Anisotropy, multivalency and flexibility-induced effects in colloidal systems

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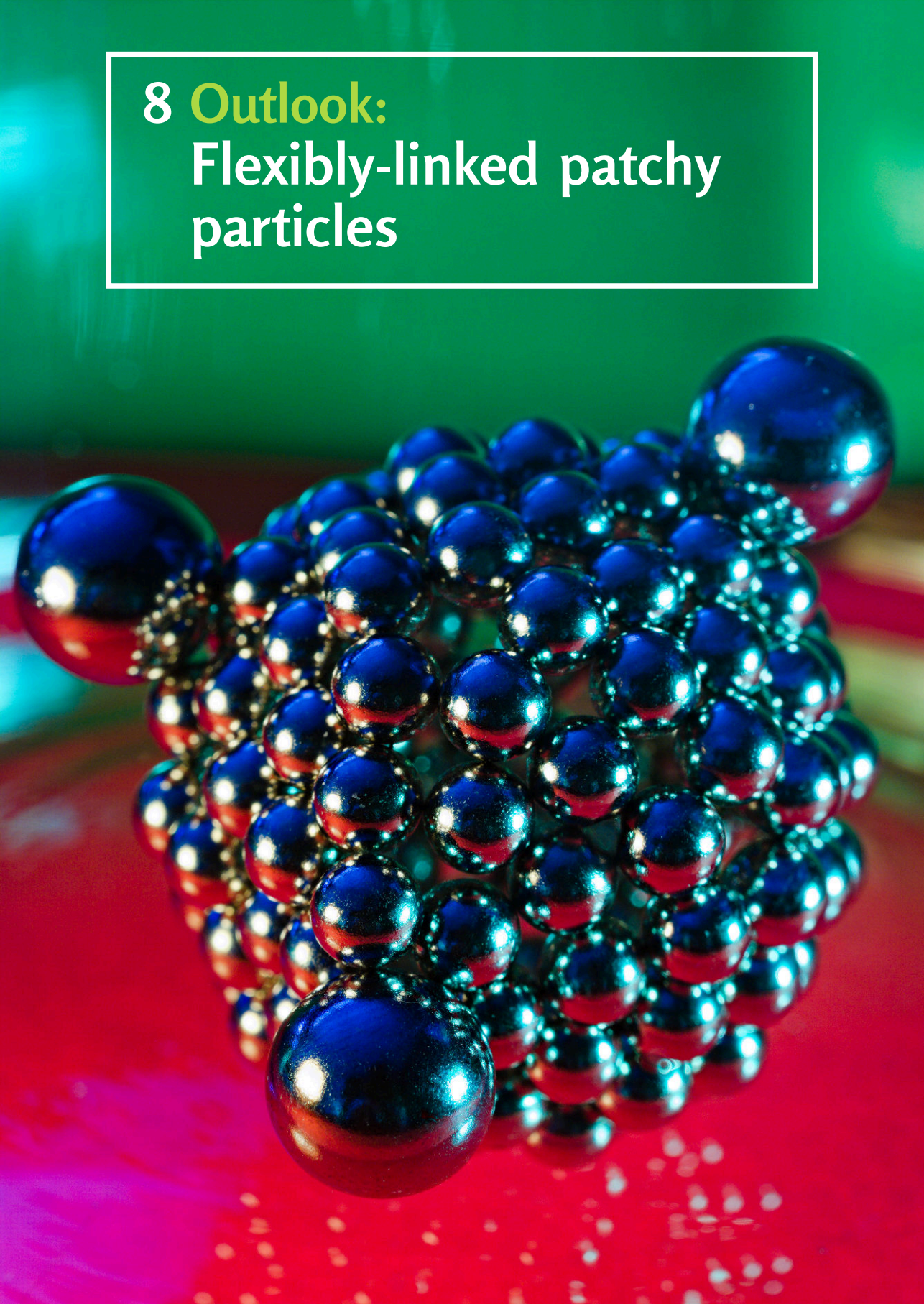
8 Outlook: Flexibly-linked patchy particles



