Supporting information: A microgel-Pickering emulsion route to colloidal molecules with temperature-tunable interaction sites

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Fig S1: Microgel-decorated 1.5 µm oil droplets by simple mixing

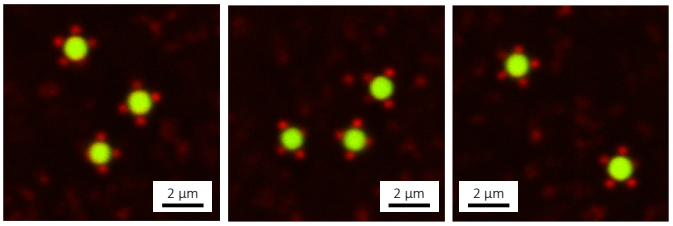

FIG. S1: *xy* CLSM micrographs showing examples of microgel-decorated oil droplets prepared by mixing the emulsion shown in Fig. 6 and Video S1 (30 µl) with microgel suspension (2.0 wt%, 10 µl). The assemblies are adsorbed to the bottom glass cover slip. Judging from observations made in bulk solution (not shown), the oil droplets carry about 7-9 microgels each. The (image acquisition) temperature was 20 °C.

Fig. S2: Growth of microgel-decorated oil droplets with time

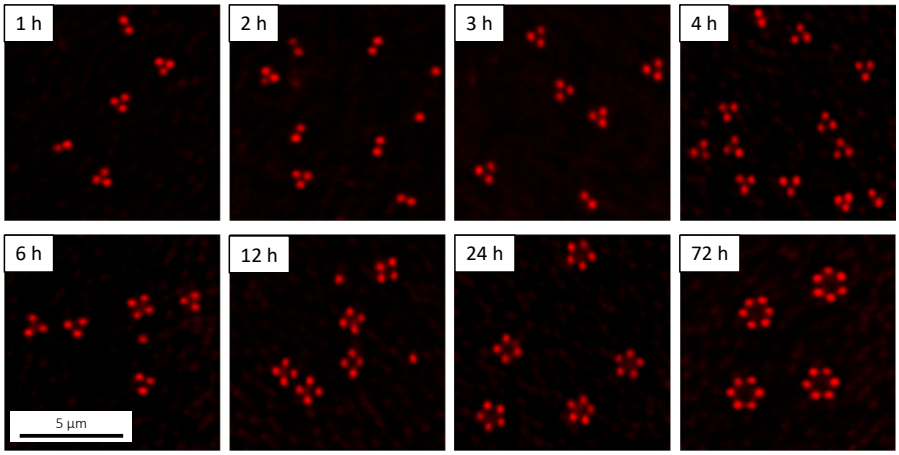

FIG. S2: *xy* CLSM micrographs showing the growth of microgel-decorated oil droplets with time, prepared from 0.01 v/v DMDES in pH 10.8 NH_3 (aq.) in the presence of very weakly positively charged (μ=+0.53·10^{-8} m^{2}V^{-1}s^{-1}), 299 nm (R_H) PNIPAM microgels. The microgels are covalently labelled with rhodamine whereas the oil droplets are non-fluorescent and therefore not visible. The assemblies are adsorbed to the bottom glass cover slip. The (image acquisition) temperature was 20 °C.

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Fig S3: Aggregates resulting from insufficient excess of microgels

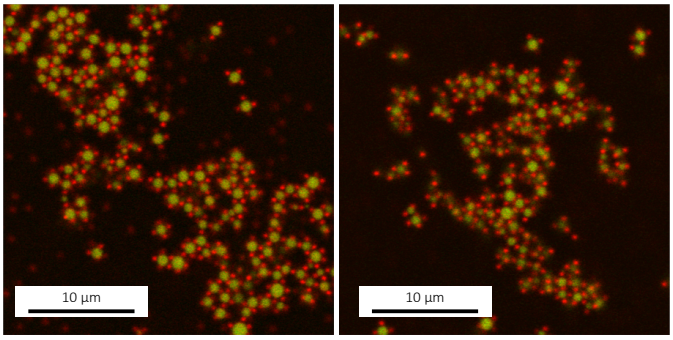

FIG. S3: *xy* CLSM micrographs showing the detrimental effect of having too few microgels in the suspension during oil droplet growth. Instead of discrete microgel-decorated oil droplets, large aggregate structures form as a result of microgels bridging the droplets. The microgel concentration was 0.05 wt%, ten times less than typically used. The oil droplets were prepared from 0.01 v/v PM546-dyed DMDES in 0.1 v/v NH_3 (aq.) and grown in the presence of the microgels for 72 hours. The (image acquisition) temperature was 20 °C.

