Simple method for the synthesis of inverse patchy colloids

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Abstract

Inverse patchy colloids (IPC’s) have recently been introduced as a conceptually simple model to study the phase-behavior of heterogeneously charged units. This class of patchy particles is referred to as inverse to highlight that the patches repel each other in contrast to the attractive interactions of conventional patches. IPCs demonstrate a complex interplay between attractions and repulsions that depend on their patch size and charge, their relative orientations as well as on charge of the substrate below; the resulting wide array of different types of aggregates that can be formed motivates their fabrication and use as model system. We present a novel method that does not rely on clean-room facilities and that is easily scalable to modify the surface of colloidal particles to create two polar regions with the opposite charge with respect to that of the equatorial region. The patch size is characterized by electron microscopy and fluorescently labeled to facilitate using confocal microscopy to study their phase behavior. We show that the pH can be used to tune the charges of the IPCs thus offering a tool to steer the self assembly.

Keywords: patchy particles, inverse patchy particles, colloids, synthesis, surface modification, heterogeneous surface charges, self-assembly

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

1. Introduction

Self-assembly is the process by which small units collectively form larger structures while minimizing the free energy. This process takes place in and between (bio-)molecules, such as proteins, but also between much larger colloidal particles. In fact, self-organization relies on thermal fluctuations to sample various configurations that may be more or less favorable from an entropic and enthalpic perspective. The addition of constraints on a self-assembling system can reproducibly guide the self-organization of simple colloidal units into highly complex structures [1, 2]. Much work has been done to study the fundamental characteristics of self-assembly using colloidal model systems [3].

The size of the colloidal particles can be chosen up to the micrometer scale, allowing easy inspection in real-time and real-space using light microscopy techniques [4]. In recent years, people have realized that their size also allows to construct colloidal particles with anisotropic interactions, making them behave more similar to molecules [5]. Anisotropic interactions can originate from heterogeneous particle surfaces, where the different areas are commonly referred to as patches [6–8], or simply from an anisotropic shape. Most experiments involving patchy particles have involved electric or magnetic dipole interactions or directional hydrophobic [9–11] or depletion attractions [12]. However, charge interactions are abundant in nature and can also be orientation dependent as the surface charge often is heterogeneous.

Heterogeneously charged particles are called inverse patchy colloids (IPCs) [13] to highlight the contrast to particles with conventional, mutually attractive patches. The term inverse is used because in IPCs the patches are mutually repulsive. One of the simplest examples of inverse patchy particles are spheres that have two polar patches with a mutually repulsive interaction (e.g. positively charged) defined...
by the solid angle of the patch and an equatorial region with the opposite and to the patched attractive property (e.g. negatively charged), while between two equatorial regions there is a repulsive interaction. Such particles have been shown to give rise to interesting behavior in bulk simulations [14–16] as well as in simulations of IPCs under confinement [17, 18].

An emerging feature of many simulated IPC systems is the formation of planar aggregates irrespective of the extent of confinement [17]. IPCs close to a homogeneously charged substrate were shown to form assemblies with well-defined translational and orientational order depending on e.g. the charge ratio of the different entities involved, the patch size and the interaction range. Depending on the system parameters, the formation of disordered gel-like structures has been observed numerically. These structures are characterized either by a large fraction of local, ring-like arrangements or by crystalline domains which themselves represent the branches of a disordered network (referred to as microcrystalline gel). The properties of these assemblies were shown in some cases to be reversibly tunable with experimentally accessible parameters, such as the pH of the solution and the charge of the substrate [18]. Despite such fascinating prospects there have so far been few experiments with heterogeneously charged particles, which mainly is explained by a lack of suitable colloidal experimental systems.

Over the years, scientists have developed various methods to synthesize patchy colloids [7]. At its simplest, heterogeneously charged core-satellite particles were produced by letting positively charged hydrogel particles stick on negatively charged polystyrene particles [19]. Depending on the size ratio between the core and satellite particles, this results in anisotropic particles with random charge distributions. Patches with different surface chemistries on otherwise spherical particles can be made by for instance glancing angle deposition after assembly of particles in 2D layers [20–22]. Such colloids have been demonstrated to give rise to the self-assembly of new structures such as the Kagome lattice using attractive hydrophobic patches [9]. Alternatively, patches can be modified after temporarily shielding off part of the particle surface. Silica particles can be made to sink into mixed polymer beds to leave part of the particle surface free to surface modification. The affinity between the particles and the polymers can be chemical [23] or physical in nature [24], while the depth of penetration and thus the patch size can be controlled by reaction time and temperature.

