

Nucleus Developer Notes

London Exchange Meeting: Liposome Protocol Survey

London Exchange Meeting Participants

Abstract

Participants in the Developer Cell Project gathered at the London Exchange Meeting and compared liposome encapsulation protocols across labs, revealing significant variation. Discussions during this meeting will help establish the engineering practice for protocol development within Nucleus and build out a framework of co-existing, category-specific protocols for benchmarking synthetic cell systems.

Request for comment

This Developer Note (DevNote) is a work in progress. Please leave comments on the Nucleus Forum thread [here](#). Dialogue there will be used to update and upgrade this DevNote.

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1. OVERVIEW

Participants in the [Developer Cell Project](#) gathered at Imperial College, London for the London Exchange Meeting. This DevNote captures key points that emerged during a discussion on the usage of the [Base Cell Protocol](#). The Base Cell protocol is an evolution of an earlier effort to create alignment around a liposome preparation [protocol](#) that emerged from the Build a Cell community in 2018.

A key goal of the Developer cell program is to build an engineering practice that enables the integration of an increasing number of engineered components into synthetic cells. This requires not only the use of community accepted protocols with clearly defined interfaces but also a community practice for modifying and extending standard protocols that evolve as the field matures to address new and greater challenges. The London exchange meeting is an important step in establishing this practice.

It is clear that advancing the field will require not a singular ideal protocol but rather category-specific protocols that enable benchmarking, along with a [framework](#) for their continued improvement.

2. STATE OF AFFAIRS

As it stands, there is significant variation in the protocols used by the members of the collaboration.

Protocol Survey

Lipid preparation

Lab	Method
Lab 1	Film

Lab 2	Lipid film
Lab 3	Lipid film
Lab 4	Lipid film
Lab 5	Thin film
Lab 6	Thin film
Base Cell Protocol	Oil-CHCl ₃ mix

Lipid composition & concentration

Lab	Composition	Concentration
Lab 1	POPC	3 mg/mL
Lab 2	90/10 POPC/Cholesterol	~0.4 mg/mL (0.5 mM)
Lab 3	DOPC or POPC	4 mg/mL
Lab 4	97/3 POPC/Biotin	2 mg/mL
Lab 5	POPC $\pm$ Chol (2:1 or 9:1), also DOPC	15 mg/mL (20 mM)
Lab 6	60/30/10 POPC/Chol/(POPE or POPA or POPG)	15 mg/mL (20 mM)
Base Cell Protocol	70/30 POPC/Cholesterol	3 mg/mL

Oil & outer solution

Lab	Oil phase	Outer solution	Osmolarity
Lab 1	Thermo Scientific (415080010)	1.1 M glucose + 50 mM HEPES	~1150 mOsm
Lab 2	Mineral oil, light (Sigma M5904)	PBS (~300 mOsm) or Tris-HEPES (~1180 mOsm)	~300–1180 mOsm
Lab 3	Mineral oil	0.5 M glucose in PBS	~500+ mOsm
Lab 4	Mineral oil (Sigma)	1086 mM glucose, 50 mM HEPES	~1136 mOsm
Lab 5	Bioultra mineral oil (Sigma 69794)	400 mM glucose in 1 M HEPES	~1400 mOsm
Lab 6	Bioultra mineral oil	sMix + 100 mM glucose + 100 mM HEPES	variable
Base Cell Protocol	Thermo Scientific (415080010)	850 mOsm (glucose)	~850 mOsm

Volumes & emulsification

Lab	Inner solution	Oil volume	Column layering
Lab 1	10 μ L	250 μ L	260 μ L emulsions, 120 μ L lipid oil, 300 μ L outer
Lab 2	10 μ L	600 μ L	600 μ L emulsions, 300 μ L lipid oil, 400 μ L outer
Lab 3	10 μ L	100 μ L	100 μ L oil on 150 μ L glucose

Lab 4	20 μ L	200 μ L	120 μ L lipid/oil on 300 μ L outer, drop emulsion on top
Lab 5	20 μ L	200 μ L	Entire L/O layered on 100 μ L outer
Lab 6	40 μ L (washboard)	200 μ L	Entire inner layered over 240 μ L outer
Base Cell Protocol	30 μ L	150 μ L	Complete transfer; 300 μ L outer

Density & monolayer		
Lab	Density medium	Monolayer incubation
Lab 1	5% Optiprep	1 hour
Lab 2	4–5% OptiPrep (CFE)	15 min
Lab 3	5% Optiprep or 0.2 M maltotriose	5–10 min
Lab 4	5% Optiprep (cytosol) or sucrose	>30 min
Lab 5	400 mM Sucrose	5–10 min
Lab 6	100 mM Sucrose	5 min on ice
Base Cell Protocol	Optiprep	5–10 min

