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Self-Assembly

Biological Soft Materials

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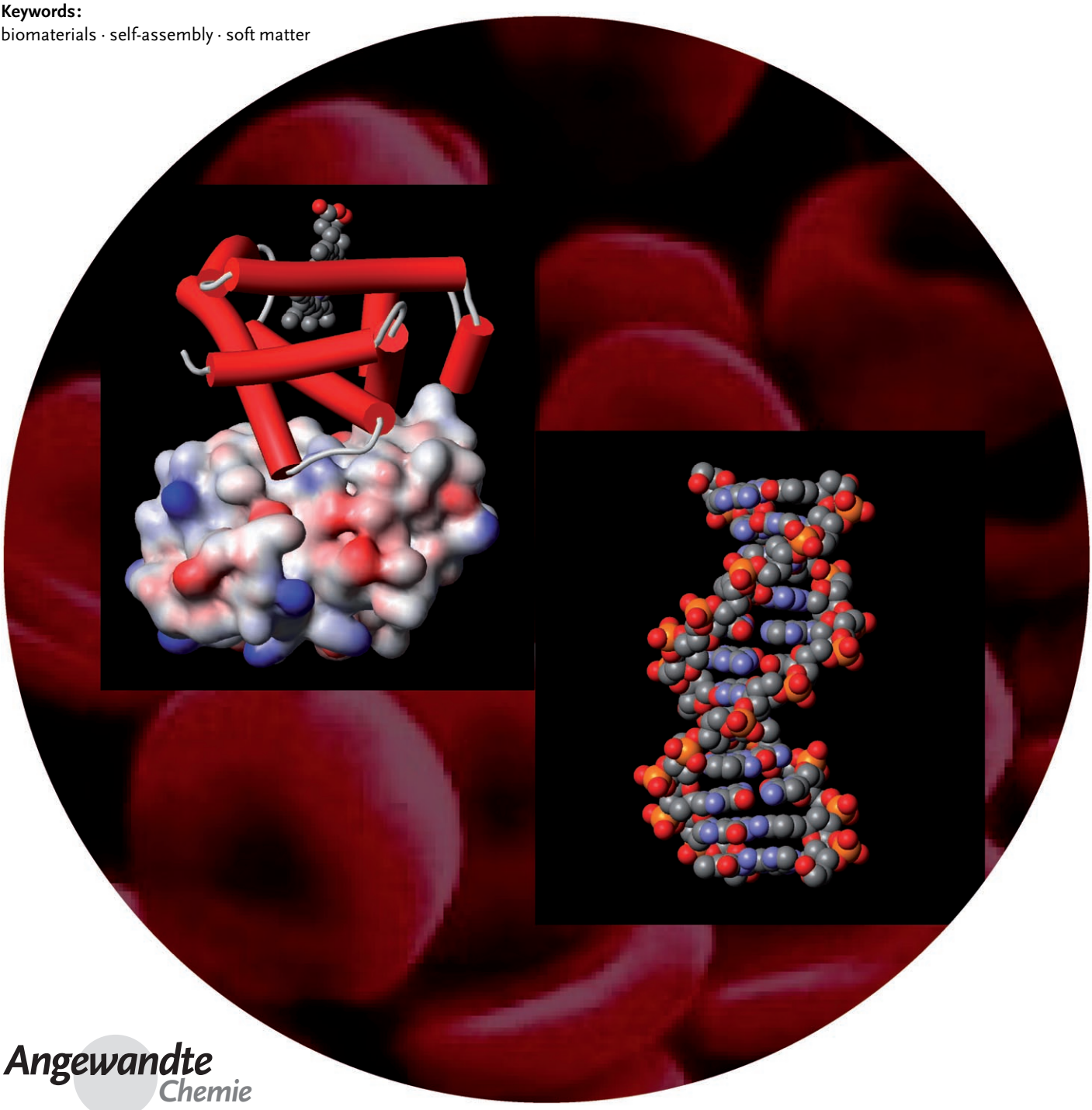

With one or two exceptions, biological materials are "soft", meaning that they combine viscous and elastic elements. This mechanical behavior results from self-assembled supramolecular structures that are stabilized by noncovalent interactions. It is an ongoing and profound challenge to understand the self-organization of biological materials. In many cases, concepts can be imported from soft-matter physics and chemistry, which have traditionally focused on materials such as colloids, polymers, surfactants, and liquid crystals. Using these ideas, it is possible to gain a new perspective on phenomena as diverse as DNA condensation, protein and peptide fibrillization, lipid partitioning in rafts, vesicle fusion and budding, and others, as discussed in this selective review of recent highlights from the literature.

From the Contents

1. Introduction

This Review is focused on selected exemplars of biological soft-matter science. What do we mean by this term? We want to show how concepts from soft-matter physics and physical chemistry are being applied to understand problems in biology. This research area is not the same as biological physics, biophysics, or biochemistry. What is "soft matter"? A lengthy discussion of this question is outside the scope of this Review and has been discussed elsewhere.[1–6] Alternative names for these systems are complex or structured fluids. In brief, soft matter is characterized by flow properties intermediate between those of a crystalline solid and a liquid (hence "soft") and a level of hierarchical molecular order that goes beyond the intramolecular covalent bond. Self-organization is central to many soft materials and is often based around hierarchical ordering due to noncovalent interactions such as hydrogen bonding or screened electrostatic forces.

Although one could argue that almost all biological matter is, by definition, "soft", we would not wish to subsume biology within soft-matter science! Rather, we mean that concepts from polymer physics, colloidal physical chemistry, and amphiphilic self-assembly can yield great insights into fibril formation, protein crystallization, or membrane structure and stability for instance. In fact, these are several of the examples discussed further herein. It is not a complete list, but is a discussion of important topics that have recently been or currently are under investigation.

The current Review is focused on soft materials based on lipids, nucleic acids, proteins, or cells. Lack of space prevents us from discussing the fascinating physics and chemistry of other biological soft materials such as polysaccharides, which as comblike macromolecules have particularly interesting rheological properties, nor of plant biomolecules based on cellulose. In addition, we do not discuss the extremely important topic of protein folding, which in our view lies in the biophysics realm. We also do not consider complexes of proteins with nucleic acids or lipids. In a short review of this type, we have to be selective, and unfortunately other topics may also be omitted. The Review starts at the level of molecular self-assembled lipid structures and leads in a logical manner through consideration of larger and more complex structures to finish with whole cells.

