Molecular bionics - engineering biomaterials at the molecular level using biological principles

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Life and biological units are the result of the supramolecular arrangement of many different types of molecules, all of them combined with exquisite precision to achieve specific functions. Taking inspiration from the design principles of nature allows engineering more efficient and compatible biomaterials. Indeed, bionic (from bion-, unit of life and -ic, like) materials have gained increasing attention in the last decades due to their ability to mimic some of the characteristics of nature systems, such as dynamism, selectivity, or signalling. However, there are still many challenges when it comes to their interaction with the human body, which hinder their further clinical development. Here we review some of the recent progress in the field of molecular bionics with the final aim of providing with design rules to ensure their stability in biological media as well as to engineer novel functionalities which enable navigating the human body.

Keyboards: molecular bionics, biomaterials, supramolecular chemistry, organic chemistry, topology, biomedicine

1. Introduction
Since Aristotle, nature observation has been translated into engineering solutions to solve human problems. Nowadays, and thanks to the huge development of science and engineering techniques, we can understand and almost imitate nature systems, with biomimetics and bionics becoming growing disciplines in materials design. The latter, although seeming related to sci-fi, is a well-established medical field, where engineering, physics and chemistry are combined to create replacement parts of the human body. The question is, can we do this at the molecular level? Can we design bionic units that mimic specific biological functions and/or introduce operations that do not exist in Nature? This is what we refer to as molecular bionics: the effort to unravel the complexity of the basic architectures of life, with the final aim of using its design principles for the construction of artificial systems sharing the distinctive characteristics of their biological counterparts. Bionic or life-like properties would comprise reversibility, dynamism, functioning under out-of-equilibrium conditions (homeostasis), adaptation and self-healing, growth self-amplification or self-reproduction, signalling, and
response to external stimuli or cues (i.e., stimuli-responsiveness), to name a few (Figure 1) [1,2]. This is definitely a collective and multidisciplinary effort that involves inputs from chemistry, physics, material science and engineering from one side, and cell and molecular biology, physiology, immunology, oncology and neuroscience from the other.

**Figure 1. Molecular bionics.** Schematics showing the general concept of molecular bionic biomaterials.

Here we review the recent progress in the field of molecular bionic biomaterials (Figure 1) with the final aim of providing with a set of design guidelines to construct functional materials able to interact with biological entities. For this purpose, we split our narrative into two interrelated blocks following the schematics of Figure 1. The first one (section 2) is connected with the manufacturing stage and the subsequent introduction of functionalities, such as stimuli-responsiveness, selectivity, control of the topology or locomotion, aimed at enhancing the biomaterial performance in biological environments (i.e., improved interaction with cells, tissue growth or tissue penetration) (Figure 1). In the second block (section 3), we briefly discuss some of the characteristics of biological environments and their interactions with self-assembled materials with a special focus on the special functionalities needed to navigate the body and to improve targeting/tissue penetration (Figure 1). We finally wrap up with some conclusions and indications about the future directions that, from our point of view, will be addressed in the coming years.
2. Engineering strategies to construct molecular bionic biomaterials

In this section we aim to provide with a toolbox for the construction of biomaterials based on a molecular bionic approach. We first focus on the combination of synthetic and supramolecular chemistry and physics to tune inter/intramolecular interactions and self-assembly processes (section 2.1 and 2.2) between the constituting building blocks, whose synthesis is also discussed (section 2.3). We then provide with some strategies to arrange these blocks into distinctive patterns or topologies (in both 2D and 3D models) which ultimately define the interaction with living cells (section 2.4).

2.1. Self-assembly and supramolecular forces

Living systems are the outcome of a very precise and balanced, ordered, organization of molecules and macromolecules. From single cells to whole organs, the human body is the result of a hierarchy of compartments continually exchanging chemical information through regulated gating systems. Such gated compartments are effectively managed by a network of protein complex transporters, vesicular carriers, supramolecular lipid aggregates, and whole cells carrying chemical information across the different barriers. These systems are formed with precise chemical signatures that direct supramolecular interactions between them and/or with water. The supramolecular interactions allow for the formation of mesoscale architectures with superb temporal and spatial control. This process, known as self-assembly, is ever-present in nature with very specific design rules, and is at the core of many biological transformations.

Supramolecular self-assembly involves the reversible association of individual components (ions, molecules, macromolecules) to form a more complex entity, mediated by supramolecular interactions [3]. The most distinctive feature of supramolecular interactions is the weakness of the bonds compared to their molecular counterparts, being typically 10 to 100 times weaker. The typical bond energies for both molecular and supramolecular bonds (shown in Figure 2a) evidence that the difference between the two is about one order of magnitude. How is it then possible that they maintain long-ranged ordered structures (i.e., nanoparticles, fibres, etc.) whose mechanical properties are sometimes comparable to those of covalently linked materials? [4] The key point is that biologically-relevant ensembles are never held uniquely by a single type of interaction, but by a combination of several types and large numbers of forces, working collectively with frequent synergistic effects [5]. For example, protein structures encompass a large number of hydrogen bonds (ranging between 4 to 8 \( kT \)), few aromatic interactions, and electrostatic interactions, all synergistically creating supramolecular structures that can withstand loads comparable to steel or Kevlar, as in the case of silk proteins. Such a combination of two or more types of interactions also minimises non-selective interactions and create non-linear responses as a function of the temperature, ligand/receptor concentration, chemical potential, etc. (as will be later described in section 2.2.5) [6]. Furthermore, the weakness of noncovalent interactions is also key in living systems: supramolecular bonds can form, break and be reformed again in a reversible manner, which is the basis of the dynamical processes essential to life [5].
Physics teaches us that every interaction can be explained by four fundamental forces: gravity, electromagnetic forces, weak and strong nuclear forces. Unless the resulting structure grows bigger than the micron scale or comprises high density materials, gravity can be ignored at the molecular level. Similarly, nuclear forces, albeit very strong, only act within the nucleus and hence, well below the length scales of molecular and supramolecular forces. Among the electromagnetic forces, electrostatic ones define most molecular interactions. Depending on the number of electrons and relative protons there are different elements we know that, except noble gases, interact between each other forming molecules. These are formed by bonds that can be ionic or covalent depending on whether electrons are fully or partially exchanged. We also know that electron-rich metals can interact via the formation of metallic bonds between each other and coordination bonds with electron-acceptor molecules. Whatever the case, the chemical bonds ultimately define supramolecular interactions.
experimental observations suggest that the distance dependence is more likely to be with \( n < 2 \)

\[ d_0 \] hydrophobic persistence length;
\[ \sigma \] radius of water;
\[ k \] Debye length;
\[ R_g \] radius of gyration.

Pair potentials and bond energies have been obtained or derived from ref.[7].

Two elements are critical to define any interaction: its intensity or strength, and how this changes as a function of the distance between two molecules, macromolecules or supramolecules. Typically, molecules comprise permanent charges (ions), permanent dipoles or induced dipoles, with the strength of the supramolecular interactions between them decreasing as the charge intensity decreases (Figure 2a). In addition, electrostatic interactions are considerably weaker in water. This comes from Coulomb law in which the influence of the
solvent is accounted in the electric permittivity, \( \varepsilon \), which is 80 times higher in water than in vacuum. In addition, molecules in solution and small particles in suspension, are subjected to thermal energy which results in rapid vibrations/rotations and Brownian motion respectively, effectively reducing the strength of the interactions [8]. For example, van der Waals interactions involving induced dipoles are almost ubiquitous in nature and can help to maintain self-assembled structures (e.g., they appear between the tightly packed bases in the double helix structure of DNA, between uncharged groups in the interior of proteins, or in the hydrophobic domain of the cell membrane) but are too weak to build assemblies on their own.

As for the dependence of the intensity of the interaction on the distance between the two entities, the binding energy decays according to a power law, i.e., \( \propto 1/r^n \) with \( n \) equal to 2 for ion-dipole, 3 for dipole-dipole, 4 for ion-induced dipole, 5 for dipole-induced dipole, and 6 for induced dipole-induced dipole (Table 1). This rule applies to almost all molecules with the exception of those bearing an electronegative atom covalently bound to a hydrogen atom (H-bond donor, typically oxygen and nitrogen in biology) which can interact with a H-bond acceptor (an atom carrying a pair of unbounded electrons, and thus negatively charged). Here the small size of the hydrogen atom gives rise to quantum mechanical effects that make a special form of dipole-dipole interaction known as the hydrogen bond (Figure 2a). This can be as strong as tens of \( kT \)s and it is very directional, with a decay of \( 1/r^2 \). In addition, the partial sharing of the electron pair (similar to covalent bonds) makes H-bond especially strong in comparison to other dipole-dipole interactions. Actually, in the case of water, for example, it has been shown that overlapping between the molecular orbitals of water molecules exists, contributing to this covalent character [9]. Finally, apparent charges can distribute in multipoles giving rise to strong interactions whose intensity and sign change considerably depending on the molecule mutual orientation (i.e., they are strongly directional interactions). The most important is the aromatic interaction or \( \pi-\pi \) bond (Figure 2a and b), involving aromatic molecules and other aromatic molecules or ions, whose strength also can reach some tens of \( kT \)s. Polar substituents in the ring alter the electron distribution creating even more complex electronic arrangements. All these molecular forces are listed in Table 1 with their intensity, directionality and distance decay.

However, if considered isolated, supramolecular forces tend to be very weak, but, as Aesop stated “in union there is strength”, and indeed supramolecular forces can synergistically combine to form very strong bonds. For example, as stated by Lifshitz theory, weak van der Waals forces integrated over the volume of larger objects, such as polymers or colloids, become orders of magnitude stronger. When doing so, the van der Waals pair potential between two spherical colloids increases one order of magnitude compared to the intermolecular interactions, and its intensity decays hyperbolically with the distance, i.e., \( \propto r^{-1} \), while van der Waals interactions at the molecular level decrease with \( r^{-6} \). Similarly, multiple ionisable groups on the surface of a colloidal particle result in a net surface charge. Such a collective effect is well represented by the double layer potential which describes the repulsive
forces arising between two charged surfaces (Figure 2c). The confinement of charges onto a surface exposed to water leads to the formation of the so-called double layer where the surface confined charges are balanced by mobile counter-ions which concentrate locally. Such an excess of ions means that when two double layers approach each other, an osmotic pressure arises between the surface, pushing them apart (i.e., electrostatic repulsion). The double layer potential depends strongly on the ion concentration and indeed it can be completely switched off at ionic strengths similar to those encountered within biological fluids. For example, for nanoparticles stabilised by electrostatic interactions, charge screening results in poor colloidal stability, clustering and aggregation, which in turn, affect the way they are processed by cells [10,11]. In addition to ionic strength, the effect of the pH on the assemblies needs to be considered. For example, peptide-based materials often comprise amphoteric groups, so that a small shift of the pH can alter the assemblies considerably, as it happens with protein denaturalization in very acidic or basic environments. Some examples of pH-sensitive materials and applications are described in section 3.3.1.

Finally, most biomaterials are designed to work in biological media, where other collective effects appear resulting from molecules or assemblies interacting with water, the universal biological solvent. These are known as solvent effects and make molecules and macromolecules interact according to forces controlled by the effect on the H-bond network of water. Molecules incapable of forming H-bonding (and these are not only non-polar molecules, see the strange case of chloroform!) distort the water hydrogen bond network forcing it to order or to disorder depending on the size. For example, for very small hydrophobic molecules (< 0.5 nm), the water molecules can still form rigid cages enclosed by a network of H-bonds, with a rate of H-bonding breakage similar to that occurring in the pure liquid. In this case, the hydrophobic units induce ordering of the water around them with a freezing-like effect, which is therefore entropy unfavourable if the cage structure is to be maintained over large areas. For this reason, for large hydrophobic molecules with low curvature extending over large areas (> 1 nm²), the H-bonding cannot be maintained, and around < 1 H-bond is lost compared to the pure liquid, with water moving away from the cavity, increasing entropy, and creating an interface similar to that between water and vapour. Similarly, for high concentration of hydrophobic molecules, the solute molecules will aggregate to reduce the surface area (and thus the surface tension), leading to phase separation and insolubility [12,13]. The strength of this interaction, known as the hydrophobic effect, of tens of kT, is stronger than that predicted by continuum theories of van der Waals forces, and the fact that it is weakly sensitive to the presence of ions shows that it is not directly based on electrostatic forces (Figure 2a and Table 1). Conversely, molecules able to form bonds with water including ions and other hydrogen binders prefer water molecules rather than themselves. For example, the same ionic bond that makes salt melting at 1000 °C, can be easily broken in water at room temperature where its ions solubilise singularly and start interacting via damped electrostatic interactions. Polar molecules can similarly interact with
and reorient water molecules so strongly that any interaction involving them has to account
this extra interaction, in some cases giving rise to actual potentials (collectively known as
hydrophilic forces, Figure 2a and Table 1). An extreme case of hydrophilic interaction emerges
when hydrophilic groups are polymerised and grafted together with high density, creating a
polymer brush that repels almost anything but water via steric forces (Figure 2c). As will be later
shown, these are extremely important to guarantee colloidal stability of the assemblies and to
avoid unspecific interactions in biological fluids. Finally, biological fluids comprise a complex
mixture of colloidal objects of very different size and surface properties which can also give
rise to an additional type of interaction known as depletion forces. These arise when large
colloids repel smaller ones from their proximity, creating a depletion zone around their surface.
When two of these zones overlap, the osmotic pressure arising from the small colloids pushes
the two large ones together (Figure 2c). For a more detailed description of colloidal forces
between particles, the reader is advised to check the reviews of refs. [14,15].

