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The rise of bottom-up synthetic biology and cell-free biology

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Spatially organized cellular processes arise from the interactions of nanometer building blocks. These emergent behaviors cannot be understood by knowing the parts alone, but rather how they *interact*. A central challenge in cell biology is thus to understand how cellular processes are organized spatiotemporally from the component parts¹. One powerful approach in such a quest is bottom-up cellular reconstitution as an attempt to recreate the complexity of cellular life². Many examples of bottom-up reconstitution have come from various cytoskeletal, molecular motors, and membrane trafficking systems and provide vivid demonstrations of large-scale biomimetic behaviors. In addition, the use of cell-free systems provides an experimental platform for connecting genetic information to modifying component parts. This special issue highlights both current cellular reconstitution efforts from purified proteins as well as cell-free systems of DNA-programmed behaviors in artificial cells.

Cellular reconstitution began as a biophysical approach to understand how self-organization and self-assembly of a small number of protein components give rise to shape changes or pattern formation that mimic those found in living cells. The interaction between membrane and cytoskeletal polymers pose many fascinating questions and remains a popular experimental system. We have seen tremendous progress in recent years in cytoskeletal reconstitution that is in part attributed to advances in techniques in vesicle encapsulation. Mulla *et al.*'s topical review from Gijsje Koenderink's group provides an excellent overview of recent advances in cytoskeletal reconstitution³. A number of encapsulation techniques were described, including swelling-based, emulsion-based, microfluidic-based methods as well as a recently developed method based on droplet-stabilized giant vesicles. The review also discusses anchoring cytoskeleton systems to the membrane and triggering their assembly, both of which can be challenging aspects when working with cytoskeletal encapsulation systems. The mechanics of encapsulated cytoskeleton provides a powerful avenue for dissecting force balance in governing shape changes and to better understand the

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mechanical roles that membrane-cytoskeleton play. It is envisioned that such research endeavor may eventually lead to the development of artificial cells that autonomously grow, divide, and move.

A specific example of the importance of membrane-cytoskeleton interaction is described by the work of Simon *et al.* from Cecile Sykes' group, where the authors reported that membrane tension and actin network structure and dynamics govern vesicle shapes⁴. Actin networks were reconstituted on the surface of giant vesicles and the effect of capping proteins on liposome deformation under different tension environments was investigated. Capping proteins bind to the barbed end of actin filaments and therefore control the lengths of the filaments and actin network topology. By osmotically deflating liposomes to reduce membrane tension, liposomes with actin network in the absence of capping proteins underwent symmetry breaking events and liposome deformations compared to non-deflated liposomes. This study highlights the importance of both membrane tension and architecture of actin networks in governing liposome shape changes induced by actin assembly.

Besides cytoskeletal reconstitution for force generation, there is also significant interest in oscillatory dynamics of proteins in cellular reconstruction. Mizuuchi and Vecchiarelli reviewed the reconstitution work of Min oscillator on supported membrane⁵. The Min proteins (MinD and MinE) selforganize into a standing-wave to help define cell division site at mid-cell in bacteria. A range of patterns, including amoebas, waves, mushrooms, and bursts, can be observed when Min proteins are reconstituted on a flat lipid bilayer. The review described a model of pattern formation that posits membrane-bound stoichiometry of MinD and MinE acts as the 'switch' from MinE-stimulated recruitment and stabilization of MinD to MinE-stimulated release of MinD. The systematic biochemical and quantitative dissection of the Min oscillatory system will provide more refine and better understanding of bacterial cell division. Moreover, this mechanism may be leveraged for positioning division site in artificial cells.

Beside cellular reconstitution with purified protein, a paradigm for artificial cell construction is the use of cell-free expression system that has the potential for bringing spatiotemporal control of gene expression to artificial cells. Among the different cell-free systems available is the *E. coli*-based PURE (protein synthesis using recombinant elements) system that consists of all purified components. Christophe Danelon's group contributed two articles to this special issue. With the goal to improve system's performance of PURE, Doerr *et al.* set out to systematically characterize three commercially available systems and investigate factors that might impeded robust gene expression⁶. Through this extensive work, the authors were able to identify the depletion of key transcription initiation factors as a possible inhibitory mechanism of robust gene expression. A mechanistic computational model was developed for the PURE system and it remains an ongoing effort to arrive at a unified system's level model. The work is of fundamental importance as it illustrates quantitatively different gene expression kinetics between different commercial PURE systems and this serves as a reminder that batch to batch variability in reagents (both commercial or home-made) can be a real challenge. The expression variability of PURE system can also be observed when encapsulated into cell-size liposomes. Blanken *et al.* reported the use of high-content imaging of gene-expressing liposomes encapsulating different PURE systems⁷.

Interestingly, a subset of liposome population had prolonged expression lifetime that resulted in remarkable expression yields. The heterogeneity of gene expression in single liposomes may be used to couple to natural selection for evolving artificial cells in the future.

Artificial cells, in the form of ‘smart’ vesicles, can be made to sense a single stimulus or multiple stimuli. These smart vesicles can process environmental cues and produce functional responses. These vesicles are promising theranostic agents when encapsulating therapeutic molecules and imaging agents. Abraham *et al.* in Cheemeng Tan’s group reviewed the significance of biosensing vesicles and described recent advances in engineering smart vesicles⁸. The release of bioactive agents can be triggered by different means (*e.g.* light, temperature, magnetic field, ultrasound, presence of certain enzyme) with the outcome often be the destabilization of the vesicles. The authors foresee exciting applications of smart vesicles in cancer treatment, particularly in the detection and targeted killing of circulating tumor cells.

In a recent commentary in *Nature*, bioengineer Dan Fletcher challenges researchers working on engineering biology with an inspiring message to focus on ambitious goals and solving grand problems⁹. A vision of artificial cells for use in biomedical application was articulated by Majumder and Liu¹⁰. The perspective article describes and discusses an approach to build artificial platelets from the bottom-up. The design is based on a shear stress-sensitive vesicle that when bound to injured vasculature triggers the exposure of negatively charged lipid phosphatidylserine to the vesicle membrane surface to promote thrombin generation, and thereby leading to fibrin clot formation. To carry out such a scheme, a modular approach is proposed but the challenges of integrating multiple modules remain. As natural cells work as a system of interconnected sub-cellular processes, engineering artificial cells with complex functions will be an exciting area for innovative solutions.

In summary, each of these articles presents the cutting-edge research at the frontier of bottom-up synthetic biology and cell-free biology. The field offers a tremendous potential for new fundamental discovery as well as biomedical applications. I hope that the readers enjoy this special issue and there is a bright future ahead for this field.

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