In this work we describe a straightforward method to fabricate inverse patchy particles with defined areas of different charge. We use polymer membranes around monolayers of silica particles that stick out of the membrane on two sides as a template for the chemical modification of the particles’ polar regions, inspired by work using microparticles to template pores through polymer membranes [25]. In figure 1, we schematically depict the procedure. First, silica particles are assembled into a 2D crystal on a flat substrate by capillary forces during the evaporation of a solvent [26] (figure 1(a)); instead of performing the assembly at a solid substrate we use the top face of an agarose gel; the hydration of the gel provides a liquid type interface onto which the particles adsorb by partial immersion due to wetting and gravity. The next step is to mask the equatorial region of the particles with a polymer film in order to enable fabrication of particles with two polar patches (figure 1(b)). This is done by depositing a polymer solution with a controlled amount of polystyrene (PS) in diethylether (DEE) on top of the 2D crystal; upon evaporation of the DEE a condensed PS membrane is formed around the particle equatorial plane (figure 1(c)). The PS membrane with the trapped particles is peeled off the lyophilized interface (figure 1(d)) and re-dispersed in ethanol. The exposed polar regions of the particles are reacted with a silane which decorates the particle surface with amine groups (figure 1(e)) that can be used for further chemical coupling of e.g. a charged dye to monitor the presence of the charged patches (figure 1(f)). We demonstrate this approach to create Rhodamine B isothiocyanate labeled, cationic polar patches, for which the charge density relative to the negatively charged equatorial silica region can be controlled by the pH. The membrane flakes are spun down after the patches have been functionalized and the solvent is exchanged for toluene to dissolve the PS and liberate the IPCs (figure 1(f)).

In the following we describe in detail how we synthesized charged inverse patchy particles with fluorescently labeled patches of the opposite charge. We describe how we characterize the size and charge of the patches using scanning electron microscopy (SEM) and zeta potential measurements. Finally we use brightfield microscopy and confocal laser scanning microscopy (CLSM) to study the interactions between these novel colloids which we compare to simulations on IPCs in literature.

2. Experimental

2.1. Gel preparation

A 35 mm soda-lime glass petri-dish (STERIPLAN®. Carl Roth) was fluorinated to promote the formation of a nearly flat meniscus using a vapor of Trichloro(1H,1H,2H,2H-perfluorooyctyl)silane (97% SIGMA-ALDRICH, USA) in a desiccator closed at ambient pressure and kept at 80°C overnight. The petri-dish was rinsed with propanol and dried in a stream of dry nitrogen. The next step was to place at 37°C at saturated humidity for 15 min before 3 ml of a 2% agarose solution (Agarose universal, PEQLAB, Erlangen, Germany) prepared in a microwave oven and stored at 55°C with nitrogen. After 30 min the agarose had formed a gel and was brought to room temperature. After another 30 min the gel was ready to be covered with a monolayer of particles (figure 1(a)).

2.2. Membrane preparation

Silica particles (81108 FLUKA) with a nominal diameter of 2 µm were used for the fabrication of IPCs. Approximately 100 µl of the stock solution was transferred to propanol and washed twice before they were dried with a stream of nitrogen and weighted. The density of the silica was assumed to be 1.9 kg l⁻¹ and the amount of particles was chosen such that they made up 80% of the surface area of the petri-dish. To this end ethanol (Ethanol absolute ≥ 99, 8%, CARL
Figure 1. Schematic of the procedure to modify the surface of silica particles to make IPCs. (a) A dispersion of silica particles in ethanol is pipetted onto the surface of an agarose gel. The particles form a crystalline monolayer upon evaporation of the ethanol. (b) A solution of PS in DEE is pipetted on top of the frozen gel. (c) After the evaporation of the DEE, the PS is deposited in between the particles forming a membrane from which the particles stick out on both sides. (d) The agarose gel is freeze-dried such that the membrane with the particles can easily be peeled off. (e) The membrane with the particles is dispersed in ethanol with APTES to functionalize the exposed polar patches with amine groups. (f) The amine patches on the particles are rendered fluorescent and more strongly positively charged by chemical binding of RITC. (g) The PS from the membrane is dissolved in toluene, leaving a solution of dispersed IPCs.