In this thesis, we have studied flexibly-linked structures of spherical colloid-supported lipid bilayers (CSLBs) that are held together by DNA linkers, which can freely move over the surface of the colloids. Because the interaction between such particles is isotropic, the number of crystal structures that could be formed using high volume fractions of these particles is limited. A great challenge in materials science is the controlled bottom-up self-assembly of materials with highly sought after properties,[311] such as materials of specific crystal lattices,[28,312] materials that have photonic bandgaps[59,71] or more general colloidal meta-materials,[313] with properties not found in naturally occurring materials. The successful fabrication of such materials using colloidal self-assembly processes requires a great amount of control over the interactions between the colloidal building blocks. In addition, it is often desirable to constrain the relative motions of two functional elements,[85] for example to enable the fabrication of nano- to micron-sized robotic devices.[121–125]

Anisotropic or directional interactions may provide a solution to overcome these issues. One way to encode anisotropic interactions into colloidal building blocks is by making use of patchy particles.[28,53] Patchy particles are colloids that have distinct patches on their surface, which are modified to serve as specific interaction sites. In other words, the patches provide a different inter-particle interaction than the bulk portion of the particle, which leads to directional interactions. A wide variety of colloidal patchy particles is available.[28,51] For example, patchy particles can be made from particle-laden emulsion droplets.[80,314] Other techniques include patchy particle synthesis by colloidal fusion[81] or by photoprinting techniques.[82] The method we focus on here is based on the induced phase separation between crosslinked polymer particles and a polymerizable monomer swelling solution, resulting in particles with protrusions.[52,83] These patchy particles can be used for further hierarchical assembly.[315]

Broad applicability of patchy particles in self-assembly processes requires linking agents that can provide bonding with a high specificity. Such specificity can be obtained by functionalizing the patches with DNA linkers that have sticky ends which can provide a high binding specificity.[80,82,316] These sticky ends are pieces of single-stranded DNA that specifically hybridize to their complementary sequence and therefore can act as an "intelligent glue" between the micron-sized patchy particles.

In this short outlook, we propose a method to functionalize liquid protrusions of colloidal particles with DNA linkers that can diffuse over the surface of the liquid patches,

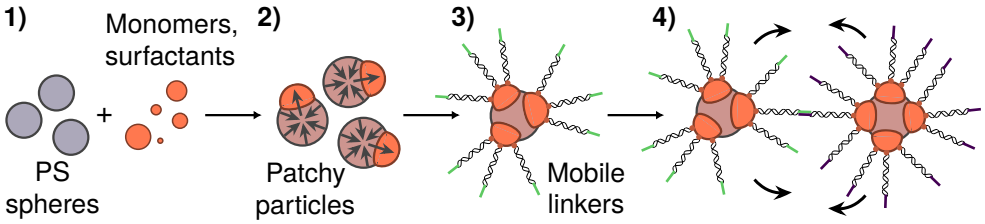


Figure 8.1: **Flexibly-linked patchy particles.** Schematic of DNA-decorated patchy particles, not to scale. **1)** Surfactant-stabilized polystyrene particles (grey) are swollen with a surfactant-stabilized styrene emulsion (orange). **2)** Elastic stress of the polymer network induced by swelling leads to patch formation. **3)** DNA linkers are inserted into the liquid patches via their hydrophobic anchors. Because the patches are fluid, the DNA linkers are expected to be mobile. **4)** Self-assembly via DNA-mediated interactions results in flexibly-linked patchy particles.

thereby paving the way towards patchy particles with both directional and mobile bonds. This combination of properties is highly desirable, because colloidal structures of reconfigurable shape are expected to be able to relax more quickly towards their thermodynamic equilibrium configuration than rigid structures, thereby mitigating equilibration issues.[77] In addition, they could provide ways to build switchable materials.[285] Here, we propose a method to realize such structures experimentally and we discuss our finding from preliminary experiments.

