Topical Review

Solid Colloids with Surface-Mobile Linkers

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Abstract. In this review we present an overview of the possibilities of using colloids with surface mobile linkers. Specifically, we focus on how the mobile linkers could influence colloidal self-assembly. A promising tool to create systems with mobile linkers is the use of lipid bilayers. These bilayers can be either used in forms of vesicles or as coatings for hard colloids and emulsion droplets. Inside the lipid bilayers molecules can be inserted via membrane anchors. Due to the fluidity of the lipid bilayer, also the anchored molecules stay mobile. The use of different lipid mixtures even allow to create Janus like particles that exhibit directional bonding if linkers are used which have a preference for a certain lipid phase. In nature mobile linkers can be found e.g. as receptors in cells. Therefore, we also briefly address the possibility to use colloids with surface mobile linkers as model systems to mimic cell-cell interactions and cell adhesion processes. Some examples of such model systems are given in the last part of the review.

1. Introduction

Since a number of years, DNA-coated colloids have been employed as versatile bio-inspired model systems to study processes of self-assembly and crystal formation. [1–6] The unique feature of these colloids lies in the fact that the nucleotide sequence of the DNA can be chosen specifically to promote interaction with colloids that are coated with complementary nucleotide sequences. In the majority of cases these model systems consist of gold, glass or plastic beads to which DNA molecules are either attached using physical absorption [7–9], or covalent chemical bonds [1, 2, 10]. By careful design of the single-strand (sticky) ends of the surface-crafted DNA molecules, interactions between colloids can be made not only specific, but also tunable [11–14]. In addition, methods have recently been developed to create solid colloids with DNA linker molecules that are mobile on the colloid surface (Fig. 1)

In this review we discuss the potential of solid colloids with surface-mobile linkers as new model systems for self-assembly processes. We first review previous experimental model systems with surface mobile linkers, and then focus on the recent development of model systems based on DNA-coated spheres. Next, we briefly discuss the potential of these systems for the experimental study of multi-bond interactions with biological relevance. Especially on this last topic, there is a vast theoretical literature on the effect of linker mobility on for example cell-cell interaction forces. We only superficially refer to this literature, and refer the interested reader to excellent previous work for more in-depth information [15–17].

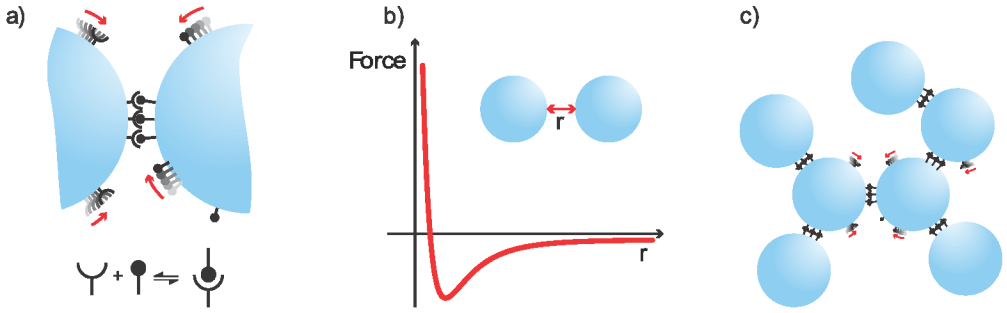

Figure 1. (a) Schematic drawing of solid colloids carrying linking molecules that can diffuse over the particles' surface. This system can be used as (b) model system for measuring multibond interactions forces or (c) for studying colloidal self-assembly.

1.1. Self-assembly processes

Self-assembly is the process where individually non-functional building blocks form higher-ordered, organized structures or patterns as a consequence of specific, local interactions among the components themselves, in the absence of external guidance.

Studying self-assembly processes is of great importance for several reasons. For instance, due to the huge developments in nano-science and the unprecedented potential applications it has, there is a high demand for practical methods to built nano-structures, which self-assembly could provide. Further, self-assembly is common to many dynamic, multicomponent systems, from smart materials and self-healing structures to netted sensors and computer networks [18]. Finally, many supra-molecular structures in living cells are the result of self-assembly processes. Hence, understanding the principles of self-assembly will also help us understanding cells.

There are a number of features that a structure should have in order for it to be considered a self-assembled structure. One of the features is that the final structure should possess a higher order than the isolated parts. Secondly, the interactions linking the components together should be weak with respect to covalent bonds. The building blocks do not necessarily have to be atoms or molecules but can also be larger mesoscopic objects like lipid vesicles or micro particles. Finally, a self-assembly process is non-dissipative and occurs spontaneously because the energy of unassembled components is higher than the self-assembled structure, which is in a (local) static equilibrium, persisting without the need for energy input [19]. There are many ways to direct self-assembly towards a preferred state [20]. For example, because the components must be able to move with respect to one another during the self-assembly process, the type of phase in which the components are dispersed dictates the motility of the components and therefore the dynamics of the self-assembly process. In addition, one can use physical boundaries and other templates to restrict the assembly to specific dimensions. Another tunable parameter is the type of interaction between components. By adjusting for example the shape, surface properties, charge, polarizability, magnetic dipole or mass of the building blocks, the direction and or the affinity with which the components bind to each other can be changed thereby affecting for example the kinetics of the self-assembly process and hence the final structure.

In this review we primarily consider specific interactions mediated by molecules. Such interactions can be achieved by chemically coupling specific linking molecules to the surface of self-assembling building blocks. Popular examples of such molecules are the biotin/streptavidin couple and the Watson and Crick (DNA) type of bonding between short oligonucleotides. Molecules are coupled to the surface of nano or micro particles in such a way that the molecular sites of interaction are left exposed, remaining free to bind to a partner. Especially the use of DNA provides a vast amount of controllability, via e.g. the number of molecules per surface area, the interaction depth (i.e. the length and flexibility of the oligonucleotide strand) and the interaction strength per molecule (i.e. the number of complementary base pairs). Explorations of this parameter space have led to the development of reliable crystallization strategies for nano-colloids (Fig. 2), in which great control is achieved over the crystal structure, the lattice spacing, and other properties [21].

However, comparable control over the self-assembly of larger micron-sized building blocks appears more challenging as reviewed by DiMichele and colleagues. Due to

kinetic issues encountered during the self-assembly process, e.g. particles irreversibly sticking together, the structures more frequently equilibrate to amorphous phases (often referred to as glass structures) than towards the expected thermodynamically more stable crystal phase. inhibiting thus far the development of technological applications such as self-assembling photonic metamaterials [22]. Fortunately, recent experimental and theoretical progress has led to the development of new rational designs that could potentially facilitate a wider range of self-assembly experiments. The roles of many of the key parameters, e.g. the length of inert spacers, the combinatorial entropy and the presence of competing linkages are now far better understood which already has led to the creation of exciting structures [23–26].