2. Biological Soft Matter: Selected Recent Examples

2.1. Lipid Rafts

Lipid rafts are phase-separated domains (typical diameter < 10 nm) observed within lipid membranes (Figure 1).[7] They

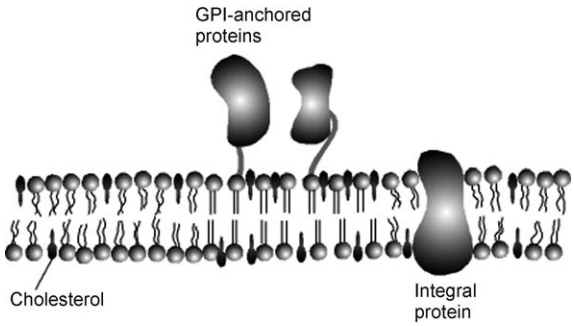

Figure 1. Structure of a lipid raft. The central raft domain contains lipids in the liquid-ordered phase, in which the acyl chains of the lipids are extended. This domain is rich in cholesterol, and many types of protein are bound to such domains.

are rich in sphingolipids (derivatives of the long-chain amino alcohols sphingosine and dihydrosphingosine) and cholesterol, and these fragments of the membrane are characteristically insoluble in cold non-ionic detergents.[8–10] A closer definition has recently been made:[11,12] "membrane rafts are small (10–200 nm) heterogeneous, dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes". Small rafts can sometimes be stabilized to form larger platforms through protein–protein and protein–lipid interactions. Other membrane heterogeneities are described as "nonraft domains", of which there is a diversity in composition and origin.[12] Lipid rafts are often detergent-

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insoluble or detergent-resistant bodies within membranes. The lipids present in rafts are believed to be in the liquid-ordered phase (l_o).[8–10,13] The acyl chains of the lipid molecules are extended and ordered as in the gel phase (below the melting temperature of the lipid) but have high lateral mobility within the membrane plane. This phase is also known as a liquid-crystalline phase (l_c), in contrast to the liquid-disordered phase (l_d). The high melting point of sphingolipids enables them to form ordered assemblies separate from the bilayer of phospholipids with lower melting temperature.

Many types of protein are known to interact with lipid rafts, in particular proteins modified by the addition of saturated lipid derivatives such as glycosylphosphatidylinositol (GPI), which anchor the proteins to the membrane. Many signaling and receptor proteins are modified in this way, pointing to the importance of lipid rafts in signal transduction.[14,15] Viruses can also exploit these receptor pathways by binding to signaling proteins in lipid rafts. Lipid rafts may therefore play a role in the transmission of certain infectious diseases.[8,15]

In addition, lipid rafts have been implicated in processes such as endocytosis,[16] exocytosis,[17] and vesicular trafficking (transport of vesicles across the cell), as discussed in Section 2.2. Exocytosis involves the fusion of intracellular fluid-filled vesicles with a cell membrane. Endocytosis involves the capture of external species by folding the cell membrane around them and budding off of the membrane to form vesicles within the cell. In all of these processes, lipid rafts may assist vesicle budding or fusion by contributing to regions of enhanced membrane curvature that result from the lateral phase separation of lipids with different degrees of acyl-chain packing.[18]

2.2. Membrane Fusion

The fusion mechanism of biological membranes depends on whether it is an intracellular, intercellular, or viral membrane-fusion event. The compartmentalization of organelles within eukaryotic cells relies on the integrity of lipid membranes; thus, intracellular fusion processes have been extensively studied. Within the cell, molecules need to be delivered between different organelles without compromising membrane integrity. The process is mediated by soluble NSF-attachment receptor (SNARE; NSF = *N*-ethylmaleimide-sensitive factor) proteins.[17,19] The mechanism is discussed in more detail below. Figure 2 illustrates the process of

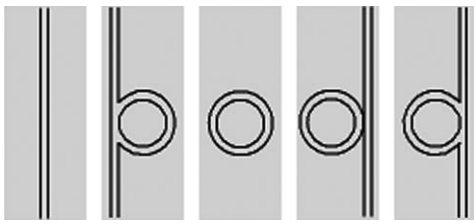

Figure 2. Vesicular transport from one cell membrane to another.[20]

membrane trafficking, which involves the budding of a vesicle from a donor organelle and then its fusion with a target organelle.[20] Membrane fusion is also essential to transmission of chemicals between synapses in the brain, because this involves the fusion of neurotransmitter-filled presynaptic vesicles with the plasma membranes.[19]

The process of membrane fusion mediated by SNARE proteins has been the subject of several reviews.[19,21–23] This class of membrane-bound proteins is divided into two subclasses: Q-SNARE and R-SNARE proteins, which have conserved glutamine-containing and arginine-containing residues, respectively.[19,21] All SNARE proteins contain conserved heptad repeats that form coiled-coil structures. Different SNARE proteins are localized within specific cellular subcompartments.[19] The most widely studied SNARE proteins are neuronal proteins functioning at the synapse. It is proposed that the SNARE proteins facilitate membrane fusion by bringing membranes into close apposition through the formation of a four-helical bundle from a combination of membrane proteins associated with a vesicle and proteins bound to the target membrane. The crystal structure of the four-helical bundle in the SNARE core complex has been studied[24] (see reference [19] for a good illustration). The core complex is exceptionally stable; it is insoluble in the detergent sodium dodecyl sulfate and is not denatured on heating.[21] The initial docking of membranes may be mediated by small guanosine triphosphatases (GTPases) of the Rab protein family.[21,22,25]

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A proposed mechanism for SNARE-mediated membrane fusion is shown in Figure 3. The "zipping" of SNARE complexes from the amino-terminal end draws the membranes into proximity and then increases the interfacial

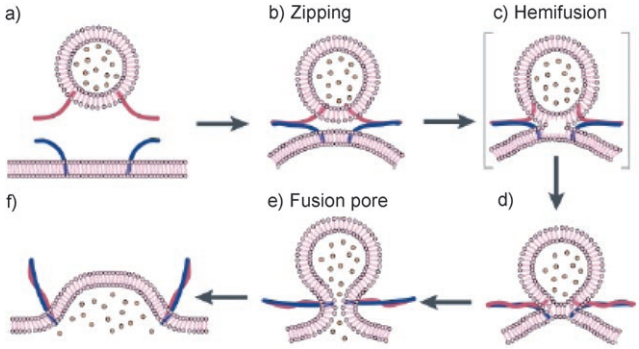

Figure 3. Model of SNARE-mediated lipid fusion.[19] The red and blue chains are SNARE proteins.

curvature and lateral tension. This step leads to hemifusion, followed by pore formation to eliminate the unfavorable void space. This process may be mediated by Ca^{2+} ions, which trigger many membrane-transport steps by changing the membrane tension. A related model for vesicle exocytosis is discussed in the same review.[19]

The so-called stalk hypothesis suggests that, when membranes fuse, the intermediate hemifusion state (Figure 3c) has a structure in which the proximal monolayers are connected by a bent stalk (Figure 4) and the distal monolayers are pulled

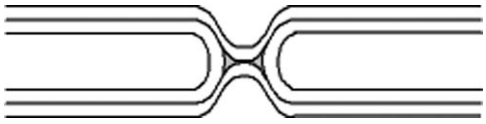

Figure 4. Connection of lipid membranes by a stalk as an intermediate structure during membrane fusion.

towards each other forming a dimple. The stalk model has been supported by theoretical and experimental observations.[26,27] The fusion of model membranes appears to occur by the same series of intermediates as the fusion of in vivo membranes,[27,28] although the approach of the membranes is not Rab/SNARE-mediated, but is driven by reduced bilayer-repulsion forces arising from hydration, electrostatic interactions, thermal fluctuations (Helfrich interaction[1]), or osmotic stress. Membrane fusion is also promoted by defects introduced into the membrane by lateral phase separation (for example, of lipid rafts), high spontaneous membrane curvature, or the addition of macromolecules or proteins into the membrane.[28]

Many viruses contain a membrane that encloses the viral nucleocapsid. When a viral membrane fuses with a host cell by endocytosis, the nucleocapsid is injected into the cytoplasm, leading to infection.[21] The membrane fusion appears to occur by a mechanism similar to that proposed for intracellular fusion, in that a central role is played by the formation of α-helical bundles of hydrophobic fusion peptides.[22] This process occurs concomitantly with the proteolytic cleavage of the fusion protein as it is inserted into the target membrane.