2.2. Design rules for molecular bionic biomaterials
Taking the overview of supramolecular chemistry and colloid science given in the previous
paragraph as a reference framework, the next question is whether there are exist any universal
rules to build assemblies for biomaterial purposes (Figure 1). Although, this will ultimately
depend on the final application, we can extract some general guidelines. First, strong
interactions, of tens of $kT$s, are needed to guarantee that the assembly is stable when placed
in biological fluids, where thermal effects and Brownian motion are relevant. The main three
candidates to ensure this are H-bond, aromatic interactions and the hydrophobic effect (see
below some examples of materials based on each interaction). The first two are interesting
because they are strongly directional and can define the final shape of the assembly, while the
hydrophobic interaction lacks directionality [6,16]. However, the hydrophobic effect has the
advantage of being very little affected by the presence of ions, which is very beneficial to
ensure stability in high ionic strength environments. Normally they all are collectively used
leading to synergistic effects.

Other interactions such as electrostatic or van der Waals interactions can also help to
maintain the integrity of the structure, although they can become screened at high ionic
strength. For example, electrostatic interactions have been used for the preparation of
polyelectrolyte capsules formed by the deposition of layers of oppositely charged molecules
onto colloidal particles, a method known as the layer-by-layer (LbL) technique (Figure 3a)[17].
Numerous molecules such as polyelectrolytes, proteins, nucleic acids, inorganic particles, dyes
and lipids have been successfully assembled by LbL methods [17,18]. However, it has been
argued that additional interactions other than the electrostatic, e.g., hydrophobic, are normally
involved in the assembly [19]. Similarly, another example of nanocompartments built by
electrostatic interactions are coacervate droplets, which are the result of liquid-liquid
separation in aqueous media giving a phase which consists of oppositely charged molecules,
normally large polyelectrolytes, although small peptide and nucleotide molecules can also be used [20,21]. Complex coacervation between hyaluronic acid and recombinant mussel adhesive fusion protein has been successfully used as coating for titanium implants [22]. Similarly, coacervation between a negatively charged elastin-like protein and a positively charged peptide led to the formation of membranes capable of morphogenesis. However, in this second case, the authors demonstrated that, even though the initial assembly is driven by electrostatic interactions, hydrophobic effects are also needed [23].

In addition, it is necessary to engineer the assembly surface in terms of both colloidal stability and interactions with biological units (e.g., proteins, cells, etc.). For example, in the case of the hydrogels used as scaffolds in tissue engineering, cell adhesion is vital. In this regard, controlling surface topology is very useful, as will be explained in section 2.4. In the case of nanocarriers for drug delivery, surface functionalisation which prevents from colloidal aggregation due to van der Waals forces and hydrophobic effects is needed. Relying on the electrostatic double layer is normally insufficient at high ionic strengths, while steric repulsion gives much better results, as already pointed out by the Whitesides rules (see section 3.1). In section 3, we will give an overview of the best strategies to achieve this. In addition to colloidal stability, nanocarriers need to be functionalised with biologically relevant ligands which enable targeting and selectivity [6,16]. In section 3 we describe some strategies to design nanoparticles able of effective navigation and selectivity in living systems. But before this, let us first briefly describe how the three strong interactions mentioned above (i.e., H-bond, aromatic interactions and hydrophobic effects) can be used for biomaterial purposes.

2.2.1. Materials based on hydrogen bonding
The hydrogen bond is one of the most relevant interactions in biological entities such as DNA or proteins, and it is responsible for many of the properties of water, which accounts for approximately 85 wt % of cells. H-bonds between the amide protons and carbonyl oxygens of amino acids contribute to stabilise the secondary structure of proteins in the form of α-helices (H-bonds between groups of a single polypeptide chain) and β-sheets (H-bonds between adjacent chains), as well as for the double helix structure of DNA, in which complementary bases interact via a very specific H-bond (Figure 2b). In synthetic systems, cooperative H-bonds are used in supramolecular peptide assemblies which can form nanotubes at the nanoscale and fibres at larger scales. As mentioned before, they are often combined with other interactions such as aromatic stacking, electrostatic, hydrophobic, and van der Waals interactions [16,24,25]. They can also give rise to cooperative effects with cation–π interactions [26]. Another example of H-bond collectivism in supramolecular biomaterials are supramolecular polymers, which constitute a family of polymers whose monomeric units are linked by highly directional supramolecular interactions, and which can be used in a number of biomaterials (see for example the reviews in ref. [27–29]). Although other types of interactions (i.e., electrostatic or aromatic interactions) can be used to link the monomers, H-bonding is especially attractive because of its strength and directionality, as mentioned above.
Indeed, using multiple arrays of H-bonding was the first strategy used to assemble these materials, as shown by the pioneer works of Lehn (Figure 3b). Since then, many strategies have been used, most of them relying on the use of urea-based motifs capable of multiple (up to six!) H-bonds [30]. These collective effects lead to biomaterials with mechanical properties similar to those of natural rubber, which in addition exhibit unique self-healing behaviour, thanks to the reversibility provided by the noncovalent nature of the interactions [31,32]. For these reasons, they have been classified under the term ‘dynamers’ [30].

2.2.2. Materials based on aromatic interactions
Aromatic interactions are responsible for the stacking (π–π stacking) of the nucleotide bases in the DNA double helix (Figure 2b), as well as for protein folding involving amino acids with aromatic groups (tryptophan, tyrosine, histidine and phenylalanine). They also contribute to the formation of amyloid fibrils, related to diseases like Alzheimer or type II diabetes [33]. π–π stacking enables small molecules such as homoaromatic dipeptides like diphenylalanine (FF) to form stable ensembles [24,33–35]. Similarly, aromatic peptide amphiphiles consisting of peptide chains linked to other aromatic groups like fluorenylmethoxycarbonyl (Fmoc), assemble into spheres, worms, sheets, tapes or fibres, thanks to π–π stacking of the aromatic group, together with other types of interactions (hydrogen bonding, etc.), see Figure 3c [34]. Peptide-based nanoarchitectures based on π–π stacking are especially robust because of the limited solubility of the aromatic groups in water [24]. The addition of aromatic interactions to supramolecular polymers based on hydrogen bonding is also responsible for the enhancement of the mechanical and self-healing properties as well as thermodynamic stability of these materials [36,37].

A sub-class of the aromatic interaction is the one occurring between aromatic groups and cations, also known as cation-π. These interactions play important roles in biology (peptides, nucleic acid, enzymes, etc.) as explained in the throughout reviews of ref. [26,38]. Although very important in other areas of supramolecular chemistry (such as the synthesis of catenane [39]), their use in self-assembled biomaterials is scarce.
2.2.3. Materials based on the hydrophobic effect

The hydrophobic effect is probably the main driving force for biologically-relevant self-assembly in water, the most distinctive example being the phospholipid bilayer of the cell membrane, which allows for compartmentalization, essential to life [41]. In fact, the hydrophobic effect allows shielding from water of some of the most important biological functions which require a hydrophobic environment (as in the interior of proteins, whose tertiary structure is largely determined by hydrophobic interactions). In addition, hydrophobic effects are much lesser influenced by the presence of salts than interactions involving...
electrostatic charges or dipoles. Nevertheless, note, that on their own, hydrophobic effects are insufficient to lead to the formation of stable assemblies in water, especially because they are non-directional [42]. Fortunately, amphiphile molecules consist of both a hydrophilic part (charged or polar groups) and a hydrophobic part (usually a mono- or di-alkyl chain or hydrophobic polymer), and it is precisely this dual nature which makes them so interesting from the self-assembly point of view. Most amphiphiles used in biomaterials fall into four categories: surfactants, lipids, block copolymers or peptide amphiphiles (they will be described in details in section 2.3). For all of them, the hydrophilic parts are well hydrated groups (sulfonate, phosphonate, carboxylates and ammonium ions in the case of surfactants and lipids, hydrophilic polymers in the case of amphiphile copolymers, or hydrophilic peptides for peptide amphiphiles) which interact between themselves and with water by electrostatic interaction or H-bonding. The hydrophobic moieties are also attracted to each other via hydrophobic effects.

When placed in water at a concentration above the critical micelle concentration (CMC) amphiphiles self-assemble in micelles, spherical aggregates in which the hydrophobic moieties of the amphiphile are shielded from water and point inwards towards a common centre via the hydrophobic effect, while the hydrophilic parts point outwards and are exposed to the water phase [3,43]. They thus incorporate a hydrophobic core, where hydrophobic cargos can be encapsulated. Long hydrophobic moieties favour micelle formation because of enhanced hydrophobic effects. Similarly, branched hydrophobic chains tend to increase the CMC because of the difficulties in packing of the chains. When the CMC is high, as it happens for small amphiphiles such as surfactant molecules, there is a rapid exchange of amphiphiles molecules (i.e., unimers) between the assembly and the pool of free unimers, which leads to stable assemblies in thermodynamic equilibrium. Larger amphiphiles such as block copolymer molecules, have virtually zero CMC, leading to kinetically-trapped assemblies which do not exchange unimers with the external environment [44]. The exchange of unimers with the external environment may result in destabilization of the assemblies, for example, if the unimers in solution are degraded by an enzyme and therefore, removed. For example, Carstens et al. found that lipase-mediated degradation of methoxy poly(ethylene glycol)-oligo(e-caprolactone) (mPEG-OCL) micelles followed a Michaelis-Menten kinetics. They provided with two potential mechanisms for degradation: (i) the enzyme degrades the unimers in solution causing a shift of the equilibrium between micellese and unimers, and/or (ii) the enzyme degrades the micellar core (i.e., the hydrophobic block). However, because both situations would follow a Michaelis-Menten kinetics according to the authors’ calculations, they could not discriminate which mechanism was predominant [45]. In any case, degradation by blood proteins would lead to side effects arising from the release on an encapsulated drug, for example, into the blood stream. However, the dynamics of the assembly can be also engineered to release the cargo in specific sites precisely by making use of specific enzymes, such as lipases (as will be discussed in more detail in section 3.3.2) [46].
In addition to the spherical shape, micelles can adopt other geometries depending on the structure of the individual amphiphile and its packing configuration when assembled (Figure 3d). To account for this, a dimensionless parameter, the packing factor, is usually defined as $p = V/a_0 l$, where $V$ is the volume of the hydrophobic moiety, $l$, the length of the hydrocarbon chain, and $a_0$ the optimal interfacial area occupied by the amphiphile [3,47]. The latter results from the balance between two opposed effects acting on the interfacial area of the amphiphile, $a$: (i) the hydrophobic effect which tends to reduce $a$, and (ii) the tendency of the hydrophilic head to maximize contact with the water phase, which tends to increase it. The optimal area, $a_0$ corresponds to the minimum of free energy (Figure 3d). If $p$ is small ($p \leq 1/3$) as for cone-shaped amphiphiles, then spherical micelles are formed. If the packing parameter increases, as for more truncated cone-shaped amphiphiles, then cylindrical micelles or worms are formed ($1/3 < p \leq 1/2$). If it is further increased ($1/2 < p \leq 1$), then the amphiphiles cannot be packed into cylinders and they assemble into bilayer structures in the form of membranes or vesicles (Figure 3e). When $p > 1$, inverted micelles are formed, which are only stable in oil. In addition to the geometry of the amphiphile, other parameters such as the pH, temperature, salinity or concentration affect the final shape of the aggregate. For example, increasing the concentration of the amphiphile leads to micelle interaction and formation of more complex architectures such as hexagonal or lamellar phases [48,49].

