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Synthetic Supramolecular Systems in Life-like Materials and Protocell Models

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SUMMARY

One of the biggest challenges in modern chemistry is the preparation of synthetic materials for the assembly of artificial cells. In recent years, numerous artificial systems that mimic cellular components and functions have been developed. Supramolecular chemistry plays a key role in such cell mimics as non-covalent interactions control the shape and function of many biomolecules, such as DNA base pairing, protein structure, ligand-receptor binding and lipid membrane packing. However, the complexity of living cells constitutes a major challenge for their bottom-up assembly from pure synthetic materials. Inspired by the building blocks of Nature, a wide range of supramolecular systems have been developed to reproduce cellular functions such as cell-cell communication, signalling cascades and dynamic cytoskeleton assemblies. This review surveys a selection of key advances in synthetic derivatives of biomolecules with supramolecular organisation and life-like behaviour, addressing their non-covalent foundation and integration as increasingly complex protocell models.

Supramolecular chemistry, self-assembly, life-like materials, synthetic cells, biomimicry, protocells

INTRODUCTION

The design of simple stimuli-responsive supramolecular systems with life-like behaviour constitutes a fundamental scientific exercise towards understanding the role of self-assembly in the origin of life. By 'life-like' we refer to the natural cellular and sub-cellular responses and mechanisms that are fundamental to life from the molecular to macroscopic level (*e.g.* energy consumption and dissipation, dynamic self-assembly, molecular replication, motion, sensing and signalling, *etc.*).[1] Replicating these complex phenomena with simple synthetic building blocks poses a daunting challenge from a supramolecular and functional standpoint. However, minimalistic systems can now be engineered from the bottom-up to imitate and tune particular biological processes.[2–4] The idea is to replicate individual cellular functions with synthetic molecules, which can be eventually integrated into multifunctional life mimicking materials with increasing complexity.[2] Inspired by natural systems, the growing efforts in this area are bringing closer the dream of engineering full living cells synthetically and unravelling the chemical foundations of life. The exciting discoveries made during the fabrication of artificial cells improve our understanding of the complex cellular machinery and allow us to install non-natural functions in synthetic surrogates of biological structures.[5,6]

Supramolecular interactions play a central role in defining the structure and function of biological systems.[7,8] The bases of protein folding, DNA hybridisation or membrane integrity rely on the interplay between inter- and intramolecular self-assembly. Therefore, artificial cells made of synthetic building blocks capitalise on mimicking, tuning and blending these interactions to design artificial suprastructures with biomimetic organisation and environmental responses.[5,9] Supramolecular forces work cooperatively in living organisms towards stronger interactions with defined spatial arrangement.[8] Importantly, artificial supramolecular systems can be rationally designed to reversibly respond to physical and chemical stimuli, being particularly well suited for mimicking the adaptive behaviour of living organisms.[10] Beyond natural biomolecules and derivatives thereof, fully artificial supramolecular designs exploit the principles of non-covalent

interactions to open new opportunities in the rational design of life-like synthetic materials and minimal artificial cells.

We here survey the latest and most remarkable developments in artificial life-mimicking systems through application of supramolecular chemistry. The following discussion covers the design of artificial building blocks and their supramolecular organisation. This review is organised based on the chemical nature of the assembling molecules. Firstly, natural biomolecules (*i.e.* peptides and proteins, nucleic acids, lipids and glycans) and derivatives are repurposed for alternative biological functions and/or to modulate their natural responses. Secondly, non-biologically related building blocks (*e.g.* synthetic polymers, molecular motors, artificial receptors, *etc.*) will further illustrate the potential of rational supramolecular designs to mimic, tune and expand the natural responses of cells and tissues. Overall, we aim to provide a broad perspective of the new synthetic suprastructures with life mimicry, also addressing related hot topics in the field, such as out-of-equilibrium self-assembly, supramolecular self-replicators and kinetic and thermodynamic control.[11,12] We have included a selection of key references to refer the reader to more detailed and specific literature on each particular topic. Besides the derived lessons learned from supramolecular systems,[13,14] new biomimicking materials can also bring important applications such as cell and tissue engineering, diagnostics and next-generation therapies. The implementation of these technologies *in vivo* is also presented here to demonstrate their current and future potential. Conceptually, the bottom-up engineering of synthetic cells helps us understand the implications of self-assembly in the origins of life, posing plausible evolutionary steps form simple building blocks to complex and highly specialised biostructures.

NATURAL BUILDING BLOCKS

Peptides and proteins

The diverse chemical functionality and inherent tendency of peptides to assemble (*e.g.* α-helices and β-sheets) offer high flexibility to design biomimetic structures.[15] Inspired by natural protein architectures, artificial peptide scaffolds have been designed to combine hierarchical self-assembly with biomimicry.[16] Importantly, the activity of many natural peptides and proteins is chemically regulated by their metabolic (de)activation, which ultimately translates into conformational changes that dictate function. For example, the eukaryotic cytoskeleton consists of proteins that show alternating cycles of growth and decay triggered by phosphorylation and dephosphorylation events.[7,17] This dynamic instability allows the active remodelling of these natural supramolecular fibres with precise spatiotemporal resolution. However, given the structural complexity of cytoskeletal proteins, chemists have focused on the established principles of peptide self-assembly to obtain artificial minimalistic scaffolds that mimic this adaptive behaviour. Due to the vast literature in supramolecular bio-inspired peptide systems, we will divide this section into two parts: (I) Artificial peptide assemblies fully based on non-covalent interactions, and (II) reaction-based systems, where the formation and break of covalent bonds controls self-assembly.

(I) Artificial peptide assemblies: The dynamic fibrillation of short synthetic peptides is one of the most studied prototypes of supramolecular biomimetic assemblies.[18] The rationally predictable non-covalent interactions of short peptides give chemists the opportunity to design supramolecular structures with complex biomimetic behaviour, such as dynamic remodelling and self-replication. One of the benchmark artificial fibrillating peptides are Stupp's peptide amphiphiles (PAs), which consist of aliphatic chains conjugated to the termini of short peptides with hydrophobic β-sheet inductors and charged residues (Figure 1A).[19] In these PAs, the aliphatic tail is the main driving force of self-assembly by hydrophobic effect in water. Additionally, the peptide region forms stabilising H-bonded networks, whereas charged residues maintain the extended conformation of the assembly by electrostatic repulsion. A recent expansion of this work exploits the conjugation of fibrillating PAs to DNA, which generates intertwined PA fibres by complementary DNA pairing (Figure 1A-B).[20] These PA-DNA hybrids self-assemble into dynamic 3D structures that allow reversible remodelling, which function as synthetic mimics of the extracellular matrix. Hence, specific DNA hybridisation endows self-assembling peptides with additional

supramolecular recognition and hierarchical organisation, imitating the different levels of non-covalent organisation found in biological systems.

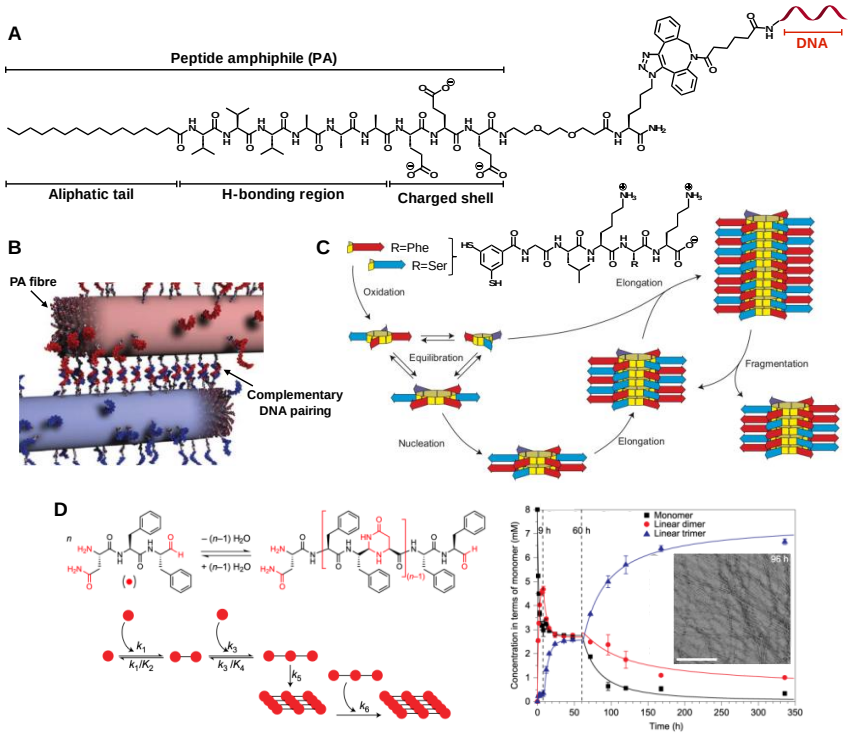

Figure 1. Dynamic supramolecular assemblies of peptide derivatives

(A) Structure of peptide amphiphile (PA) linked to a DNA strand. (B) Hierarchical self-assembly of PA fibres and complementary DNA hybridisation to cross-link PA fibres. Reprinted with permission from Freeman *et al*.[20] Copyright 2018 AAAS.

(C) Dynamic cyclic peptide oligomers that evolve from non-assembling short oligomers to hexameric macrocycles, which self-replicate via supramolecular fibrillation. Reprinted with permission from Sadownik *et al*.[21] Copyright 2016 Springer Nature.

(D) Left: Structure and condensation model of a tripeptide (NFF-CHO) dynamic network where reversible *N,N*-acetal linkages are represented by black lines connecting tripeptide monomers (red dots). The constants indicated in the model represent forward reaction (k_1, k_3), equilibrium (K_2, K_4), nucleation (k_5) and autocatalytic growth (k_6). Right: Dynamic network composition over time and TEM image of linear trimer fibres found after 96 h (scale bar = 200 nm). Reprinted with permission from Chen *et al*.[22] Copyright 2017 Springer Nature.

Supramolecular recognition is proposed as one of the mechanisms that may have guided the chemical evolution of life, as certain molecules may have been selected and replicated through self-assembly from pools of structural analogues.[11,23] Otto and co-workers have developed intriguing peptide-based self-replicating dynamic libraries, which arise from short synthetic peptides connected to an aromatic di-thiol that oligomerises into macrocyclic products (Figure 1C).[21] The kinetic products obtained from these peptides are short macrocycles (3-mer and 4-mer), which over time rearrange into larger ones (*e.g.* 6-mer) that self-assemble into fibres driven by β-sheet interactions. The supramolecular stacking of 6-mers templates shorter macrocycles and drives their evolution to the self-assembling product. Interestingly, the self-replication of the 6-mer only takes place when the system is agitated, as shear stress causes pre-formed fibres to fragment into nucleation points for the selection and elongation of 6-mer fibres.[24] These dynamic combinatorial libraries not only allow the study of molecular Darwinian evolution, as just described, but also other complex inter-species relationships such as parasitic and predatory behaviour with the same chemical foundation.[25] Alternatively to disulphide chemistry, Lynn *et al*. developed analogous dynamic peptide networks using reversible *N,N*-acetal linkages for oligomer exchange (Figure 1D).[22] Over time, the ability of the linear trimers to fibrillate as

β-sheets drives their self-replication and supramolecular selection from a pool of oligomers in equilibrium. Template-directed growth was confirmed by the rapid fibrillation of the monomer when seeded with pre-assembled trimer. Remarkably, this dynamic system could also grow supramolecular nanotubes from seeds of a related amyloid peptide, demonstrating the flexibility of this network to replicate different templates. The group also demonstrated the on-surface catalytic activity of these supramolecular phases, which despite unable to read or write specific sequences, can perform templated polymerisations that resemble the function of natural polymerases.[26]