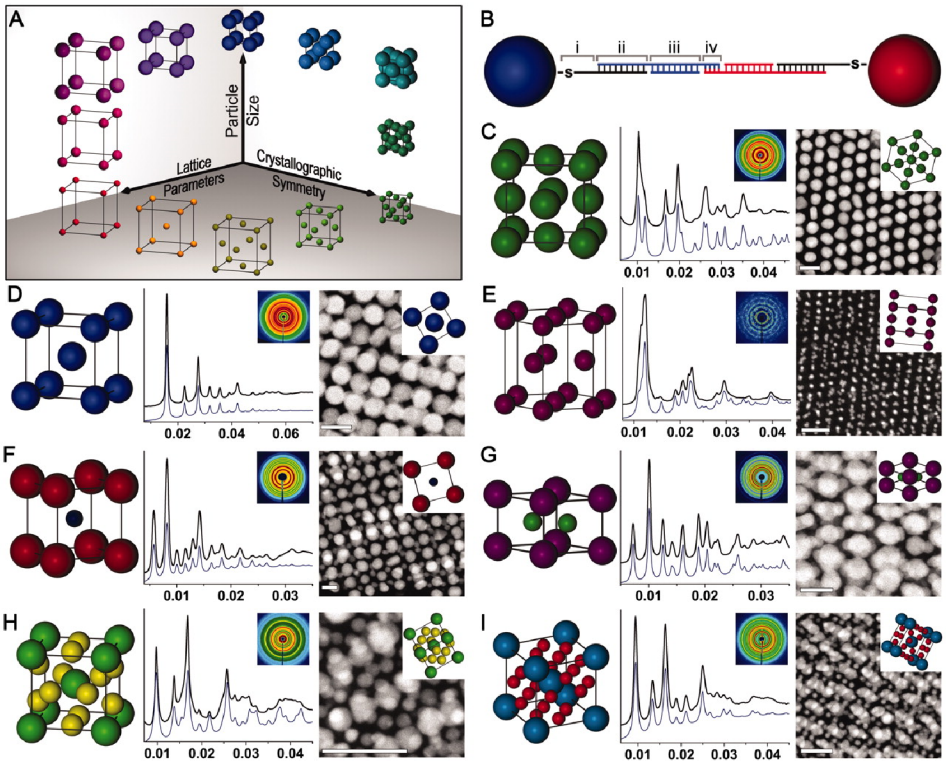

Figure 2. Examples of crystal structures that are accessible by self-assembly of nanoparticles mediated by nucleic acid linkers [5]. (A) By varying the parameters that control the assembly of the nanoparticle building blocks (e.g. particle size and the length of the nucleic acid linkers), it has been shown that over 100 different crystal structures, covering 17 distinct crystal symmetries, can be made. (B) The DNA strands mediate these nanoparticle assemblies, are composed of (i) an alkyl-thiol moiety and 10-base nonbinding region, (ii) a recognition sequence that binds to a DNA linkers, (iii) a spacer sequence of programmable length to control interparticle distances, and (iv) a "sticky end" sequence that drives nanoparticle assembly via DNA hybridization interactions. The superlattices displayed here are isostructural with (C) fcc, (D) bcc, (E) hcp, (F) CsCl, (G) AlB_2,(H) Cr_3Si, and (I) Cs_6C_{60} lattices. Figure reprinted from [5].

1.2. The need to equilibrate

As illustrated in the last paragraph of the previous section, an essential requirement for the self-assembly process to successfully take place is the ability of the system to equilibrate. A common equilibration method is to allow for the building blocks to be able to transiently switch between aggregated and non-aggregated states. In the case of DNA functionalized colloids, this can be achieved by successively crossing the temperature at which the DNA strands melt [24]. During the melting of the DNA strands, the colloids inside the disordered aggregates get more loosely connected and hence have more freedom to relocate to finally get to their expected ordered ground state. However, calculations, simulations and measurements on colloidal pair potentials have revealed an extreme sensitivity on environmental variables like temperature, which makes the formation of ordered structures a very difficult process [1, 9, 10, 27, 28].

A strategy that could assist in overcoming the challenge of equilibrating the aggregates, but that has not been much looked into yet, is to allow for particles that are already attached to move with respect to each other. Adjusting their position relative to their neighbors within an aggregate gives the colloids the freedom to maneuver to their ordered ground state location within the aggregate without the need for the interactions to transiently open and close. One could for example achieve this by coupling the linking molecules to the surface of the building blocks in such a way that they can still undergo lateral motion along the surface. When in this situation two complementary particles bind, they can move with respect to each other by dragging the binding molecules along the surface while remaining at close proximity. Currently, there is no direct experimental evidence that proves that surface-mobility of linking molecules facilitates the organization of crystal structures. However, there are studies that have reported surface mobility of ligands coupled to gold nanoparticles [29–32]. Consequently, the crystallization presented in the studies on DNA-coated gold nanoparticles might in part be explained by the DNA linkers being able to move position on the particles' surface.

An alternative way to functionalize colloids with surface mobile linkers is to coat them with a lipid bilayer into which binding moieties can be embedded. Lipid bilayers or liposomes spontaneously self-assemble from lipids, which are molecules consisting of hydrophilic, water-loving, heads and hydrophobic, water-hating, tails [33, 34]. Due to the fluidic nature of typical lipid bilayers at room temperature, the embedded linkers can freely diffuse along the bilayer surface. Prior to the combination with colloids, lipid-based model systems have indeed been successfully used in a number of self-assembly studies. These model systems, as well as the insight they provided, are reviewed in some detail in the next section.

2. Lipid-based model systems with surface-mobile linkers

2.1. Self-assembling liposomes

Liposomes, or vesicles, which are themselves the result of a self-assembly process, can undergo a secondary self-assembly process mediated by their lipid composition, solution properties, or the presence of lipid bound proteins or other active molecules. Similar to colloidal particles, the bilayers of liposomes can be functionalized with molecules like streptavidin/biotin or oligonucleotides. Then, under the appropriate solvent conditions, vesicles displaying complementary molecules or single-type vesicles mixed with linking molecules, will self-assemble into multi-vesicle aggregates. The multi-vesicle aggregates can serve as idealized model systems for biological tissues or can function as multicomponent or multifunctional drug delivery systems [35–37]. In addition, their spontaneous formation could lead to new methods for developing artificial tissues and soft composite materials [38].