In contrast to intracellular vesicle fusion and viral membrane fusion, cell–cell membrane fusion does not appear to be mediated by α-helical bundles. The details of the process are not yet fully clear and may be system-specific. Several examples are given in the review by Chen and Olson.[22] One common feature is the role played by the actin cytoskeleton in the process, in particular, in transporting proteins to fusion sites.[22] Actin polymerization and network formation are discussed further in Section 2.6.

The fusion of model liposome membranes has been induced by various processes that involve the use of electric fields (electrofusion), chemicals, or photochemistry, as discussed in a recent review.[29] Electroporation is the process of applying a voltage across a tensioned membrane in a micropipette to create pores. These pores can subsequently link separate vesicle compartments. Chemically induced fusion occurs when repulsive interactions between liposomes are reduced, for example, by the attachment of poly(ethylene glycol) (PEG), which may also lead to depletion-type attractive interactions. Divalent cations such as Ca^{2+} can screen charges at the membrane surface, reducing the electrostatic interaction. These ions may also change the hydration of the lipid membrane. Photochemical fusion can be induced by incorporating a photochromic lipid into the membrane. Light of the appropriate wavelength may then trigger a conformational change in the photochromic species, inducing membrane fusion.

2.3. Membrane Instabilities

Membrane-curvature instabilities give rise to the fusion or budding of vesicles. The related process of vesicular trafficking has already been discussed in Section 2.2. Concentration gradients can also give rise to membrane instabilities. The example considered here is the formation of myelins when an amphiphilic lamellar (L_α) phase comes into contact with excess solvent. Myelins are multilayer tubules that grow from the interface between the lamellar phase (or pure surfactant) and the solvent (Figure 5). These myelins should not be confused with myelin sheaths, which are electrically insulating lipid layers (with a small protein content) that surround the extended parts of nerve cells (axons).

Buchanan and co-workers have investigated the mechanism of myelin formation. They suggest that it involves water flux into the lamellar phase at the base of the myelins (Figure 6a), rather than diffusion into the tips (Figure 6b) as in a fingering instability. This hypothesis is supported by experiments using tracer particles.[30,31] However, they did not provide a detailed model for myelin formation. Several recent models attempt to explain the origin of myelin formation. Huang et al. proposed a simple geometrical model, in which the bilayer repeat spacing is larger (and hence the bilayer repulsion is smaller) in the confined myelin cylinder than in a multilamellar disk.[32] When the disk is converted into a tube, the central cylindrical tube has a smaller perimeter than the

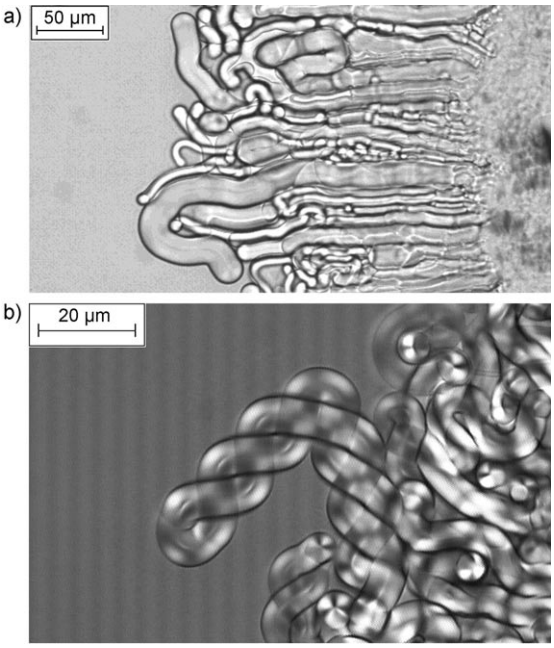

Figure 5. Optical micrographs of myelins formed at the interface between the surfactant *n*-dodecyl tri(ethylene glycol) ($C_{12}E_3$) and water.[179] a) Diffusive growth in an early stage. b) Coiling in a later stage.

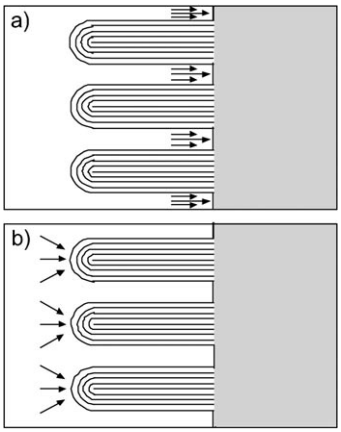

Figure 6. Myelin growth occurs a) by fluid flow into the lamellar phase between the myelins, rather than b) by diffusion into the tips of the myelins.[30]

disk, and hence the membrane spacing increases.[32,33] However, it was later suggested that such a model based on free-energy minimization around a local equilibrium is inappropriate, because myelins require the presence of a chemical-potential (hydration) gradient or external fluid flow, otherwise they retract.[34] It was instead proposed that myelin formation could be caused by an elastic instability of stressed bilayer stacks.

After the initial growth period, in which Fickian diffusive growth (whereby the thickness of the contact region is proportional to the square root of time) occurs, instabilities such as coiling appear in the myelins (Figure 5b). These instabilities may arise from the increased intermembrane attractions when the myelins are helically coiled.[35,36] Several theories have been put forward to describe the instability of tubular myelins on the basis of membrane buckling.[35,37,38] The formation of single and double helices, as well as intricate globular structures, has recently been reported in myelins formed by phospholipid membranes decorated with hydrophobically modified polymers (that is, with hydrophobic groups distributed statistically at a wide spacing along their backbone). The formation of these structures was ascribed to the spontaneous curvature induced by the selective aggregation of the polymers around the inner rim of the curved tubules.[36]

Myelin formation has recently been observed in a swollen amphiphilic block-copolymer film.[39] Myelin destabilization mechanisms, including coiling, budding, and clewing, were also observed. In addition, novel structures were observed in an emulsion of the copolymer in a chloroform/water mixture. Figure 7 shows these so-called myelinsome structures, which comprise a core of interconnected amphiphilic membranes and a corona of myelins.

