Controlled self-assembly of nucleotide–lanthanide complexes: specific formation of nanofibers from dimeric guanine nucleotides†

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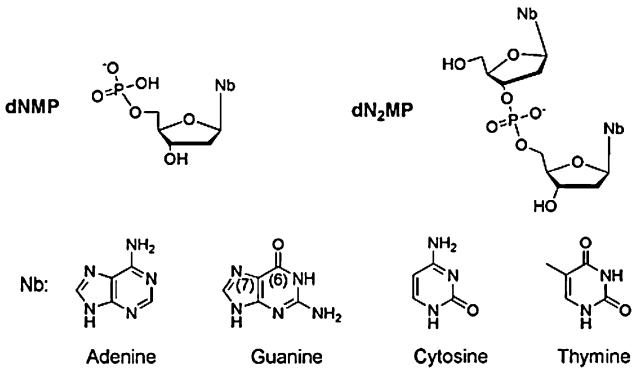
Monomeric and dimeric guanine nucleotides monophosphate spontaneously self-assemble into nanoparticles and nanofibers in the presence of lanthanide ions, which reflects differences in the unit coordination structures and their hierarchical assembly.

The self-assembly of oligonucleotides is driven by cooperative and multiple non-covalent interactions (hydrogen bonding, hydrophobic, van der Waals, stacking, electrostatic and ion–dipole interactions). Their self-assembling properties render them interesting candidates for the design of nano- to micrometric structures with a broad diversity of morphologies (DNA origami strategy).[1] On the other hand, little is known about the self-assembly of mononucleotides and small oligonucleotides. One exception concerns the self-assembling behaviour of guanine based G-quartet systems, which were first identified in the early 1960s.[2] Formation of alkali metal ion-mediated G-quartets of 5′-guanosine monophosphate was reported for highly concentrated water solutions.[3] While G-quartets focus attention notably because of their biological implications,[4,5] self-assembly between other nucleotides and cationic species has comparatively been left over, in spite of their rich structural diversity. Indeed, although we previously reported the self-assembly of adenosine 5'-triphosphate and cyanine dyes into supramolecular nanofibers,[6] nucleotides and small oligonucleotides have not been considered as building blocks for the design of new supramolecular systems. In this context, nucleotides have rather been used along with self-assembling building blocks, such as surfactants.[7]

We make use of the bifunctional properties of nucleotides to self-assemble into nano-architectures. Our focus is to investigate and compare self-assembling characteristics of dimeric deoxynucleotides monophosphate (dN_2MP) with those of monomeric dNMP (Scheme 1). dN_2MPs are dimeric derivatives bearing one anionic phosphodiester group and two identical nucleobases. According to the computational calculation (MacroModel/Maestro 8.0.314, Schrödinger, LLC), dN_2MP phosphodiester bond constraints molecules to adopt a unique pincer-like structure. Indeed, the phosphodiester bond constraints the two bases in parallel orientation with a fixed distance, inducing strong aromatic stacking interactions between the bases (Fig. 2(a) and Fig. S1, ESI†). It is interesting to see if such unique biomolecular structure leads to characteristic self-assembling properties. In this work, the behaviour of small nucleotidic building blocks has been investigated in the presence of terbium (Tb^{3+}) ions, which were selected because of their high affinity to phosphate groups and potential luminescent properties.[8] Unless otherwise mentioned, dNMP and dN_2MP are investigated at a fixed concentration of 0.5 mM in the presence of 0.25 mM terbium chloride in 0.05 M Hepes buffer. The nucleobase unit has been systematically varied so as to study all four nucleobases adenine, guanine, cytosine and thymine. Simply by mixing dNMP or dN_2MP with Tb^{3+} ions in aqueous solution, supramolecular nano-architectures were spontaneously formed.



Scheme 1 Molecular structure of mono (dNMP) and di-deoxynucleotides monophosphate (dN_2MP) bearing one of the nucleobases (Nb): adenine, guanine, cytosine, and thymine.

