Self-templating assembly of soft microparticles into complex tessellations

The soft microparticles used in our experiments are monodisperse poly(N-isopropylacrylamide) (PNIPAM) microparticles synthesized by one-pot precipitation polymerization. We synthesized three batches of microparticles of different bulk sizes ranging from 600 nm to 900 nm in deionized water (MilliQ, 18.2 MΩ cm−1). Such microparticles develop a marked core–corona architecture upon adsorption and confinement at a water–oil (hexane) interface22, because the portion in contact with the interface stretches out radially22. For our particles, the cross-sectional diameter at the interface σ is about 1.5 to 1.8 times their diameter in bulk aqueous suspensions (Extended Data Table 1).

The microparticles were first allowed to self-assemble into a hexagonally packed monolayer via a modified Langmuir–Blodgett deposition technique (for more details see the Methods). Such a monolayer is a close-packed crystal insofar as the microparticles are in corona-to-corona contact but without much overlap between coronas23,24. Its packing fraction ϕ is then approximately equal to that of identical circles in a close-packed crystal insofar as the microgels are in corona-to-corona contact but without much overlap between coronas23,24. Its packing fraction ϕ is then approximately equal to that of identical circles in a hexagonally close-packed crystal (Eq. 1). The deposited monolayers are then dried and imaged by atomic force microscopy (AFM). Because the coronas have a thickness of just a few nanometres, large-area AFM images such as the ones in Fig. 1c, d mostly reveal the cores of the microparticles (~0.6σ).

Using an analogous protocol, we deposited a second monolayer onto the same substrate to combine monolayers of different packing fractions (Fig. 1a). In particular, we realized orthogonal gradients of packing fraction ϕi along the two axes of a substrate (Fig. 1b) by rotating the substrate by 90° between depositions, where i denotes either the first i = 1 or the second monolayer i = 2. By lifting the substrate across the water–hexane interface before injecting the microparticles, we also obtained two regions on the substrate with only one monolayer from each deposition, which we then used to estimate the position-dependent ϕi across the whole substrate (Extended Data Fig. 2).

We find that such a sequential assembly leads to non-hexagonal two-dimensional patterns that would not otherwise emerge upon compression and deposition of an individual monolayer (Extended Data Fig. 3). AFM images reveal that the microparticles of the first and the second monolayer are co-planar, rather than being stacked out-of-plane (Extended Data Fig. 3 and Fig. 1d). Moreover, while the microparticles of the monolayers that are subjected to different surface pressures Π, and thus possessing different ϕi, are seamlessly transferred to different locations of the same substrate21,22. The deposited monolayers are then dried and imaged by atomic force microscopy (AFM). Because the coronas have a thickness of just a few nanometres, large-area AFM images such as the ones in Fig. 1c, d mostly reveal the cores of the microparticles (~0.6σ).

Encoding Archimedean and non-regular tessellations in self-assembled colloidal crystals promises unprecedented structure-dependent properties for applications ranging from low-friction coatings to optoelectronic metamaterials1–7. Yet, despite numerous computational studies predicting exotic structures even from simple interparticle interactions8–12, the realization of complex non-hexagonal crystals remains experimentally challenging13–18. Here we show that two hexagonally packed monolayers of identical spherical soft microparticles adsorbed at a liquid–liquid interface can assemble into a vast array of two-dimensional micropatterns, provided that they are immobilized onto a solid substrate one after the other. The first monolayer retains its lowest-energy hexagonal structure and acts as a template onto which the particles of the second monolayer are forced to rearrange. The frustration between the two lattices elicits symmetries that would not otherwise emerge if all the particles were assembled in a single step. Simply by varying the packing fraction of the two monolayers, we obtain not only low-coordinated structures such as rectangular and honeycomb lattices, but also rhomboidal, hexagonal and herringbone superlattices encoding non-regular tessellations. This is achieved without directional bonding, and the structures formed are equilibrium structures: molecular dynamics simulations show that these structures are thermodynamically stable and develop from short-range repulsive interactions, making them easy to predict, and thus suggesting avenues towards the rational design of complex micropatterns.
first monolayer retain their hexagonal arrangement, owing to their strong adhesion to the underlying substrate\textsuperscript{25}, the microgels of the second monolayer can re-arrange and break their hexagonal ordering. As we will show, the degree of mismatch between \( \phi_1 \) and \( \phi_2 \), as well as the total packing fraction \( \phi_1 + \phi_2 \), regulates the formation of a wide spectrum of non-hexagonal patterns.