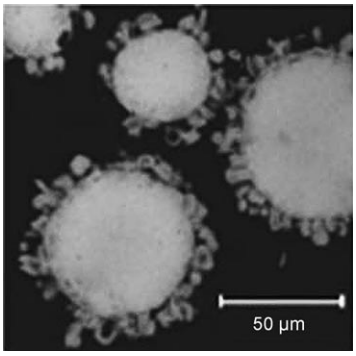

Figure 7. Laser scanning confocal micrographs of myelinsomes formed by a poly(oxyethylene)-*b*-poly(oxybutylene) diblock copolymer in a water/chloroform mixture.[39]

2.4. DNA Condensation

The persistence length of DNA restriction fragments (64–900 base pairs) in 1–2 mM NaCl is approximately 50–60 nm, depending on the salt concentration.[40] DNA condensation refers to the electrostatic attraction between negatively charged DNA and cationic species which leads to partial collapse of the DNA chains as the charge is neutralized and counterions are released.

DNA has a charge density of $1e$ per 1.7 Å^2, arising from two negative charges per base pair.[41] Thus, DNA can be condensed in the presence of divalent cations, which bind preferentially to grooves in the DNA chain (Figure 8). However, some types of cation that have a strong affinity to phosphate groups bind to the DNA strand and do not induce condensation.[42] An intriguing observation for charged species such as DNA is the existence of attractive interactions between the counterions. These interactions have been ascribed to fluctuation-driven counterion condensation[43] or to the selective condensation of counterions in grooves (the so-called electrostatic zipper motif, Figure 8).[44] In the latter model, an attractive interaction can arise because the

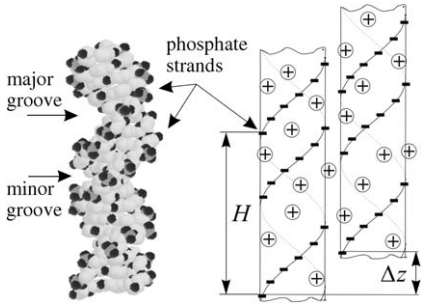

Figure 8. Inhomogeneous charge distribution on the surface of DNA. The phosphate residues are negatively charged, and the counterions are positively charged. According to the electrostatic zipper model, the strength of the attraction between the positively charged grooves on one DNA chain and the negatively charged parts of another depends on the distribution of the counterions and on the ratio of the axial shift (Δz) to the helical pitch (H).[44]

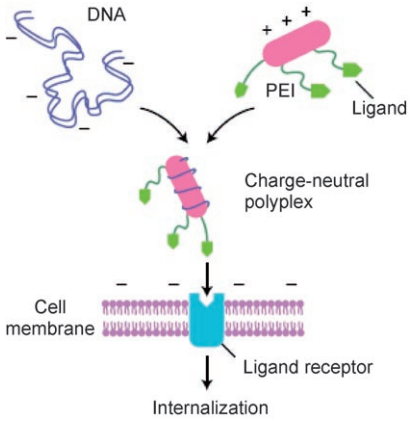

Figure 9. Nonviral gene transfection using the cationic polymer PEI.[180]

positively charged grooves on one DNA chain can approach the negatively charged parts of another when the molecules have a suitable orientation.

Gene therapy requires DNA to be condensed before it is transported across the cell membrane. Gene therapy is promoted as a potential treatment of certain disorders, especially those caused by genetic anomalies or deficiencies, and involves the introduction of specific engineered genes into a patient's cells. There is some evidence that gene therapy may be efficacious in the treatment of medical conditions due to the deficiency of single genes.[45] DNA condensation is also relevant to membrane technology and drug encapsulation.

Both viral and nonviral approaches to gene therapy have been used in clinical trials to treat illnesses such as cystic fibrosis and several forms of cancer. Viruses have evolved efficient ways of targeting cells, delivering genetic material, and expressing it. However, inflammatory and immunological responses induced by viruses may limit their utility for repeated administration.

Numerous systems have been studied for nonviral gene delivery,[46–48] including cations, polyamines, polymers such as polylysine,[49–51] PEG-based block and graft copolymers,[52,53] biologically derived liposomes,[54] and cationic lipids.[46,50] Block copolymers of PEG and one or more cationic blocks are a recent alternative to viral vectors. They form a nanoshell polyplex with an outer PEG shell when the cationic block associates with plasmid DNA (pDNA) in solution.[47] PEGylated polyplex micelles from cationic triblock copolymers with a core of condensed pDNA, an intermediate shell with buffering capacity, and an outer biocompatible PEG shell have recently been prepared.[48] Dendrimeric photosensitizers have also been used to package DNA, since they proved to be effective for light-induced gene-transfer therapies.[55]

A very widely studied cationic polymer for DNA condensation is poly(ethylene imine) (PEI),[51,53,56–62] because it has a high charge density. This polymer has also been conjugated with ligands specific to a target cell type. The ligand receptor inserted in the cell membrane acts as a channel that allows the passage of the DNA–polymer complex into the cell (Figure 9).

Another mechanism for nonviral gene transfection is the endocytosis of a block copolymer containing PEG and a cationic block (Figure 10).[63–67] The PEG block forms a steric-

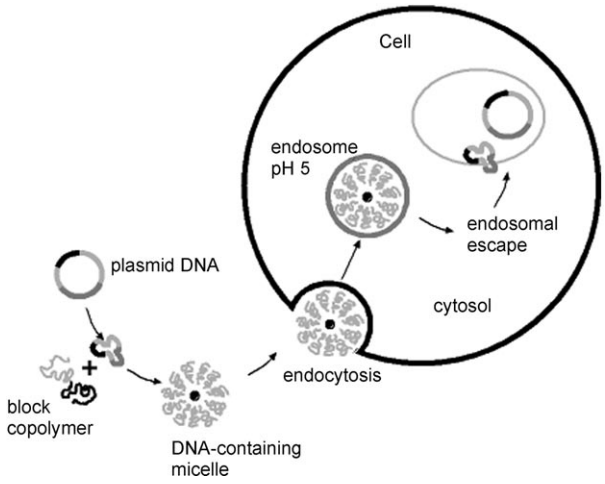

Figure 10. Transfection of plasmid DNA into a cell by endocytosis using a block polyelectrolyte. Redrawn from reference [181] with permission.

stabilization layer, while the cationic block encapsulates the DNA in the core of the micelle. In endocytosis, the encapsulated DNA gains entry into a cell without passing through the cell membrane. Rather, the endocytosis results in the formation of an intracellular vesicle by virtue of the invagination of the plasma membrane and subsequent membrane fusion

Recently, the site-specific cleavage of supercoiled pDNA condensed by a PEG-*b*-PLys (PLys = poly(L-lysine)) diblock copolymer was reported.[68] Regulated fragmentation of the DNA was achieved using S1 nuclease, an enzyme that cleaves single-stranded DNA.[68] It was proposed that the pDNA is condensed into a structure containing regular cruciform loops by the block copolymer and that cleavage occurs at loops in the topologically stressed double helix.

