

Well-Defined DNA Nanoparticles Templated by Self-Assembled $M_{12}L_{24}$ Molecular Spheres and Binding of Complementary Oligonucleotides

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Abstract: Perfectly monodisperse DNA nanoparticles were prepared via the self-assembly of 12 Pd(II) ions and 24 bidentate ligands functionalized with oligonucleotides of varying length ($n = 1-3$). The DNA nanoparticles formed hydrogen bonds with cDNA strands and an insoluble aggregate formed only with matching trimeric nucleotide strands (tri-A).

DNA-coated nanoparticles¹ are promising materials as biological sensors in DNA/RNA detection,² gene regulation,³ and biological screening.⁴ Recent applications have focused on their use in material applications where the subsequent recognition and binding of cDNA strands can template further structural elaboration.⁵ Gold clusters^{2a-k,3,4,5a-c} and polymer micelles^{2l,m} are typically utilized as platforms for DNA nanoparticles, but as the starting templates are ill-defined and structurally disperse, the resultant DNA nanoparticles exhibit a larger range of properties. If the DNA nanoparticles were isostructural, an enhancement of material properties and functions should result.

Here we report well-defined, perfectly monodisperse DNA nanoparticles exploiting self-assembled coordination nanospheres as nanoparticle templates to control the number, spacing, and alignment of the peripheral DNA strands. The assembly of $M_{12}L_{24}$ complexes⁶ from 12 Pd(II) ions (M) and 24 bidentate ligands (L) is well established. Our present strategy relies on the attachment of a single DNA strand at the convex of the bidentate ligands and their subsequent assembly into a nanosphere coated by precisely 24 DNA oligonucleotides (Figure 1).

Ligands **1a-c** were synthesized by coupling the desired protected oligo-thymine (T) derivative, prepared by the liquid-phase phosphoramidite method⁷ (see Supporting Information), to the $-OCH_2CH_2OH$ tether of the bispyridyl ligand. Protecting the phosphate is necessary, as the unprotected anion interferes in the self-assembly process. $M_{12}L_{24}$ sphere **2a** was constructed by treating ligand **1a** (10 μ mol) with $Pd(NO_3)_2$ (6 μ mol) in DMSO- d_6 (1 mL) at 70 °C for 2 h. The quantitative formation of $M_{12}L_{24}$ coordination nanosphere **2a** was suggested by 1H NMR spectroscopy (Figure 2) as the downfield shifts of the pyridine α and β proton signals ($\Delta\delta = 0.82$ and 0.59 ppm, respectively) are indicative of metal coordination. The phosphodiester tethers survived the complexation reaction, but $\sim 10\%$ of the terminal TBS protecting groups were hydrolyzed. Spheres **2b** and **2c** were also prepared in the same way.

Cold-spray ionization mass spectrometry (CSI-MS)⁸ of complexes **2a** and **2b** in CH_3CN revealed molecular weights of 21 693 and 30 267, respectively, after anion exchange of nitrate (NO_3^-) for tetrafluoroborate (BF_4^-) ions (see Supporting Information) and clearly demonstrated sphere identity and their precise fidelity. The low solubility of **2c** in CH_3CN precluded CSI-MS experiments,

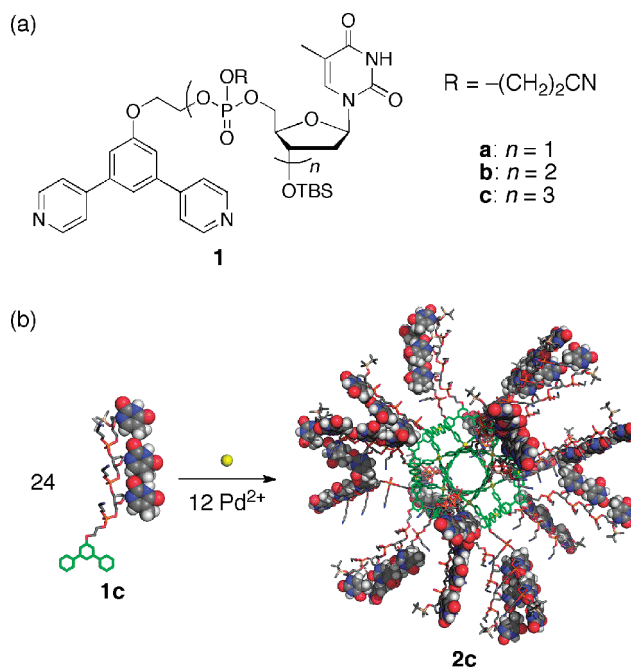


Figure 1. Self-assembly of DNA-displaying coordination nanospheres. (a) Structures of ligands **1a-c**. (b) Self-assembly of DNA-conjugated molecular sphere **2c** from 12 Pd(II) ions and 24 **1c** ligands. Ligands **1a** and **1b** also give the corresponding $M_{12}L_{24}$ spheres (consisting of 12 Pd(II) ions and 24 ligands).