We rationalize the formation of such non-hexagonal structures by hypothesizing that the two sequential depositions are equivalent to the annealing of a colloidal monolayer comprising two populations of particles that are identical except for the fact that one population is fixed into a hexagonal lattice (Fig. 1e). The immobile fraction therefore acts as a template that frustrates the ordering of the mobile particles, thereby dictating their spatial organization. In particular, we assume that the two populations interact via repulsive short-range pairwise interactions within the same plane. In this framework, the ground-state configuration depends solely on the packing fraction of both monolayers (\( \phi_1 \) and \( \phi_2 \)), and on the functional form of the pair potential.

The effective interactions between microgels adsorbed at liquid–liquid interfaces remain largely unexplored. Nonetheless, the behavior of microgels in bulk suspensions is often captured by the Hertzian potential\textsuperscript{26}. The latter is an excellent approximation in the limit of small deformations, but it becomes inaccurate for large overlaps\textsuperscript{27,28}. Because of the large extent to which we compress the microgels and the possible deviations introduced by their confinement at a liquid–liquid interface, we choose to relax the constraint on the power-law exponent of the potential energy and the area of a unit cell of hexagonally packed disks are \( F_{\text{hex}} = 3U(r) \) and \( A_{\text{hex}} = r^2/\sqrt{3} \) (ref. \textsuperscript{29}), respectively, we can write the surface pressure \( \Pi \) as:

\[
\Pi|_{r=0} = -\frac{\partial F_{\text{hex}}}{\partial A_{\text{hex}}} = \frac{\varepsilon}{3} \left( 1 - \frac{r}{\sigma} \right)^{\alpha-1} \Theta \left( 1 - \frac{r}{\sigma} \right)
\]

by neglecting Brownian contributions (\( \varepsilon \approx k_BT \)). Equation (2) gives an excellent description of the \( F(r) \) compression curves measured for the three different types of microgels studied. Letting \( \sigma \) be a fitting parameter, we find a good agreement with the diameters of the microgels measured by AFM (Extended Data Table 1). Normalizing \( r \) by the respective \( \sigma \) causes all the experimental data to collapse onto a single master curve, which is best described by \( \alpha = 1.8 \pm 0.2 \), indicating a marked departure from the Hertzian model (\( \alpha = 2.5 \)) (Fig. 1f). This also suggests that, for our particles, the effective interactions do not depend on particle size and synthesis protocol.

Determining that \( \alpha \approx 2 \) is particularly important because \( \alpha = 2 \) demarcates a qualitative change in the shape of the generalized Hertzian potential, and thus in the topology of the phase diagram with respect to the structural variety of the ground states\textsuperscript{30}. This is because the repulsive force \( F(r) = -\partial U/\partial r \) is convex (\( \partial^2 F/\partial r^2 > 0 \)) for \( \alpha > 2 \), and concave

\[
U(r) = \frac{\varepsilon}{\sigma} \left( 1 - \frac{r}{\sigma} \right)^{\alpha-1} \Theta \left( 1 - \frac{r}{\sigma} \right)
\]
(∂²ϕ/∂²r < 0) for α < 2 (Extended Data Fig. 4). In other words, for α < 2, the force experienced by two approaching particles increases more rapidly for small overlaps r = 0 than it does for large overlaps r = 0. This feature can translate into the stabilization of asymmetric and low-coordinated structures such as rectangular lattices that would otherwise be inaccessible for α > 2 (refs. 10,11,13).

