Topical Review

Filamentous phages as building blocks for reconfigurable and hierarchical self-assembly

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Received 1 December 2016, revised 23 October 2017
Accepted for publication 3 November 2017
Published 17 November 2017

Abstract
Filamentous bacteriophages such as fd-like viruses are monodisperse rod-like colloids that have well defined properties of diameter, length, rigidity, charge and chirality. Engineering these viruses leads to a library of colloidal rods, which can be used as building blocks for reconfigurable and hierarchical self-assembly. Their condensation in an aqueous solution with additive polymers, which act as depletants to induce attraction between the rods, leads to a myriad of fluid-like micronic structures ranging from isotropic/nematic droplets, colloid membranes, achiral membrane seeds, twisted ribbons, π-wall, pores, colloidal skyrmions, Möbius anchors, scallop membranes to membrane rafts. These structures, and the way that they shape-shift, not only shed light on the role of entropy, chiral frustration and topology in soft matter, but also mimic many structures encountered in different fields of science. On the one hand, filamentous phages being an experimental realization of colloidal hard rods, their condensation mediated by depletion interactions constitutes a blueprint for the self-assembly of rod-like particles and provides a fundamental foundation for bio- or material-oriented applications. On the other hand, the chiral properties of the viruses restrict the generalities of some results but vastly broaden the self-assembly possibilities.

Keywords: colloid rods, reconfigurable self assembly, depletion, chirality

(Some figures may appear in colour only in the online journal)

1. Introduction

Self-assembly is a phenomenon in which a collection of particles spontaneously arranges into mesoscopic structures [1–5]. The complexity of the mesoscopic structure is encoded in the building blocks and the physics that drive its condensation. Although the fundamental and driving questions in self-assembly have persisted from the birth of the field up to the present—can the global behavior of a system be engineered using local rules set by the building block properties? How do local rules interlace with physics principles and determine global behavior?—the complexity has drastically increased over the years.

Complexity is in part driven by the properties of the building blocks. In this respect colloidal building blocks have a peculiar flavor. In contrast with polymers, copolymers, surfactants or molecular liquid crystals [6, 7], colloids can be considered as giant atoms [8, 9], where the solvent mediates the interactions. The consequences are many. First, it is a convenient experimental system; since colloids can be quite big, both the shape and the structure of the condensate can be visualized at the colloidal level under a microscope. Second, colloids and their condensates may be manipulated using microfluidics and external fields such as optical traps. Third, due to their large size, the colloid dynamics is also quite slow, which permits the local kinetics and mechanisms
to be probed, leading to their condensation and restructuring. Finally, colloids come with a large toolbox that permits their shape and interactions to be engineered. The last decades have seen an explosion of strategies to obtain synthetic and biological colloids. Colloids may come in various shapes from spheres to anisotropic geometries [10, 11] such as colloidal polyhedras [12, 13], tetrapods [14], dumbbells [15] and triangles [16] or cubes [17, 18]. Classical techniques to tailor isotropic interactions include tuning the van der Waals attraction via the Hamaker constant, grafting polymers to the colloidal surface for entropic repulsion, screening the surface charge with salt, the use of polymers to induce depletion interactions [19], etc. Anisotropic interactions are common in proteins [20] and may be induced just by the shape of the colloid [21] or by functionalizing the particle surface [22] with lock and key groups, such as topological patches, DNA oligonucleotides [23–26], protein-based cross-linkers like biotin–avidin or antibody–antigen binding pairs [27] and metallic patches [28]. Depending on the strength and topology of the patchy interactions, the binding of two colloids may lead to super colloids with internal degrees of freedom, such as the articulated bonds obtained in topological patches, for instance, [29] or by grafting DNA onto the liquid interfaces of emulsions [30]. Anisotropic interactions may also come from the solvent. Small water droplets dispersed in a nematic liquid crystal exhibit short-range repulsion and a long-range dipolar attraction, which lead to the formation of anisotropic water droplet chain-like structures [31]. Interactions that can be triggered externally are essential knobs in the study of reconfigurable self-assembly and sequential or layer-by-layer self-assembly. They allow the phase diagram of colloidal dispersions to be navigated in a continuous way, providing a reversible pathway to induce the transition between different structures. Diver strategies are adopted to build interactions that respond to temperature (DNA coated colloids [32, 33], proteins [34]), magnetic fields [35–37], or electric fields [38, 39].

Complexity also comes from the interplay between thermodynamics and kinetics. Self-assembly is a stochastic process and the thermal energy $k_BT$ plays a particular role. It enables the particles to diffuse and probe the energy landscape of the dispersion. In principle, according to thermodynamics, the preferred self-assembled structures are the ones that minimize the free energy of the dispersion. However, the actual structures obtained may depend on nonequilibrium effects, local fields, kinetic traps, and pathway-dependent ordering. Hard sphere crystallization is a simple case of self-assembly directed by entropy that can be hindered by the kinetic effect, namely the glass transition [40]. Assembly strategies have complexified in recent years. In directed assembly, external fields are used as a template to order the colloids [39, 41, 42]. In template-assisted self-assembly, a substrate is used to order colloids [43, 44]. In reconfigurable self-assembly, tunable interactions permit the transition from one state to another [45, 46]. In programmable self-assembly, information is added to the colloids to direct their organization [26, 47–50]. Sequential self-assembly is based on colloids with selective interactions and their sequential activation to form material through multistep kinetics [51, 52]. These strategies may lead to simple structures like homogeneous crystals, or to hierarchical assembly where the building block organization takes place over distinct multiple levels leading to material structuring at length scales that are much larger than the building blocks [46, 53–58].

In this review paper, we first focus on filamentous phages—fd-like viruses—as model colloidal rods, section 2. We then show that in the presence of depletants, their condensation leads to a myriad of self-assembled structures, section 3. Finally, we discuss a road map using isotropic aqueous suspensions of filamentous phages and depletion to rationalize the hierarchical and reconfigurable self-assembly of the phages upon variations of attraction via the depletion interaction, chirality and rod composition, section 4.

2. Filamentous phages as versatile building blocks for self-assembly

A bacteriophage or phage is a virus that infects and replicates within a bacterium. They were discovered by Twort [59] and d’Hérelle [60] in the early 20th century. Bacteriophages are among the most common and diverse entities in the biosphere [61] and are widely distributed in locations populated by bacterial hosts, such as soil, the intestines of animals or sea water. In the latest study, up to $\sim 10^8$ virions per milliliter were found in microbial mats at the water surface [62]. The impact of phage research in biology has been huge: from novel biochemical mechanisms for replication, maintenance and expression of the genetic material, as well as new insights into the origins of infectious disease, to their use as therapeutic agents [63]. Here, we focus on fd-like phages and their use as building blocks for self-assembly [64].