2.2. With biotin/streptavidin bonds

The high affinity and specificity of the biotin-streptavidin interaction makes this pair attractive for steering liposomal self-assembly. Biotin can be conjugated to lipids without affecting the vesicles' properties [39], and subsequent inter-vesicle bridging can be induced by the addition of streptavidin. This self-assembly process can easily be controlled by e.g. changing the density or affinity of the interactions, and is fully reversible by adding competing binding molecules with a higher affinity for streptavidin in solution. For small vesicles ($\varnothing \sim$ 50-100 nm) controlling the inter-vesicle binding by biotin/streptavidin interactions additionally minimizes the contribution of the whole lipid bilayer surface as compared to vesicles interacting through for example charged lipids, thereby reducing the consequential stresses and deformations and stabilizing the vesicle aggregates (see Ref. [40] and Fig. 3).

Investigation of the self-assembly of streptavidin-biotin presenting liposomes showed that the aggregation could be very well predicted by a kinetic model based on Smoluchowski kinetics, fractal concepts and Rayleigh and Mie scattering theory. Together with agreeing experimental results, Lynch *et al.* theoretically showed that the aggregation probability of liposomes carrying low amounts of biotin depends on the relative amounts of bound streptavidin and free biotin molecules on the liposome surface [42]. Whereas, for liposomes carrying high densities of biotin, the probability depends upon the relative liposome surface area covered with streptavidin molecules. By tuning the fraction of streptavidin to biotin-lipids and monitoring the clustering very carefully, Kisak *et al.* were able to create compact, limited-sized aggregates of vesicles [41]. A process that is due to a competition between biotin-binding to streptavidin free in solution and streptavidin already bound to another biotin.

Although they did not include it in their model, it was additionally observed that the rearranging intra-aggregate bonds are responsible for the compact shapes of

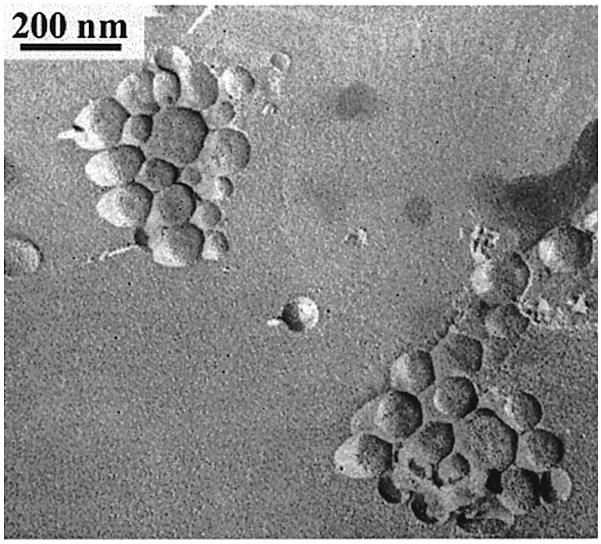

Figure 3. Self-assembled clusters of unilamellar vesicles mediated by biotin - streptavidin interactions. Freeze-fracture electron micrograph of aggregates obtained for a streptavidin to biotin ratio of 1.5, after aging for 3 weeks. As the aggregates age, intra-aggregate binding continues and the aggregates grow increasingly compact. Image adopted from [41].

the vesicle-aggregates, making this system different from other aggregation models. Interestingly, at relatively few biotin-ligands per vesicle, intra-aggregate binding can become faster than inter-aggregate bonding meaning that the mobility of the linkers enhances the self-limited aggregation.

2.3. With oligonucleotides

Inspired by the successes of employing DNA-mediated adhesion for assembly of binary hard-sphere colloids, strategies have been developed to also direct the self-assembly of liposomes with complementary nucleotides. Beales and Vanderlick incorporated short oligonucleotides into the bilayers of vesicles (hydrodynamic radius ~50 nm) by means of a cholesterol group on one end of the DNA strand [43]. To induce self-assembly, the other end of the DNA strands comprised a short sequence of nucleotides which was complementary to the end of DNA strands incorporated to a second set of vesicles. In close enough proximity, two vesicles that carry the complementary sequences are bound together by the oligonucleotides forming double stranded DNA duplexes. The self-assembly is reversible since the DNA duplexes dehybridize at temperatures above their melting transition temperature or low ionic strength of the suspension.

One observation they made was that a minimum number of DNA strands per vesicle was required at which the vesicles started aggregating. This suggests that the strength of only one DNA duplex is insufficient for maintaining vesicle adhesion. More strands must diffuse into the binding site and form a duplex in order to realize a stable coupling. Furthermore, at an intermediate number of strands per vesicle they observed

that the aggregate size after some time ceased to increase. Plausibly, too much of the DNA eventually resides in the contact area between two adhered vesicles leaving too few free strands available for a third vesicle to adhere. They support their explanation by measuring the kinetics of aggregation and comparing the effective time for two vesicles to bind with the time for a strand to diffuse into the binding site. The former clearly exceeds the latter by several orders of magnitude meaning that there is ample time for the majority of the strands to collect in the binding site. At high DNA densities they observed unrestricted aggregation.

The same group obtained similar results for larger vesicles, so called giant unilamellar vesicles (GUVs) having a diameter the size of several micrometers. Again at low DNA densities no aggregation was seen, limited aggregation was observed at intermediate densities and unceasing aggregation occurred at high densities. Yet, compared to the smaller vesicles, GUVs carry many more DNA strands per vesicle. Therefore, these different aggregation regimes cannot be explained by the same reasoning as for the regimes seen for smaller vesicles. Other aspects like the deformability of the membrane start to play a role. As soon as two GUVs adhere, a large osculating area forms. The growth of the adhesion plaque is driven by the fluid nature of the membrane which allows for the DNA to diffuse into, and enhance the binding site. With a sufficient number of DNA linkers this growth is only limited by the tension stresses it creates on both GUVs. Unlimited aggregation can therefore take place when the maximum plaque size is reached and there is enough DNA on the membrane to saturate the contact zone, while leaving sufficient DNA on the unbound area available to form a stable coupling with another GUV. Also multi-vesicle assemblies of higher complexity have been realized this way [44].

2.4. "Janus" vesicles or domain carrying vesicles

Recently, there has been an increasing interest in the development of patchy particles and the structures into which they self-assemble. A major consequence of the anisotropy of these particles is that their interactions do not only depend on the inter-particle separation but also on the particles' relative orientation. This provides an additional complexity degree that allows for greater control and sophistication, which allow for new structures to form.

Janus particles are a subclass of such anisotropic particles, which comprise two opposing faceswith different surface chemistry [45, 46]. Self-assembled architectures composed of amphiphilic molecules like lipids turn out to be very useful for the realization of particles that present two distinct phases. In particular, with vesicles comprising lipid mixtures that demix into coexisting liquid-like phases, the initial membrane domains eventually fuse together until the vesicles exhibit two opposing surfaces each having different chemical compositions. The two chemically different phases themselves can have properties that favor the binding of two particles together, for example if the two surfaces have opposing charge. Moreover, one can load the Janus

vesicles with specific molecules which preferentially reside in one of the two phases [47]. Including highly unsaturated lipids such as cardiolipin(CL) in the phase separating lipid compositions enhances the partitioning of chol-DNAs into the liquid ordered (L_o) phase. A possible explanation of this effect is that the structure of CL creates large lateral packing stresses in the hydrophobic regions of the membrane. It is likely that these stresses increase the free energy cost of inserting a cholesterol modified DNA strand into the liquid disordered (L_α) phase and hence drive the partitioning of such molecules into the L_o domains [48,49]. Either way, the splitting of the surface chemical properties into two phases provides a means to realize asymmetric surface distributions of adhesive functionalities.