One step beyond one-dimensional peptide fibrillation was taken with the design of *de novo* coiled-coil peptide assemblies, which opens access to more complex hierarchical structures and life-like functions. Woolfson and co-workers have contributed extensively to this area with *de novo* coiled-coil peptide barrels that work as synthetic receptors for small hydrophobic molecules[27] and display artificial esterase activity[28] (Figure 2A-B). These multimeric coiled-coils consist of amphiphilic α-helical peptides that follow the heptad design 'hpphppp', where 'h' and 'p' correspond to hydrophobic and polar amino acids, respectively, thus creating a hydrophobic face in the helix that packs into a pore upon oligomerisation. Furthermore, *de novo* coiled-coil peptides could direct the self-assembly of natural proteins into artificial supramolecular scaffolds within living bacteria, where tagged enzymes could be accumulated with improved turnover rates.[29] Jerala *et al*. have encoded multiple coiled-coil interactions into long peptides -over 700 amino acids- that fold into defined polyhedra, such as tetrahedra, pyramids and prisms (Figure 2C).[30] Beyond the fundamental expansion of non-natural supramolecular peptide assemblies, the authors demonstrate the expression and correct folding of these coiled-coil peptide origami from plasmids *in vivo*, and hence their potential application in biological settings and *in situ* generation. This group has also implemented *de novo* coiled-coil peptides as substrates for proteolytic logic circuits, which are versatile tools to emulate cellular signalling pathways and thus study the dynamics and evolution of biological regulation.[31] Engineering supramolecular protein interactions *de novo* in coiled-coil systems has also allowed the expression and orthogonal assembly of coiled-coil heterodimers in living bacteria.[32] Unlike most hydrophobic-driven protein oligomers, these coiled-coil heterodimers were established by highly specific H-bonding patterns between side chains reminiscent to natural DNA base pairing. Moreover, Ghadiri *et al*. first demonstrated the autonomous self-replication of coiled-coil peptide dimers via supramolecular templation of activated precursor fragments through leucine zippers.[33] This concept was recently applied to a reversible self-replicating coiled-coil network, where dynamic thioester bonds between peptide fragments allowed the transition between coiled-coil and unfolded precursor states.[34]

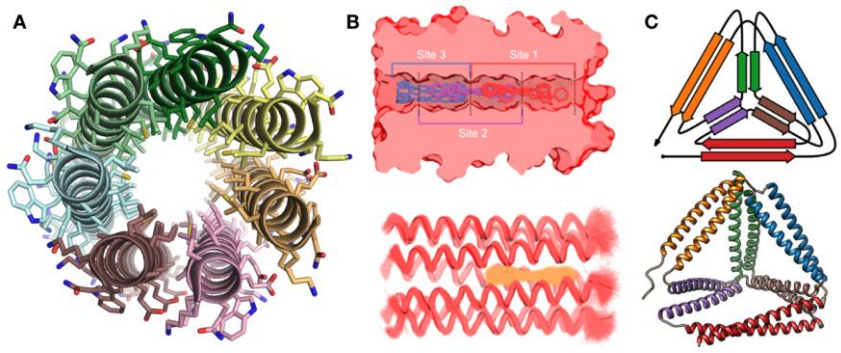

Figure 2. *De novo* coiled-coil peptide assemblies
(A) Crystal structure of a multimeric coiled-coil barrel with hydrophobic pores and polar surfaces (Protein Data Bank: 5EZ8).[28] (B) Application of these multimeric coiled-coil barrels as artificial receptors with multiple binding sites within their hydrophobic centre. Reprinted with permission from Thomas *et al*.[27] Copyright 2018 American Chemical Society.
(C) A single peptide sequence that self-assembles into tetrahedra by complementary coiled-coil segments. Reprinted with permission from Ljubetič *et al*.[30] Copyright 2017 Springer Nature.

The alternative use of D-amino acids as peptide building blocks opens access to interesting non-natural supramolecular architectures, such as one-faced β-sheets[35] and cyclic peptide nanotubes.[36] Our group has worked extensively on D/L-alternating cyclic peptides that stack by H-bonding into nanotubes, allowing additional supramolecular interactions encoded on their superficial side chains to control self-assembly.[14] Thus, the fibrillation of cyclic peptides can be controlled with pH, when charge repulsions between protonated histidine residues are neutralised by the addition of base (Figure 3A).[37] In this example, lateral hydrophobic and π-π interactions between pendant pyrene units drove the bundling of peptide nanotubes into larger fibres. Alternatively, charge repulsions between cyclic peptides can be screened by addition of small electrolytes to induce nanotube formation and bundling.[38] The confined and localised fibrillation of these cyclic peptides at the centre or interface of droplets emulates the spatially controlled supramolecular polymerization of a cytoskeleton (Figure 3B). Furthermore, segregation of polar and hydrophobic amino acids in cyclic peptides allows the amplification of this amphiphilic character in one dimension along the resulting nanotubes (Figure 3C).[39] These amphiphilic nanotubes can further self-assemble as bilayers in aqueous medium by hydrophobic packing, with leucine zippers providing directional growth in this second dimension. Hierarchical 1D and 2D self-assembly can be thus engineered into D/L-alternating cyclic peptides to form giant supramolecular nanosheets with stimuli-responsive behaviour in the high micron scale. These novel 2D materials provide alternative supramolecular non-lipid membranes, also accessible from other peptide-based building blocks such as peptide amphiphiles,[40] triple helices[41] and peptoids.[42]

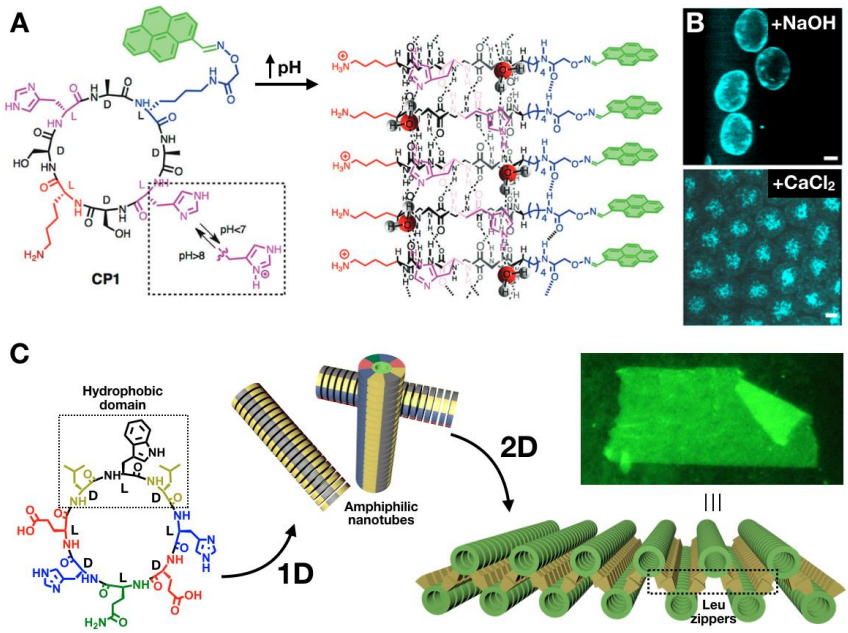

Figure 3. Self-assembling D/L-alternating cyclic peptide nanotubes

(A) Representative D/L-alternating cyclic peptide with pH-responsive self-assembly. Neutralisation of charged histidines induces the oligomerisation of peptide monomers through H-bonding as nanotubes with side chains projected outwards. Reprinted with permission from Méndez-Ardoy *et al*.[37] Copyright 2018 Royal Society of Chemistry.

(B) Localised fibrillation of cyclic peptides within droplets at their interface or lumen in response to base or electrolytes, respectively. Reprinted with permission from Méndez-Ardoy *et al*.[38] Copyright 2020 Wiley-VCH.

(C) Sequential 1D-to-2D self-assembly of amphiphilic cyclic peptides into supramolecular nanosheets stabilised by lateral leucine zippers between adjacent nanotubes. Reprinted with permission from Insua *et al*.[39] Copyright 2020 American Chemical Society.

(II) Reaction-based systems: The fibrillation of short synthetic peptides driven by enzymatic transformations represents a minimal model to study the regulated dynamics of biological suprastructures. For example, intracellular fibrillation of a synthetic peptide was

achieved *in vitro* by sequential dephosphorylation by alkaline phosphatase (ALP) and disulphide reduction of the peptide precursor; modifications that removed Coulombic repulsions and thus triggered intracellular self-assembly (Figure 4A).[43] Similarly, cellular production of ALP can also trigger the extracellular fibrillation of short aromatic peptides, which undergo π-π and hydrophobic packing upon removal of sacrificial phosphate groups.[44] These are excellent examples of how rational design and self-assembly can exploit cellular metabolism to engineer artificial supramolecules in living cells.

Enzymes catalysing both amide formation and hydrolysis can be employed to generate transient peptide fibres regulated by the kinetics of these two enzymatic steps. Ulijn *et al.* illustrated this concept with the conversion of soluble aspartame into fibrillating tripeptides by action of α-chymotrypsin, which also catalysed the hydrolysis of the self-assembling tripeptide to give an inactive product (Figure 4B).[45] This group later employed α-chymotrypsin to control the self-assembly of a chimeric dipeptide, either as transient helical fibres or nanotubes, by feeding amino acids of specific polarity and chirality that determined enzymatic hydrolysis and amidation rates.[46] Thus, the competition between the supramolecular or covalent (*i.e.* enzymatic) attachment of the fed amino acids to the parent dipeptide, selected for specific supramolecular pathways with kinetic control based on α-chymotrypsin's reactivity. Importantly, the sustained activity of this enzyme accumulates reaction by-products that lead to a progressive drop in turnover rates. The inhibition of enzymatic pathways by their own by-products is a problem solved in living organisms with waste clearing mechanisms. To mimic the natural perpetuation of fuelled supramolecular systems, Hermans and co-workers developed a peptide-based supramolecular dissipative system, where enzymatic (de)phosphorylation of a perylenediimide (PDI) peptide derivative induces the formation of transient fibres inside a dialysis chamber, which can be sustained over time by constant supply of fuel (*i.e.* ATP) and waste removal.[47] In this work, the enzymatic phosphorylation of the peptide (PDI, Figure 4C) triggers its supramolecular elongation into helical fibres as transient products (p2-PDI), while hydrolytic phosphatase activity causes the reversion of the system to its original state with release of inorganic phosphate waste. Different non-equilibrium states could be accessed and/or maintained over time by controlling the supply of ATP and constant waste clearance (Figure 4C, right). Both the elongation and decay of these peptide fibres is controlled by enzymatic reactions, regulated at the same time by their substrates' availability, in an excellent example of non-natural supramolecular homeostasis. In general, the supramolecular motifs selected for these systems and other fibrillar analogues are based in the directional control of H-bonding between peptide backbones in β-sheets[48] or α-helices,[49] which are usually aided by lateral π-π and hydrophobic interactions[43,45] or even electrostatics[47] between the corresponding peptide side chains. Several enzymatic reactions can be coupled to generate artificial biocatalytic pathways, where peptide precursors transition between different supramolecular structures based on the ratio or sequential addition of enzymes.[50] Alternatively, indirect methods of fuelled enzymatic peptide self-assembly have been reported, such as pH oscillations triggered by urease[51] or pH/redox regulation by glucose oxidase,[52] which offer an interesting approach to control dynamic self-assembly without the direct chemical modification of peptides.

