipids/ Polymers

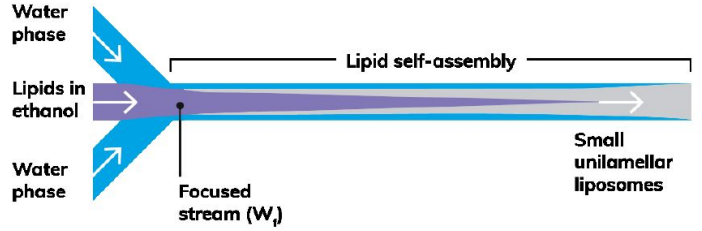
- **There is a clear and urgent need for methods for automated, effective and efficient production of LNPs for screening, optimization and manufacturing**

Microfluidic LNP Generation

- LNP are generated at the interface of two flows:
  - Lipid dissolved in Ethanol
  - Payload dissolved in an aqueous buffer

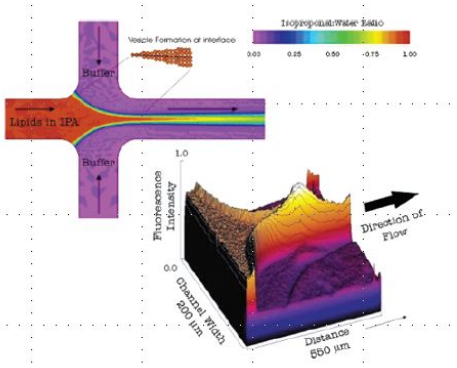
- Size is typically a function of the Flow Rate Ratio between the fluids
- PDI, Encapsulation Efficiency and other parameters can be optimized by flow conditions and precursor selection
- Automation of this process allows users to accelerate screening and optimize performance



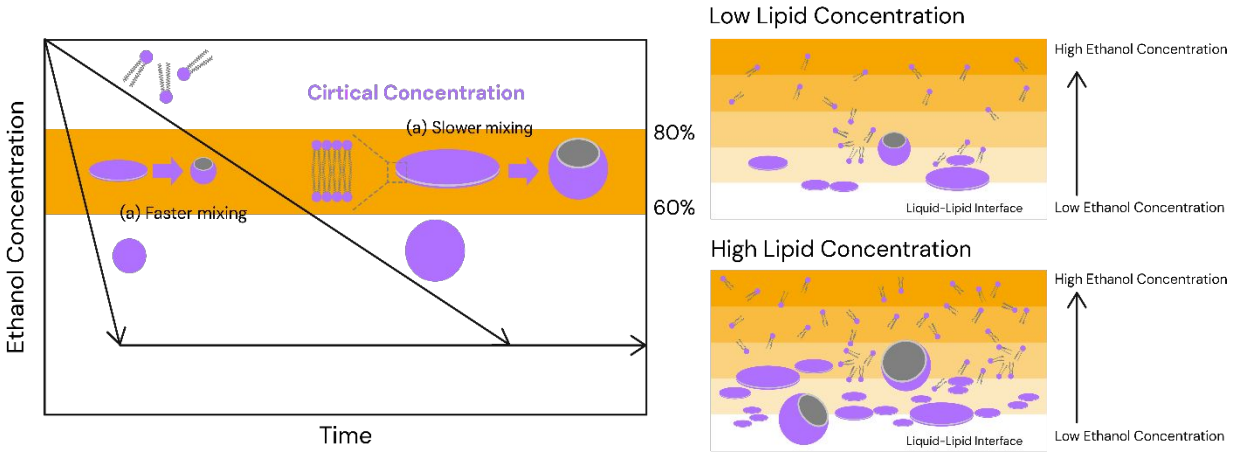


TFR = total flow rate

FRR = $\frac{\text{water phase flow rate}}{\text{ethanol flow rate}}$



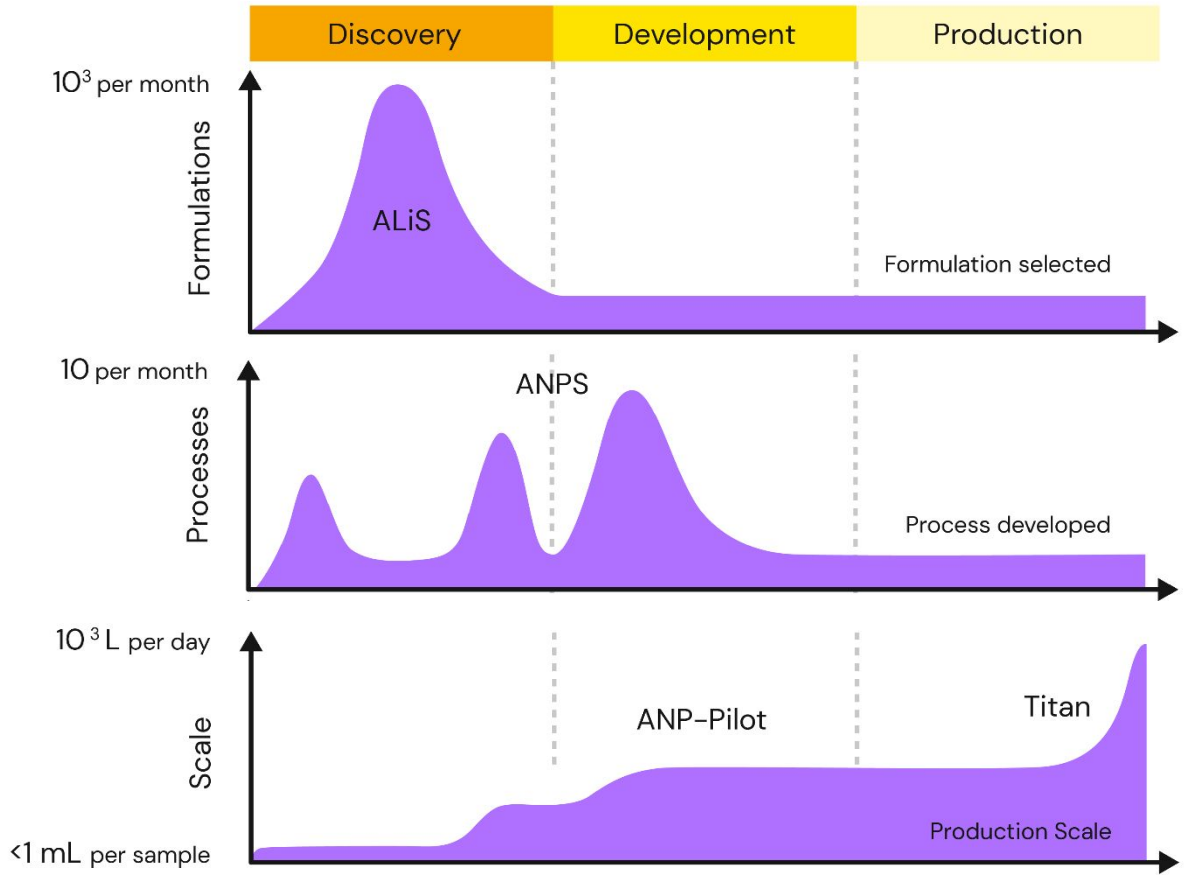
J. Am. Chem. Soc. 2004, 126, 9, 2674–2675



https://doi.org/10.1371/journal.pone.0187962.g007

Covering the full development pathway

- As Covid-19 has shown, rapid product development requires accelerated steps and parallel task execution
- Scalable automation is a key solution to address both requirements – the same core technology can cover the whole range
- Microfluidics is key to achieving automation and scalability
- Particle Works has developed our 4 core systems to cover the range of tasks required



