Repurposing ESCRT-III machinery for fabrication of eukaryote-like artificial cells

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Introduction

The bottom-up design and fabrication of artificial cells is a challenge of significant current focus in Synthetic Biology. Inspired by natural cells, these are compartmentalised chemical systems, usually utilising repurposed biomolecular components and reconstituting native biological machineries for new purposes. Synthetic vesicles composed of lipids or amphiphilic polymers have been a primary approach to the design of the chassis of an artificial cell. However these structures usually comprise a single compartment, akin to a prokaryote, and hence lack the advantages associated with the highly compartmentalised architectures of a eukaryote that facilitate multiple incompatible chemical processes to occur in parallel through spatially separated physical confinement. To address this challenge, our approach is to repurpose the natural membrane remodelling machinery of ESCRT-III, which is involved in the generation of multivesicular bodies in native cells, for controllable generation of new intraluminal compartments within giant lipid vesicles towards the feasible fabrication of next generation artificial cells with the desired internal complexity. ESCRT-III components are known to self-assemble in a spiral complex on the membrane surface that is responsible for membrane invagination and scission at the neck of the resultant vesicular buds (Figure 1). In order to gain this control in a practical system, we need a more in-depth understanding of ESCRT-III biophysics and we are reengineering this complex to simplify the number of distinct components required for predictable, reproducible *in vitro* function.

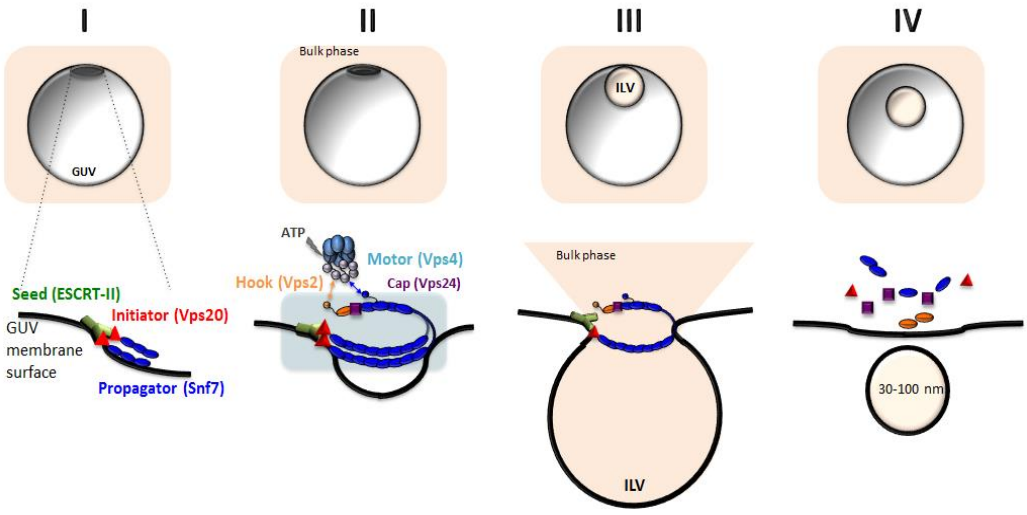

Figure 1: Cartoon of a proposed mechanism for ESCRT-III assembly and

Results

We have successfully reconstituted intraluminal vesicle (ILV) formation in an *in vitro* giant unilamellar vesicle (GUV) model system. As a first step to harnessing this process in controlled artificial cell fabrication, we need to understand which experimental parameters dictate the size and number of ILVs that form in each GUV. Our initial hypothesis was that this regulation would arise from controlling the concentration and stoichiometric ration of the different protein components that can crudely be assigned to individual functions of nucleation, growth, termination and ATP-dependent activation of the complex in prevalent models in the molecular biology literature.

To our surprise, this approach was not as predictable as would have been expected. Within a broad range of concentrations above a threshold required for function, only weak control of ILV size and number was attainable. This strongly suggests that ESCRT-III biophysics is more

complicated than current theories account for. Furthermore, we observed a greater variation in ILV size and number in an individual experiment than was evident in the average variability across the parameter space we explored. Most provocative was the realisaton that distinct “species” of GUV could be identified (Figure 2): (i) no ILVs; (ii) a large number of <2 μm ILVs; (iii) a few very large >3 μm ILVs; (iv) buds stalled at the outer membrane. GUVs did not tend to be observed containing populations of small and large ILVs, just one or the other: i.e., the heterogeneity in size and number of ILVs in a sample was primarily a heterogeneity between different GUVs rather than within individual GUVs. This strongly suggests that heterogeneities in GUV properties within a sample dominate the outcomes of ESCRT-III function more so than the concentration or stoichiometry of this complex.

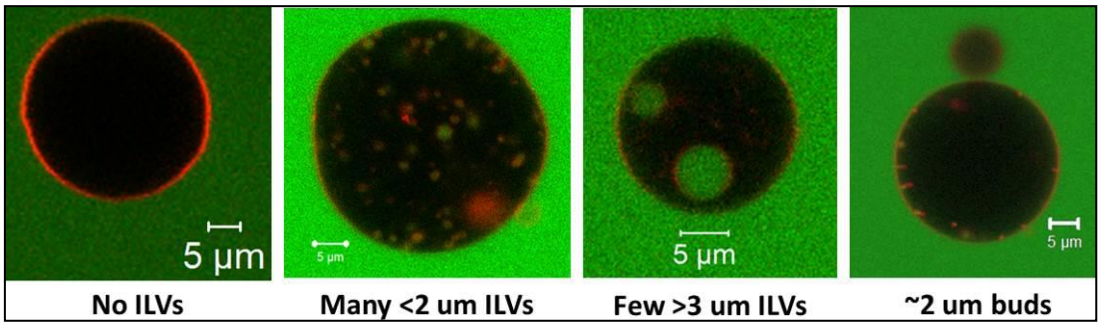

Figure 2: Examples of different ILV morphologies. Rh-DOPE (red) labels the

Having found that there was no strong correlation between vesicle size (an obvious heterogeneity in GUV samples) and these distinct ILV morphologies, other variables were sought. It is well established that considerable variation in membrane tension exists within GUV populations and therefore this was our next point for consideration. Firstly, we found that osmotic relaxation of membrane tension in a GUV population increased the number of ILVs that formed by over an order of magnitude, providing strong evidence that membrane mechanics are a significant regulating factor in ESCRT-III function. Using membrane flicker spectroscopy to determine the mechanical properties of individual GUVs, we have shown that GUVs where ILVs do not form represent that high tension population of our samples, lending further support to our hypothesis of strong mechanical regulation. This may also have significant biological implications with regards to how cells might regulate local membrane tension to exert control over ESCRT-III function.

In further work, we have early evidence to suggest that chimeric proteins can be successfully created and used to reduce the number of individual protein components in a functional complex. We have also successfully demonstrated formation of two distinct ILV populations within an individual GUV: an important step towards fabrication of complex artificial cell architectures. Ongoing and future work seeks to use membrane engineering for enhanced control over artificial cell structures and fluidic technologies are being developed to enhance the ease of fabrication and analysis of these systems.

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