2.1. Synthesis

The fd-wt virus was originally isolated from sewage [66]. fd-wt ($M_w = 16.4 \times 10^6 \text{ g mol}^{-1}$) are identical to one another and composed of a single strand of DNA surrounded by a protein layer of about 2700 identical p8 protein subunits. The protein p8 has a molecular mass of 5240 g mol$^{-1}$ and accounts for about 99% of the total protein mass. The rest of the protein mass belongs to the minor coat proteins which are located at the tips of the virus [67]. At one end of the filament, there are five copies of proteins p9 and p7. At the other end of the phage, there are five copies of p3 and p6. p3 proteins are the first to interact with the Escherichia coli host during infection, and is also the last point of contact with the host as a new phage buds from the bacterium, figure 1.

Bacteriophage viruses are named based on their observed ability to lyse bacterial cells (in Greek, ‘bacteria eaters’). However not all phages lyse bacteria. In particular, fd-wt use a lysogenic cycle. Lysogeny is characterized by the integration of the bacteriophage nucleic acids into the host bacterium’s genome or formations of a circular replicon in the bacterial cytoplasm. In this condition, the bacterium continues to live and reproduce normally. The genetic material of the
bacteriophage is transmitted to the daughter cells at each subsequent cell division. Once infected, the cell and its descendants thus turn into a virus manufacturer.

fd-wt are grown using standard biological techniques [68, 69]. In short, an overnight starter culture taken from a single colony of the bacterium E. coli ER2738 is incubated for 12h at 37°C and shaken at 250rpm in 5ml of sterile 2xYT (yeast extract tryptone) growth medium. From the resulting overnight E. coli culture, 200μl is then grown in 5ml of a fresh growth medium until it reaches an optical density of OD = 0.5 at 600nm measured with a UV–vis spectrophotometer. The sample is then inoculated with 10μl fd-phage stock at approximately 1 mg ml⁻¹.

The suspension is incubated and shaken for 30min then transferred to a 250ml conical flask with 30ml of broth media for 2h and finally transferred to a 2l flask with 500ml of growth media and grown until OD = 1. From the 500ml growth cycle, the E. coli cells and debris are removed by centrifuging the cultures twice at 8300 g for 15 min, harvesting the supernatant, the rest is isotropic. Longer rods preferentially dissolve in the nematic phase [71]. The isotropic fractions are isolated and used as a stock of monodisperse viruses. Such a preparation with 500ml of growth media yields approximately 200mg of virus. For the self-assembly experiments, the viruses are dispersed in a buffer that contains 100mM NaCl and 20mM ml⁻¹ Tris at pH = 8.05.

For the study presented in this review, in addition to fd-wt, the filamentous phages fd-y21m and M13KO7 are also used. Their synthesis follows the same protocol as for fd-wt and their unique properties are detailed in table 1.

2.2. A model system

There are unique advantages of this particular system. First, fd-wt are monodisperse, which eliminates complications related to the polydispersity of rods and facilitates direct quantitative comparison with theory. Second, fd-wt have a diameter of 6.6nm for a contour length of 880nm [76, 77], conferring them a large aspect ratio, ~130, which is similar to that of spaghetti (~150). Finally, the viruses are quite rigid: fd-wt has a persistence length of ~2.8 μm and the mutant fd-y21m has an even greater persistence length, ~9.9 μm [73]. Therefore, viruses, and fd-y21m in particular, can be considered as a model liquid crystal system in the framework defined by Onsager. At low concentrations, colloidal rods form an isotropic phase with no direction or orientation order. However, as the concentration is increased, the isotropic dispersion becomes metastable or unstable: orientation fluctuations drive concentration gradients, which lead to phase separation into an isotropic state in coexistence with a nematic state where the rods have no positional order but tend to point in the

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**Figure 1.** Phage-virus-like fd-wt uses the machinery of E. coli to reproduce itself. (a) A schematic of the reproduction cycle of fd-wt. (B) A schematic [65] and electron microscopy image of the fd-wt virus; scale bar 200nm.

**Figure 2.** The gel electrophoresis of a typical fd-wt preparation [46]. (a) Ethidium bromide stained gel viewed under UV illumination. The right bright band consists of 880nm long fd-wt monomers, while the middle and left bands contain fd-wt dimers and trimers, respectively. (b) Virus polydispersity is quantified by plotting the normalized intensity profile of the gel [46].

| Table 1. The properties of the filamentous phages fd, fd-y21m and M13KO7; diameter D, contour length L, persistence length Lp, charge density at pH = 8.05, C and chirality [72–75]. |
|---|---|---|---|
| | fd-wt | fd-y21m | M13KO7 |
| D (nm) | 6.6 | 6.6 | 6.6 |
| L (μm) | 0.88 | 0.88 | 1.2 |
| Lp (μm) | 2.8 | 9.9 | 2.8 |
| C (e⁻ nm⁻¹) | 10 | 10 | 7 |
| Chirality | Right | Left | Right |
and eventually vanishes at 360° throughout the sample. The axis of this rotation is normal to the axis of the corkscrew-shaped nematic phase and leads to two homogenous isotropic and nematic phases separated by a single interface, and (c) a schematic of the nematic phase. Due to their higher flexibility, the coexistence region for fd-wt is narrower and shifted toward higher concentrations: $c_{Iso} = 19.8$ and $c_{Nem} = 22.6 \text{ mg mL}^{-1}$.

The same direction, which defines the nematic director. During this transition, transient nematic droplets or tactoids nucleate in an isotropic background and coalesce to minimize the interface between the isotropic and nematic phases. This leads to a thermodynamically stable state: two homogeneous phases, namely the isotropic and nematic phase separated by a single interface, figure 3. Onsager has established that this transition is purely entropic in nature [78, 79]. The entropy loss due to the orientation ordering in the nematic phase is over-compensated by the increase in translational entropy: the free volume for any one rod increases as the rods align. Moreover, he established that the transition volume fractions for rigid rods with an aspect ratio larger than 75 and repulsive interactions are: $\phi_{Iso} = 3.289 D/L$ for the isotropic phase and $\phi_{Nem} = 4.192 D/L$ for the nematic phase [78], where $D$ is the diameter of the rod and $L$ is its contour length. For rods with an aspect ratio of fd-y21m, the transition concentrations are $c_{Iso} = 14.2$ and $c_{Nem} = 18.1 \text{ mg mL}^{-1}$. These predictions are remarkably close to the experimental results [73], figure 3.

2.3. A versatile library of colloid rods

A challenge associated with hierarchical assembly is to control the final macroscopic assembly by specific modification of the relevant microscopic parameters. Thanks to nature’s diversity, genetic engineering and bio-chemistry—on top of having properties that remain unmatched by chemical synthesis—it is possible to create a large library of monodisperse fd-like particles with slight variations in their physical properties, like their contour length, diameter, rigidity or interactions [64, 80].

2.3.1. Chirality. fd-wt is chiral and left-handed: in close contact with one another, fd-wt tend to twist preferentially clockwise. Therefore, fd-wt form a cholesteric instead of a nematic phase at room temperature [46, 81], figure 4. The cholesteric phase shows nematic ordering but its director rotates throughout the sample. The axis of this rotation is normal to the director and the distance over which the director rotates by 360° is called the cholesteric pitch. Moreover, fd-wt chirality is temperature sensitive. Chirality decreases with temperature and eventually vanishes at $T \approx 60 ^\circ C$, figure 4. Understanding virus chirality and its temperature dependence as well as its propagation at the macroscopic length scale remains a challenge [67, 82–85]. Day and Meyer proposed that the cholesteric twist derivates from the ‘cork screw’ shape of the virus due the interplay between its major coat proteins and its DNA backbone [67].