We investigate the validity of α < 2 by quantifying the degree and type of crystalline order found in both experimental and simulated structures in terms of the average bond-orientation order parameter ψ (Figs. 2, 3):

$$\psi_k = \frac{1}{N} \sum_{l=1}^{N} \frac{1}{N_l} \sum_{m=1}^{N_l} e^{ik\theta_{lm}}$$

where N is the total number of particles, Nl is the number of neighbours of particle l, θlm is the angle between the unit vector (1,0) and the ‘bond’ vector rlm connecting the reference particle l and its neighbour m, and k is the natural number defining the k-fold symmetry against which the order parameter is computed. Thus defined, ψ is a scalar between 0 and 1 that describes the average degree of k-fold symmetry of the local environment of each particle. For example, a honeycomb lattice corresponds to ψ = 1 because each particle is surrounded by three neighbours that are placed at 120° from each other. Analogously, square or a rectangular lattices correspond to ψ = 1.

We systematically experiment with simulations in the parameter space [0.85,1.65]ψ × [1,4,2.5]ϕ (Fig. 4b), by quantifying the discrepancy between the predicted and observed ψ (ϕ, ψ) in terms of the overall percentage error ε (Methods). We find that the observed structures are best described for ψ = 1.9 (Fig. 2b), in remarkable agreement with the α = 1.8 ± 0.2 extracted from the compression experiments of individual monolayers (Fig. 1f). In particular, not only does α = 1.9 provide a good qualitative description of our data, but also captures qualitative changes in the type of structures emerging across the (ϕ, ψ) diagram (Fig. 3).

A key feature captured by the simulations for α = 1.9 is the transition from rectangular to honeycomb lattices along the diagonal of the (ϕ, ψ) diagram, that is, upon increasing ϕ with ψ = ϕ (Figs. 2c, 3a, b). By comparing the energy density of honeycomb and rectangular lattices, we find that the latter are energetically favourable for ϕ = ψ, only if α < 2 (Fig. 2c). This is in good agreement with the observed high values of ψ > 0.6 in the bottom-left corner of the (ϕ, ψ) diagram and the growth of ψ at the expense of ϕ along the ϕ = ψ diagonal (Fig. 2a). Decreasing α below 1.9 causes further changes in the topology of the phase diagram, namely the emergence of a high-ϕ region in the upper-right corner of the (ϕ, ψ) diagram (Extended Data Fig. 4). This region is not observed in the experimental data, hence the existence of an optimal value of α.

If combining monolayers with the same packing fraction results in structures with a single type of short-range and long-range order, mismatched packing fractions (ϕ ≠ ψ) bring about a wide range of structures of far greater complexity: superlattices. We identify, in both simulations and experiments, three crystalline structures in different regions of the (ϕ, ψ) diagram: interlocking-S structures, hexagonal and honeycomb superlattices. These are periodic structures consisting of unit cells spanning several σ, which present varying symmetries at different length scales, as evident in the real space images, structure factors, and positional autocorrelation functions shown in Fig. 3c–e.

The interlocking-S structures are superlattices occurring at ψ > 1.4 > ϕ = 0.9 characterized by a staggered tessellation of rhomboid unit cells (Fig. 4a), comprising particles with a coordination number ranging from 3 to 8, which in turn encode tessellations of irregular polygons ranging from triangles to octagons. Perturbing the packing fraction in the neighbourhood of (1.4,0.9)ϕ,ϕ breaks the long-range order, with changes in ϕ1 or ϕ2 resulting in superstructures of different nature (Fig. 4b, c and Fig. 4e–f). For instance, decreasing ϕ1 leads to disordered tessellations of broken dodecagons (the interlocking-S) formed by chains of alternating triangles and rectangles enclosing octagons or hexagons, which locally resemble the Archimedean (3.4.6.4)-tiling also known as the rhombitrihexagonal tiling (Fig. 4). On the other hand, decreasing ϕ2 results in disordered tessellations reminiscent of the 3-uniform (3.3.3.4;3.4.6.4)-tiling, in that they consist of triangles arranged into...
triangular superstructures bounded by lines of rectangles interrupted by octagons (Fig. 4c, f).

Hexagonal superlattices arise in the same range of $\phi_1 + \phi_2$ as the interlocking-S structures, but for $\phi_2 > \phi_1$. These are lattices where high-coordinated sites are concentrated in regions that are arranged on a hexagonal lattice formed by triangular superstructures comprising low-coordinated sites. Interestingly, we find that increasing $\phi_2$ translates not only into a higher fraction of high-coordinated sites, particularly the number of pentagons in the Voronoi tessellation, but also into a decrease in the lattice spacing of the hexagonal superlattice (Fig. 4g–l).