Higher order valencies may be realizable if the lipid mixture does not separate into two opposing, equally sized surfaces, but separate into multiple spots or rafts. By preparing vesicles or polymersomes using a mixture of neutral and anionic amphiphiles, Christian and colleagues showed to be able to make liposomes that have stable spots or rafts upon adding divalent cations like calcium or copper [50]. The domain sizes were shown to be controllable by changing the pH, divalent cation concentration, and fraction of anionic amphiphiles.

2.5. Self-Assembling Emulsion Droplets

When more mechanical stability of the self-assembling structures is desired, it is possible to use emulsion droplets instead of liposomes. Emulsion droplets form when two immiscible fluids are mixed in the presence of surface active agents, so that one of the fluid phases gets dispersed into the other. Emulsion droplets exhibit all the classical aspects of colloids: Brownian motion, reversible phase transitions as a result of droplet interactions and irreversible transitions that generally involve their destruction [51]. In addition, just like with liposomes they can be functionalized with ligand-receptor like pairs such as biotin-streptavidin or DNA oligonucleotides [52,53].

Detailed studies of the self-assembly of DNA-functionalized emulsion droplets have given hints to what the most important control parameters are, and how one can predict the self-assembling structure. One crucial aspect of these systems, just as with vesicle systems, is that the linking molecules can move freely over the surface to which they are attached. As a consequence, during binding of two complementary droplets more linkers are likely to move into the connecting area.

When complementary droplets carry respectively red and green fluorescently labeled linker molecules, the accumulation of binding molecules is clearly seen in confocal micrographs by the yellow color in the area where bound droplets overlap, see Ref. [54] and Fig. 4. In these experiments, the degree of accumulation could be tuned by adjusting the number of binders per droplet, which was quantified by measuring the size of the patches as a function of linker density. The results fitted nicely to a mean field thermodynamic model that is based on the assumption that the recruitment of binders continues until the translational entropic cost of the immobilized binders in the patch

plus the energy paid to deform the emulsion droplet matches with the energy gained by binding.

Although the number of binders inside a patch with a diameter of for example 1.6μm was estimated to be large ($2{\cdot}10^3$ connections), the patches remained able to freely diffuse. The motion of the patches was quantified by coupling multiple nanoparticles to emulsion droplets with DNA linkers and following their respective motion. Despite the hydrodynamic drag which is expected to be much larger for an adhesive patch than for a single lipid, the nanoparticles (i.e. the patches) exhibited an average diffusion constant of $D \sim 0.012\ \mu\mathrm{m}^2 s^{-1}$.

According to the thermodynamic model, a maximum patch size exists due to the dramatic sensitivity of the deformation energy cost on the patch angle θ: $E_{\mathrm{deform}} \sim \theta^4$, where $\theta = r_{\mathrm{p}}/R$, $\mathrm{r_p}$ is the radius of the patch, and R is the radius of the droplet. Controlling the DNA density therefore controls the number of patches to a droplet can maintain. Both theoretically and experimentally it was shown that with DNA surface densities of 7.5, 15 and 30%, respectively monovalent, divalent and multivalent structures are formed (see Fig. 4a). In addition, self-arresting structures could be formed using a droplet/nanoparticle hybrid system. The nanoparticles are large enough to make it energetically favorable to form a ring around the contact points of two emulsion droplets without actually reaching the contact points. Because this maximizes the number of nanoparticles in the contact zone, the geometry of the final compact structure can be controlled (by tuning the number of droplets and the nanoparticle surface density) to favor the formation of linear chains, triangular lattices or flowers (see Fig. 4b-d).

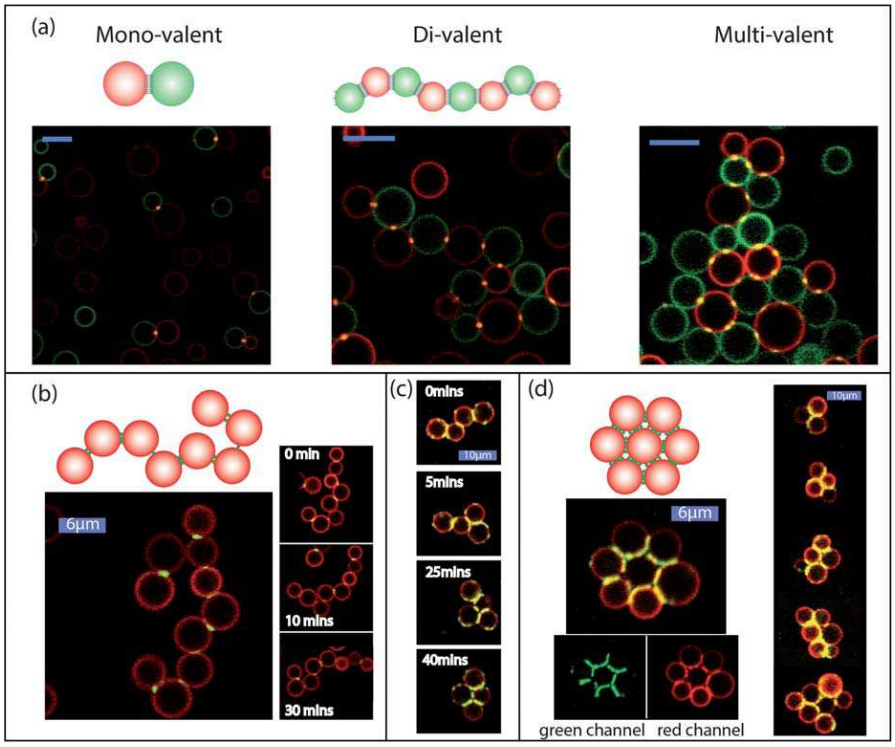

Figure 4. Controlling the valency of clusters of emulsion droplets. (a) By varying the number of linkers per emulsion droplet one can create (left) monovalent doublets, (center) di-valent droplet chains and (right) multi-valent aggregates. The scale bars are 10 μm. The same mono- (b), di- (c) and multi-valent (d) clusters can be realized by using DNA-coated nanoparticles as linkers and changing the droplet to nanoparticle ratio. Figure adopted from Ref. [54]

3. Solid colloids with surface mobile

As demonstrated in the previous sections, introducing mobility to the molecules that mediate the self-assembly of larger entities uncovers new interesting strategies to control the organization of the final equilibrium assembly. It was shown with systems of vesicles and emulsion droplets that by carefully tuning the binding interactions, it becomes possible to induce for example self-limited clustering and even to control the valency. The latter is attractive because it potentially allows for the development of open structures that do not form in systems with isotropic interactions. Examples of similar structures in nature are carbon atoms that form diamonds as a consequence of the well-defined electronic valency. Getting colloids to self-assemble into a diamond lattice could potentially lead to the production of photonic band gap materials [55]. A photonic band gap is a location within a material where electromagnetic wave propagation at a specific band of wavelengths is forbidden in all directions. Controlling the properties of such photonic band gaps allows one to control the flow of light inside the structure and hence have a wide range of potential applications in optics.