Automation via microfluidic “packet” technology

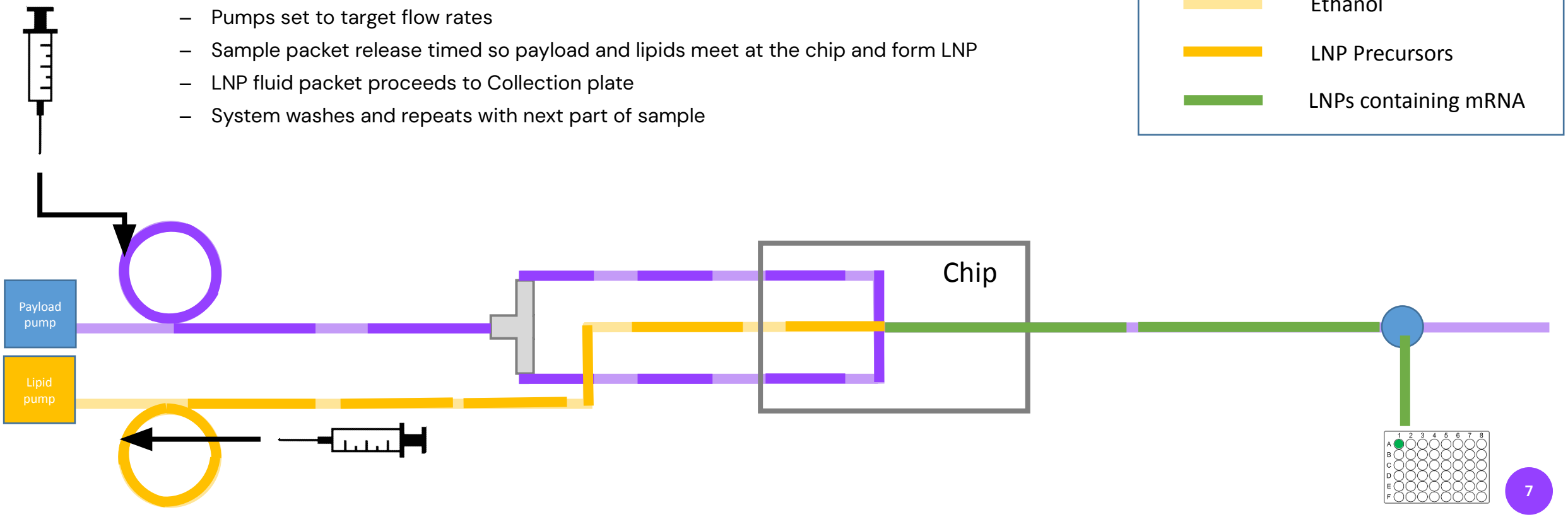
Our automated systems use driving fluids to move small volumes of reagent through the flow path to the microfluidic chip

ANP system experiment sequence:

- Samples manually injected into loops
- Pumps set to target flow rates
- Sample packet release timed so payload and lipids meet at the chip and form LNP
- LNP fluid packet proceeds to Collection plate
- System washes and repeats with next part of sample

Key

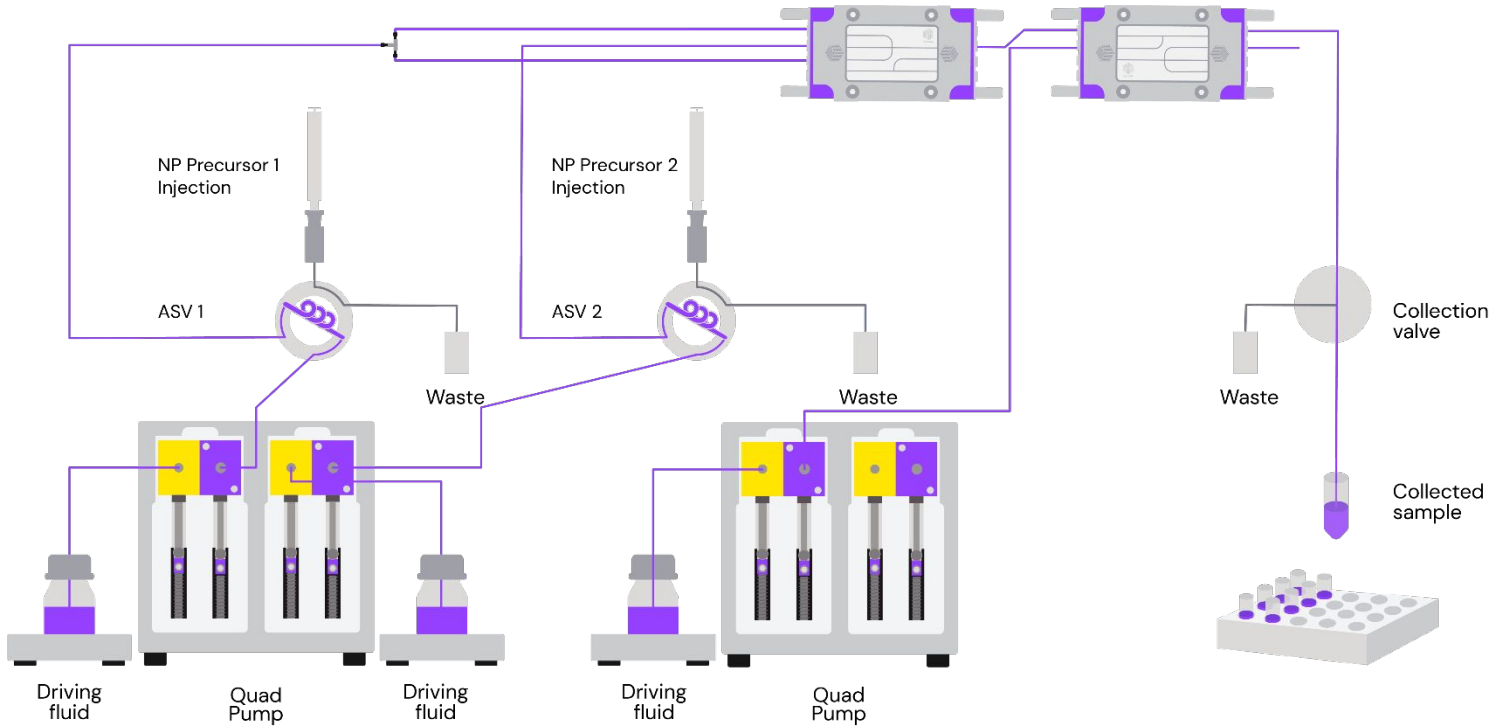
- Aqueous Buffer
- mRNA
- Ethanol
- LNP Precursors
- LNPs containing mRNA



The Automated Nanoparticle System (ANP System)

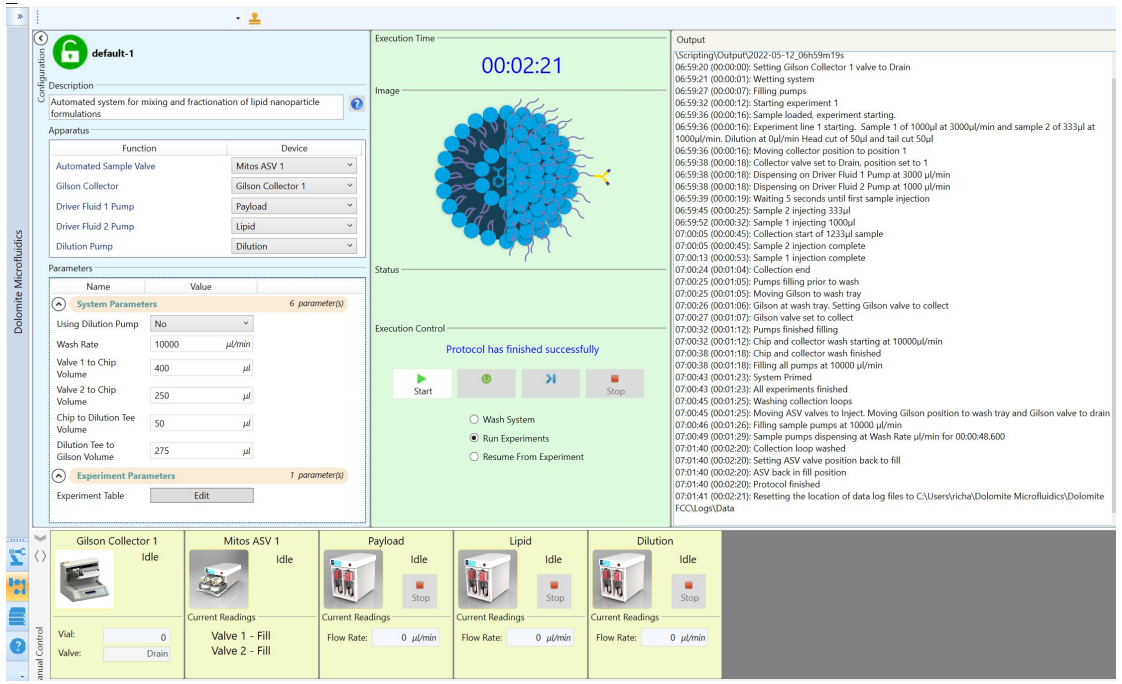
Maximize protocol development efficiency using microfluidics with walk away automation