The herringbone superlattice is, instead, a simpler structure occurring at high total packing fractions and for $\phi_1 \approx 1.4$ and $\phi_2 \approx 1.2$. This structure bears similarities to both honeycombs and rectangular lattices, in that it consists of staggered lines of particles connected by honeycombs. Intermediate but more disordered structures are also found in different regions of the diagram ($\phi_1, \phi_2$), specifically in the neighbourhood of the diagonal (Extended Data Fig. 6).

Each of the above structures can be accessed experimentally by controlling the surface pressure throughout both evaporations (Extended Data Fig. 9). Our approach therefore lends itself to the fabrication of micropatterns over large areas. The stability of the patterns upon re-immersion in water also offers opportunities for template-assisted growth of three-dimensional colloidal structures33.

Our findings also suggest that the range of superstructures that can be accessed experimentally can be further expanded through the rational design of microparticles that realize interaction potentials of tailored steepness. This could be done, for example, by engineering the architecture of soft colloids33,34, or by controlling their equilibrium position.
with respect to the interface through a careful balance between elastic and capillary forces as well as between the chemical affinities with the two fluids. 

Online content
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Fig. 4 | Simulated and experimental interlocking-S structures (ϕ₁ > ϕ₂) and hexagonal superlattices (ϕ₁ < ϕ₂) for different (ϕ₁, ϕ₂) pairs. a–c. Snapshots and Voronoi tessellations of ground-state interlocking-S structures obtained for ϕ₁ = 0.9 and varying ϕ₂, namely 1.1 (g), 1.3 (h) and 1.4 (i). j–l. AFM images, structure factor (top right) and positional autocorrelation function (bottom right) of the experimental counterparts of g–i obtained with 3CS1 (j and 3CS0 (k–l) microgels. Scale bars, 5 µm.
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Methods

Microgel synthesis

We synthesized PNIPAM microgels following an existing aqueous one-pot precipitation polymerization approach. Two amounts of the crosslinker N,N-methylenbisacrylamide (BIS) were added to 180 mM N-isopropylacrylamide (NIPAM) monomer solutions to obtain microgels with crosslinker-to-monomer ratios of 3 wt% and 5 wt%, respectively. NIPAM and BIS were dissolved in MilliQ water at 80 °C with the aid of magnetic stirring. The solution was then deaerated with N₂, before the addition of 1.8 mM potassium persulfate (KPS) to initiate the reaction. The temperature was maintained at 80 °C for 5 h to ensure that it ran to completion. The resultant microgels were then cleaned with three ultracentrifugation cycles at 20,000 rpm for 1 h. At the end of each ultracentrifugation cycle, the supernatant was removed and replaced with fresh MilliQ water, and the microgels were re-dispersed by 1 h of ultrasonication. Microgels obtained via this synthesis process present a core–shell morphology, with a degree of crosslinking that decreases radially.

Additional particle types were synthesized by further extending the PNIPAM shells of the microgels via an extra growth step. NIPAM and BIS in the corresponding crosslinker-to-mass ratios of 3 wt% and 5 wt% were dissolved in 6 ml of MilliQ water under N₂ flow with the aid of magnetic stirring. In a separated vessel, 0.25 of the previously synthesized, freeze-dried microgels were re-dispersed in 30 ml of MilliQ water under a flow of N₂, and heated to 80 °C under magnetic stirring. The reaction was performed in four steps within the vessel containing the dispersed microgels, by adding 1 ml of 1.2 mM KPS in MilliQ water and 1.25 ml of the crosslinker-monomer solution every 10 min, for a total of 40 min. Afterwards, the reaction mixture was kept at 80 °C under magnetic stirring for 5 h. By following this protocol, we could vary the microgel size at a given crosslinking mass ratio. In particular, we label our particles as CXY, where X = 3 or 5 is the crosslinking mass ratio and Y = 0 or 1 is the number of steps for additional shell growth. The size of the microgels in bulk MilliQ water was measured by dynamic light scattering (Malvern Zetasizer) at 25 °C (see Extended Data Table 1).