2.3.2. DNA backbone. The contour length of a phage virus scales linearly with its genome size. The virus length impacts the dynamics of the virus. Maguire et al have shown that the rotational diffusion coefficient of rods in the isotropic phase scales linearly with the length [86]. The virus length also affects the phase diagram. It shifts the location of the isotropic–nematic phase transition toward higher volume fractions and it stabilizes the smectic phase [72, 87, 88]. So far physicists have used viruses whose lengths range from ~0.4 to 1.2 μm. However, using molecular cloning techniques it is possible to engineer viruses that are as short as 50 nm and as long as 8000 nm [89–93].

2.3.3. Major coat proteins. The major coat proteins confer to fd-wt a net linear charge density of $10 e^- nm^{-1}$ at pH = 8.05 [72, 75]. It is possible to label the major coat proteins with chemical compounds. This is very convenient for making the virus fluorescent and tracking their individual dynamics within an assemblage. Coatings such as PEG [94], SiO2, TiO2, [95], PNIPAM [96], DNA [97], gold [98], carbon nanofiber [99] and fluorescent dyes [100] obviously increase the diameter of the virus, but may also drastically change the interactions between them, and therefore the way they self-assemble.

Genetic mutation represents another way of acting on the major coat proteins [101]. For example, structural biologists have genetically engineered fd-wt into fd-y21m, which is a mutant virus in which the 21st amino acid out of the 50 composing the major coat protein changes from tyrosine to methionine [102]. fd-y21m is not only stiffer, as mentioned above, but it also has a left-handed cholesteric state, unlike fd-wt which forms a right-handed cholesteric state [73]; furthermore, in contrast with fd-wt, fd-y21m chirality is temperature-independent [46]. By mixing fd-wt and fd-y21m at a controlled ratio $x_{fd}$, it is possible to design cholesteric phases with the desired the pitch and chirality, figure 5. The phase space of all possible mutations of the major coat protein is huge. This could be investigated using phage display technology [103] to better understand the impact of the coat protein structure on the coarse-grained properties of the filament [104].

2.3.4. Cap proteins. Another attractive feature of filamentous bacteriophages is the presence of cap proteins, which are distinct from the major coat proteins, thus enabling selective labeling of the end of the virus, and in particular normal anchoring of the phage, i.e. attaching the virus perpendicular to a surface. On the one hand this feature can be used to create new materials; for instance, using phage display, filamentous phages are organized into smectic layers that are intercalated with layers of end-bound inorganic nanoparticles [105, 106]. On the other hand, it can also be used to design new particles.
such as star colloids, where filamentous phages are pinned to the colloid surface [107] or filamentous ring-like structures by labeling the two ends of the virus with distinct labels that stick to each other [108]. This last example paves the road toward specific and sequential self-assembly.

### 3. Condensation of colloidal rods

#### 3.1. Colloidal rod and depletion

Depletion interaction is an effective attraction that arises between large colloidal particles that are suspended in a dilute solution of depletants. Except for excluded volume effects, the depletant and the colloids do not interact and are considered as hard spheres. Usually, the depletant is a polymer which is much smaller than the colloid. In this configuration, there is a region surrounding each colloid which is unavailable to the centers of mass of the depletants. Therefore, as the two colloids approach each other, the excluded volumes overlap and an additional free volume becomes available to the polymers, thus increasing the overall entropy of the mixture [109]. This results in an effective attractive (depletion) potential between the colloids, whose strength and range can be increased by increasing the polymer concentration and size, respectively [109].

The depletion principle can be transposed to mixtures of viruses and polymer depletants. This has been tested under a relatively high salt content so that hard-core repulsive interactions dominate and using different polymers like Dextran or polyethylene glycol, whose size is always smaller than the rod length $L$, but can be greater than its diameter $D$ [110, 111]. In contrast with colloid/polymer mixtures, the depletion interaction becomes anisotropic with rod-like colloids. It tends to align the viruses [112] so that the overlap volume is maximized figure 6. The obvious consequence in the introduction of polymers in suspensions of phages is that it tends to shift the isotropic boundary to lower volume fractions. Due to the level rule, the nematic state is consequently shifted to higher volume fractions. Using fd-wt and dextran mixtures, this behavior is quantitatively confirmed and modeled [110], figure 7. Depletion is an ideal tool for promoting entropic condensation.
corresponds to the approximate intermolecular potential between interaction; the dots are the results of computer simulation. The line potential is the sum of the electrostatic repulsion and the depletion viruses separated by a distance $r$ and oriented at 90° with attraction (SVTA). Inset: the interaction potential between two line corresponds to the predictions from the second virial theory delimit the $I^N$ coexistence region from the nematic state. The blue – this approximated potential as an input.

fits the potential obtained through computer simulation. SVTA use penetrable spheres, whose effective radius and concentration best – attractive part of the potential is modeled by Asakura – aqueous dispersion of fd-wt at a concentration of $c_v [115]$. Circles delimit the isotropic state from the $I \!-\! N$ coexistence region. Squares delimit the $I \!-\! N$ coexistence region from the nematic state. The blue line corresponds to the predictions from the second virial theory with attraction (SVTA). Inset: the interaction potential between two viruses separated by a distance $r$ and oriented at 90°. The interaction potential is the sum of the electrostatic repulsion and the depletion interaction: the dots are the results of computer simulation. The line corresponds to the approximate intermolecular potential between rods with an effective hard core diameter $D_{eff} = 10.5$ nm. The attractive part of the potential is modeled by Asakura–Oosawa penetrable spheres, whose effective radius and concentration best fits the potential obtained through computer simulation. SVTA use this approximated potential as an input.

3.1.1. Nematic droplets. Nematic droplets, rather than being spherical, display a spindle shape [113–115]. This shape is due to the interplay between the interfacial tension and the splay and bend elastic constants of the inner nematic phase [78]. Tuning the morphology and order within the droplets represent a corner stone for applications such as light modula-

3.12. Colloidal membranes. Figure 7 shows that depletion interactions promote rod condensation, but it does not reveal any new phases compared to the case without depletant. Barry and Dogic extended this phase diagram to higher dextran concentrations ($M_p = 500,000 \text{ g mol}^{-1}$) [123] and showed that starting from an isotropic rod suspension at $c_v = 1–10 \text{ mg ml}^{-1}$, it is possible to assemble a new phase: 2D colloidal membranes composed of a monolayer of aligned rods one rod length thick. The membrane diameter is not controlled and varies from a few microns to hundreds of microns, figure 9. On a coarse grain level the self-assembled fluid-like and equilibrium monolayers have the same symmetry as lipid bilayers and one can develop many analogies. First, like lipid bilayers, the instantaneous and average projected colloidal membrane area $A$ are proportional, $(A - \langle A \rangle)^2 = k_BT(A)/\chi$, where the compressibility is $\chi \sim 4500 \text{ kgT } \mu \text{m}^{-2}$) [123–127]. For comparison, the compressibility of the lipid membrane is $2–3$ orders of magnitude higher $\sim 10^7 \text{ kgT } \mu \text{m}^{-2}$ [128]. Second, the colloidal membranes viewed in edge-on configurations exhibit thermal undulations. The Fourier analysis of these fluctuations can be modeled using the elastic free energy written down by Helfrich, originally developed for lipid bilayers [129]. Finally, the stability of colloidal membranes is similarly related to the way in which lipid bilayers interact [130–132]. Indeed, from $D_{ext} = 45$ to 53 mg ml$^{-1}$, the colloidal membranes remain isolated from each other: as two membranes approach each other in the suspension, protrusion fluctuations lead to an effective repulsive interaction and promote the stability of isolated membranes. At higher dextran concentrations, the depletion interaction becomes sufficiently large to overcome this effective repulsion and colloidal membranes stack on top of each other [123].