As mentioned above, amphiphile membranes and vesicles are essential to life because they allow compartmentalization of cells and other organelles inside them. The formation of a vesicle can be explained as if a bilayer disk was closed as a pulled bag, enclosing a volume of water, which involves a balance between the energy needed to bend the disk and the energy arising from the line tension associated with the rim of the bilayer disk [44]. Vesicle formation arises when the bending elasticity is low and the surface tension high. Vesicles consist of an inner aqueous phase and a amphiphile membrane, whose thickness can vary from 3-5 nm for liposomes [47,50-52] (i.e., lipid vesicles), to 3-50 nm for polymersomes [53-55] (i.e., block copolymer vesicles) [56]. Vesicles are usually kinetically trapped structures, meaning that they are not at thermodynamic equilibrium. For this reason, their morphology strongly depends on the method of preparation. Different preparation techniques can give rise to multi-layered, tubular, oblate, or starfish vesicles [47,56]. The final shape is given by an interplay between the areas of the inner parts of the bilayers (which can vary due to exchange of molecules between the monolayers which results in bilayer asymmetry) and the vesicle volume (which can be changed by swelling or deswelling). Factors such as amphiphile composition, electrolyte concentration, adsorption of third species, etc. also affect the shape of the vesicles. In the particular case of block copolymers, stable high-genus vesicles can also be obtained. Remarkably, under out-of-equilibrium conditions, the lifetime of vesicles can be externally controlled by the application of an external fuel such as adenosine triphosphate, ATP, showcasing nanoreactor capabilities coupled to transient behaviours [57].
2.2.4. Engineering kinetics and thermodynamics of self-assembly
Although many supramolecular materials are the result of equilibrium assemblies (i.e., corresponding to an absolute minimum of energy, and thus thermodynamically stable over time), living systems are often governed by non-equilibrium behaviours. Living cells are dynamically assembled systems which work in far-from equilibrium conditions (dissipation of energy, maintenance of concentration gradients, etc.), but manage to keep in steady state conditions (homeostasis) by a continuous supply of energy and matter [58]. Designing materials based on out-of-equilibrium assemblies offers the possibility of mimicking features of nature systems such as dynamism, transient behaviours, etc.

Essentially, non-equilibrium self-assembly (also known as dynamic self-assembly [59]) represents that either the system is kinetically trapped in a local energy minimum (and thus can persist over a transient period of time before moving to the absolute minimum of energy), or there is an external supply of energy from a drive, which is in part dissipated to increase entropy, able to maintain the assembly in conditions far from equilibrium, i.e., steady-state [42,60]. In addition, there can exist a combination of both mechanisms, as shown for example in the work by Tena-Solsona et al. [61] where dynamic supramolecular assemblies such as colloids, hydrogels or inks were formed thanks to the supply of an external drive, (i.e., a fuel). However, the assemblies, based on Fmoc-peptides, did not return to the original state upon fuel removal, being therefore kinetically trapped. The materials showed transient behaviours of tunable lifetime such as self-erasing in the case of inks [61]. The exploration of these non-equilibrium assemblies is relatively new in the field of biomaterial design and engineering, but it is already leading to the discovery of emergent life-like behaviours (such as adaptation, self-repairing, etc.) [62] and even complete new areas of research such as supramolecular systems chemistry [42,60].

Many examples of kinetically-trapped self-assembled structures have been reported in the literature for building blocks such as peptide amphiphiles [24,63], supramolecular polymers [64], or amphiphilic diblock copolymers (assembling into disks [44], tubes [65], neuron-like tubular membranes [66], or lamellarsomes [67]). However, there are still very few examples of assemblies driven by the supply of an external source of energy. Indeed, one of the challenges associated with externally-driven self-assembly is the noisiness arising from the multitude of possible states for the system, due to the supply of energy. This makes especially difficult to control the spatiotemporal conditions of the process as well as to predict the final assemblies (something relatively easy in the case of equilibrium assemblies). In this sense, Prigogine established that steady-state assemblies are the outcome of the minimization of the rate of entropy production, although this can only be applied in the linear response regime. More recently, England [68,69] has demonstrated that out-of-equilibrium assemblies excited by an external drive are more likely to be maintained over time if the energy supplied in the form of work is dissipated by the system. The more efficient this energy dissipation is, the more irreversible the assembly will be. Indeed, if the externally-supplied work which maintains the system far-from-equilibrium is dissipated, then it would not be available to bring the system back to the initial state. In the field of materials with interest for biomedical purposes, energy
can be supplied, for example, from sources such as light [70–72], an external chemical fuel [57,73–78], or microfluidic environments (see for example, the recent tutorial review of ref. [79]). In addition, England coined the term dissipative adaptation to describe the fact that out-of-equilibrium systems have memory and tend to adopt configurations where dissipation occurs in a more effective way, something which can help design the conditions leading to a particular assembly [68]. This pioneer concept has been demonstrated in open compartments (cocervate droplets) encapsulating a protein (FtsZ) which assembled into filaments in the presence of guanosine triphosphate (GTP). Coacervate droplets behaved as open systems in which dissipation of energy in the form of hydrolysis of GTP (catalysed by FtsZ) to guanosine diphosphate, GDP, led to filament destabilization. The possibility of supplying GTP (drive) thanks to the absence of a membrane around the droplets resulted in dynamic droplets which adopted different configurations depending on the drive availability (see Figure 4. Out-of-equilibrium self-assembly and cooperativity. a) and b) Self-assembly of FtzZ protein filaments (red) within coacervate droplets (formed by GFP-K72, green colour) driven by GTP hydrolysis. The GTP (drive) concentration increases over time in a), leading to deformation and fragmentation of the droplets into dynamic fibrils. b) corresponds to snapshots at different distances from the GTP source (x = 0). These results demonstrate that self-assembly is adapted to the available drive. Reprinted by permission from Springer Nature and Copyright Clearance Center: [Springer Nature, Nature Nanotechnology, Esra te Brinke, Joost Groen, Andreas Herrmann, Hans A. Heus, Germán Rivas, Evan Spruijt and Wilhelm T. S. Huck, Dissipative adaptation in driven self-assembly leading to self-dividing fibrils, 13, 849-855 Copyright (2018)[62] c) Schematics showing different forms of cooperativity and corresponding examples: (1) cooperative aggregation, (2) allosteric cooperativity, (3) intramolecular cooperativity and (4) interannular cooperativity. Republished with permission of Royal Society of Chemistry, from [Assessing cooperativity in supramolecular systems, Larissa K. S. von Krbek, Christoph A. Schalley and Pall Thordarson, 46, 2622-2637, 2017]; permission conveyed through Copyright Clearance Center, Inc. [82]a) [62].

2.2.5. Collective effects and cooperativity

Despite their complexity, biological systems exhibit exquisite selectivity, as shown, for example, by the pairing between the complementary bases of DNA or by the specificity between enzymes and their substrates [80]. Mimicking the self-selection and self-recognition occurring in nature to avoid non-selective interactions, so that building blocks assemble in an almost bio-orthogonal manner, is thus very desirable [6]. In addition to the advantages from an assembly point of view, high selectivity and specificity are very valuable when it comes to the self-assembled materials and living systems to interact.

Nature attains selectivity and specificity by making use of collective effects and cooperativity. Cooperativity implies that two or more interactions are involved in the formation of an assembly, in such a way that the interplay of the different interactions is different than the contribution of each interaction alone [81]. For example, DNA base pairing is highly specific (cytosine-guanine, thymine-adenine), even though the involved supramolecular interactions (H-bonding, π-π stacking and van der Waals) are not highly specific themselves (Figure 2b). In energetic terms, this means that after the first interaction (or binding event) the free energy of the system either decreases or increases, giving rise to positive or negative cooperativity, respectively [82]. Indeed, cooperativity enables and regulates many biological processes from the molecular to the intercellular levels, because it allows circumventing some of the constraints of physical and chemical laws, such as entropic costs [82–84].
Figure 4. Out-of-equilibrium self-assembly and cooperativity. a) and b) Self-assembly of FtzZ protein filaments (red) within coacervate droplets (formed by GFP-K72, green colour) driven by GTP hydrolysis. The GTP (drive) concentration increases over time in a), leading to deformation and fragmentation of the droplets into dynamic fibrils. b) corresponds to snapshots at different distances from the GTP source (x = 0). These results demonstrate that self-assembly is adapted to the available drive. Reprinted by permission from Springer Nature and Copyright Clearance Center: [Springer Nature, Nature Nanotechnology, Esra te Brinke, Joost Groen, Andreas Herrmann, Hans A. Heus, Germán Rivas, Evan Spruijt and Wilhelm T. S. Huck, Dissipative adaptation in driven self-assembly leading to self-dividing fibrils, 13, 849-855 Copyright (2018) [62] c) Schematics showing different forms of cooperativity and corresponding examples: (1) cooperative aggregation, (2) allosteric cooperativity, (3) intramolecular cooperativity and (4) interannular cooperativity. Republished with permission of Royal Society of Chemistry, from [Assessing cooperativity in supramolecular systems, Larissa K. S. von Krbek, Christoph A. Schalley and Pall Thordarson, 46, 2622-2637, 2017]; permission conveyed through Copyright Clearance Center, Inc. [82].

In the field of supramolecular chemistry there has been some debate in the last decades, but essentially four forms of cooperativity (see Figure 4b) can be described as recently reported by von Krbek et al. [82] The first one, allostery (also known as intermolecular cooperativity), appears when a monovalent ligand binds to a receptor with multiple binding sites. In this case, the binding of a first ligand favours the binding of a second separate ligand because it induces a change of the conformation of the receptor (e.g., a protein), as exemplified by the binding of oxygen to haemoglobin. For some authors, allostery necessarily means a structural communication between binding sites to favour or hinder the subsequent binding [82,83]. The second mechanism, known as chelate cooperativity, intramolecular cooperativity or multivalency, involves binding of two or more ligands which are part of the same entity, to a receptor bearing multiple binding sites. Therefore, while in allostery the two ligands are independent, in multivalent interactions they belong to the same protein, nucleic acid, lipid, artificial nanoparticles or polymer chain. In this second case again, the first binding event can favour or hinder subsequent, intramolecular, binding contacts, leading to positive (avidity) [83] or negative cooperativity, respectively [82-84]. In this case, when the first binding event takes place, the whole entity (e.g., the nanoparticle) and therefore, the rest of the ligands attached to it, come closer to the host, which eases or impedes the subsequent interactions (free host and guest model). In energetic terms, the first event reduces the number of possibilities for the following, thus reducing the entropic cost. In addition, once the nanoparticle is bound, if one ligand-receptor pair unbinds, it is more likely that it re-binds again.
If individual bonds between ligands and receptors form independently, so that many complexes with similar energy state can be formed at a given time, then an entropic contribution called the avidity entropy is introduced. In section 3.2.1., we describe how this feature can be exploited to achieve superselective binding useful in targeted drug delivery. The third mechanism is known as interannular cooperativity and it involves a third element, like a chaperone protein, which promotes the interaction. Usually, for all these forms of cooperativity, a parameter which compares the non-cooperative binding with a cooperative interaction (normally in the form of the ratio of association constants) is defined for quantification. The reader is advised to check refs. [81,82,84] for more details in the calculation of these.

Finally, the fourth form of cooperativity is known as cooperative aggregation, which normally leads to the formation of larger aggregates. When positive, cooperative aggregation leads to an OFF-ON behaviour. For example, in amphiphile self-assembly, the molecules conforming the assembly (i.e., unimers) are either free in solution or fully assembled, with almost absence of intermediate states [81]. This implies, that the transition from the free and assembled states takes place sharply for a small change in the conditions promoting the assembly (e.g., a change in temperature or pH). This is the case, for example, of the assembly of pH-sensitive amphiphilic diblock copolymers (such as poly(2-(methacryloyloxy)ethyl phosphorylcholine)-poly(2-(diisopropylamino)ethyl methacrylate, PMPC-PDPA) for which a step-wise increase in the assembly size, involving transition from free unimers to micellar or vesicular structures, is obtained when the pH is raised above the pKa of the molecule (around pH 5-7.5 depending on the length of the hydrophobic block, DPA) [85].

2.3. Synthetic strategies at the molecular level: the starting building blocks

Now that we have introduced the main interactions for the construction of the assemblies, we want to provide here with some synthetic routes to produce the building blocks which are most commonly used to construct them. We specially emphasize the biocompatibility (or lack of biocompatibility) in each case because this will ultimately dictate whether the material is suitable or not to be used in biological environments. We also indicate how the building blocks can be engineered to provide functionalities such as stimuli-responsiveness.

2.3.1. Small molecules (low-molecular weight building blocks)

a. Phospholipids

Amongst the building blocks employed to produce supramolecular assemblies, low-molecular-weight amphiphilic phospholipids are amongst the most traditional. Phospholipids are molecules bearing a hydrophilic head group and hydrophobic acyl chains that are linked together through an alcohol. Variations in the polar heads, hydrophobic tails and alcohol linkers are at the base of their versatility. Phospholipids can thus interact via hydrophobic effects to build lipid vesicles, also known as liposomes. Phospholipids are the endogenous constituents of cell membranes, hence their supramolecular assemblies are favourably biocompatible as well as biodegradable [86–94].
Figure 5. Phospholipids and non-ionic surfactants. General chemical structure of a) glycerophospholipids, sphingomyelins and b) non-ionic surfactants commonly used for supramolecular applications, including the different polar -X groups and the acyl groups. PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, PA: phosphatidic acid, PI: phosphatidylinositol, PG: phosphatidylglycerol, CL: cardiolipin.

