biContainers Working Group

Build-A-Cell Workshop #3
6 August 2018, U. Minnesota

If you would like to add thoughts to this document, please add comments at the BOTTOM

Scope: unifying and creating reproducibility of liposome formation

Expected outcomes:

- List of protocols available for building liposomes: protocol list (work in progress)
- Agreement on common protocol (or two) that multiple labs will test and report back on within a week or two after the meeting: v0.1 protocol (work in progress)
- Agreement on IT infrastructure to test for developing and maintaining common protocol(s): https://github.com/BuildACell/liposome-kit
- Volunteer or two to serve as “benevolent dictator” for Build-A-Cell container. list of labs willing to perform interlab study
    - Testers: agree to try out the common protocol(s) and report back
    - Curators: agree to help maintain protocol, review changes, answer questions

Resources (places where we have been collecting information and experimenting with approaches)

- Slack: #containers. Please join discussion on slack channel, or comment in this doc.
- GitHub repository: https://github.com/BuildACell/liposome-kit
- ~~Protocols.io: protocol site that allows release of protocols with ability to comment, etc~~ (deprecated)
- ~~OpenWetWare: MediaWiki site~~ (deprecated)
- Workshop notes (this document)
- Protocols list
- v0.1 protocol

Agenda

0. Agree on the outcomes and agenda
1. Talk about whether it makes sense to have one or more “standard” protocols for making liposomes (see original Slack post).
    - Motivation #1: make it easier for new people to get something that works without reinventing the wheel
    - Motivation #2: create a common chassis on which results from multiple groups can be combined without "incompatibilities" that prevent operation
    - Note: goal is not to "enforce" a standard, but rather provide a standard that others use because it works and there is a community who is improving it over time
2. If we agree it makes sense, sort out where (and how) to “host” the protocol and why (some possibilities: github.com, protocols.io, osf.io, docs.google.com, openwetware.org)

    - Do we want to use a single site or support multiple sites?
    - Should there be a "curator" (or group of curators)?
    - What features do we want the IT infrastructure to support (group editing? curation? versioning/history?)
3. If we decide on one or more places to host, come up with an agreed upon v0.1 protocol that we think should work and that participants agree to go home and try.
    - To complete the protocols, one needs also the preparatory steps, that generally are not described in the papers (how to store lipids, how to solubilize in the various solvents, the minimization of lipid degradation, where to buy lipids, etc). This can be refined later. [slack]
    - Having a reference object or positive control could be helpful in terms of enabling and coordinating. Is there anything that might serve/work as such in this case? [slack]

Workshop participants (add your name below if you attending one of the working sessions):

1. Neha Kamat (Northwestern- github:npkamat)
2. Jan Gregrowicz (Caltech)
3. Zoila Jurado (Caltech)
4. Dhasharath (Dhash) Shrivathsa (MIT/Self)
5. Akshay Maheshwari (Stanford)
6. Richard Murray (Caltech)
7. Milena Popovic (BMSIS) OneScientista
8. Aaron Engelhart (University of Minnesota) github: aaronengelhart
9. Paola Torre (UPenn)
10. Kazuhito (Kaz) Tabata(U of Tokyo)
11. Joseph Heili (University of Minnesota)

Agreement on agenda and outcomes

- Need to sort out what is a “good” protocol. Need a consistent quantification.
- Need reproducible liposome; how do we measure?
- What are the categories of liposomes that people are interested in.
- What is the minimally viable product: works in anybody’s hands, then move from there.
- What are the “unit tests” to know that things are working? FRET pairs? Benchmarks to measure “performance” over revisions

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Topic #1: Discussion about whether to have a common protocol

Does it make sense to have a “common” protocol? What does this mean?

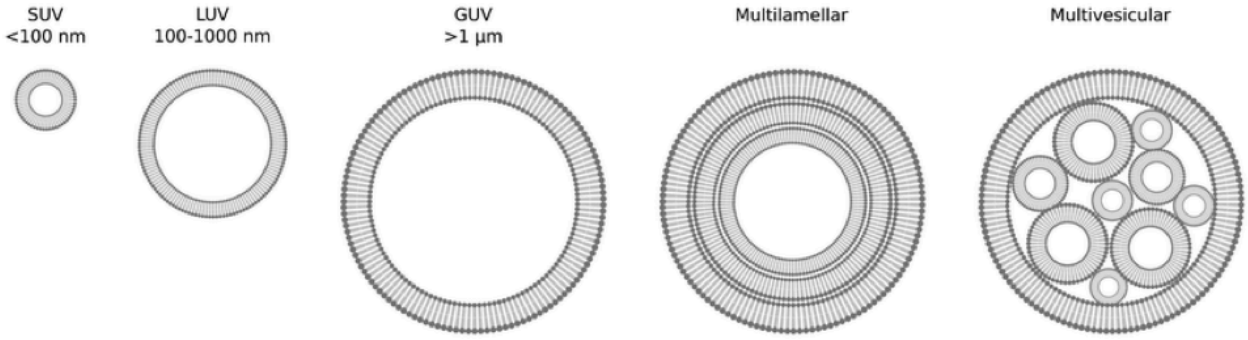
- What are the properties that are required? Different categories?
- Would like to see an automated system; eg, OpenCV pipeline for checking on this - program that you run against the image