Deposition of monolayers from liquid–liquid interfaces

Monolayers of microgels were deposited onto 2 × 2 cm² silicon substrates (Siltronix, <100>, wafer diameter 100 mm, with only one side polished) following the procedure described in a previous work. In brief, we prepared 0.1 wt% microgel suspensions in 4:1 water/isopropanol mixtures. The presence of isopropanol assists the spreading of microgels at the fluid interface. Prior to deposition, the silicon substrates were rinsed in three consecutive ultrasonic baths of toluene, isopropanol and MilliQ water and then dried with pressurized N₂. We positioned the silicon substrates inside a customized liquid–liquid Langmuir–Blodgett trough (KSV5000, Biolin Scientific), by connecting them to the dipping arm at an angle of 30° relative to the interface plane. We filled the trough with MilliQ water until the substrate was fully immersed and the water stabilized for 10 min. Finally, the wafer intersected the interface was used as a reference to reconstruct the value of surface pressure as a function of position. We then added the desired amount of the microgel dispersion to the interface with a Hamilton glass microsyringe (100 µl) and let the system stabilize for 10 min. Finally, the wafer was gradually increased by compressing the interface with the barriers from 197.5 cm² to 59.5 cm² at a rate of 2.3 mm min⁻¹, while the dipping arm was raised at 0.5 mm min⁻¹. The second deposition was repeated with the same protocol by using the same substrate with the previously deposited monolayer but rotating it by 90° with respect to the direction of the first compression. In this way, we created two regions where only a single monolayer was present (Fig. 1b), which can be used for the estimation of the surface densities of the first φ₁ and second φ₂ depositions, as well as the total density φ₁ + φ₂. The deposition of target structures over large areas (Extended Data Fig. 9) was achieved by keeping the surface pressure constant throughout the deposition process via the feedback control loop of the Langmuir–Blodgett trough.

The microstructure of the dried structures was imaged via AFM (Brucker Icon Dimension) in tapping mode (cantilever resonance frequency 300 kHz, spring constant 26 mN m⁻¹). AFM images of 40 × 40 and 88 × 88 µm² were taken at a rate of 1 Hz. The diameter of the microgels at the interface was measured via AFM of isolated deposited microgels by limiting the height in the images to 5 nm to increase the contrast of the thinner corona. The coordinates of all particle centres from AFM images were extracted using the IDL version of the Crocker and Grier particle tracking code.

Compression curves were constructed by relating the position-dependent Π and area per particle A_r, obtained by extracting the number of particles in each image with ImageJ, and converted to Π versus r/σ (Fig. 1f) by assuming hexagonal packing. The latter was found to be a good approximation up to r/σ ~ 0.75. Therefore, we was estimated by fitting log(F/η(σ)/r) to the natural logarithm of equation (2) for 0.75 ≤ r/σ ≤ 1 using the Curve Fitting Toolbox of Matlab.

Reagents

PNIPAM (TCI 98.0%), BIS (Fluka 99.0%), KPS (Sigma-Aldrich 99.0%), isopropanol (Fisher Chemical, 99.97%), toluene (Fluka Analytical, 99.7%) and n-hexane (Sigma-Aldrich, HPLC grade 95%). The monomer was purified by recrystallization, in 60/40 v/v toluene/hexane. The rest of the reagents were used without further purification.

Simulations

The ground-state structures were estimated through two-dimensional molecular dynamics simulations carried out with the open-source libraries of the simulation toolkit HOOMD-blue. We ran simulations for 10 × 10 evenly spaced (φ, φ₁) pairs in the interval [0.85,1.6] × [0.85,1.65], for each of the following values of φ: 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.25 and 2.5. The equations of motion were integrated in the canonical NVT ensemble using the Nosé–Hoover thermostat with a time step dτ = 0.01 and a coupling constant τ = 1, both expressed in terms of normalized time units. Where M and σ are the mass and diameter of the particles, and ε is the energy scale of the interaction potential, which, without loss of generality, were all set equal to 1. For each (φ, φ₁) pair, the simulations were initialized by placing two populations of equally sized spherical particles in a two-dimensional box: one of immobile particles constrained to a hexagonal lattice, and the other of randomly distributed mobile particles. Periodic boundary conditions were enforced on all the sides of the box. The total number of particles varied in the range 6,238–11,250. The positions of the mobile particles were allowed to evolve towards the minimum energy configuration by varying their reduced temperature kT/ε from 10⁻² to 0 over a period of 1.2 × 10⁶ steps. In particular, the ground-state configurations were obtained via the Fast Inertial Relaxation Engine (FIRE) algorithm, which was used to bring kT/ε from 10⁻² to 0 while minimizing the total potential energy. At all times, the mobile particles interacted with each other and with the immobile particles according to the same interaction potential, namely the generalized Hertzian potential defined in equation (1).