According to the alcohol molecule contained in their structures (either glycerol or sphingosine) phospholipids can be categorised in glycerophospholipids and sphingomyelins (Figure 5a). The chemical structures of glycerophospholipids can be classified depending on: (i) nature of the head group (e.g., phosphatidylcholine (PC), phosphatidylethanolamine (PE)); (ii) length and saturation of hydrophobic tails; (iii) chemical bond between glycerol and hydrophobic chains [95]; (iv) number of aliphatic chains [96]. Due to the relatively short paraffin residues on the sphingosine in comparison to the acyl chains, sphingomyelins exhibit a certain degree of asymmetry [97,98]. Moreover, sphingomyelins exhibit a high degree of saturated hydrophobic chains, and are capable of forming intermolecular and intramolecular hydrogen bonds. This ultimately results in more rigid assemblies, characterised by higher phase-transition temperatures.
Many of the phospholipids employed to produce supramolecular assemblies can be obtained from natural sources, although egg yolk and soybean are the most important ones [94]. The phospholipids are obtained through validated non-toxic solvent-extraction and chromatographic procedures, with low consumption of energy and low production of waste, which in turn keeps the costs to a minimum [99]. An important aspect to be considered though, is the purity of the final phospholipid composition. Indeed, different sources exhibit different contents and species of phospholipids [86,100,101]. Raw materials sourced with consistent quality, though, usually result in products with excellent batch-to-batch reproducibility with respect to composition. For extremely high-purity grades, however, chemical synthesis processes might be considered [99,102]. In this case, the synthetic approach can entail either minor modifications on an existing structure (semi-synthesis) or a total synthesis ex novo starting from the commercially available precursors.

In the case of the semi-synthetic modifications of glycerophospholipids, these can be summarised in: (i) reduction of the insaturations of natural phospholipids to increase the resistance to oxidation and the melting point; (ii) change of the tail groups via deacylation of natural PC (using phospholipase A1 and/or A2) and subsequent reacylation via a suitable activated acyl-derivative; (iii) change of the polar head group (e.g., from choline to serine) using phospholipase D [99]. The total synthesis of glycerophospholipids, on the other end, entails the attachment of the apolar moieties to the glycerol precursor via ester or ether bonds, followed by the attachment of the polar head group. However, for sphingomyelins, the semisynthetic route to deacylate and reacylate with the acyl derivative of choice usually causes a partial change in the configuration of the final product, whilst the total synthesis of the stereochemically pure sphingomyelin is extremely complex and thus impractical [103].

A paramount synthetic modification, though, carried out on phospholipids which are to be used in biomedical supramolecular assemblies is the grafting to a polyethyleneglycol (PEG) chain. The reason at the base of this modification is to increase the half-life of the supramolecular assemblies in vivo by reducing their opsonisation and clearance by the mononuclear phagocyte system (MPS) thanks to the steric hindrance as well as the hydration shell provided by the PEG corona. This modification is achieved by creating a link between the PEG chain and the polar head (e.g., by esterifying a PEG-COOH with the primary amino group of ethanolamine in PE) [102,104-107]. A similar stealth effect can be obtained by adjusting the composition of the phospholipids that constitute the self-assemblies. In particular, a surface composition which resembles the surface of red blood cells (i.e., richer in PC and sphingomyelin) is more resistant to MPS clearance [108,109]. Similar chemical modifications to the head groups can also be exploited to conjugate other substances to the phospholipids, e.g., proteins or peptides for biotargeting purposes.

Stimuli-responsiveness such as pH-sensitivity and thermoresponsivity are inherently present within the phospholipid building blocks. However, careful selection of the composition
of the supramolecular preparations has to be achieved to prepare physico-chemically stable supramolecular assemblies that exhibit these properties in useful ranges for biological applications. Alternatively, conjugation of pH-sensitive or thermo-responsive polymers to the polar heads of the phospholipids can also be exploited [110-112].

b. Non-ionic surfactants
Non-ionic surfactants are also amphiphilic molecules that can be exploited to produce supramolecular assemblies [113-115]. Similar to the vesicular liposomes produced by phospholipids, the vesicles made of non-ionic surfactants are called niosomes. In comparison to phospholipids, non-ionic surfactants are easier to derivatise and exhibit lower costs. Their assembling capability mainly depends on a structure-related parameter called hydrophilic-lipophilic balance (HLB), i.e., an expression of the degree to which the molecule is hydrophilic or lipophilic. In the case of non-ionic surfactants, the hydrophilic and hydrophobic portions of the molecule can be linked either by ether, amide or ester bonds; hence, we can distinguish amongst alkyl ethers, alkyl amides and esters of fatty acids [114], although alkyl ether and ester surfactants are perhaps the most used for supramolecular applications (Figure 5b) [116-119].

Alkyl ethers (e.g., Brij®) are characterised by high physico-chemical stability, and their assemblies can be exploited to load biological molecules [120,121], although the most hydrophilic ones are susceptible to oxidation, which could lead to chromatic alterations (e.g., discoloration) [122,123]. Ester type of surfactants are less toxic than the ethers and are biodegradable by esterase enzymes. Amongst the most widely used alkyl esters we can distinguish Span® and Tween® esters, which are respectively fatty acid esters of sorbitan or of a multi-PEGylated version of sorbitan [115,124]. As in the case of phospholipidic supramolecular assemblies, modification with PEG can enhance the half-life of niosomes by reducing their uptake from the MPS [125-127].

Although there is a plethora of publications regarding non-ionic supramolecular assemblies [119,128], few data are available about their potential toxicity, especially long-term. Thorough metabolic studies on the fate of these surfactants should be performed if these assemblies have to realistically compete with phospholipid-based supramolecular systems towards biomedical applications.

2.3.2. Block-copolymers and polypeptides (high-molecular weight building blocks)

a. Polymers and block-copolymers
Polymeric materials are extremely versatile and convenient when it comes to the production of supramolecular architectures. The advances in the polymerisation processes over the past 20 years allow us to obtain polymer biomaterials with well-defined molecular weights and polydispersity. Living polymerisation approaches have been developed, thus resulting in uniformly growing polymer chains.
Figure 6. Living radical polymerisation. General scheme of controlled/living radical polymerisation and chemical structures of the most popular monomer units used to prepare block copolymers and polypeptides employed for supramolecular applications. Independently from the reaction mechanism, the establishment of a dynamic equilibrium between the propagating and "dormant" species (i.e., reversibly end-capped) results into a significant reduction of the termination reactions.

Controlled polymerisation protocols include living anionic polymerisation, living cationic polymerisation, living ring-opening polymerisation (ROP), ring-opening copolymerisation (ROCOP), and reversible deactivation radical polymerisation (also known as living radical polymerisation, LRP). Within this latter category, amongst the most popular methods we can further encounter nitroxide-mediated LRP (NMP), reversible addition/fragmentation transfer polymerisation (RAFT), and metal-catalysed living radical polymerisation, which includes atom-transfer radical polymerisation (ATRP) with all its variations [129]. The chemical structures of the different types of monomers used in these methods are depicted in Figure 6, whilst the advantages and disadvantages of each method are summarised in Table 2. Summary of main advantages and disadvantages of the most common controlled polymerisation methods.
Table 2. Summary of main advantages and disadvantages of the most common controlled polymerisation methods.

<table>
<thead>
<tr>
<th>Polymerisation method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Living ionic polymerisation</td>
<td>• Extreme control over the molecular weight (1 kDa to 1000 kDa)</td>
<td>• Technical expertise required and equipment (e.g., glove box and handling of air-sensitive reagents)</td>
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<tr>
<td></td>
<td>• Low polydispersity ($M_w/M_n &lt; 1.05$)</td>
<td>• Incompatibility with certain functional groups (e.g., OH, SH, SiOH, NH₂, C≡CH, CHO, COR, and COOH)</td>
</tr>
<tr>
<td></td>
<td>• Branched and linear architectures</td>
<td></td>
</tr>
<tr>
<td>Living anionic/cationic polymerisation[130-132]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reversible/fragmentation transfer polymerisation (RAFT)[139]</td>
<td>• Simple experimental procedures (oxygen removal and inert atmosphere)</td>
<td>• Purification problems (potential cytotoxic effects by transition metals in ATRP and chain transfer agents in RAFT) [141-144]</td>
</tr>
<tr>
<td>Atom-transfer radical polymerisation (ATRP)[140]</td>
<td>• Tolerance for many functional groups</td>
<td>• Colour might hinder the patient compliance [145,146]</td>
</tr>
<tr>
<td></td>
<td>• Applicability of a wide-range of structural monomers</td>
<td>• Systematic safety studies still lacking for modified methods</td>
</tr>
<tr>
<td></td>
<td>• Extremely good control over the molecular weight</td>
<td>• Non-biodegradable carbon-carbon backbone (long-term accumulation adverse effects) [147,148]</td>
</tr>
<tr>
<td></td>
<td>• Control over architecture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Low polydispersity</td>
<td></td>
</tr>
<tr>
<td>Nitroxide-mediated polymerisation (NMP)[149]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerisation-induced self-assembly (PISA)[152-154]</td>
<td>• Same as for RAFT, ATRP and NMP</td>
<td>• Same as for RAFT, ATRP and NMP</td>
</tr>
<tr>
<td></td>
<td>• Allows obtaining supramolecular assemblies in high concentrations (up to 25% w/v solids)</td>
<td>• Non-biodegradable carbon-carbon backbone (long-term accumulation adverse effects) [147,148]</td>
</tr>
<tr>
<td></td>
<td>• Compatible with large-scale production</td>
<td></td>
</tr>
<tr>
<td>Living ring-opening polymerisation (ROP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed polymerisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radical ring-opening polymerisation (RROP)</td>
<td>• Extremely mild conditions</td>
<td>• Poor compatibility with side-chain functionality</td>
</tr>
<tr>
<td></td>
<td>• Ease of execution</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Copolymer composition can be tailored to adjust its degradation rate</td>
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A number of examples of supramolecular assemblies produced using polymeric materials made by living ionic polymerisation methods can be found in the literature [130-132,156-162]. The synthetic efforts and potential dangers [163] linked with these polymerisation techniques, however, paved the way for the exponential rise in popularity of the living radical polymerisation processes (Figure 6) [133-138,150]. Perhaps the most popular living radical polymerisation methods are atom transfer radical polymerisation, ATRP, and reversible addition-fragmentation chain transfer, RAFT[139,140]. Numerous examples of supramolecular assemblies produced using polymeric materials obtained via these methods have been
reported, ranging from spherical micellar systems to worm-like micelles up to vesicles and even genus-like structures [65,85,164-168].

In a particular case, the so-called polymerisation-induced self-assembly (or PISA) [169], supramolecular assemblies can be obtained directly during polymerisation in high concentrations (up to 25% w/v solids), thus compatibly with large-scale production [152-154,169-178]. Despite their popularity, supramolecular biomaterials obtained by polymers made via ATRP and RAFT suffer from a purification drawback, which might encumber their safety [141-144] and/or the patient compliance for therapeutic applications due to the exhibition of a certain colour or odour in the final product [145,146]. From this point of view, nitroxide-mediated polymerisation (NMP) deserves to be mentioned [149]. The residual presence of the alkoxyamine moieties used to control the polymerisation process is not toxic if the product is intended for biomedical applications [150,179-181]. Nevertheless, the reduced compatibility of this technique for certain monomers [150,151] hindered its development in favour of ATRP and RAFT. In spite of their practicality, all the methods discussed so far exhibit a common flaw: they all rely on the generation of a non-biodegradable carbon-carbon backbone, which might cause long-term accumulation adverse effects in vivo [147,148].

Perhaps the most important characteristic of supramolecular assemblies for biological applications is that, ideally, they should be biodegradable, hence their structure should be suitable for the human enzymes to break them and digest them into harmless excretable components. For this reason, although they are not as new and attractive as the living radical polymerisation processes, ring-opening polymerisation (ROP) approaches of cyclic esters and anhydrides give rise to products such as biodegradable polyesters, polypeptides and polycarbonates [140,155,182-191]. An advantage of these type of polymers is that they bear functionalities within their backbone structure which are inherently pH or enzymatically-sensitive, and hence they can be degraded in the body and release their cargo (e.g., drugs or proteins). ROP, unfortunately, is poorly compatible with side-chain functionality [155,192-194]. From this point of view, a modified polymerisation method called ring-opening copolymerisation (ROCOP) of commercially available epoxides and anhydrides seems to be more advantageous, bypassing the need for complicated synthetic procedures [193,195-197]. Despite this advantage, in all cases the usage of metal catalysts could hinder the product applications in vivo, and although the residual presence of some of them is FDA-approved below certain levels (e.g., tin octanoate), still too little is known about their potential long-term toxicity or accumulation effects [198]. Organic catalysts (e.g., organobases) seem to be a better alternative [188,199-206], but they are less efficient [207-209]. Also enzymes have been successfully exploited as catalysts for ROP processes [210,211].