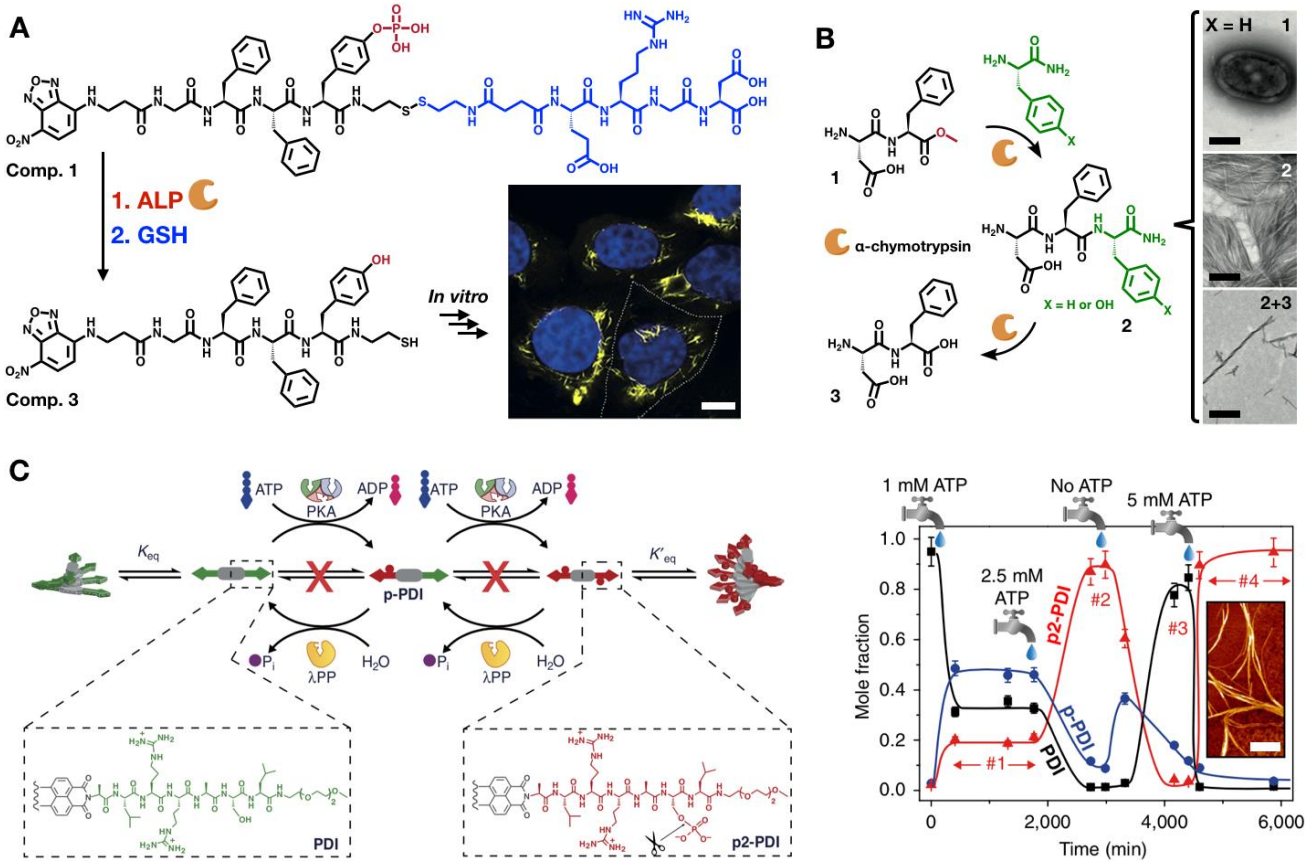

Figure 4. Enzyme-triggered self-assembly of peptides (and derivatives thereof) into fibrillar structures

(A) Sequential enzymatic dephosphorylation by extracellular alkaline phosphatase (ALP) and disulphide cleavage by intracellular glutathione (GSH) of Comp. 1 to generate Comp. 3 *in situ*, which self-assembles into fibres selectively inside living cancer cells with cytotoxic effects. Scale bar = 10 μm. Reprinted with permission from Zhan *et al*.[43] Copyright 2018 Wiley-VCH.

(B) Biocatalytic conversion of aspartame (1) to self-assembling peptide 2 and subsequently to soluble peptide 3 by α-chymotrypsin. Representative TEM images show the disassembled state of the system composed of 1, fibrillation upon its conversion into 2, and gradual disassembly of 2 as it is digested to 3. Scale bars = 0.5 μm. Reprinted with permission from Pappas *et al*.[45] Copyright 2015 Wiley-VCH.

(C) Left: Simplified structure of the dipeptide PDI and its mono- (p-PDI) and di-phosphorylated (p2-PDI) products by protein kinase A (PKA) and reverse dephosphorylation by α-protein phosphatase (αPP) with supply of fuel (ATP) and release of waste products (ADP and Pi = inorganic phosphate). Right: Composition of a PDI solution inside a dialysis chamber over time with different levels of fuel supply and constant waste removal. Representative AFM image (inset) of p2-PDI's fibrillated state. Scale bar = 500 nm. Reprinted from Sorrenti *et al*.[47]

To fully mimic natural supramolecular fibrillation synthetically, enzymatic regulation must be replaced for man-made catalysts and entirely artificial self-assembling pathways. Recent efforts to this end have focused on chemically fuelled dissipative self-assembly by transformation of inactive precursors into self-assembling products.[53] Thus, a transient supramolecular system is generated outside its thermodynamic equilibrium, which eventually returns to its original disassembled state by the spontaneous degradation of the product into its precursor. As an example, Boekhoven *et al*. employed carbodiimide chemistry to neutralise the electrostatic repulsions between short acidic peptides as transient cyclic anhydrides that self-assembled into fibres (Figure 5A).[54] Spontaneous hydrolysis of the cyclic anhydride caused the recovery of charge repulsions and hence the disassembly of the supramolecular fibres. Thus, fibre (de)polymerisation was kinetically controlled by the relative cyclisation-hydrolysis rates of the different carbodiimides and peptides tested. More specifically, fibrillation is determined by fuel concentration and pH,

as these parameters dictate the rates of precursor conversion and product hydrolysis, respectively.[55] Das and co-workers described another exciting carbodiimide-fuelled dissipative system, where *N*-octadecylhistidine was employed to assemble fibres via autocatalytic esterification (Figure 5B).[56] Here, histidine's imidazole catalysed both the esterification and the hydrolysis steps at the *C*-term, thus creating a transient self-immolative fibrillar state. The fibrillation of the reaction product could template and co-assemble its precursor, which further accelerated the conversion of substrate. This self-dissipative histidine-promoted esterification was recently applied to the reversible fibrillation of amyloid peptides with temporal control.[57] The (auto)catalytic activity of some of these artificial supramolecular systems imitates the natural function of many histidine-based enzymes, which has found application in a plethora of man-made catalytic peptide assemblies.[58]

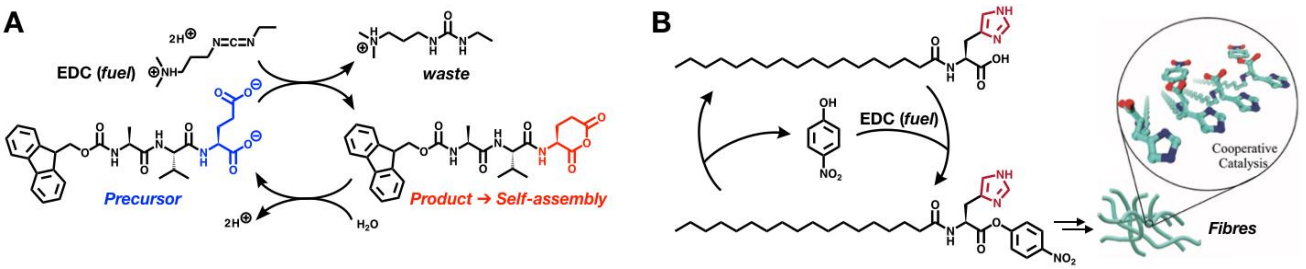

Figure 5. Fuelled dissipative self-assembly of fibrillating peptide scaffolds

(A) Cyclisation of a dicarboxylate precursor with consumption of EDC (*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide) as fuel into a fibrillating cyclic anhydride, and its spontaneous hydrolysis and disassembly in water back into the disassembled precursor. Reprinted from Tena-Solsona *et al*.[54]

(B) EDC-fuelled activation of histidine amphiphiles into a fibrillating *p*-nitrophenol ester that catalyses both the esterification and hydrolysis steps. The supramolecular fibres also template precursor molecules, thus also accelerating the reaction rates. Reprinted with permission from Bal *et al*.[56] Copyright 2019 Wiley-VCH.

Nucleic acids

The main role of nucleic acids in living organisms is the storage, expression and propagation of genetic information, although some nucleic acids play a catalytic role (*i.e.* DNA/RNAzymes). From a supramolecular perspective, the anionic backbone of nucleic acids and their complementary H-bonded base pairing offer highly specific and multivalent self-assembly possibilities.[59] DNA hybridisation is a reversible process that increases nucleic acid stiffness nearly two orders of magnitude, noteworthy from a structural standpoint, where invader DNA/RNA molecules can displace duplexed strands of lower affinity.[60] Pendant single stranded domains (*i.e.* toeholds) can initiate strand displacement in duplexes by providing additional attachment points, with toehold length and sequence specificity being determinant for the kinetics of this process.[61] Therefore, the dynamic hybridisation of nucleic acids allows reversible strand-displacement reactions and thus supramolecular remodelling, which can be exploited for adaptive processes such as sensing, signalling pathways and responsive self-assembly. Most applications of DNA tagging, recognition and folding use either single strands for dynamic exchange, or multiple strand folding into so-called 'tiles' or 'bricks', which can oligomerise by complementary sticky ends (*i.e.* unpaired segments) into higher order networks.[62] Whereas DNA origami folds a long 'scaffold' strand with multiple short 'staple' strands,[63] DNA tile folding offers higher modularity to control the structure and function of the final ensemble. Unlike peptides, where the sequence of amino acids directly affects secondary structure and thus supramolecular self-assembly, nucleic acids directly hybridise from unfolded strands to generate duplexes, tiles and origami structures – although some designs may include self-folded DNA hairpins. This distinction makes it easier to design long complementary DNA strands than long coil-coiled peptides, as the former do not rely on pre-assembled blocks and thus provide more freedom in sequence design without affecting multimeric assembly. On the other hand, amino acids offer a wider palette of pendant functional groups for specific chemical and physical properties such as bioconjugation, pH-response and amphiphilic domains.

DNA self-assembly has been successfully applied to the formation of non-peptide mimics of a cytoskeleton. An illustrative example by Franco *et al*. used pentameric DNA junctions as tiles, whose complementary sticky ends drove their polymerisation into hollow DNA nanotubes (Figure 6A).[64] The reversible dynamics of DNA hybridisation allowed the disassembly of these nanotubes by addition of an invader strand, and their subsequent re-assembly with an anti-invader strand. DNA nanotubes tolerated repeated (dis)assembly cycles, as the invader and anti-invader strands combine into an unreactive waste product. This system was further coupled to a dual enzymatic pathway whereby RNA invader strands are generated *in situ* by polymerase activity, while RNase action digests this invader, thus creating an oscillatory fibrillating system with metabolic control.[64] Another interesting example used trimeric DNA junctions to assemble supramolecular networks as cytoskeletons for minimal cell mimics like liposomes and lipid droplets.[65] The anionic backbone of these DNA tiles drives their accumulation on the surface of cationic lipid membranes. This artificial cytoskeleton enhanced the mechanical stability of these lipid colloids, allowing a more realistic study of such model compartments as minimal cell mimics.

