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Supplementary information

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

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Experimental Section

Chemicals: 2'-deoxy nucleotides 5'-monophosphate (dNMP) and lanthanide ions were purchased from Across Organics, Wako pure chemical industries, LTD and used without any further purification. Dimeric 2'-deoxy nucleotides 5'-monophosphate (dN_2MP) were purchased from SIGMA Aldrich Japan, k.K. Life Science Division and were used without any further purification. Water was purified with a Direct-Q system, Millipore Co (18.2 MΩ.cm).

Sample Preparation: The nucleotide of interest was first suspended in Hepes buffer before addition of a solution of lanthanide in pure water (final pH=7.4). At this pH, dNMP and dN_2MP derivatives are both singly charged. The mixture was then briefly stirred and supramolecular architectures allowed forming for about 6 hours. Because of the low concentration of both nucleotides and lanthanides, no precipitate could be observed macroscopically.

Transmission Electron Microscopy (TEM), and High Resolution TEM. A small drop of dGMP-Tb^{3+} or dG_2MP-Tb^{3+} samples in aqueous solution was deposited on carbon-coated copper grids. After one minute, the excess liquid was blotted with filter paper. Neither staining nor Pt vaporization were used prior to sample observation. TEM images were recorded using a JEOL JEM-2010 electron microscope operating at 120 kV and a Gatan ssCCD camera. HR-TEM experiments were performed on a TECNAI-20 FEI electron microscope operating at 200 kV.

Scanning Electron Microscopy. A small drop of dGMP-Tb^{3+} or dG_2MP-Tb^{3+} samples in aqueous solution was deposited on carbon-coated copper grids. After one minute, the excess liquid was blotted with filter paper. Samples were coated by Pt vaporization on a HITACHI E-1030 ion sputter. SEM images were recorded using a HITACHI S-5000 electron microscope operating at 15 kV.

Fluorescence Measurements. Emission spectra of dGMP-Tb^{3+} and dG_2MP-Tb^{3+} samples in water were recorded on a Perkin Elmer LS55 fluorescence spectrometer, using quartz cells with 1 mm path lengths. For luminescence measurements, excitation and emission wavelengths were set at 260 nm and between 400 and 700 nm respectively. Excitation and emission slit widths were 10 nm. For ANS titration experiments, 5 mM ANS stock solution was prepared in DMSO to ensure complete solubilization of the dye. ANS was added to dGMP/dG_2MP aqueous solution prior to Tb^{3+} addition to allow ANS to be incorporated into the nanostructures. Starting from a sample containing [dGMP/dG_2MP] = 0.5 mM in presence of [Tb^{3+}] = 0.25 mM in pure water, sample with gradually increasing ANS concentration (from 0 to 120 µM) were prepared by addition of 0.6 to 7.38 µl ANS stock solution. dGMP/dG_2MP and Tb^{3+} concentration deviation due to ANS addition (up to [dGMP/dG_2MP] = 0.49 mM in presence of [Tb^{3+}] = 0.24 mM for [ANS] = 120 µM, V_{ANS} = 7.38 µl) were considered as non significant. Excitation and emission wavelengths were set at 370 nm and between 400 and 600 nm respectively. Excitation and emission slit widths were 5 nm. Absorbance at λ_{em} = 485 nm was then plotted against ANS concentration.

Molecular Modeling. Molecular modeling was carried out with MacroModel/Maestro 8.0.314, Schrödinger, LLC. Conformational searches on moneric dGMP and dimeric dG_2MP were carried out using the OPLS-2005 force field, in water. The starting geometries for these searches were obtained from minimization using the same force field in water. All structures within 50 kJ/mol were retained from a sampling of 50 000 structures, obtained by randomly changing a torsion angle in the starting geometry, and minimizing the resulting structures to convergence.

Calculated conformation of dG_2MP in water.

