

Electronic Supplementary Information

DNA-Functionalised Blend Micelles: Mix and Fix Polymeric Hybrid Nanostructures

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1. Materials and Methods

1.1 Materials

Solvents and reagents for DNA synthesis were purchased from Novabiochem (Merck, UK) and SAFC
15 (Sigma-Aldrich, The Netherlands). Solid supports, Primer SupportTM (200 $\mu\text{mol/g}$) from GE
Healthcare, were used for the synthesis of **22PPO**. ÄKTA oligopilot plus (GE Healthcare, Uppsala,
Sweden) was used for DNA synthesis. ÄKTA explorer (GE Healthcare, Uppsala, Sweden) was used
for purification and analysis of DNA materials. After synthesis DNA amphiphiles were purified by
anion exchange chromatography using a HiTrapTM Q HP 1 ml or 5 ml column (GE Healthcare, The
20 Netherlands) through custom gradients using elution buffers (A: 25 mM Tris-HCl pH 8.0, B: 25 mM
Tris-HCl pH 8.0 and 1.0 M NaCl). Fractions were further desalted by dialysis membrane (MWCO
2000, Spectrum[®] Laboratories, The Netherlands).

The oligonucleotides (5'-TAA CAG GAT TAG CAG AGC GAG G-3', cDNA) modified with
carboxyfluorescein (FAM, absorption max = 494 nm, emission max = 520 nm) at either the 5'- or 3'-
25 end (FAM-5'-cDNA or FAM-3'-cDNA), or thiolated at the 5'- end (thiol-5'-cDNA) were purchased
from Biomers, Germany.

Gold colloid (5 nm) in citrate was purchased from BBinternational (UK).

Pluronic F127 ($M_w = 12,600$ g/mol), carbonyldiimidazole, ethylene diamine, pentaerythritol
tetraacrylate (PETA) and pyrene were purchased from Sigma-Aldrich (The Netherlands) and were
30 used as delivered if further purification is not described below. Extra dry tetrahydrofuran (THF) in
AcroSeal[®] bottles was purchased from Acros organics (Belgium). 5-(and-6)-
carboxytetramethylrhodamine succinimidyl ester (TAMRA-SE, 5(6)-TAMRA mixed isomers) was
purchased from Invitrogen, The Netherlands.

Ultrapure water (18 M Ω) dispensed through 0.22 μm membrane filter using arium[®] 611 UF (Sartorius,
35 The Netherlands) was used during all experiments.

1.2 Equipment and Techniques

Absorption and Fluorescence Spectroscopy. Absorption and fluorescence spectra of stabilised micelles were measured on a SpectraMax M2 spectrophotometer (Molecular Devices, USA) using either a 1 cm light-path quartz cuvette or 96-well Abs/FLU plates (Greiner, Germany / NUNC, Denmark). For FRET experiments a Cary Eclipse fluorescence spectrophotometer (Varian, The Netherlands) was used with a 96-well fluorescence plate.

Photo-cross-linking. A RayonetTM photochemical reactor (The Southern New England Ultraviolet Company, USA) was used for the photo-cross-linking of PETA in the micelle core.

Atomic Force Microscopy (AFM). AFM images were captured in Tapping Mode in air using a MultiMode-II SPM with a Nanoscope IIIa controller (Veeco, France). V-1 grade mica plates were purchased from Electron Microscopy Sciences (USA). N-doped single-crystalline Si Tapping Mode cantilevers with a spring constant of 25 – 75 N/m (ACTA) were purchased from ST Instruments. MgAc₂ buffers were prepared by diluting a 1 M stock solution (Sigma Aldrich) with ultrapure water to the desired concentration. In order to maintain relative concentrations, all micelle samples were prepared under the same procedure. Sample solutions were diluted with 50 mM MgAc₂ to 1/5 of initial concentration, i.e. to 160 μM F127 and/or 32 μM **22PPO**. Freshly cleaved mica was exposed to 40 μL of 50 mM MgAc₂ buffer for 5 minutes, after which the mica surface was blown dry under a N₂ stream. This preparation strongly favours the immobilization of negatively charged materials. A volume of 30 μL of diluted sample solution was immediately applied, and the sample was covered for 90 minutes to prevent evaporation. After two careful rinses with 100 μL ultrapure water, the sample was blown dry once more under a N₂ stream and promptly imaged.