ROTH, Germany) was added to the particles to bring the concentration to 6.85 mg ml\(^{-1}\). The dispersion was sonicated for 1 h to make sure all particles were in suspension. Then 200 \(\mu\)l of this dispersion was pipetted onto the agarose gel at room temperature (figure 1(a)). After 2 h the ethanol had all evaporated and the particles formed a monolayer of mainly hexagonal order on the gel, see figure 2(a). The gel with the particles on top was then put at \(-20\) °C to fix the particles more firmly to the gel and to lower the surface tension of the DEE. After one hour the gel was frozen and 600 \(\mu\)l of cold DEE was pipetted on the tilted gel. The evaporation of the DEE was suppressed by the lid of the petri-dish which was left on top for 1 h to reduce the risk of the Cassie–Baxter state [29]. Then 400 \(\mu\)l of a cold solution of PS (Polystyrene Mw 35 000, SIGMA-ALDRICH, USA) at 1 mg ml\(^{-1}\) was pipetted into the DEE on top of the particle monolayer and the DEE was gently mixed (figure 1(b)). The total amount of PS was calculated to correspond to the volume required to mask the desired region of a close-packed particle film, leaving two 40° patches (see figure 2(c) for the definition of the opening angle \(\gamma\)). The lid was left open to let the DEE evaporate over the course of 3 h (figure 1(c)). The top of the particles was left free of PS due to the contact angle that formed between the silica of the particle, the polymer rich DEE and the air above the sample. Then the frozen gel was freeze-dried overnight.

2.3. Patch modification

The formed PS membrane with encapsulated silica particles was peeled off from the lyophilized agarose gel and put in an eppendorf tube (figure 1(d)). To this 100 \(\mu\)l of ethanol with 0.005% v v\(^{-1}\) (3-Aminopropyl)trimethoxysilane 97% (SIGMA-ALDRICH, USA) was added (figure 1(e)) and the tube was shaken for 5 min before the membrane flakes were spun down and washed with ethanol 5 times, to remove any possible remains of the gel. Then a few grains of Rhodamine B isothiocyanate (RITC, SIGMA-ALDRICH, USA) were added and left to bind to the patches overnight (figure 1(f)). The particles where then again washed 3 times with ethanol which was then replaced with toluene. The PS was left to dissolve overnight in the sonicator (figure 1(g)). The IPCs were then washed twice in acetone, twice in ethanol and twice in milliQ water.

2.4. Scanning electron microscopy

To study the formation of the patches, some flakes of the membrane were peeled off the lyophilized gel and prepared for inspection using scanning electron microscopy. To this end they were deposited on a SEM-stub. Care was taken that the orientation of the flakes with respect to the gel was known.
Figure 2. (a) A brightfield micrograph of a 2D crystal of 2 \( \mu \)m silica particles formed on top of an agarose gel. (b) SEM of the bottom face of the PS membrane incorporating equatorially encapsulated particles. The inset shows a sideview of the membrane. (c) SEM of the top face of the same membrane. The inset shows how the patch opening angle \( \gamma \) is determined from the size of the exposed silica in SEM images.

A 5 nm gold layer was sputtered on the membranes to prepare them for inspection with an Inspect S50 from FEI, using an EM SCD005 from Leica.

2.5. Epifluorescence

The IPCs were transferred to a 10 mM HEPES or TRIS buffer at pH 5, 6, 7, 8 and 9. Some of the dispersions were placed in rectangular borosilicate capillaries (W5010 Hollow Rectangle Capillaries ID 0.10 \times 2.00 mm, CM Scientific, UK) that were used as received and studied using an inverted Nikon Eclipse TE 2000-S microscope using epi fluorescence.

2.6. Confocal microscopy

The samples were turned upside down to allow inspection using an upright confocal laser scanning microscope (Leica SP5). For confocal inspection on an inverted confocal laser scanning microscope (Leica SP8) the dispersions were instead pipetted in a SensoPlateTM Plus, 96 well from Greiner Bio-one.

2.7. Zeta potential measurements

To measure the Zeta potential of the patch and bare region separately, we prepared one set of bare particles by subjecting them to all the washing steps, but not to the assembly on the gel and the labeling procedure and one set of particles that was labeled on the entire surface with APTES and RITC using the same conditions as for the particles masked partially by a polymer membrane. These two batches were dispersed in the same buffer solutions as those used for the epifluorescence and confocal studies. The electrophoretic mobility and thus the zeta potential at room temperature was measured using a Zetasizer Nano (Malvern).