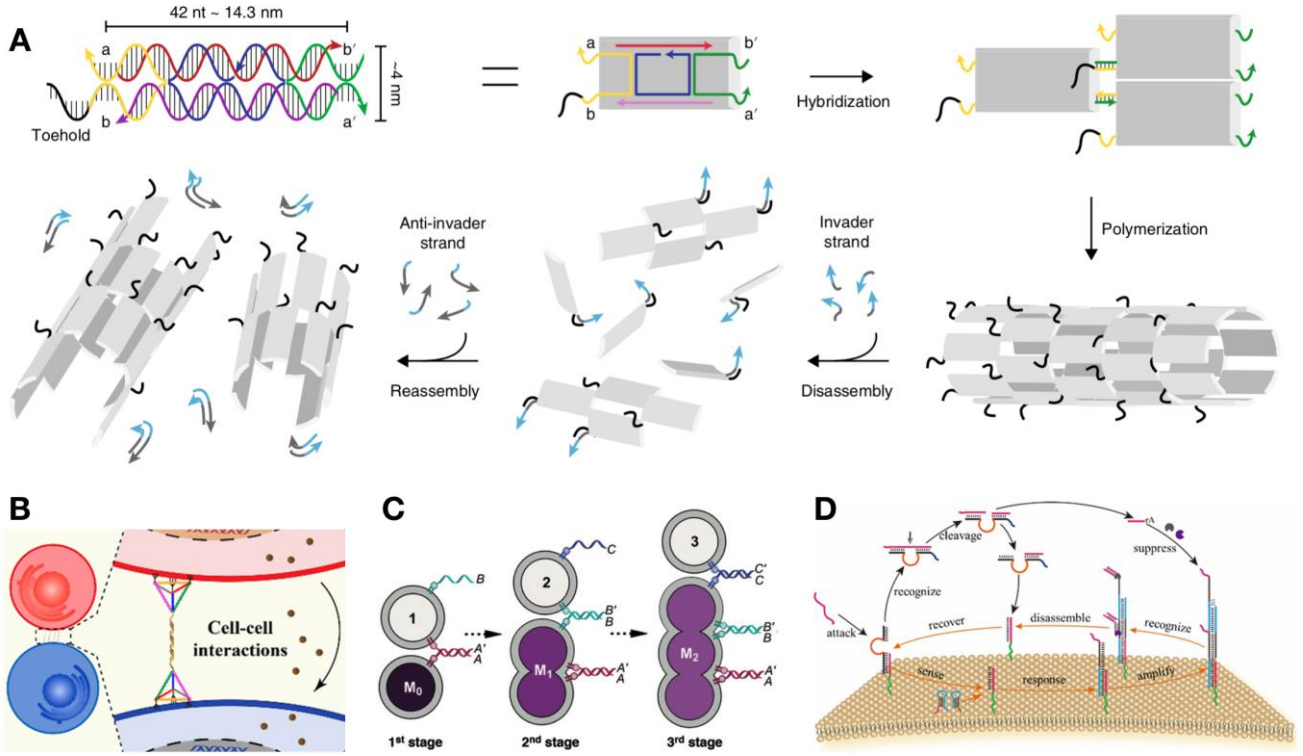

Figure 6. New biomimetic applications of nucleic acid hybridisation

(A) Five-way DNA tiles that polymerise into nanotubes via hybridisation by their sticky ends (a, b, a', b'). A toehold-complementary invader strand triggers nanotube disassembly, while an anti-invader strand displaces the invader and causes the reassembly of the nanotubes. Reprinted with permission from Green *et al*.[64] Copyright 2019 Springer Nature.

(B) Membrane-anchored DNA tetrahedra with pendant DNA strands from their top vertices to mediate cell-cell attachment and communication. Reprinted with permission from Li *et al*.[66] Copyright 2019 American Chemical Society.

(C) Sequential vesicle fusion by hybridisation of DNA strands presented on their surface. Reprinted with permission from Löffler *et al*.[67] Copyright 2017 Wiley-VCH.

(D) Logic circuits based on multi-step DNA hybridisation, displacement and enzymatic digestion, engineered on the surface of lipid membranes. Reprinted with permission from Liu *et al*.[68] Copyright 2019 American Chemical Society.

Other biological structures beyond the cytoskeleton have been also mimicked with DNA tile self-assembly. DNA nanotubes formed by longitudinal hybridisation of 40-mer DNA cylinders were designed as mimics of natural myosin filaments to study their motor function with actin.[69] These nanotubes presented pendant DNA strands on their surface spaced with nanometric precision for the attachment of DNA-decorated myosin motors. This artificial DNA scaffold allowed the study of multimeric motor proteins outside the complex structural matrix of natural myosin filaments. Cylindrical DNA scaffolds have been also applied to direct the oligomerisation of pendant proteins[70] and peptides[71] into uniform membrane nanopores. In these cases, supramolecular DNA assemblies serve as templates to guide the spatial organisation of conjugated peptides and proteins, demonstrating the potential of DNA to engineer more specific non-covalent interactions than their amino acid-based pendants. Circular DNA constructs can also template the growth of highly monodisperse lipid vesicles using internal lipid pendants as nucleation points, thus creating a DNA exoskeleton that imposes supramolecular control over membrane size.[72] Outside hybrid DNA conjugates, fully DNA-based membrane nanopores have been engineered via origami assembly of six double-helical DNA domains, anchored to the membrane of lipid vesicles with cholesterol pendants, with pore sizes of *ca.* 2 nm that mimic the conductivity of transmembrane ion channels.[73] Specific cell-cell interactions have been also established with 3D DNA assemblies, where tetrahedral DNA constructs were hydrophobically bound to the surface of living cells via cholesterol anchors, while DNA strands presented on their surface allowed specific cellular recognition (Figure 6B).[66] Overall, the production of these synthetic DNA scaffolds opens new possibilities to simplify the study of complex biological processes with spatially-defined DNA frameworks, while also providing a pool of non-natural DNA structures for new supramolecular designs, such as cubes, octahedra and tetrahedra.[62]

Outside the multimeric organisation of DNA tiles, single oligonucleotide hybridisation has been applied to reproduce a wide variety of life-like processes. Although natural DNA hybridisation is mainly driven by the multivalent H-bonded pairing of complementary nucleobases, DNA self-assembly can be also directed by non-natural moieties such as metal complexes, hydrophobic groups and secondary H-bonding scaffolds.[62] Applications of single stranded DNA recognition include the targeted binding and fusion of liposomes to model the natural traffic of vesicles in cells (Figure 6C).[67] In this example, four different liposome populations were sequentially fused by multiple DNA tags, highlighting the potential of this simple labelling method for cascade reactions similar to those in natural metabolic pathways. Further into DNA labelling of membranes, DNA-based artificial signalling systems have been engineered on the membrane of giant vesicles as auto-regulated protocells. In this example, an invader DNA displaces capping DNA from membrane-anchored initiator strands, thus triggering a hybridisation chain reaction that polymerises DNA from the surface of protocells outwards as protective shells (Figure 6D).[68] Several enzymes then mediate the digestion of the stimulus and disassembly of the polymer, which returns this adaptive system to its original state and allows repeated sensing-transduction-recovery cycles. The H-bonded polymerisation of complementary DNA strands just described can be designed to follow step-growth or chain-growth pathways depending on DNA sequence and conformation, allowing the DNA-driven supramolecular polymerisation of pendant proteins with architectural control.[74] Note that in many of these examples nucleic acids are attached to the surface of vesicles by insertion of hydrophobic pendants (*e.g.* cholesterol) into lipid membranes.[66–68,73] Thus, the hierarchical self-assembly of DNA conjugates with orthogonal supramolecular scaffolds offers modular design opportunities in structural and functional bottom-up cell engineering.

DNA signalling circuits have been also studied between synthetic microcompartments to mimic cellular communication and transduction, as communities of living organisms need to sense, adapt and distribute information between them and their environment. de Greef and co-workers engineered semipermeable microcapsules (*i.e.* proteinosomes) loaded with DNA duplexes that could sense DNA inputs by toehold strand displacement, so the release and diffusion of the displaced strand to neighbouring protocells can trigger collective responses such as reaction cascades, signal amplification and feedback inhibition between protocell populations.[75] Another remarkable example used DNA logic circuits to mimic the

natural humoral and cellular immune responses in protocells: Fusion of vesicles containing so-called pathogenic DNA with vesicles encapsulating the artificial immune response simulator (AIRS) displayed recognition, tolerance, killing and memory responses to pathogenic DNA.[76] In brief, recognition of pathogen strands releases T-cell-mimicking DNA that catalyses the production of antibody DNA to capture the pathogen, while DNA strands released along this supramolecular signalling cascade remain to provide memory to the AIRS. Artificial enzymatic regulation and metabolic homeostasis have been also reproduced in protocell models, where ribozyme activity was suppressed by high concentrations of short complementary RNA sequences, displaced upon dilution by vesicle fusion with recovery of ribozyme activity.[77] Interestingly, the observed ribozyme inhibition by partially complementary RNA sequences might explain the natural selection of RNA inhibitors from pools of random primal oligonucleotides. Together, these examples demonstrate the high versatility of nucleic acids to perform non-canonical tasks, all based on the highly specific and reversible hybridisation of complementary strands, opening exciting opportunities to engineer dynamic responses in life-mimicking supramolecular systems.

Evolution has selected the quaternary nucleobase alphabet of DNA as we know it today, but recent strategies are being explored to expand the genetic code and modify its basic structure into functional artificial analogues. One remarkable example is the alkylation of DNA's polyphosphate backbone to give uncharged *P*-methyl and *P*-ethyl phosphonate derivatives, which could hybridise natural or uncharged complementary strands (Figure 7A).[78] The authors also engineered artificial polymerases with small hydrophobic amino acids to allow the growth of complementary uncharged DNA strands from natural DNA sequences. Due to their lower solubility in water, these neutral DNA analogues could pave the way towards non-aqueous nucleic acid encoding, metabolism and catalysis. Other backbone modifications were introduced by Szostak *et al.*, who developed a non-enzymatic approach to copying natural RNA sequences by H-bonded templation of complementary imidazole-activated nucleotides.[79] This method can generate copies of the RNA template with artificial backbones based on the chosen imidazolyl precursors, such as glycan-free or arabino nucleotides.[80] For more information, the variety of artificial DNA backbones developed thus far has been recently reviewed elsewhere.[81]

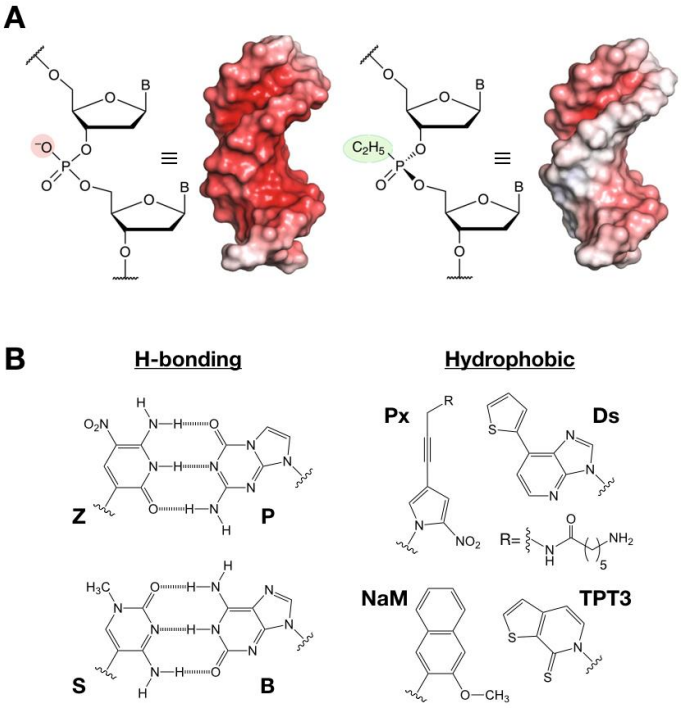

Figure 7. Chemical modifications of nucleic acids

(A) Chemical structure and calculated surface electrostatic potential of a natural DNA double helix (left) versus hybrid DNA/(*P*-ethyphosphonate)DNA analogues (right). Surface electrostatic potential increases from white to red. 'B' stands for natural nucleobases. Reprinted with permission from Arangundy-Franklin *et al*.[78] Copyright 2019 Springer Nature.
(B) Functional artificial nucleobases incorporated into natural DNA backbones based on complementary H-bonding or hydrophobic effects.

Great efforts have been also devoted to expanding the natural genetic alphabet, where two main nucleobase analogues have been explored: Predominantly hydrophobic and non-natural H-bonding scaffolds (Figure 7B).[82] Hirao and co-workers developed a high-affinity hydrophobic base pair, Ds-Px, which displayed high fidelity and efficiency in polymerase chain reaction (PCR) in presence of natural nucleotides.[83] Romesberg *et al.* reported the *in vivo* transcription of DNA containing the artificial hydrophobic nucleobases NaM and TPT_3 into messenger RNA, and its subsequent translation by transfer RNA with the complementary artificial codons.[84] This process resulted in the biosynthesis of a functional green fluorescent protein in bacteria supplemented with these artificial hydrophobic nucleobases, thus demonstrating genetic expression including synthetic genes and codons without H-bonded pairing. Although simple hydrophobic and π-π interactions can be thus incorporated into functional nucleic acid derivatives, lacking a defined recognition pattern (*e.g.* H-bonding array), the current challenge lies in encoding several hydrophobic base pairs with orthogonal recognition. Alternatively, Benner and co-workers have expanded the natural genetic alphabet with two new pairs of orthogonal H-bonded nucleobases, Z-P and S-B.[85] Not only this larger eight letter code was able to generate artificial DNA duplexes without nucleotide mismatch or unfolding, but also 'hachimoji' (*i.e.* eight letters) DNA could be transcribed into functional RNA aptamers. Hence, the tolerance of nucleic acids in chemical composition and non-covalent recognition opens access to new bio-coding languages, with important biological and supramolecular implications such as new ways to control DNA and protein metabolism and non-natural DNA architectures.

