Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

Supplementary Information for **"Specificity, flexibility and valence of DNA bonds guide emulsion architecture"** by L.Feng[+], L-L.Pontani[+], R.Dreyfus, P.M.Chaikin, J.Brujic[@].

Movie S1: (figure 1c in the manuscript)
DNA mediated emulsion aggregate melt upon heating. This movie is 5x sped up.

Movie S2: (figure 1d in the manuscript)
DNA mediated colloid-emulsion hybrid system melts upon heating. This movie is 5x sped up.

Movie S3: (figure 4c in the manuscript)
Droplet network connected by specific DNA bonds. The adhesive patches are mobile owing to the liquid interface.

Movie S4: (figure 5a in the manuscript)
Thermal fluctuations of a polymer chain of complemetary divalent emulsion droplets, formed by limiting the amount of binders on the surface.

Movie S5: (figure 5b in the manuscript)
Thermal fluctuations of a polymer chain of emulsion droplets branched in a string through complementary nanoparticles.

Movie S6: (figure 5c in the manuscript)
At higher nanoparticle/droplet ratios, more than two adhesion patches form, leading in this case to a trivalent 2D structure.

Movie S7:
A confocal scan through the 3-d static emulsion-colloid structure. The colloidal nanoparticles assemble into rings between droplet contacts to maximize the amount of droplet/particle adhesive area with little droplet deformation.

DNA CONSTRUCTS

Sequence S (61bp)
5-GGA TGA AGA TGA GCA TTA CTT TCC GTC CCG AGA GAC CTA ACT GAC ACG CTT CCC ATC GCT A/BiotinTEG/-3

Sequence S' (61bp)
5'-CAT CTT CAT CCA GCA TTA CTT TCC GTC CCG AGA GAC CTA ACT GAC ACG CTT CCC ATC GCT A-/BiotinTEG/-3'

Long sticky end sequence A (74bp)
5'-AAG TTC TCA GGT TAA CGT ATG ACA AGC ATT ACT TTC CGT CCC GAG AGA CCT AAC TGA CAC GCT TCC CAT CGC TA-/BiotinTEG/-3'

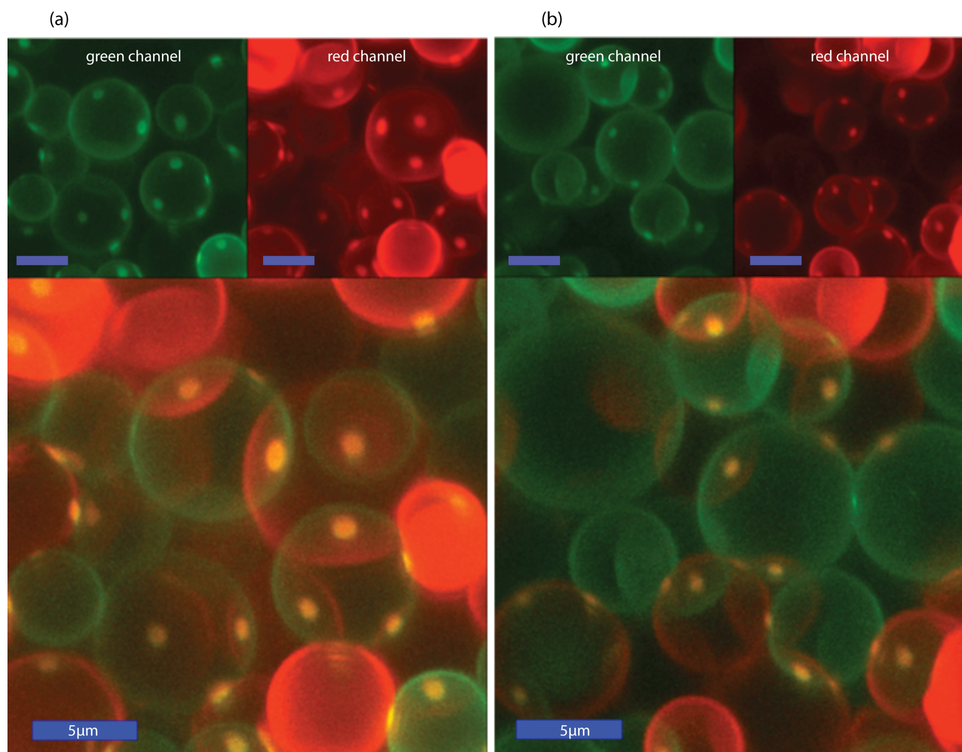
Long sticky end sequence B (74bp)
5'-TGT CAT ACG TTA ACC TGA GAA CTT AGC ATT ACT TTC CGT CCC GAG AGA CCT AAC TGA CAC GCT TCC CAT CGC TA-/BiotinTEG/-3'