However, from a microscopic perspective the forces driving the assembly of colloidal membranes and lipid bilayers are very distinct. Colloidal membranes are assembled from micron-length hydrophilic rod-like molecules, whereas lipid bilayers are assembled from nanometer amphiphilic lipids. This leads to orders of magnitude difference in their compressibility, a lateral bending modulus or lateral tension. These differences can, in a first approximation, be attributed to the size differences of the building blocks. Indeed, the distance $d$ between the constituent particles in the colloidal membrane is $\sim 10$ nm and $\sim 1$ nm is the lipid bilayer. Assuming that $\chi \sim 1/d^2$ [135], we roughly recover the ratio between the compressibility of colloidal membranes and the lipid bilayer. The same holds for the lateral bending modulus which scales as $(D/L)^2$ [136]. Colloidal membranes, being robust assemblages that are stable over a wide range of parameters [111], represent a unique opportunity to investigate membrane biophysics from an entirely new perspective on length scales where it is possible to visualize and follow the constituent building blocks under a light microscope, the membrane dynamics or reconfigurable processes.
We first discuss the edge properties of colloidal membranes which are described at a macroscopic level by the interfacial tension $\gamma$ [137]. For 2D colloidal membranes, $\gamma$ is 1D and is the equivalent of surface tension for 3D objects like emulsion, for instance. This a thermodynamic quantity that results from the greater affinity of the colloidal membrane particles to each other than to the particle isolated in the solvent. The net effect is an inward force at the membrane circumference that causes the edge to behave elastically. The control of interfacial tension is manifold, justifying the fact that the colloidal membranes adopt a circular shape. Its control, in analogy with micro-emulsion, could lead to the fine-tuning of the colloidal membrane size.

The edge structure of the achiral colloidal membrane is determined using three complementary imaging techniques, namely two-dimensional (2D) and three-dimensional (3D) polarization microscopy and electron microscopy [46], figure 10. The 2D-LC-PolScope [138] of a membrane lying normal to the $z$-axis of the microscope produces images in which the intensity of a pixel represents the local retardance and indicates the local tilt of the rods with respect to the $z$-axis. The rods in the bulk of a membrane are aligned along the $z$-axis, and it follows that the 2D LC-PolScope images appear black in that region. By contrast, the bright, birefringent ring along the membrane’s periphery reveals the local tilting of the rods at the edge. The 3D reconstruction of the membrane structure using electron tomography [139, 140], shows that the virus tilts by 90°, from being normal to the membrane surface in the bulk to tangential to the edge along the membrane periphery. This behavior is corroborated by a 3D-LC-PolScope [141]. This twist goes with a hemi-toroidal

Figure 8. The condensation of nematic droplets in dispersions of colloidal rods with thermo-sensitive depletants [120]. (a) Phase contrast images of a suspension of M13K07 viruses at $c_v = 1 \text{ mg ml}^{-1}$ mixed with PNIPAM microgel particles at $c_P = 30 \text{ mg ml}^{-1}$. As the temperature is lowered (0.09 °C min$^{-1}$), the attraction increases and condensation of the nematic droplets is observed. The process is fully reversible when the temperature is increased; scale bar, 5 $\mu$m. (b) Measurements of the long and short semi-axis of the droplet, $r_1$ and $r_2$ as a function of temperature $T$. $T_1$ and $T_2$ are respectively the temperatures that separate the isotropic state, the spherical dense isotropic droplets in an isotropic background regime and the tactoids in an isotropic background regime.

Figure 9. fd-wt colloidal membranes, $T = 22$ °C, $D_{ext} = 45 \text{ mg ml}^{-1}$ [123]. (a) The differential interference contrast (DIC) micrograph of colloidal membranes; the scale bar is 10 $\mu$m. (b) The fluctuation spectrum resulting from the Fourier analysis of a sequence of uncorrelated membrane conformations. The red line is the best fit to the Helfrich equation, which yields a lateral bending modulus of 135 $k_B T$ and a surface tension of 100 $k_B T \mu m^{-2}$. For comparison, the lateral bending modulus and surface tension of lipid membranes are respectively $\sim 1 \text{ k_BT}$ and $\sim 10^6 \text{ k_BT} \mu m^{-2}$ [133, 134]. Inset: DIC micrograph of a colloidal membrane edge-on; scale bar 2 $\mu$m. (c) A sketch of the lipid bilayer and colloidal membrane, which on a coarse grain level are similar and obey the Helfrich equation.
k is the bare line tension of a membrane edge composed of Dext. In the range of temperatures κing rigidity of the interface, (temperature): the effect of attraction (dextran concentration) from chirality thus have a unique system where it is possible to decorrelate ral interaction which depends solely on the temperature. We alone, and therefore athermal, as apposed to the fd-wt chi-

3.2. Colloidal membranes and chirality

3.2.1. Tuning the edge chirality. A classical way of measuring the interfacial tension consists of analyzing the membrane’s edge thermal fluctuations in the Fourier space [144–146]. A typical fluctuation spectrum for an achiral edge is shown in figure 11. In the thermodynamic limit, which corresponds to small wave vectors, q, the mean square Fourier amplitudes of the edge fluctuations, \( \langle a_q^2 \rangle \), are q-independent and yield the effective line tension, \( \langle a_q^2 \rangle = k_B T / \gamma \) [144]. In the large-q limit, fluctuations scale as 1/q^2 and yield the bending rigidity of the interface, κ. In the range of temperatures and dextran concentrations explored, for fd-wt colloidal membranes, κ \( \sim \) 100 k_B T · μm while γ varies from \( \sim \) 100 to 800 k_B T μm^-1.