To minimize the formation of metastable grain boundaries and to test the reproducibility of the ground-state configurations, we carried out two consecutive annealing cycles: kT/ε is first brought to zero from 10⁻² over a period of 2 × 10⁶, then brought back to 0.5 × 10⁻², and eventually decreased to 0 after a long period of 1 × 10⁶ steps. As shown in Extended Data Fig. 7, the bond orientational order parameters φ₂ attain the same values at the end of both annealing cycles, attesting to reproducibility of the observed symmetries.
Structural analysis
The bond-orientation order parameters $\psi_i$, the positional correlation function, and the Voronoi tessellations were computed using several modules of the freud library\textsuperscript{42}. The structure factors were constructed by calculating the fast Fourier transform of the particles’ positions. Because the observed structures present more than one type of symmetry, and thus different characteristic length scales, the nearest neighbours of each particle were identified based on the Voronoi tessellation rather than on a single cut-off distance. The nearest neighbours of the $i$th particle are defined as the $j$ particles whose Voronoi cell share an edge with the cell of the $i$th particle. Only edges greater than 8% of the perimeter of the $i$th cell are considered. This threshold was introduced to reduce the sensitivity of the computation to small lattice distortions, and thus to consider only the most representative bonds. Simulation snapshots were visualized with the software Ovito\textsuperscript{43}.

The values of $\psi_i$ for 3CS0 and 3CS1 were estimated based on the particle’s positions extracted from 250 - 350 40 x 40 µm$^2$ AFM images per sample. The corresponding values of $\phi_i$ and $\phi_i$ were extrapolated from AFM images taken in the corresponding lateral regions of the wafer where only one monolayer was deposited. To test the validity of this approximation we measured the ($\phi_i, \phi_i$) pairs of 40 - 50 AFM images where the particles of the first and the second deposition could be singled out on the basis of a slight height difference between the two populations. As shown in Extended Data Fig. 3, the actual values of $\phi_i$ and $\phi_i$ are in good agreement with the extrapolated ones, with a root-mean-square error of approximately 0.05. The latter was used as a measure of the uncertainty in $\phi_i$ and $\phi_i$. Assuming the true values of the latter to follow a normal distribution centred in the extrapolated values with standard deviation equal to the root-mean-square error, the 95% confidence intervals were estimated to be equal to the extrapolated $\phi_i \pm 0.1$.

To reconstruct the best estimate of $\psi_i(\phi_i, \phi_i)$ we propagated the uncertainty in $\phi_i$ and $\phi_i$ via the Monte Carlo method by averaging 10,000 realizations of $\psi_i(\phi_i + \xi, \phi_i + \eta)$, where $\xi$ and $\eta$ are normally distributed random numbers with zero mean and a standard deviation of 0.05. In particular, all the realizations of $\psi_i$ were interpolated and averaged at 100 x 100 evenly spaced points in the interval [0.85,1.65] x [0.85,1.65] via thin plate splines. The values of $\psi_i$ thus estimated were then compared with the ones obtained for the simulated ground-state structures, which were also interpolated at the same ($\phi_i, \phi_i$) pairs.