The CS strand complementary to the backbone (49bp)
5'-TAG CGA TGG GAA GCGTGT CAGTTA GGT CTCTCG GGA CGG AAA GTA ATG C-3'

TEG: Tetra-Ethylene Glycol

This journal is © The Royal Society of Chemistry 2013

STATISTICAL MODEL FOR DNA ADHESION PATCHES



S-Fig1: When two complementary emulsions interact, the green droplets (Alexa Fluor 488 streptavidin) coated with the S sequence adhere to the red droplets (Alexa Fluor 633 streptavidin) coated with the S sequence. They then form an adhesion patch that is enriched equally in each complementary DNA binder as shown in the red and green channels for both the double stranded (A) and single stranded DNA (B). Merging the channels therefore shows a yellow adhesion patch in both cases.

Let us consider two interacting droplets of the same radius R and coated with complementary strands of DNA. When two DNA strands from opposite surfaces bind together, they gain binding energy but lose entropy due to the spatial constraint into the patch. The system also endures a deformation energy cost to allow the droplets to deform and form the adhesion patch area.

First we use the simple 'lattice model' (or 'box model') to get the entropy of molecules in a non-dilute solution. Given the total area S_{total}, the molecule (streptavidin) size A_{strep}, the number of streptavidins N and the total number of sites available on the droplet surface $N_0 = \frac{S_{total}}{A_{strep}}$ where $N \leq N_0$, the total number of configurations reads:

$$\Omega = \frac{N_0!}{(N_0 - N)!N!} \tag{1}$$

Using the Stirling's formula the entropy is approximated to:

$$S = k\ln(\Omega) = k[N\ln(\frac{N_0 - N}{N}) + N_0\ln(\frac{N_0}{N_0 - N})] \tag{2}$$

With the binding energy for a pair of DNA sticky ends $\Delta G_{DNA} = \Delta F_{DNA} - T\Delta S_{DNA}$ [1], the deformation energy of the droplet[2] and the entropy term derived in equation (2), we can write down the global free energy difference between the bound state and the non-interacting droplets state as follows:

$$\Delta F = E_{DNA,\beta} - 2(TS_\beta + TS_\alpha) + E_{deformation} - F_{unbound} \tag{3}$$

Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

The subscript β refers to the adhesive patch region while α refers to the non-interacting region on the rest of the droplet surface. Since two droplets interact to form a patch, the entropy term has to be taken into account twice which justifies the prefactor.

The energy terms in equation (3) can be written, similar to what was used in ref[3]:

$$E_{DNA,\beta} = -kT\ln[(1+\exp(-\frac{\Delta G_{DNA} - T\Delta S_r - T\ln(A_w C_\beta)}{kT}))^{N_\beta} - 1] \tag{4}$$

The entropy and the deformation energy in equation (3) can be written:

$$S_\beta = k[N_\beta \ln(\frac{N_{\beta 0} - N_\beta}{N_\beta}) + N_{\beta 0}\ln(\frac{N_{\beta 0}}{N_{\beta 0} - N_\beta})] \tag{5}$$

$$S_\alpha = k[N_\alpha \ln(\frac{N_{\alpha 0} - N_\alpha}{N_\alpha}) + N_{\alpha 0}\ln(\frac{N_{\alpha 0}}{N_{\alpha 0} - N_\alpha})] \tag{6}$$

$$E_{deformation} = \frac{1}{2}\sigma\pi R^2\theta^4 = \frac{1}{2}\sigma\pi\frac{r_p^4}{R^2} \tag{7}$$