ANP System : Setup and execute 10 experiments, with samples ready in 15 minutes



Software

- ALiS and ANP system software very similar
- The primary variable is an Experiment List, defining desired flow rates and sample volumes
- The List will also define the well location for the Lipid and Payload
- The Experiment List can be prepared off-line in Excel and then uploaded at run time



- The Protocol software allows easy setup and execution of protocols
- Protocols can be paused and resumed
  - Eg This is useful to allow a few samples to be run an analyzed ahead of completing the main run

Particle Works

Add Experiment

Edit/Delete Experiment

Export CSV Table

Sample 1 Loop Volume (mL)	5	**Sample 1 Loop - Volume Required (µL)**	**Sample 2 Loop - Volume Required (µL)**
Sample 2 Loop Volume (mL)	5	1700	500
Pump Syringes	Red syringe (2.5 mL/10 mL)		

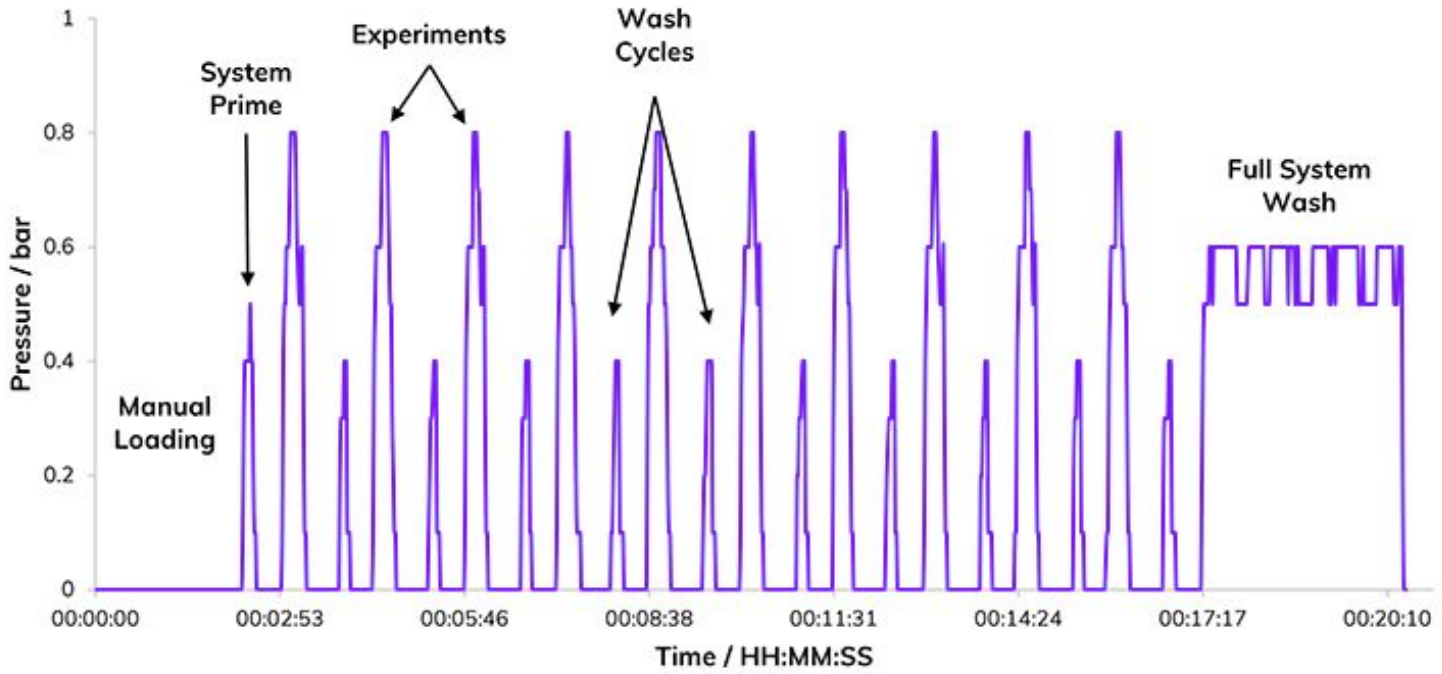
Experiment Number	Sample1 - Pump Flow Rate (µL/min)	Sample2 - Pump Flow Rate (µL/min)	Dilution Pump Flow Rate (µL/min)	Total Flow Rate (µL/min)	Sample 1 Volume (µL)	Sample 2 Volume (µL)	Dilution Volume (µL)	Fluid Packet Volume (µL)	Head Cut (µL)	Tail C (µL
1	4000	1000	0	5000	800	200	0.0	1000	50	50
2	3000	1000	0	4000	900	300	0.0	1200	50	50

Key benefits of Automated Nanoparticle System

Particle Size	Particles ranging from 40 nm to 800 nm.
Particle monodispersity	Extremely monodisperse with PDI typically ~0.1
Sample Volume	200 µl to continuous production.
Speed	10 samples collected within 15 minutes.
Automation	The system software allows easy creation of protocols to automatically process many different experiments from a single sample loading which will be run without human interference.
Flexibility	The modular system can be paired with a range of glass chips and sample loops tailored to your specific application. Can be used to manufacture a wide range of nanoparticles from LNPs to polymers.
Scalability	From screening to production using same chip allowing production volumes from µl to liters per day.
Budget-friendly	Excellent encapsulation efficiency coupled with very low sample use and reusable glass microfluidic chips provide a very cost effective and environmentally responsible solution.

