Electronic Supplementary Material (ESI) for Soft Matter.

Supporting information: Preparation of colloidal molecules with temperature-tunable interactions from oppositely charged microgel spheres

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Figure S1: Examples of unwanted clusters resulting from mixing under deionised conditions

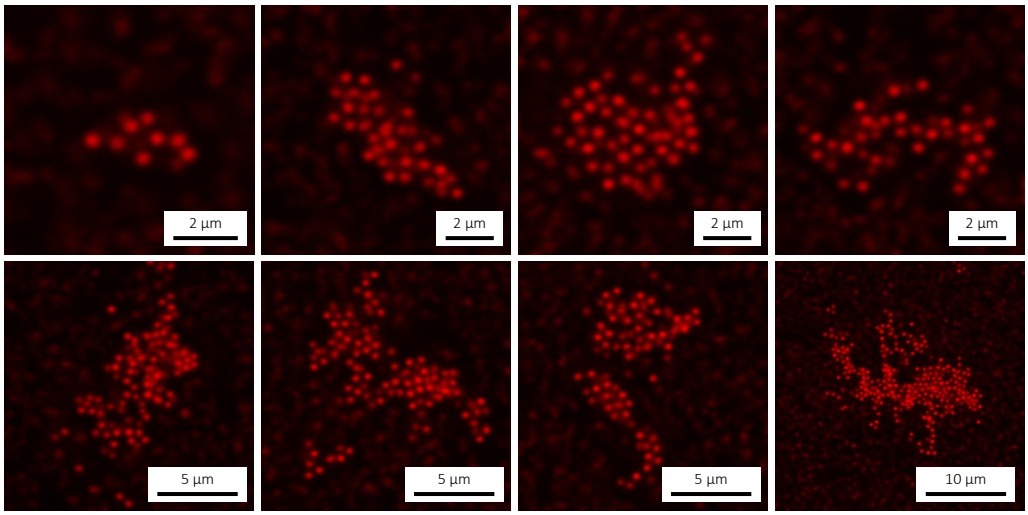

FIG. S1: *xy* CLSM micrographs showing examples of unwanted aggregates formed when A220 cores were added to B474 satellites under deionised conditions. The *B:A* number ratio was ≈ 48. The core suspension (43.5 µl) was slowly added (0.5 µl/min) to the satellite suspension by means of a syringe pump, under vigorous stirring. The CLSM micrographs were recorded 4 hours after completed core addition. The (image acquisition) temperature was 20 °C.

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Figure S2: Analytical ultracentrifugation (AUC) analysis of B474, and of B474+A220

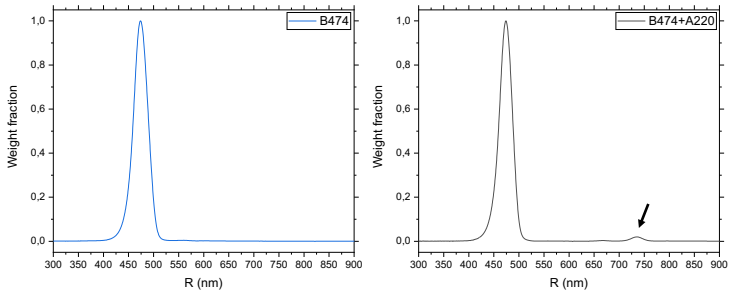

FIG. S2: Particle (cluster) size distributions measured by AUC for **(left)** the pure B474 satellite suspension, and **(right)** for the suspension harvested from the dialysis bag following assembly of B474 satellites and A220 cores. The arrow points to the peak corresponding to AB_4 clusters.

Figure S3: Clusters remain intact in acid and salt solutions

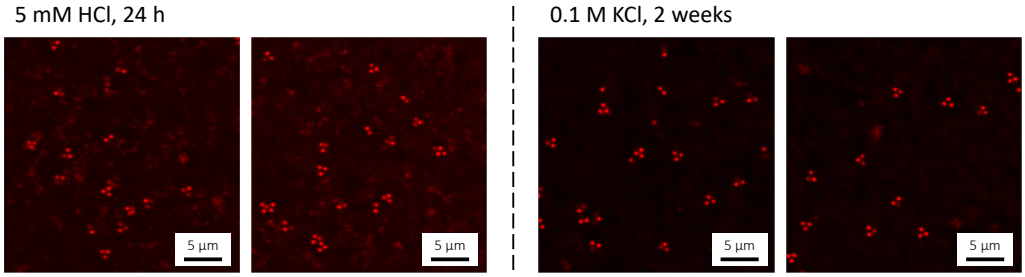

FIG. S3: xy CLSM micrographs of preserved AB_4-type, colloidal molecule-like clusters adsorbed to the cover slip in the presence of excess free satellite microgels at (left) 5 mM HCl and (right) 0.1 M KCl. 24 hours had passed since the addition of HCl, 2 weeks since the addition of KCl. The (image acquisition) temperature was 20 °C.

Video S1

Three CLSM videos compiled, containing the snapshots shown in Fig. 6A (A115 cores/B474 satellites), 6B (A220 cores/B474 satellites) and 6C (A394 cores/B474 satellites), showing AB_n-type, colloidal molecule-like clusters in the presence of excess B474 satellites. The (image acquisition) temperature was 20 °C. Each video consists of 150 frames recorded over 10.237 s. Frame rate is 15 fps.