Nucleic acids have been also employed to assemble highly permeable polymeric nano/microcompartments (*i.e.* coacervates or polyion complexes) by electrostatic complexation with oppositely charged small and macro molecules.[86] These colloids behave as membraneless protocells that can exchange charged and hydrophobic molecules with their medium. Applications of these protocell models include the dissipative fibrillation of tubulin cytoskeletons within RNA-protein coacervates,[87] photo-responsive azobenzene-DNA complexes for non-natural genetic exchange,[88] and RNA catalysis inside droplets as primal metabolic microenvironments.[89]

Lipids

The structural role of lipids in living organisms is the establishment of physical boundaries (*i.e.* membranes) between cells and their environment. Cellular membranes are constituted by bilayers of amphiphilic phospholipids with embedded proteins that perform vital functions like nutrient transport and respiratory metabolism. Cells also anchor oligosaccharides to the surface of their membranes as identity and recognition patterns. In water, the hydrophobic effect drives the supramolecular arrangement of lipid bilayers, which can be easily decorated by membrane insertion of molecules bearing hydrophobic pendants. With these considerations, cells can be simplified to confined microreactors that communicate and reproduce, and so researchers aim to mimic these tasks with synthetic components and non-natural chemical routes.[9]

Natural lipids undergo enzymatic remodelling of their hydrophobic tails and polar head groups to trigger changes in membrane morphology and function. Most synthetic membranes, such as liposomes, are kinetic traps of lipids unable to exchange components with their medium. Devaraj and co-workers have developed several biomimetic approaches for lipid remodelling in synthetic vesicles that span from purely chemical to enzyme-driven methods. Native chemical ligation (NCL), was employed for the reversible exchange of the hydrophobic lipid tails with competing lysolipids triggering non-enzymatic remodelling of vesicles (Figure 8A).[90] Alternatively, azobenzene-containing lipids have also allowed reversible changes in membrane organisation, stiffness and microdomain generation with

light in synthetic vesicles.[91] While natural phospholipid synthesis is catalysed by membrane-bound enzymes, a soluble bacterial ligase, FadD10, has been repurposed to generate phospholipids by thioester activation of fatty acids and their subsequent reaction with amine-functionalised lysolipids.[92] This elegant enzymatic route allowed the *de novo* formation and growth of phospholipid vesicles in solution without pre-existing membrane-bound biocatalysts.

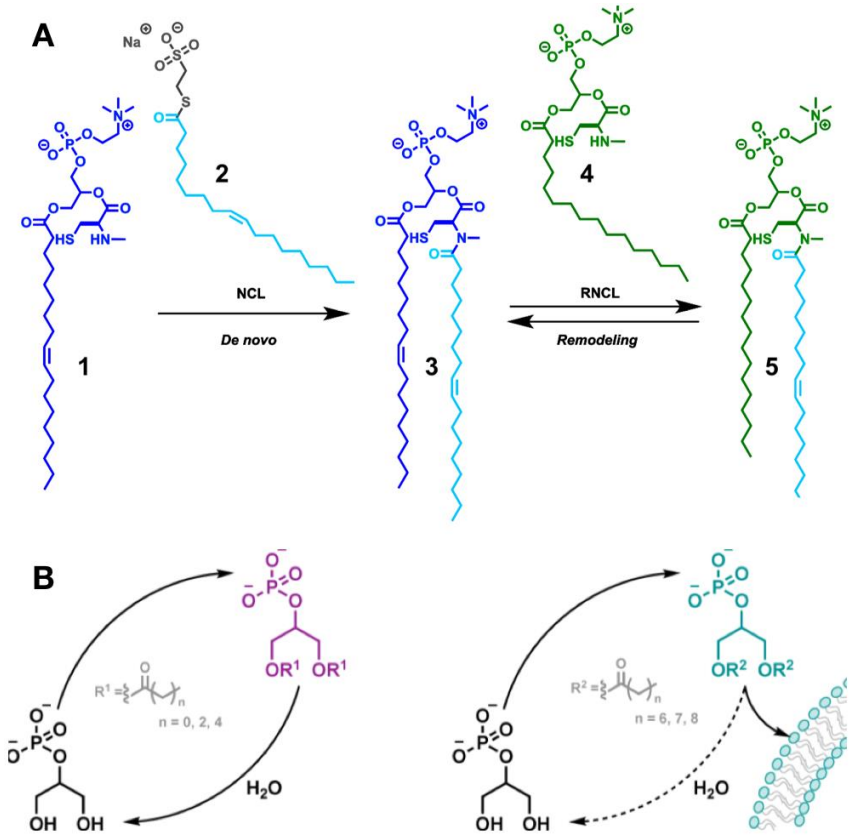

Figure 8. Artificial phospholipid membrane synthesis and remodelling

(A) *De novo* formation of unnatural self-assembling phospholipid (3) from non-assembling artificial lysolipid (1) by native chemical ligation (NCL). Exchange between lysolipids (4) and (1) allowed the artificial remodelling of pre-formed lipid membranes between states (3) and (5) by reversible NCL (RNCL). Reprinted with permission from Brea *et al*.[90] Copyright 2016 National Academy of Sciences.

(B) Selective synthesis of longer chain (n = 6,7,8) phospholipids over shorter analogues (n = 0,2,4) from the corresponding *N*-acylimidazoles and glycerol-2-phosphate. Selection takes place by self-assembly and accumulation of the former as membranes. Reprinted with permission from Bonfio *et al*.[93] Copyright 2019 American Chemical Society.

The chemical evolution of biological membranes, their lipid composition and biosynthesis are currently under intense research.[94] For example, non-enzymatic acylation of glycerol-2-phosphate by activated fatty acids (*i.e. N*-acylimidazolides) of varying chain length generated a small library of diacylglycerides screened for vesicle formation and compartmentalisation of nucleic acids (Figure 8B).[93] Since only longer chain diacylglycerides could form stable vesicles, sequential acylation-hydrolysis cycles accumulated these self-assembling products, demonstrating how supramolecular organisation can direct the evolution of lipid membranes. However, single chain amphiphiles are proposed as the original building blocks of primal lipid vesicles due to their simpler structure, as compared to diacylglycerides.[94] Szostak and co-workers have extensively studied the assembly of primal lipid vesicles from fatty acids, which can form vesicles at pH values close to the pK_a of their polar heads due to H-bonding between neutral

and ionised carboxylates.[95] Importantly, these single chain lipid vesicles are destabilised by divalent cations through metal-carboxylate coordination, which are on the other hand required as cofactors for the plausible primitive RNA catalysis. To address this incompatibility, the group developed fatty acid vesicles that generated *in situ* amide derivatives of their constituting amphiphiles, which improved vesicle tolerance to divalent cations to support RNA catalysis in their lumen.[96] This group also demonstrated that peptide catalysis inside oleate vesicles could generate a hydrophobic dipeptide (Phe-Leu) that accumulates in the membrane, inducing Darwinian selection through vesicle growth, division and survival versus non-catalytic competitors.[97] Additionally, the reaction of fatty acid vesicles with cyclic amino acid precursors allows their functionalisation with cationic residues, which drive the electrostatic binding and entrapment of RNA by these minimal protocells.[98] The incorporation of peptides to the polar head of lipids can also direct their accumulation at specific membrane domains, providing topological control in vesicle composition.[99]

As previously described for peptide derivatives (Figure 5), the fuelled assembly of out-of-equilibrium supramolecular structures is one of the trademarks of living organisms. This principle has been applied to the assembly and biological function of transient lipid vesicles. An artificial metal amphiphile C_{16}TACN·Zn^{2+} was assembled into vesicles in presence of adenosine triphosphate (ATP), whose multivalent interaction with surfactant molecules diminished their critical aggregation concentration and stabilised the supramolecular structure (Figure 9A).[100] Enzymatic hydrolysis of ATP by potato apyrase yields monovalent adenosine monophosphate (AMP) and two molecules of orthophosphate (P_i), which are much less efficient in stabilising the non-covalent C_{16}TACN·Zn^{2+} bilayers, which triggers vesicle disassembly. Additional supply of ATP allowed up to seven cycles of dissipative vesicle assembly, eventually hampered by the accumulation of AMP and P_i. Importantly, this fuelling process is fully supramolecular (*i.e.* multivalent Coulombic interaction) and takes places without formation of any new covalent bonds unlike previous examples of dissipative self-assembly (Figure 5). The authors later demonstrated that energy dissipation could be autonomously triggered by the cooperative action of the assembled supramolecular building blocks without need of an external dissipator (*i.e.* enzyme).[101] This self-regulated system consists of C_{16}TACN·Zn^{2+} vesicles assembled with a monophosphate scaffold, which can be cooperatively templated and hydrolysed by two proximal Zn^{2+} centres, as found in their assembled state.

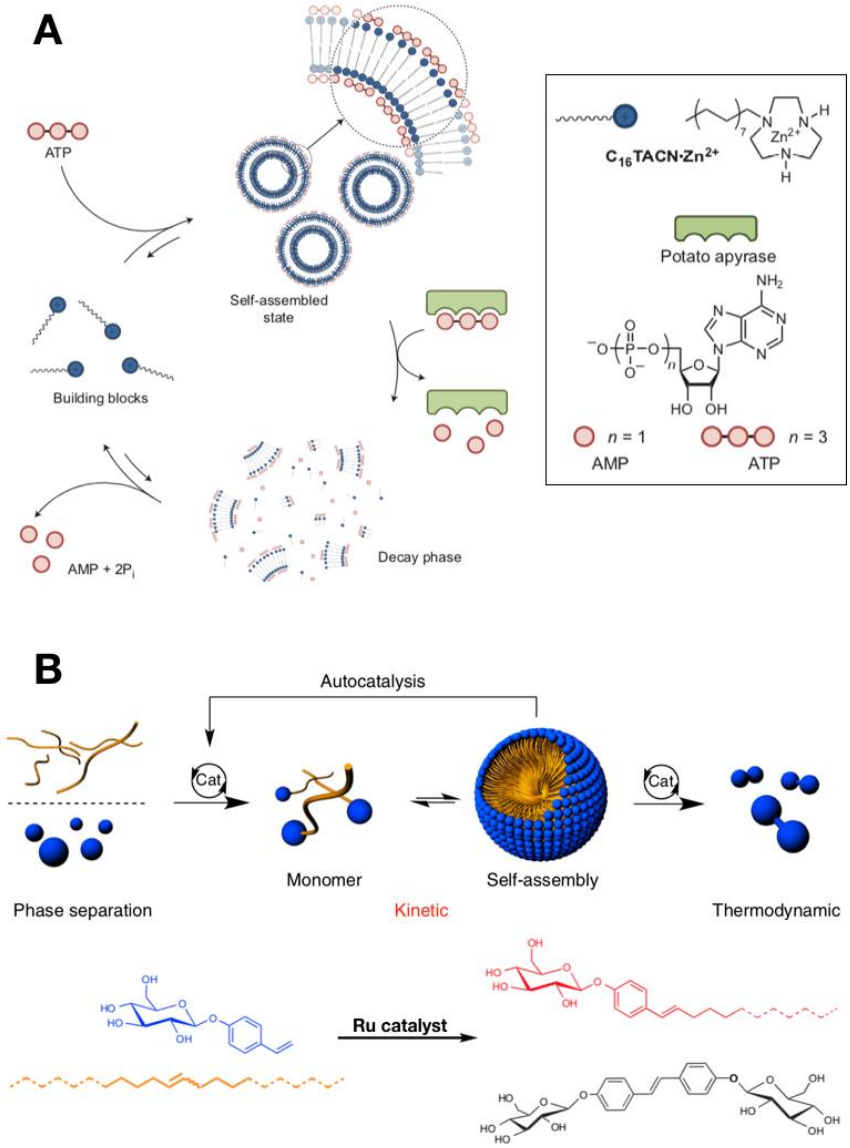

Figure 9. Synthetic out-of-equilibrium vesicles

(A) Self-assembly of the metallo-amphiphile C_{16}TACN·Zn^{2+} in the presence of ATP by multivalent Coulombic interactions. Hydrolysis of ATP by potato apyrase leads to vesicle disassembly, which can be re-assembled with further supply of fuel (*i.e.* ATP). Reprinted with permission from Maiti *et al.*[100] Copyright 2016 Springer Nature.