The condensation of DNA can lead to phase separation into a concentrated liquid-crystalline phase containing the DNA and cations and a more dilute phase containing the excess species.[69] Before precipitation, the formation of DNA aggregates is often observed. Rodlike, flower-like, fibrillar, and toroidal structures have all been detected.[70–73]

Several studies on DNA condensation have used block copolymers, in particular, PEG-*b*-polycation systems, where the polycation is PLys,[74–76] PEI, poly[2-(dimethylamino)ethyl methacrylate], or polyspermine.[69–73,77–80] The electrostatic self-assembly of DNA with long-chain linear polyelectrolytes such as PLys, polyspermine, and poly(L-arginine) has also been reported.[72] PEG-based graft copolymers such as PEG-*g*-PLys[81] and PEG-*g*-PEI[52,80] have also been used.

DNA condensation induced by block copolymers with a hydrophobic block and a cationic block has been less well studied. One example is the condensation of calf thymus DNA by amphiphilic PS_m-*b*-$PLys_n$ (PS = polystyrene) block copolymers composed of a very short, non-ionic, hydrophobic PS block and a hydrophilic, charged PLys block. The stability and structural characteristics of the DNA/(PS_m-*b*-$PLys_n$) polyplexes, and the influence of the PLys block length on the DNA condensation process were studied.[82] The DNA condenses, owing to electrostatic interactions between PLys and DNA, in the form of fibrillar precipitates with an irregular local packing. The local packing is not hexagonal (as it is for highly condensed DNA in the presence of NaCl[83]) and is possibly perturbed by the presence of the hydrophobic PS block. The polyplexes became more compact upon increasing the degree of polymerization of the PLys block (n). It was concluded that for DNA/(PS_m-*b*-$PLys_n$) polyplexes, the balance between the length of the PLys block and the excess charge in the system plays an essential role in the formation of a liquid-crystalline phase.[82]

2.5. Protein Crystallization

The crystallization of proteins from solution is vital to the preparation of samples for crystallography experiments, which are a central aspect of structural biology. Protein crystallization can also occur in vivo.[84] Herein, we do not provide a review of the whole field, which is vast. We discuss instead some very close analogies which exist between the crystallization of globular proteins and that of colloids,[85,86] which are classical systems in soft-matter science. The crystallization of lysozyme has been particularly well studied.[85,87–91] We consider the crystallization of proteins by the addition of an electrolyte or the addition of a non-adsorbing polymer to the solution (there are many other methods, such as the addition of organic solvents or an increase in temperature). This crystallization method leads to screening of long-range repulsive electrostatic interactions, so that short-range attractive interactions can cause aggregation of the proteins into clusters, which may act as nuclei for crystallization. Crystallization occurs when the second virial coefficient becomes sufficiently negative (Figure 11).[91–93] Gel formation may occur before crystallization for sufficiently high concentrations, when the solution is supersaturated with respect to the fluid–crystal phase boundary.[94] Gelation can precede crystallization when the attractive interactions are weak, because under these conditions nucleation is slow. The gel boundary can be described using a simplified version of mode-coupling theory.[94] It should be noted that gelation can lead to a glass transition, as a result of either attractive or repulsive interactions.[95]

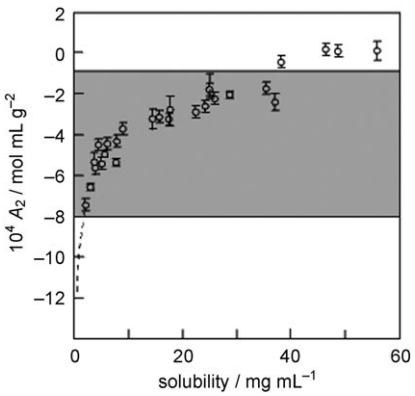

Figure 11. Correlation of the osmotic second virial coefficient (A_2) with solubility for globular proteins. The shaded region is the "crystallization zone". Adapted from reference [90]. Bhamidi et al.[90] redrew data from Wilson and co-workers[91] and measured additional data.

The interaction between proteins can, in the simplest approximation, be described as that between charged hard spheres, and the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory can be used to describe the interparticle interactions and their dependence on salt concentration.[87] Later, more sophisticated models were developed to allow for "sticky" patches on the surface of the protein.[96]

An interesting feature of suspensions of colloidal particles or globular proteins is the formation of equilibrium clusters as a result of the balance between short-range attractions and long-range electrostatic repulsions.[88,97] The conditions for the formation of such clusters were investigated using an analytical theory.[97] The formation of strongly interacting equilibrium clusters in lysozyme solutions was probed by small-angle neutron scattering (SANS).[88] Figure 12 presents examples of scattering profiles in which the structure factors show maxima on two length scales, corresponding to the clusters (q_c^*) and the "monomers" (that is, protein globules) within the clusters (q_m^*), which are quite closely packed. The peak at q_c^* is independent of concentration; however, it is temperature-dependent because the attractive interactions are temperature-dependent. The peak at q_m^* is independent of both concentration and temperature. The effect of ionic strength was also probed: increasing salt concentration leads to increased screening and hence enhanced attractive interactions.[88]

2.6. Fibrillization of Peptides and Proteins

The formation of fibrils is symptomatic of many amyloid diseases, such as Alzheimer's and Creutzfeldt–Jacob disease.[98] It is now thought that protofilaments formed in the

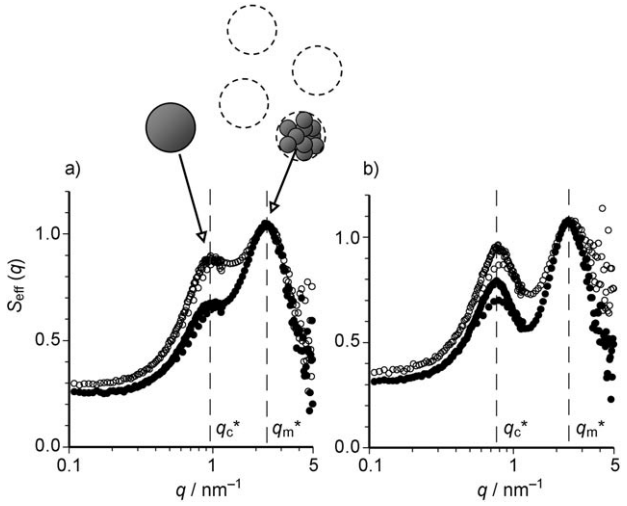

Figure 12. Effective structure factors ($S_{eff}(q)$) obtained by SANS for aqueous solutions of lysozyme at concentrations of 254 mg mL^{-1} (●) and 169 mg mL^{-1} (○) a) at 25 °C and b) at 5 °C.[88] The dashed lines indicate that the positions of the peaks due to the clusters (q_c^*) and the "monomers" (q_m^*) are independent of the lysozyme concentration.