Similar polyester and polycarbonate backbones might also be obtained radically through a ring-opening mechanism. This method is called radical ring-opening polymerisation (RROP)
and, as any radical polymerisation, it is characterised by extremely mild conditions and ease of execution. Its main drawback is represented by the need of using peculiar monomers (e.g., high-strain rings [212] such as cyclic ketene acetals (CKA) [212,213] which are difficult to synthesise/purify and store. Nevertheless, a number of reports of supramolecular assemblies (spherical/worm-like micelles and vesicles) bearing copolymers produced using this technique are starting to appear in literature for drug delivery applications [214–219].

b. Polypeptides
Amongst the biodegradable and biocompatible materials to be used for supramolecular self-assembly, polypeptides are perhaps the most investigated since they exhibit high versatility in functionality and biodegradability. In addition, the possibility of establishment of secondary structures greatly helps in predicting and designing polymeric materials which assemble into specific architectures and can potentially transition depending on the environmental conditions (e.g., pH or ionic strength) [220–222]. Many examples of peptide-based block copolymers used for supramolecular assembly are actually hybrids between synthetic and peptidic blocks [220,221,223,224]. These syntheses are usually performed via ligation (e.g., click chemistry) between the synthetic polymeric portion and the preformed peptide [225] or alternatively via ROP of N-carboxyanhydrides (NCAs) using a synthetic polymer as a macroinitiator [220,221,223,224,226]. This last type of synthesis, however, requires expert chemists and strictly anhydrous conditions as well as thorough purification of the starting materials [227–229].

An easier possibility would be to exploit either recombinant biotechnology techniques, i.e., genetically engineered bacteria which express the peptide sequences of interest, or alternatively resort to solid-phase synthesis techniques which are nowadays quite established and allow to obtain polypeptides up to 100 and more aminoacids. The first approach is advantageous on an industrial scale, when the optimal conditions for transfection and protein expression and isolation have been identified. However, at a laboratory scale, the lengthy optimisation phase and the low amount of material obtained could hinder the subsequent supramolecular assembly investigations [230,231].

Solid-phase synthesis has been optimised to obtain peptides, oligonucleotides and other molecules in an efficient fashion [232–234]. Everything starts from an aminoacid or nucleotide which is immobilised on a suitable solid phase (e.g., polystyrene). All the monomers used exhibit specific protecting groups, and several cycles of deprotection, coupling and finally washing, allow the desired product to grow on the resin, potentially without contamination from other species and maximised yield. At the end of the synthesis, the product is cleaved from the solid phase and further purified (e.g., via chromatography). The whole process is straightforward, and because of its simplicity it can be easily automated using ad hoc flow reactors. Nevertheless, “all that glisters is not gold”, because in reality the yields of this type of synthesis are limited by the amount of initial material that can be loaded on the solid phase.
Moreover, the reactive protected monomers are quite expensive and their shelf-life is limited. Furthermore, although there is an optimal control of the product sequence, side reactions might take place, which might result in “dead” sequences (i.e., peptides or nucleotide strands that are shorter than the desired product). This depends on the length of the sequence to be synthesised. To overcome these issues, convergent synthetic strategies can be exploited, such as native chemical ligation (NCL) and other ligation chemistries (e.g., ketoacid-hydroxylamine (‘KAHA’) amide-forming ligation chemistry or Ser/Thr ligation). Independently from the chemistry involved, these strategies allow for the synthesis of larger peptides by coupling two peptide fragments [235–238]. When used in conjunction with solid-phase synthesis, these convergent strategies can allow for total chemical synthesis of much longer proteins and polypeptides (up to ~300 aminoacids). However, these strategies still remain fairly limited for producing polypeptides due to both cost and the synthetic efforts required (e.g., need for specific orthogonal reactions) which might favour the usage of other synthetic approaches [239,240].

2.4. Synthetic strategies at the supramolecular level: controlling topology

2.4.1. Topology in living systems

In the context of living systems, topology refers to the spatial arrangement of the various chemical component integrating their parts. In 3D, topology describes how shapes change from one to the other, i.e., how a sphere evolves into a torus. In 2D, topology refers to how different components distribute on the surface creating different patterns (not to be confused with topography, which refers to the surface roughness). Finally, in 1D, chain topology of DNA and proteins describes the coiling and folding of the single chain. Here we focus on 2D topology and particularly on how patterning creates specific energy binding profiles. Biological surfaces are not homogeneous systems and their constituents are arranged according to specific regular patterns fit for purpose. Typical regular patterns can be observed in innumerable macroscopic systems, for example in the distribution of hair follicles on the skin, which are not only equidistant, but also suggest hexagonal packing. Spacing of feathers in birds, shells in marine organisms, cloud formation, butterfly wings and snake scales, or animal markings as in zebra stripe skin patterns are just but a few examples of regular repetition of identical elements on surfaces found in nature [241,242]. These organised arrays of elements or patterns are typically constructed through local interactions between the different components in the systems. The investigation of non-linear dynamics and self-organisation in complex systems involves several scientific fields spanning from fracture mechanics and zoology to sociology and mathematics (Figure 7a) [243].
We now know that natural systems exhibit common features that can be described by mathematical analogies expressed as continuum equations of diffusion and materials transport. These non-linear equations come with certain boundary conditions responsible for the appearance of defects, flaws or symmetry-breaking features in the solutions. An explanation for the patterning appearing in animal skins, like the zebra stripes or the leopard spots, arises from the investigation of oscillating chemical reactions, also called chemical clocks [247,248]. In oscillating reactions, a complex mixture of compounds appears to react in one direction and then converse backwards to its initial state. In this fashion the concentration of reactants and products changes with time in a periodic or quasi-periodic oscillating manner [248]. The whole process, out-of-equilibrium, is an exquisite balance between reaction, which destroys the reagents, and diffusion, which refills them. The break in symmetry, in other words, the formation of patterns arises from the reaction and diffusion processes opposing each other. Mathematically this was first formalized by Alan Turing in 1952 [249,250].

The formation of patterns at the micro- and nanoscale is also found in much smaller biological assemblies such as viruses, lipoproteins, synaptic vesicles, and bacteria. Structural subunits can be combined into topologies with highly symmetric arrangements, like in most nonenveloped viruses,[251] into semi ordered topologies, such as in lipoproteins[252], or into
Turing-like patterns as in most enveloped viruses [253] and endogenous trafficking vesicles [250]. A useful tool to describe aperiodic natural assemblies is by fractal methods. A fractal can be defined as a mathematical set that displays a recursive self-similar pattern with a fractal dimension, the Hausdorff dimension, which exceeds its topological dimension [254]. The family of shapes described by fractals, tend to be scaling. This implies that their irregularity is identical in all dimensions. Fractals offer a powerful strategy of analysing and understanding naturally occurring patterns, and ultimately designing synthetic artificial machines using self-assembly. A typical example is found in DNA, one of the most used building blocks for the design of assembly nanoscale architectures aiming to construct biomimetic systems. Various DNA self-assembly methods [255] have been reported for fabricating surrogate architectures with pronounced geometrical complexity and precision. Actually, the invention of DNA origami in 2006 was a great advancement in the field of DNA nanotechnology [256].

Surface topology is not stochastic and is the outcome of an adaptive change often correlated with a specific function. Viruses are a perfect example of this process as they alter their surface topology during maturation from a noninfectious, practically inactive assembly to an infectious cell-active structure capable of rapidly entering cells [257]. The new tiling models are able to predict the surface structure and topologies of viral capsids by exploiting the concept of symmetry to the full extent [258]. To date viruses have proved to be the most efficient vectors for gene therapy, although they come with several practical, ethical and safety concerns about their use [259]. It could be hypothesized that viruses, endogenous lipoproteins and trafficking vesicles have tailored their surface topology to match that of the cell surface they aim to target. This would indicate that cellular targeting and signalling are not only controlled at a molecular level, as single ligand-receptor interaction, but also at a mesoscale level in the way in which ligands and/or receptors are arranged. Today we have a better understanding of these supramolecular interactions and of how to exploit them to engineer structures that mimic the complexity of biological systems. This has enabled the translation of underlying design principles into synthetic surrogates that can be used for engineering novel biomaterials and biomimetic devices.

2.4.2. Engineering surface topology in artificial systems
Multicompartment systems are very commonly found in Nature. Blood proteins, such as serum albumin, offer circulatory conveyance for soluble compounds such as lipids, hormones, vitamins, and metals [260]. Eukaryotic cells also possess compartmentalisation characteristics in the form of subdivided domains, with different physical and chemical properties performing several cellular functions. These discrete compartments although in close proximity have diverse physical environments. They facilitate complex chemical and biological exchange processes without mutual interference. The late eighties saw a real advancement in cross-disciplinary research in the fields of medicine, biology, physics, chemistry and polymer science. Helmut Ringsdorf, a german polymer chemist with expertise in self-assembly of polymers into functional aggregates, had the vision of polymer science being able to simulate cellular
processes unlike any other material [261]. Later on, multicompartment micelle systems were designed in an attempt to mimic biological structures and features [262].

Multicompartment micelle research has greatly developed in recent years. This is due to advances in novel methods to synthesize triblock copolymers with different block sequences, or add stimuli-responsive polymers and certain moieties. In general, an AB diblock copolymer in a selective solvent for the A block will adopt one of the three morphologies: spherical, worm-like micelles, or vesicles. In each case the solvophobic B block will form a single nanoscale domain. When a third component C is included in the system, as in an ABC triblock copolymer, B and C core domains can be formed, thus generating multicompartments at nanoscale sizes [263]. The block sequence in linear triblocks, i.e., ABC, BAC, or ACB, can play a crucial role in dictating the self-assembly behavior and thus the distribution of the solvophobic (hydrophobic) B and C core domains [264, 265]. The ABC miktoarm (μ) star triblock copolymer structure also provides a powerful method to generate multicompartment micelles. This is when the three blocks meet at a common point, thus forcing the resulting A, B, and C nanodomains to segregate at their point of contact [266]. Lodge et al have reported a vast variety of multicompartment micelle morphologies by synthetizing miktoarm stars containing three mutually immiscible blocks, poly(ethylene glycol) (PEG), poly(ethylene glycol) (PEE), and poly(perfluoropropylene oxide) (PFPO). By changing the molecular weights of the three blocks as well as their volume fractions, a myriad of conformations could be obtained such as hamburger micelles, segmented worm-like micelles and nanostructured bilayer vesicles [267].

Multicompartment micelles have the ability to encapsulate hydrophobic compounds in their hydrophobic compartments. However, the encapsulation of hydrophilic agents in multicompartments micelles still remains a challenge. In addition to this, current synthetic and assembly methods are not quite in place yet to mimic biological processes in multicompartment micelles. This is because the current methodology typically generates polydisperse micelle aggregates, which can be prone to further aggregation or rearrangement. Biological systems do not display this size distribution issue [263]. New alternatives need to be put in place to avoid polydisperse systems and overcome the challenge of encapsulating hydrophilic agents as described above. These alternatives need to also comply with compartmentalization and efficient encapsulation of both hydrophilic and hydrophobic compounds.

Amid the latest biomimetic efforts, polymersomes are possibly one of the few examples that encompass simultaneously both compartmentalization and positional self-assembly. Membrane permeability and the ability to separate extreme pH gradients are fundamental conditions a successful biomimetic system must comply with [268]. As described previously, polymersomes also have the capability of efficiently encapsulate both hydrophilic and hydrophobic compounds in their hydrophilic core and hydrophobic corona inner membrane, respectively. This characteristic becomes critical when designing biomimetic systems for
controlled release and delivery applications. Research in polymersomes engineered with topologically controlled functional motifs and surface properties is a relatively new area that has generated great interest in the last decade. This is because surface engineered polymersomes find use in numerous applications ranging from separation catalysis, to artificial muscles and drug delivery vectors [269-271]. From a biological perspective, polymersome cellular uptake i.e., the number of polymersomes per cell over time, is strongly dependent not only on the polymersome size, shape and surface chemistry [11,272], but also on its surface topology. For example, in PEGylated nanoparticles, the molecular engineering of polyethylene glycol (PEG) on the nanoparticle surface is a key factor of nanoparticle-cell interactions [271]. In this fashion, the spatially configurational freedom and area of occupancy of the PEG chains on a particle surface play a significant role [273].