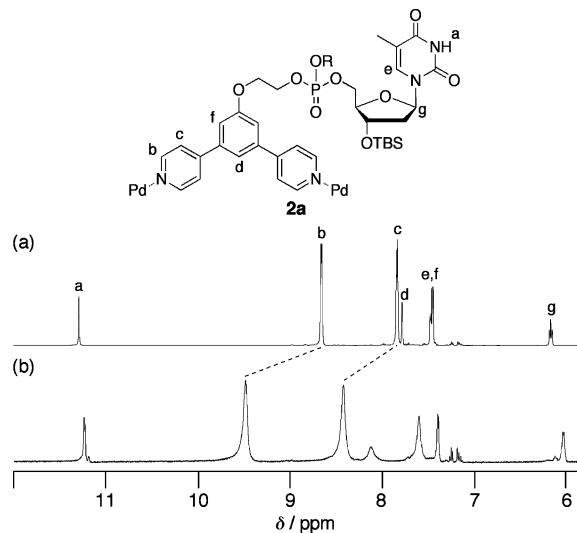


Figure 2. 1H NMR spectra of (a) **1a** and (b) **2a** (500 MHz, DMSO- d_6 , 300 K). Spectra displaying all the protons at a range of -1 to 12 ppm are shown in the Supporting Information.

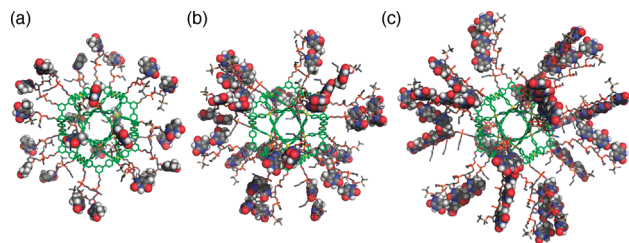


Figure 3. Molecular models of DNA coordination nanospheres (a) **2a**, (b) **2b**, and (c) **2c**.

but diffusion ordered NMR spectroscopy (DOSY) of **2c** in DMSO confirmed the formation of a single product with a diffusion coefficient of $(3.4 \pm 0.2) \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$. From the diffusion coefficient, the estimated diameter of **2c** is $4.4 \pm 0.2 \text{ nm}$, larger than the diameter of the core framework (3.5 nm in diameter) but indicates the peripheral DNA strands are not fully extended.⁹ Optimized structures of **2a–c** with 24 fully extended oligonucleotides predicted diameters of 6.5, 7.2, and 8.7 nm, respectively (Figure 3). The calculated surface density of DNA chains ($0.59 \text{ chains nm}^{-2}$) is higher than the previously reported value for DNA-covered gold nanoparticles ($0.23 \text{ chains nm}^{-2}$).^{3b}

The peripheral DNA strands of **2c** form Watson–Crick base pairs with complementary oligodeoxyadenosine trimers (tri-A). When 24 equiv of tri-A **3c** was added to a DMSO- d_6 /CDCl₃ (1:2) solution of **2c** (0.167 mM), the signals of the thymine amide protons shifted downfield ($\Delta\delta = 0.06 \text{ ppm}$), indicating the formation of a base pair between T and A (Figure 4a, red line).^{10,11} The downfield shift increased linearly with **3c** and was of comparable magnitude to pairs of free tri-T and tri-A (Supporting Information). In contrast, the amide proton signals of mono-T and di-T spheres, **2a** and **2b**, showed no notable downfield shifts when mixed with the complementary mono-A **3a** or di-A **3b**, thus indicating that the singly or doubly bound hydrogen bond pairs of **3a** and **3b** are insufficient and that the triply bonded H-bond pairs of **3c** are the minimum under these conditions (Figure 4a, green and blue lines).

When the solvent polarity was decreased (DMSO- d_6 /CDCl₃ = 1:4) and the concentration of complementary oligo-A **3a** and **3b** increased, hydrogen bonds with spheres **2a** and **2b** were observed. After 120 equiv of the di-A **3b** were added to the solution of sphere **2b** (0.167 mM), the signals of the thymine amide protons shifted downfield ($\Delta\delta = 0.51 \text{ ppm}$) (Supporting Information). At 120 equiv the thymine amide signals of mono-T sphere **2a** also shifted downfield but to a lesser extent ($\Delta\delta = 0.14 \text{ ppm}$) and indicate mono-A **3a** is only weakly bound (Figure 4b, red line).