Fig S4: 12-hour-old microgel-decorated oil droplets

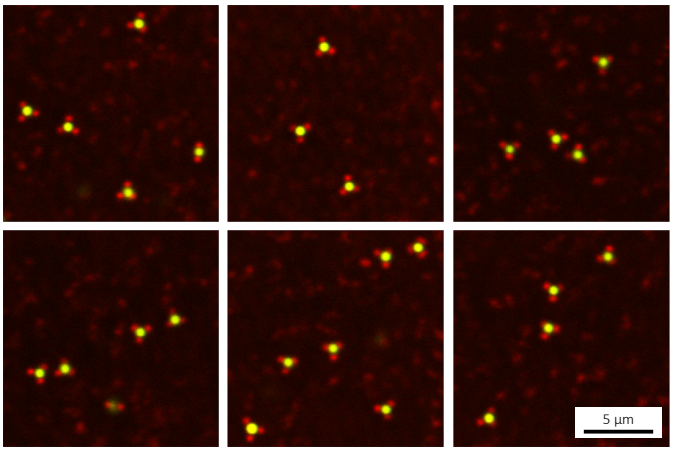

FIG. S4: Additional *xy* CLSM micrographs showing microgel-decorated oil droplets after 12 hours of oil droplet growth in the presence of microgels. The assemblies are adsorbed to the bottom glass cover slip; Video S2 shows assemblies in bulk solution. The oil droplets were prepared from 0.01 v/v PM546-dyed DMDES in 0.1 v/v NH_3 (aq.). Microgel concentration was 0.5 wt%. The (image acquisition) temperature was 20 °C.

The DLD device

DLD device description

DLD device design was carried out using the empirical formula for the critical diameter D_c derived by Davis[1]

$$D_c = 1.4G \cdot N^{-0.48} \tag{1}$$

where $G = \lambda - D_{post}$ is the distance (gap) between two posts and $N = \lambda/\Delta\lambda$ is the period of the array (Fig. S5). When designing the device, the gap was kept relatively large with respect to the particles (excess microgels and microgel-decorated oil droplets) in order to minimise clogging and thereby enable longer run times. Given that the diameter of a microgel-decorated oil droplet is about 2 μm, we decided to use a gap of 6 μm.

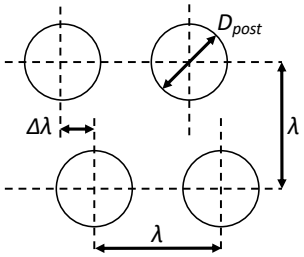

FIG. S5: Schematic drawing showing the arrangement of posts in a DLD device, and the meaning of D_{post}, λ and $\Delta\lambda$.

Another important factor to consider in device design is related to the manufacturing grid used during UVL mask fabrication. Our supplier (Deltamask B.V. The Netherlands) uses a grid size of 0.2 µm, which is the positioning accuracy of a feature on the mask. This means that the posts can only be shifted by an integer multiple of this number. With this in mind, the post diameter D_{post} was varied from 16 to 22 µm while extracting the number of periods of the array and the resulting critical size (Fig. S6). In order to simplify the design, an integer period was desired. In the end, the chosen parameters for the array were $D_c = 1.18$ μm, $\lambda = 24$ μm, $D_{post} = 18$ μm and $N = 60$.

