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DNA-driven self-assembly of gold nanorods

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Specific organization of gold nanorods into anisotropic 3D-aggregates is obtained by DNA hybridisation.

Symmetry breaking and the associated introduction of anisotropy in nanoparticle systems have been a major challenge for chemists and physicists working on nano-materials in recent years. Two distinct approaches have been explored. First, lower symmetries can be obtained by assembling spherical particles in anisotropic environments. Mixtures of different types of particles,[1] microcontact printing,[2] 1D arrays of particles along biolipid tubules[3] or in block copolymers[4] are some examples of this approach. The synthesis of anisotropic nanoparticles constitutes a second strategy. A wide variety of materials has recently been made available in the shapes of elongated rods[5–8] or more complex morphologies.[9] The anisotropy of these building blocks can be transferred to superstructures even by isotropic interactions such as hydrophobicity.[7,10] Although these interactions are efficient in producing large architectures, they are neither specific or selective, thus preventing a rational design of the self-assemblies. In this report, we show how the selective and reversible DNA-driven assembly of nanoparticles can be applied to gold nanorods.

The formation of networks of spherical gold nanoparticles crosslinked by well-defined oligonucleotide duplexes has been recently demonstrated.[11–13] In this approach, two non-complementary strands of DNA are immobilised on the surface of two batches of 13-nm particles. Upon mixing the two populations, no recognition occurs but adding a third strand half complementary to each of the grafted sequences induces hybridisation, which drives the self-assembly.[11,12] Not only is this method selective enough to allow the detection of a single base mismatch in the oligonucleotide sequence,[12] but the self-organisation is also reversible as the DNA duplex can be dissociated by heating.[13] By transferring this technique to non-spherical nanoparticles, we define a method to organise anisotropic materials that may exhibit orientation-dependant physical properties.

Gold nanorods used in this work were prepared by the electrochemical conversion of a gold anodic material into particles in an electrolytic co-surfactant system as described elsewhere.[5] The surfactants were hexadecyltrimethylammonium bromide (CTABr, Sigma, 99%) and tetradodecylammonium bromide (TDABr, Fluka, 98%). After synthesis, the colloidal suspensions were repeatedly centrifuged and washed with deionised water to remove excess surfactant. The concentration of nanorods was then adjusted to an optical density of *ca.* 0.5 at 800 nm in 5-mm lightpath UV cells. The average aspect ratio of the rods was 4.7 (*ca.* 10 nm in diameter and 50 nm in length) as inferred from transmission electron microscopy (TEM) images as well as from the position of the longitudinal plasmon band (850 nm).[5]

Two oligonucleotide systems were examined and all sequences were purchased from Oswell DNA Service, Southampton, UK. The first one, which is shown in the insert of Fig. 2, was identical to the complementary three-strand system reported by Mirkin and coworkers.[12] The second system was composed of two directly complementary strands of thiolated oligonucleotides. The sequence of this duplex is shown in Fig. 3.

Functionalisation of the nanorod surface was performed according to the reported method.[12] Briefly, the thiol group of the DNA strands was activated by treatment in 0.1 M of dithiothreitol (DTT, Aldrich, 99%) at pH 8.4 and room temperature for 1 h. The solutions were then purified through a desalting column (NAP-5, Sephadex G-25 Medium). The different thiolated oligonucleotides were then left to react at room temperature with the nanorod solutions for up to 72 h. Typical final concentrations were *ca.* 5×10^8 nanorods per mL (*i.e.* 1 pM) and 1 nM of DNA. After incubation, the suspensions were centrifuged at 5000 rpm for 20 min, decanted and redispersed in double distilled water to the same concentration.

For hybridisation experiments, identical volumes of equimolar suspensions of functionalised nanorods were mixed in a 5-mm lightpath UV cell at room temperature. Aggregation was monitored by recording UV–VIS spectra as a function of time on a Lambda 11 Perkin-Elmer spectrophotometer. For the complementary two-strand system, duplexation occurred immediately upon mixing the complementary nanorods suspensions. For the three-strand system, aggregation was initiated by adding the free, non-thiolated oligonucleotide, the final concentration of which was typically 10 nM.