Proposed method for patchy particles with mobile linkers

We propose the following method, as schematically depicted in Figure 8.1. First, particles with liquid protrusions are obtained by swelling crosslinked polymer particles with an emulsion of monomers and surfactants (Figure 8.1 steps 1 and 2). DNA linkers with hydrophobic anchors are inserted into the protrusions, which can be used for further hierarchical self-assembly of the particles (Figure 8.1 steps 3 and 4). Specifically, in Figure 8.1 step 1, crosslinked polystyrene (PS) microparticles are swollen with a surfactant-stabilized styrene monomer emulsion.[52,83] In our experiments, we have used 1.5 %v/v divinylbenzene (DVB) crosslinked polystyrene spheres* (diameter (1.42 ± 0.04) µm) containing 10 % 3-(trimethoxysilyl) propyl methacrylate (TPM), as prepared by a dispersion polymerization procedure.[317] We have tested particles that were dispersed in a 1 %w/w aqueous solution of sodium dodecyl sulfate (SDS) or in a polyvinyl alcohol (PVA) solution of the same weight percentage. The styrene swelling emulsion was prepared by mixing 8.5 %v/v styrene in water and the mixture was subsequently emulsified for 2 min at 8000 rpm and then for 10 s at 10 000 rpm, using a IKA T18 Ultra Turrax homogenizer. As a surfactant, we have used both PVA, SDS and a combination of 99 mol % unsaturated DOPC ((Δ9-Cis)-

*Particle synthesis was performed by Vera Meester, see subsection "Cross-Linked Particle Synthesis" of the Experimental Section of Meester and Kraft[317] for details.

1,2-dioleoyl-sn-glycero-3-phosphocholine) phospholipids with 1 mol % of the lipopolymer DOPE-PEG(2000) (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]). Additionally, for some of the experiments where we used lipids as surfactants, we have used 0.2 mol % of the fluorescently-labeled phospholipid DOPE-Rhodamine (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)).

Then, as depicted in Figure 8.1 step 2, the swelling of the particles with monomer solution induces an elastic stress in the crosslinked polymer network of the particles. This elastic stress leads to the formation of a liquid patch of monomer solution, which protrudes from the particle.[318] The size of the protrusions can be tuned by varying the concentration of the surfactant or by changing the swelling ratio S of particle to swelling emulsion volume.[52,83] Here, we have typically used $S = 8$. After the protrusions have formed, in Figure 8.1 step 3, DNA linkers with hydrophobic anchors are inserted into the hydrophobic liquid protrusions. We have used double-stranded DNA with a double stearyl anchor, where a single-stranded overhang can function as a sticky end. We used two sets of DNA strands with complementary sticky ends that can act as linkers, of respectively strands DS-H-A and DS-H-B of Table A.1. We expect that since the protrusions are fluid, the linkers can diffuse on the protrusion surface, similarly to systems of emulsion droplets functionalized with DNA linkers.[56,84,194,216,265] Finally, in Figure 8.1 step 4, we schematically show how two such patchy particles decorated with DNA linkers with complementary sticky ends might self-assemble into a flexible structure.

Balancing colloidal stability and patch functionalization

We have tested the proposed procedure by swelling PS particles with a styrene emulsion stabilized by various SDS concentrations. As shown in Figure 8.2a, anisotropic particles with multiple protrusions were formed. We reproduced earlier studies, in which it was shown that by increasing the surfactant concentration, more numerous and smaller protrusions can be obtained,[83] as can be seen from Figure 8.2a. Additionally, we found that, as expected, increasing the surfactant concentration improves the colloidal stability of the particles. Specifically, as shown in Figure 8.2a, we found that the particles aggregated for SDS concentrations below the critical micelle concentration (CMC) of SDS but showed good colloidal stability above the CMC.

