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The artificial cell: biology-inspired compartmentalisation of chemical function

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Over the past decade, the engineering and redesign of biological systems as novel functional materials for useful applications has accelerated and matured into the field of Synthetic Biology. Within this broad field, the construction of an artificial cell from its constituent molecular components has emerged as an ambitious challenge. In principle, an artificial cell could act as a minimal chassis for the integration and coordination of biological and biomimetic functionalities to achieve desired and useful properties. At a more fundamental level, understanding how to construct an artificial cell will lead to a deeper understanding of cell biology and living systems, as well as provide deep insights into the emergent properties necessary for the origins of life.

Compartmentalisation of chemical function is a fundamental characteristic of cellular life. This aspect of artificial cell research was the focus of a Royal Society Theo Murphy meeting at the Kavli Royal Society Centre, Chichelely Hall in Buckinghamshire, UK, 26th-27th February 2018. This meeting brought together life and physical scientists to explore mechanisms of biological compartmentalisation and how these principles can be harnessed to develop smart technologies. The meeting consisted of a multidisciplinary line up of speakers from the fields of organelle function in cell biology, biophysical reconstitution of biological function, membrane biophysics and the chemical engineering of minimal cell systems. The breadth of disciplines aimed to stretch all participants beyond their usual comfort zones to facilitate the inception of new ideas and paradigms for future advancement towards the ambitious challenge of a truly artificial cell. The reviews and articles in this issue are contributions from some of the invited speakers at this meeting that set the context of the state of the art and point towards challenges for the future.

Compartmentalisation is not the only hallmark of living systems. Jan van Hest and coworkers take a more holistic look at the requirements for an artificial cell by defining five key features of living systems, of which compartmentalisation is only one (Yewdall et al.). While each of these features have individually been partly mimicked in synthetic systems, all have yet to be combined into a single system that could be considered as artificial life. Significant hurdles still remain in integrating these properties.

In designing compartmentalised cellular architectures, it is informative to first look to Nature for inspiration. Cargo capture and sequestration is essential for the assembly of an artificial cell. One mechanism by which the cell achieves this is through the autophagy pathway, where large molecular complexes and organelles are encapsulated and targeted to the lysosome for degradation. Reviewed by Doreswamy and Wollert, ubiquitin-like ATG8 family proteins act as molecular tags for recognition in this process and are versatile by not only acting in cargo capture but also contributing in downstream development and maturation of the

autophagosomes (Doreswamy & Wollert). In vitro reconstitution has been invaluable in understanding these processes; in future these complexes, or similar mimics, might be repurposed to find use in artificial cell systems for specific cargo capture.

Another cellular machinery important to cellular compartmentalisation is based on the ESCRT proteins. These proteins form supramolecular complexes involved in a wide range of membrane remodelling processes, including vesiculation and repair. In particular, ESCRT-III is important in the formation of multivesicular bodies, which broadly mimic the compartmentalised architecture of a eukaryotic cell. However ESCRT-III is a multicomponent complex that requires the expression and purification of multiple protein partners for in vitro reconstitution to be effective. Marklew and coworkers describe a first attempt to develop a chimeric ESCRT protein that incorporates the functional of multiple ESCRT-II sub-units in one molecule, making the system more tractable as an in vitro membrane remodelling tool (Marklew et al.). This chimera shows improved activity compared to the core Snf7 sub-unit of ESCRT-III, demonstrating that this approach is feasible for developing a minimal toolkit for generating multicompartment giant vesicles as artificial cell architectures.

Biological membranes are fundamental to much of the compartmentalisation in the cell and so the understanding and control of their properties is of fundamental importance. Lateral fluidity in a biological membrane plays an important role in cell signalling and organisation of surface functionalities. Diffusion in biomembranes is well known not to follow traditional Stokes-Einstein diffusion behaviour but exhibits anomalous sub-diffusion. Weatherill et al. explore diffusive processes in an artificial surface supported membrane and find that urea, a chaotrope, induces sub-diffusive behaviour in these supported membranes on a length scale relevant to biological cells (Weatherill et al.). Several possible mechanisms for this surprising result are discussed, and further investigation of these is proposed.

In other studies, Trantidou et al. encapsulate engineered bacterial cell modules (a lactate biosensor) within an artificial vesicle (Trantidou et al.) as a step towards developing functional protocellular materials. The vesicle provides protection and delineates an optimal environment for the bacterial biosensor to function. This approach is referred to as “cellular bionics”, where the artificial cell provides enhanced functionality to the living cell.

Despite a strong focus of membranes, it is now clear that not all compartmentalisation in the cell follows this paradigm. In particular, there is burgeoning interest in the formation of non-membrane bound “organelles” within living cells by a process of spontaneous liquid-liquid phase separation. Such structures can occur via associative interactions between different polymers (e.g. oppositely charged macromolecules) to produce coacervate micro-droplets, or through segregative interactions between like polymers (e.g. Flory-Huggins-type phase separation). The distinct liquid phases can preferentially sequester biological complexes into functional membrane-less “organelles” both in vivo and in vitro. Crowe and Keating review the use of these phenomena in generating artificial cell systems (Crowe & Keating).

Together, the articles in this edition of *Royal Society Interface Focus* reveal the rich diversity of research currently being undertaken in the field of artificial cell design and construction, and highlight the challenges that lie ahead. The continued development of a cross-disciplinary dialogue between the life and physical sciences is of key importance if the many obstacles are to be surmounted and the final ambition of constructing an artificial cell is to be achieved. This will be best facilitated by networks and further meetings between scientists with broad relevant expertise but common goals.