Electronic Supplementary Information

One-pot synthesis of oligonucleotide-grafted polymeric nanoparticles

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Electrophoretic light scattering of oligonucleotide-grafted polymeric nanoparticles after purification

An experiment to detect unbound DNA was designed. In this experiment, different volumes of an aqueous solution of non-functionalized single stranded DNA (ssDNA) with concentration 2.0 mg mL\(^{-1}\) were added to a dispersion of PNIPAm-coated mesoglobules (2 mL, 0.5 mg mL\(^{-1}\)), as follows: 0.15 mL (mixture 1) and 0.02 mL (mixture 2). Electrophoretic mobilities were measured and the results are presented in Figure S1 as $\zeta$ potential distributions. Figure S1 shows also results for PNIPAm-coated mesoglobules and final oligonucleotide-functionalized particles as reference samples. The two reference samples exhibit narrow monomodal distributions in sharp contrast to the mixtures, in which ssDNA exists as a separate species non-bound to the coated mesoglobules. The magnitude of the peaks varies consistently with the ssDNA/PNIPAm-coated mesoglobules ratio. The results clearly indicate that unbound ssDNA would be immediately detected by electrophoretic light scattering and indirectly prove the purification efficiency of dialysis as the final oligonucleotide-functionalized particles invariably exhibit monomodal size and $\zeta$ potential distributions.

Figure S1. $\zeta$ potential distributions from electrophoretic light scattering of mixtures of ssDNA and PNIPAm-coated mesoglobules, PNIPAm-coated mesoglobules and final oligonucleotide-functionalized particles after extensive dialysis.
Simulation of the reaction conditions of oligonucleotide attachment

Incubation of non-functionalized oligonucleotide with free radical-generating initiator for different time intervals at 65 °C.

Figure S2. Polyacrylamide gel electrophoresis of non-functionalized oligonucleotides. Lane assignments are as follow: molecular marker (A); pure oligonucleotide solution (B); the oligonucleotide solution from B incubated at 65 °C for 45 min (C); the oligonucleotide solution from B in the presence of the initiator AAPH incubated at 65 °C for 15 min (D), 30 min (E), and 45 min (F). See Experimental for the contents of the oligonucleotide and initiator.

Characterization data of oligonucleotide-grafted polymeric nanoparticles

In this section representative characterization data from UV-Vis, AFM, TEM, DLS, SLS, and electrophoretic light scattering are shown to complement the derived picture of the oligonucleotide-grafted polymeric nanoparticles.
Figure S3. (a) Size distribution functions from DLS of the initial PDEGMA mesoglobules (1), intermediate PHEMA-coated mesoglobules (2), and final oligonucleotide-functionalized ACH1 particles (3). Measurements were performed at 65 °C; ζ potential values of (2) and (3) are indicated as well. (b) AFM images and cross-sectional profiles of final oligonucleotide-functionalized ACH1 particles. (c) UV-vis spectra of intermediate PHEMA-coated mesoglobules, and final oligonucleotide-functionalized ACH1 particles (black and red traces, respectively).
Figure S4. (a) Size distribution functions from DLS of the initial PDEGMA mesoglobules (black), intermediate PHEMA-coated mesoglobules (green), and final oligonucleotide-functionalized ACH2 particles (red). Measurements were performed at 65 °C; ζ potential values are indicated as well. (b) UV-vis spectra of intermediate PHEMA-coated mesoglobules, and final oligonucleotide-functionalized ACH2 particles (black and red traces, respectively). (c) and (d) AFM images of final oligonucleotide-functionalized ACH2 and ACN2 particles, respectively.
Figure S5. Representative relaxation time distribution (a), angular dependence of the relaxation rate (b), concentration dependence of diffusion coefficients (c), and Berry plot (d). (a) – ACN2 at an angle of 100°, c = 0.61 mg mL\(^{-1}\) and 25 °C; (b) – ACH1 at c=0.093 mg mL\(^{-1}\) and 37 ºC; (c) – ACN2 at 25 °C; (d) – ACN2 at 37 °C. The lines through the data points in (b) and (c) represent the linear fit to the data.

**Numerical example of the calculation of the average number of oligonucleotide strands per particle**

The sample abbreviated by ACN1 was grafted with 0.3 mg of oligonucleotide. Given the molar mass of the oligonucleotide of 6707 g mol\(^{-1}\) and using the Avogadro's number, the total number of the oligonucleotide molecules is 2.69x10\(^{16}\).

The total solute content in 2.43 mL for ACN1 is 2.1 mg (c = 0.86 mg mL\(^{-1}\)). Given the molar mass of 136.0x10\(^6\) g/mol and using the Avogadro’s number, the number of particles is 9.27x10\(^{12}\). Hence, for the average number of oligonucleotide strands per particle we obtain:

\[
\frac{\text{Number of oligonucleotides}}{\text{Number of particles}} = \frac{2.69 \times 10^{16}}{9.27 \times 10^{12}} = 2902
\]

**Calculation of the Flory radius, \(R_F\)**

The Flory radius is the end-to-end distance of a free polymer coil in a theta solvent, resulting from the balance between the expanding steric forces and the counteracting entropic forces from stretching of the coils. In its simplest form it is given by equation (3):\(^1\)

\[
R_F = an^{3/5}
\]  

(3)

Here \(a\) is the length of the monomer unit and \(n\) is the degree of polymerization. The methacrylamide functionalized oligonucleotide is composed of a hexaethylene glycol spacer and 21 bases. If the lengths of ethylene oxide unit and nucleotide unit are taken as 0.39 nm and 0.34 nm, respectively, and the total degree of polymerization is 27 (= 6 + 21), then for \(R_F\) we obtain:

\[
R_F = \left(\frac{6}{27} \times 0.39 + \frac{21}{27} \times 0.34\right) \times (6 + 21)^{3/5} = 2.54 \text{ nm}
\]

**References**