Next we provide evidence of the role of chirality on γ. The self-assembly of colloidal membranes is driven by entropy alone, and therefore athermal, as apposed to the fd-wt chiral interaction which depends solely on the temperature. We thus have a unique system where it is possible to decorrelate the effect of attraction (dextran concentration) from chirality (temperature): \( \gamma(Dext, T) = \gamma_{bare}(Dext) + \gamma_{chiral}(T) \), where \( \gamma_{bare} \) is the bare line tension of a membrane edge composed of achiral rods and \( \gamma_{chiral} \) is the chiral contribution to the line tension [46]. In figure 11, we observe that the effect of chirality drastically modifies the fluctuation spectrum of the edge of a colloidal membrane, \( \langle a_q^2 \rangle \), as expected from the edge structure. First, \( \langle a_q^2 \rangle \) is shifted upward at a low q, which indicates that the line tension decreases with chirality as hypothesized. This is further demonstrated using the dextran series, which shows that γ decreases with the same slope as the temperature decreases confirming that the two contributions to γ are uncorrelated. Second, a peak appears at intermediate q which is attributed to the Gaussian curvature, enabling out-of-plane fluctuations. Red lines are fit using equation (3).

![Figure 10](image1.png)

**Figure 10.** The structure of a colloidal membrane, \( T = 22 \) °C, \( Dext = 45 \text{ mg mL}^{-1} \) [46]. (a) A 2D LC-PolScope retardance map of a colloidal membrane. The bright band associated with the edges indicates local rod tilting. (b) The electron micrograph cross section of a membrane directly visualizing the tilt of the rods and the curved edge profile. (c) A sketch of a colloidal membrane indicating that its edge adopts a curved profile, forcing the rods to locally twist.

curved edge. The twisted edge makes the membrane a chiral object. For achiral virus dispersions, the spontaneous twist at the edges is equally likely to be clockwise or anticlockwise [46]. For chiral virus suspensions, the edge adopts the chirality of the virus. By comparison with an untilted edge, the curved edge structure lowers the area of the rod–polymer interface, thus reducing interfacial tension, at the cost of increasing the elastic energy due to twist distortion.

![Figure 11](image2.png)

**Figure 11.** The chiral control of the interfacial tension of colloidal membranes composed of fd-wt [46, 142, 143]. (a) The fluctuation spectrum of the membrane edge \( \langle a_q^2 \rangle \) at \( T = 20 \) °C (square) and 50 °C (circle) for \( Dext = 36 \text{ mg mL}^{-1} \). For small wavenumbers q, \( \langle a_q^2 \rangle \) is independent of q and inversely proportional to the effective line tension γ. For large q, \( \langle a_q^2 \rangle \) are independent of Dext and T and scale as 1/q^2. For very chiral samples a peak is observed at intermediate q which is attributed to the Gaussian curvature, enabling out-of-plane fluctuations. Red lines are fit using equation (3). Inset: DIC images, taken 1s apart, illustrating the fluctuations of the membrane’s edge; scale bar, 5 μm. (b) The line tension as a function of temperature for different dextran concentrations. In the achiral limit at 60 °C, \( \gamma_{chiral} = 0 \) and \( \gamma_{bare} = \gamma \). Increasing the Dextran concentration increases \( \gamma_{bare} \). Decreasing the temperature reduces γ because \( \gamma_{chiral} \) decreases. The red lines of the fixed slope are guides to the eyes illustrating the universal scaling of γ with chirality.
3.2.2. Twisted ribbons.

Positive Gaussian curvature, in the $\z$-direction \cite{151}, which vouches for the existence of a positive Gaussian curvature, $\bar{k} \sim 150k_B T$ \cite{142}.

Twisted ribbons. The chiral control of line tension raises the possibility of the chiral contribution to the interfacial energy dominating the bare line tension at sufficiently low temperatures, lowering the energetic cost of creating edges and leading to the control of the size of the membrane or to spontaneous edge formation. With decreasing temperature, membranes remain polydisperse in size. However, the membrane edge eventually becomes unstable, resulting in a remarkable polymorphic transition, figure 12. Twisted ribbons grow along the entire periphery of the disk from the out-of-plane fluctuations of the membrane edge, generating a starfish-shaped membrane. This polymorphic transition is reversible and twisted ribbons form equilibrium structures at high chirality and low dextran concentrations. Twisted ribbons are a beautiful example of hierarchical assembly, figure 13. They consist of a twisted monolayer of aligned rods, which form a helicoidal structure perpendicular to the rod twist. As observed by Efrati and Irvine, such objects are simultaneously right- and left-handed \cite{152}. The rod twist at the edge is left-handed, while on larger length scales the helicoidal structure of the ribbon is right-handed. As such it differs from other twisted ribbons observed in the literature \cite{153–158}. The twisted ribbons can be seen on a coarse grain level as polymers with a persistence length of the order of the pitch of the helicoidal structure, and may form a zoology of structures ranging from a branched polymer to a looped polymer or entangled phone-cord-like structures reminiscent of a DNA double helix \cite{46}. The twisted ribbon stability with respect to the colloidal membranes is attributed to two factors. First, chirality is frustrated in the colloidal membranes as viruses in the bulk cannot twist due to their virus neighbors, whereas in twisted ribbons all the viruses twist and chirality may be naturally expressed. Second, twisted ribbons are edge objects with low interfacial energy compared to the membranes. Given the 3D structure of the ribbons, Gaussian curvature may also be an important parameter that justifies the twisted ribbon stability \cite{142}.

It is possible to use laser tweezers to manipulate the self-assembled structures. For instance in figure 14, the two opposite sides of a colloidal membrane are trapped with a dual-beam optical trap. The viruses align with the electric field of the laser and the membrane turns sideways. Using a static trap and providing an extensional displacement with the other optical trap, the membrane is stretched, causing the transition to a twisted ribbon. This mechanically induced disk-to-ribbon transition is reversible; on removal of the optical trap, the
3.3. Colloidal membranes and chiral coalescence

Driven by the balance between interfacial tension and bulk energy, a pair of liquid droplets—when sufficiently close to one another—may coalesce to form a single daughter droplet. The coalescence process is complex and involves the rupture and fusion of the droplet surfaces associated with the energy barrier and local rearrangements [160–168]. In most cases, it is an ‘all-or-nothing’ process; once initiated, the reaction proceeds to completion. However, there is also the possibility of incomplete coalescence. For example, vesicles coalesce into a hemi-fused state [169] and nanotubes into a defect-ridden structure [170]. Taking advantage of the chiral edge, colloidal membrane coalescence enlightens the role of geometrical frustrations [171] in the self-assembly of new structures [172, 173].

The edge chirality of the colloidal membranes can be controlled in various ways. First, if the colloidal membrane is small enough—a diameter smaller than the virus length—the viruses stand straight at the edge and those membrane seeds are achiral, figure 15. Second, for larger membranes the edge adopts the chirality of the virus. Third, it is possible to compose achiral virus suspensions, for instance using fd-wt with a diameter smaller than the virus length—which may coalesce to form a single daughter drop—figure 15. Second, for larger membranes the edge chirality of the colloidal membranes can be controlled either with a left- or right-handed edge. For chiral membranes, we can divide the coalescence into two families: homo- and hetero-chiral coalescence, figure 16. In homo-chiral coalescence, one coplanar membranes have the same chirality. The rods at the coalescence point are tilted in opposite directions, trapping 180° of the twist between the two membranes. In hetero-chiral coalescence, both coplanar membranes have the opposite chirality and the viruses at the edge need only to straighten in the z-direction at the coalescence point.