Having established the conditions required to form anisotropic particles with protrusions, we proceeded with the functionalization of the liquid protrusions. This was tested by adding DNA linkers with hydrophobic anchors to the particles. The DNA linkers are expected to spontaneously insert into the hydrophobic protrusions because of their hydrophobic double stearyl anchors, as has been demonstrated for comparable systems of emulsion droplets.[56,84,194,216,265]

As a first step, we functionalized SDS-stabilized styrene emulsions with DNA linkers, before swelling the polymer particles. As shown in Figure 8.2b, for SDS concentrations well below the CMC, we observed a fluorescent signal stemming from the fluorescently-labeled DNA linkers on the outer surface of the emulsion

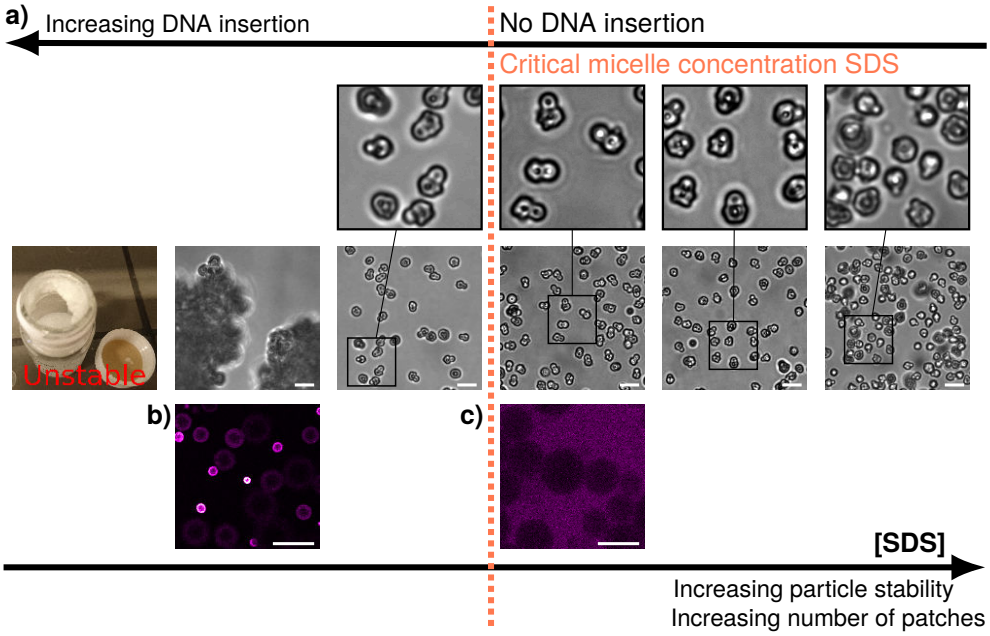


Figure 8.2: **DNA linker insertion into SDS-stabilized liquid protrusions. a)** Anisotropic particles with protrusions were obtained by swelling crosslinked polystyrene particles with an emulsion of styrene monomers, which was stabilized by SDS. Increasing the SDS concentration leads to smaller protrusions and a higher colloidal stability. On the other hand, DNA linkers can only be inserted into the styrene phase when the concentration of SDS is below the critical micelle concentration (CMC, 0.26 %w/w), but this leads to nonspecific aggregation of the particles. **b)** Fluorescence stemming from dyed DNA linker strands indicates that below the CMC of SDS, DNA can be inserted into an emulsion of styrene. **c)** Above the CMC of SDS, no DNA linkers are inserted into the styrene phase, as evidenced by the lack of fluorescent signal. Scale bars are 10 µm.

droplets, as expected. However, for higher concentrations of SDS, we found that the DNA linkers could no longer be inserted into the emulsion droplets, as evidenced by the lack of fluorescent signal on the outside of the droplets in Figure 8.2c. We therefore conclude that it is likely that SDS forms micelles with the hydrophobic anchors of the DNA linkers, as we had observed for CSLBs in Chapter 3. This would slow down or inhibit the insertion of the DNA linkers into the emulsion droplets. Alternatively, at these high surfactant concentrations, the emulsion droplet surface could already be fully covered by SDS molecules, which would also prevent the insertion of DNA linkers.