Centrifugation & temperature		
Lab	Centrifugation	Temperature
Lab 1	30 min, 9000 $\times$ g	RT
Lab 2	10 min, 3000 $\times$ g	RT (emulsion); 15°C (CFE centrifuge)
Lab 3	30 min, 9000 $\times$ g	RT
Lab 4	10 min, 9000 $\times$ g (no PURE); 30 min (with PURE)	—
Lab 5	15 min, 18,000 $\times$ g	4°C
Lab 6	15 min, 18,000 $\times$ g	4°C
Base Cell Protocol	10 min, 9000 $\times$ g	RT

Original Table

Name	Lipid preparation (film vs oil-CHCl3 mix)	Lipid composition concentration	Oil phase	Outer solution (matched to cytosol)	Density medium	W/o emulsions	Column conditions	Monolayer incubation	Centrifuge	Temperature
Lab 1	Film	POPC - 3 mg/mL	Thermo Scientific	1.1 M glucose + 50 mM HEPES (~1150 mOsm)	5% Optiprep	10 µL inner solution, 250 µL oil	260 µL emulsion, 120 µL Lipid oil, 300 µL outer solution	1 hour	30 mins, 9000 x g	R/T
Lab 2	Lipid film	90/10 POPC/Cholesterol - 0.5mM (~0.4mg/mL)	Mineral oil, right oil (sigma-Aldrich) Cat # M5904	non-CFE: PBS (~300mOsm) CFE: 0.5M Tris-1.7M HEPES buffer stock (resulting pH 7.4 with ~2400 mOsm) diluted to ~1180 mOsm	OptiPrep (4% CFE) for CFE: 0.5M	10 µL inner solution, 600 µL oil-lipid mix	600 µL emulsions, 300 µL lipid oil, 400 µL outer solution	15 min	10 mins, 3000 x g	R/T (emulsion); 15C for CFE encapsulation (centrifuge)
Lab 3	Lipid film	DOPC or POPC - 4mg/mL	Mineral oil	0.5M glucose in PBS solution	5% optiprep or 0.2M maltotriose	10uL inner solution in lipid oil	100uL oil on 150uL glucose solution	5-10mins	30 minutes at 9000g	RT
Lab 4	Lipid film	2 mg/ mL, 97% POPC, 3% Biotin by mass	Mineral oil (sigma-Aldrich)	1086mM Glucose, 50mM HEPES if buffering necessary	5% Optiprep when using Cytosol, sucrose when not	20uL inner in 200uL L/O	120uL lipid/ oil on 300uL outer solution, drop emulsion on top	>30 min	10m, 9000g (without pure), 30m with pure	
Lab 5	Thin Cell Protocol	20/30 POPC/Cholesterol	Brown Ultra	800mM Glucose (100mM HEPES)	5% Optiprep	20 µL inner solution	Entire lipid transfer, 5-10 min	5-10 min	10 minutes, 9000g (rcf)	RT
April 27, 2026		POPC/Cholesterol		Glucose + 100 mM HEPES	Sucrose solution	200 µL lipid oil	Layered on top			4

Table 2. *Idealized protocol.*

Parameter	Idealized Protocol
Lipid preparation	Thin film
Lipid composition / concentration	POPC:Chol 70:30, 3 mg/mL
Oil phase	Bioultra (CAS 8042-47-5)
Outer solution	Glucose 0.5 M in DPBS 1X
Density medium	5% Optiprep
W/O emulsions	TBD
Column conditions	TBD
Monolayer incubation	TBD
Centrifugation	TBD
Temperature	TBD

An “[idealized](#)” protocol was discussed that incorporated common features from the protocols in use following some discussion.

3. IMMEDIATE NEXT STEPS

We did not have enough time during the exchange meeting to finish defining an ‘ideal’ protocol. Instead it was agreed to capture the state of affairs and convert the ideas discussed into the present DevNote and continue the work asynchronously. As such the DevNote remains a work in progress. We expect and encourage comments on this DevNote on the associated [Nucleus Forum thread](#).

3.1. *Distributed testing*

We recognize that the current protocol may not be perfect but improvements must be rooted in data. DevNotes and the Nucleus Forum provide venues for sharing data that can inform improvements to the protocol - this is data that would normally fall outside of the scope of existing modes of scholarly communication and exists significantly as remembrances.

An example of this kind of work can be found in a recent [DevNote](#) by Mary Kelly.