(B) Interfacial Ru-catalysed synthesis of self-assembling glycolipids, whose micelles template the precursors and thus physically catalyse the formation of more monomer (*i.e.* glycolipid). The kinetic glycolipid product is eventually converted by the same Ru catalyst into non-assembling disaccharides, causing the thermodynamic evolution to a disassembled state. Reprinted from Colomer *et al.*[102]

Transient self-replicating systems are able to catalyse the formation of copies of their supramolecular structure until their spontaneous degradation into disassembled non-catalytic products. The first synthetic autopoietic (*i.e.* self-perpetuating) micelles were developed by Giuseppone *et al.*, who demonstrated the supramolecular basis of self-replication and autocatalysis in dynamic combinatorial amphiphile libraries.[103] Transient behaviour was shown by Fletcher and co-workers on a ruthenium-mediated alkene metathesis platform that formed single chain glycolipids, whose micelles physically catalysed (*i.e.* templated) the conversion precursors into twin micelles (Figure 9B).[102] The

same Ru catalyst also mediated the degradation of these glycolipids and the resulting disassembly of the self-replicator, but the slower kinetics of this second step ensured the initial micelle formation. As such, the supramolecular state of this system is controlled by the kinetics and thermodynamics of the assembly and disassembly steps, respectively. Moreover, competition between glycolipid precursors leads to selection for the self-assembling products with faster formation kinetics.[104] In later work, the authors introduced disulphide chemistry to link and exchange the head and tail of similar single chain amphiphiles, leading to chemically fuelled dissipative self-replicators.[105] Here, hydrogen peroxide works as fuel by regenerating micelle precursors from their degradation products to close the out-of-equilibrium self-assembly cycle. In essence, these minimalistic systems help understand the origin and fate of cellular membranes by imitating complex persistence and adaptability responses found in biology.

It has been discussed before how vesicle fusion can be directed by decorating their surface with complementary DNA strands,[67] but more challenging is the fission of artificial vesicles and transmission of their contents to daughter vesicles in a mitosis-like process. Han *et al.* developed a vesicle-in-vesicle (VIV) system by osmotic deformation of precursor giant unilamellar vesicles, whose inner compartment was loaded with DNA as a eukaryotic cell model.[106] PCR could be performed inside these VIVs, leading to an internal osmotic stress that triggered their division into two identical VIVs with the same genetic information as their mother VIV.

Overall, lipid vesicles are versatile tools to study the compartmentalisation of life-like processes and the supramolecular determinants of membrane metabolism, adaptation and cell division.[9] As shown here, artificial vesicles offer great flexibility in design and function by incorporation of lipid or polar head surrogates, ranging from metal complexes[100] to photo-switches[91] and peptides.[98] For more details, the preparation of liposomes and reconstituted cellular membranes for biomedical applications has been thoroughly reviewed elsewhere.[107] Finally, we must highlight the growing importance of microfluidics in the development of more complex liposome formulations and other artificial microcompartments (*e.g.* emulsion droplets),[108] which provides high precision and reproducibility to control the structural features of model protocells such as shape,[109] encapsulation efficiency and polydispersity.[110]

Carbohydrates

Cells display a crown of oligosaccharides on their surface (*i.e.* glycocalyx) that serve as supramolecular recognition points for cell-cell interactions and surface attachment.[111] Several natural and synthetic molecules can recognise and bind glycans with high affinity and selectivity, including proteins such as antibodies and lectins (*i.e.* specialised carbohydrate-binding proteins), nucleic acid aptamers and boronic acid derivatives.[112] These molecules bind glycans through supramolecular interactions, mainly H-bonding and hydrophobic interactions, with the exception of reversible covalent bonds established between boronic acids and saccharides. Lectins stand out amongst other glycan binders due to their capacity to oligomerise and thus create multimeric interactions, which improves their monomeric affinity and selectivity, also allowing the binding of multiple glycosylated (bio)materials into supramolecular networks.

Artificial glycosylated membranes have been obtained by supramolecular attachment of saccharides to pre-formed membranes that mimic natural cell recognition phenomena. For example, supported lipid bilayers with biotin handles allowed streptavidin-mediated decoration of their surface with sialoglycans as modular platforms to study the multivalent attachment of viruses to host cells.[113] Alternatively, hydrophobic insertion of saccharides with cholesterol pendants also generated artificial vesicle-supported glycocalyces, which could be selectively and reversibly linked by multimeric lectins to other protocells as communities or prototissues.[114] Artificial hydrophobic pendants, such as fluoroalkyl-pyrenes, have been also used to attach glycans to vesicle membranes, whose recognition and uptake by human cells *in vitro* could be tuned via enzymatic modification of their sugar composition.[115]

Glycan scaffolds have been also covalently conjugated to artificial vesicles as biomedical tools and cell mimics. Rather than following sequential vesicle assembly and glycosylation steps, these systems are usually prepared from amphiphilic glycans that readily assemble into the final structure by one-faced hydrophobic packing. Percec and co-workers have developed synthetic amphiphilic glycodendrimers that spontaneously self-assemble in water as glycodendrimersomes (GDSs).[116] These are polymeric vesicles with surface-bound saccharides that mimic the organisation of a glycocalyx in a protocell (Figure 10A). Glycodendrimers with different branching and glycan substitution self-assemble as GDSs with distinct cell-like glycan microdomains on their surface, allowing control over GDS affinity for specific lectins.[117] The authors further developed onion-like multilamellar GDSs that mimic the organisation of natural multilamellar vesicles, where the size and number of layers of GDSs can be adjusted with glycan topology and concentration.[118] The lack of electrostatic repulsion between their polar faces allows the multilamellar assembly of GDSs by H-bonding between ethylene oxide pendants in water.[119] Remarkably, isolation and reconstitution of bacterial membranes with glycodendrimers allowed the assembly of hybrid bioactive membranes that combined natural and artificial surface glycans.[120] Not only these semisynthetic vesicles could incorporate bacterial membrane proteins that retained transporter function, but also bacterial surface lipopolysaccharides to replicate and modulate natural glycan-lectin recognition. Linear amphiphilic block-glycopolymers can also self-organise in water as cell-sized polymeric vesicles, consisting of bilayers of their hydrophobic block with inner and outer surfaces covered with their glycan segments.[121] These polymersomes could be recognised by biological receptors, such as lectins and bacterial cells, with adjustable affinities based on their carbohydrate composition.

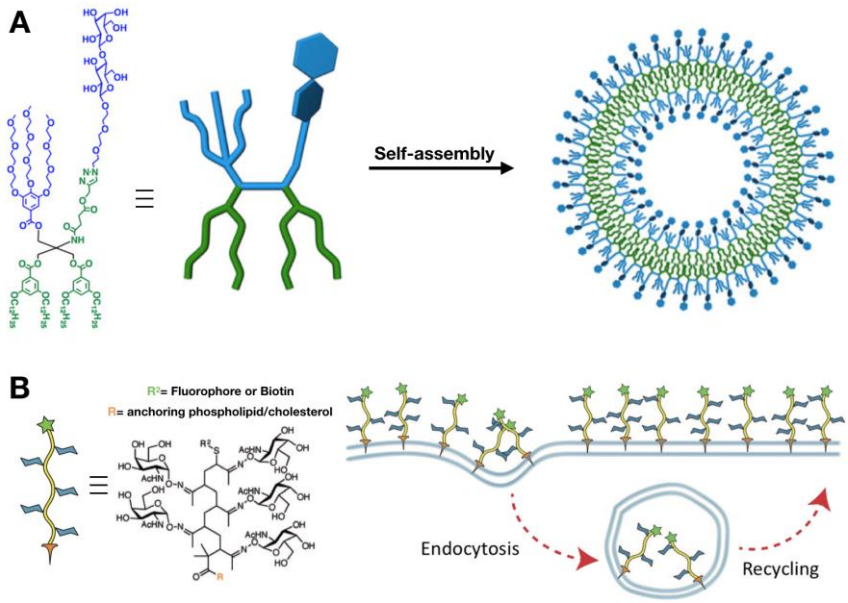

Figure 10. Artificial decoration of synthetic and cellular membranes with glycans
(A) Amphiphilic dendrimer with hydrophobic aliphatic tails (green) and polar glycosylated poly(ethylene glycol) chains. This structure self-assembles in aqueous solution into synthetic vesicles (*i.e.* glycodendrimersomes) with inner and outer surface glycans. Reprinted with permission from Xiao *et al.*[120] Copyright 2016 National Academy of Sciences.
(B) Lipid-anchored glycopolymers with fluorescent or biotin tags. Glycolipids with cholesterol anchors can be internalised by cells into vesicles that recirculate into the membrane, thus working as depot to maintain glycopolymers on the surface of living cells for days. Reprinted with permission from Woods *et al.*[122] Copyright 2015 Wiley-VCH.

The artificial modification of membrane glycans *in vivo* can be employed to tune recognition and function in living cells. For example, synthetic glycosylated phospholipids could be taken up by bacteria and incorporated into their membrane through natural metabolic pathways, allowing artificial edition of their cellular envelopes.[123] Likewise,

synthetic glycopolymers with cholesterol pendants could be incorporated into the glycocalyx of mammalian cells to improve the survival of non-cancerous cells in a metastatic animal model.[122] Capitalising on the natural traffic of cholesterol in cells,[124] these cholesteryl-glycopolymers could be endocytosed into depot vesicles from where they could be recycled to the cellular membrane, allowing long-lived glycocalyx modifications for at least ten days (Figure 10B). Alternatively, the glycocalyx of human cells could also be modified semi-synthetically by transfection with DNA encoding artificial mucin synthesis.[125] Mucins are natural heavily glycosylated proteins, which in this example contained hydrophobic anchors for membrane attachment and display on the surface of cells. Modular DNA design allowed the variation of glycan structure and other mucin domains, such as cytoplasmic motifs and reporting groups, proving the versatility of this semi-synthetic approach.

Outside membranes, synthetic oligosaccharide scaffolds have been also developed for supramolecular recognition and self-assembly in solution. Bifunctional Janus glycopeptides bearing two distinct glycan faces were synthesised for complementary recognition by chimeric lectins with two oppositely oriented binding surfaces.[126] These two synthetic biomaterials self-assembled by specific recognition into ordered networks that mimic the extracellular matrix, while chimeric lectins could also cross-link glycosylated vesicles by imitating the cellular junctions found in natural tissues. Given their length and flexibility, polymers can interact via multiple pendants with one or more targets, allowing multivalent interactions and the establishment of large supramolecular networks. For example, some glycopolymers allow modulation of sugar-lectin binding by adjusting glycan density and incorporation of proximal non-binding units.[127]

As shown, new synthetic and semi-synthetic approaches have allowed the replication and modulation of supramolecular glycan recognition. These methodologies have generated vesicles with artificial glycocalyces, which allowed the rewriting of surface glycans in living cells, and opened new possibilities in biomolecular self-assembly. Importantly, accessible mono and short oligosaccharides are here used to decorate vesicles for sugar recognition, but new synthetic methods are still required to access the complex structure of natural glycocalyces efficiently.