Unfortunately, the surfactant concentrations for which DNA linkers could be inserted into the emulsion droplets have no overlap with the surfactant concentrations for which the protrusion-decorated particles were found to be stable. Therefore, to overcome this problem, we have tried to first functionalize emulsions with DNA linkers at lower surfactant concentrations. Using 0.1 %w/w SDS, we found that the DNA linkers could be successfully inserted into the emulsion droplets (Figure 8.2b). Then, we added these DNA-decorated emulsions to the polystyrene seed particles in order to swell the particles and thereby induce the formation of protrusions. This approach was not successful, most likely because the emulsion droplets were sterically stabilized by the DNA linkers and therefore were not able to swell the colloids. Namely, after adding the DNA-functionalized emulsion to the particles, we did not observe swelling of the seed particles or the formation of protrusions. However, in future experiments, this method could be tried again using either lower DNA linker concentrations or longer incubation times.

As an alternative to using SDS as a surfactant, other surface active compounds may be employed, such as phospholipids. Phospholipids have been used to form fluid monolayers on emulsion droplets, into which DNA linkers have been successfully inserted.[56,84,194,216,265] Indeed, by using phospholipids as surfactants during the preparation of the styrene swelling emulsion, we found that particles with protrusions could be formed. This is shown by the fluorescent signal stemming from dyed phospholipids in Figure 8.3a and b, where two examples of particles with a protrusion are shown. It can be seen that in both examples, one lobe of the particle has a brighter signal than the other, indicating the presence of a protrusion of styrene monomer stabilized by the lipid molecules, of which a small fraction was fluorescently-labeled. To prevent the formation of nonspecific aggregates in this SDS-free system, before swelling, the particles were additionally stabilized by a low amount (0.1 %w/w) of polyvinyl alcohol (PVA) and we added 1 mol % of DOPE-PEG(2000) to the emulsions for additional steric stability.

First mobile structures

Subsequently, these protrusion-decorated particles were functionalized with DNA linkers. As a requisite for successful self-assembly of these particles via DNA-mediated binding, a buffer of sufficient ionic strength is needed.[223] This allows for the proper hybridization of the complementary sticky ends that are responsible for binding, as

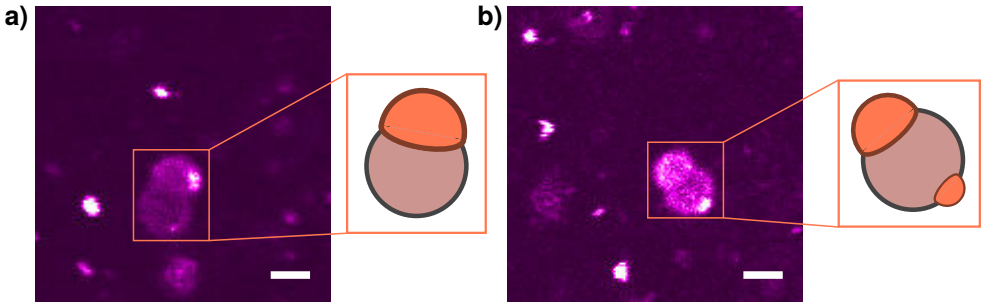


Figure 8.3: **Colloids with lipid-stabilized protrusions functionalized with DNA linkers.** Confocal images of particles with protrusions, the fluorescent signal stems from dyed lipids. Schematics indicate the possible arrangement of the protrusions (orange) on the swollen seed particle (grey/orange). Scale bars are 2 μm.

the negative charges of the DNA backbone need to be electrostatically screened by counter-ions. We observed that the protrusions on the particles that were formed while using a buffer were smaller than the protrusions we had observed by using only ultra-pure water. However, the protrusion size could be increased by using a higher swelling ratio.