- Encapsulation efficiency, unimellarity, getting rid of oil or not, adding fluorophores or not, integrating membrane proteins (decorate the protein)
    - Encapsulation efficiency: how much of the water solution ends up inside the vesicle
- Membrane structure that is most similar to live cell membranes (eg, not oil droplet). Easier to talk living cells
- Possible category in non-natural containers?
- Size: small (50-200 nm), large (?), giant (> 1 um). Size can affect ability to get into cells (therapeutics). 100 nm filter => 99% of vesicles have 1 membrane.
- Small vesicle in a large vesicle?
- Model of the liposome: composition, quality
- Mimicking cells
- Size uniformity (cryoET <400nm, Confocal >10μm); membrane charge
- What will we use to visualize and assess (microscope versus garage). What about artifacts that are introduced in measurement.
    - Use lipid stains? (Not rhodamine) Dyes can insert into aggregates as well; how to avoid. Spin filters? Look for a dye inside the vesicle? pH sensitive dye? (add amino acid that brings proton into the vesicle).
    - How to capture liposomes onto a slide? (sometimes they are floating). Imaging protocols. Slide prep.
- Size and shape consistency. What goes on the inside (and does it matter). Should membrane be optimized/customized based on contents?
- Can we use extruders to get uniform distribution of sizes?
- Characterizing physical properties of the membranes; be careful about whether dyes change these properties. Scalability (trillions, not hundreds of thousands)
- Amount of time it takes
- FACS protocols (making sure they don't leak, right size)
- Minimum size
- Green fluorescence is seen even without any proteins due to lipid aggregation; need to make sure that isn't happening

Properties for a minimally viable product (MVP)

- Efficient production of giant (~10 um) unilamellar vesicles capable of encapsulating either TX-TL or PURExpress
    - Giant vesicles to make it easy to see under microscope
    - Probably have to give up on size consistency
    - ?? to write up first draft
- Set of controls that can be used to measure efficiency, uniformity, unilamellarity
    - Some fraction of fluorescent probes that shows vesicles have formed (POPC, fluorescent cholesterol or DNA, fluorescent protein) [Paola to write up?]
    - Size exclusion; use a plate reader? Can use this to see whether things have leaked. May not work at 10 um? [Aaron to write up?]
    - Encapsulate some fluorescent protein or some other fluorescent component
    - Efficiency: may not be able to measure for GUVs? ~1 um might be the magic size? Can probably do something with a spin column. [Neha to write up?]
    - Perhaps add the dyes after, so that we don't restrict use of the vesicle
- Liposome should be capable of integrating membrane proteins (control = ?)
    - Alpha hemolysin is standard. Very composition specific (perhaps that is good)?

    - Membrane protein with a GFP tag that fluoresces when folded.
    - Can check for function by measuring leakage.
    - Is there a way to have the controls work on a portion of the sample. Extract a fraction of liposomes and add aHL + dye.
    - Use DiI/DiO/DiL dyes? Standard in cell biology.
    - Is there a dye that binds to the head group?
- Should take a 1 hour or less from start to imaging; should last for 24 hours (properly stored)
    - Swelling: overnight? Temperature?
    - Lyophilized POPC?
    - Has to work in a few hours if we want to use TX-TL or PURExpress
    - How to store?
    - 15 minute prep using n-Hexane: Easy and Fast Preparation of Large and Giant Vesicles from Highly Confined Thin Lipid Films Deposited at the Air–Water Interface
    - Vesicle protocols (fatty acid focused, but most apply to POPC) https://www.jove.com/video/57324/preparation-purification-and-use-of-fatty-acid-containing-liposomes
- Customization/characterization/optimization of protocols can be done on top of this
    - How many vesicles of a given composition?
    - How much encapsulation?



Topic #2: Governance and infrastructure

Discussion: How do we host, curate, edit a common protocol (or small set of protocols)

- Possibilities:
    - Protocols.io: very nice interface, but doesn't quite support changes in forks (everything has to be done manually)
    - OpenWetWare: anyone can write => hard to manage changes.
    - Google Doc: no forking/merging capability
    - GitHub: has all of the features we need, but can be complicated for new users
- Decision: try using GitHub for now
    - Fork a local copy to make changes
    - Submit pull requests that will be reviewed by the group
- (lunch break)

- Discussion: how do we maintain/curate the protocol?
    - Testers: people who agree to try out the parts of the protocol they can implement and report
        - Volunteers: Neha, Zoila/Jan, Milena, Akshay, (Kaz)
    - Curators: keep track of the testing and accept the changes that work
        - Volunteers: (Neha), (Milena), Zoila, Aaron, Paola, Kaz

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Topic #3: Draft protocol

Discussion: V0.1 protocol (aka mini-prep kit)

- Q: what version should we start with?
    - Protocol list as a google doc: use this to quickly review protocols that might be compatible with our requirements (GUV, 1 hour start, etc)
- Q: what language are we going to use for the protocol?

[moved the suggested protocol that was here to a protocols list]

Other things people would like us to consider

Programmable template for better programmability of size, shape, and spatial organization, e.g. lithographically fabricated templates or DNA nanotech-based template, e.g. origami shell (greg ti)

Automated protocol, or tests of how to make liposomes using automation

Question regarding liposomes in the context of our discussion about democratizing of the SynBio (i. e. development of the protocols that anyone/anywhere can implement):

- What is the "objective" of the testing experiments? i. e. what would be the parameter(s)/output(s) that you want me to measure to help you further improve the protocol?