FULLY ARTIFICIAL BUILDING BLOCKS

Synthetic molecules not bearing the basic biological monomers (*i.e.* amino acids, nucleotides, phospholipids and monosaccharides) have been also studied as biomimetic supramolecular architectures.[1,6,8,10] Instead of implementing biomolecular self-assembly, these strategies capitalise on the advances of artificial supramolecular systems to guide structural design and function.[128] Thus, the possibilities to access life-like behaviour synthetically have been expanded well beyond the building blocks evolved by living organisms. The vast chemical and functional diversity found in these synthetic systems is revolutionising supramolecular chemistry from fundamentals to applications. In this section, some of the most remarkable examples of fully artificial biomimetic supramolecular architectures are summarised.

Motion is an essential trait of living organisms from the molecular to the cellular and tissue levels. Bacterial flagella and muscular fibres are two examples of natural motors that transform chemical fuel into motion. Many synthetic approaches have been explored to amplify molecular movement along supramolecular assemblies into higher-order motion.[129] Feringa *et al.* reported an amphiphilic photo-responsive rotor that could self-assemble in water as aligned nanofiber bundles, resembling the hierarchical organisation of natural muscular fibres (Figure 11A).[130] UV light induced molecular rotation and thus mechanical stress within these nanofiber bundles, causing the coordinated muscle-like contraction of the ensemble with directional control towards the light source. Remarkably, liquid crystal films doped with very small amounts of such photorotors (1% w/w) induced rotary motion in supported submillimetre glass rods when irradiated with UV light, demonstrating non-covalent large scale movement amplification from a molecular to microscopic level.[131] Katsonis and co-workers have developed self-propelling droplets made from an achiral liquid crystal doped with small amounts of chiral photorotors (Figure

11B).[132] In this system, the chirality of the small molecular rotors is transmitted to the achiral liquid crystal by supramolecular packing, which amplifies the helical ordering of the dopant across the whole droplet. These droplets swim in helical trajectories due to the Marangoni effect, which is generated by solvent thrust at heterogenous droplet surfaces. Irradiation of these droplets with light induced the inversion of the rotor's chirality and by extension switched the sense of helical motion. The group of van Hest developed another remarkable example of artificial cell-like motion device by employing supramolecular bowl-shaped polymeric vesicles, known as stomatocytes, loaded with platinum nanoparticles that catalysed the production of propelling oxygen from hydrogen peroxide (Figure 11C).[133] Movement speed and direction could be tuned with temperature by reversibly opening and closing the catalytic centre with thermo-responsive polymers. This self-propelled supramolecular nanomotor can thus sense its environment and regulate motion with artificial valves. Korevaar *et al*. recently developed an elegant droplet system capable of autonomously organising the disposition of so-called 'source' and 'drain' populations in the mesoscale by self-assembly and gradient flows (Figure 11D).[134] Here, droplet movement relies on the interplay between repulsive Marangoni flows and attractive uptake of supramolecular filaments, which together determine the dynamic positioning of droplets, analogous to the spatiotemporal regulation of microtubule filaments in cells. This group also engineered propagating movement (*i.e.* waves) in poly(acrylic acid) hydrogels, which showed transient contraction and expansion of the polymer network.[135] This behaviour is based on three distinct supramolecular states, which evolve by localised supply and diffusion of acid and metal ions: i) Electrostatic repulsion between anionic carboxylates, ii) contraction by metal-carboxylate coordination, and iii) protonation of carboxylates and metal release.

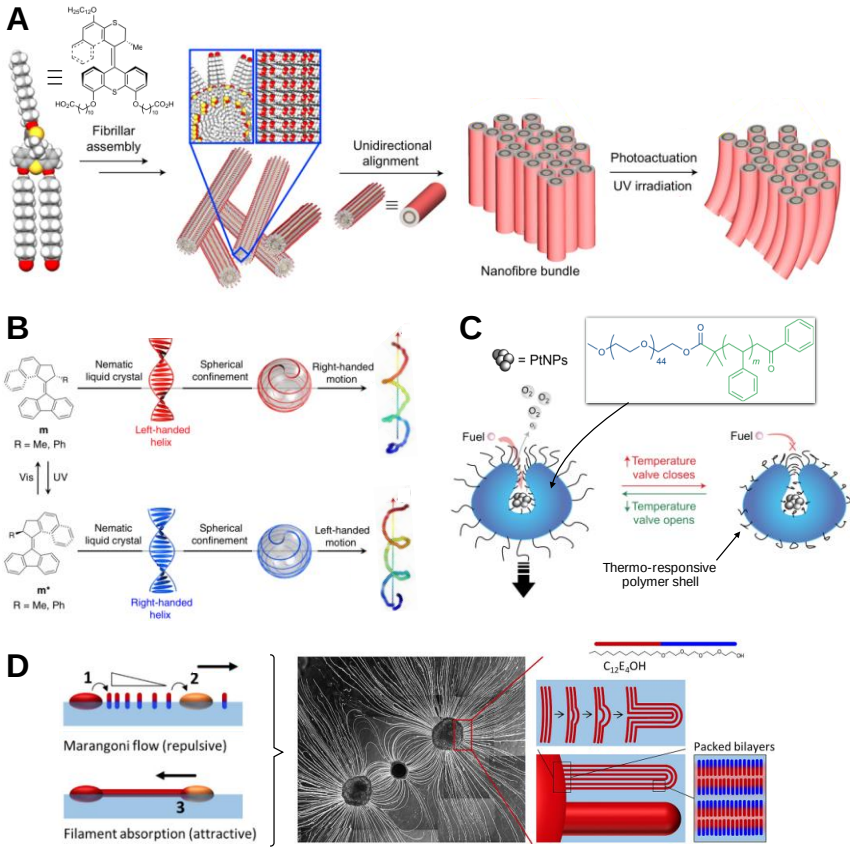

Figure 11. Motion in synthetic supramolecular systems

(A) Self-assembly of amphiphilic molecular rotors into nanofibrillar bundles that contract in a muscle-like motion when irradiated with UV light due to conformational changes in the rotor. Reprinted with permission from Chen *et al*.[130] Copyright 2018 Springer Nature.

(B) Chiral liquid crystal droplets with spirally organised molecular motors (m, m*). UV/visible light irradiation induces a helical sense inversion due to m-m* interconversion, which translates into the reorientation of the Marangoni helical motion of the droplets. Reprinted from Lancia *et al*.[132]
(C) Polymeric stomatocytes bearing a thermo-responsive polymer shell that opens/closes the access of fuel (hydrogen peroxide) to the inner cavity, where platinum nanoparticles (PtNPs) catalyse the formation of oxygen that propels the stomatocytes directionally (arrow). Reprinted with permission from Tu *et al*.[133] Copyright 2017 Springer Nature.
(D) Autonomous spatiotemporal organisation of droplets. Left: Interplay between repulsive Marangoni flows by release of amphiphile molecules from source amphiphile droplets (red, 1) that can be taken up by drain droplets (orange, 2), and attraction of drain droplets by absorption of amphiphile filaments (3). Straight arrows indicate movement directionality. Right: Optical microscopy image of a centred drain droplet absorbing supramolecular amphiphile filaments generated by two surrounding source droplets. Reprinted from van der Weijden *et al*.[134]

As mentioned earlier, the dynamic supramolecular fibrillation of cytoskeletal proteins controls important cellular functions such as phagocytosis and cell division.[17] Non-equilibrium supramolecular polymerisations are currently under thorough study to unravel the determinants of dynamic self-assembly, shedding light into supramolecular thermodynamics, cooperativity, energy dissipation and pathway selection, amongst other complex behaviour.[11,53,136] Artificial supramolecular systems have been developed to mimic and control dynamic protein fibrillation with alternative materials to natural amino acids. For example, George *et al.* designed transient supramolecular fibres assembled from dormant charge-transfer complexes (CTCs) made from coronene and an aldehyde-equipped viologen scaffold (Figure 12A).[137] Imine formation upon reaction with a series of alkyl amines induced the supramolecular assembly of fibres, whose formation and disassembly could be tuned with pH via amine (de)protonation and imine hydrolysis, respectively. Kudernac and co-workers employed V-shaped amphiphiles bearing azobenzene photoswitches to trigger the strain-driven disassembly of supramolecular nanotubes (Figure 12B).[138] The *cis* isomerisation of azobenzenes deplanarised the supramolecular building blocks and thus induced the accumulation of strain until a threshold breaking point. Notably, full conversion to the *cis* isomer was not required to trigger disassembly, as these nanotubes preferably dissipate strain by amplifying local defects along the supramolecular structure.[139] Another intriguing example has been recently introduced by van Esch and co-workers, who mimicked the strain-stiffening response of natural cytoskeletal fibres with synthetic fibrillar networks, which from hydrogels that can be orthogonally self-assembled with liposomes as cells-in-matrix models of biological tissues.[140]

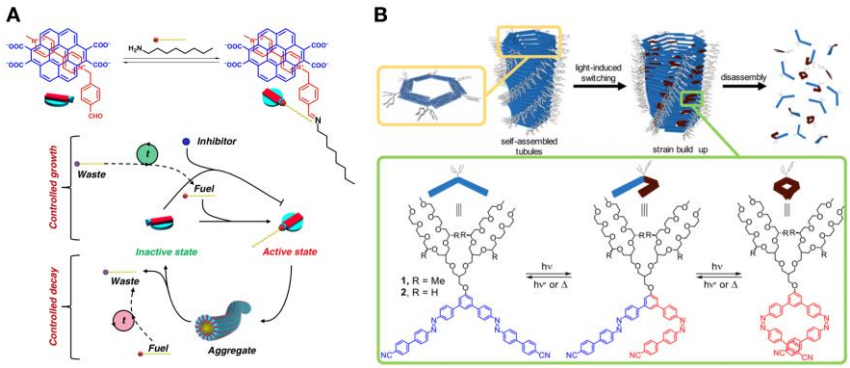

Figure 12. Dynamic fibrillation of synthetic scaffolds
(A) Self-assembly of coronene (blue) and viologen (red) stacks triggered by conjugation with aliphatic amines (fuel) via dynamic imine bonds. Polar non-fibrillating amines can be used as inhibitors that delay fibre growth in competition with aliphatic amines. Temporally controlled (t) acidification caused the disassembly of the fibres due to the protonation of fuelling amines into unreactive ammonium salts (waste). Likewise, controlled pH increments converted waste into fuel and trigger fibrillation. Reprinted from Jain *et al*.[137]
(B) Self-assembly of chiral nanotubes from amphiphilic *trans-trans* azobenzene dimers. Light-induced *trans*-to-*cis* transitions built up strain up to a rupture point, where the strain is amplified

along the supramolecular structure causing its rupture. Reprinted with permission from Fredy *et al*.[138] Copyright 2017 National Academy of Sciences.