For AFM studies of mixed micelles hybridised with Au-NP-cDNA conjugates, samples were prepared from a stock mixture of 1 μL stabilised mixed micelles (800 μM F127, 160 μM **22PPO** and 0.4% PETA by weight) and 40 μL Au-NP-cDNA conjugate (590 nM Au). Freshly cleaved mica was exposed to 40 μL of 50 mM MgAc₂ for 5 minutes, after which the surface was blown dry and 15 μL of the stock mixture and 10 μL of 50 mM MgAc₂ were deposited. After 20 minutes, the surface was carefully rinsed once with 50 μL of ultrapure water, blown dry under N₂ and promptly imaged.

Transmission electron microscopy (TEM). Onto a carbon coated copper grid, treated with glow-discharge prior to use, samples were prepared by placing a drop of micelle sample followed by addition of a drop of the staining solution (uranyl acetate). Excess solution was carefully blotted off using filter paper and samples were air dried for at least 5 minutes before analysis. TEM images were obtained using a CM10 (Philips, Eindhoven, The Netherlands) electron microscope.

FT-IR. Attenuated Total Reflection (ATR) infrared measurements were performed using a Specac Golden GateTM accessory with diamond top-plate on a Bruker IFS88 spectrometer equipped with a MCT-A detector at 2 cm⁻¹ resolution. Each final spectrum of F127 and F127-NH₂ is averaged intensity of 1,000 measurements.

10

2. Stabilisation of the F127 and Mixed Micelles

22PPO was synthesised and purified as previously reported by our group.¹ The purity of **22PPO** (96%) was determined by anion-exchange HPLC (data not shown). General preparation and stabilisation of pure or mixed micelles are described here.

15 **Mixed micelle preparation.** Dry F127 (2.5 g) was dissolved in 50 mL of ultrapure water under gentle agitation to prepare 5 wt % concentration. F127 5 wt% (4.0 μM, 400 μL), **22PPO** (800 μM, 400 μL), and H₂O (1.2 mL) were mixed. The mixture was heated to 95 °C for 30 min and then allowed to cool to r.t. overnight using a thermocycler (Eppendorf, The Netherlands) to make uniform-size micelles in the solution.

20 **Loading PETA/pyrene and stabilisation.** To the bottom of a glass vial, PETA (40 wt % of F127) and pyrene (200 μg / 1 mL of total volume) in acetone were added, and evaporated under vacuum. Annealed mixed micelle solution was added to the vial and was mixed using an orbital-shaker (IKA, The Netherlands) allowing micelles to contact hydrophobic small compounds. After shaking overnight, the vial was placed in a reaction vessel and the inside was filled with argon. The reaction vessel was UV-irradiated for 2 hr at 50 to 60 °C.² After irradiation, the reaction mixture was filtered through 0.23 μm PVDF syringe filter (Whatman, The Netherlands). Resulting stabilised micelles here were used for further analysis such as UV/Vis and fluorescence spectroscopy (Fig. S1) and AFM (Fig. S2).