Starting from dNMP-Tb^{3+} complexes, transmission (TEM) and scanning (SEM) electron microscopy revealed the formation of nanoparticles having a diameter of about 20 nm.[9] Similar nanoparticles were observed, whatever the nucleobase considered (Fig. S2, ESI†). Thus, no morphological diversity could be generated by varying the nucleobase in dNMP. These nanoparticles are stable, since no morphological variation could be observed up to several months. Dimeric dN_2MP were investigated in identical conditions. Nanoparticles having a diameter of about 10 nm were observed for dA_2MP, dC_2MP and dT_2MP upon coordination with Tb^{3+} ions (Fig. 1(a), (c) and (d)). Very interestingly, nanofibers were formed shortly after mixing dG_2MP and Tb^{3+}, instead of nanoparticles (Fig. 1(b)). These nanofibers were observed at varied nucleotide : lanthanide (Tb^{3+}) molar ratio from 1 : 1 to 3 : 1 (Fig. S3, ESI†).

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† Electronic supplementary information (ESI) available: Detailed experimental section, calculated conformation, dNMP-Tb^{3+} self-assembly, energy dispersive X-ray spectrometry, possible models for nanofiber and nanoparticle formation, influence of nucleotide : lanthanide ratio and solvent. See DOI: 10.1039/b815779k

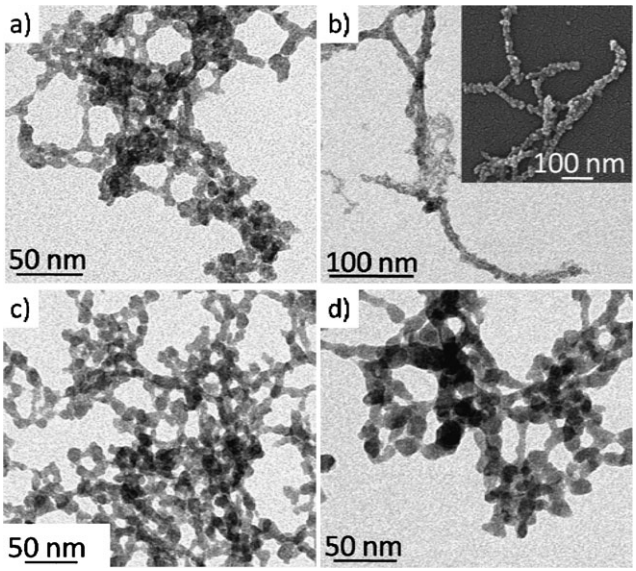

Fig. 1 TEM and SEM (insert) micrographs of the supramolecular structures formed by dN_2MP coordination with Tb^{3+} ions in Hepes buffer. Nanoparticles are formed except in the case of dG_2MP, where nanofibers are observed: (a) dA_2MP, (b) dG_2MP, (c) dC_2MP, (d) dT_2MP.

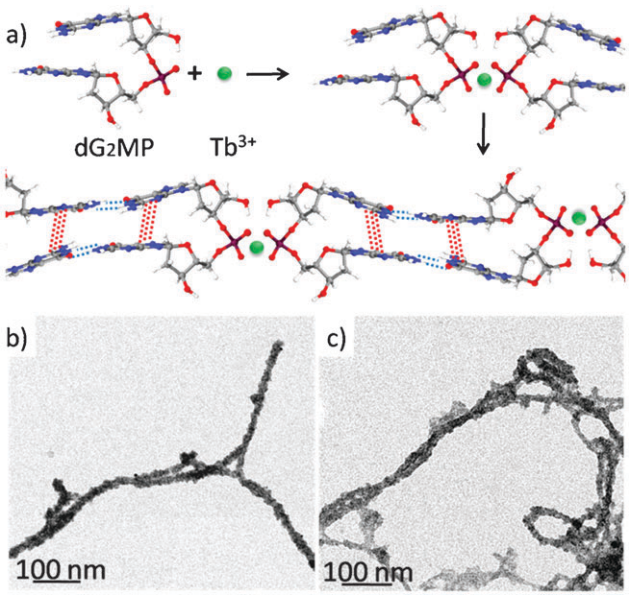

Fig. 2 (a) A model proposed for the self-assembly of pincer-like dG_2MP and Tb^{3+} ions into a unit structure of nanofibers (counter-anions are omitted for clarity). Red and blue dots represent stacking interactions and hydrogen bonding, respectively. TEM micrographs of nanofibers formed from dG_2MP-Ln^{3+} in Hepes buffer: (b) Eu^{3+}, (c) Gd^{3+}.