Video S2

Video showing AB_4-type, colloidal molecule-like clusters entering the outlet reservoir of the DLD device following sorting, at room temperature. The video consists of 262 frames, recorded during 8.18 s. Frame rate is 30 fps. The video was recorded using a Nikon Eclipse Ti (Nikon Corporation) inverted microscope in combination with a scientific CMOS camera (Flash 4.0 V2, Hamamatsu).

Video S3

Two CLSM videos compiled (bright-field and fluorescence, respectively), containing the snapshots shown in Fig. 9, showing association of two AB_4-type, colloidal molecule-like clusters in 5 mM HCl and

35 °C. Each video consists of 109 frames recorded over 7.422 s. Frame rate is 15 fps.

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. S4). When designing the device, the gap was kept relatively large with respect to the particles (satellites and clusters) in order to minimise clogging and thereby enable longer run times. Given that the diameter of an AB_4 cluster is about 2 μm, we decided to use a gap of 6 μm.

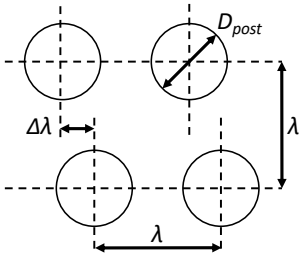

FIG. S4: Schematic drawing showing the arrangement of posts in a DLD device, and the meaning of D_{post}, λ and $\Delta\lambda$.

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. S5). 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 $N = 60$.

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. S6). Samples inlets were positioned at the sides of the array, and the water inlet in the centre. With this design, the AB_4 clusters are expected to be focused in the centre of the array through lateral displacement, exiting into their dedicated outlet, while the smaller satellite microgels follow the overall direction of the fluid and exit the device into the side outlets.

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 cluster outlets are $o_c = 2 \cdot 15\lambda$ while the satellite 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 cluster 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 cluster 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 AB_4-clusters in this case, can reach the cluster outlet no matter where they are injected into the device. At the same time, particles smaller than D_c, the excess satellite microgels in this case, need to travel laterally at least 6λ in order to reach the cluster 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 clusters while maintaining the sorted fraction free from the smaller satellite particles.

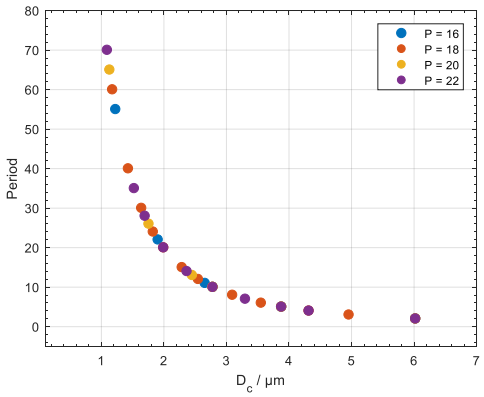

FIG. S5: Number of periods N and the resulting critical diameter D_c for different post diameters D_{post} (P) (µm) in the DLD device.

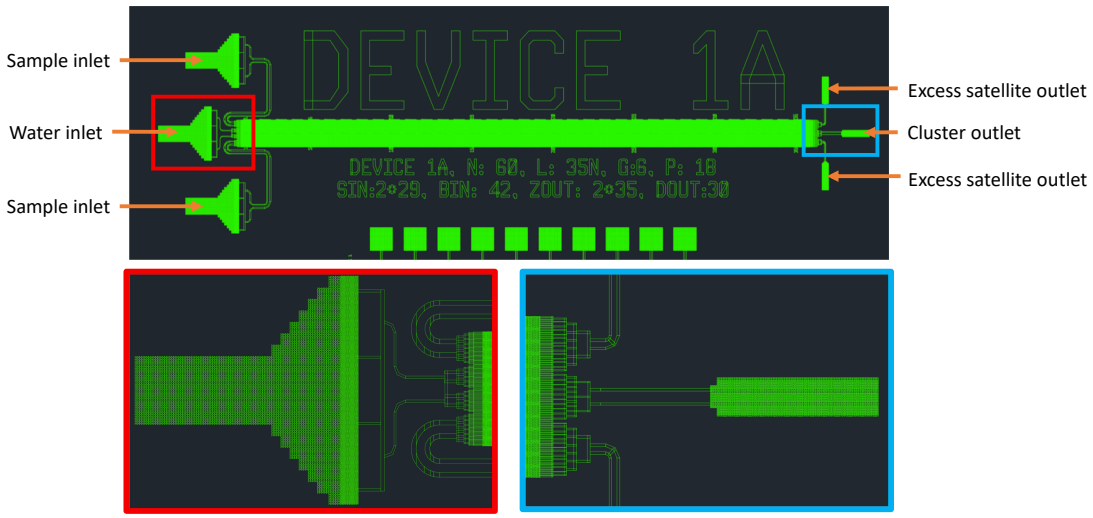

FIG. S6: The DLD device has a mirrored design to allow for greater sample throughput.

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 (Plasma Preen II-862, Plasmatic Systems, Inc, North Brunswick, NJ, USA). In order to minimise the immobilisation of particles the device was passivated us-

ing 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 satellites and clusters. 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.

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[1] Davis, J. A. *PhD thesis* (Princeton University, 2008).

[2] Beck, M. *et al.* Improving stamps for 10 nm level wafer scale nanoimprint lithography. *Microelectron. Eng.* **61**, 441–448 (2002).