With the aim of synthetically recreating the outstanding properties of virus surface-confined assembly we have demonstrated how the polymersome surface topology can be arranged into organized nanosized domains. This is accomplished by mixing different membrane-forming block copolymers and triggering membrane-confined separation [244,272,274,275]. Such process leads to the formation of ‘patchy’ polymersomes. In patchy polymersomes the size and morphology of the surface domains are controlled by the molar ratio of the two block copolymers employed in the mixture and their respective molecular weight. The nanoscopic domains can be generated by mixing chemically different copolymers either as AB/CD where phase separation is driven by both hydrophobic and hydrophilic blocks, AB/CB where phase separation is driven by just the hydrophilic blocks, and AB/AD, for which phase separation is driven by the hydrophobic blocks. The domains are formed by the minor copolymer component in the mixture. When the domains are made of cellular binding (i.e., poly(2-methacyloyloxyethyl phosphorylcholine, PMPC) and cellular-inert (i.e., PEO:PEG) copolymers, the cellular uptake kinetics is strongly controlled by the domain geometry and size [244,272,276]. For example, the rate of polymersome uptake by cells was monitored as a function of surface domain morphology and size on PEG$_{23}$-PDPA$_{10}$ and PMPC$_{25}$-PDPA$_{70}$ mixtures at different molar ratios (Figure 7b). The average size of these domains increased linearly as the polymersome dimensions increased [244]. The copolymers PEG and PMPC in the mixture micro phase-separated on the surface to form 10-nm domains within a 100-nm polymersome, while larger (40-nm) domains were formed within a 400-nm polymersome. Furthermore, the findings suggested that the endocytosis efficiency can be effectively improved by the size of the PMPC or PEG rich-domains, with polymersome cell internalization reaching a maximum at optimal domain size between 30-40 nm [272]. These results show that not only the polymersome dimensions and surface chemistry are essential for efficient intracellular delivery, but also the topology of the polymersome surface plays an important role in polymersome cellular interaction. Deserno and Kremer [273] used coarse-grained membrane simulations to show that curvature-inducing model proteins adsorbed on lipid bilayer membranes experience attractive interactions that arise purely as a result of membrane
curvature. They found that the binding energy needed for membrane deformation was reduced when two capsids, modelled as single spheres were separated a certain distance when compared to a single capsid deforming the membrane. This finding supports well the enhanced endocytosis effect observed when using polymersomes with topological surfaces as compared to pristine polymersomes [277].

Extensive evaluation on the polymersome patches, i.e., internal structure, morphology and shape, becomes essential for the development of strategies, on a bottom-up approach, aimed at generating novel topological features in polymersomes. We have reported direct imaging of polymersome domains by transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) with unprecedented single-chain resolution to screen nanoscale clustering formation at a molecular level [275]. This was accomplished by labelling one of the copolymers in the binary mixture with an electron-dense transition metal (indium). This metal and its associated copolymer could be visualized directly by TEM without the need of any additional staining. The system under study was a binary mixture of Indium-PMPC-PDPA and PEG-PDPA. In doing so, statistical distribution of the number of chains per PMPC domains was achieved.

More recently Ruiz-Perez et al. [274] studied the use of triblock copolymers PEG-PDPA-PMPC in patchy polymersomes. Binary and ternary mixtures of diblock copolymers were employed, with the addition of the triblock copolymer acting as an ABC lineactant. The formation of domains in the confined self-assembled polymersome were again driven by the interaction between the A and C blocks. The size and shape of these domains were dependent on their ratio as well as on simple geometrical considerations. In the case of a triblock-containing binary mixture, the two polymers created an interface whose curvature is the result of their respective occupancy in the membrane. This results in a two-dimensional version of the packing factor, which efficiently predicts the self-assembly of amphiphiles in water, as explained in section 2.2.3 [7]. The findings indeed suggest that by devising a simple course-grained model of the patterns observed experimentally, the system can be recreated in silico. Similarly, atomistic and mesoscale simulations of the behavior of immiscible ligands adsorbed on metal (i.e., gold and silver) nanoparticles demonstrated phase separation (up to the point of forming Janus-like structures) or stripe-like patterns depending on the entropic gain of the system (Figure 7d) [246].

Exploiting polymersome surface asymmetry Van Hest and co-workers reported a polymersome nanoreactor with controlled permeability induced by pH and sugar-responsive block copolymers [278]. The polymersomes were formed by a mixture of PEG-b-PS and boronic acid containing polymer PEG-b-PSBA. Boronate was used to ionize boronic acid in basic media thus improving the solubility of the PSBA block in water, which was further enhanced by the binding of glucose to the boronic acid moiety. Glucose addition and raise of pH allowed the hydrophobic PSBA block to switch to a hydrophilic domain and be removed.
This mechanism led to creation of membrane pores that allowed the substrate to enter and be converted by enzymes already encapsulated in the polymersomes. By varying the ratio between PEG-\(b\)-PS and PEG-\(b\)-PSBA the permeability and porosity of the polymersomes could be controlled. Nanoreactors have also been prepared by the incorporation of a channel protein in the polymersome membrane to create porous membranes [279–281]. Montemagno et al reported the design of hybrid nanoreactors comprising membrane proteins stabilized with block copolymer membranes for energy conversion applications [282–284].

Mesoscale domain formation within assembled mixtures of neutral and anionic polymer amphiphiles driven by divalent cations has also been investigated. The study was done with neutral poly(ethylene glycol)–poly(butadiene) (PEG-PBD) and anionic poly(acrylic acid)–poly(butadiene) (PAA–PBD) by Discher and co-workers [160]. Divalent cations had the ability to crossbridge polyanionic PAA-PBD, that then demixed from neutral PEG-PBD and formed spots or rafts within polymersomes, as well as stripes within cylindrical micelles. These striking results indicate the phenomenon is electrostatic in nature and can occur in a variety of systems. Nanoparticle surface structural and chemical heterogeneity is being reported to also be important in determining protein-nanoparticle surface interaction and subsequent protein conformation [285]. Lau et al prepared chemically similar gold nanoparticles with different surface structures and investigated their interaction with bovine serum albumin (BSA). BSA adsorption onto particles with nanoscale stripe-like polar and nonpolar domains behaved differently from the particles with randomly distributed domains [285].

Nanoscale surface patterns are not only relevant to nanoparticles but also affect substrate surfaces as a significant factor affecting cell adhesion, motility, and morphology [286]. In physiological conditions, the cells in our body have to adhere to the underlying non-cellular environment, called the extracellular matrix (ECM) so as to survive and maintain their functions. The ECM comprises a three-dimensional (3D) cellular scaffold with structural widths and lengths at the nano- and micrometer scales on which tissues are structured. Although the responses vary between different cell types, it has become clear that physical properties of the ECM such as topography, topology, elasticity, stiffness and anisotropy play an important role in cellular response [287]. The next section will focus on the effect that substrate anisotropy and/or topology have on cell adhesion and subsequent responses. The design of biomimetic nanoparticles with surface topology takes inspiration on virus exquisite surface features as we have mentioned above. Likewise, spatial analysis of the ECM components in organs and tissues have been widely investigated with the aim of obtaining templates or blueprints that are useful for the design and fabrication of tissue engineering scaffolds [288,289]. This has allowed the design of novel ECM-mimicking biomaterials for new therapeutic approaches and regenerative medicine among others. It is therefore imperative a profound understanding of the ECM structure, composition and behaviour so that the basic principles can be recognised and extrapolated into tools to mimic the ECM development and regenerative processes.
a. 2D models

The investigations performed on 2D systems have allowed us to comprehend many aspects of the cell-matrix interactions and how specific substrate properties affect cell behaviour. However, the ability to control cell behaviour in 3D is especially important if tissue engineered scaffolds are to be used as artificial implants. Parameters such as surface topography (roughness), stiffness, chemistry and wettability have been widely studied, yet, more and more properties have been coming into focus recently [290–294].

Surface topology, has gained considerable attention in the last decades as most of the early work on ECM did not account for this parameter. The exclusion of topological features on substrates implies that the surfaces are rendered homogeneous and hence exhibit the same properties on all areas. This setting does not represent the heterogeneous complexities existing in cell-ECM interfaces. Nowadays it is widely accepted that the variations of spatial patterns and arrangements of substrate surfaces or conjugated molecules (at the same length scale) offer important cues for investigating cell-ECM interfaces. One way to explore topological features is the fabrication of surfaces where chemistries are arranged in different patterns. Such patterns then create distinctive topological cues which are exploited to control cell adhesion and signaling.

Molecules able to form self-assembled monolayers (SAMs) are widely used as the type of material to create patterned surfaces on substrates. Self-assembled monolayers are constructed on glass or metal by deep UV photolithography. In this fashion, the substrate surface displays motifs at molecular level. Primary cell lines and various types of cancer cells were cultured on spatial regions of hydrophobic SAMs with 1-200 µm feature size and they were shown to attach and grow restrictively on SAMs patterns [295]. This was the result from differential protein adsorption between modified and unmodified substrate areas, which can preferentially promote or resist cell attachment [296]. Similar studies were conducted later on using different SAMs, materials, preparation methods and cell types [297–300].

Substrates without any biologically active sites can be treated with biomolecules involved in cell attachment. One of the most used ligands for this purpose is the RGD (arginine-glycine-aspartate) peptide, a recognition sequence for integrin presence in several fibrous ECM proteins. The arbitrariness as well as ordering of RGD patterns created on SAM surfaces were also found to affect the degree of cell attachment [301,302]. Roberts et al [303] reported that the cells adhere to a gold-coated glass slide with mixed (EG)₃OH and (EG)₆OGRGD SAMs depending on the mixture molar ratios. Cell spread and attachment increased directly proportional to the mole fraction of RGD [303]. A combination between cell specific adhesion cyclic (-RGDFK-)ₐ(R: arginine, G: glycine, D: aspartic acid, f: D-phenylalanine, K: lysine), and non-specific adhesion positively charged KKK peptide, on SAM also demonstrated a synergistic effect with the ability to enhance cell adhesion more efficiently than cell culture plate [304]. The findings revealed that the cells can adhere and spread well on lower RGD intermolecular
spacing and disordered patterns. Finally, hyaluronic acid, galactose, heparin, insulin and EGF (epidermal growth factor) are good examples of biomolecules that were also micropattern-immobilised on substrates and displayed differential cell adhesion when compared to non-immobilised substrates areas [305-308].

b. 3D Scaffolds
The design of 3D scaffolds or matrices for applications in tissue engineering and regenerative medicine must comply with certain criteria as suitable constructs. The criteria conditions encompass an interconnected porous or fibrous structural network with the ability to sustain cellular proliferation and survival through sufficient cell-matrix and cell-cell contacts [309]. In addition, biocompatibility and biodegradability are critical features of designed scaffolds aimed for in vivo applications. The engineered scaffold must also support the diffusion of nutrients and metabolic wastes by means of porosity and permeability [310].

Synthetic biocompatible and biodegradable polymers have been widely employed to investigate the effect that the 3D matrix porosity has on cell migration and proliferation. The most commonly used synthetic polymers for in vivo applications approved by The Food and Drug Administration (FDA), include: poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(caprolactone) (PCL), poly(lactic acid) (PLA), poly(hydroxyethyl methacrylate) (PHEMA), poly(acrylic acid) (PAA), poly(glycolic acid) (PGA), and poly(propylene fumarate) (PPF). The working modes for creating topological features on 3D polymer substrates are identical to those for nanoparticle surfaces mentioned before in this review.

Porous scaffolds of poly(l-lactide-co-glycolide) polymer were fabricated by addition of poly(ethylene glycol) (PEG) at different concentration as the second phase [311]. Cell adhesion, growth and migration of osteoblasts cultured on these scaffolds increased inversely proportional to PEG concentration. Along the same lines, low cell adhesion was clearly observed in PEG regions [311]. Patchy surfaces were constructed on 3D polystyrene foams exploiting the nature of amphiphilic block copolymer self-assembly in combination with the high internal phase emulsion (HIPE) templating technique [312]. By mixing two amphiphilic block copolymers, polystyrene-b-poly(ethylene glycol) (PS-b-PEG) and/or polystyrene-b-poly(acrylic acid) (PS-b-PAA), in different molar ratio, micro-clusters of PAA and PEG can be formed on the surface of the polystyrene foams. As for polymersomes, the second phase is the less abundant copolymer in the binary copolymer mixture and is responsible for the topological features assembled on the matrix. By tuning the mixture molar ratios, clusters of PAA assembled on PEG matrix or the inverse scenario i.e., clusters of PEG on PAA matrix can be tuned. The cells are able to attach and grow only on a certain mixture of block copolymer scaffolds [312].