The agreement between simulated and experimental structures was quantified in terms of the median symmetric accuracy\textsuperscript{44}: $\zeta = 100(\exp[\exp(\psi_{i\phi})] - 1)$, where $\psi_{i\phi}$ and $\psi_{i\phi}$ are the predicted and observed values of $\psi_i$, respectively, and $M$ is the median function. This metric, which can be interpreted as a percentage error, is insensitive to outliers and gives the same weight to overprediction and underprediction, and thus provides a robust and unbiased measure of the accuracy of our predictions. The overall percentage error $\varepsilon$ is then defined as the weighted average of $\zeta_i : \varepsilon = \sum_{i=1}^{3} w_i \zeta_i$, with weights proportional to the interquartile range (IQR) of the respective observed $\psi_i : w_i = \text{IQR}_i / \sum_{j=1}^{3} \text{IQR}_j$. This is to give more weight to the most representative observables, that is, the ones that vary the most across ($\phi_i, \phi_i$) pairs. In fact, the experimental $\psi_i$ attain the highest values and degree of variation for $k = 3$ and $k = 4$ across all ($\phi_i, \phi_i$) pairs, and rapidly drop for $k > 4$, approaching an almost constant value in the range 0.15 - 0.2 for $k > 8$ (Extended Data Fig. 5).

Data availability
The data that support the findings of this study are available at https://doi.org/10.3929/ethz-b-000402331 under Creative Commons Attribution-NonCommercial 4.0 International license.

Code availability
Numerical simulations and analysis code that support the findings of this study are available at https://doi.org/10.3929/ethz-b-000402331 under Creative Commons Attribution-NonCommercial 4.0 International license.

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Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | Compression of individual monolayers. Surface pressure $\Pi$ versus area per particle $A_p$ and representative AFM images obtained by the simultaneous compression and deposition of a monolayer of 3CS0 microgels from a water–hexane interface onto a silicon wafer. Scale bar, 3 µm.
Extended Data Fig. 2 | Agreement between measured and extrapolated values of $\phi_1$ and $\phi_2$. The deposition process implies that $\phi_1$ and $\phi_2$ are expected to vary only along $x$ and $y$, which correspond to the respective compression directions. This is particularly advantageous because $\phi_1(x)$ and $\phi_2(y)$ can be reliably estimated from AFM images taken along the regions where only a single hexagonal monolayer was deposited ($y < 0$ or $x < 0$). To test whether extrapolating $\phi_1(x)$ and $\phi_2(y)$ from such regions to the entire Si wafer provides a good estimate, we systematically compared extrapolated ($\phi_1, \phi_2$) pairs with the values measured from AFM images taken in regions where two monolayers were deposited onto each other ($x, y > 0$), where a slight difference in height between the particles from the two depositions could be leveraged to measure $\phi_1$ and $\phi_2$ independently. The dots denote the different measurements based on AFM images taken for $x$ and $y > 0$. RMSE, root-mean-square error.

$$RMSE = \sqrt{\frac{\sum_j^{N_j} (\phi_1(x > 0)_j - \phi_1(x < 0)_j)^2}{N_j} + \frac{\sum_j^{N_j} (\phi_2(y > 0)_j - \phi_2(y < 0)_j)^2}{N_j}} \approx 0.05$$
Extended Data Fig. 3 | Depositing two monolayers onto the same substrate leads to in-plane packing. AFM images of particle monolayers with similar area per particle (a, 0.3 µm²; b, 0.4 µm²) but obtained after single (a, and red curve in c) and double (b, and blue curve in c) deposition, respectively. c, The height profiles along two representative line scans show that particles from both single and double depositions sit at the same height. The patterns arising from double depositions are therefore not the result of an out-of-plane stacking, but originate from the re-arrangement of the particles of the second monolayer into in-plane interstitial positions. Furthermore, the maximum height of the particles from the second deposition (second, fourth and sixth peak of the blue curve) is systematically higher (by about 10 nm) than the ones from the first deposition (first, third and fifth peak of the blue curve), owing to the in-plane compression exerted by the previously deposited particles. This fact was leveraged to estimate $\phi_1$ and $\phi_2$ from individual images, as particles arising from different depositions can be singled out via a height threshold (see Extended Data Fig. 2). Scale bar, 3 µm.
Extended Data Fig. 4 | Effect of $\alpha$ on the shape of the interaction potential and on bond-orientation order. Top, generalized Hertzian potential $U(r)$ (left) and corresponding interparticle force $F(r)$ (right) as a function of the normalized separation distance $r/\sigma$ for different values of $\alpha$. Bottom, $\psi_3$, $\psi_4$ and $\psi_5$ of the simulated ground-state structures as a function of $\phi_1$ and $\phi_2$ for different values of $\alpha$. 