However, so far it has not been straightforward to control the coordination number in solid colloidal systems. Wang *et al.*, made considerable progress by developing a method to deposit patches on colloids at precise locations onto which DNA could be grafted [56]. By limiting the position of DNA strands to specific locations on the colloids they were able to generate small aggregates with controllable coordination number (valency). Although very promising, in order to produce bulk colloidal phases of practical interest the yield of their method needs to be scaled up substantially, which as of today remains a challenge.

3.1. Controlling valency with mobile linkers

Recent theoretical and computational studies indicate a potential alternative and less-involved strategy to control the valency. Angioletti-Uberti *et al.*, reported calculations on colloidal particles functionalized with surface-mobile DNA strands [57]. Their results predict that the many-body effects that naturally arise in the DNA-coated colloids with surface mobile linkers can be used to enforce the valency. In addition, they demonstrate that the valency can be tuned by changing the density of inert strands or the interaction between individual strands.

Their predictions are based on calculations on a binary model system of colloids, A and B, functionalized with surface-mobile double DNA strands. The sequence of the DNA on the colloids of type A is complementary to the DNA sequence on the particles of type B. At the same time, same-type particles repel each other due to the steric hindrance caused by the non-binding DNA strands. When two colloids (A and B) bind, their attraction increases as the number of bonds increases due to their mobility. If a second colloid B_2 binds, any of the mobile DNA strands on A can now bind with B or B_2. As a consequence of the symmetry in the system, both particle connections will eventually contain the same number of DNA bonds. Assuming that the number of DNA

strands per particle remains constant, the more particles bind to A the fewer strands remain per particle connection and hence the effective attraction between two particles decreases.

This many-body effect by itself is however not enough to impose valency: even though the addition of an additional particle makes the inter-particle interactions weaker, it does add one more interacting pair. This can be quantified by adapting a previously developed expression for the binding of DNA-coated particles to account for the mobility of the DNA strands [14, 58]. The authors could show that indeed the binding free energy per pair continuously increases upon increasing number of neighbors, confirming the weakening of the attraction between particles upon cluster growth. However, the final coordination number of the cluster is controlled by the free energy of the cluster, which was shown to be a continuously decreasing function upon increasing number of neighbors. Only through the addition of non-specific double stranded DNA, they identified a region in parameter space where the free energy of the clusters exhibits a minimum at coordination numbers below the physically attainable maximum. After running Monte Carlo simulations the authors showed that this free energy minimum was enough to give the colloids valency. Furthermore, this valency could be tuned by changing the binding energy of each individual strand, e.g. by adjusting the temperature or salt concentration, see Fig. 5. The introduced valency led to the self-assembly of the colloids into more open structures with limited long-range order, which are potentially good enough to inhabit photonic band gaps [59].

3.2. Experimental model systems for realizing valency control

The theoretically obtained guidelines for realizing clusters with controlled valency encourages the search for model systems that provide experimental verification. A potential candidate could be the droplet system developed by Feng *et al* . [54]. In the low-valency structures that they reported, the required repulsion is likely to originate from the deformation of the droplet interfaces. However, whether the deformations alone are responsible for the valency effect has still to be confirmed.

In addition, Lee *et al.* who studied the self-assembly of gold *nano*particles functionalized with a monolayer containing a mixture of hydrophobic and hydrophilic ligands, were able to guide the self-assembly from micellar clusters, to extended chain-like assemblies in aqueous solution [32]. They could explain their findings by formulating an assembly mechanism in which the hydrophobic ligands reorganize on the surface to form patches between neighboring particles.

Silicia microparticles coated with DNA-functionalized lipid bilayers such as the ones developed by van der Meulen *et al.*, provide another promising experimental system that may allow for the control of valency [60]. Here, double stranded DNA with short single stranded overhangs are first coupled to small unilamellar vesicles (SUVs) by two cholesterol anchors. Next, the vesicles are mixed with a suspension of monodisperse silicon oxide microparticles. The contact of the SUVs with the surface