Functionalization of fibre scaffolds has also been extensively explored due to the benefits of fibrous structures resembling ECM backbone (fibrous proteins). The design process of topological features on fibre scaffolds aims to mimic the ECM physiological conditions. The
design process in this case follows the parallelism of topologically engineered polymersomes mimicking viruses. One of the most commonly employed techniques to create fibrous scaffolds is electrospinning [313]. This fabrication technique allows the functionalization to be done in a fairly simple way just by using amphiphilic diblock copolymers [314]. Mixing homopolymers with additives containing different degrees of hydrophobicity can also create polymer segregation occurring at the fibre surface and subsequent surface property changes [315].

Although it is not a simple task to achieve functionalization and spatial control, several research groups have successfully obtained surface functionalized electrospun fibres or hydrogels using different approaches [316-319]. Amphiphilic diblock copolymers were mixed at different molar ratios inducing confined microphase separation at the fibre-water interface. This confined microphase separation rendered heterogeneous topologically defined surfaces with different compositions. In the amphiphilic block copolymers RGD was conjugated to the hydrophilic block of the diblock containing divinyl sulfone group. Microphase separation was clearly visualized on the fibres in terms of PMPC and POEGMA molar quantities in the binary block copolymer mixture (Figure 7c). The cells displayed better spreading and attachment on mixed compositions than on other compositions containing only inert or adhesive surfaces [245,318].

Hydrogels have also been used as an important type of 3D tissue-engineered scaffolds by using natural and synthetic polymers. Hydrogel biocompatibility and biodegradability, high water content and easy architecture of structural parameters confer hydrogels tremendous advantages over other polymeric scaffolds [320]. The hydrogel network provides a soft tissue-like matrix for cell growth permitting diffusion of nutrients and cellular waste through the network structure. While many biophysical parameters have been reported as important factors for controlling cell behavior, hydrogel stiffness has been found to be a decisive parameter in cell fate [321,322]. Stiffness in this context does not refer to static but to dynamic stiffness. This is the ability of hydrogels to change their viscoelastic characteristics in time-dependent stress/strain changes, matrix degradation and posterior formation in the interaction with cells. Current synthetic polymers are still limited in mimicking the multiple functions of the ECM. Future strategies for successful tissue engineering ECM-like hydrogels involve the fabrication of hydrogels with biophysical parameters that allow them to undergo dynamic changes both spatially and temporally, in response to the interactive cells [322].

3. Interactions between molecular bionic materials and living systems
Despite all synthetic efforts, artificial systems are still far from the perfection achieved by biology in producing biomolecules. The design of biomaterials should be tailored to the intended application, taking into account the biological challenges that the biomaterials will face. The following section will provide an overview of the key biological aspects to be considered for the production of nanocarriers for drug delivery based on molecular bionics, and possible strategies to take advantage of biological mechanisms for this purpose.
3.1. Navigating the body

One of the most important challenges in the field of biomaterials is the fabrication of nanocarriers which are stable in the chemical complexity of biological media, such as blood, interstitial fluid or cell cytosol. Upon systemic administration, nanocarriers are immersed in a crowded environment mainly comprised of proteins (~3700 different types) and cells. Almost instantaneously, these carriers create spontaneous supramolecular interactions with the protein components of the biological milieu, which affect their surface appearance. The layering of proteins on the surface of the system, often referred to as ‘protein-corona’, is extremely important as it dictates the fate of the biomaterial at a systemic and cellular level [10,323,324]. For example, proteins such as the third complement component (C3) or the apolipoproteins, which are abundant in the blood plasma, are often the major component of the protein-corona and promote an immunological response that results in the clearance of the material from the circulation [323,325]. In this context, DNA-based supramolecular biomaterials are emerging as a novel drug delivery strategy since they can be engineered to have predefined 3D shapes and to be responsive to various endogenous stimuli [326,327]. However, they are particularly immunogenic, which is a limiting factor that still needs to be completely addressed [328]. One way to limit the formation of a protein-corona is the functionalization of the biomaterials with non-fouling materials on the surface. These are generally chemical groups which fulfill the “Whitesides design rules”: I) extremely hydrophilic, II) include hydrogen bond accepting groups, III) lack of hydrogen bond donor groups, and IV) lack of net charge [329]. The classical example of non-fouling molecule is PEG, which is clinically approved and has been historically used to reduce protein adsorption and opsonisation in circulation. However, recent investigations demonstrated that the density of PEG exposed on the surface is crucial for prolonging blood half-life [330], and hence it is a crucial parameter to consider when designing nanocarriers. Also, it has also been suggested that the human immune system can produce anti-PEG antibodies, creating an immunologic memory that could minimise the efficacy of PEGylated therapeutics [331]. Nevertheless, further investigations adopting standardized and sensitive detection methods regarding the immunogenicity of PEG are still required [331,332].

Although in principle the formation of a protein-corona can be seen as a drawback, the interaction with proteins and other entities such as enzymes can be elegantly used for navigation purposes. For example, nanoparticles coated with albumin on their surface have shown a prolonged circulation time [333]. Alternatively, by tuning the surface properties of the delivery system it is possible to control the components of the protein corona creating an indirect targeting effect [334]. Furthermore, the analysis of the protein-corona could be exploited as a diagnostic tool for early detection of pathological conditions creating theranostic nanocarriers.
Figure 8. Chemotactic synthetic vesicles and superselectivity. **a)** Representation of the chemotactic polymersomes formed using a combination of amphiphilic polymers and encapsulating glucose oxidase and/or catalase enzymes. **b)** Transmission electron microscopy micrograph showing the patchy structure of the chemotactic vesicle. **c)** Graph showing the significant increase in brain parenchyma distribution of chemotactic polymersomes. **d)** and **e)** Simulation snapshots showing binding of monovalent (d) and multivalent (e) nanoparticles (green spheres) to host surfaces exhibiting different degrees of receptor (red spots) density. Increasing the density of receptors does not significantly change the number of adsorbed species for the monovalent case, while there is a 10-fold increase for the multivalent nanoparticles, typical of a superselective behaviour [336]. **f)** Simulation results showing the fraction of bound guests as a function of the receptor density for monovalent (for both weak, green curve, and strong, blue curve, ligand-receptor interactions) and multivalent particles (for weak ligand-receptor interaction, red curve). Superselective behaviour, which corresponds with a sigmoidal (i.e., off-on response) curve, only takes place for multivalent particles decorated with ligands which interact weakly with the surface receptors. **d)**-**f)** are reprinted with permission from Designing super-selectivity in multivalent nano-particle binding, F.J. Martinez-Veracoechea and D. Frenkel, Proc Natl Acad Sci USA, 108 (27) 10963-10968 (2011) [336].

Together with their biochemical complexity, biological fluids change considerably over the different locations of the body, creating gradients of chemicals which can be smartly exploited to transport drug carriers. For example, the daily high consumption of glucose by the brain inherently creates a gradient of glucose in the blood. Quite recently, a strategy making use of such a gradient has been developed, which allowed to overcome the blood brain barrier (BBB), an exceptional filter and a highly complex “obstacle” which prevents successful delivery of drugs to this organ. Indeed, accumulation of drug delivery systems in the brain tissue is observed when the BBB is disrupted by inflammation or malignancies [337]. However, in case of an intact BBB, alternative strategies such as conjugation of targeting peptides or direct injection of therapeutics into the brain via catheters (i.e., convection enhanced delivery) are needed [338]. The extreme difficulty of targeting this organ is summarized by the current paucity of stimuli-responsive materials designed for applications in the brain tissue [339]. Nevertheless, small progress has been made in recent years through the development of
nanoswimmers. These are a novel class of smart synthetic particles able of self-propelling into the body through tissues following, for example, chemical gradients via chemotaxis [340]. At the nanoscale, flows take place in the Stokes regime, in which inertial forces are much smaller than viscous forces. In addition, nanoscale objects are subjected to thermal agitation and thus Brownian motion. For this reason, to achieve self-propulsion it is necessary to induce some slip velocity on the surface of the object, for example, by creating a gradient of a chemical around it (diffusiophoresis). Joseph and colleagues created brain targeting polymersomes (more specifically, asymmetric patchy polymersomes as those described in section 2.4.2) containing two enzymes (glucose oxidase and catalase), which were able to self-propel following glucose gradients through the metabolism of the substrates (Figure 8a and b). Due to the asymmetry in the polymersome topology, the flow of the enzyme substrates (glucose) and products (d-glucono-δ-lactone and water) in and out the polymersomes became polarised, leading to two propelling effects: an osmotic torque that aligned the polymersome towards the glucose gradient, and a self-diffusiophoretic drive from the product that propelled the polymersomes toward high concentrations of glucose. One of the challenges to achieve propulsion in biological environments would be the flow inside blood vessels. However, in their work, Joseph et al. showed that the polymersomes did not deviated from the glucose gradient when a flow rate of 10 μm s⁻¹ (perpendicular to the glucose gradient) was applied. In addition, simulations of the flow inside a blood vessel demonstrated that the chemotactic polymersomes increased the nanoparticle distribution at the interface of the endothelial wall, compared to non-propelled polymersomes. Actually, when tested in a rat model with an intact BBB, these polymersomes had almost a 4-fold increased penetration in the brain parenchyma compared to non-chemotactic polymersomes (Figure 8c) [341].

3.2. Targeting in the body
Undoubtedly, the most prominent application of biomaterials for drug delivery is cancer treatment, whereby the aim of the system is to efficiently deliver therapeutic molecules at the tumour site. The majority of the nanocarriers administered systemically take advantage of the leakiness of the tumour vasculature and the concurrent poor drainage of the lymphatic system, classically defined as enhanced permeability retention (EPR) effect [342], to passively accumulate in solid tumours. However, targeting tumours merely exploiting the EPR effect has shown limited success due to the lack of passive penetration in the deepest regions of the tumour [343,344]. Furthermore, exploiting the EPR effects is no longer possible when the target tissue is not a solid tumour, but a tissue to regenerate for example. In this context, colloidal biomaterials can be engineered with targeting motifs on their surface (e.g. antibodies, peptides, small molecules, aptamers) in order to confer targeting to specific tissues. For example, the integrin αβ₃ is highly expressed in proliferating tumours and can be targeted using the three amino acids peptide RGD [345]. Other examples of commonly aimed targets are the folate receptor alpha in solid tumours [346], the low density lipoprotein receptor-related protein-1 (LRP-1) and the transferrin receptor for brain targeting [347,348]. However,
in order for the active targeting to be specific it is indispensable that the targeted molecules are accessible and ideally exclusively expressed [349]. A possible solution to augment specific targeting is the design of “superselective” nanocarriers, as discussed next.

Super-selective binding is characterised by an on-off association profile for which binding only takes place when a control parameter exceeds a threshold value, something that would allow minimising the side effects of chemotherapy, for example. This could be achieved if the control parameter would be the density of membrane receptors in the cells. Therefore, binding of a drug or carrier drug would be limited to high density of receptors (i.e., above the threshold) as it happens for cancer cells, while no binding would happen to healthy cells even though they both exhibit the same type of (endogenous) receptors [351]. As we mentioned in section 2.2.5, nature achieves selectivity thanks to cooperativity and multivalency. In the case of nanoparticles used in drug delivery, they can be decorated with multiple ligands able to attach to the receptor of the cell membrane. As also explained in that section, in such case, and provided that the ligand-receptor bonds are form independently, there is an entropic contribution to the energy of association called the avidity entropy. For this second situation, and as shown recently by simulations of the Frenkel group [336,352] the number of bound states grows non-linearly with the receptor density if the ligand-receptor interactions are weak (Figure 8d and e), giving rise to on-off association profiles, distinctive of a super-selective behaviour, as mentioned above (Figure 8f). However, this super-selectivity theory has two main drawbacks. The first one is that multivalent particles are also more sensitive to non-specific interactions than monovalent ones, as show recently by Angioletti-Uberti, a problem which could be solved by attaching some protective receptors to the nanoparticle surface which would compete with those on the cells [353,354]. Also, the ligand-receptor affinity required to achieve super-selective profiles is weaker (1 kT compared to few kTs, Figure 8f) than usual supramolecular interactions (Table 1), which limits its practical applications. In this regard, we have recently proposed the use of multivalent polymersomes decorated with targeting ligands which are embedded in the polymer brush of the hydrophilic block. This insertion of the ligand in the polymer brush creates a steric repulsion for binding to the respective cell membrane receptor, which effectively reduces the strength of the interaction, leading to superselective binding profiles [355]. In addition, the combination of different types of ligands in the same polymersome (multiplexing) allows targeting cells which overexpress unique combinations of receptors [355].