initial self-assembly process are the toxic agents.[99,100] Because of its relevance to diverse medical conditions that affect a large number of people, fibril formation by the amyloid peptide has been widely studied. There are two variants of the amyloid peptide in humans, Aβ(1–40) and Aβ(1–42), of which the latter is believed to be more susceptible to fibrillization.[101]

Protein fibrillization can occur by two polymerization mechanisms.[102] Isodesmic polymerization,[103] as observed for tubulin, for example, occurs by the sequential addition of monomers in an unrestricted self-assembly process in which monomers, dimers, trimers, and larger species are in equilibrium. The process is noncooperative, and each addition of monomer leads to a constant decrease in the free energy.[104] In contrast, nucleated polymerization, as observed for actin, for example, is based on the formation of a thermodynamically unstable nucleus. In the literature of surfactant self-assembly, isodesmic self-assembly is also known as open association,[105,106] to be contrasted with closed association,[105,106] as observed during micellization, which is characterized by a sharp transition as the equilibrium shifts to a coexistence of micelles and unassociated molecules. Thermally or chemically initiated polymerization also occurs by a non-isodesmic process, that is, via an activated state, which leads to a discontinuity in specific heat at a critical temperature.[107,108]

The self-assembly of chiral peptides into helical aggregates has been shown to be non-isodesmic, as revealed by pronounced (concentration) thresholds for the formation of aggregates (tapes or fibrils of de novo designed peptides).[109] This process has been described by a detailed theoretical model, in which the elastic cost of twisting ribbons is balanced against the energy gained by fibrillar association.[103] The main parameters are the relative helix pitch of the isolated ribbons and the relative side-by-side attraction energy between the ribbons. A scheme of the hierarchical self-assembly and a calculated phase diagram are shown in Figure 13.

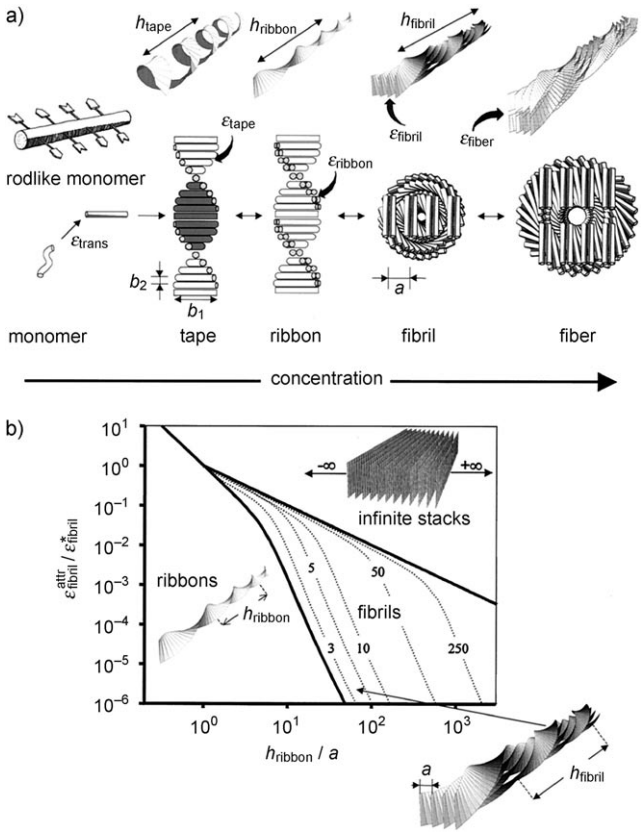

Figure 13. Self-assembly of peptides to form chiral tapes.[109] a) Successive stages in the self-assembly process and the associated interaction energies (ε). b) Calculated phase diagram in terms of the relative side-by-side attraction energy between the ribbons ($\varepsilon^{attr}_{fibril}/\varepsilon^*_{fibril}$) and the relative helix pitch of the ribbons (h_{ribbon}/a). The thick lines divide regions where different types of aggregates are stable. The dotted lines are lines of stability for fibrils containing the indicated number of ribbons. In the calculation, the ratio of elastic constants was $k_{bend}/k_{twist} = 0.1$.[103]

Recent work on the self-assembly of oligomers of *p*-phenylenevinylene derivatives, bearing one moiety with multiple hydrogen-bond donors and one moiety with multiple hydrogen-bond acceptors per molecule, indicates that they can form helical aggregates by a similar non-isodesmic process.[110] A sharp transition in association number as a function of temperature is observed, indicating nucleation with a high activation barrier. The non-isodesmic self-assembly process was modeled using an approach similar to the Oosawa–Kasai model[111] for the helical assembly of proteins in solution. This model predicts a critical concentration for the formation of helical aggregates.

Actin is one of the major cytoskeletal proteins implicated in cell motility. Actin is a constituent of several cell types and is involved in many cellular processes, including locomotion, secretion, cytoplasmic streaming, phagocytosis, and cytokine-

sis. Actin polymerization involves the transformation of the globular protein G-actin into F-actin fibers. On the basis of studies on the spontaneous self-assembly of G-actin and on the polymerization following sonication of either F- or G-actin, it was concluded, as mentioned above, that polymerization is a nucleated process.[112] Seeding by the addition of F-actin accelerates the polymerization of G-actin. Actin also plays an essential role in muscle structure. In the sarcomeres of muscles, thick and thin filaments overlap. The thick filaments contain the protein myosin, whereas the thin filaments contain several proteins and have cores composed of polymerized F-actin fibrils.[113]

Networks of cross-linked and bundled F-actin exhibit exceptional elastic behavior that reflects the mechanical properties of the individual filaments. The local viscoelasticity has been investigated by microrheology techniques using embedded colloidal probe particles and optical interferometry[114,115] or magnetic tweezers.[116] The elastic modulus is a strong function of the actin concentration and, thus, cross-link density.[117,118] Actin can also be cross-linked to form a network coating the surface of a vesicle.[119–121] The actin is polymerized in the presence of streptavidin at the surface of biotinylated vesicles. The viscoelastic and deformation properties of the actin-coated vesicles, which provide a model system for cell membranes, have also been examined.[119,122]

Microtubules are another major component of the cytoskeleton. The mechanical properties of single microtubules have been tested by lateral indentation with an atomic force microscopy (AFM) tip.[123,124] These studies showed that the indentation can cause substantial damage to the walls of the microtubule. The compressive forces sustained by cytoskeletal microtubules were also studied in vitro,[125] which led to the conclusion that the maximum load that the microtubules can bear under physiological conditions in the cells is increased thanks to their mechanical coupling to the surrounding cytoskeleton.