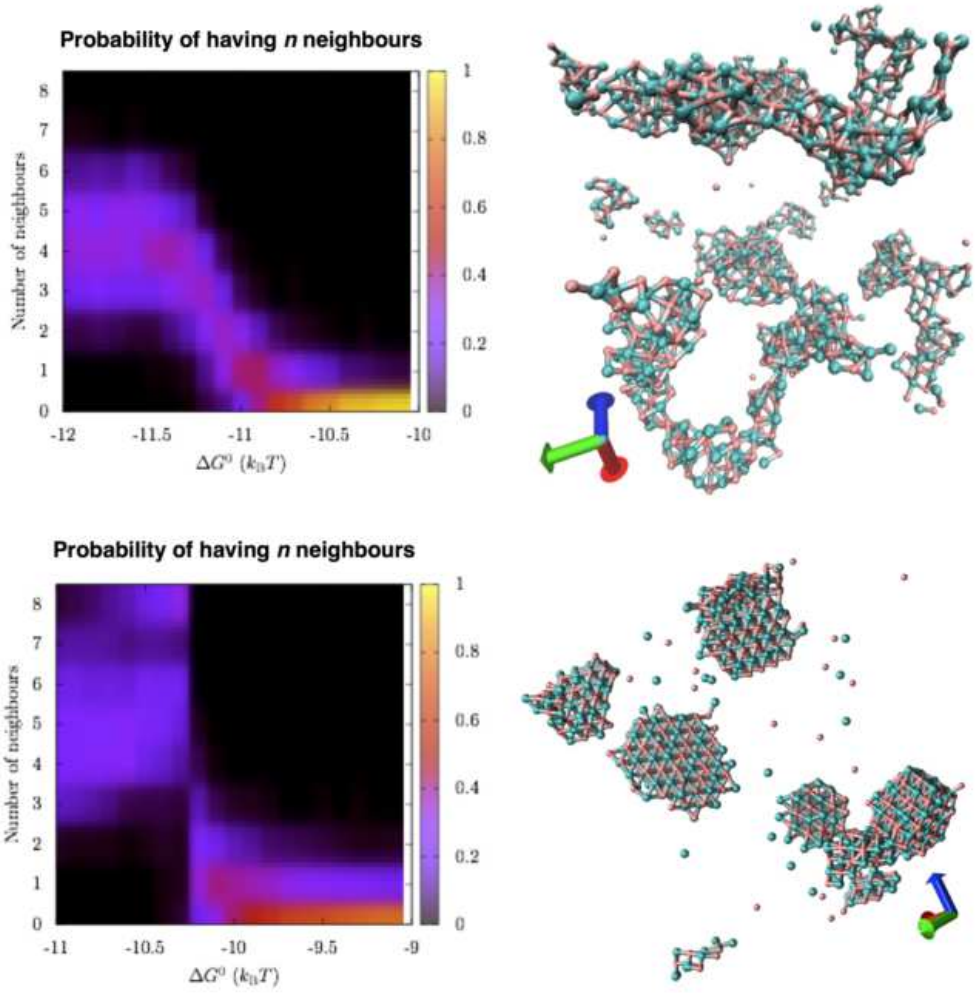

Figure 5. Valency distribution as a function of ΔG^0, for colloids with (top) and without (bottom) inert strands. ΔG^0 corresponds to the hybridization free-energy for two DNA strands in solution and depends only on the DNA sequence and is a function of temperature and ionic strength of the solution. The repulsive free-energy at the equilibrium distance between colloids is basically zero except when inert strands are present. The snapshots show typical configurations at low ΔG^0 in the two cases, where the system either assembles open structures of tetrahedral coordination (top, with inert strands) or a more compact NaCl structure (bottom, no inert strands). Figure adopted from Ref. [57].

of the microparticles in combination with the high surface tension of the SUVs triggers the rupture and spread of the vesicles around the particles until they are completely enveloped by a lipid bilayer. The DNA strands incorporated in the bilayer maintain their mobility on the surface and have shown to remain inside the bilayer when a strong enough hydrophobic anchor is used [61, 62]. See Fig. 6 for a schematic overview of the model system.

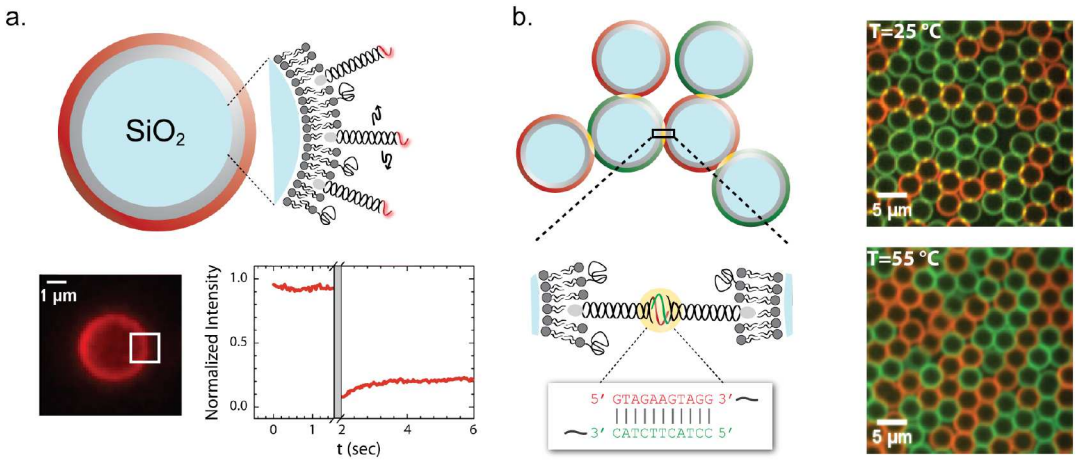

Figure 6. Schematic representation of solid microparticles coated with surface-mobile DNA binding groups. (a) Silica microparticles are coated with a lipid bilayer comprising predominantly DOPC and a small percentage of PEGylated DPPE. The DNA interpenetrates the bilayer through a hydrophobic anchor, e.g. cholesterol. The partial recovery of the fluorescence intensity after bleaching on one side of a DNA functionalized particle indicated the surface-mobility of the DNA [60]. (b) Controllable self-assembly was achieved by mixing two particle populations carrying DNA with complementary ends. Below the melting temperature of the DNA bonds, the complementary particles bound together indicated by the appearance of bright yellow spots at the contact zone. The particles dissociated upon increasing the temperature indicated by the disappearance of the bright yellow spots.

Van der Meulen *et al.* studied the self-assembly properties of their mobile-DNA particles by designing a similar binary mixture of particles decorated with either A or the complementary B strands. These colloids self-assembled into clusters at 25 °C, which dissociated at temperatures of 55 °C. Because both DNA strands were fluorescently labeled with different colors, it could be shown that at low temperatures the DNA molecules accumulated in the contact zone whereas they diffused to a homogeneous surface distribution at high temperatures (see Fig. 6b). The packing of the mobile-DNA-coated particles directly upon mixing appeared already more dense than for immobile-DNA-coated particles. Nevertheless, the packing could be enhanced by melting the clusters and subsequently slowly cooling (~9 °C/hr) the suspension, which led to slightly more hexagonally packed clusters, see Fig. 7. Furthermore, during melting of the clusters of mobile-DNA-coated particles, the transition from a bound to an unbound state is far less sudden as compared with the transition for immobile-DNA-coated particles. From these observations it is speculated that the particles have more freedom to move with respect to each other and rearrange due to a collective mobility of the bonds in the contact zone. The mobility of the linkers and the occurrence of a hexagonal-like crystal phase indicate that the system reproduces the predictions for particles with

no additional repulsion reported by Angioletti-Uberti et al. The effect of repulsion is still to be tested, but could easily be realized by adding inert DNA strands of two or three times the length of the complementary DNA strands. As the simulations suggest, tuning the amount of repulsion should than produce particles that obtain valency upon binding. These particles should subsequently self-assemble into 2D structures with a lower coordination number than the 2D hexagonal close-packed structure that has already been experimentally observed.

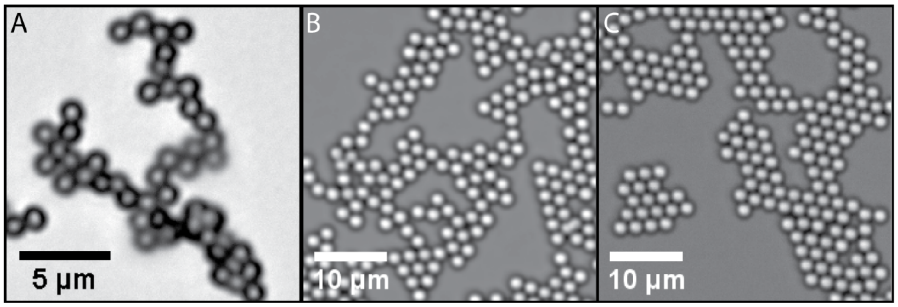

Figure 7. Self-organization of complementary microparticle mixtures functionalized with either immobile or mobile DNA linkers. 1:1 mixtures of S- and S'-functionalized particles were cooled quickly from a fully dissociated state at 52°C to a fully associated state at 25°C in 15 min. The bright-field snapshots show the resulting structures for (A) 1.0 μm diameter particles with immobile DNA linkers and (B) 2.0 μm diameter particles with mobile DNA linkers. (C) The same sample as in panel B after redissociating and slowly cooling from 52 to 25°C in 3 h. Figure adopted from Ref. [60].