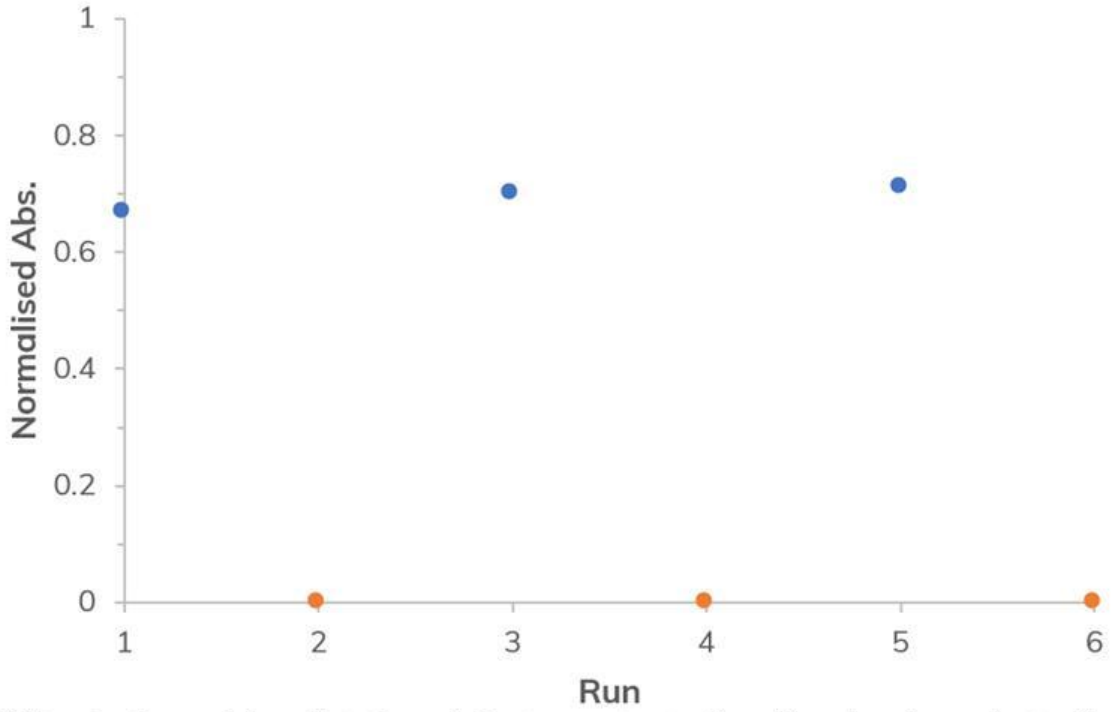
Automated Process optimization

Setup and execute 10 experiments, with samples ready in 15 minutes



Automated System wash ensures no cross contamination between samples

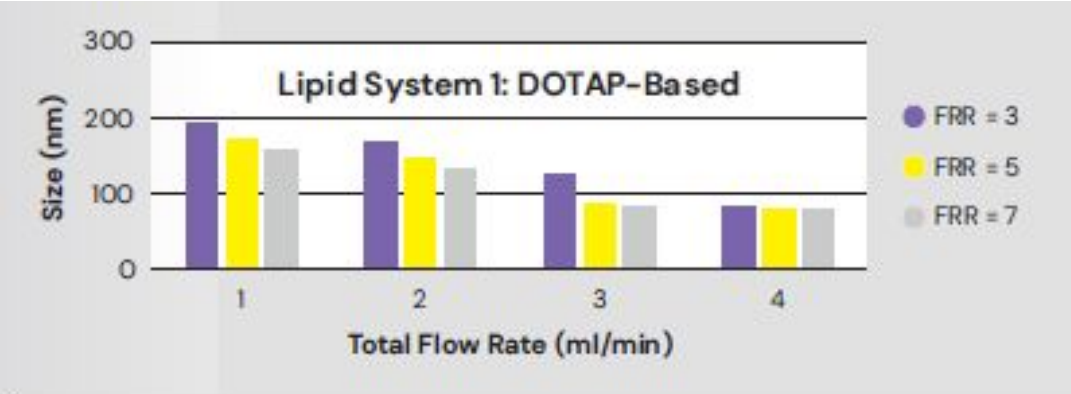
- All wetted parts of the system downstream of the sample loops are washed between samples
- To test for cross contamination, we spike alternate samples in the sample loop with fluorescein and then measure collected fluorescein in the collection vial via UV-Vis
- The graph shows no measurable cross contamination



UV/vis absorbance of the collected sample for six experiments. The odd numbered runs, depicted by blue symbols indicate fluorescein experiments and the even numbered runs, depicted by orange symbols indicate experiments carried out using water.

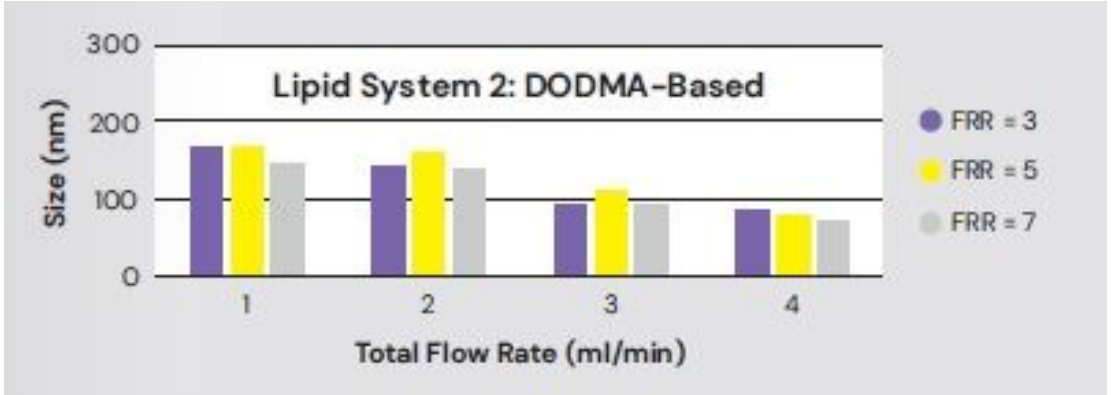
Controlling Size & Encapsulation Efficiency

Particle size and EE can be controlled by optimizing total flow rate (TFR) and flow rate ratio (FRR) whilst also minimizing PDI



Encapsulation Efficiency (%)

		TFR (ml/min)			
		1	2	3	4
FRR	3	98	98	98	97
	5	97	97	97	97
	7	97	97	96	96



Encapsulation Efficiency (%)

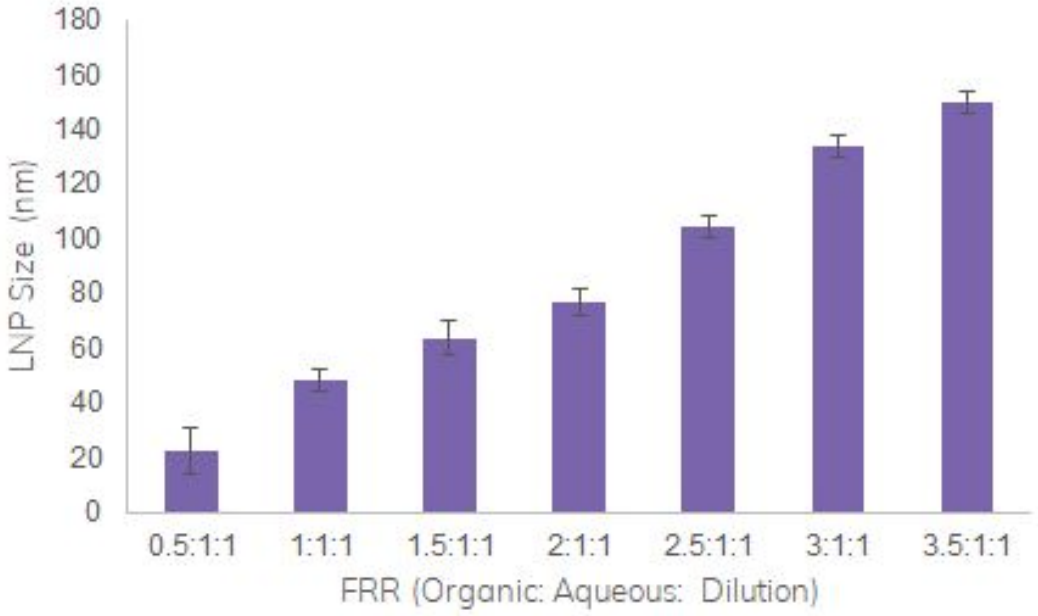
		TFR (ml/min)			
		1	2	3	4
FRR	3	85	90	94	94
	5	86	89	95	97
	7	91	93	94	94

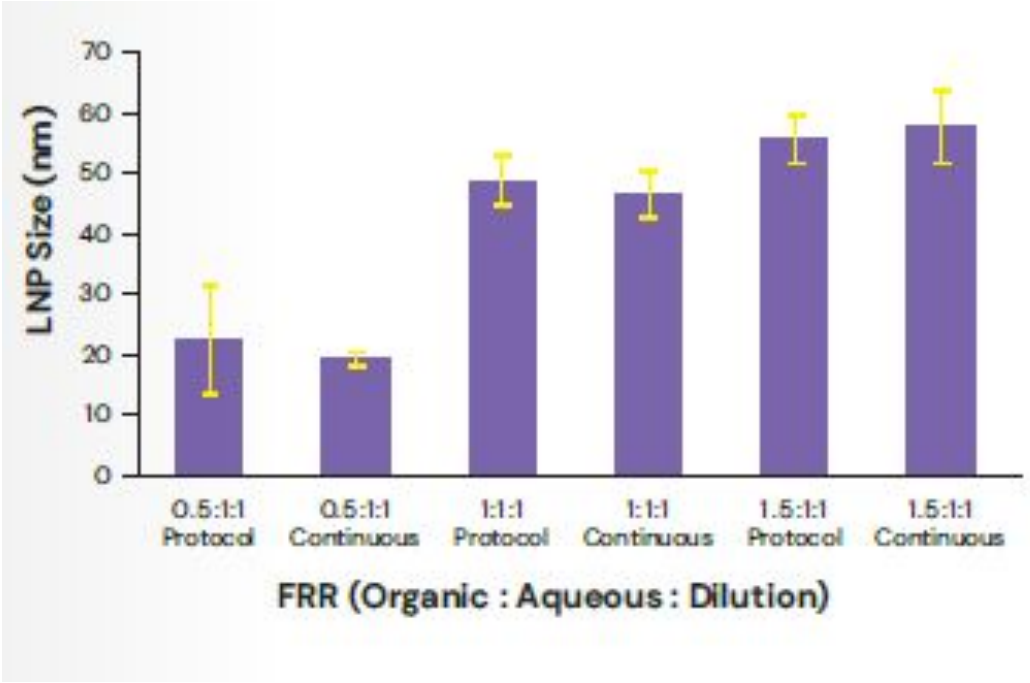
Data courtesy of Phosphorex

Optimize & Scale up

Our flexible and powerful software provides you with total control of key parameters.