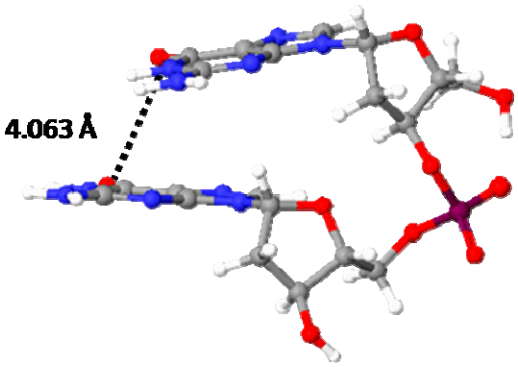

Figure S1: Image representing the calculated conformation of dG_2MP in water (MacroModel/Maestro 8.0.314, Schrödinger, LLC), and showing the pincer-like structure adopted by dG_2MP.

Self-Assembly of dNMP-Tb^{3+} complexes

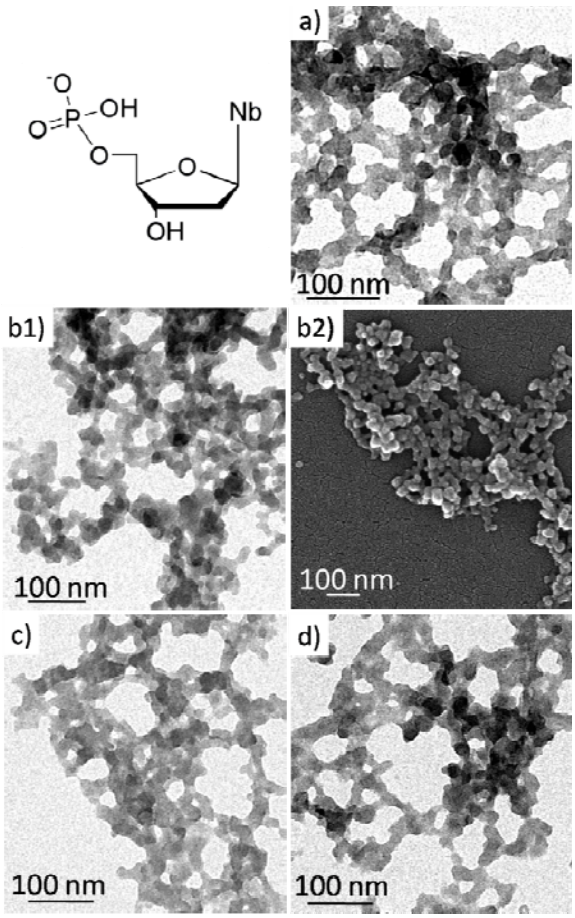

Figure S2: TEM (a, b1, c, d) and SEM (b2) micrographs of the supramolecular structures formed from monodeoxynucleotides monophosphate (dNMP) and Tb^{3+} ions in Hepes buffer. These photos show that nanoparticles are formed whatever the nucleobase. a) dAMP, b1-b2) dGMP, c) dCMP, d) dTMP.