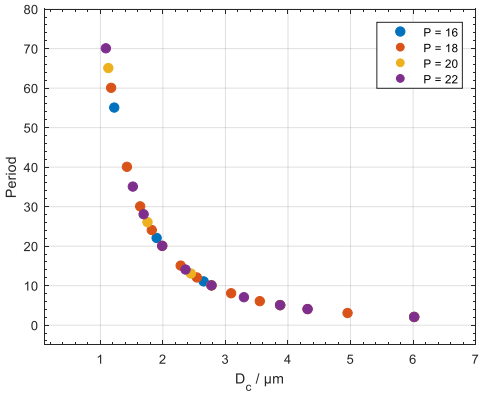

FIG. S6: Number of periods N and the resulting critical diameter D_c for different post diameters D_{post} (P) (µm) in the DLD device.

With the size constraint of a glass slide, the array could be repeated 35 times. This gave a length of 50 mm, leaving sufficient space for inlets and outlets. In order to increase the throughput, a mirrored design was chosen (Fig. S7). Samples inlets were positioned at the sides of the array, and the water inlet in the centre. With this design, the microgel-decorated oil droplets are expected to be focused in the centre of the array through lateral displacement, exiting into their dedicated outlet, while the smaller excess microgels follow the overall direction of the fluid and exit the device into the side outlets.

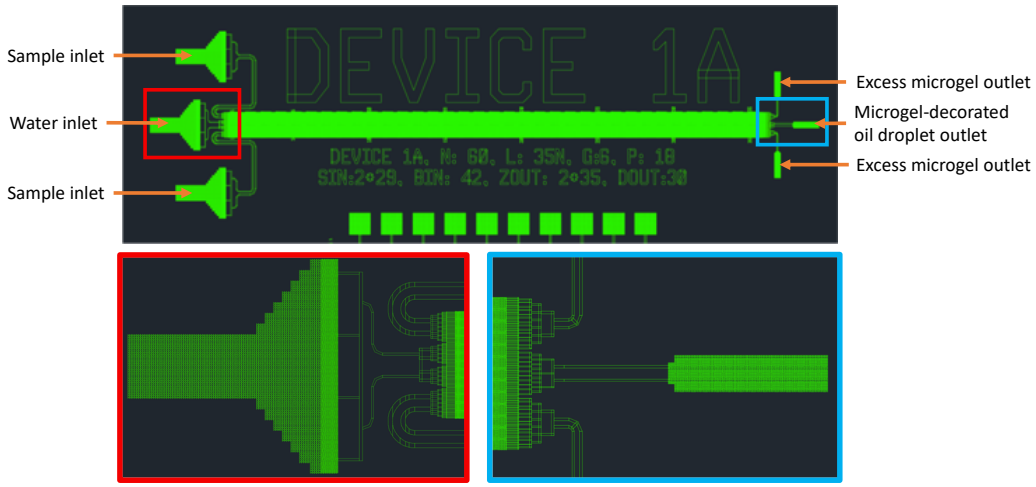

FIG. S7: The DLD device has a mirrored design to allow for greater sample throughput.

In the current device, the width of the sample inlets are $i_s = 2 \cdot 29\lambda$ while the water inlets are $i_w = 2 \cdot 21\lambda$, giving a total width of 100λ (2.4 mm). The width of the microgel-decorated oil droplet outlets are $o_c = 2 \cdot 15\lambda$ while the excess microgel outlets are $o_s = 2 \cdot 35\lambda$. This means that a particle has to be displaced at least 6λ in order to end up in the microgel-decorated oil droplet outlet. At the same time, a particle that is injected at the wall of the device needs to travel 35λ in order to end up in the microgel-decorated oil droplet outlet. The array in the device consists of 35 repeated periods, where each period has a length of 60λ (1.44 mm). Each period laterally displaces particles larger than D_c a distance 1λ. Consequently, particles larger than D_c, the microgel-decorated oil droplets in this case, can reach the microgel-decorated oil droplet outlet no matter where they are injected into the device. At the same time, particles smaller than D_c, the excess microgels in this case, need to travel laterally at least 6λ in order to reach the microgel-decorated oil droplet outlet. Considering 1D diffusion, this distance would on average take 6.5 hours for a particle with a radius of 500 nm. We therefore expect to be able to extract the vast majority of the microgel-decorated oil droplet while maintaining this sorted fraction free from the smaller excess microgels.