- Freedom to explore any FRR/TFR/Dilution to identify optimal production criteria for any given microfluidic chip type.
- Scale up from µl to liters with the same system and same chip in continuous mode





mRNA Testing

- Moderna lipid formulation

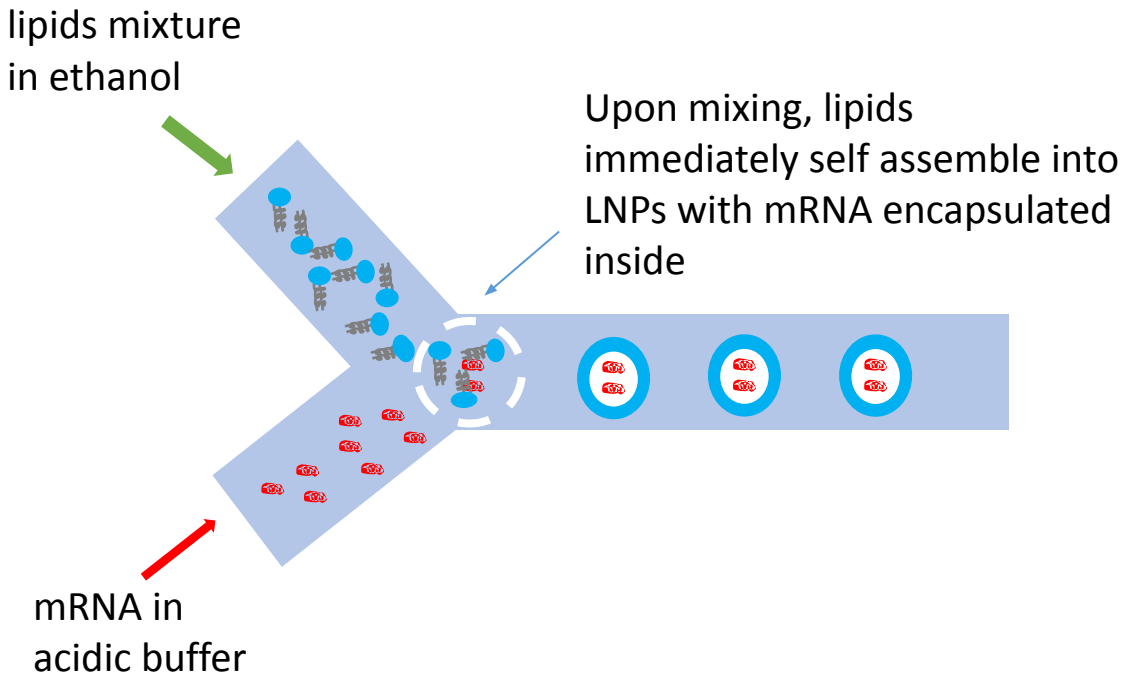
  SM-102: DSPC: Cholesterol: PEG-2000

  50% 10% 38.5% 1.5%

  Lipids dissolved in ethanol (10mM, 6.2mg/ml)
- Luciferase mRNA
  - In sodium acetate buffer (pH 4.0, 50mM)

- 3:1 Flow Rate Ratio (FRR) of Aqueous (mRNA) to Organic (lipid)
- 190/190um droplet junction chip
- Varied Total Flow Rate (TFR) and N/P ratio

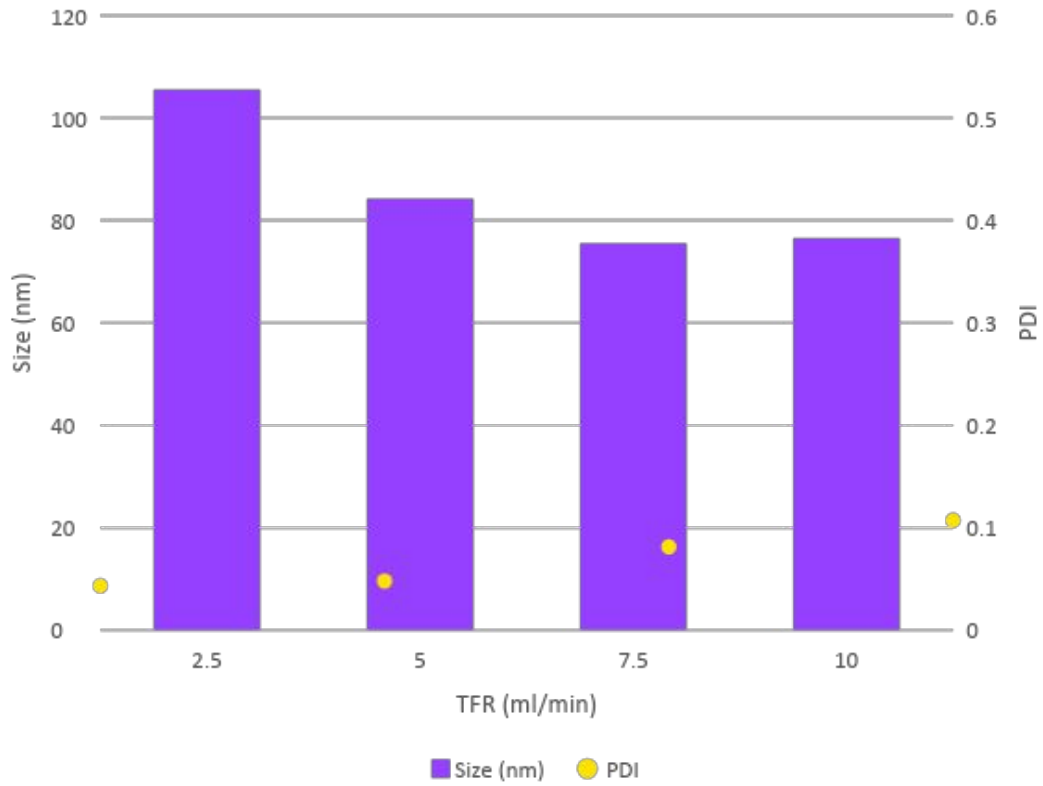
- Achieved PDIs of between **0.05-0.17**
- Achieve Encapsulation efficiency **>95%**



Varying Total Flow Rate (TFR)

- The TFR was run at 2.5ml/min, 5ml/min, 7.5ml/min and 10 ml/min.
- A higher TFR leads to smaller LNP size, due to more rapid mixing and faster self-assembly of mRNA-LNPs
- PDI of 0.1 or below achieved for whole range

Data shown is at N/P ratio of 8 (mRNA concentration 0.067 mg/ml) and size was determined by DLS pre-dialysis

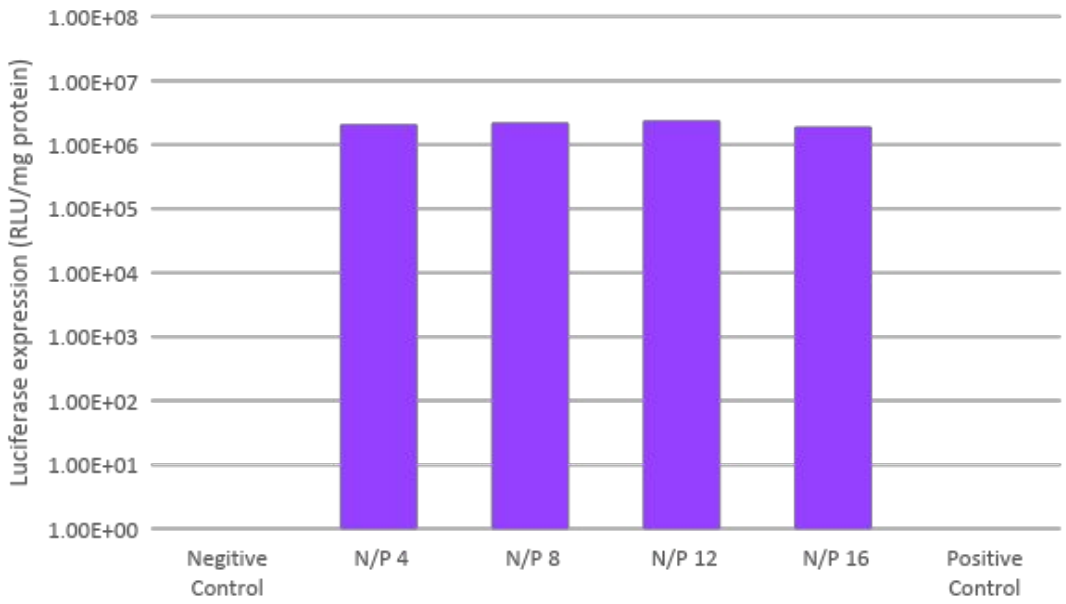


Luciferase expression in HEK293 cells transfected by mRNA-LNPs

- HEK293 cells were transfected with the mRNA-LNPs which contained mRNA encoding the Firefly Luciferase gene
- Luciferase expression of the cells was measured by bioluminescence, compared to a negative control (un-transfected cells) and a positive control (cells transfected with the commercial available reagent Lipofectamine 2000 (L2K))