Upon the addition of tri-A **3c** to sphere **2c** in the DMSO- d_6 /CDCl₃ (1:4) solvent mixture, a white solid immediately precipitated. Precipitation did not occur when **3a** or **3b** was added to **2c** nor did spheres **2a** and **2b** precipitate with **3c**. Only spheres **2c** and trinucleotide **3c** formed an insoluble hydrogen-bonded network structure.¹²

The DNA nanospheres recognized and selectively formed hydrogen bonds with the complementary nucleobase. Mono-T sphere **2a** bound mono-A **3a** (120 equiv) in DMSO- d_6 /CDCl₃ = 1:4 solution ($\Delta\delta = 0.14 \text{ ppm}$; Figure 4b, red line). Hydrogen bonds with mismatched G (**4**)¹³ or C (**5**)¹⁴ derivatives were not observed. Even the addition of excess **4** or **5** (120 equiv) to a solution of **2a** (0.167 mM, DMSO- d_6 /CDCl₃ = 1:4) produced only minor shifts in the thymine amide proton signals on **2a** ($\Delta\delta = 0.01$ and 0.04 ppm ; Figure 4b, blue line and green line, respectively). These results show that oligonucleotide-modified spheres prepared here will be promising materials as molecular DNA nanoparticles, which selectively recognize cDNA strands.

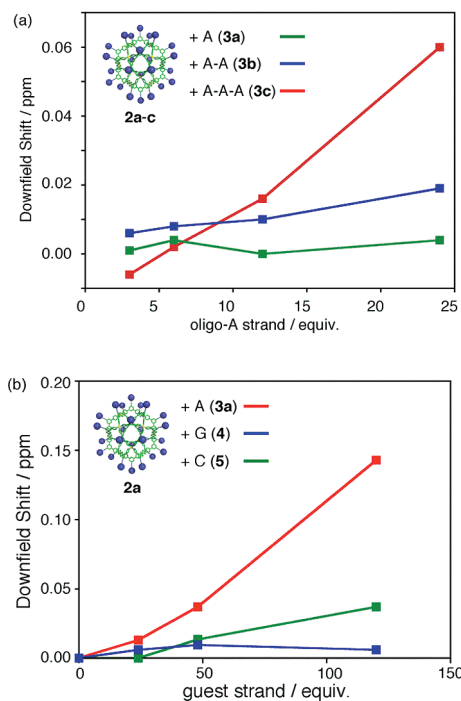
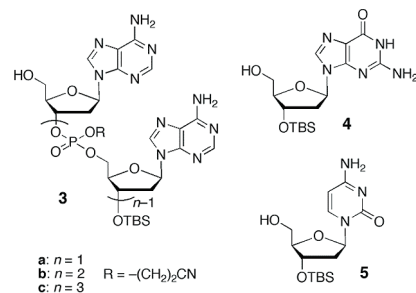


Figure 4. (a) Values of downfield shifts of thymine proton signals after the addition of oligo-A strand **3a–c** to the solutions of corresponding oligo-T spheres **2a–c** as a function of the equivalent of the oligo-A strand (green line: **2a** + **3a**; blue line: **2b** + **3b**; red line: **2c** + **3c**) ([**2**] = 0.167 mM, DMSO- d_6 /CDCl₃ = 1:2, 500 MHz, 300 K). (b) The values of downfield shift of thymine proton signals of **2a** after the addition of mono-A (**3a**), G (**4**), C(**5**) (red line: **2a** + **3a**; blue line: **2a** + **4**; green line: **2a** + **5**) ([**2**] = 0.167 mM, DMSO- d_6 /CDCl₃ = 1:4, 500 MHz, 300 K).

In summary, we have achieved the facile preparation of the M₁₂L₂₄ spherical complexes with their surface modified with 24 oligonucleotide chains by attaching the oligonucleotide to the convex of the bidentate ligand. We have also succeeded in the selective recognition of complementary oligonucleotides by using the oligonucleotide-coated spheres through the formation of the hydrogen bonds. The DNA nanoparticles prepared here have well-defined platforms of perfect fidelity on which precisely 24 oligonucleotide chains are densely arranged. In due course, the attachment of longer DNA chains with anionic phosphate backbones will lead to a new recognition platform for natural DNA in aqueous media.

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Supporting Information Available: Synthesis and characterization data of the compound and additional figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (9) The diameter of **2c** (4.4 ± 0.2 nm) was estimated from the Stokes–Einstein equation (standardized with X-ray structure of a relevant complex^{6b}). Since molecular modeling reveals 8.7 nm for the diameter of **2c** with extended DNA strands (Figure 3c), the DNA strands seem to adopt a shrunken conformation rather than an extended one.
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