The misfolding of certain polypeptides can lead to the formation of β-strand structures, which can further aggregate into elongated fibrils and then into larger plaques symptomatic of disease.[183] This mechanism underpins amyloid diseases such as Alzheimer's, type II diabetes, and bovine spongiform encephalopathy (BSE). In particular, many globular proteins, such as β-lactoglobulin (β-lg),[126] α-lactalbumin,[127] bovine serum albumin (BSA),[128] and lysozyme,[129] form amyloid fibrils when partially denatured. The protein β-lg forms fine-stranded, transparent gels upon heating at pH 2 and low ionic strength. At this pH value, β-lg has a positive surface charge, and the monomers interact through repulsive electrostatic interactions, leading to the formation of transparent gels of amyloid fibers with a relatively open network structure.[126] The milk protein α-lactalbumin forms amyloid fibrils at low pH values. The transformation of this protein into an essentially unfolded and highly flexible conformation is required for successful fibril formation.[127] BSA can form fibrillar gels upon heating. The gelation process results from the heat-induced unfolding of the BSA monomer. The BSA fibrils self-assemble into amyloid fibers, the thickness of which depends on the concentration of the system.[128] Amyloid fibrils can be generated from lysozyme by dissolving the protein at pH 2 and heating the sample for a period of time. The fibrils obtained in this way consist of protein filaments twisted along the fibril axis.[129]

The formation of nanostructures, including nanofibrils, from block copolymers containing peptide sequences has been the focus of much recent activity, as summarized in several reviews.[82,130,131] Fibrils result from intermolecular hydrogen bonding of the polymers into β-sheet structures. The conjugation of β-sheet peptides to PEG confers enhanced solubility and is of interest in peptide therapeutics, because PEG can provide steric stabilization of a peptide drug. In fact, PEG can stabilize the secondary structure against changes in concentration, pH, and temperature as shown by recent work on hybrid block copolymers containing short de novo designed peptide sequences.[132,133]

Block copolymers in which one block is an α-helical peptide have also been studied. These may aggregate into coiled coils if an appropriate heptad sequence is incorporated into the polypeptide chain.[134–136] The one-dimensional self-assembly process can be hindered by the amorphous block (for example, PEG), so that association is restricted to finite aggregates (for example, dimers or tetramers; Figure 14). The

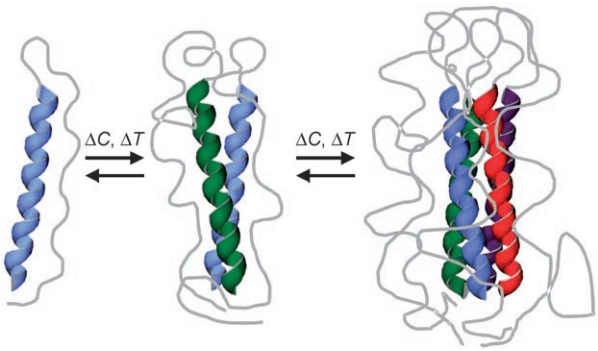

Figure 14. Self-assembly of PEG-*b*-peptide diblock polymers containing the heptad sequence LAEIEAK.[134,182]

incorporation of PEG chains in hybrid diblock copolymers significantly enhances the stability of coiled-coil superstructures towards denaturing, an effect ascribed to the formation of a hydrophilic, protective shell of PEG around the hydrophobic interior.

Switch peptides are designed with amino acid sequences such that a pH change induces a change in conformation from β sheet to α helix.[137] Whereas fibrillization is generally observed for β-sheet peptides, fibril formation from α helices has, surprisingly, been observed for switch-peptide homopolymers and related PEG-*b*-peptide diblock copolymers at low pH values.[132] The α-helical coils have hydrophilic and hydrophobic faces; this "Janus" structure can lead to their one-dimensional self-assembly into individual fibrils in solution, which aggregate into thicker fibrils when the solution is dried (to prepare samples for transmission electron microscopy (TEM), for example; Figure 15).

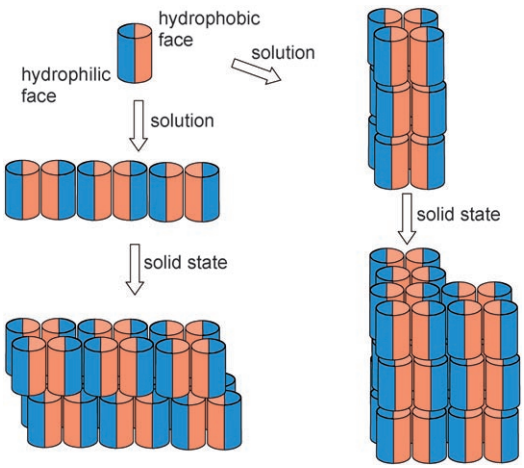

Figure 15. Two models for the fibrillization of "Janus"-faced α-helices.[137] The left-hand model is favored, because the measured width of the tapes in solution corresponds to the length of the α-helical peptide chains.

2.7. Cell Adhesion and Forces between Biomolecules

The cell envelope comprises lipid–protein bilayers tethered to a several hundred nanometer thick macromolecular network formed of the protein spectrin cross-linked with actin. The development of artificial tethered membranes[138–140] has allowed the isolation of membranes from the influence of a solid substrate.

Despite their complex composition, cell envelopes behave as elastic shells with linear viscoelastic responses.[141] The bending moduli (κ) are approximately $1000\,k_B T$, and the shells exhibit pronounced fluctuations of approximately 100 nm in amplitude, a phenomenon termed "flickering". This Brownian motion impedes adhesion, as shown by interference contrast microscopy experiments imaging the envelope of white blood cells on glass.[141] Analysis of the root-mean-square (rms) fluctuation amplitude enables the bending modulus to be calculated. The adhesion energy can be estimated from κ and the contact-area contour of the adhering part of the cell surface.

A number of methods can be used to probe the strengths of bonds between biomolecules,[142] which are relevant to cell adhesion (among other things), as well as the strengths of bonds within molecules, which are relevant to protein (un)folding (not discussed herein). For a complex biomolecule under extensional force, multiple bond rupture occurs at different points depending on the loading rate. AFM can be used to measure the average force as a function of the extension of a molecule tethered between a surface and the tip.[143,144] The difficulty of determining whether only a single molecule is tethered can lead to complications in the analysis of the force–distance profiles. Optical tweezers can be used to measure forces on (bio)molecule-coated beads trapped in a narrowly focused beam of laser light.[145] Finally, Evans and co-workers have pioneered the biomembrane force probe (BFP) where force is measured from the axial displacement of a (biomolecule-coated) glass bead when it is moved towards the target cell.[142,146,147] A red blood cell acts as a linear force transducer of determinate spring constant. The displacement of the glass bead attached to the blood cell (by biotin–streptavidin or biotin–avidin) enables the force of pushing or pulling the BFP tip to be determined when the target body is driven to or from the contact using a piezo-controlled motor.[146,147] The BFP technique has enabled dynamic force spectroscopy where the bond-rupture forces can be probed as a function of loading rate. The rupture of the multiple weak bonds in biomolecules can be described in terms of the traversal of a cascade of activation barriers.[148] The force at which bond rupture occurs under steadily increasing force is most frequently determined by the rate of loading. The process of overcoming the activation barriers can be analyzed to a first approximation using a Kramers rate equation.[142,148]

2.8. Blood: A Complex Rheofluid

Blood is a concentrated suspension of individual elements, including red blood cells (RBCs), white blood cells (WBCs), and platelets. RBCs are flexible, biconcave disks and constitute approximately 45% of blood by volume. Circulating WBCs are approximately spherical in shape and are approximately 1000 times less abundant than RBCs. Platelets are discoid particles and constitute 1/800 of the total cell volume of blood. These cellular components are suspended in plasma, leading to the general classification of blood as a two-phase suspension.