After mixing two batches of these anisotropic particles with protrusions functionalized with complementary DNA linkers, we observed various mobile structures. First, as shown in Figure 8.4a, we could observe a large number of particles that were moving on emulsion droplets left in the mixture after the swelling of the particles. From these observations, we concluded that the emulsion droplets were still fully fluid and that adhered particles could move on the surface of these droplets. However, it is unclear whether the particles were attached via DNA-mediated bonds or through adsorption.

In addition to these Pickering-like emulsions, several clusters and chain-like aggregates could be observed, as shown in Figure 8.4b, that displayed some flexibility. For those structures, we observed overall shape changes and changes in the relative orientations of the individual particles. These results demonstrate that in principle, assemblies of anisotropic particles with mobile bonds can be formed by using particles with liquid protrusions as building blocks. However, for these structures as well, it is unclear how they are bonded, i.e. by the DNA linkers or via other nonspecific interactions, which has to be further tested in future experiments.

Conclusions and next steps

In conclusion, we have shown that it is possible to synthesize anisotropic particles with liquid protrusions and to functionalize these protrusions with DNA linkers with hydrophobic anchors. Future experiments are necessary, in order to characterize the self-assembly behavior of this type of particles, as well as the flexibility of the resulting structures.

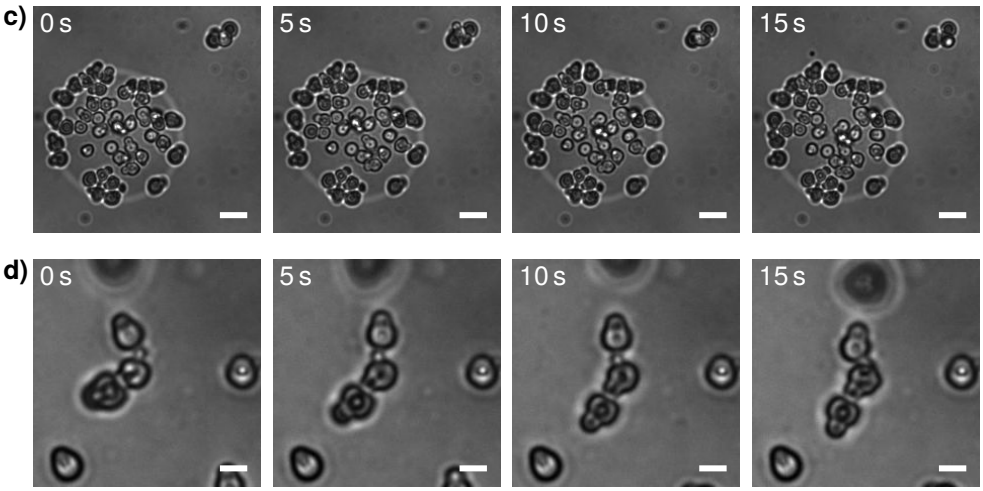


Figure 8.4: **Flexible aggregates of protrusion-decorated particles. a)** Time series of protrusion-decorated particles diffusing on an emulsion droplet, together with a flexible structure in the top right corner. **b)** Example of a flexibly-linked chain-like aggregate of anisotropic particles with protrusions. Scale bars are 5 μm.

Alternative methods to synthesize patchy particles of reconfigurable shape should be explored as well. We will discuss some examples, which we characterize by the combination of body and patch phases, where we consider liquid or solid phases. In that sense, the particles we have discussed so far consist of a solid body, which is formed by the polystyrene sphere, and of multiple liquid patches, which are formed by the styrene protrusions, as indicated in Figure 8.5b.