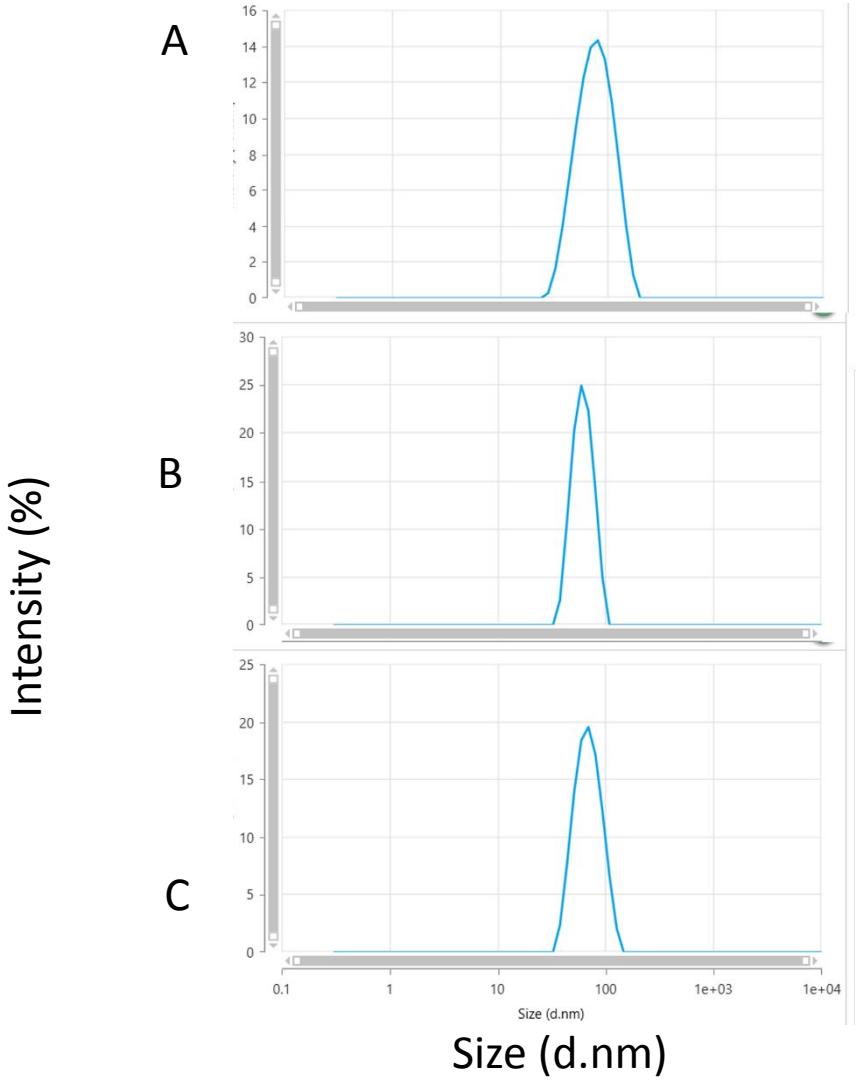
mRNA-LNPs were prepared at TFR of 5 ml/min

The equivalent of 300ng of mRNA was transfected per well, and each condition transfected in triplicate



	Size (nm)		PDI	
	Pre-dialysis	Post-dialysis	Pre-dialysis	Post-dialysis
N/P 4	86.32	105.6	0.09	0.09
N/P 8	84.21	83.56	0.05	0.12
N/P 12	113.3	131	0.14	0.16
N/P 16	113.4	112.5	0.14	0.17

Further tests on Size control and Encapsulation Efficiency



	After dialysis	mRNA EE(%)
Sample A	84.0	95.56
Sample B	80.2	95.55
Sample C	79.6	96.14

Experimental details:

- 3 independent runs
- Moderna lipid formulation

  SM-102: DSPC: Cholesterol: PEG-2000

  50% 10% 38.5% 1.5%

  Lipids dissolved in ethanol (10mM, 6.2mg/ml)
- Luciferase mRNA in sodium acetate buffer (50mM, pH 4)
- Collected sample size 800ul
  - 1ml dispensed with 100ul head and 100ul tail cut
- 3:1 Flow Rate Ratio (FRR) of Aqueous (mRNA) to Organic (lipid)
  - No in-ine dilution
- 190 um junction chip
- Total Flow Rate (TFR) at 5ml/min and N/P ratio 8

Note: Samples are prepared by ANP system and analysed by DLS after dialysis. All samples show consistent size 81.3±2.4 nm in diameter. mRNA EE(%) was quantified using RiboGreen assay.

Automating High Throughput Screening

Requirement to screen many formulations and/or payload variations

For screening our customers told us they needed to be able to;

- Process up to 96 samples per run
- Produce 250 µl to 2 ml per sample for downstream analysis
- Pierce sealed well plates to avoid cross contamination/ evaporation
- Scale seamlessly to process & protocol optimisation

These features, and more are built into our Automated Library Synthesis (ALiS) systems which will be shipping in Q4 2022. Pre-ordering now available.

ALiS microfluidic “packet” technology

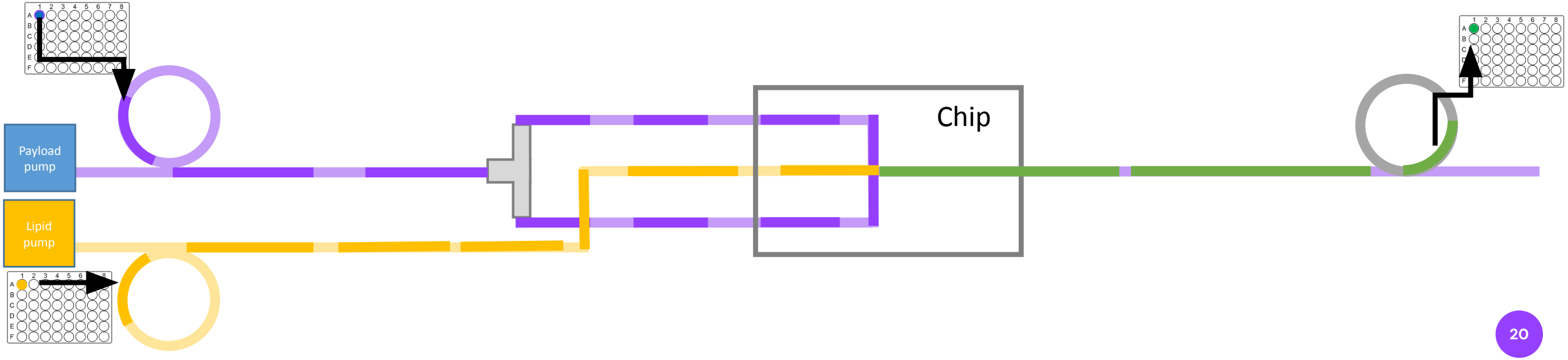
The ALiS automated system adds liquid handling units to allow 96 wellplates of different lipid and payload samples

ALiS Experiment sequence:

- Samples collected from wellplate into loops
- Pumps set to target flow rates
- Sample packet release timed so payload and lipids meet at the chip and form LNP
- LNP fluid packet proceeds to collection loop
- LNP packet transferred from loop to Collection plate
- System washes and repeats

Key

- Aqueous Buffer
- mRNA
- Ethanol
- LNP Precursors
- LNPs containing mRNA



ALiS Automated Library System

System Purpose

- Automated reaction of large numbers of different formulations loaded into well plates
- High throughput screening of payload candidates and LNP formulations in early stage development

System Specification

- Up to 96 different experiments varying lipids, payload, protocol
- Offline setup of protocols using Excel
- Reusable chips with automated washing to prevent cross-contamination
- 200uL to 2ml per sample

Key Benefits

- Small sample size minimises materials costs
- Sealed reagent and collection plates avoid evaporation or cross contamination
- High collection precision with optional heart cut
- Excellent monodispersity and encapsulation efficiency