3. Results

The first step to be characterized was the masking of the particles by the PS polymer membrane. The ‘bottom’ and ‘top’ side of the flakes were studied using scanning electron microscopy (SEM), see figures 2(b) and (c), which provided high resolution images of the exposed polar caps. The PS clearly has a different contrast with respect to the particles. In the inset of figure 2(b) we provide a look on the cross-section of the membrane as visible at an edge that curled up. The inter-particle distance can be measured to determine whether the particles are in contact with each other. At a center-to-center distance of 2.0 \( \mu \)m most particles were still in direct contact with their neighbors, but due to the polymer membrane that partially covered them they appeared smaller and slightly separated by a dark area providing the contrast to the PS in the micrographs.

From the diameter of the polar cap of the particles that sticks out it is straightforward to determine the opening angle of the patch that will result from a subsequent chemical modification. The inset of figure 2(c) shows the equation for the patch opening angle \( \gamma \) as function of the particle diameter \( D \) and the diameter of the polar cap that sticks out of the membrane \( D_p \). Analyzing the SEM images this way we found that the bottom patches, those parts of the particles that were wetted by the gel, had an average opening angle of 39 \( \pm 7^\circ \) while the top patches on average were slightly larger with 47 \( \pm 6^\circ \). The polydispersity might be due to local variations in the gel density which is also evident from its slightly turbid appearance. We determined the top and bottom patch angles on separate membrane samples, since it was not possible to remove and flip the same sample from the carbon sticker on the SEM-stub. Thus, we could not verify any correlation between the size of the bottom and top patches.

The translation of the polymer mask to functionalized patches was investigated by the attachment of a fluorescent dye to the APTES-coated particles as described in the Experimental section. The micron-sized fluorescent patches are suitable for inspection using a fluorescence microscope. The IPCs dispersed in water clearly reveal their patchy nature in epifluorescence, although in a single snapshot not all the particles appear to have a black equatorial region, see figure 3(a). When the particles are in focus, they appear as a luminous circle that is interrupted if the pole-to-pole axis lies in the image plane (with the interruptions perpendicular to the pole-to-pole axis). Some of the particles for which this was the case have been highlighted with a white circle. The
Figure 3. (a) Epifluorescence image of IPCs using a false color table to demonstrate the fluorescence of the patches and the absence of fluorescence on the unmodified equatorial region. On properly oriented particles (some of them highlighted in white) two fluorescent polar patches are observed. (b) Zoom in of a confocal micrograph, showing two fortunately oriented particles for which the polar caps and equatorial regions are clearly discerned. The fluorescent polar caps roughly correspond to the expected sizes from the SEM analysis of particles encapsulated in the polymer membrane mask. All particles are 2 µm in diameter.

Figure 4. (a) Overlaid brightfield and confocal micrograph of IPCs sedimented at a negatively charged glass surface at pH 7 just after the particles sedimented to the bottom of the cell. Upper inset: a measurement of the zeta potential as function of pH of bare silica and particles that were modified over the entire surface with APTES and RITC. Lower inset: close up on the fluorescence channel. (b) A similar micrograph of the same sample three days later. Square, pentagonal and hexagonal arrangements of particles are observed; some of these are highlighted in respectively blue, yellow and green as a guide to the eye.

particles in the confocal snapshots in figure 3(b) were oriented optimally for visualizing the opening angle of the patch. The optically resolved patch is in general agreement with the results of the SEM imaging. The diffraction limited resolution of fluorescence imaging of the patches allows us to confirm the existence of patches of similar size to what was found by SEM, but not to estimate the opening angle with the same precision.

The functionality of the IPC patches is determined not only by the size of the patches, but also by the surface charge density or zeta potential. Due to the different pKa and IEP of the RITC and silica surface respectively, their absolute and relative zeta potentials should be possible to adjust to control the interactions between the particles and steer the self-organization. This was investigated by measuring the zeta potential as function of pH for fully RITC-functionalized and unfunctionalized silica particles. The zeta potential of the bare silica was found to be fairly constant in the pH range between 5 and 9 at about −70 mV, see the inset in figure 4(a). Silica has an IEP close to 2, which predicts a stable zeta potential in the investigated range as found. The zeta potential of the particles with the same surface chemistry as the patches was found to decrease roughly linear with increasing pH from 80 mV at pH 5 to 7 mV at pH 9; this suggests that the pH is a good parameter to control the charge of the patches independent of the charge of the equatorial region of the particle surface.