3.3. Penetrating the target tissue

Besides the targeting of the specific organ, another major challenge in the field of drug delivery is tissue penetration. Indeed, in order to have selectivity, the drug delivery system has to be in close proximity (~30 nm) of the target. Advances in the design of biomaterials have been impressively pushed forward in the last decades, and new “smart” strategies have been adopted to enhance tissue penetration. Smart or stimuli-responsive supramolecular
Biomaterials can be designed by simply predicting the behaviour of the building blocks conforming the supramolecular entity in a defined biological microenvironment. Broadly, supramolecular biomaterials can respond to two types of stimuli: exogenous and endogenous. The former comprise forces applied from external sources such as magnetic fields, light, ultrasounds and electric fields, whilst the later are stimuli that can be found within the human body resulting from a pathology or disease [356]. Here we will focus on self-assembled nanomaterials that are sensitive to endogenous stimuli and that can be administered systematically.

Tumors, particularly the ones of a solid nature, present a series of distinct biological conditions such as low pH [357], hypoxia [358], and over-expression of enzymes [359], that allow for the proliferation of the cancer cells. However, these peculiar features are also present in the majority of inflamed tissues and can be used as triggers for increasing tissue penetration and cellular internalization of biomaterials, as will be discussed next.

3.3.1. pH-responsive delivery systems

pH sensitive materials can be designed either using building blocks that change solubility according to the protonation state or through the insertion of acid-cleavable bonds [360]. Attractive pH sensitive biomaterials can be produced using peptides or polymers. In fact, peptides these represent versatile building blocks as tuning their amino acidic sequence gives the ability to control the final geometry and supramolecular properties [361]. pH sensitivity can be combined to other endogenous stimuli in order to create multi-stage delivery platforms able to respond to a sequence of biological microenvironments in order to increase tissue penetration. For example, iCluster nanoparticles formed by small pH sensitive prodrugs assembled into bigger anti-fouling nanoparticles (~100 nm in size) undergo disassembly in the tumour microenvironment favouring deep tissue penetration of the prodrugs (see Figure 9) [362]. Alternatively, high penetration and cellular uptake have been increased by employing an acidic-responsive copolymer as a sheddable layer of a polymeric prodrug conjugated with a cell penetrating peptide (iRGD) [363].
Figure 9. Preparation and physicochemical properties of the clustered nanoparticles. a) Chemical structure of the components of the iClustered nanoparticle. b) Cartoon depicting the 3D morphology and the mechanism of action in response to tumour acidity. c) In vivo antitumor activity showing growth inhibition. d) Kaplan-Meier plots showing the increased survival of the mice treated with iCluster nanoparticles. Reproduced with permission from Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. Hong-Jun Li, Jin-Zhi Du, Xiao-Jiao Du, Cong-Fei Xu, Chun-Yang Sun, Hong-Xia Wang, Zhi-Ting Cao, Xian-Zhu Yang, Yan-Hua Zhu, Shuming Nie, and Jun Wang. Proc Natl Acad Sci USA, 113(15), 4164-4169 (2006) [362].

In a different context, pH-responsive nanoparticles have been employed for the delivery of drugs to tissues exposed to bacterial infections. PLGA-b-polyhistidine-b-PEG nanoparticles were shown to switch to a cationic surface charge through the selective protonation of the polyhistidine in acidic environments. This led to the increased bacterial uptake of nanoparticles and could be used for selectively targeting infected cells [364]. Supramolecular self-assembly has also been exploited for the creation of multi-responsive antifungal delivery systems sensitive to the low pH of the infected tissue as well as to the presence of intra- and extracellular reductase enzymes. This system showed promising results for the treatment of infected mice and it is a novel alternative to other ‘non-responsive’ systems present on the market (Abelcet®, AmBisome® and Ampotheck) [365].

3.3.2. Enzyme-responsive delivery systems
The tumour microenvironment also presents a high density of enzymes, in particular, matrix metalloproteinases (MMPs). These are proteases that degrade the extracellular matrix and are particularly active in aggressive tumours, facilitating the migration and further metastasis of the cancerous cells [366]. Enzymes represent another endogenous stimulus that has been used for triggering smart materials. Torchilin and colleagues created a sheddable multi-stage delivery system. Here, the sensitive block was represented by an MMP-responsive octapeptide, which linked a long chain of PEG with paclitaxel (chemotherapeutic). Following cleavage of the
peptide by the MMPs, a cell penetrating peptide (TAT) or a poly-cation polymer were exposed on the surface of the micellar preparation. This ‘onion-like’ strategy resulted efficacious in increasing cellular penetration and promoting drug accumulation compared to the non-responsive micelles [367,368]. Lipases are another type of enzyme present in the tumour microenvironment that have also been used as trigger for the controlled release of therapeutics from liposomes [369].

3.3.3. Hypoxia-responsive self-assembled structures

Hypoxia is another a metabolic state associated to tumours, as well as various other vascular diseases [370]. Nitroaromatic groups are often used to create hypoxia-responsive self-assembling structures because under low oxygen conditions they are converted into hydrophilic groups. For example, Thambi and colleagues conjugated a nitroimidazole derivative to the backbone of carboxymethyl dextran (CM-Dex) creating an amphiphilic polymer that self-assembled in micelles. These micelles were shown to selectively accumulate in hypoxic tumour tissues and effectively delivered a cytotoxic payload [371]. A multistage-delivery strategy based on hypoxia sensitivity has been also created employing azobenzene bonds to link a protective shielding layer with a polyethyleneimine (PEI) small interfering RNA (siRNA) complex. Upon shedding of the protection within the tumour, this complex is released and internalized by cells inducing gene silencing [372].

3.3.4. Shear stress

A final type of endogenous stimulus often present in injured tissue is an alteration of the shear stress. The obstruction or lumen-restriction of blood vessels is symptomatic of atherosclerosis and coronary heart disease. However, these diseases could be attenuated or prevented by acting with the correct timing at the site of injury. The impediment present produces a significant increase in fluid shear compared to healthy vessels. The stress created, called shear stress, has been used as a trigger for targeting obstructed blood vessels [373]. One of the first shear-stress responsive material tested was a lenticular liposome made of 1,3-diaminophospholipid. These nanoparticles were shown to release a model payload in conditions of high rheological stress due to the transient formation of pores in the membrane of the vesicle [374,375]. Another smart material design which involves pressure gradients is the creation of micrometer sized aggregates of poly (lactic-co-glycolyc acid)-PLGA) nanoparticles coated with tissue plasminogen activator. These aggregates are held together by hydrophobic effects, and given the weak nature of these, upon experiencing a high shear stress in the proximity of the vessel obstruction, they disaggregate releasing the payload and inducing the dissolution of blood clots [376]. Shear forces have also been exploited in order to create thinning supramolecular materials that change their state of matter in a controlled fashion. These materials rely on selective and directional supramolecular forces in order to become liquid-like, following application of a shear stress, while turning into a solid phase at equilibrium [377]. Examples of these materials are self-assembling peptides which form nanofilaments exploiting the supramolecular interactions created by amino acid charges and
protein secondary structures [378]. Nano self-assembled filaments have been used to deliver a variety of molecules including vascular endothelial growth factor (VEGF) mimic peptide and a heparin-binding domain for either ischemic tissue repair [379] and bone tissue regeneration [380]. However, the nature of these types of materials limits their administration to local injections precluding a wide-spectrum implementation.

3.4. Intracellular drug delivery

The final challenge of any drug delivery system is represented by the intracellular delivery. The continuous progress in understanding the process of endocytosis by cells uncover novel mechanisms by which colloidal systems are taken up and trafficked inside the cells. Generally, all the nanocarrier preparations, with and without targeting motifs, are internalised by cells via endocytic pathways and the uptake mechanism varies according to the cell type and the properties of the colloidal system used [381]. Upon internalisation, nanocarriers are physiologically trafficked intracellularly into vesicular compartments where the pH progressively drops to ~4.7 in order to favour their degradation. Ideally, the drug loaded in a given nanocarrier should be released from these endocytic compartments in order to reach the final target and avoid lysosomal degradation. In this context, it is crucial to promote the ‘endosomal escape’, whereby the payload is released into the cytosol before getting degraded. Here we propose a brief overview of the most common approaches adopted to promote endosomal escape.

The hypothesis of ‘proton sponge’ effect associated to the use of polycationic molecules, such as PEI, has been described for years as the main mechanism behind the endosomal/lysosomal escape of nonviral vectors. This hypothesis is based on the idea that polycationic molecules with buffering capability are continuously protonated in the endosome, with a parallel active pumping of counter ions to balance charges. This effect would increase the osmotic pressure inducing the rupture of the endocytic vesicles and the release of the cargo. However, the ‘proton sponge’ hypothesis is under debate, as the ability of altering the lysosomal pH by polycations is questionable [382]. Hence, it is possible that payload delivery by polycations vectors is mediated by different and uncharacterised mechanisms. Nevertheless, changes in osmotic pressure at the endosomal level are also induced by pH sensitive materials. For example, polymersomes including PDPA in their composition undergo rapid protonation with a consequent supramolecular disassembly in the presence of an acidic microenvironment. The swift increase in osmotic pressure within the endosome results in the formation of pores on the vesicle membrane that favour the endosomal escape [383]. This approach has enabled the delivery of various cargos ranging from plasmid DNA to antibodies [348,384]. Alternative strategies have been inspired by biological mechanisms occurring during viral infection. Generally, pores are formed onto the bilayer of the endosome in order to favour the acidification of vesicle. The opening and closing of these pores is regulated by the membrane tension, and some peptides such as TAT, haemagglutinin and aurein can either
preclude the pore from closing or can self-assemble themselves creating transmembrane pores [385]. Therefore, tethering the surface of nanocarriers with these molecules has been reported to be an efficient mechanism to induce endosomal escape. Another interesting approach has been proposed by Akita and colleagues who coated colloidal nanoparticles with a lipidic bilayer that promotes membrane fusion. This strategy has been implemented to induce endosomal escape and nuclear targeting for gene delivery. The authors produced a multi-layered DNA-polycation complex coated with nuclear and endosomal fusogenic lipids which are sequentially shed enabling for endosomal escape first, and nuclear targeting with gene delivery after [386]. Nuclear targeting, has also been reported following tethering of nanoparticles with small peptides functioning as nuclear localisation signals [387]. However, the nucleus is only one of various intracellular organelles that can be targeted by nanoparticles in order to promote more specific therapies. Another example are mitochondria, as they are the site of specific diseases called ‘mitochondrial diseases’. The classic strategy to target mitochondria is the use of cationic molecules, particularly triphenylphosphonium (TPP), as they counter balance the high membrane potential present on the surface of the organelle membrane [388]. One successful example was proposed by the Dhar group, where polymeric nanoparticles dressed with TPP showed increased localisation in mitochondria compared to unfunctionalised structures [389]. An alternative to TPP is represented by mitochondria-penetrating peptides. These are cationic sequences which act in a similar fashion to TPP, but that can be easily tuned by altering their sequence in order to better control final targeting [390].

Although this type of intracellular targeting could open to new and exciting therapeutic possibilities, it has to be noted that any structural modification of the colloids surface area (e.g. lipid coating, conjugation of polycations and small molecules), affects the ability of the nanocarriers to travel around the body (see section 3.1) and to penetrate the tissue (see section 3.3). Therefore, despite the great results achieved in vitro, a careful evaluation of these systems in vivo is needed.

As we have developed in this review article, the design of materials aimed at biomedical applications requires a careful selection of building blocks and of interactions to assemble these into functional biomaterials. In doing so, bionic materials with life-like properties, able to mimic some of the features of biological compartments or tissues can be constructed. Here we have given some guidelines or design rules to design materials able of adapting to the specific conditions of the human body and human tissues (as for tissue substitutes), or of navigating the body and targeting specific cells (as in drug delivery vehicles). As evidenced along the article, there are many disciplines involved in the development of these biomaterials (ranging from mathematical modelling to clinical applications or surgery) and the future development of biomaterials will for sure require even more fluid communication between them. We also
envisage that many new functionalities will be introduced if we move towards far-from-equilibrium systems. Moving towards far-from-equilibrium systems allows the development of more complex behaviours such as chemotaxis, morphogenesis or self-healing as we have shown here [23,335]. For sure, these systems will play a definitive role in the construction of biomaterials. Finally, the design of systems able of evolve and adapt to changing conditions in a Darwinist manner is still a challenge which will be for sure addressed in the coming years [15]. Indeed, biological systems and mechanism have had almost 4 billions of years to evolve, and their perfection is still far from being reproduced in synthetic systems [42]. Yet, this (maybe unbridgeable?) gap should encourage us to keep on studying and understanding the fundamentals of molecular bionics to use them for the future design of biomaterials [4].

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