While all small nucleotidic building blocks successfully assemble into these nanostructures upon coordination with Tb^{3+} ions, the nanofiber structure is formed specifically from the dimeric dG_2MP. Electrostatic interactions between dG_2MP and Tb^{3+} would favor the coordination of Tb^{3+} ions with multiple dG_2MP molecules. However, the number of dG_2MP molecules coordinating to a small Tb^{3+} ion (ionic radius, 1.78 Å)[10] would be limited, because of their bulky pincer-like structures. We assume from energy dispersive X-ray spectrometry that two dimers are complexed with Tb^{3+}, along with Hepes molecules (Fig. 2(a) and Fig. S4–S5, ESI†). These anionic phosphodiester ligands would be arranged so as to minimize the steric hindrance, with stacked guanine bases of dG_2MP molecules oriented far from the central Tb^{3+} ions (Fig. 2(a)). This unit supramolecular structure would be able to self-assemble anisotropically into nanofibers, *via* multiple intermolecular interactions (hydrogen bonding, van der Waals and stacking) (Fig. 2(a) and Fig. S5, ESI†). Meanwhile, the other dimers dA_2MP, dC_2MP and dT_2MP gave nanoparticles (Fig. 1(a), (c) and (d)). It was against our expectation that nanofibers were not formed, particularly from dA_2MP, which contains another pair of puric bicyclic nucleobases. However, while adenine and guanine have similar stacking propensities,[11] guanine exhibits edges having self-complementary hydrogen-bond donors and acceptors. In addition, the large aromatic surface of guanine unit possesses a high dipole moment and is hence highly polarizable. These factors may account for the higher degree of intermolecular interactions observed for dG_2MP.[4,11] In parallel, we also investigated the effect of lanthanide ions (Ln^{3+} = Eu^{3+}, Gd^{3+}) on supramolecular structures formed from dGMP-Ln^{3+} and dG_2MP-Ln^{3+} complexes in aqueous solution. Nanoparticles were formed from monomeric dGMP-Ln^{3+} (Fig. S6, ESI†), while nanofibers were formed from dG_2MP-Ln^{3+} complexes (Fig. 2(b), (c)). Thus, formation of nanofibers from dG_2MP and lanthanide ions is a general phenomenon.

To obtain information on the coordination environment in dGMP-Tb^{3+} nanoparticles and dG_2MP-Tb^{3+} nanofibers, luminescence properties were investigated. It is known that emission intensity of Tb^{3+} ions is sensitized by energy transfer from an excited state of a donor (*e.g.* nucleobase) to the emissive $^5D_4$ state of Tb^{3+} ions.[12] Because nucleic acids exhibit strong π–π* absorption in the 250–280 nm range, coordination of nucleobases to Tb^{3+} ions allows UV light absorbed by the nucleobases to be converted to the luminescence of Tb^{3+} ions. Luminescence measurements in dGMP-Tb^{3+} and dG_2MP-Tb^{3+} systems were performed in pure water (after having made sure that similar morphologies were obtained, Fig. S7, ESI†). Fig. 3(a) compares luminescence spectra obtained for aqueous dGMP-Tb^{3+}, dG_2MP-Tb^{3+} and Tb^{3+} ions (in the absence of nucleotides). dGMP-Tb^{3+} nanoparticles showed strong emission bands in the visible region at 489 ($^5D_4 \rightarrow {}^7F_6$), 544 ($^5D_4 \rightarrow {}^7F_5$), 586 ($^5D_4 \rightarrow {}^7F_4$) and 621 nm ($^5D_4 \rightarrow {}^7F_3$). The sensitized luminescence observed for dGMP-Tb^{3+} nanoparticles indicates that dGMP molecules act as bidentate ligands. The O6 and N7 atoms of guanine bases and anionic phosphate groups are coordinated to Tb^{3+} ions, giving amorphous coordination networks.[9] On the other hand, dG_2MP-Tb^{3+} complexes show much weaker luminescence, whose intensity is almost comparable to that in the absence of nucleotides. This indicates that guanine bases are not coordinating to Tb^{3+} ions, because of steric hindrance exerted by dG_2MP molecules. As excited states of lanthanide ions are nonradiatively deactivated by water molecules in the inner coordination sphere,[12] the weak luminescence observed for dG_2MP-Tb^{3+} nanofibers could