Where r_p is the radius of the enriched patch; θ is defined as the deformation angle r_p/R; σ is the surface tension of the emulsion[2]; $N_\alpha + N_\beta = N$ gives the total number of streptavidins, N_β of them being in the binding patch; $N_{\alpha 0} + N_{\beta 0} = N_0 = 4\pi R^2/A_{strep}$ gives the total number of biotin sites on a emulsion, while $N_{\beta 0} = \pi r_p^2/A_{strep}$ is the number of sites available in the patch area; $C_\beta = N_\beta/(\pi r_p^2)$ is the concentration of streptavidin in the patch; A_w is the area over which two bound DNA strands could move relative to each other while remaining hybridized; ΔS_r [3] and $k\ln(A_w C_\beta)$ are the configurational entropy losts due to rotational and translational confinement of hybridized DNA sticky ends, respectively.

We now minimize this global free energy ΔF with respect to two independent parameters in the equations: $N_{\beta 0}$ and N_β. Note that we could conversely use the two independant parameteres C_β and r_p instead, which would result in the same equations.

The first equation leads to the chemical potential equilibrium. In the strong binding case where $\Delta F_{DNA} - T\Delta S_{DNA} - T\Delta S_r$ is at least a few kT, this first equation can be simplified as follows:

$$\begin{aligned} &\Delta G_{DNA} - T\Delta S_r - kT - kT\ln(A_w C_\beta) \\ &-2T[k\ln(\frac{N_{\beta 0} - N_\beta}{N_\beta}) - k\ln(\frac{N_{\alpha 0} - N_\alpha}{N_\alpha})] = 0 \end{aligned} \tag{8}$$

The second equation reads:

$$\begin{aligned} kT\frac{N_\beta}{N_{\beta 0}} - 2kT[\ln(\frac{N_{\beta 0}}{N_{\beta 0} - N_\beta}) - \ln(\frac{N_{\alpha 0}}{N_{\alpha 0} - N_\alpha})] \\ + \frac{\sigma N_{\beta 0} A_{strep}^2}{\pi R^2} = 0 \end{aligned} \tag{9}$$

The resulting N_β and $N_{\beta 0}$, directly leading to values of r_p and C_β/C_α, can be solved numerically which allows the comparison with our experimental values for the patch size r_p and contrast C_β/C_α. These analytical solutions are obtained under the approximation that there is an infinite dilute reservoir with a constant supply $N_\alpha/N_{\alpha 0} = Const = d$. This approximation is reasonable for our experimental condition, since $N_\alpha/N_{\alpha 0} < 0.1$ and the relative change in C_α is less than 10%, even with the most enrichment condition. As a result, the approximate solution to equation (8) is :

$$\frac{N_\beta}{N_{\beta 0}} = \frac{2cd^2 + e^{a/b} - e^{\frac{a}{2b}}\sqrt{4cd^2 + e^{a/b}}}{2cd^2} \tag{10}$$

Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

Where $a = \Delta G_{DNA} - T\Delta S_r - kT$, $b = kT$, $c = A_w/A_{strep}$. This expression is directly linked to the measured patch intensity contrast $C_\beta/C_\alpha = \frac{N_\beta}{N_{\beta 0}} \cdot \frac{N_{\alpha 0}}{N_\alpha} = \frac{N_\beta}{d \cdot N_{\beta 0}}$. Since we know the relation $N_{\beta 0} = \pi r_p^2/A_{strep}$, equation (9) directly gives us:

$$r_p^2 = R^2 \frac{kT[2\ln(\frac{N_{\beta 0}}{N_{\beta 0}-N_\beta}) - \frac{N_\beta}{N_{\beta 0}}]}{\sigma A_{strep}} \tag{11}$$

or

$$\theta = \sqrt{\frac{kT[2\ln(\frac{N_{\beta 0}}{N_{\beta 0}-N_\beta}) - \frac{N_\beta}{N_{\beta 0}}]}{\sigma A_{strep}}} \tag{12}$$

We can now compare our experimental values to the ones found analytically here.

