Supplementary Material (ESI) for Chemical Communications

DNA-coated microcrystals

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Supplementary Information

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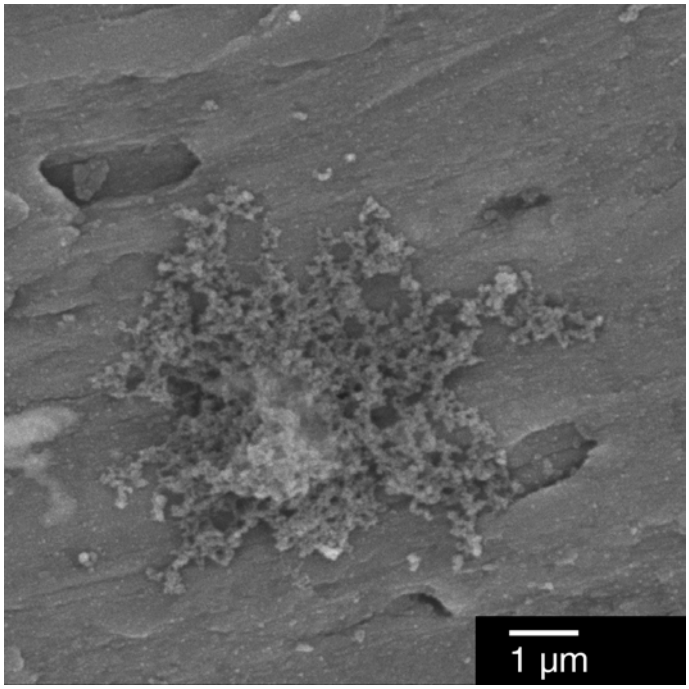


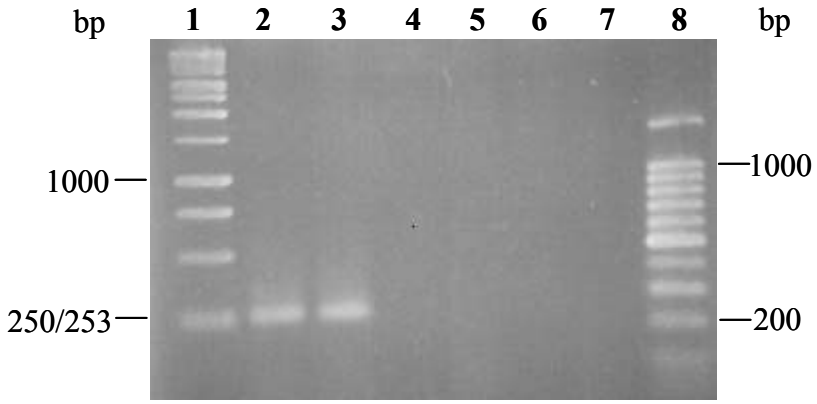
Fig. 1. SEM image of oligonucleotides (< 50 bp) precipitated in 2-propanol in the absence of any carrier.

Re-dissolution experiment

Further evidence that the majority of oligonucleotides are located on the surface of the carrier crystals was obtained from a redissolution experiment. DCMC made with DL-valine and crude oligonucleotides were prepared, dried and resuspended in a saturated aqueous solution of DL-valine. The carrier crystals are insoluble in this solution and so although DNA exposed to solvent on the surface will dissolve, DNA entrapped within the carrier crystal will remain bound. Known amounts of DCMC made of crude oligonucelotides and DL-valine (DNA loading: 10 wt%) were re-suspended in 1 ml saturated aqueous DL-valine solution. The suspension was incubated at 1000 rpm for 2h. The remaining solids were separated by centrifugation and the oligonucleotide concentration in the supernatant determined (OD_{260nm}). It was found that >90% of the DNA was found in the saturated solution while the core crystalline material remained as a crystalline suspension. This is consistent with the fluorescence microscopy and shows that the DNA is primarily on the surface of the carrier crystals.

PCR and Sequencing

PCR was performed, using the primers DQA-HEX and Rev 254. These primers were designed to amplify a 254 bp product from the human MHC class II HLA-DQ gene located on chromosome 6.[7] Both treated DQA-HEX (rehydrated from DCMC prepared with DL-valine as co-



precipitant, 0.1% DNA loading) and untreated DQA-HEX resulted in DNA amplification, as followed by gel electrophoresis (Fig. 3). There was no discernable difference in the intensity of the product bands in the gel, using either untreated (lane 2) or treated DQA-HEX (lane 3). This demonstrates that biological functionality of DQA-HEX was not compromised during the DCMC formation.

The sequence of both types of PCR products, produced with treated DQA-HEX primer (DCMC) or untreated DQA-HEX primer, was identical and in a BLAST search matched the human MHC class II HLA-DQ gene, as expected; (for gene sequence see S. Beck et al., *Nature* 1999, *401*, 921-923).