Influence of Nucleotide:Lanthanide ratio

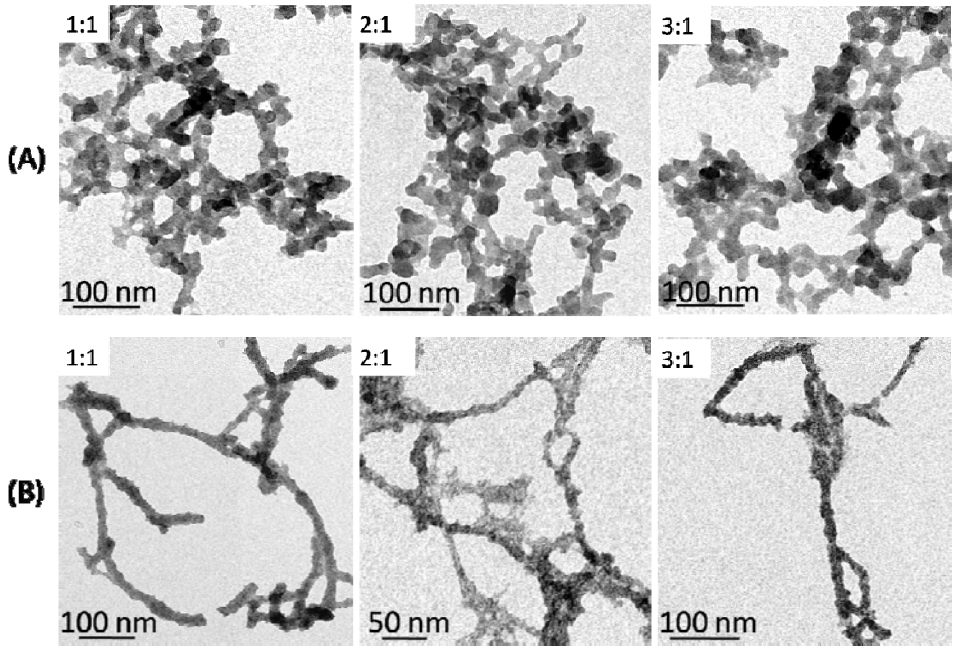

Figure S3: TEM micrographs of (A) dGMP (0.5 mM) and (B) dG_2MP (0.5 mM) in the presence of Tb^{3+} at different concentrations. $[Tb^{3+}]$ = 0.5 mM (1:1 molar ratio), 0.25 mM (2:1 molar ratio) and 0.17 mM (3:1 molar ratio), in 0.05 M Hepes buffer. These pictures show that nanoparticles (dGMP-Tb^{3+}) and nanofibers (dG_2MP-Tb^{3+}) are formed in a wide range of nucleotide:lanthanide mixing ratio. Although nanoparticles shown in (A) are somewhat randomly agglomerated, nanofibers in (B) clearly show their basic tendency to form one-dimensional structures.

High Resolution TEM (HRTEM) and Energy Dispersive X-ray spectrometry (EDS)

HRTEM-EDS was used to determine the composition and stoichiometry of dG_2MP-Tb nanofibers. In EDS spectra, two different ratios were measured over three different scanning regions. It indicates the presence of distribution in stoichiometry and thus precise determination of the dG_2MP:Tb ratio was difficult. In the following EDS measurements, oxygens are reporter atoms for the presence of dG_2MP dimers. Indeed, while both oxygen and phosphor have a light electron density, oxygen is 10 times more abundant than phosphor in dG_2MP structures, allowing for its detection. Taking into account that one dimer contains 10 oxygens, the two first regions show dG_2MP:Tb ratios of 1.6:1 (O: 92.84 ± 3.60 %, Tb: 5.83 ± 3.85 %, Figure S4-1) and 1.7:1 (O: 94.34 ± 4.31 %, Tb: 5.65 ± 4.39 %, Figure S4-2) respectively. However, the third region shows a higher dG_2MP:Tb ratio of 3.1:1 (O: 95.20 ± 3.77 %, Tb: 3.11 ± 3.24 %, Figure S4-3). In addition, sulfur atom coming from Hepes molecule was detected (Figure S4-3), though its amount was very low. Considering that chloride ion was not detected in the EDS measurement, it is likely that Hepes molecules are partly incorporated in nanofibers as counterions.