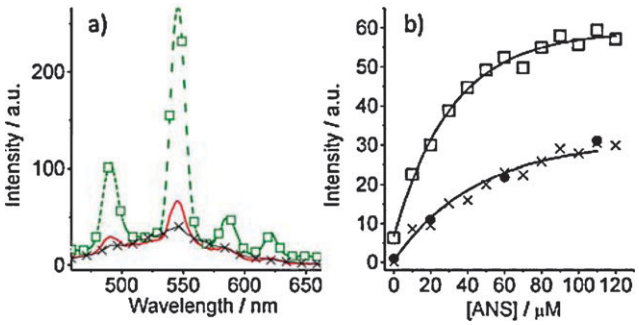

Fig. 3 (a) Luminescence measurements of dGMP-Tb^{3+} (dashed green line with open squares), dG_2MP-Tb^{3+} (solid red line), and Tb^{3+} (0.25 mM) (crosses) in water (λ_{exc} = 260 nm). (b) Dependence of ANS fluorescence intensity on its concentration in presence of dGMP-Tb^{3+} (white squares), and dG_2MP-Tb^{3+} (black circles) ($C_{dGMP/dG2MP}$ = 0.5 mM, C_{Tb} = 0.25 mM), and in water (crosses); λ_{exc} = 370 nm, λ_{em} = 485 nm.

also be ascribed to the presence of water molecules completing the coordination sphere. These results are consistent with the unit supramolecular models described in Fig. 2(a). In the case of dGMP-Tb^{3+} nanoparticles, on the other hand, coordination of dGMP phosphate groups allows Tb^{3+} ions to be further coordinated by neutral guanine bases. Such coordination networks would provide hydrophobic microenvironment as indicated by the sensitized luminescence of Tb^{3+} ions.[9]

To evaluate the microenvironment of dGMP-Tb^{3+} and dG_2MP-Tb^{3+} assemblies, we used the fluorescent probe 1-anilino-8-naphthalene sulfonate (ANS). In hydrophobic microenvironments, ANS fluorescence increases, along with a fluorescence blue shift from *ca.* 520 to 485 nm.[13] To aqueous mixtures of each nucleotide and ANS, Tb^{3+} ions were added to induce self-assembly. Fig. 3(b) shows fluorescence intensity of ANS obtained at increasing ANS concentrations. In the aqueous dispersion of dGMP-Tb^{3+}, the fluorescence intensity at λ_{em} = 485 nm increased steadily with increasing ANS concentration until 50 μM ANS. Beyond this concentration, the intensity starts levelling off. Apparently, ANS is incorporated into dGMP-Tb^{3+} nanoparticles that provide a hydrophobic microenvironment. This is in agreement with the strong luminescence observed for nanoparticles. On the other hand, fluorescence intensity of ANS measured in aqueous dG_2MP-Tb^{3+} follows the same trend but with a much lower intensity. As an identical intensity profile was observed for ANS in pure water, it seems that ANS molecules are remaining in water without being incorporated into dG_2MP-Tb^{3+} nanofibers. These observations indicate that dG_2MP-Tb^{3+} nanofibers do not provide a suitable hydrophobic microenvironment for ANS binding and sensitized luminescence of Tb^{3+} ions.

In conclusion, nanofibers were successfully obtained from dimeric dG_2MP upon coordination with lanthanide ions. It is noteworthy that monomeric and dimeric forms of guanine nucleotides give totally different coordination environments for Tb^{3+} ions and specific supramolecular architectures. Dimeric dG_2MP adopts a unique pincer-like conformation, and the difference in nucleotide molecular structures is expressed as characteristic hierarchical self-assemblies and luminescent properties. The controlled hydration of lanthanide ions in supramolecular nanostructures is crucial to develop magnetic resonance contrast agents and we envisage potential applications of these nanofiber systems in such imaging technologies.

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