Electronics and 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 (Sigma-Aldrich, The Netherlands). Solid supports, Primer Support™ (200 µmol/g) from GE Healthcare, were used for the synthesis of 22PPO. ÅKTA oligopilot plus (GE Healthcare, Uppsala, Sweden) was used for DNA synthesis. ÅKTA 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 HiTrap™ Q HP 1 ml or 5 ml column (GE Healthcare, The 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'-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 (Mₜ = 12,600 g/mol), carbonyldiimidazole, ethylene diamine, pentaerythritol tetraacrylate (PETA) and pyrene were purchased from Sigma-Aldrich (The Netherlands) and were 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 MΩ) dispensed through 0.22 µm membrane filter using arium® 611 UF (Sartorius, 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 Rayonet™ 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 Gate™ 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-NH\(_2\) is averaged intensity of 1,000 measurements.

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.

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 H\(_2\)O (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.

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 ($\lambda_{\text{ex}} = 330$ 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 ($\lambda_{\text{ex}} = 330$ 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 ± 2.3 nm. Stabilised F127 micelles (middle) had a total population of 234 and a mean height of 6.9 ± 2.6 nm. Non-stabilised 22PPO micelles (left) had a total population of 349 and a mean height of 2.3 ± 1.4 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, 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 µL, 6 mmol) and N,N-diisopropylethylamine (DIPEA) (277 µL, 1.6 mmol) were added to the reaction mixture and further stirred for 48 h at r.t. resulting in 1-amino-2-ethancarbamate 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.

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.** To a solution of 200 mg (~15.7 µmol) 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 µmol) 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 (ε = 65,000 cm⁻¹M⁻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.](image-url)
**5. FRET on Blend Micelles**

**Fig. S4** FT-IR spectra of amino-functionalised F127 (red) and pristine F127 (black). Characteristic a and b bands of F127-NH$_2$ were assigned. Other bands assigned are identical to those of pristine F127.\textsuperscript{5}

**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).

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 µL in 0.5× TAE buffer with 100 mM NaCl and 60 mM...
MgCl$_2$. 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

<table>
<thead>
<tr>
<th></th>
<th>FAM 3’ FRET</th>
<th>FAM 3’ control</th>
<th>FAM 5’ FRET</th>
<th>FAM 5’ control</th>
<th>F127-TAMRA reference</th>
<th>F127-TAMRA reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F127</td>
<td>160 µM</td>
<td>200 µM</td>
<td>160 µM</td>
<td>200 µM</td>
<td>160 µM</td>
<td>180 µM</td>
</tr>
<tr>
<td>F127-TAMRA</td>
<td>20 µM</td>
<td>20 µM</td>
<td>20 µM</td>
<td>20 µM</td>
<td>20 µM</td>
<td>0 µM</td>
</tr>
<tr>
<td>22PPO</td>
<td>40 µM</td>
<td>0 µM</td>
<td>40 µM</td>
<td>0 µM</td>
<td>40 µM</td>
<td>40 µM</td>
</tr>
<tr>
<td>FAM-3’-cDNA</td>
<td>40 µM</td>
<td>40 µM</td>
<td>0 µM</td>
<td>0 µM</td>
<td>40 µM</td>
<td>40 µM</td>
</tr>
<tr>
<td>FAM-5’-cDNA</td>
<td>0 µM</td>
<td>0 µM</td>
<td>40 µM</td>
<td>0 µM</td>
<td>0 µM</td>
<td>0 µM</td>
</tr>
</tbody>
</table>

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. The isolated Au-cDNA was hybridised with mixed micelles in 0.5× TAE buffer with 100 mM NaCl and 60 mM MgCl$_2$.

![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.](image)

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 \( d_{NN} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2} \), 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


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).


6. The yield of F127-NH2 was calculated assuming that the both hydroxy ends of F127 were converted into the 1-amino-2-ethancarbamate groups.

7. During whole TAMRA labelling, all experiments were performed in dark atmosphere.

% Random particle generator runs on Matlab2009a
% as a part of a manuscript "DNA-Functionnalised Blend Micelles: Mix
and Fix Polymeric Hybrid Nanostructures"
% by M. Kwak, A. J. Musser, J. Lee and A. Herrmann
% submitted to Chem. Commun. (Royal Chemical Society) in April, 2010.
% sourcecode of rparticle.m

noPixels = 1024;    % image size (px)
noParticles = 722;  % number of particles in a square
sizeNP = 5.0;       % size of the used particle (nm)
imageLength = 915.75; % real size of the image calculated with
                      % magnification of microscope (nm)
tableD = zeros(noParticles);   % empty matrix for table of distances
tableCount = zeros(11);       % empty matrix for counts
cdnt = floor(noPixels * rand(2, noParticles));  % random x,y
coordinates in the defined area
maxD = sqrt(2)* noPixels;    % diagonal length of the image (px)

% calculate all distances between possible 2 (x,y) coordinates
for i=1:noParticles
    for j=1:noParticles
        tableD(i,j)=sqrt(power(cdst(1,i)-cdnt(1,j), 2) +
                        power(cdst(2,i)-cdnt(2,j), 2));
        % replace the distance of itself (0 px) to the maximum
        % diagonal distance
        if tableD(i,j) == 0
            tableD(i,j) = maxD;
        end
    end
end

% find and save the d_NN (center to center, pixel) of each
coordinates
tableMinD = min(tableD, [], 1);

% count particles in range
for i=1:noParticles
    l = imageLength / noPixels * tableMinD(i) - sizeNP;
    if l >= 20
        tableCount(11)=tableCount(11)+1;
    elseif l > 18
        tableCount(10)=tableCount(10)+1;
    elseif l > 16
        tableCount(9)=tableCount(9)+1;
    elseif l > 14
        tableCount(8)=tableCount(8)+1;
    elseif l > 12
        tableCount(7)=tableCount(7)+1;
    elseif l > 10
        tableCount(6)=tableCount(6)+1;
    elseif l > 8
        tableCount(5)=tableCount(5)+1;
    elseif l > 6
        tableCount(4)=tableCount(4)+1;
    elseif l > 4
        tableCount(3)=tableCount(3)+1;
    elseif l > 2
        tableCount(2)=tableCount(2)+1;
    else
        tableCount(1)=tableCount(1)+1;
    end
end

% draw a graph
bar(tableCount);