For the DNA sequence used in the exepriments, $\Delta G_{DNA} = \Delta F_{DNA} - T\Delta S_{DNA}$ is $\approx -20kT$ at room temperature [1], and the experimental value for $T\Delta S_r$ are $-14.6kT$ for the double-stranded backbone DNA and $-14.8kT$ for the single-stranded backbone one [3]. We therefore use the same fitting parameters for both the ssb and dsb case. $A_w/A_{strep} \cong 34$ for dsb while it is only $A_w/A_{strep} \cong 1$ for ssb DNA. This discrepancy is due to the different rigidities of the DNA strands: double stranded DNA is more rigid and rod-like and can thus reach a large number of strands on the opposite surface, whereas single stranded DNA behaves as a very flexible polymer in our buffer conditions, with a persistent length of $\sim 2nm$ leading to a smaller end-to-end distance of $\approx 6nm$. $\sigma \cong 15mN/m$ for phospholipid emulsions co-stabilized with 1mM SDS. With a streptavidin size of $A_{strep} = 60nm^2$ and the initial streptavidin surface concentration of $1400/\mu m^2$ [2], this leads to $d_{max} \cong 0.09$.

Experimentally we vary the DNA surface density $d = N_{DNA}/N_{max}$ by changing the amount of DNA introduced in the system $N_{DNA} \sim$ 1pmol, 2pmol, 4pmol, 8pmol, 20pmol or 80pmol. An ideal emulsion packing of $30\mu L$, as used in this experiment, can bind up to $N_{max} \sim 30$ pmol of DNA. Nevertheless the experiements require two washing steps of the emulsions before DNA conjugation, which is suspected to significantly reduce this number.

As a result, all the data in main text figure2c and figure3b&c can be fitted with only two fitting parameters: $\Delta S_p = -16R$ and $N_{max} = 12$ pmol.

POLYDISPERSE EMULSION DROPLETS INTERACTION

In the approximation of infinite reservoir, the only radius dependent term in the above set of equations are from the deformation energy. The deformation energy of the emulsions should be corrected as: $E_{deformation} = \frac{1}{4}\sigma\pi\frac{r_p^4}{R_1^2} + \frac{1}{4}\sigma\pi\frac{r_p^4}{R_2^2}$ with different radius of contacting emulsions, R_1 and R_2, in the lowest order approximation[2]. We define a square-averaged radius $< R >= \sqrt{\frac{2R_1^2R_2^2}{R_1^2+R_2^2}}$, so that $E_{deformation} = \frac{1}{2}\sigma\pi\frac{r_p^4}{<R>^2}$ and we can use all the equations in the previous section replacing R with $< R >$, as plotted or used in main text figure2c and d.

LINEAR REGRESSION, ADDITIONAL GEOMETRY AND DIFFRACTION LIMIT

We fit d_p as a function of $< R >$ with a simple linear regression relation rather than a line $d_p = \theta R$ going through the origin as suggested by our model. The origin of this choice lies in geometrical arguments. Indeed the DNA constructs can be stretched, which leads to enrichment outside of the geometrically predicted adhesion patch. This additional area leads to a geometric factor $\frac{\Delta L}{\theta}$ contributing to the patch size. Both double-stranded and single-stranded DNA can extend up to $\Delta L \sim 12nm$ as estimated respectively from [3] and a worm like chain model. This leads to an entropy loss of $\sim 2-3kT$, which reduces DNA concentration by half.

Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

A a result the model is modified to include this additional term:

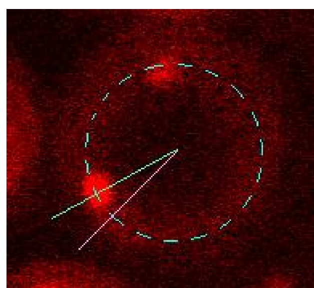
$$d_p = 2\theta R + \frac{\Delta L}{\theta} \tag{13}$$

The estimate for the respective intersections for dsb and ssb DNA give the values of $\Delta L/\theta \sim 80nm$ and $\sim 160nm$, which are smaller than the experimental values of $\sim 150nm$ (dsb) and $\sim 210nm$ (ssb) that are certainly fixed by our experimental diffraction limit of $\sim 150nm$.