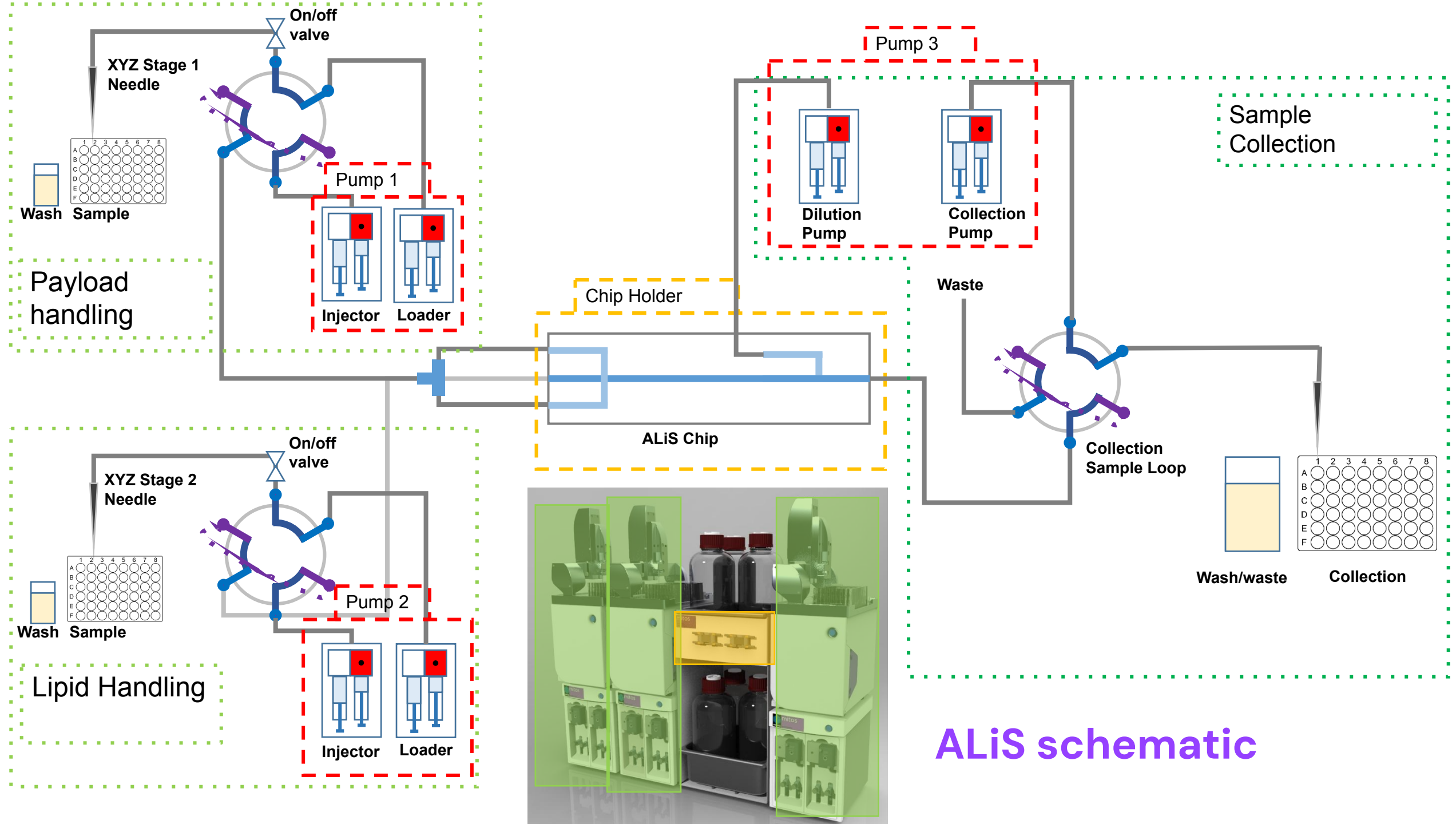
Status

- System validated at US biotech site 2020->
- Beta Systems now under test at Dolomite
- ALiS available for delivery Q4 2022

ALiS Module design

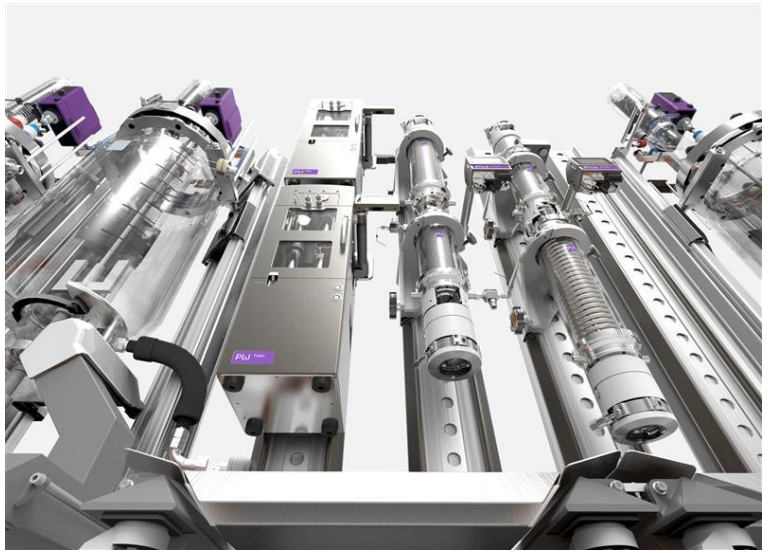
- ALiS is build from 3 identical fluidic modules, plus a central Fluid Store/ Chip holder
- This simplifies build and setup

- Each “stack” consists of a 2-channel pump, an automated sample loop, and a 96 well liquid handling unit


On/off valve
XYZ Stage 1 Needle
Wash
Sample
Pump 1
Injector
Loader
Payload handling
Pump 3
Dilution Pump
Collection Pump
Sample Collection
Chip Holder
ALiS Chip
Waste
Collection Sample Loop
Wash/waste
Collection
On/off valve
XYZ Stage 2 Needle
Wash
Sample
Pump 2
Injector
Loader
Lipid Handling
ALiS schematic

Automating GMP Manufacturing

- A wide range of manufacturing scale is required: from 1 to 1,000 liters per day
- GMP requires ground up planning and execution for validation processes, chip design, pumps, control systems, etc
- Particle Works has the core capability in each of these areas and is demonstrating performance with key users
- 2 Systems for use in GMP environments will be available depending on the scale required: ANP-Pilot and Titan



The ANP Pilot System – Overview

System Purpose

For users conducting final process development in a manufacturing environment, and for initial manufacture for clinical trials/low volume applications

System Specification

- Continuous sample manufacturing
- ~1 to 2 liter per hour scale
- Plan to be GMP compliant in the coming 12 months
  - Customers already using current units for manufacturing GMP products using post production sterile filtration

Key Benefits

- Direct translation from Discovery to Development
- Scalable by running systems in parallel