Since blood is present in a state of continuous flow, a large number of papers have focused on blood rheology (hemorheology) and RBC deformation. The flow behavior of blood has many features in common with the rheology of colloidal suspensions or polymer solutions.[149,150] In addition, under strong flow, the blood cells are deformed analogously to vesicles under flow.[151]

Plasma is an aqueous solution that follows Newtonian flow behavior. In contrast, although plasma is a major constituent of blood, blood is a non-Newtonian fluid. The non-Newtonian nature of the hemorheology is due to the two-phase nature of blood and to the interactions between blood cells, especially under microcirculation conditions, where vessel dimensions become comparable to cell diameters.[152]

Many theoretical works have focused on predicting the flow properties of blood for a given vessel geometry and blood composition. For example, the interactions between RBCs and WBCs in capillaries have been simulated using a lattice Boltzmann model, in an attempt to estimate the magnitude of the forces involved in these interactions.[153,154] The lattice Boltzmann model has also been used to analyze the flow of concentrated RBC suspensions in blood vessels.[155] The flow resistance was calculated for various RBCs and vessel radii. In addition, WBCs were also included in the model, allowing the increase in resistance due to WBC rolling and adhesion to be modeled.[155]

The mechanical properties of RBCs play a major role in the blood flow. In particular, the following three factors are important: RBCs can be damaged under flow (hemolysis); they can undergo reversible aggregation; and they can be highly deformed (Figure 16).

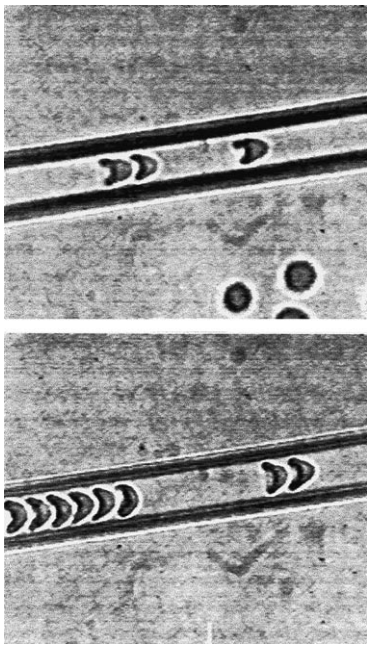

Figure 16. Video images of RBCs passing through a glass capillary tube, showing the parachute shape adopted.[159]

Experimental studies demonstrated that turbulent stresses contribute strongly to mechanical blood damage; numerical predictions of hemolysis obtained by computational fluid-dynamic modeling were in good agreement with the experimental data.[156]

The tendency of RBCs to undergo reversible aggregation is an important determinant of apparent viscosity, because the size of RBC aggregates is inversely proportional to the magnitude of shear forces; the aggregates are dispersed with increasing shear forces and then reform under low-flow or static conditions. RBC aggregation also affects the in vivo fluidity of blood, especially in the low-shear regions of the circulatory system.[157]

RBCs are highly deformable, and this physical property significantly aids blood flow, both under bulk flow conditions and in microcirculation. Deformability refers to the ability of the RBC to undergo a change in shape in response to a deforming force,[158] and it is an important index of blood flow and oxygen transport in microcirculation.[159]

Under normal physiological conditions, RBCs have a biconcave disc shape with a diameter of approximately 8 μm.[160] It is well-known that RBCs undergo a biconcave–parachute shape transition when flowing through channels with a diameter equal to or slightly smaller than their own diameter (Figure 16).[159,161–164] The parachute shape plays a dominant role in the dynamics of RBCs in microvessels. The influence of this particular shape on the elastic properties of the cytoskeleton has been theoretically studied using a three-dimensional mesoscopic simulation.[160]

The deformation of RBCs and fluid vesicles in capillary flows were studied by lubrication theories[163,164] and boundary-integral methods.[165,166] The effect of the initial shape on the dynamics of the parachutes has been theoretically investigated.[167] In these calculations, it was assumed that the RBCs initially have a convex ellipsoidal front part and an off-center ellipsoidal indentation at the rear (the so-called slipper-like shape observed experimentally).[161,168] However, it was found that an initial non-axisymmetric shape does not noticeably influence the dynamics of the parachutes.[167] The capillary flow deformation of elastic capsules consisting of biconcave discs has been studied using boundary-integral methods.[166] These studies show that discs with initial coaxial and noncoaxial orientations deform into parachute and slipper-like shapes, respectively.[166]

Besides these theoretical studies, there have been several experimental investigations of RBC deformation in different flow vessels, such as a rheoscope,[169,170] a micropipette,[171,172] a glass capillary,[168] and polycarbonate sieves.[173] However, the results from these experiments can be affected by the quality of the filter material or by RBC aggregation. Capillary models based on microchannel systems have been developed as alternatives to study RBC deformation and to measure blood rheology. Microvascular flow systems have been fabricated by etching glass plates with images of the vascular pattern,[174] by forming microchannels on a silicon substrate,[175–178] or by fabricating transparent microchannel capillaries.[159] In particular, transparent microchannel capillary models allowed the observation of the deformation of RBCs to a parachute shape, in a similar manner to in vivo observation using a high-speed video camera.[159]

3. Conclusions

Taking concepts from soft-matter physics and chemistry (as applied to synthetic materials) is a fruitful route to new insights into the self-assembly of certain biological soft materials. We have discussed examples where the crystallization of proteins, vesicle budding and trafficking in the cell, and protein fibrillization have been related to principles of the self-assembly of colloidal suspensions, models for the deformation of membranes, and models for one-dimensional self-assembly. We have also discussed DNA condensation and have considered blood as a complex fluid, which has rheological properties that resemble those of colloidal suspensions or polymer solutions, and which is composed of cells whose shapes transform under flow in a manner reminiscent of vesicles.

We have barely touched on dynamic and kinetic aspects of self-organization, which must be promising future directions for research. We also have not considered the structure of plant-based soft materials (for example, plant cells, cellulose, or polysaccharides). There also remain outstanding questions related to designing more complex hierarchically ordered soft materials with inbuilt functionality, such as DNA-, protein-, or polyelectrolyte-decorated membranes or vesicles, vesicles with internal compartments, or polyelectrolyte complexes with internal structures. There are many other examples.

Future research will no doubt focus on the interface of synthetic and biological materials, for example, in cell-growth media, tissue scaffolds, or DNA condensates. The greatest challenges must lie in the application of such materials to the

development of new therapies to prevent amyloid fibril formation, to improve gene-delivery systems, and to control vesicular trafficking and other transport and signaling processes within and between cells.

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