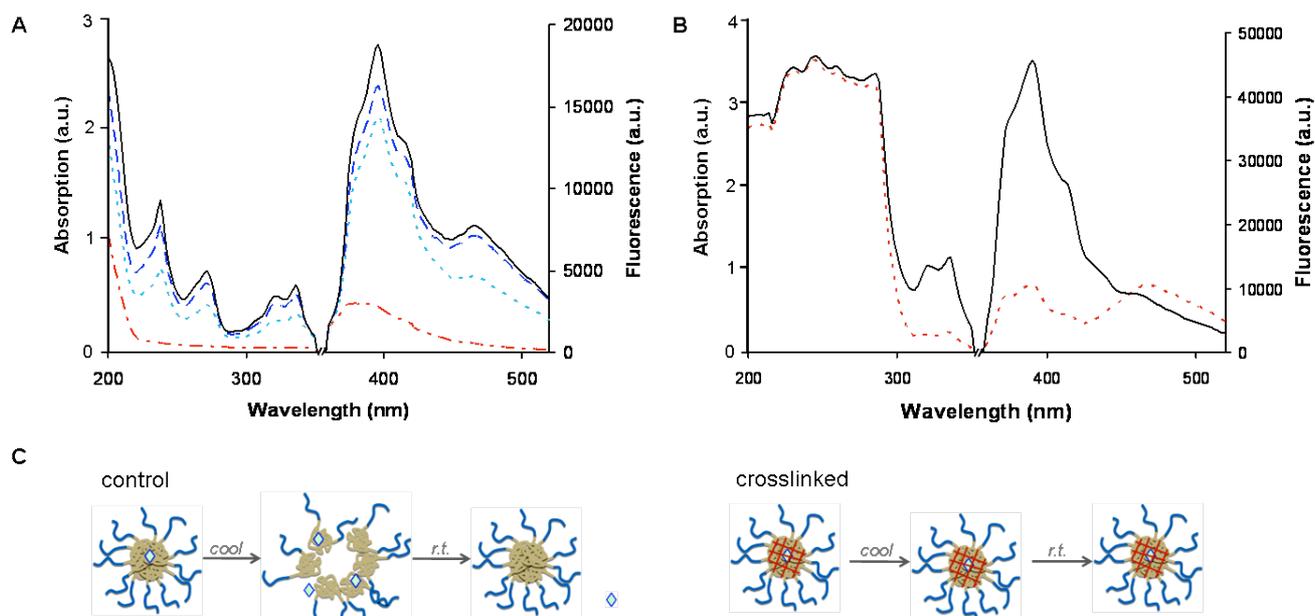


Fig. S1 Stabilised F127 micelles. (A) Absorption and fluorescence ($\lambda_{\text{ex}} = 330 \text{ nm}$) spectra of pyrene in the core of 1% F127 micelles with varying PETA concentration after overnight storage at 4°C : solid, 40% PETA; dashed, 20% PETA; dotted, 10% PETA; dot-dash, control without PETA. Incorporation of PETA drastically enhances the retention of pyrene, with increasing concentrations yielding progressively smaller effects. (B) Absorption and fluorescence ($\lambda_{\text{ex}} = 330 \text{ nm}$) spectra of pyrene in the core of mixed micelles with (solid) and without (dotted) PETA cross-linking after overnight storage at 4°C . The large sub-300 nm absorption is due to the presence of DNA. (C) Schematic of pyrene loss and retention due to storage below 4°C of non-stabilised and stabilised micelles, respectively.

3. Additional AFM Data

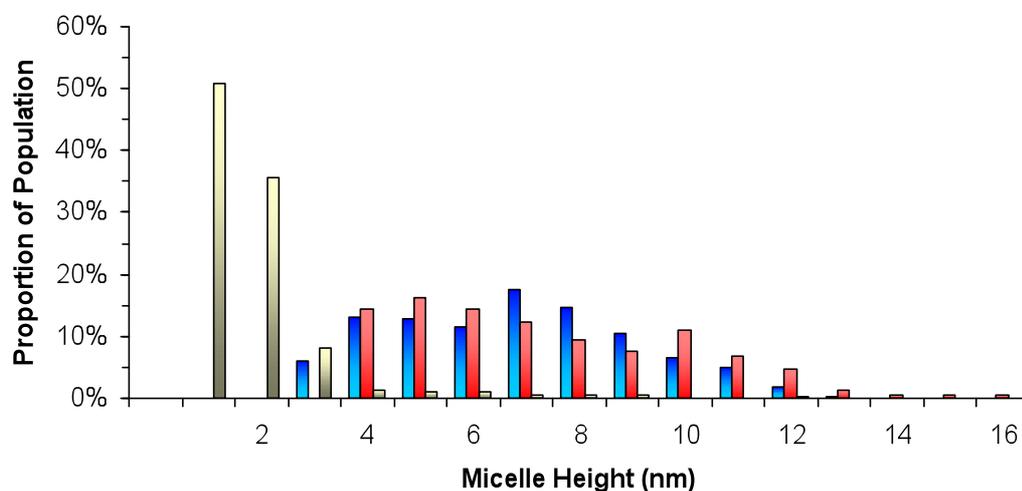


Fig. S2 Micelle height statistics collected from AFM images. Stabilised mixed micelles (left) had a total population of 561 and a mean height of $7.1 \pm 2.3 \text{ nm}$. Stabilised F127 micelles (middle) had a total population of 234 and a mean height of $6.9 \pm 2.6 \text{ nm}$. Non-stabilised **22PPO** micelles (left) had a total population of 349 and a mean height of $2.3 \pm 1.4 \text{ nm}$. Note a major effect of PETA cross-linking is to give a much greater proportion of large, well-formed micelles. The values differ from typical hydrodynamic radii due to the flattening of micelles on the mica surface and the predicted collapse of PEO and DNA chains in the corona due to drying.

4. Synthesis of TAMRA-labelled F127

Following a reported strategy for labelling Pluronic (Fig. S3) with modification,^{3,4} the terminal hydroxy groups of F127 were converted to amino groups (see Fig. S4 for the FT-IR spectra) and labelled with TAMRA.