Titan System

Particle Works

System Purpose

– Particle manufacturing in a GMP environment for large scale applications

System Specification

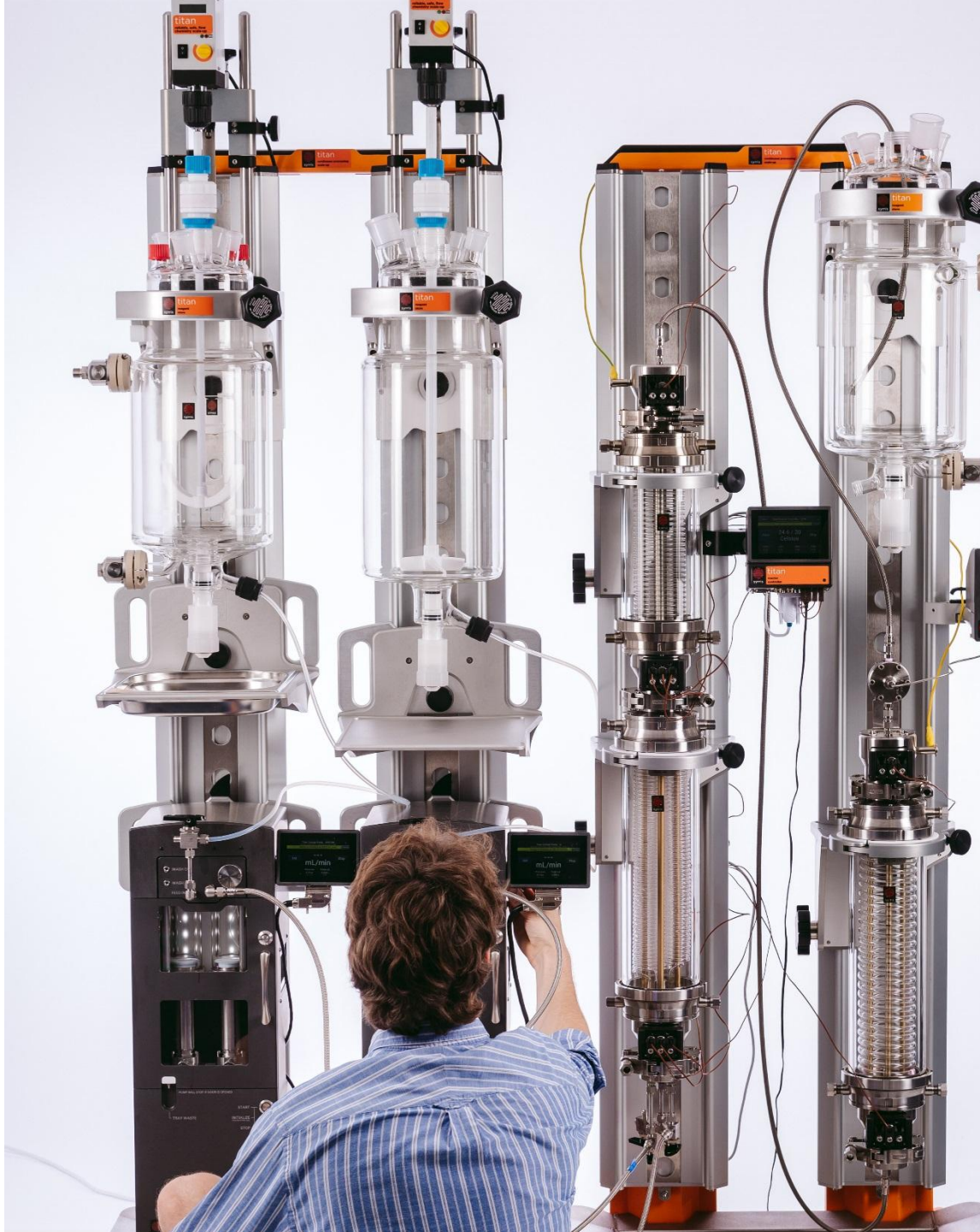
– Continuous manufacturing
– 1440L in 24h (~400 g of LNPs, 40M Vaccine Doses)

Key Benefits

- Modular structure enables process flexibility
- Scalable by running systems in parallel

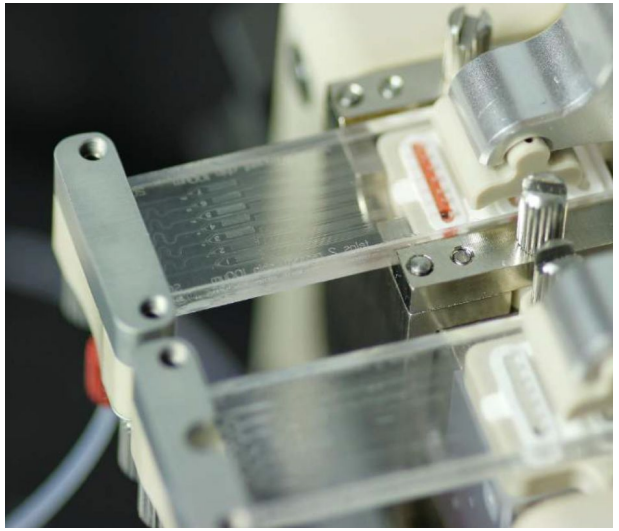
Status

- Modules largely developed
- Expect commercial release in 2023



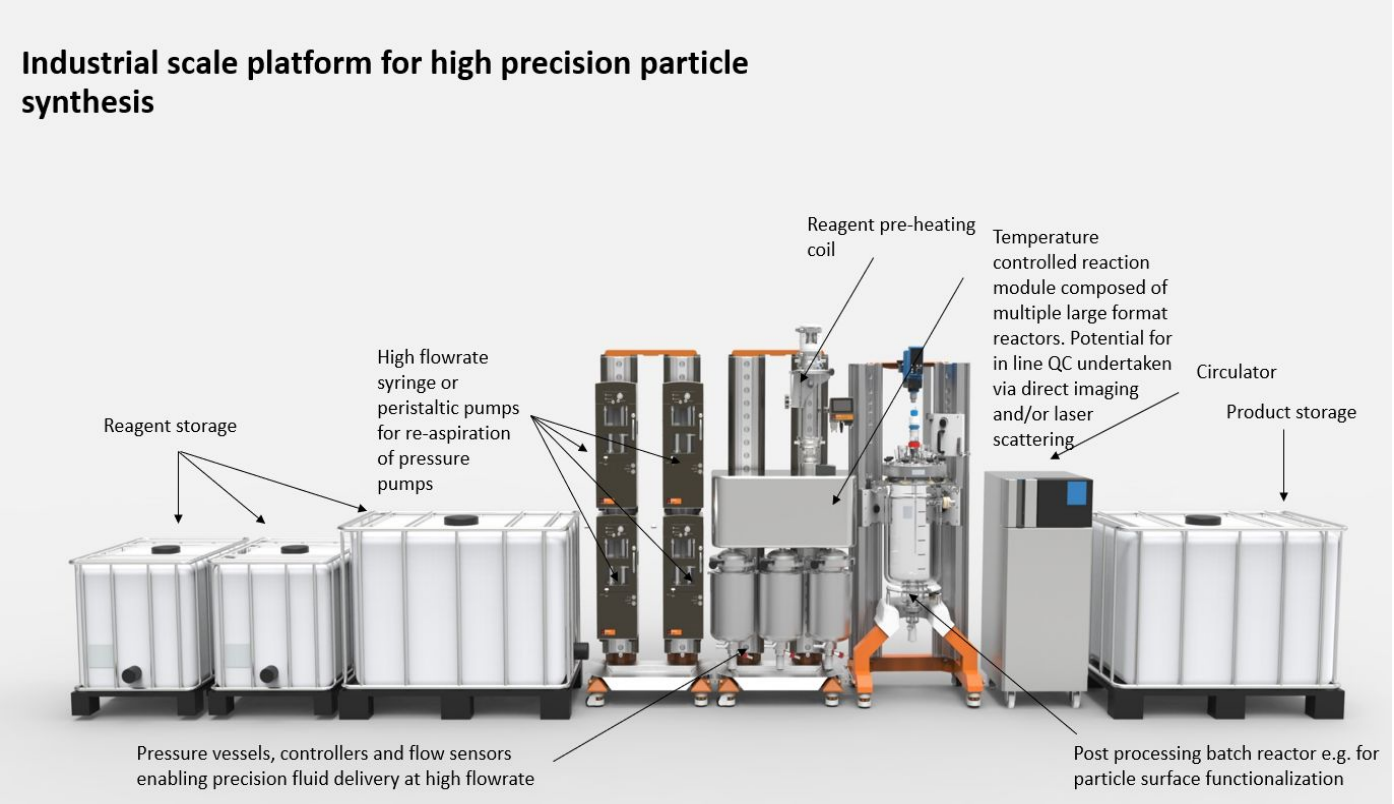
Titan LNP production approaches

- Two primary options for scaling up flow in a microfluidic system:
  - **Scale up chip** design
    - We have developed single channel chips for Titan flow rates of ~ 1 liter/min
  - **Scale out** standard chips with parallel fluidics
    - We have Telos parallel channel systems for 70x single channel flow rate
- There are potential pros and cons to each approach
- We are evaluating both methods for a range of LNP formulations and will potentially offer both options for the Titan system to give users maximum flexibility



Automating Manufacturing – GMP available 2023

- An example rendering of a full Titan system with examples of the key modules.



Summary

The Particle Works product family meets user's needs for nanoparticle process development and production.

Our game-changing automated technology allows you to produce LNP and other nanoparticles with unrivalled precision, quality, consistency and control, while reducing your development time and cost.

Speed
Rapid optimization timeframes.

Monodispersity
Excellent PDI and encapsulation efficiency.

High throughput
Aspirate from and dispense into 96 well plates.

Automation
Walk away during experiments increasing lab efficiency.

Scalability
From 200 µl to continuous production.

Cost saving
Reduced reagent use and reusable chips.

Precision
Accurate control, robust & reliable results.

Flexibility
Easy to set up and modify parameters.

No IP Licensing
No tying in to licencing models.

Thank you for your attention

Any questions?

Particle Works