### 3.3.1. Homo chiral coalescence—π-walls, pores, Möbius anchors and colloidal skyrmions

Homo-chiral coalescence [159] is at the center of chiral topological frustration. In figure 17, the coalescence between two homo-chiral membranes may result in a defect-free daughter membrane. Similar to liquid droplets, the thermal fluctuations are sufficient to form a bridge between the two membranes. In this bridge, which is one rod length wide, the rods twist by 180° to match the orientations of the joining edges. The twisted bridge induces a torque, which enables the two membranes to rotate along the axis formed by the bridge and expel the trapped twist. As the membranes twist around each other, the connecting bridge expands in width, eventually leading to a circular defect-free daughter membrane. In an other pathway, coalescence is initiated by the formation of two twisted anchors, which bind the membranes together and initiate the nucleation of a continuous 1D line defect. This line defect, named a π-wall, quickly grows to its equilibrium size, pushing the two anchors apart. Once the π-wall is fully formed, it remains indefinitely. The π-walls are stable with respect to the two free membrane edges: the measurements of the π-wall interfacial tension γπ and the membrane edge interfacial tension γ show that γ < γπ < 2γ.

However, in fd-wt systems where chirality can be controlled with temperature, the π-wall can be continuously brought to regimes with high chiral interactions at low temperatures. In this case, γπ > 2γ and π-walls become metastable with respect to isolated membranes. In high chiral regimes, we...
do not observe the spontaneous dissociation of a π-wall into two defect-free membranes. We instead observe the opening of pores in the π-walls, figure 18. These pores may form an alternating bridge-pore array (ABPA) structure, which can be closed back into a π-wall by increasing the temperature. This behavior remains to be understood but seems reasonable, as pores create a large number of edge interfaces, which are favored at high chirality. More importantly, it empirically proves that with the proper ingredient, it is possible to actuate pores upon external signaling in self-assembled membranes. Pore actuation is of primal importance throughout the cell lifecycle [174, 175].

Finally, we discuss two structures related to π-walls: Möbius anchors and colloidal skyrmions. Both of these structures rely on a robust on-demand method for imprinting defects into colloidal membranes with arbitrary spatial precision. Taking inspiration from recent work with thermotropic liquid crystals [176–178], we also use an optical trap. A simple Möbius strip is a one-sided continuous surface, formed by twisting a long narrow rectangular strip of material through 180° and joining its ends. Such a structure can be made in liquid-crystal by knotting the microscopic topological defect lines with optical tweezers about the colloids [179]. The Möbius strips we observe in the colloidal membrane are Möbius anchors [172]. The Möbius anchor is associated with the way π-walls are anchored to the membrane edge, figure 19, and is mandatory for the π-wall to remain stable. For instance, with optical tweezers it is possible to imprint a π-wall on a colloidal membrane. However, if the optical trap is released before the π-wall is anchored, the defect retracts. Based on 2D-LC-PolScope micrographs, it seems that the viruses follow a simple Möbius strip, which tightens the

Figure 18. Reconfigurable self-assembly and chirality—the π-wall to the alternating bridge-pore array (ABPA) transition in fd-wt colloidal membranes [173]. (a) A temperature quench induces a transition from a π-wall to an ABPA. The colloidal membrane assembled at $D = 45 \text{ mg ml}^{-1}$ is quenched from $T = 55$ to 22 °C. During the quench, the π-wall morph into alternating bridge-pore arrays. Similarly to the membrane/twisted ribbon transition, this transition is reversible; DIC microscopy images; scale bar, $5 \mu m$. (b) The π-wall structure; from left to right—a time sequence illustrates the rotation of a fluorescently labeled virus (green) by 180° as it diffuses through a π-wall. Fluorescence images are superposed with simultaneously acquired phase-contrast images (red). The 2D-LC-PolScope image of a π-wall is compatible with the 180° twist of the viruses. A fluorescence image of a π-wall where all the rods are fluorescently labeled. The defect appears darker in its center, which corresponds to a decrease of the defect thickness. Bottom: a sketch of the π-wall. (c) The ABPA structure; from left to right—the simultaneous fluorescence (green) and phase-contrast (red) imaging reveals that the rods twist by 180° within a bridge. A 2D-LC-PolScope confirms this twist. An ABPA image, where all the rods are fluorescently labeled, shows that the pores are empty of viruses. Bottom: a sketch of the ABPA; scale bars, $2 \mu m$.

Figure 19. Möbius anchors in fd-wt colloidal membranes, $D = 45 \text{ mg ml}^{-1}$, $T = 22 \degree C$ [159]. (a) A DIC micrograph of a π-wall; scale bar 5 μm. Inset: the 2D-LC-PolScope anchor point of the π-wall at the edge of a colloidal membrane. We guess that this anchoring point forms a Möbius loop which prevents the π-wall from vanishing. (b) A DIC image of the Möbius anchors before and after the π-wall is severed with optical tweezers; $z+$ and $z−$ correspond to the images focused slightly above and below the membrane plane. (c) A sketch of the Möbius anchor; scale bars 2 μm.

Figure 20. The colloidal skyrmion in fd-wt colloidal membranes, $D = 45 \text{ mg ml}^{-1}$, $T = 22 \degree C$ [159]. The evolution of the radius of the colloidal skyrmion over time; $t = 0$ corresponds to the time the colloidal skyrmion is imprinted with optical tweezers. Inset: a 2D-LC-PolScope image of a colloidal skyrmion using laser tweezers to close a π-wall on itself. Scale bar 1 μm.
π-wall to both edges of the daughter membranes, figure 19. At this point the anchoring structure is only a guess. This hypothesis is, however, supported by the fact that it is necessary to produce a back and forth motion with the optical trap to create the anchor, which is reminiscent of the pathway depicted in soap film to create a Möbius loop [180].

Colloidal skyrmions [172] are obtained using optical tweezers to cleave a π-wall in two places, and then quickly joining the two ends to form a closed ring embedded within the membrane, figure 20. The colloidal skyrmion shrinks to an equilibrium diameter of about \( \sim 1 \, \mu m \). Note that for a similar size, an isolated colloidal membrane displays an untwisted edge, figure 15. The colloidal skyrmions share properties with skyrmion excitations encountered in hard condensed matter physics [181–185]. It is topologically protected [186, 187], having positive energy compared with the background field, although the π-wall forming the skyrmion cannot be untrapped unless the π-wall is severed. Moreover, it is a 2D structure characterized by a vorticity \( m = 1 \) and a phase helicity \( \varphi = \pm \pi/2 \), whose sign depends on the chirality of the π-wall. As such, it is very similar to the single-out skyrmions of the hexagonal SkX state on MnSi [182] and Fe\(_{1-x}\)Co\(_x\)Si [188–190] and seems to be the closest realization of a theoretical nematic skyrmion restricted to straight infinite lines in unbounded ideal materials [191]. It does, however, differ from other liquid crystal skyrmions such as double twisted cylinder ‘baby-skyrmions’ [192, 193], and skyrmions in cholesteric blue phases subjected to strong external fields [194, 195].