4. Application to the study of cell-cell interactions

As explained above, solid colloids with surface-mobile bonds provide a promising route to creating self-assembled structures with controlled valency. At the same time, these model systems hold the potential of providing a new model system for the study of multi-bond interactions with biological relevance. In fact, one of the earliest appreciations of mobile interactions originates from the study on interactions between cells: immediately when Singer *et al.* pointed out the fluid nature of lipid membranes and their embedded proteins [33], it was realized that the mobility of surface receptors should influence the way cells interact with their surroundings [63]. See Fig. 8 for a 3D representation of interacting cells. Since then numerous studies have revealed great variability in the modes of lateral mobility of proteins inside the membrane, elaborately reviewed by [16]. The different modes of motion have been proposed to play crucial roles in multiple processes, e.g. cell adhesion [17], signal transduction, cell activation and cell differentiation [64, 65], yet dissecting the precise reason for the existence of all the different motility modes in cells remains a challenging task. Any of the processes outlined above are a result of an enormously complex network of many components accurately orchestrated to perform the designated task.

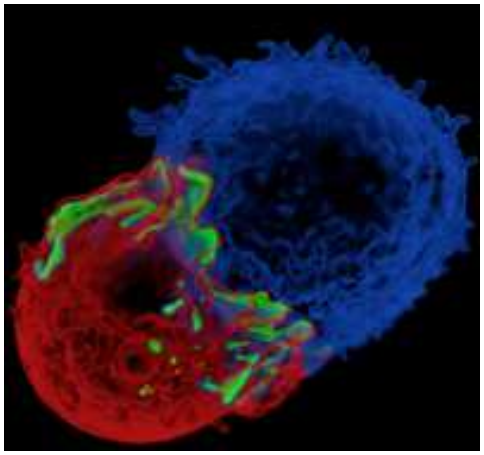

Figure 8. 3D rendering of an HEK-293 cell (biochemically engineered to express T cell genes) (red) interacting with an antigen-presenting cell (blue). Signaling between the two cells is initiated by the recruitment of kinase ZAP70 (green) to the area where the two cells interact. Figure adopted from Ref. [66].

Despite the great complexity, there are several strategies available to narrow down the roles of each individual player in a particular action of the cell. One could remove or mutate a specific protein of interest and monitor the response. If a change is observed, the manipulated protein is most likely involved in that particular process. By repeating the same process for related proteins or for different mutations the mechanism of interest may become clear. An alternative method is to transfer all the components responsible for a biological phenomena occurring in a specific cell into a cell that normally does

not express the proteins of interest, see for example Refs. [66, 67]. In this way, one can specifically study a particular process in the absence of interfering processes that occur in the original cell, whilst still taking place within the realistic conditions of a cell. This, at the same time poses a major complication for both described strategies. Proteins often have multiple functions and introducing unfamiliar proteins or manipulated ones can have detrimental consequences for the behavior of the cell. Furthermore, living cells are dynamic systems where energy is consumed to up or down regulate protein expression levels and hence molecular concentrations are never constant, thereby complicating quantitative modeling. Therefore, over the years an increasing number of simple model systems have been designed that do not suffer from these variabilities but still provide valuable information about the mechanism of a specific cellular process.

4.1. Model systems based on supported lipid bilayers

A proven-to-be essential workhorse for such model systems is the supported lipid bilayer (SLB). An SLB is a lipid bilayer which is formed on a solid substrate, such as glass, silicon oxide or mica. Because a thin layer of water exists between the substrate and the bilayer, the bilayer itself remains fluidic and allows for the embedding of other molecules like membrane proteins or other kinds of lipids. SLBs can be prepared either by the Langmuir Blodgett technique [68] or by deposition and spreading of lipid vesicles on the surface [69, 70].

Once a SLB is formed, one can functionalize it with specific proteins or receptors. By subsequently adding cells one can not only monitor the cell's response to the SLB presenting molecules, but the responses can also relatively easily be quantified, given the quasi two dimensional geometry of the SLB. For example Zhu *et al.* examined the interactions between the cell-receptors CD2 and CD58, both mobile in the bilayer, by monitoring the adhesion of CD2-expressing T-cells with a CD58 containing SLB [71]. It appeared that adhesion is associated with the accumulation of both receptors in the contact area (schematically represented in Fig. 9). Furthermore, FRAP analysis of the contact area revealed that the bleached molecules were quickly replaced by molecules from outside the bleaching spot, indicating that the CD2-CD58 dissociation rather leads to partner-exchange than rebinding of the same pairs. Further examples for the interactions of supported lipid bilayers with cells can be found in the papers of Groves and Dustin [72, 73].

To reduce the complexity further one can replace the cells by vesicles. This way one has precise control over all the components that could take part in adhesion, e.g. membrane composition and the density of cell-adhering-molecules (CAMs), which allows to study only those interactions that one is interested in [74–76]. Because all the ingredients of the system are known, quantifying the free energy of adhesion becomes easier. A major result of studying these particular biomimetic model systems is the observation that the formation of adhesion domains is an inevitable result of a delicate interplay between short range attraction forces between pairs of cell adhesion molecules

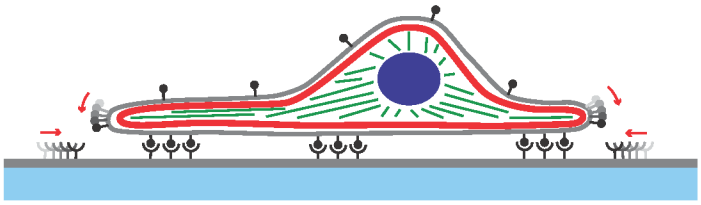

Figure 9. Schematic representation of a cell carrying cell-adhering-molecules (CAMs) that bind the cell to a receptor-decorated supported lipid bilayer (SLB). Because the linking molecules in both the cell-membrane and the SLB are mobile, binding groups can cluster to strengthen the adhesion [71].

(CAMs), long range repulsion forces mediated by the glycocalyx, and the elastic stresses of the lipid protein bilayer [17].