DLD device simulation

In order to ensure that the flow is split up symmetrically into the respective outlets at the end of the device, computational fluid simulations were carried out using COMSOL Multiphysics 5.3. By adjusting the widths of the centre outlets in small increments, the exact size resulting in a correct hydraulic resistance could be determined.

DLD device fabrication

To make a master for replica moulding, SU-8 (MicroChem, Newton, MA, USA) was spin coated onto 3" silicon wafers at varying thicknesses (10 and 22.3 µm) and patterned using UV light in a contact mask aligner (Karl Suss MJB4, Munich, Germany). A chrome mask was fabricated by Delta Mask (Delta Mask, Enschede, The Netherlands) with a design drawn in L-Edit 11.02 (Tanner Research, Monrovia, CA, USA). Before casting, the master was given an anti-adhesion layer of 1H,1H,2H,2H-perfluorooctyltrichlorosilane (ABCR GmbH & Co. KG, Karlsruhe, Germany) to facilitate demoulding.[2] PDMS monomer and hardener (Sylgard 184, Dow Corning, Midland, MI, USA) were mixed at a ratio of 10:1, degassed, poured onto the master and baked for 1 hour at 80°C. Connection tubes were cast directly into the PDMS to avoid the need for any additional adhesive. The patterned PDMS slab was bonded to a blank PDMS slide following surface treatment with oxygen plasma (Zepto W6, Diener electronic, Ebhausen, Germany). In order to minimise the immobilisation of particles the device was passivated using PEG-silane. A pressure gradient was used to drive flow through the DLD devices. Outlets were kept at atmospheric pressure and

the overpressure at the inlets was controlled individually using an MFCS-4C flow controller (Fluigent, Paris, France).

As mentioned, DLD is known for its excellent size resolution. However, like many other microfluidic particle sorting techniques, the throughput is often limited. Consequently, an important feature for the device was to achieve a high throughput while still maintaining a sufficient resolution to discriminate between the excess microgels and the microgel-decorated oil droplets. This was achieved by fabricating the device as deep as possible given the feature sizes and the aspect ratio constraints of the fabrication techniques used (PDMS replica moulding of SU8 patterned silicon wafers) and by not only using a single device but two devices in parallel. The expected throughput is calculated to 10 µl/h.

Synthesis and characterisation of microgels used to make patchy colloidal molecules

PNIPAM-*co*-AAc microgels were prepared by free radical precipitation polymerisation. In a three-necked flask, 3.02 g (26.6 mmol) NIPAM, 0.22 g (1.40 mmol, 4.8 mol%) BIS, 4.0 mg MRB and 0.9 mg SDS were dissolved by stirring in 130.0 g of water. Upon complete dissolution of the reactants, the necks of the flask were fitted with a septum, a nitrogen inlet and a condenser, after which the flask was immersed into an oil bath at 70 °C. During warm-up, the solution was purged with nitrogen under stirring. After 30 min, when the temperature was equilibrated and the solution was well purged, polymerisation was initiated through addition of 56.9 mg KPS in 2.0 g of water. 15 min after KPS addition, 0.10 g (1.40 mmol, 4.8 mol%) AAc in 2.0 g of water was added. The reaction was allowed to proceed for 2 hours following AAc addition, under a nitrogen mantle, after which the suspension was allowed to cool down down to room temperature. The suspension was then filtered through glass wool to remove any traces of coagulum, and was thereafter purified by repeated cycles of centrifugation, decantation and redispersion. Following purification, the microgels' electrophoretic mobility was measured to $-5.05 \pm 0.11 \cdot 10^{-8}$ $m^2V^{-1}s^{-1}$ at 20 °C and under deionised conditions (Malvern Zetasizer Nano-ZS, 17° scattering angle, 15 min equilibration time, 5 measurements of 60 s). The swelling curve obtained from DLS measurements (Malvern Zetasizer Nano-ZS, 173° scattering angle, 15 min equilibration time, 5 measurements) is shown in Fig. S8.