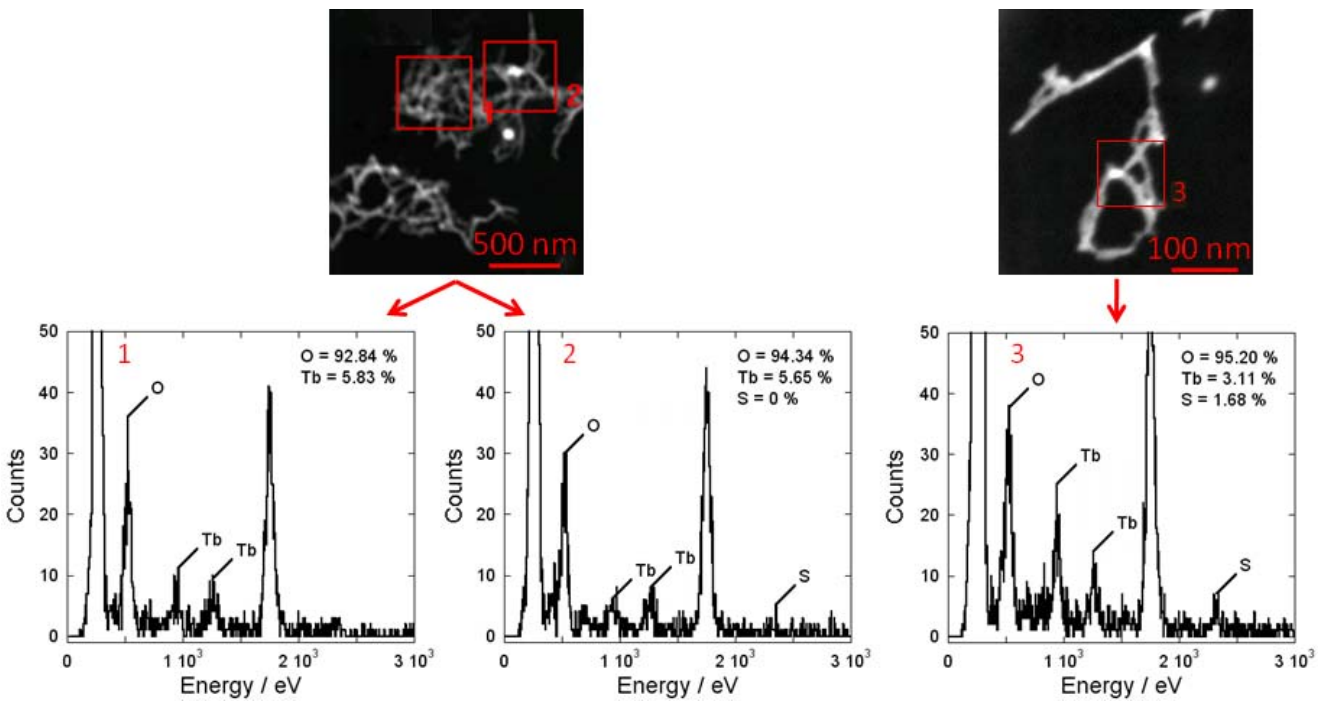

Figure S4: Dark field TEM images and elemental analysis (EDS) of dG_2MP (1 mM)-Tb (0.5 mM) in 0.05 M Hepes buffer in three different scanning regions. The C or Si peaks (at 2.58 10^2 and 1.75 10^3 eV respectively) are due to the TEM grid.

Taking all these data together, it seems that dG_2MP molecules interact with trivalent Tb^{3+} ions *via* coordination of the anionic phosphodiester units, with a dG_2MP:Tb ratio between 1.6:1 and 3.1:1. Because of the bulkiness of dG_2MP molecules, it is natural to assume that most of the trivalent lanthanides is coordinated by two dimers, which is compatible with the above described EDS data. Moreover, this is reinforced by the detection of Hepes molecules that would electrostatically bind to Tb^{3+} ions.