The figures illustrate the effect of varying $\alpha$ on the interaction potential and bond-orientation order. The graphs show how the potential and force change as the separation distance increases for different $\alpha$ values. The bottom part of the figure demonstrates the orientation parameters $\psi_3$, $\psi_4$, and $\psi_5$ as functions of $\phi_1$ and $\phi_2$.
Extended Data Fig. 5 | Distribution of the observed bond orientational order. $\psi_k$ (with $k \in 3,.., 12$) is represented through box and whisker plots for 3CS0 (a) and 3CS1 (b). The bottom and top edges of the box indicate the 25th and 75th percentiles, the central red line indicates the median, and the lower and top whiskers indicate values equal to the median $\pm 1.5$ times the interquartile range. The inset shows the interquartile range (IQR) of the observed $\psi_k$. c, Measured values of $\psi_3$, $\psi_5$, and $\psi_7$ for 3CS0 microgels as a function of $\phi_1$ and $\phi_2$. 


Extended Data Fig. 6 | Experimental and simulated intermediate superstructures. AFM images of intermediate superstructures obtained with 3CS0 microgels and their corresponding simulation snapshots. The values in brackets indicate the respective (ϕ₁,ϕ₂) pairs. The simulations were run with α = 1.9. The scale bars in the AFM images are 5 µm.
Extended Data Fig. 7 | Estimating ground-state structures by molecular dynamics simulations. Analysis of molecular dynamics simulations showing the structural evolution towards minimum energy configurations as a function of time and reduced temperature $kT/\varepsilon$ for two different $(\phi_1, \phi_2)$ pairs, namely $(1.4, 1.4)$ in panels a and c, and $(0.9, 1.3)$ in panels b and d. For illustration purposes, the disks in panels a and b are drawn with a diameter of $0.6\sigma$. The FIRE algorithm was used to minimize the potential energy while bringing $kT/\varepsilon$ from $10^{-4}$ to 0. The insets in c and d show simulations snapshots taken after $2 \times 10^7$ steps.
Extended Data Fig. 8 | Relaxing the constraint on the mobility of the first monolayer leads to simpler structures. Simulated ground-state structures obtained at different packing fractions $\phi_1 + \phi_2$ for $\alpha = 1.9$ when all the particles are allowed to re-arrange freely. For illustration purposes, the particles are drawn with a diameter of 0.6σ.
Extended Data Fig. 9 | Realization of target structures over centimetre-scale substrates. AFM images of honeycomb lattices obtained from double depositions of 5CS1 (ϕ₁ ≈ ϕ₂ ≈ 1.2) (a), 3CS0 (ϕ₁ ≈ ϕ₂ ≈ 1.4) (b) and 3CS1 (ϕ₁ ≈ ϕ₂ ≈ 1.5) (c) microgels. d–i, Optical microscope images of two different superstructures deposited over two different 2 cm × 2 cm substrates by keeping the surface pressure constant throughout each deposition. The optical images were taken at random locations that were more than 1 mm apart from each other. The scale bars are 10 µm for the optical microscopy images and 2 µm for the AFM image in the insets. The surface pressures during the sequential depositions were Π₁ = Π₂ = 18 mN m⁻¹ for d–f and Π₁ = 26 mN m⁻¹ and Π₂ = 9 mN m⁻¹ for g–i.
Extended Data Table 1 | Diameter \( \sigma \) of the microgels

| Microgel | \( \sigma \) DLS [nm] | \( \sigma \) AFM [nm] | \( \sigma \) fit [\( \mu \)m] |
|----------|-----------------|-----------------|-----------------|
| 3CS0     | 618 ± 83        | 923 ± 64        | 1.11            |
| 3CS1     | 879 ± 121       | 1578 ± 46       | 1.52            |
| 5CS1     | 620 ± 204       | 1066 ± 71       | 1.10            |

\( \sigma \) was measured in bulk with dynamic light scattering and at the interface with AFM (from 25 isolated deposited microgels). \( \sigma \) was also extracted from the compression of individual monolayers by fitting equation (2) to experimental values of \( \Pi (r/\sigma) \).