Fig. 2. Agarose gel (2.5%) electrophoresis of HLA DQ PCR products. Lanes: 1, 1 kb ladder; 2, 254 bp product using untreated DQA-HEX as primer; 3, 254 bp product using re-hydrated DCMC made of DL-valine and DQA-HEX as primer; 4, control: in absence of DQA-HEX; 5, control: in absence of the reverse primer Rev 254, 6, control: in absence of both primers; 7, control: in absence of genomic DNA; 8, 100 bp ladder.

Experimental details

Materials. DNA from herring sperm (< 50 bp), termed "crude oligonucleotides" was from Sigma, UK. Oligonucleotide Rev 254 (5'-CAC GGA TCC GGT AGC AGC GGT AGA GTT G) was synthesized by MWG Biotech (Germany). Oligonucleotide DQA-HEX (5'-HEX $(T^*C)_6$ GTG CTG CAG GTG TAA ACT TGT ACC AG) was kindly provided by Prof. D. Graham (University of Strathclyde, UK). (HEX = 2,5,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein, T* = 5-(3-aminopropynyl)-2′-deoxyuridine).[7] Herring sperm DNA (~2000 bp) and Lipofectamin 2000 were from Invitrogen, Paisley, Scotland. DL-Valine was from Fluka.

Production of DCMC. DCMC were prepared on a mg scale. DNA was used as an aqueous solution as obtained or if the starting material was a powder (crude oligonucleotides), this was dissolved in 10 mM Tris buffer (pH = 7.6). A wide range of DNA concentrations were applicable (0.2 to 10 mg/ml). The aqueous DNA solution was boiled for 1 min. Upon cooling to RT, a concentrated aqueous DL-valine solution (60 mg/ml) was added. The desired DNA loading was obtained by adding the required volume of a 60 mg/ml DL-valine solution to the quantity of DNA used for DCMC formation. It was also possible to dissolve the oligonucleotide powder directly in the aqueous DL-valine solution. The aqueous solution containing the DNA and DL-valine was mixed and then added dropwise with a Gilson pipettor to a vial with 2-propanol pre-saturated with DL-valine, which was vigorously stirred with a magnetic stirrer bar. The ratio of the volume of the aqueous DNA/DL-valine solution : volume of 2-propanol was 1:15. The resulting DCMC were separated by filtration (Durapore membrane HVLPO4700, Millipore, UK) and thoroughly washed with 2-propanol (pre-saturated with DL-valine). Then the DCMC were either air-dried or resuspended in 2-propanol (pre-saturated with DL-valine) and stored as such.

Microscopy. Scanning electron microscopy (SEM) was performed with a JEOL JSM 6400 Scanning Microscope. Suspensions of the DCMC in 2-propanol were air-dried and sputter-layered with gold. Confocal laser scanning microscopy was performed with a ZEISS LSM 5 Pascal microscope. Suspensions of DCMC (0.3 wt% DNA loading) in 2-propanol were deposited on a glass slide and air-dried. Excitation of HEX was at 535 nm and the emitted fluorescent light was detected at 556 nm.

Particle size analysis. The particle size of the DCMC (suspended in 2-propanol) was measured by laser light scattering (Malvern 2000, Malvern Instruments, Malvern, UK).

Differential Scanning Calorimetry (DSC). DSC of air-dried DCMC prepared of crude oligonucleotides and D,L valine (18.4wt% loading) was performed with a DuPont Instruments, 910 Differential Scanning Calorimeter. Temperature profile: Heat at 10°C/min from 30°C to 375°C; N_2 flow: 80 ml/min.

PCR. DCMC composed of DQA-HEX and DL-valine (0.1 wt% loading) were dissolved in H_2O, and without further purification used to provide the DQA-HEX primer for PCR. The primers DQA-HEX and Rev 254 were designed from the human HLA-DQ gene and generate a 254 bp product using PCR.[7] The PCR reaction mixture used contained 100 ng of human genomic DNA (D-5037, Sigma, UK), 10 mmol of each dNTP, 21 pmol forward primer DQA-HEX (treated primer: aqueous solution of DCMC made of DQA-HEX and DL-valine, 0.1wt% DNA loading;), 11 pmol reverse primer Rev 254, 2.5 units of AmpliTaq DNA Polymerase (Promega, UK), 10 mM TRIS-HCl, pH = 9.0, 50 mM KCl, 2 mM $MgCl_2$ and distilled water to a volume of 50 µl. For DNA amplification, the temperature program was initiated at 95°C for 3 min and subsequent temperature steps were as follows: 95°C for 15 s, 55°C for 15 s, and 72°C for 10 s, with 30 cycles followed by a temperature hold at 4°C. Products were visualized using gel electrophoresis to ensure amplification had occurred

(2.5% agarose). 1 kb and 100 bp DNA ladders were used as molecular weight markers (Promega, UK).

Sequencing. Sequences of 4 PCRs, using either untreated (as received) or treated primer DQA-HEX (DCMC) were obtained from MBSU Sequencing Service, University of Glasgow. Rev 254 was used to obtain the sequence data.