Understanding the principles that support or prevent membrane coarsening and defect formation, such as the π-wall, is essential for growing large defect-free membranes and considering their applications. As chirality is at the center of π-walls, this suggests that producing achiral colloidal membranes would lead to defect-free coalescence and uniform monolayers.

3.3.2. Hetero-chiral coalescence—scalloped membranes and Gaussian curvature. To study hetero-chiral coalescence [173], colloidal membranes composed of homogeneous mixtures of fd-wt and fd-y21m are used. For 0.04 < \( x_{fd} < 0.45 \), in the early stages of sample maturation, we observe colloidal membranes of either edge handedness, indicating spontaneously broken achiral symmetry. Over time, intermediate-sized membranes with a mixed edge twist continue to coalesce. Both homo- and hetero-chiral coalescence is observed. In both cases, coalesced membranes display homogeneous mixing of fd-wt and fd-y21m. In hetero-chiral coalescence, as the two proximal edges of a pair of coplanar membranes merge, the twist of the edge-bound rods is expelled by aligning the constituent rods with the membrane normal. Hetero-chiral coalescence leads to scalloped membranes. As compared to homo-chiral coalescence, scalloped membranes form easily. Moreover, they are defect free in their bulk and may reach a millimeter diameter. The hallmark of scalloped membranes is located on its edge. It displays two outward protrusions which separate the left- from the right-handed edge, figure 21. Using confocal microscopy, it is observed that the two protrusions escape in the z-direction in opposite directions. This 3D point-like singularity on the vertical axis vouches for the presence of the Gaussian curvature \( \kappa_G \) associated with its Gaussian elastic modulus \( k \).

The distance \( \delta s \) between two adjacent edge protrusions greatly depends on \( x_{fd} \), figure 22. Close to the achiral limit, at \( x_{fd} = 0.26 \), adjacent protrusions freely move along the edge and the dynamics of \( \delta s \) is diffusive. In contrast, close to the boundary of the stability region of scalloped membranes at \( x_{fd} = 0.04 \) or 0.45, adjacent protrusions pair and remain bound to each other at a well-defined distance \( \delta s_0 \). To measure the entire binding potential, active experiments are performed: one defect is moved by \( \delta s \) using an optical trap, while simultaneously measuring the force \( F \) exerted on the adjacent defect. For this purpose, the 1.5 μm diameter colloidal beads are embedded into two adjoining cusp defects. The force is negative below \( \delta s_0 \), and positive above \( \delta s_0 \). \( \delta s_0 \) is the stable equilibrium position. The force steeply increases for small separations and saturates at large separations, indicating that a pair of defects is permanently bound, figure 22.

These observations can mainly be explained by the chirality of the edges. For instance, at \( x_{fd} = 0.07 \) the system is mostly composed of fd-y21m and right-handed chirality is favored. Therefore, the right-handed edges have lower energy than the left-handed ones. This leads, in scalloped membranes,
to a finite difference in the line tension $\Delta \gamma$ between the left-handed and right-handed outward protrusions. The edge-free energy is minimized by reducing the length of the outward protrusions with the unfavored twist and the amplitude of $\Delta \gamma$ accounts for the strength of the long-range attraction between two adjacent protrusions. Bringing the two protrusions close together has two consequences. It tends to over-bend the edge, separating the two adjacent protrusions and flattening the protrusion in the $z$-direction. This again works the bending rigidity of the edge $\kappa$ and against a negative Gaussian curvature $\kappa_G$, which lowers the free energy of elastic deformations if the Gaussian modulus is positive and sufficiently large, $\bar{k} = 200 k_B T$ [173, 196, 197].

Figure 22. Measurement of the protrusion interactions in fd-wt/fd-y21m scalloped membranes, $T = 22 \, ^\circ\mathrm{C}$, $\text{Dext} = 45 \, \text{mg ml}^{-1}$ [173]. (a) A phase contrast image of scalloped membranes at $\text{Dext} = 40 \, \text{mg ml}^{-1}$. Decreasing the ratio of $x_0$ (increasing the chirality) leads to tighter coupling between two adjacent protrusions which then pair; scale bars, 2 $\mu$m. (b) Force measurements $F$ (dots), obtained with laser tweezers, and fitted with a theoretical model (full curves) as a function of $\delta s$, the distance between two adjacent protrusions.

Figure 23. Phase separation and membrane rafts in a mixture of viruses fd-y21m and MK13KO7 [74, 210]. (a) A sketch of the depletion interaction in a mixture of long and short rods. (b) Dual view fluorescence images of fd-y21m (yellow) and MK13KO7 (red), and a DIC micrograph of the colloidal membrane as a function of the dextran concentration. At $\text{Dext} = 34 \, \text{mg ml}^{-1}$, the membrane is homogeneously mixed. At $\text{Dext} = 38 \, \text{mg ml}^{-1}$, we observe the formation of finite-sized clusters enriched in fd-y21m embedded in a background enriched in MK13KO7. At $\text{Dext} = 52 \, \text{mg ml}^{-1}$, complete separation of the bulk phase is observed; scale bar, 5 $\mu$m. (c) A sketch of the colloidal membrane in (b).

Figure 24. Equilibrium rafts and their interaction in fd-y21m/ M13KO7 colloidal membranes [74]. (a) The dependence of raft expansion rates on raft size directly reveals the critical nucleus size and equilibrium size. Inset top: expansion of an undersized raft and contraction of an oversized raft. Inset bottom: fluorescently labeled fd-y21m rods associate and dissociate from the raft, revealing binding kinetics at the single-molecule level. (b) The effective pair interaction potential obtained using the blinking trap technique for clusters with diameters of $1.6 \, \mu$m. Inset: a 2D-LC-PolScope image of rafts embedded in a colloidal membrane.
require in-plane heterogeneities or an external force to be folded or wrinkled. Finally, achiral symmetry breaking has been observed in diverse soft systems with orientational order, ranging from lipid monolayers and nematic tactoids to confined chromonic liquid crystals [116, 117, 206–208]. In particular, the measured structure and interactions of the cusp-like defects in colloidal membranes resemble the studies of point defects moving along a liquid crystalline dislocation line in the presence of chiral additives [209]. The main difference is that in colloidal membranes, the achiral symmetry breaking leads to out-of-plane 3D membrane distortions that couple the liquid-crystal physics to membrane deformations. This is not possible for inherently confined liquid crystalline films.

3.4. Asymmetric mixtures of colloidal rods

3.4.1. Phase separation in colloidal membranes. Phase separation can be triggered by asymmetric forces between the colloids. This force configuration can be achieved by mixing depletant with viruses of different lengths: the fd-y21m virus (880 nm long) and M13KO7 virus (1200 nm long) 19. The strength of the depletion force is proportional to the overlap of the excluded volume. In figure 23, two short rods with a short and a long rod share the identical overlap of the excluded volume, while two long rods have a large overlap with the excluded volume and therefore display greater attraction.