Possible Models for Nanofiber and Nanoparticle Formation

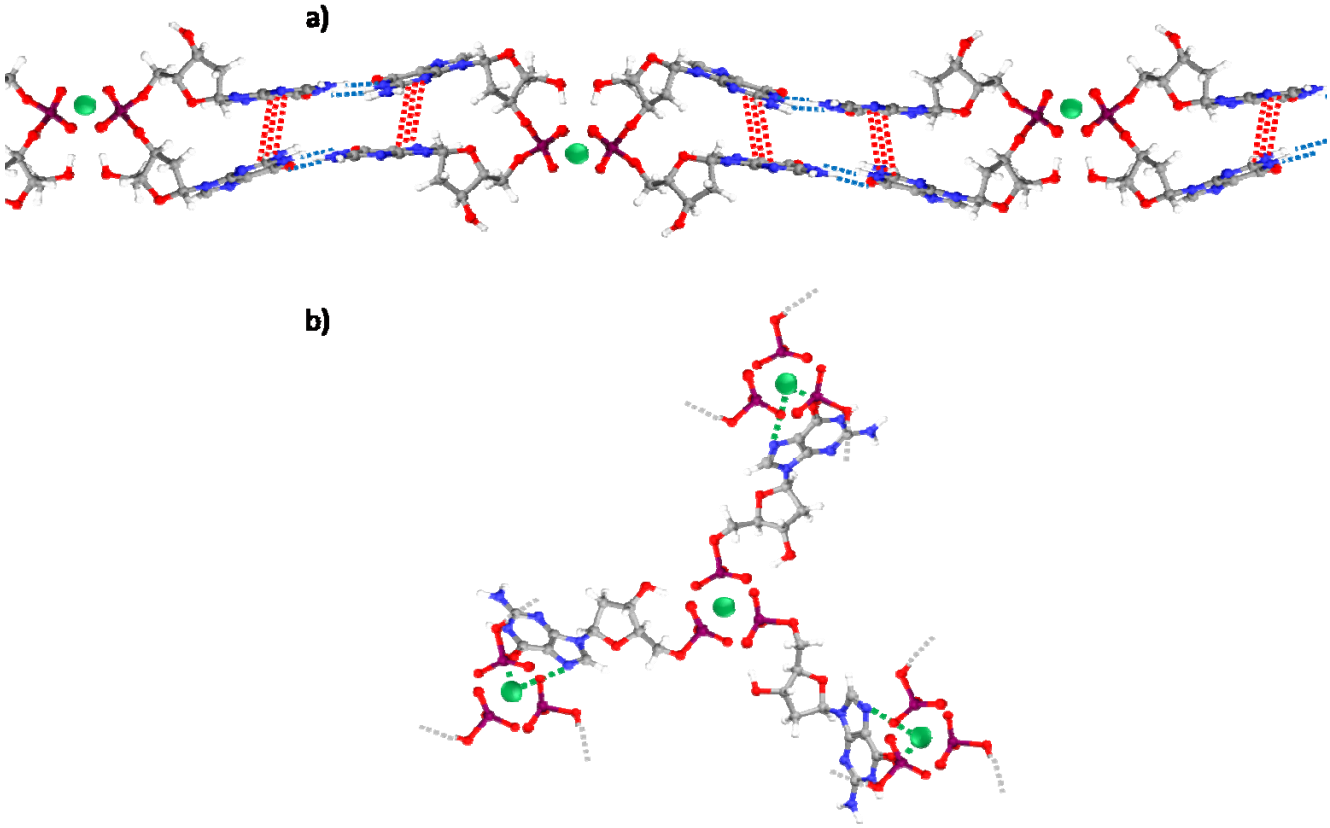

Figure S5: Possible models for (a) dG_2MP-Tb^{3+} and their assembly via hydrogen bonding and stacking to nanofibers, (b) coordination networks self-assembled from dGMP-Tb^{3+}. Counterions are omitted for clarity.

In (a), the pincer-like structure of dG_2MP (Figure S1) induces stable stacking between the two guanine bases of a dimer (red dots). In addition, guanine exhibits edges having self-complementary hydrogen-bond donors and acceptors responsible for strong hydrogen bonding (blue dots). From the above described HRTEM-EDS measurements (Figure S4), it seems that two bulky dG_2MP molecules interact with trivalent Tb^{3+} ions *via* coordination of the anionic phosphodiester units. The resulting steric hindrance would prevent coordination of another phosphate group or guanine bases of dG_2MP molecules. Instead, water molecules are likely to be completing the coordination spheres together with counterions such as Hepes, as supported by weak luminescence observed for dG_2MP-Tb^{3+} complexes (Fig. 3a). This structure model is consistent with the absence of ANS fluorescence intensity enhancement (Fig. 3b), indicating rather polar microenvironment in the dG_2MP-Tb^{3+} nanofibers. Taken together, these factors well explain the anisotropic self-assembly of dG_2MP and Tb^{3+} ions into nanofibers.