Synthesis of F127-NH₂. Dry F127 (5 g, 0.4 mmol) was activated with carbonyldiimidazole (CDI) (257 mg, 1.6 mmol) in anhydrous dichloromethane (DCM) (50 mL) at 37°C for 4 h. After cooling to r.t., ethylenediamine (398 μ L, 6 mmol) and *N,N*-diisopropylethylamine (DIPEA) (277 μ L, 1.6 mmol) were added to the reaction mixture and further stirred for 48 h at r.t. resulting in 1-amino-2-ethanocarbamate functionalised F127 (F127-NH₂). The reaction mixture was dialysed using a 2kDa cutoff membrane against 15% ethanol for 4 days changing the buffer 7 times, and freeze-dried to yield 4.7 g (93%) of F127-NH₂ as a white solid.⁶

FT-IR (powder / cm^{-1}) = 3230 (N-H, broad), 2970, 2877, 2861, 2742, 2696, 2360, 1722 (C=O, sharp), 1466, 1373, 1360, 1342, 1280, 1241, 1145, 1095, 1060, 962, 947.

Synthesis of F127-TAMRA.⁷ To a solution of 200 mg (~15.7 μ mol) of F127-NH₂ in acetonitrile (2 mL), 2 mL of 0.1 M sodium tetraborate buffer (pH 8.5) was added and further stirred for 30 min. A solution of TAMRA-SE (19 mg, 31.8 μ mol) in DMSO (1 mL) was added to the mixture and subsequently stirred at r.t. for 18 h. The reaction mixture was diluted with 20% ethanol and dialysed with 2kDa cutoff membrane against 20% ethanol for 5 days at 4°C, changing the buffer 12 times. Residual TAMRA was again removed by gel filtration on NAP-25 column (GE Healthcare) and F127-TAMRA was eluted with ultrapure water. The polymer fraction was lyophilised yielding 124 mg (57%) in 80% coupling efficiency as determined by optical density of F127-TAMRA ($\epsilon = 65,000 \text{ cm}^{-1} \text{ M}^{-1}$) in 25 mM Tris buffer (pH 8.0).

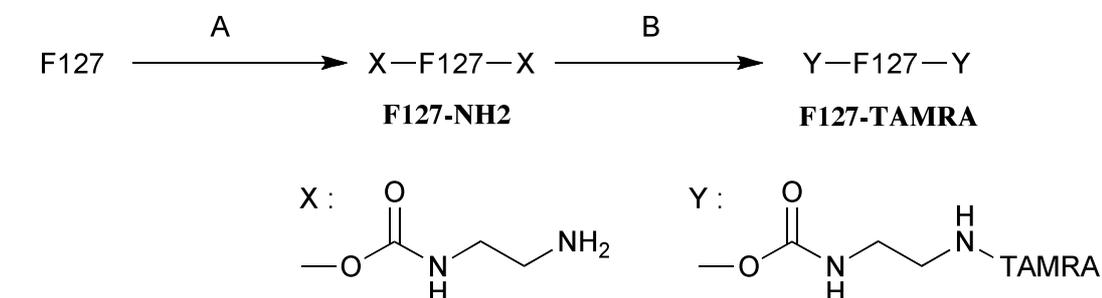


Fig. S3 Synthetic scheme of labelling F127 with TAMRA. (A) 1. CDI, DCM, 37°C, 4 h. 2. ethylenediamine, DIPEA, DCM, r.t., 48 h. (B) TAMRA succinimidyl ester, sodium tetraborate buffer (pH 8.0), r.t., 18 h.