As hybridisation progressed, the colour of the suspension changed from red to purple and macroscopic precipitation was observed after 24 h. The nature of the organisation of the nanorods in the purple suspensions was elucidated by TEM. In contrast to as-synthesised or DNA-covered but non-duplexed nanorod suspensions, which showed totally random aggregates when examined under the same conditions, numerous organised aggregates, often several micrometers in size, were observed for the hybridised suspensions. The samples were composed of 3-D bundles of hundreds of nanorods aligned in parallel stacks (Fig. 1). Both alternate and superimposed vertical stacking of the rods could be seen. The inter-particle distance was about

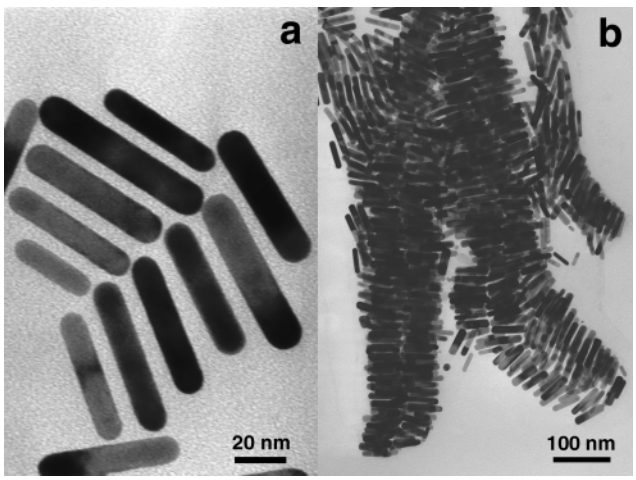

Fig. 1 Transmission electron micrographs of bundles of DNA-linked gold nanorods. Topological characteristics of the three-strand (a) or two-strand (b) DNA linking systems were similar.

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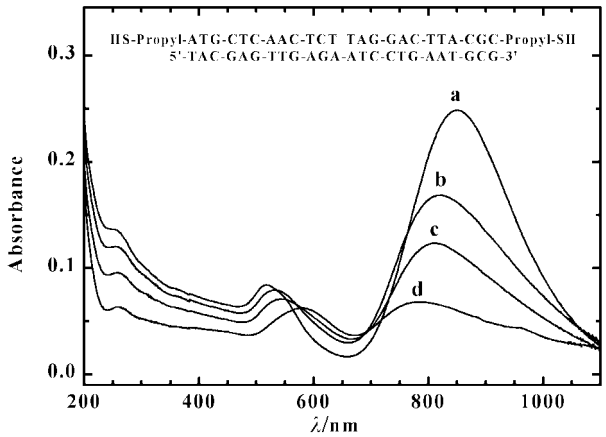

Fig. 2 UV–VIS spectra of a suspension of non-complementary DNA-functionalised nanorods. (a) 0 s, (b) 10 min, (c) 1 h, (d) 2 h after duplex initiation arising from the addition of the third complementary strand. Sequences of the three oligonucleotides are shown in the insert.

6 nm (Fig. 1a), which is longer that an interdigitated CTA bilayer (*ca*. 3 nm)[14] but commensurate with the length of a 24-base pair helix with two propyl spacers (6.5–9.0 nm). This is consistent with an aggregation process resulting from DNA duplex formation rather than from hydrophobic interactions between the CTA alkane chains.