Other kinds of patchy particles that consist of a solid body with liquid patches may be used to obtain reconfigurable clusters. For example, it may be possible to encode patchy interactions into colloid-supported lipid bilayers (CSLBs), by inducing phase-separation of the lipid membrane surrounding the particles.[319] Here, the body is formed by the solid colloid that supports the fluid lipid bilayer. This is an integral part of the patchy particle because it fixes its overall shape. Then, the particles can feature multiple patches, formed by the different lipid domains. As shown in Figure 8.5a, phase separation results in the formation of specific lipid ordered (green) and lipid disordered domains, into which DNA linkers (purple) with suitable lipid anchors are expected to preferentially partition.[319] If the linkers are mobile in the phase-separated bilayer, flexible structures could be formed using these particles.

The partitioning of linkers into different lipid phases was also demonstrated for linker-functionalized Janus vesicles,[320,321] as shown in Figure 8.5c. These Janus vesicles can be thought of as patchy particles, where both the body and the patch are liquid: namely, they are both formed by different lipid domains. Various types of linkers can be employed, such as DNA linkers[320] or biotin-Avidin based linkers.[321] In

contrast to the phase-separated CSLBs in Figure 8.5a, the shape of the vesicles is not fixed and the Janus vesicles therefore deform upon binding, as shown in Figure 8.5c. This deformation could potentially affect their reconfigurability because of geometric constraints and an increase in inter-particle friction because the size of the contact area becomes larger upon deformation of the vesicles. While the successful formation and assembly of these type of Janus vesicles has been firmly established,[320,321] the degree of reconfigurability of the formed structures remains to be further investigated.

Instead of using liquid patches, solid patches may alternatively be used. For example, as shown in Figure 8.5d, solid polystyrene particles that can act as patches can be bound to liquid oil emulsion droplets,[216] which then form the liquid body of the patchy particle. In this specific system, the solid patches were found to be immobile on the emulsion droplet surface because of gelation of the emulsion droplet.[216] We expect that this problem can be overcome by using a different oil as the emulsion droplets, as was already demonstrated for isotropically-functionalized emulsion droplets that can form reconfigurable structures via DNA linker-mediated binding.[56,84,194,265] For emulsion droplets decorated by mobile solid particles, hierarchical assembly of the patchy particles via bonding of the solid patches on different patchy particles may lead to reconfigurable structures.

Lastly, for all systems discussed so far, either the body or the patch was required to be liquid in order for the structures to show flexibility. In contrast, reconfigurable assemblies can also be formed by using patchy particles that are completely solid. We discuss two examples: first, as shown in Figure 8.5e, patchy particles that are bonded by attractive critical Casimir forces between the patches can be used to obtain reconfigurable structures.[322] The reconfigurability stems from the fluidity of the solvent and the fluctuating nature of the critical Casimir interaction based bond. Second, by using attractive depletion interactions, reconfigurable clusters can be obtained from particles that have specific indentations (the "locks") into which spherical particles of the correct shape (the "keys") can fit via a lock and key interaction,[323] as shown in Figure 8.5f. In this system, the lock particles can be thought of as being patchy in the sense that they have a specific patch where the curvature differs from the body of the particle. Furthermore, this patch can be employed for directional bonding, as shown in Figure 8.5f. Both of these systems rely on specific properties of the solvent to ensure stable bonding and reconfigurability, which may limit their applicability in biological systems.

To conclude, we hope that the type of particles discussed here, because of their unique combination of directional interactions with flexible bonds, will lead to a higher degree of control over the self-assembly of reconfigurable colloidal structures in future studies. Ultimately, this may facilitate the formation of synthetic structures with currently unavailable designer material properties, resulting in smart and switchable colloidal structures.

