CHEMICAL COMMUNICATION BETWEEN CONFINED MICRO-SPACES

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Chemical confinement and selective accessibility

Life as we know it, is an embodied phenomenon. The cell, as the basic unitary system of biology, inhabits a spatially and functionally delineated microspace, typically many micrometres in size. The incarcerated biochemistry operates under non-equilibrium conditions in a molecularly crowded milieu laced with complex molecular networks. Arguably, the most important principle of cellular confinement is not the microscale isolation of biochemical reactions but the *selective accessibility* of these reactions to the external environment and other organisms. Consequently, the confinement chemistry of living cells is best regarded as neither closed or open but as an openable chemical microsystem that enables the coextension of endogenous reaction networks and the surrounding external environment. By selectively coupling these two domains, evolvable entropy producing systems of organized complexity are maintained by the selective exchange of matter, energy and information.

In contrast to the living cell, the confinement of chemical reactions within synthetic microspaces is unremarkable. As a first approximation, the confined chemistry is equivalent to that reported in corresponding bulk (open) systems. There may be rate and pathway modulations due to interfacial interactions (membrane effects) and molecular crowding (proximity effects) but the direct effects due to microscale confinement are minimal when compared to analogous processes operating at the nano or sub-nano length scales (see other contributions in this theme). However, developing chemical communication channels *between* confined microspaces opens new scientific areas (colloid systems chemistry/protobiology) that explore how selective accessibility to endogenously produced chemical signals can be implemented in networks of artificial cells (protocells). Thus, while both microcapsules and protocells involve chemistry in confined microspaces, the former are essentially inert (unless activated by external stimuli), while the latter are endowed with programmable features (gene/enzyme circuits for example) and operate in a minimal self-referential manner via rudimentary mechanisms of self-maintenance, self-production, sensing and communication. Consequently, the operation of a protocell may alter the state of the microscale object by internal chemical modification (artificial phenotypes), selectively change the surrounding environment (artificial niche formation), or reconfigure the behaviour of other synthetic cells in a mixed population via chemical communication (population dynamics). Together, these emergent behaviours provide a step

to distributed networks of interacting chemically confined microsystems with minimal forms of autonomy, agency, information storage and self-renewal.

Our contributions to synthetic cellularity

In recent years, we have set out to engineer cell-like materials via the design and construction of a range of new protocell models using novel and established methods for generating spatially confined chemical microsystems. In many cases, aqueous micro-environments containing functional molecules are isolated from and connected to the bulk phase by enclosure within semi-permeable membranes assembled from amphiphilic building blocks such as protein-polymer nanoconjugates (proteinosomes), partially hydrophobized silica nanoparticles (colloidosomes), and polymer/ATP, DNA/organoclay or polymer/polyoxometalate polyelectrolyte complexes. Alternatively, we have used liquid-liquid phase-separation to prepare coacervate droplets as the basis for the construction of a wide range of membraneless, molecularly crowded protocell models. The focus is to explore how the chemistry within the confined microspaces can be used to develop individuated semi-autonomic microdevices capable of movement and sensing [1], for example. These studies clearly indicate that microscale confinement can be exploited in numerous ways to generate self-integrated colloid systems with semi-independent structures and functions. Moreover, individual enzyme-containing protocells can be chemically aggregated (using Click chemistry, for example [2]) to generate artificial tissue-like materials (prototissues) exhibiting properties such as coordinated contraction/expansion (beating) and chemo-mechanical actuation.

Our contributions to synthetic protobiology

Arguably, the individual cell is a necessary but not sufficient entity to define the living state; rather, life is an emergent phenomenon that is instantiated at the population level. This realization has brought a new perspective to our recent work where we have shifted focus from the structural and functional aspects of individual semi-permeable protocells to the interactivity and dynamics of distributed protocell networks. This has led to the pursuit of synthetic protobiology, in which protocells are considered as spatially confined but chemically accessible nodes in an artificial signalling environment. For example, spatially ordered 1- or 2-D networks have been prepared using microfluidic traps [3] or acoustic pressure fields [4], and operated by the diffusive transfer of ssDNA/RNA or enzyme substrates between different members of the protocell community. Typically, the trapped protocells are functionalized using encapsulated dsDNA motifs or enzyme circuitry such that molecular inputs initiate DNA strand displacement or enzyme cascades housed within the semi-permeable protocells to generate programmable outputs. The resulting communication channels include different types of protocell-embodied DNA/enzyme logic gates, negative feedback pathways, pulsing chemical reactions and the spatial integration of signalling pathways.

As an alternative approach, we have used aqueous dispersions of interacting protocells to establish dynamic 3D networks that operate by contact-dependent mechanisms. This approach enables the communication pathways to be coupled with changes in the local organization of the protocells (Figure 1).