Colloidal membranes containing both fd-y21m (right-handed) and M13KO7 (left-handed) are assembled by adding depletant to a dilute isotropic mixture of fd-y21m and M13KO7, [74]. After reaching a large enough size, the membranes settle as sediment at the bottom of the sample chambers; the constituent rods point in the z direction, figure 23. At low depletant concentrations, the thermal energy is sufficient to overcome the attraction between rods of different sizes and the rods remain homogeneously mixed in the membrane. At high depletant concentrations, the rod within the membrane separates into two phases: an enriched M13KO7 phase surrounded by an enriched fd-y21m phase. Both phases conserve the symmetry of the colloidal membrane. At intermediate concentrations, micro-phase separation is observed: colloidal rafts, which are highly monodisperse micrometer-sized 2D droplets enriched in fd-y21m, float in the background of M13KO7.

3.4.2. Membrane rafts. Colloidal rafts [74] do not coarsen with time, suggesting that they are equilibrium structures, figure 24. Particle tracking experiments show that the rods diffuse in and out of these rafts, allowing for equilibration to a preferred size. Using optical tweezers to create a raft population with heterogeneous radii, the raft growth rate is measured. Below a critical radius the rafts melt, and above it the rafts converge toward an equilibrium radius of $\sim1\ \mu m$.

Colloidal rafts seem similar to the equilibrium clusters found in protein and colloidal dispersions [211–213]. The stability of equilibrium clusters is attributed to the mixed potential of the particles forming the cluster. This mixed potential is composed of short-range attraction and long-range repulsion. For colloidal rods, the attraction is due to the depletion interaction. In contrast to equilibrium cluster particles, the electrostatic interactions of the colloidal rods are fully screened and the long-range repulsion is attributed to virus chirality.
Two rafts are indeed in a homo-chiral coalescence configuration, which is not propitious for merging in 2D. The raft edge twist is further transmitted by the twisted structure of the background membrane, which mediates long-range elastic repulsion between rafts. This interaction is measured quantitatively by bringing two rafts close together with optical traps and tracking their trajectories upon release of the traps [74]. This chiral repulsion stabilizes small rafts against an interfacial line tension that would otherwise promote coarsening to a single raft domain and establishes a preferred depletant-concentration-dependent raft size [210].

These results fuel the ongoing discussion on lipid rafts, whose structure, properties and function constitute ongoing research [214–217]. These membrane raft structures have evolved from controversial detergent-resistant entities to dynamic, nanometer-sized membrane domains formed by sterols, sphingolipids, saturated glycerophospholipids and proteins [215, 216, 218–220]. Provided that the analogy between the colloidal raft and the lipid raft hold, it seems that short-range attraction and chirality are the essential ingredients. A systematic study of the role of chirality in colloidal rafts with respect to the chiral molecule present in lipid rafts remains to be undertaken for a more refined analogy.

4. Conclusion and perspectives

Filamentous phages, such as fd-like viruses, are rod-like colloids that have well-defined properties such as their diameter, length, rigidity, charge and chirality. Engineering these viruses leads to a library of rods with slightly different properties, which can be used as building blocks for self-assembly, section 2. Their condensation in an aqueous solution with additive depletants produces a myriad of structures ranging from isotropic/nematic droplets [120], colloidal membranes [111, 123, 221], achiral membrane seeds [143], twisted ribbons [46], π-wall [159], pores, colloidal skyrmions, Möbius anchors and scallop membranes [173] to membrane rafts [74], section 3. First, these structures reinforce the general notion that through careful choice of particle shapes, sizes and concentrations it is possible to ‘engineer entropy’ [4] and build structures of ever-increasing complexity. Second, the entropy driven condensation of millions of rods in finite liquid-like objects leads to dynamic equilibrium and allows the structures to permanently rearrange themselves and test their energy landscape. Therefore, these structures are very sensitive to externally tunable interactions like chirality and attractions which trigger shape-shifting transitions. Third, external forces like optical tweezers may be utilized to manipulate these structures, and probe their mechanical properties as well as the transition between multiple metastable polymorphic forms with complex topologies. Fourth, these structures represent a showcase of analogies between objects which belong to different fields of science such as colloidal membranes and lipid bilayers, chiral pore actuation and pores in cells, colloidal rafts and membrane rafts, colloidal skyrmions and solid state skyrmions, the twist penetration length at the edge of colloidal membranes and the penetration depth of the magnetic field in a superconductor, or Möbius anchors and Möbius strips. Fifth, this experiment combined with the theoretical input make it a well-established field in self-assembly. Many theoretical approaches have been proposed. A de Gennes framework accompanied by appropriate surface energy terms was used to characterize the colloidal membranes, twisted ribbons and π-wall [159, 196, 197, 222–225]. Sakhadande et al adopted a continuum Ginzburg–Landau theory to study raft stability [226]. Xie et al considered a functional density theory constructed on the free volume theory for depletant–rod interactions, and a third order virial expansion for rod–rod interactions, with the equation of state for a hard disk system to constrain the areal rod density to study 2D colloidal membranes composed of binary mixtures of rods with opposing chiralities [227]. Kang et al formulated an entropically motivated theory using three simple considerations to characterize colloidal membranes and membrane raft stability: the depletant-excluded volume, rod fluctuations perpendicular to the membrane, and rod twisting as described by the Frank free energy [143, 210]. For all these reasons, fd-like phages constitute an attractive model system in soft matter physics, figure 25.

The subject is clearly open and many questions remain unanswered. Two-dimensional colloidal membranes do not form vesicles—would it be possible to reduce the lateral bending rigidity of the membranes with smaller viruses and have them form vesicles? We have seen that chirality tends to produce 3D structures with Gaussian curvature—is it possible to enhance this effect and make 3D leather-pouch-like membranes? Rafts are stabilized due to chirality—what happens to the micro-phase separation in homo-chiral mixtures and in achiral mixtures? As this review is only based on three different phages (fd-wt, fd-y21m and M13K07), it is a long way from being representative of phage diversity [228], and there are many more structures to be discovered in such systems.

The phenomenology described in this review article should be relevant to diverse colloidal and nanosized rods that interact through excluded volume interactions. Indeed, as demonstrated in section 2, fd-like viruses are an excellent experimental realization of hard rods. The challenges for applications, especially in materials science, are threefold. Firstly, there is the development of monodisperse rods with interactions that exclude aggregation and permit equilibrium self-assembly. Secondly, robust rods that conserve their integrity in harsh conditions are needed. Thirdly, large-scale production is required. Progress in these directions is clearly on its way [229–233], and the material and bio-applications are lining up [234–237]: templates for cell growth [238], colorimetric sensors [239], photovoltaic devices [240, 241], batteries [242–244], etc.

Acknowledgment

I sincerely thank Zvonimir Dogic, who introduced me to the subject of self-assembly and filamentous phages. Many thanks to Edward Barry, Anna Modlinska, Prerna Sharma, Andrew Ward and Mark J Zakhary for the countless hours
spent together in the lab making these experiments work; to C Nadir Kaplan, Louis Kang, Tom C Lubensky, Robert B Meyer, Robert A Pelcovits, Thomas R Powers and Hao Tu for their theoretical insight; and Seth Fraden, Eric Grelet, Pavlik Lettinga and Rudolf Oldenbourg for useful discussions.

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