In addition, these mimetic systems have revealed fundamental differences between systems where both the binding partners are mobile versus a situation where one of the two is immobile [74]. A fine balance between mixing entropy and binding enthalpy allows for much larger areas of tight adhesion and increases the spreading pressure of each domain. Furthermore, both the number of bonds and their organization are significantly different when the binding partners are mobile. Many more bonds form and the ability to adjust their location on the membrane allow CAMs to optimize their inter-bond distance, which lowers the free energy of the bond state as a result of the lower entropic penalty the linkers have to pay for the coupling. A property which, in contrast to immobile linkers, allows for the stable formation of domains at physiological linker densities.

Another important difference between vesicles that are bound to a SLB with immobile or mobile bonds becomes clear when a force is applied to the vesicle to break the coupling [77]. As opposed to immobile bonds, mobile linkers close to the contact line transverse laterally without unbinding the moment you switch on the force. Sparse domains reorganize into densely packed structures accompanied by the increased probability of forming new bonds due to cooperative effects. As a result, the adhesion strength rises and together with the force-induced linker condensation, repeatedly applying these external forces causes the contact zone to enlarge monotonously. The rapid strengthening of cell adhesion upon an applied external force mediated by the mobile linkers can play an important role in situations of changing elastic stresses that require a cell to quickly adapt. An example of such a situation is the attachment of white blood cells to endothelial cells during which the linkages have to withstand high shear stresses governed by the blood flow.

4.2. Model systems based on vesicles and emulsion droplets

Although the systems in which cells or vesicles adhere to supported lipid bilayers appear very suitable for studying the effects of mobile binding groups on the adhesion strength,

they have limitations for adhesion-induced membrane deformations. As the solid support causes the SLB to be nearly fully restricted from any form of deformation, only the adhering cells or vesicles undergo deformations. This is interesting for mimicking events where cells adhere to rigid surfaces like the extracellular matrix, but for cell-cell interactions it does not suffice. Biomimetic model systems that are more resembling cell-cell interactions are those made of two liposomes or giant unilamellar vesicles (GUVs) that have receptor/ligand type of molecules incorporated in their membranes. Studying the binding of two GUVs that carry complementary molecules allows one to examine, in a very controlled manner, the binding process and all its potentially important aspects in great detail. For example, with two micropipettes one can hold two vesicles and bring them together, while keeping the surface pressure of both vesicles constant. This way Noppl-Simson *et al.* have shown that for biotin-coated vesicles cross-linked by fluorescently labeled avidin at low surface densities of biotin and avidin on the membrane ($<$5 mol %), the accumulation of linkers in the contact-zone is driven by the spreading pressure resulting from the clustering avidin molecules [78]. By measuring the contact angle between the two adhered vesicles one can estimate the density of linkages in the contact zone. In contrast, at high surface densities, the spreading-pressure argument breaks down, because the chemical driving force for adhesion far exceeds the mechanical counterforce against spreading [79].

Cell-cell contacts in real tissues are continuously subject to mechanical forces due to homeostatic pressure and active cytoskeleton dynamics. To study the role of pressure Pontani *et al.* used a dense packing of functionalized emulsion droplets in which surface interactions were tuned to mimic those of real cells [53]. The authors used an emulsion system because compression of the individual droplets with an interfacial tension of ~10mN/m leads to pressures in a similar range to those found in tissues. Furthermore, the emulsion droplets can easily be modified to reproduce the attractive and repulsive interactions that govern cell adhesion. Their system of emulsion droplets coated with biotin-streptavidin complexes, negatively charged SDS and PEG-lipids resulted in a mixture of droplets that show homogeneous coverages of streptavidin. No interaction between droplets takes place. Only when applying a pressure by centrifuging, the droplets are pressed sufficiently close together for the biotin-streptavidin bonds to form. Further examples of this approach can be found in Fataccioli *et al.* [80], who used biomimetic emulsion droplets to study specific wetting processes, and in Bourounina *et al.* [81], who studied the formation of specific receptor-ligand bonds between liquid interfaces.

A general limitation of both the vesicle and emulsion systems is that it is hard to contol the size distributions. The developments of microfluidic devices nowadays offer great potential [82], yet are still limited in their yield. Ill-defined knowledge of the sizes of the interacting vesicles complicates quantitative analysis of the processes taking place. Ideally, the density of binding molecules is accurately controlled in order to calculate the interaction forces and subsequently enable the systematic comparison between different conditions, e.g. different linker densities. Furthermore, since vesicles and emulsion

droplets are out-of-equilibrium structures, they are unstable and will eventually coalesce with each other. This in turn also limits quantitative analysis, as the structures do not remain constant in size, which constraints the experimenter to a limited time window in which measurements can be done. Using the system of solid colloids with surface mobile linkers introduced above can be a potential candidate to fill in this niche. Silica particles can be synthesized with very narrow size-distributions [83] and the lipid coating has been verified to remain stable for several days [84–86].

5. Future use of colloids with surface-mobile linkers

In the review we presented an overview of model systems with surface mobile linkers. We focused on the influence of linker mobility on the self-assembly process of colloids. In these systems, the use of mobile linkers prevents so called "hit and stick" situations and adds another degree of freedom or control parameter. A very promising method for preparing model systems with mobile linkers is to embed these linkers into lipid bilayers. The use of lipids allows a wide range of system variations. They can be used in form of vesicles, solid supported lipid bilayers, or as coatings for emulsion droplets. And besides acting as model systems for studying the self-assembly of colloids into larger structures, they are also an ideal tool to mimic cell-cell interactions and cell adhesions.

In the future one can imagine that the use of lipid-coated colloids with surface mobile linkers offers a wide range of research applications, as they provide a system which can be easily modified. Not only the linker molecules and their anchors can be varied but also the lipid matrix itself, which allows for the preparation of patchy particles or particles with different linker mobility. Furthermore, the solid support itself can have an influence of the mobility of the linkers. The use of a solid colloid allows an easy handling of the particles. They can be handled for example with optical tweezers or attached to atomic force microscopy cantilevers which then allow to probe the influence of the linker mobility on binding forces. With these possibilities we think it will be possible to create increasingly powerful model systems that enable new strategies for the self-assembly of ordered structures and mimic cell-cell interactions.

Besides enabling valency control on interacting colloids, the mobile-DNA-coated particles have other potential applications. For example Conway *et al.* reported the successful functionalization of lipid bilayer coated microparticles with DNA nanocages [87]. Also these DNA structures showed to be fully mobile on the particles surface. By introducing competing DNA strands they could selectively remove parts of the DNA cages, reorient them thereby changing their accessibility, and dimerize the cages with neighboring cages. These developments provide a leap towards the design of methods for programmable dynamic control of protein binding, cell signaling, and drug delivery, as well as methods to control nanoelectronic and optical properties of lipid bilayers, using easy to construct DNA scaffolds.

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