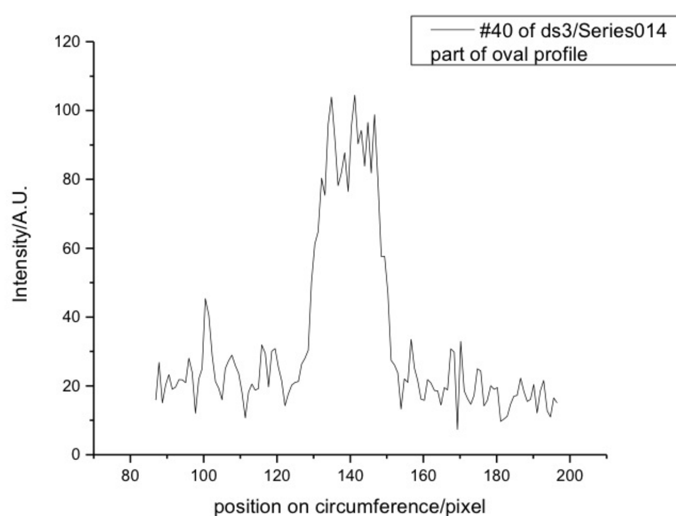
Nevertheless, the fitting curves with either $d_p = 2\theta R + \frac{\Delta L}{\theta}$ or $d_p = 2\theta R$ are similar with the data shown in Fig. 2C and the fitting parameter ΔS_p only changes by $\sim 5\%$ to fit the data. Therefore the limits of our experimental accuracy do not allow us to discriminate between the two relationships.

Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

IMAGE ANALYSIS



S-Fig2: Confocal imaging of the adhesion patches between the droplets. The fluorescence intensity along the droplets surface is analyzed with the Oval Profile plugin in ImageJ. Once the patch is identified from the circular intensity profile (see next figure), the fluorescence intensity along two radii inside and outside of the patch are also measured for contrast measurements.

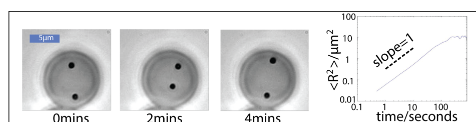


S-Fig3: Oval profile along the circumference of a droplet. The central plateau has an average intensity of $I_{patch} + I_{noise} = 88 \pm 9$ AU. We define the width of the peak as the diameter of the patch, which is here of 20 pixels or $d_{patch} = 1.2\mu m$. The average intensity in the peripheral region is $I_{emulsion} + I_{noise} = 20.3 \pm 4.5$ AU. The background noise estimated from the droplets central intensity is evaluated to be $I_{noise} = 9.8 \pm 3$ AU. Averaging this among different slices significantly reduces the error bar. Assuming that the concentration of streptavidin is linearly proportional to the measured fluorescence intensity, the streptavidin enrichment in the patch is $\frac{C_{patch}}{C_{emulsion}} = \frac{I_{patch}}{I_{emulsion}} = 7.5$. This value as well as d_{patch} are used in the main text in figure2 and 3, using statistics from different slices, patches and droplets.