What data would be most useful to share? What benchmarks should be used to compare liposomes across labs, yield or module functionality?

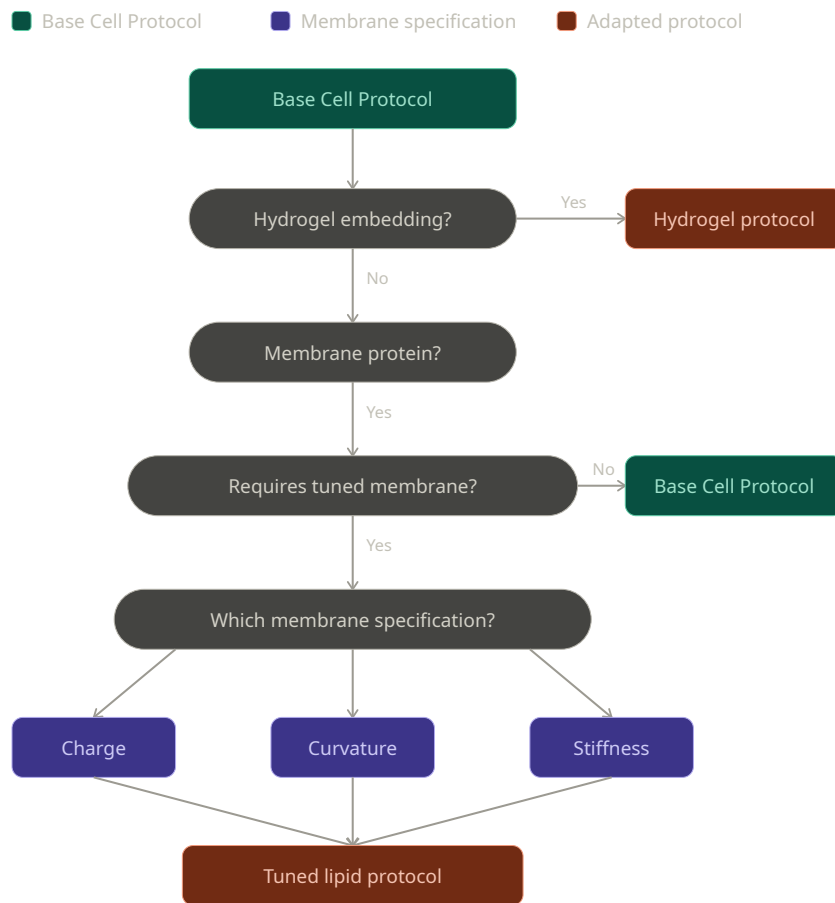
3.2. *Assistance from an Integration Node*

As the integration node in the DevCells project and maintainers of Nucleus, b.next can play an important role in synthesizing insights from the project and broader community running controlled experiments across protocol variations using a single lab's hands. As a starting point, this may involve evaluating some of the suggestions embodied in the [idealized protocol](#).

What other roles can b.next play as an integration node to support protocol convergence across the project?

4. LONG TERM VISION

One thing that is clear from discussion is that the solution is not a single protocol but rather a framework for deciding between protocols that are adapted to specific use cases.



The base cell protocol is an important first step to enable benchmarking across labs. However, it may need to be adapted to produce liposomes that can be incorporated in a specific hydrogel. Since the [Chicago](#) and [London](#) Node demonstration projects both involve embedding synthetic cells into hydrogels, there is an opportunity to define the relevant benchmarking protocol.

Similarly, it may be the case that tweaking lipid composition is needed for incorporating different membrane proteins. In this case, it may be useful to define a panel of membrane specifications that are used representing specific physico-chemical properties such as charge, stiffness, or thickness that go beyond the existing [Base Membrane Specification](#) based on POPC and cholesterol used in the Base Cell Protocol.

Participants of the London Exchange Meeting: Michael Booth (University College, London), Samuel Chen (University of Michigan), Oscar Cees (Imperial College, London), Claudia Contini (Imperial College, London), Yuval Elani (Imperial College, London), Sung-Won Hwang (University of Michigan), Anton Jackson-Smith (b.next), Mary Kelly (Northwestern University), Matthew Lucia (Northwestern University), Akshay Maheshwari (b.next), Jonah McDonald (King's College, London), Niall McIntyre (Imperial College, London), Anton Molina (b.next), Richard Murray (Schmidt Sciences), Charlie Newell (University College, London), Ojaswita Pant (Northwestern University), Julia Purrinos De Oliveira (Imperial College, London), Surendra Yadav (b.next)