To verify the presence of directional interactions controlled by the patches, a preliminary investigation was performed to see whether the behavior of the IPCs revealed indications of the recently predicted behavior of IPCs at charged interfaces [17]. Samples with particles prepared at
pH 7 and at pH 9 (not shown) were prepared and inspected immediately after preparation and three days later using confocal microscopy. Figure 4(a) shows a snapshot of the particles soon after they sedimented to the bottom of the well. Although most particles are still single, there are clearly some smaller aggregates visible. After three days the particles had formed a large 2D gel-like layer separated by large areas of relatively empty space (figure 4(b)). Unmodified particles repel due to the high negative surface charge at this pH and do not form any aggregates. During observation some bonds were broken and formed. Thin branches of the gel were seen to wiggle due to thermal excitation (see motion pictures in the online supplementary data, available at stacks.iop.org/JPCM/27/234105/mmedia). The combination of attraction and repulsion clearly indicates the existence of directional interactions since attractions are not present between uniformly charged particles. Furthermore, the sample observed at pH 9 showed freely diffusing particles even after three days, as expected due to the loss of positive charge on the patches at this pH. At both pH 7 and at pH 9 we expect the zeta potential of the bottom of the gel (SiO₂) capillary to be negative. In the gel at pH 7 (figure 4(b)) there are many regions with a square-like positional order that is reminiscent of the structures reported for IPCs with patches with an opening angle of 60° [18]. Such square-like structures should not form with exclusively isotropic interactions. We also observe large hexagonal regions that are reported to be formed by IPCs with patch extensions with opening angles of 45° [17]. There were also open ring like structures consisting of groups of five particles which were observed in simulations of IPCs with 30°.

4. Discussion

We have demonstrated that masking of the equatorial zone of particles supported on an aqueous gel by a polymer membrane can be used to modify homogeneously charged colloids into IPCs. One advantage with this method is that it allows for easy scale up. Large amounts of IPCs can be fabricated since the particles and polymer solution can be applied to arbitrarily large areas of gel interface. We observed no important differences between particles that were modified after assembly on a 35 mm diameter gel and particles that were modified after assembly on a 100 mm diameter gel. The processing can also be performed several times in parallel for scale up, since no expensive equipment or facilities are required for the process steps.

The method can also be applied to fabricate colloids with only one patch by a shorter process. Surface functionalization can be performed already on the particles assembled at the gel interface, in analogy to methods already presented for particle functionalization at liquid-liquid interfaces [27, 28]. Compared to liquid-liquid interfaces our method has the advantage that also particles with an Eötvös or Bond number too high to allow trapping and therefore functionalization at a water interface can be modified; the underlying gel prevents the colloids from leaving the interface through sedimentation, but still allows partitioning between the hydrophilic and hydrophobic phases. Thus, larger and denser particles can be modified by our method than at a traditional liquid interface. If for chemical reasons a liquid–liquid interface is required, the second solvent can be pipetted onto the particles assembled on the gel, even if the particles as such would not spontaneously stably bound to an interface between water and the added solvent. To be able to control the size of the top patch, from none to a size corresponding to the bottom patch, it is important that a sufficiently dense monolayer can be assembled on the gel.

A major problem encountered during the preparation of the polymer membrane meant to mask the particles was uneven wetting of the deposited particles by the polymer solution; this could vary over the gel substrate. The spreading of the PS solution, which only poorly wetted the surface of the silica particles and the aqueous gel interface, frequently resulted in the formation of a Cassie–Baxter state [29]. The masked region and thereby the opening angle of the patches, is controlled by the total amount of PS added under the assumption of complete space filling in between the colloids in a close-packed film. Thus, removal of air pockets to avoid the Cassie–Baxter state was of paramount importance for uniform formation of patches on the particles over the entire crystal. Improved wetting of the DEE solution and therefore suppression of the Cassie–Baxter state was achieved by lowering the temperature to −20°C.