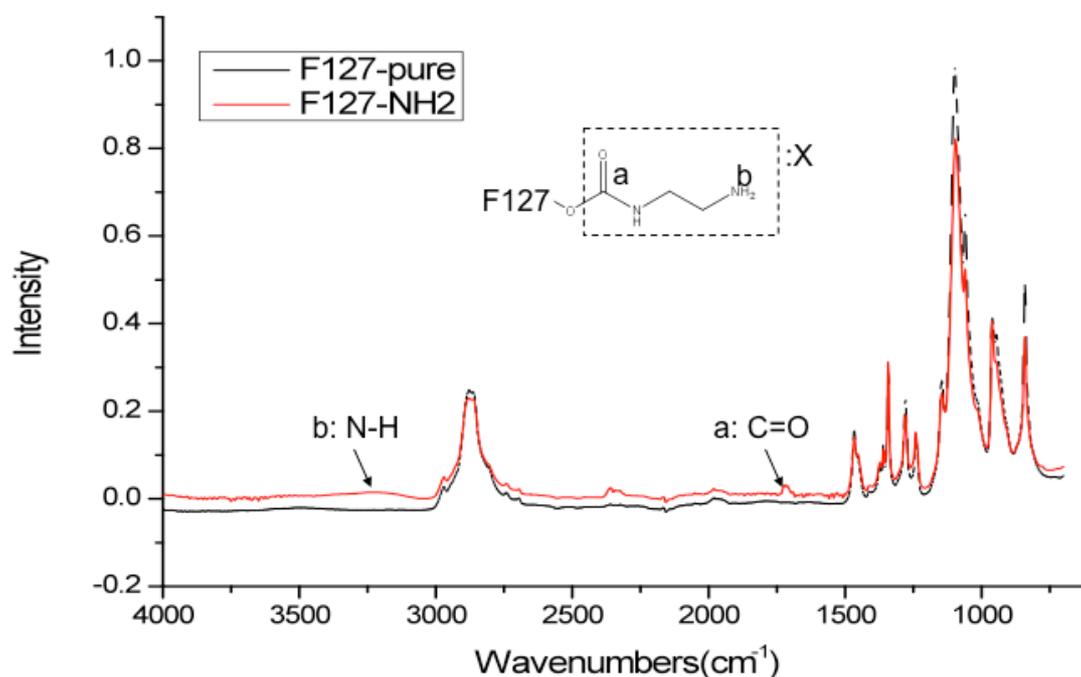


Fig. S4 FT-IR spectra of amino-functionalised F127 (red) and pristine F127 (black). Characteristic a and b bands of F127-NH2 were assigned. Other bands assigned are identical to those of pristine F127.⁵

5. FRET on Blend Micelles

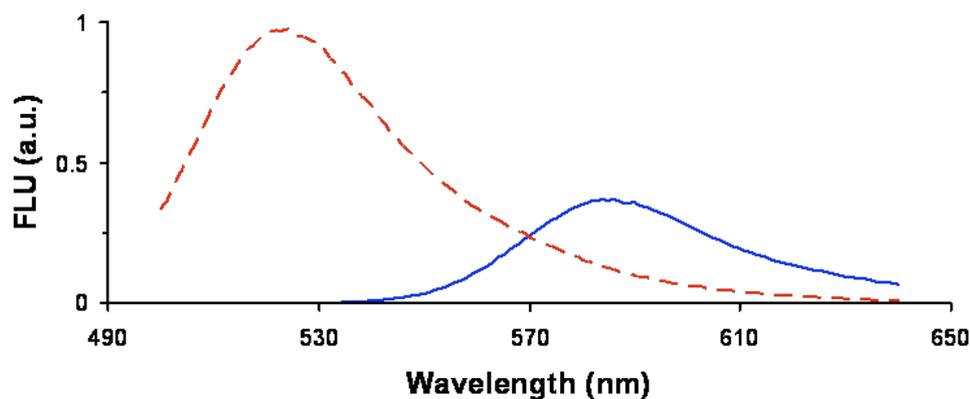


Fig. S5 Reference spectra for FRET experiments. Mixed micelles containing F127-TAMRA hybridised with pristine cDNA (solid) and mixed micelles containing unmodified F127 hybridised with cDNA-FAM (dashed).

10 A FRET pair of carboxyfluorescein (FAM) and 5-(and-6)-carboxytetramethylrhodamine (TAMRA) was selected to determine the close proximity of individually labelled polymer molecules and DNA. Mixed micelles were prepared for FRET by simple mixing. Hybridization between **22PPO** and cDNA (with or without FAM-cDNA) was accomplished by thermocycler (Eppendorf, The Netherlands). Each sample was prepared to a volume of 110 μ L in 0.5 \times TAE buffer with 100 mM NaCl and 60 mM

MgCl₂. The final concentrations of all relevant species are presented in Table S1. Note that the concentration of TAMRA in solution is approximately double the concentration of F127-TAMRA.

Table. S1 Final concentrations of mixed micelle components for FRET

	FAM-3' FRET	FAM-3' control	FAM-5' FRET	FAM-5' control	F127-TAMRA reference	FAM-3' reference
F127	160 μM	200 μM	160 μM	200 μM	160 μM	180 μM
F127-TAMRA	20 μM	20 μM	20 μM	20 μM	20 μM	0 μM
22PPO	40 μM	0 μM	40 μM	0 μM	40 μM	40 μM
FAM-3'-cDNA	40 μM	40 μM	0 μM	0 μM	0 μM	40 μM
FAM-5'-cDNA	0 μM	0 μM	40 μM	40 μM	0 μM	0 μM

6. Preparation of Au-NP and cDNA Conjugate

Single DNA (thiol-5'-cDNA) modified Au-NP at 5'-end (band B of Fig. S6) was prepared according to the protocol of Claridge *et al.* and resulted 590 nM solution.⁸ The isolated Au-cDNA was hybridised with mixed micelles in 0.5× TAE buffer with 100 mM NaCl and 60 mM MgCl₂.