Fig. 2 shows a typical series of spectra taken at various times after the addition of the third complementary oligonucleotide in the three-strand system. The colour change, which the nanorods share with the DNA-driven assembly of isotropic particle suspensions, was confirmed by the decrease in intensity and the red-shift of the transverse plasmon band (520 nm).[12,15] The UV–VIS spectra also showed a longitudinal band at 850 nm that decreased markedly in intensity and was blue-shifted with aggregation. A shoulder peak at 260 nm confirmed the presence of the nucleotides. Moreover, the intensity of this peak was reduced with time, consistent with the onset of base-pairing.[13] In the two-strand system, the decrease in intensity of the three bands upon hybridisation was also observed (Fig. 3, solid and dotted lines). However, the plasmon bands undergo almost no wavelength shift.

Further evidence that the aggregation was driven by DNA duplex formation was given by 'melting' analysis. This consists in dissociating the duplex by heating the sample above a temperature, which, in the case of the three-strand system, is 53 °C.[12] For this purpose, the purple suspensions were heated up to 70 °C by a thermostated bath and UV–VIS spectra were recorded at regular intervals. Hence, upon heating the duplexed suspension of the two-strand system, the intensities of the three bands were essentially recovered at 60 °C (Fig. 3, dashed curve), indicating reversible duplex formation between the complementary nanorod surfaces. Surprisingly, no change in the spectrum of the duplexed three-strand system could be observed and the suspension stayed irreversibly self-assembled.

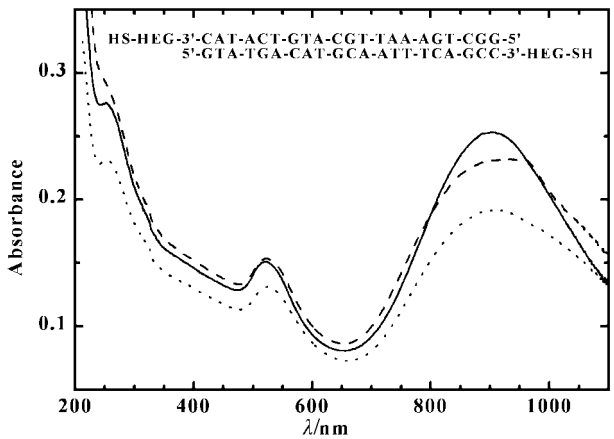

Fig. 3 UV–VIS spectra of the two-strand nanorod system before (—) and after (···) duplexation at 25 °C. The dashed curve was obtained after melting the DNA above 60 °C. The mismatch at higher wavelengths between the initial and denatured spectra is attributed to imperfect background compensation at high temperature. The sequences of the two strands are shown in the insert; HEG = hexaethyleneglycol.

Although further experiments are needed to explain this irreversibility, it is probable that the aspect ratio of the rods and/or the residual CTA molecules on the gold surface play a role. Indeed, whereas the base pairing is certainly the driving force of the self-assembly in both systems, a 12-base oligonucleotide with a propyl spacer (three-strand system) would barely emerge from a residual CTA bilayer. Once the aggregates are formed by duplexation, a multilayered CTA structure could rearrange between neighbouring rods and hydrophobic interactions could then become dominant. Upon heating for the 'melting' experiments, increases in the hydrophobic forces by dehydration of the alkane chains as well as electrostatic interactions between the $-NMe_3^+$ headgroups and the now unpaired DNA strands would reinforce this multilayered structure making rod aggregation irreversible. By contrast, the 21-base HEG-coupled oligonucleotides used in the two-strand system are sufficiently long to extend well beyond a residual CTA bilayer. Thus, in this case, the surfactant layers are not close enough to rearrange and the base pairing is therefore reversible. In addition, as no free DNA strand is released upon 'melting', the multilayered CTA structure would not be stabilised by electrostatic interactions.

In conclusion, this report has shown that large scale uniaxial organisation of metallic nanorods can be tailored by using the specific DNA duplex formation. Beyond DNA, antigen/antibody or protein/substrate bindings can be envisioned to obtain similar programmable assembly of anisotropic building blocks.[16] Systematic organisation of nanorods made of different materials is a straightforward development of this approach that should lead to anisotropic nanomaterials with more complex properties.

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