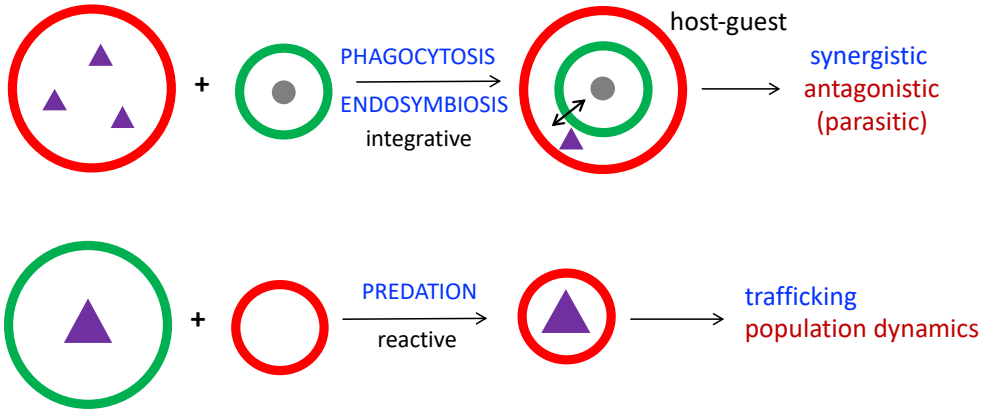

Figure 1: Contact-mediated interactions in protocell consortia.

For example, under complementary surface charge conditions, membraneless coacervate droplets spontaneously engulf membrane-bounded proteinosomes such that the initial binary population is amalgamated into a single population of nested host-guest hybrids. Interestingly, switching on certain chemical reactions in the captured proteinosomes provides an escape mechanism, which releases the guest protocells by destroying the host droplet from within [5]. Alternatively, reactions inside the captured proteinosomes are used to structurally reconfigure the host coacervate droplet; for example, by transforming the coacervate droplet into a membrane-bounded coacervate vesicle, thereby increasing the mutual stability of the host-guest construct [6]. More dramatically, ingestion of individual proteinosomes by protease-loaded coacervate droplets leads to artificial phagocytosis [7] or predator-prey behaviour [8] in which lysis within the hybrid construct results in dismantling of the guest proteinosomes and trafficking of its contents into the invading coacervate droplets.

Significantly, spontaneous contact-driven changes in the organization and distribution of mixed protocell populations can be rendered programmable by employing signal-induced mechanisms (Figure 2).

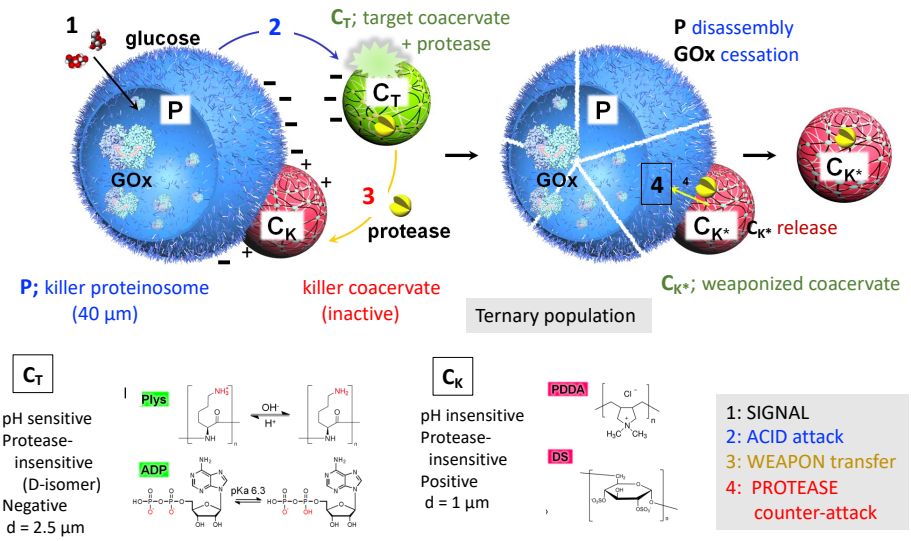

Figure 2. Response-retaliation mechanism in a ternary protocell population (see text for details). Adapted from reference [9].

For example, a predator-prey system was implemented in a ternary protocell population to produce spatiotemporally dependent response-retaliation ("tit-for-tat") behaviour [9]. Specifically, the consortium consists of: (i) negatively charged "killer" glucose oxidase-containing proteinosomes (**P**), (ii) negatively charged pH-sensitive, protease-containing target coacervate droplets ($\mathbf{C_T}$) and (iii) positively charged pH-insensitive retaliatory coacervate droplets ($\mathbf{C_K}$). The latter spontaneously adhere to the proteinosome outer membrane due to the complementary surface charges, while the target coacervate droplets are unattached and in free solution. Addition of glucose results in diffusive transfer of protons into the network such that the target coacervate droplets disassemble, followed by the release of protease, which in turn is sequestered and concentrated within the pH-insensitive retaliatory coacervate droplets attached to the proteinosome membrane ("weaponization"). Consequently, the local build-up of protease in the membrane-attached coacervate droplets results in rupture of the protein-polymer membrane and complete disintegration of the killer proteinosomes.

Outlook and future work

Combining spatial confinement and selective accessibility opens a pathway to novel distributed systems that participate in programmable network connectivity based on diffusive signaling between microscale chemical processors. Currently, protocell networks operate in water as pre-organized spatial arrays or as spontaneously colliding mixed populations of protocells. In terms of future work, we aim to use confinement at the microscale to develop chemical strategies for establishing programmable protocell

networks that are initially *segregated* into discrete regions of space. This will allow spatiotemporal dynamics to be implemented into the communication channels operating between the separated populations such that the network becomes reconfigurable as it operates. An example of this approach, involving signal-induced processes of protocell-mediated recruitment and dispatchment in a liquid-liquid phase-separated cytomimetic network, will be discussed in the symposium.

Acknowledgments
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References
Due to limited space, only references related to research contributions specifically mentioned above are listed. Key contributions from other research groups can be found in the primary papers cited below.

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