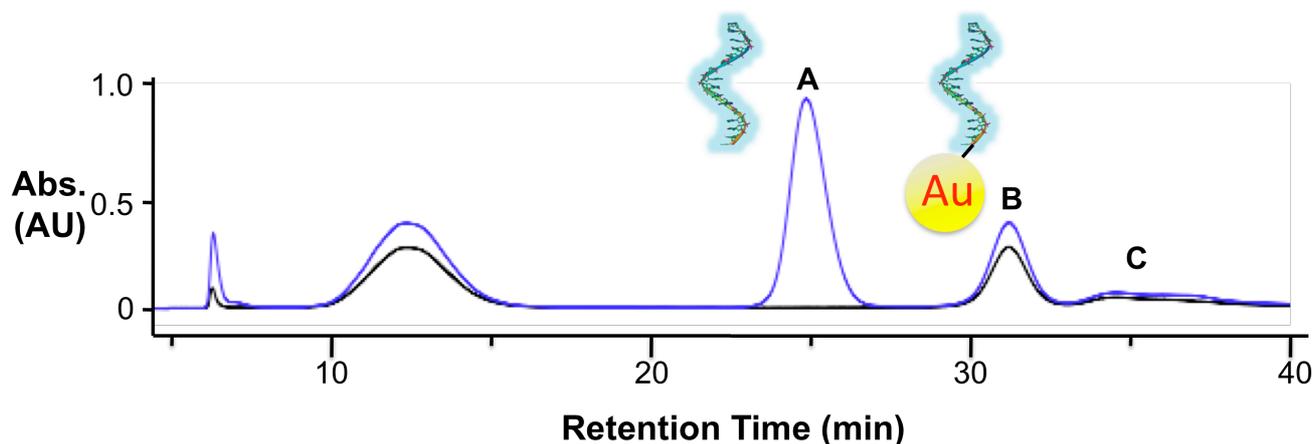


Fig S6 Anion exchange chromatogram of Au-cDNA crude mixture. Blue curve is elution monitored absorbance at 260nm (DNA) and black is 520nm (Au-NP). (A) Residual thiol-5'-cDNA which was not coupled to Au. (B) Single Au-cDNA band. (C) Elution of gold particles conjugates with multiple cDNAs. Fraction B was collected for TEM and AFM studies.