The opening angle of the bottom patches is influenced both by the contact angle between the gel and the particles and by the compressibility of the gel. The contact angle between the PS-rich DEE used to deposit the membrane between the particles and the amount of the polymer that was deposited on the other hand determine the opening angle of the top patch. When too much polymer is added, the excess that does not fit between the particles is collected in local aggregates of polymer on the membrane. Most likely, the surface tension of the solution drives the aggregation into receding solvent droplets. When too little polymer was added, it was observed that over local regions the patch sizes were similar, while the patches could vary between regions, with some regions showing patches with significantly larger patches due to a lack of PS to form the masking membrane. The patch size and the distribution of patch sizes can thus be influenced by letting the polymer solution wet the particles longer before the solvent is allowed to evaporate and the choice of polymer and solvent as well as the composition of the gel.

To produce a charged IPC already the silanization step with APTES suffices. A dense layer of positively charged amines is obtained at the particle poles. However, the positive charge of amines is low in the typical experimental pH range, because amine groups are only weakly positively charged at pH values below 6.5 [30]. Our two-step functionalization which uses the amine groups only for subsequent coupling of RITC therefore fulfils two purposes: in addition to making imaging of patches possible it also increases the effective positive zeta potential that could be obtained and controlled over a larger pH range.

Although we characterized the zeta potential that is obtained by this protocol on uniformly surface functionalized particles, one cannot with certainty know if the patches have the same zeta potential; especially close to the edge between the patches and the equatorial region the charge is likely to
deviate due to the locally different electrostatic potential [31]. However, the patch zeta potential clearly was positive, which was supported by the preliminary investigation of the IPC aggregation behavior showing both attraction to the negatively charged substrate and IPC-like aggregation of the particles in a monolayer.

The aggregates at the capillary wall formed only very slowly and showed arrested, although clearly reversible, dynamics. Therefore, it is unlikely that during our study we were observing equilibrium structures. Longer equilibration times are likely needed. The effect of insufficient time is convoluted with the polydispersity of patch sizes within one sample. This polydispersity might give rise to different equilibrium structures within one sample, since the type of arrangement has been shown to be governed by patch size in numerical simulations [17]. It is not trivial to predict the influence of polydispersity in the opening angle on the equilibrium structures and the assembly dynamics. Further refinement of the presented method or a post-synthesis purification of particles with differently sized patches is required to rule out this effect.

5. Conclusion
We presented a simple and scalable method for the fabrication of patchy particles or inverse patchy particles. This method can be applied to many different kinds of colloidal particles, including large inorganic colloids, without making use of advanced clean-room equipment or chemistry facilities. Two combined innovations made it possible to fabricate inverted patchy colloids with close to identical polar caps. First, assembling a close-packed particle monolayer crystal on an aqueous gel allowed large silica particles to be assembled uniformly at the interface without sedimentation. This in turn enabled the calculation and addition of the correct amount of polymer to form a dense polymer layer that only masked the equatorial region of the particles. Secondly, we demonstrated that this procedure allowed us to create IPCs with a negatively charged equatorial region and cationic polar caps that can be distinguished using fluorescence microscopy. The attraction between polar patches and equatorial regions between IPCs was demonstrated by their aggregation behavior at a negatively charged surface. Direction dependent attractions and repulsions between the positively charged poles and the negatively charged equatorial regions were indirectly observed under the microscope. Our method can easily be extended to other particle and surface chemical functions, which could encode for more types of directional and attractive interactions. More monodisperse IPCs with a larger range of possible patch angles are expected to be created with additional fine-tuning of the wettability contrast.