This journal is © The Royal Society of Chemistry 2013

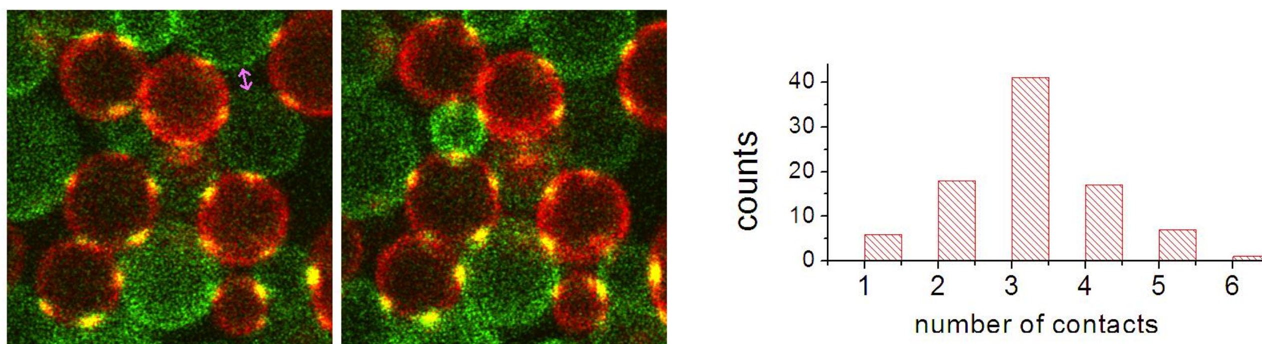
DIFFUSION COEFFICIENT MEASUREMENT

We use hybrid systems of particles and emulsions to quantify the diffusion of adhesion patches. The beads serve as reporters for the lipid motion on the monolayer surface. To measure relative motion, two colloidal particles coated with the S DNA sequence are attached onto the surface of a S' functionalized droplet through at least 200 DNA bonds [3,4]. The mean square displacement of one bead with respect to the other yields a diffusion constant of $D \approx 0.012 \mu m^2/s$. This value is significantly smaller than both the diffusion of a single lipid of size $\approx 1nm$ in a fluid model membrane with $D \approx 1 - 10 \mu m^2/s$ [5-8] and that of a $1\mu m$ colloidal particle with $D_{particle} \approx 0.5 \mu m^2/s$. This slow diffusion of the particle is due to the strong hydrodynamic drag of an adhesive lipid patch of larger radius $\approx 100nm$ [3], which is expected to be two orders of magnitude lower than that of a single lipid [7,8].



S-Fig4 Two S coated particles diffuse on the surface of a S' coated droplet. The mean square displacement of the particles with time reveals a diffusive behavior and the measured diffusion coefficient yields a value of $\approx 0.012 \mu m^2/s$ on the surface of phospholipid stabilized emulsions.

FLOPPY EMULSION NETWORK



S-Fig5 The bonds between complementary red and green emulsions are mobile, even after the structure reaches the maximum droplet connectivity (left). The distribution of the number of red-green sticky contacts can be measured with many confocal scans as in main text figure2b.

VALENCY CONTROL THROUGH THE CONTROL OF DNA BINDERS SURFACE DENSITY

The thermodynamic model suggests that the adhesion patch between two droplets can reach a saturation DNA density that is limited by the size of the streptavidin, provided that the binding energy of the DNA is strong enough (-40kT). The patch will then show a DNA density of $C_{\beta,sat} \approx 1.5 * 10^4/\mu m^2$. The maximum patch angle then is $\theta \approx 0.15$, mostly determined by the dramatic deformation energy cost ($E_{deform} \propto \theta^4$) and higher order terms. The valency n, i.e. the number of available patches is then $n \approx \frac{C_\alpha 4\pi R^2}{C_{\beta,sat}\pi r_p^2} = \frac{4C_\alpha}{C_{\beta,sat}\theta^2}$. The assembly of monovalent (n=1) and divalent (n=2) structures therefore requires DNA surface densities C_α of $\approx 84/\mu m^2$ and $\approx 168/\mu m^2$ respectively, which corresponds to 6% and 12% relative strands coverage based on our calibration of the total available streptavidin groups on the surface, as described below.

Experimentally we increase the DNA binding energy by increasing the length of the sticky ends to 24bp, using therefore the complementary sequences A and B [1]. The DNA strands are first incubated with streptavidin at a molar ratio of 1:1 for an hour in the buffer (5mM PBS, 4mM MgCl_2, and 1mM SDS). Then 2.5pmol, 5pmol and 10pmol of DNA-Streptavidin were incubated with $10\mu L$ of emulsion in $200\mu L$