7. Statistical Analysis of Au-NPs in TEM Image

7.1. The nearest neighbour distance (d_{NN}) of particles

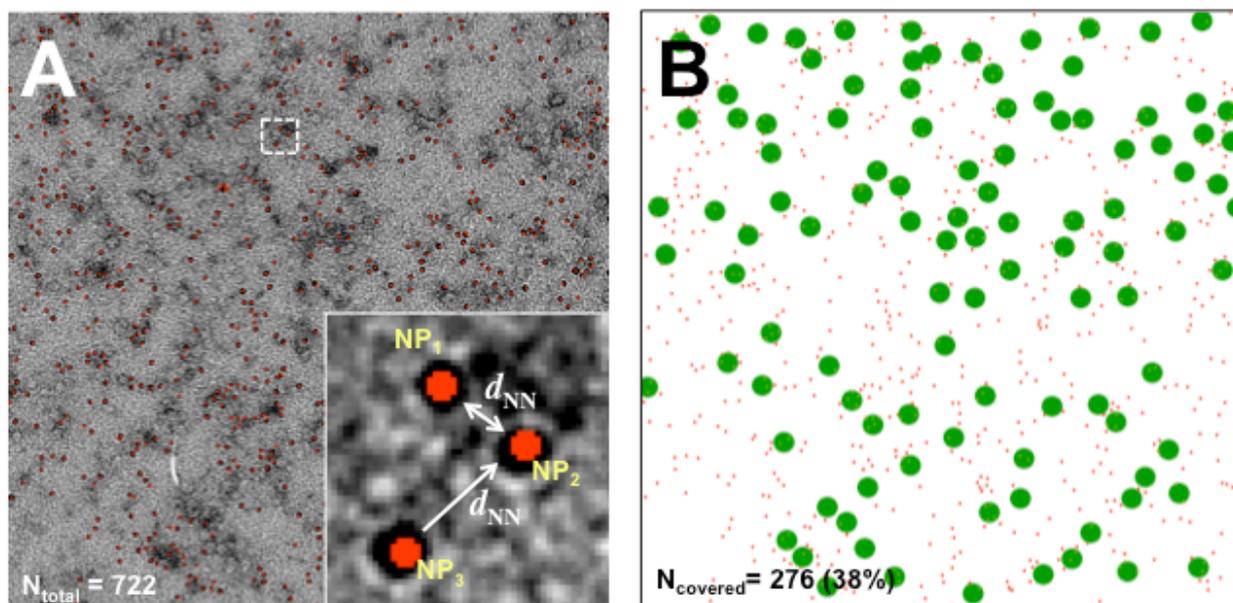


Fig. S7 Counting d_{NN} of Au-NPs in a TEM micrograph. (A) The edge-to-edge separation between NP3 and NP2 was attributed to NP3, while same, smaller edge-to-edge separation was recorded for both NP₁ and NP₂. Once counted, particles were coloured red for convenience. (B) The best-defined dark shadows representing micelles were coloured with 33 nm circles (green) and the AuNPs (red) covered by these circles were counted.

Nearest-neighbour separation statistics were extracted from the image in Fig. S7A by the following procedure. Using standard image processing software and high manual zoom as depicted in the inset, nearest-neighbour pairs were manually identified and the edge-to-edge distance (in pixels) between the two particles was recorded. One nearest neighbour separation was measured for each particle, i.e. in the inset of Fig. S7A the separation between NP₁ and NP₂ was counted twice, while the larger distance shown was attributed to NP₃. For convenience, nanoparticles were coloured red once they had been counted. In addition, the faint black shapes of micelles were covered by circles of 33 nm diameter. The number of Au-NPs covered by these circles was then counted (Fig. S7B). The total area of the circles, 0.091 μm^2 , represents 10% of the total scan area, and 276 (38%) of the AuNPs are then covered.

7.2. d_{NN} Simulation for randomly-generated particles

Because of the strong dependence of the distribution of nearest-neighbour separations on the number of particles in the scan area and the difficulty of preparing a TEM sample with the same coverage of random particles, a negative control experiment (*i.e.* without mixed micelles) could not be performed. Instead, a set of 722 Au-NPs (the same number as in Fig. S7A) were randomly placed on a field of 1024 by 1024 pixels using Matlab software (version 2009a, Mathworks) (Fig. S8A, the source code is attached). The nearest-neighbour distribution was then automatically calculated from the particle

coordinates as $d_{NNij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$, selecting the smallest d_{NN} per each particle. The columns in Fig 4C represent the averaged results of 100 such distributions. We set two thresholds established by our experimental data shown in Fig 4; 1) 50% of particles with d_{NN} of 6 nm or less, 2) 92% of particles with d_{NN} of 20 nm or less. The critical numbers of random particles (N_{Au}) required to meet 50% of each threshold are $N_{Au1} = 1530$ and $N_{Au2} = 1125$ for 1) and 2), respectively, (Fig. S8B) well above the particle number in the experimental data which established the thresholds ($N_{Au} = 722$).

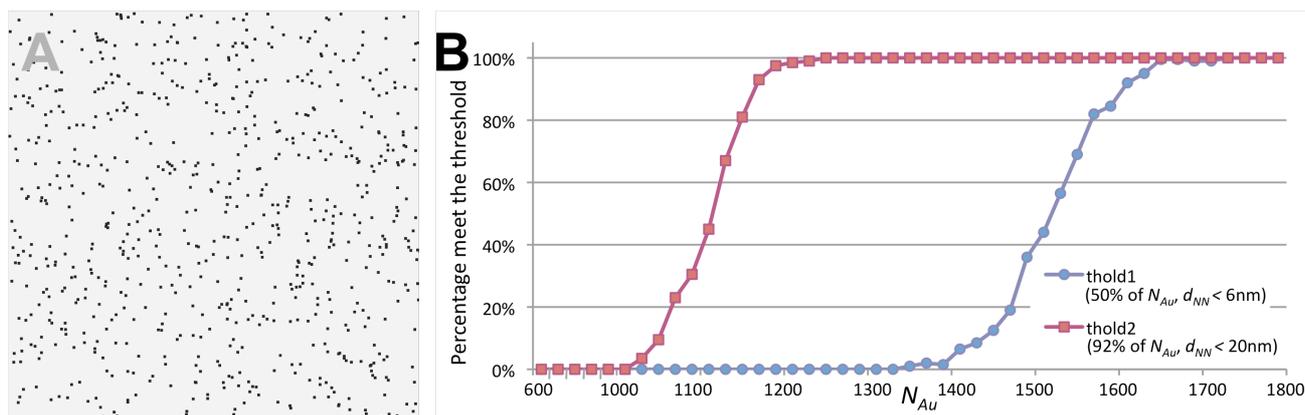


Fig. S8 Visual representation of simulated random particles. (A) An image of randomly generated particle distribution. On a 1024 by 1024 pixel (i.e. 916 by 916 nm) field, 722 particles with a diameter of 5 nm were placed as a simulated negative control experiment. (B) The percentage of N_{Au} -particle distributions which met each of the two thresholds. Each data point represents the percentage which meets the thresholds out of the 200 sets generated for the corresponding N_{Au} .