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References
[1] Coluzza I, van Oostrum P D J, Capone B, Reimhult E and Dellago C 2013 Design and folding of colloidal patchy polymers Soft Matter 9 938–44
[2] Coluzza I, van Oostrum P D J, Capone B, Reimhult E and Dellago C 2013 Sequence controlled self-knotting colloidal patchy polymers Phys. Rev. Lett. 110 075501
[3] Furst E M 2013 Directed self-assembly Soft Matter 9 9039
[4] Van Blaaderen A, Imhof A, Hage W and Vrij A 1992 Three-dimensional imaging of submicrometer colloidal particles in concentrated suspensions using confocal scanning laser microscopy Langmuir 8 1514–7
[5] Blaaderen A v 2003 CHEMISTRY: colloidal molecules and beyond Science 301 470–1
[6] Bianchi E, Blaa R and Likos C N 2011 Patchy colloids: state of the art and perspectives Phys. Chem. Chem. Phys. 13 6397–410
[7] Pawar A B and Kretzschmar I 2010 Fabrication, assembly and application of patchy particles Macromol. Rapid Commun. 31 150–68
[8] Yi G-R, Pine D J and Sacanna S 2013 Recent progress on patchy colloids and their self-assembly J. Phys.: Condens. Matter 25 193101
[9] Chen Q, Bae S C and Granick S 2011 Directed self-assembly of a colloidal Kagome lattice Nature 469 381–4
[10] Ramírez L M, Smith A S, Unal D B, Colby R H and Velegol D 2012 Self-assembly of doublets from flattened polymer colloids Langmuir 28 4086–94
[11] Ramírez L M, Michaelis C A, Rosado J E, Pabón E K, Colby R H and Velegol D 2013 Poloidal chains from self-assembly of flattened particles Langmuir 29 10340–5
[12] Ramírez L M, Milner S T, Snyder C E, Colby R H and Velegol D 2010 Controlled flats on spherical polymer colloids Langmuir 26 7644–9
[13] Bianchi E, Kahl G and Likos C N 2011 Inverse patchy colloids: from microscopic description to mesoscopic coarse-graining Soft Matter 7 8313–23
[14] Noya E G, Koloves I, Doppelbauer G, Kahl G and Bianchi E 2014 Phase diagram of inverse patchy colloids assembling into an equilibrium lamellar phase Soft Matter 10 8464–74
[15] Ferrari S, Bianchi E, Kalysuzhnyi Y V and Kahl G 2015 J. Phys.: Condens. Matter 27 234104
[16] Noya E and Bianchi E 2015 J. Phys.: Condens. Matter 27 234103
[17] Bianchi E, Likos C N and Kahl G 2013 Self-assembly of heterogeneously charged particles under confinement ACS Nano 7 4657–67
[18] Bianchi E, Likos C N and Kahl G 2014 Tunable assembly of heterogeneously charged colloids Nano Lett. 14 3412–8
[19] Tagliazucchi M, Zou F and Weiss E A 2014 Kinetically controlled self-assembly of latex-microgel core-satellite particles J. Phys. Chem. Lett. 5 2775–80
[20] Pawar A B and Kretzschmar I 2008 Patchy particles by glancing angle deposition Langmuir 24 355–8
[21] He Z and Kretzschmar I 2012 Template-assisted fabrication of patchy particles with uniform patches Langmuir 28 9915–9
[22] Song J H, Harbottle D and Lee J W 2011 Rapid assembly of colloidal monolayer for the synthesis of surface anisotropic particles ACS Appl. Mater. Interfaces 3 2392–7
[23] McConnell M D, Krauelter M J, Yang S and Compusto R J 2010 Patchy and multiregion janus particles with tunable optical properties Nano Lett. 10 603–9
[24] Lin C-C, Liao C-W, Chao Y-C and Kuo C 2010 Fabrication and characterization of asymmetric janus and ternary particles ACS Appl. Mater. Interfaces 2 3185–91
[25] Yan F and Goedel W A 2004 Polymer membranes with two-dimensionally arranged pores derived from monolayers of silica particles Chem. Mater. 16 1622–6
[26] Denkov N, Velev O, Kralchevski P, Ivanov I, Yoshimura H and Nagayama K 1992 Mechanism of formation of two-dimensional crystals from latex particles on substrates Langmuir 8 3183–90
[27] Lattuada M and Hatton T A 2011 Synthesis, properties and applications of janus nanoparticles Nano Today 6 286–308
[28] Andala D M, Ra Shin S H, Lee H-Y and Bishop K J M 2012 Templated synthesis of amphiphilic nanoparticles at the liquid–liquid interface ACS Nano 6 1044–50
[29] Marmur A 2003 Wetting on hydrophobic rough surfaces: to be heterogeneous or not to be? Langmuir 19 8343–8
[30] Lin W-C, Lee S-H, Karakachian M, Yu B-Y, Chen Y-Y, Lin Y-C, Kuo C-H and Shyue J-J 2009 Tuning the surface potential of gold substrates arbitrarily with self-assembled monolayers with mixed functional groups Phys. Chem. Chem. Phys. 11 6199–204
[31] Boon N and van Roij R 2011 Charge regulation and ionic screening of patchy surfaces J. Chem. Phys. 134 054706