The same protocol as for PNIPAM-*co*-AAc microgel synthesis was used to prepare PNIPMAM-*co*-AAc microgels, but with the following chemicals and amounts: 3.02 g (23.7 mmol) NIPMAM, 0.20 g (1.28 mmol, 4.9 mol%) BIS and 44.5 mg SDS. MRB was excluded. 0.09 g (1.25 mmol, 4.8 mol%) AAc was added after 112 minutes, after which the reaction was allowed to proceed for 2 additional hours. The microgels' electrophoretic mobility was measured to $-4.83 \pm 0.13 \cdot 10^{-8}$ $m^2V^{-1}s^{-1}$ at 20 °C. The swelling curve obtained from DLS measurements is shown in Fig. S8.

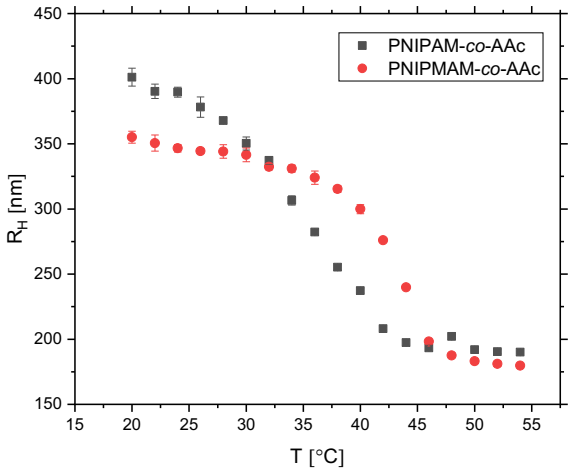

FIG. S8: Evolution of R_H with T for the PNIPAM-*co*-AAc and PNIPMAM-*co*-AAc microgels (used for the preparation of patchy colloidal moelcules), measured by DLS in water. The error bars are typically smaller than the symbol size.

Video S1: 48-hour-old PDMS oil droplets

CLSM videos showing monodisperse PDMS oil emulsion droplets in bright-field (left) and fluorescence mode (right). The oil droplets were prepared from 0.01 v/v PM546-dyed DMDES in 0.1 v/v NH_3 (aq.). The (image acquisition) temperature was 20 °C. Each video consists of 100 frames recorded over 6.804 s. Frame rate is 15 fps.

Video S2: 12-hour-old microgel-decorated oil droplets

CLSM videos showing microgel-decorated oil droplets after 12 hours of oil droplet growth in the presence of excess microgels. The oil droplets were prepared from 0.01 v/v PM546-dyed DMDES in 0.1 v/v NH_3 (aq.). Microgel concentration was 0.5 wt%. The (image acquisition) temperature was 20 °C. Each video consists of 100 frames recorded over 6.804 s. Frame rate is 15 fps.

Video S3: 12-hour-old microgel-decorated oil droplets following dialysis

CLSM videos showing microgel-decorated oil droplets after 33 hours of dialysis following 12 hours of oil droplet growth in the presence of excess microgels. The oil droplets were prepared from 0.01 v/v PM546-dyed DMDES in 0.1 v/v NH_3 (aq.). Microgel concentration was 0.5 wt%. The fluorescence of the oil droplets is considerably weaker than in Video S2 due to the non-covalent PM546-dye being partially washed away in the dialysis process. The (image acquisition) temperature was 20 °C. Each video consists of 100 frames recorded over 6.800 s. Frame rate is 15 fps.

Video S4: Sample harvested after DLD sorting

CLSM videos showing the sample harvested after DLD sorting during which the vast majority of excess microgels was removed. The (image acquisition) temperature was 20 °C. Each video consists of 100 frames recorded over 6.803 s. Frame rate is 15 fps.

Video S5: 1:1 PNIPAM-*co*-AAc and PNIPMAM-*co*-AAc mixture at 35 °C

CLSM videos showing a 1:1 mixture of PNIPAM-*co*-AAc and PNIPMAM-*co*-AAc at 35 °C, in fluorescence (left) and bright-field mode (right). The overall microgel concentration was 1.5 wt%. Each video consists of 150 frames recorded over 10.240 s. Frame rate is 15 fps.

[1] J. A. Davis, *Microfluidic separation of blood components through deterministic lateral displacement*, Princeton University, 2008.

[2] M. Beck, M. Graczyk, I. Maximov, E.-L. Sarwe, T. Ling, M. Keil and L. Montelius, *Microelectron. Eng.*, 2002, **61**, 441–448.