References and Notes

1. F. E. Alemдарoglu, K. Ding, R. Berger and A. Herrmann, *Angew. Chem., Int. Ed.*, 2006, **45**, 4206-4210.
- 15 2. Stability of DNA under the UV-irradiation: The same pristine single-stranded DNA (ssDNA) without PPO block was UV-irradiated for the same duration (2 h). Denatured polyacrylamide gel electrophoresis after the irradiation confirms that there is no fragmentation or change of molecular weight of ssDNA under the condition (result not shown).
3. Z. Yang, G. Sahay, S. Sriadibhatla and A. V. Kabanov, *Bioconjugate Chem.*, 2008, **19**, 1987-1994.
- 20 4. R. L. Wolin, H. Venkantesan, L. Tang, A. Santillan Jr., T. Barclay, S. Wilson, D. H. Lee and T. W. Lovenberg, *Bioorg. Med. Chem.*, 2004, **12**, 4477-4492.
5. Y.-L. Su, J. Wang, H.-Z. Liu, *Macromolecules*, 2002, **35**, 6426-6431.
6. The yield of F127-NH₂ was calculated assuming that the both hydroxy ends of F127 were converted into the 1-amino-2-ethanocarbamate groups.
- 25 7. During whole TAMRA labelling, all experiments were performed in dark atmosphere.
8. S. A. Claridge, H. Y. W. Liang, S. R. Basu, J. M. J. Frechet and A. P. Alivisatos, *Nano Lett.*, 2008, **8**, 1202-1206.

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1 % Random particle generator runs on Matlab2009a
2 % as a part of a manuscript "DNA-Functionalised Blend Micelles: Mix
  and Fix Polymeric Hybrid Nanostructures"
3 % by M. Kwak, A. J. Musser, J. Lee and A. Herrmann
4 % submitted to Chem. Commun. (Royal Chemical Society) in April, 2010.
5 % sourcecode of rparticle.m
6
7 noPixels = 1024;          % image size (px)
8 noParticles = 722;      % number of particles in a square
9 sizeNP = 5.0;          % size of the used particle (nm)
10 imageLength = 915.75;  % real size of the image calculated with
  magnification of microscope (nm)
11 tableD = zeros(noParticles); % empty matrix for table of distances
12 tableCount = zeros(1,11); % empty matrix for counts
13 cdnt = floor(noPixels * rand(2, noParticles)); % random x,y
  coordinates in the defined area
14 maxD = sqrt(2)* noPixels; % diagonal length of the image (px)
15
16 % calculate all distances between possible 2 (x,y) coordinates
17 for i=1:noParticles
18     for j=1:noParticles
19         tableD(i,j)=sqrt(power(cdnt(1,i)-cdnt(1,j), 2) +
  power(cdnt(2,i)-cdnt(2,j), 2));
20         % replace the distance of itself (0 px) to the maximum
  diagonal distance
21         if tableD(i,j) == 0
22             tableD(i,j) = maxD;
23         end
24     end
25 end
26
27 % find and save the d_NN (center to center, pixel) of each
  coordinates
28 tableMinD = min(tableD, [], 1);
29
30 % count particles in range
31 for i=1:noParticles
32     % unit conversion after applying size of the NP (pixel to nm)
33     l = imageLength / noPixels * tableMinD(i) - sizeNP;
34     % count the d_NN distribution
35     if l >= 20
36         tableCount(11)=tableCount(11)+1;
37     elseif l > 18
38         tableCount(10)=tableCount(10)+1;
39     elseif l > 16
40         tableCount(9)=tableCount(9)+1;
41     elseif l > 14
42         tableCount(8)=tableCount(8)+1;
43     elseif l > 12
44         tableCount(7)=tableCount(7)+1;
45     elseif l > 10
46         tableCount(6)=tableCount(6)+1;
47     elseif l > 8
48         tableCount(5)=tableCount(5)+1;
49     elseif l > 6
50         tableCount(4)=tableCount(4)+1;
51     elseif l > 4
52         tableCount(3)=tableCount(3)+1;
53     elseif l > 2
54         tableCount(2)=tableCount(2)+1;
55     else
56         tableCount(1)=tableCount(1)+1;
57     end
58 end
59
60 % draw a graph
61 bar(tableCount);

```