In the case of dGMP-Tb^{3+} (b), the smallest size of the monomeric nucleotides allows for the self-organization of a flexible network responsible for the formation of nanoparticles.[9] Contrary to dG_2MP-Tb^{3+}complexes, such lowest hindrance allows Tb^{3+} coordination with guanine base (green dots), thus preventing Tb^{3+} coordination with water molecules. As a result, dGMP-Tb^{3+} complexes provide a suitably hydrophobic environment for Tb^{3+} luminescence and ANS fluorescence enhancement.

Influence of the lanthanide ion (Ln^{3+}) species on the self-assembly of dGMP-Ln complexes.

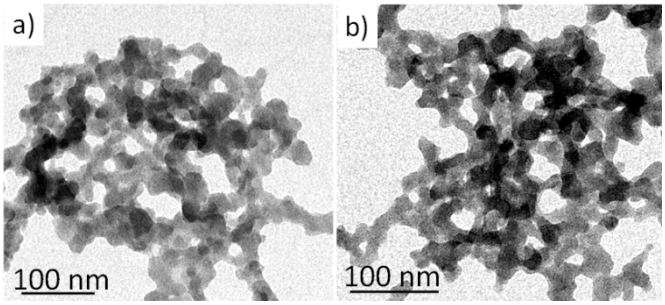

Figure S6: TEM micrographs of the supramolecular structures formed upon dGMP coordination with different lanthanide ions in Hepes buffer: (a) Eu^{3+}, (b) Gd^{3+}.

Influence of buffer

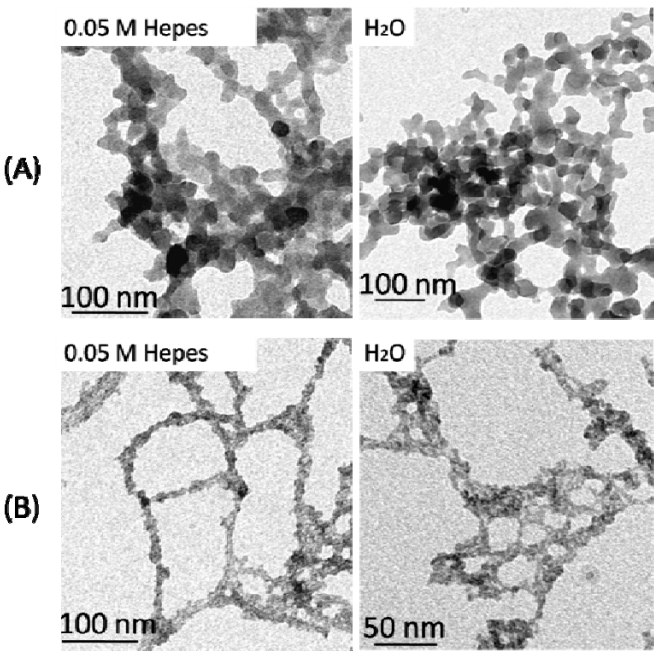

Figure S7: TEM micrographs of (A) dGMP and (B) dG_2MP (0.5 mM) in the presence of Tb^{3+} (0.25 mM), in Hepes buffer and in pure water. These pictures show that nanoparticles (dGMP-Tb^{3+}) are formed in both solvents. On the other hand, fibrous structures are formed from dG_2MP-Tb^{3+} complexes, although more entangled networks are seen in pure water. It would be reflecting uptake of Hepes molecules into nanofibers as observed in the EDS measurement. The difference in supramolecular morphologies obtained from dGMP and dG_2MP upon coordination with Tb^{3+} is maintained in pure water.