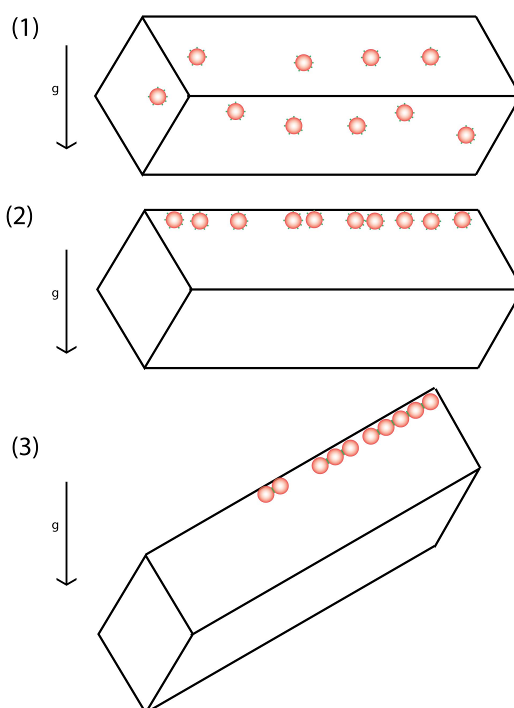
Electronic Supplementary Material (ESI) for Soft Matter
This journal is © The Royal Society of Chemistry 2013

of buffer, in order to achieve mono-valent, di-valent, and multi-valent assemblies. The calibration from [2] shows that the above mentioned protocol with 2.5pmol DNA yields a DNA-streptavidin surface density of $\approx 1/(100nm)^2$, which corresponds to $\approx 7.5\%$ of the maximum coverage. Experimentally we used slightly higher DNA coverage than the approximated number from our model, $\approx 7.5\%$ vs 6% in the model for monovalent structure, and $\approx 15\%$ experimentally vs $\approx 12\%$model for divalent structure, to compensate for the potential impurity of the DNA strands and the calibration errors in DNA coverage and streptavidin concentration.

DNA COATED PARTICLES

To measure the diffusion coefficient on the droplets surface we used $1\mu m$ streptavidinated particles, the Dynabeads MyOne Streptavidin C1 from Invitrogen. We coat the particles with biotinylated DNA to reach a final DNA surface coverage of $\approx 20000 DNA/particle$. For the self-assembly of hybrid structures, $0.2\mu m$ neutravidin labeled microsphere were used, the FluoSpheres NeutrAvidin from Invitrogen.The final DNA surface coverage reaches $\approx 300 DNA/particle$.

EMULSION POLYMER FORMATION



S-Fig6: a dilute suspension of S' coated emulsion droplets is mixed with the complementary S coated particles top a ratio of particle/droplet $\sim 5\%$. The capillary is first tilted horizontally to force the creaming of the droplets in the top corner. The droplets are then brought together by an additional slight vertical tilting that makes them slide together and adhere through the particles to form emulsion polymeric chains.

[1] Santalucia, J. Jr., 'A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics' Proc. Natl Acad. Sci USA **95**, 1460-1465(1998)
[2] Pontani, L.-L., Jorjadze, I., Viasnoff, V. and Brujic, J. 'Biomimetic emulsions reveal the effect of mechanical forces on cell-cell adhesion' Proc. Natl Acad. Sci USA **109**, 9839-9844(2012)
[3] R. Dreyfus, M.E. Leunissen, R. Sha, A.V. Tkachenko, N.C. Seeman, D.J. Pine, P.M. Chaikin, Phys Rev Lett 2009, 102, 4

This journal is © The Royal Society of Chemistry 2013

[4] R. Dreyfus, M.E. Leunissen, R. Sha, A.V. Tkachenko, N.C. Seeman, D.J. Pine, P.M. Chaikin, Phys Rev Lett 2010, 81, 4 Pt 1
[5] R.M. Clegg, W.L. Vaz, Progress in Protein-Lipid Interactions 1985, 1
[6] W.L. Vaz, R.M. Clegg, D. Hallmann, Biochemistry 1985, 24, 3
[7] M. Negishi, H. Seto, M. Hase, K. Yoshikawa, Langmuir 2008, 24, 16
[8] R.B. Walder, A. Honciuc, D.K. Schwartz, J Phys Chem B 2010, 114, 35