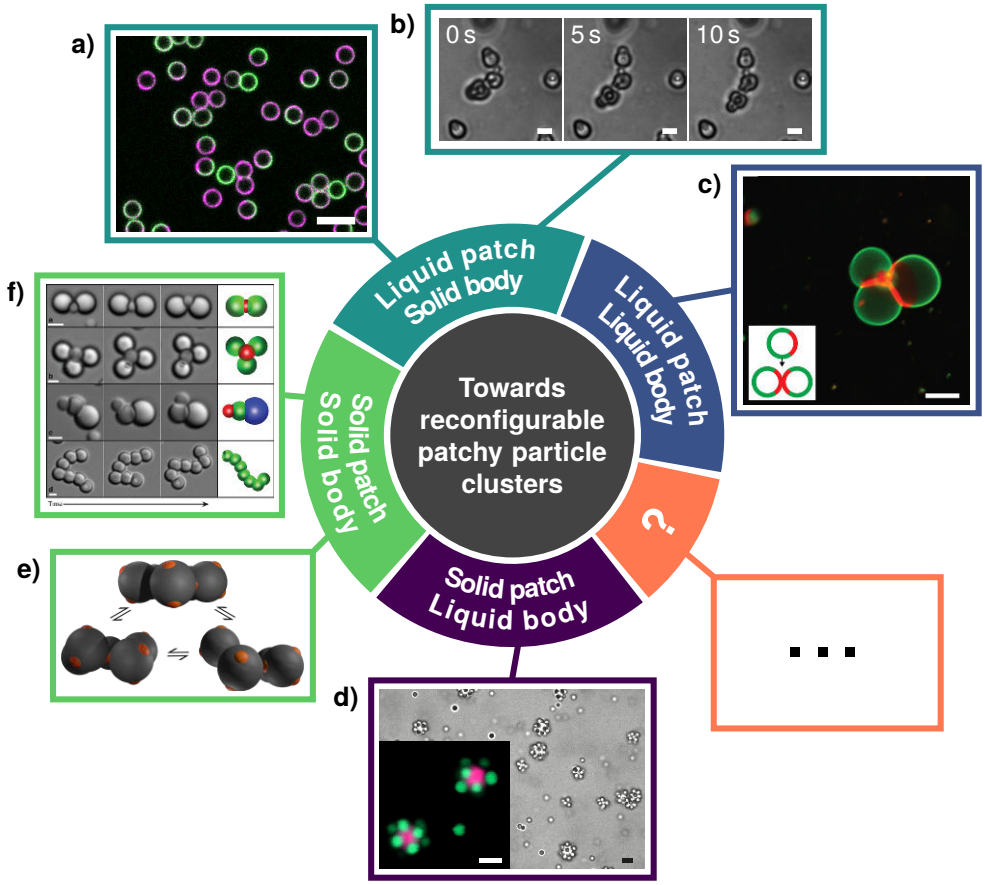


Figure 8.5: **Towards reconfigurable colloidal clusters of patchy particles: existing and envisioned methods. a)** Patchy particles from phase-separated CSLBs. DNA linkers (purple) could preferentially partition into one of the two phases: a liquid ordered phase (green) or a liquid disordered phase, thereby allowing for directional bonding. Scale bar is 5 µm. **b)** Particles presented here, as discussed in Figure 8.4. Scale bars are 5 µm. **c)** Aggregation of biotin-decorated Janus (phase-separated) liposomes upon incubation with avidin. Scale bar is 20 µm. **d)** Bright field and confocal (inset) microscopy images of colloidal clusters consisting of oil droplets (inset: magenta) surrounded by multiple attached polystyrene particles (inset: green) and freely dispersed polystyrene particles. Scale bars are 2 µm. **e)** 3D reconstruction of colloidal cyclopentane. The patchy particles remain bonded via critical Casimir interactions. **f)** Flexible structures of colloidal spheres and patchy particles with a spherical cavity, that bind spontaneously and reversibly via depletion interactions. Scale bars are 2 µm. *Attribution: a) Adapted with permission from Rinaldin.*[319] *c) Reprinted (adapted) with permission from Wang et al.*[321] *Copyright 2018 American Chemical Society. d) Republished with permission of IOP Publishing, from Van Der Wel et al.*[216] *e) Adapted with permission from Swinkels et al.*[322] *f) Reprinted (adapted) by permission from Springer Nature: Sacanna et al.*[323] *Copyright Springer Nature 2010.*

Acknowledgements

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