Life processes take place in confined microenvironments (*i.e.* cells and organelles) that coordinate structural and functional tasks in response to their medium. Cell-like communication and adaptation has been engineered synthetically between many supramolecular compartments.[9] The modular assembly of these responsive cell mimics opens exciting opportunities to design protocell communities with complex life-like responses. For example, predatory behaviour was installed in coacervates containing proteinase K that could electrostatically bind and digest proteinosomes by action of this enzyme, thus transferring the prey's cargo into the predator compartment (Figure 13A). Proteinosomes can also be guests of supramolecular coacervates, where both synergistic and antagonistic responses can be established through chemical communication between compartments (Figure 13B).[142] In this system, enzymatic digestion of glucose by the guest releases hydrogen peroxide into the host that triggers a positive fluorescence response, while high glucose turnover caused a drop in pH that induced the disassembly of the coacervate into small vesicles. Bayley and co-workers have developed 3D clusters of emulsion droplets that imitate cellular communication in tissues, where light induces the patterned expression of membrane proteins that allow electrical communication between these protocells.[143] Another noteworthy development are phagocytic dendrimersomes (Figure 10A), which can take up living bacteria solely by non-covalent membrane adhesion, so the flexibility of the polymeric vesicles allowed invagination and endocytosis of the pathogen (Figure 13C).[144] Besides these interesting responses, it is also important to synthetically access the variety of cellular morphologies required for biological function. To this end, polymeric vesicles have been assembled in several cell-like shapes (*e.g.* spheres, rods and discs) by kinetically trapping the desired morphologies during osmotic changes.[145] This technology has recently translated into structural and functional mimics of red blood cells, obtained by loading haemoglobin into stomatocytes (*vide supra*) for oxygen transport.[146]

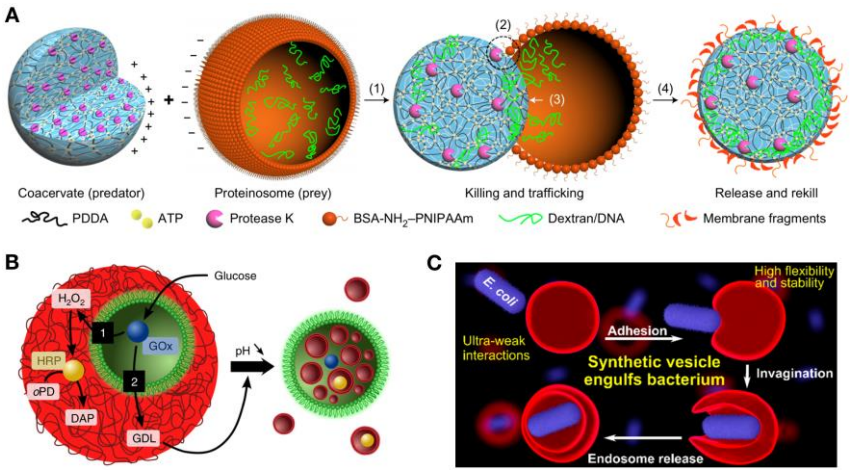

Figure 13. Biomimetic interspecific relationships by artificial supramolecular compartments

(A) Predatory behaviour of coacervates composed of poly(diallyldimethylammonium) (PDDA), ATP and proteinase K against prey proteinosomes assembled from bovine serum albumin (BSA)-poly(*N*-isopropylacrylamide) (PNIPAAm) conjugates. The electrostatic binding of predator and prey (1) is followed by proteinase-induced digestion of the prey (2), transfer of the prey's payload (dextran or DNA) into the predator (3), and release of the predator with prey components (4). Reprinted with permission from Qiao *et al*.[141] Copyright 2017 Springer Nature.

(B) Synergistic (1, fluorescence emission) and antagonistic (2, disassembly) responses of host coacervates to guest proteinosomes loaded with glucose oxidase (GOx), which triggers either response by production of hydrogen peroxide or acidification, respectively. Reprinted from Martin *et al*.[142]

(C) Phagocytic response of supramolecular dendrimersomes (Figure 10A) against living bacteria by weak adhesive forces and reorganisation of these flexible polymeric vesicles. Reprinted with permission from Kostina *et al*.[144] Copyright 2019 American Chemical Society.

Specific ligand-receptor binding is the basis for deciphering cellular recognition and communication. Given the central role of carbohydrates in direct cell-cell interactions, many efforts have focused on developing synthetic glycan receptors with high specificity. Davis and co-workers have developed 'synthetic lectins' based on cages with top and bottom hydrophobic caps, connected by polar urea likers projected towards the cavity (Figure 14A).[147] Thus, glucose can bind to this cage with good affinities by segregation of hydrophobic and hydrophilic interactions in aqueous media. Alternatively, long-range cellular communication is mainly mediated by membrane-bound receptors, which bind and respond to chemical signals produced by other cells. Mechanistically, ligand binding induces a conformational change in its receptor that triggers a specific signalling pathway within the cell. This is a highly interconnected and complex network that orchestrates cellular metabolism, and so the mimicking of these systems with fully synthetic materials constitutes an exciting challenge. An artificial helical receptor has been embedded in lipid membranes, where the metal complex exposed on the surface recognises chiral ligands that induce a preferential helix conformation, translating into a fluorescence response at the opposite face (Figure 14B).[148] It is important to note the achiral structure of the transmembrane α-aminoisobutyric folded oligomer (foldamer), which allows a uniform induction of its helical configuration by the 'controller' metal complex.[149] This structural change mimics that of G protein-coupled receptors, with different ligands inducing opposite agonist-antagonist responses with selective and competitive binding profiles. Beyond this peptide-based design, abiotic foldamers (*i.e.* not based on biological molecules) are currently under study as fully artificial mimics of biomacromolecules, displaying dynamic structures with secondary, tertiary and quaternary protein foldings. For example, Huc and co-workers have developed abiotic helical foldamers based on aromatic amide backbones, where a strategic disposition of H-bonding groups promoted the cooperative folding of this aromatic helices into distinct helical domains with multimeric bundling.[150] Multiple parallel and antiparallel helix-helix interfaces could be established via H-bonding within one single foldamer chain, demonstrating the vast potential of this synthetic structure to adopt complex bio-mimetic tertiary structures *à la carte*.[151]

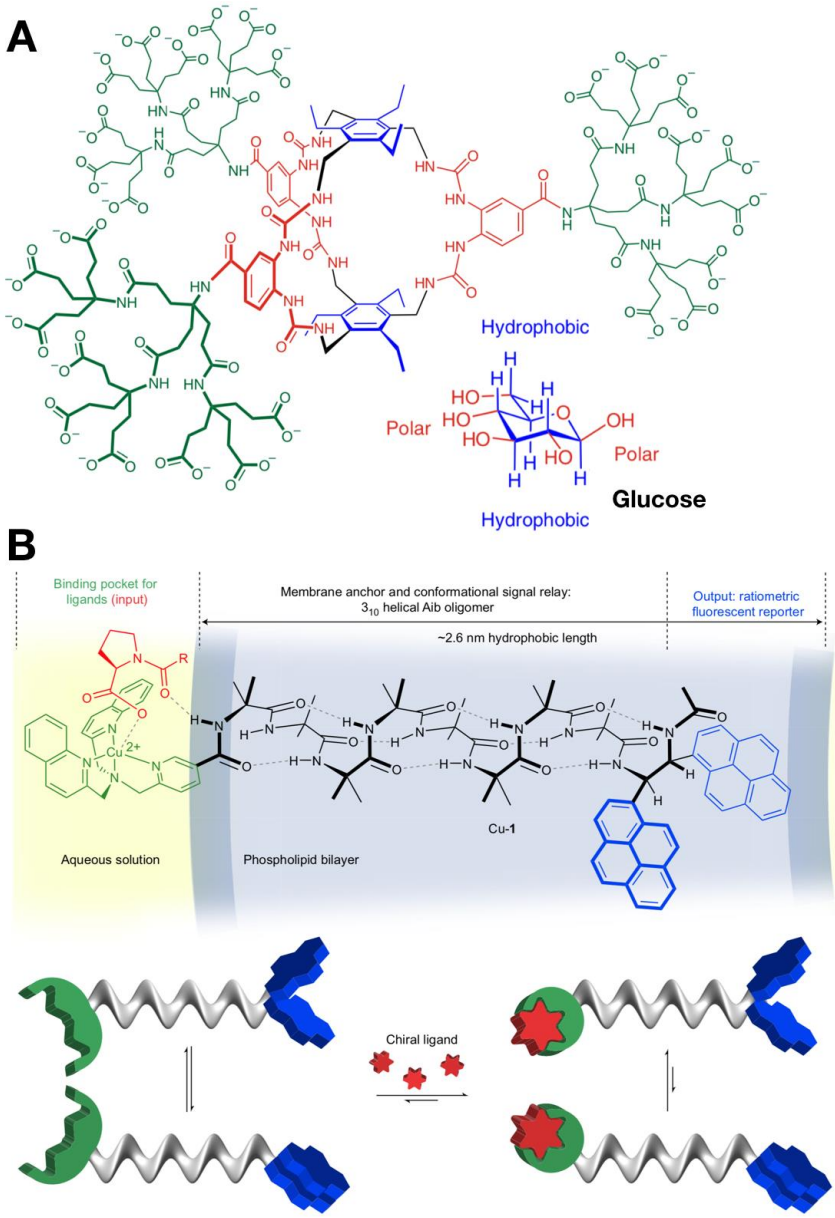

Figure 14. Artificial receptors for supramolecular recognition and transduction

(A) Macrocyclic cage with top/bottom hydrophobic faces (blue) and polar urea linkers (red) complementary to the axial and equatorial disposition of glucose's hydrogens and hydroxyl groups, respectively. Reprinted with permission from Tromans *et al*.[147] Copyright 2019 Springer Nature.

(B) Membrane-embedded helical receptor that binds at its superficial metal complex (green), inducing a preferential helical sense that translates into conformational and emissive changes of fluorescent pyrene dimers (blue). Aib: α-aminoisobutyric acid. Reprinted with permission from Lister *et al*.[148] Copyright 2017 Springer Nature.

CONCLUSIONS AND PERSPECTIVE

This review covers the chemical foundation of minimalistic supramolecular systems with like-like behaviour, addressing the connection between molecular design, self-assembly and function. We put in context the supramolecular interactions between the natural biomolecular scaffolds (*i.e.* amino acids, nucleotides, lipids and glycans) and the rational design of derivatives that tune their native function and open access to new applications.

Thus, for example, DNA derivatives can perform non-canonical tasks, such as dynamic fibrillation,[64] cell-cell junctions,[66] membrane pores[73] or immune responses,[76] all based on the natural self-assembly of DNA. We also show how fully synthetic molecules and their supramolecular ensembles can perform biological functions and even establish complex interspecific relationships within communities. Importantly, this review does not focus on one particular building block or technology that enables life-like behaviour, but rather provides a broad overview of the variety of molecules, chemistries and supramolecular assemblies that mimic all sorts of biological responses, from minimalistic systems to *in vivo* cellular engineering.

Supramolecular systems offer great design flexibility to engineer *de novo* the multivalent, reversible and directional non-covalent interactions that orchestrate life at the molecular level.[6] Thus, dynamic stimuli-responsive behaviours can be mimicked and controlled artificially, like reversible (de)polymerisations and competitive binding, by rational designs inspired in natural supramolecular structures. Alternatively, we have also shown how natural supramolecular interactions can be replaced for alternatives of very distinct character, such as hydrophobic surrogates of DNA's backbone[78] and nucleobases,[83,84] demonstrating the potential of artificial systems to expand the functionality of living organisms with scaffolds not evolved naturally.

The recent and growing developments in this field are making a strong impact in supramolecular and systems chemistry,[11] providing valuable insights into current hot topics such as dissipative[53] and out-of-equilibrium[136] self-assembly, self-replication[12] and mechanical transduction.[129] For many decades, supramolecular chemistry has focused on frozen thermodynamic assemblies, while the interest now lies in life-mimicking kinetic assemblies and their spatial and temporal control. Understanding and engineering these dynamic processes *de novo* is key to unravel the supramolecular basis on life, which will ultimately drive innovation in biomedicine, nanotechnology and advanced materials.

Despite the numerous breakthroughs presented here, many challenges remain ahead to fully mimic cellular life with artificial supramolecular structures. Further developments of proof-of-concept systems would be required to translate model prototypes into realistic settings and applications. For example, artificial small-molecule receptors could be implemented in the reading of sequence-specific biopolymers (*e.g.* glycocalyces and DNA), on-membrane ligand binding and logic gates could also be coupled to internal signalling and transduction cascades, and communication between minimal life-like systems is needed to integrate multiple tasks orthogonally. A feasible approach to access this level of complexity would be the convergent bottom-up assembly of increasingly sophisticated supramolecular units with interconnected functions.

Chemistry, the discipline of science that designs and creates new matter, plays a key role in the bottom-up assembly of minimal organelles and artificial cells. Beyond the generation of the primal building blocks and chemical reactions plausible in prebiotic conditions,[152] the attention is being focused on the role of supramolecular chemistry in the most basic systems from where life could have emerged. In our days, we have witnessed the outstanding pioneering discoveries that have shown how simple supramolecular systems could mimic the basic processes that sustain life. Energy dissipation, chemotaxis, communication, predatory behaviour, or even Darwinian evolution are just a few examples of the latest achievements. Clearly, the rational design of the next generation of biomimetic synthetic supramolecular systems will be critical to understand the origins of life and the derived industrial and therapeutic applications of the most fundamental principles of living systems.

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AUTHOR CONTRIBUTIONS

I.I. and J.M. contributed equally to the writing of this manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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