

Nucleic acid and nucleotide-mediated synthesis of inorganic nanoparticles

Since the advent of practical methods for achieving DNA metallization, the use of nucleic acids as templates for the synthesis of inorganic nanoparticles (NPs) has become an active area of study. It is now widely recognized that nucleic acids have the ability to control the growth and morphology of inorganic NPs. These biopolymers are particularly appealing as templating agents as their ease of synthesis in conjunction with the possibility of screening nucleotide composition, sequence and length, provides the means to modulate the physico-chemical properties of the resulting NPs. Several synthetic procedures leading to NPs with interesting photophysical properties as well as studies aimed at rationalizing the mechanism of nucleic acid-templated NP synthesis are now being reported. This progress article will outline the current understanding of the nucleic acid-templated process and provides an up to date reference in this nascent field.

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Inorganic nanoparticles (NPs) are a class of novel materials whose properties are defined by their submicrometre dimensions. The physico-chemical properties of NPs are both size- and shape-dependent, and differ significantly from those of the corresponding bulk material¹. A pertinent illustration of this dependency is the case of photoemissive semiconductor nanocrystals or quantum dots, where the optical properties are dictated by their dimensions. As a consequence of this tight correlation, NP dimension and dispersity must be controlled during their preparation in order to obtain populations with the desired characteristics. Therefore, synthetic protocols for the production of monodisperse populations of NPs whereby the size and shape can be intimately controlled is an area of research that is intensely studied². Although careful control over the experimental conditions permits the synthesis of fairly monodispersed populations, NP synthesis generally results in the formation of polydispersed populations, which are then focused into more monodisperse populations through efficient separation methods³.

Alternatively, by taking cues from nature, the process known as biomineralization can be mimicked. Biomineralization is a naturally occurring process by which a living organism excretes an inorganic material formed through a biologically guided process. These processes exploit biomolecular templates that interact with the inorganic material throughout its formation resulting in extremely efficient syntheses⁴⁻⁹.

BIOTEMPLATED NANOPARTICLE SYNTHESIS

The role of the templating molecule in biomineralization is to provide a synthetic microenvironment in which the inorganic phase

morphology is tightly controlled by a range of interactions. A variety of organisms exploit biotemplated mineralization for the formation of a wide range of materials. Bones, teeth and shells are typical examples of structural materials produced by natural biomineralization processes. Additionally, nature produces a variety of biomineralized materials exhibiting functions well beyond those of the structural materials mentioned above. Representative examples are the biomineralization of highly ordered and monodispersed magnetic¹⁰ and semiconductor^{11,12} NPs. Naturally occurring polypeptides such as metallothioneins¹³ and phytochelatin¹⁴ are examples of biomolecular templates where their interactions with an appropriate inorganic precursor guide the biomineralization process.

Materials scientists have extensively exploited both natural and synthetic polypeptides as templates for the synthesis of inorganic NPs. The selection of novel peptidic sequences relies on powerful biochemical methods such as the creation of phage display libraries¹⁵, where millions of unique polypeptides are displayed at the end of a viral (phage) coat protein and screened simultaneously for their affinity for a specific substrate. The expression and screening cycles are repeated multiple times, and at each cycle the peptide mixture is enriched with more effective binders. Phage display methods have already given access to peptidic sequences showing very high affinity for a number of inorganic materials¹⁶⁻¹⁸.

NUCLEIC ACIDS AS TEMPLATES

Nucleic acids consist of chains of monomeric units, termed nucleotides, linked together by phosphodiester bonds. From the standpoint of a materials scientist, nucleic acids can also be defined as programmable polymers whose physico-chemical properties are dependent on the type of constituting nucleotides, their sequence and the type of linkage holding them together. The nucleotides most commonly found in nucleic acids are shown in Fig. 1. However polymer chains containing less common nucleotides, and linkages other than the phosphodiester bond, can also be easily attained.

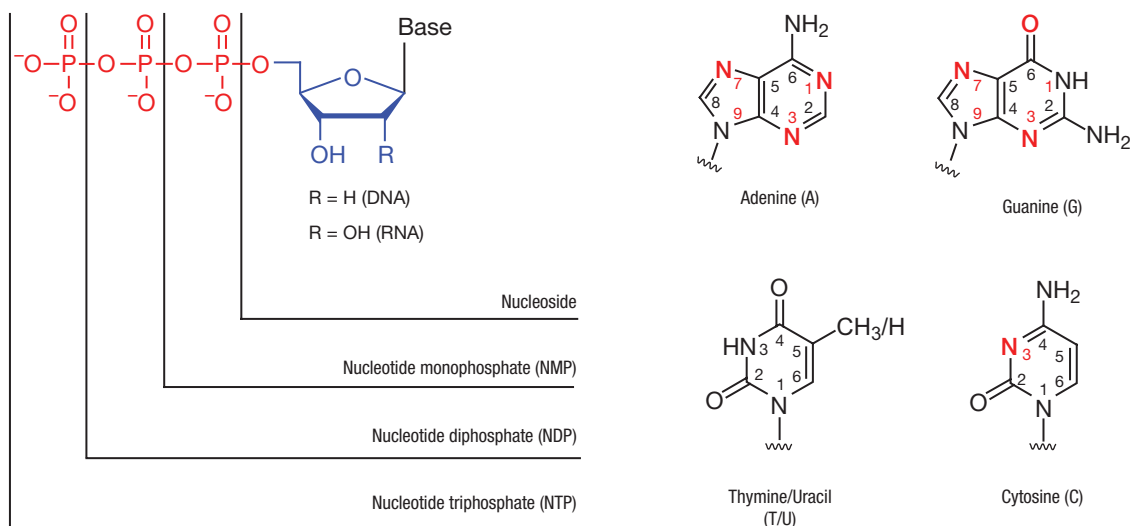


Figure 1 Nucleosides are the building blocks of nucleic acids. They consist of a nucleobase (adenine (A), thymine (T), uracil (U), guanine (G), cytosine (C)), and a ribose (in RNA) or 2'-deoxyribose (in DNA) sugar ring, highlighted in blue. Nucleotides are monophosphorylated nucleosides and nucleotide diphosphates (NDP) and triphosphates (NTPs) are, respectively, diphosphorylated and triphosphorylated versions of nucleosides. Nucleobases, nucleosides and nucleotides comprise many different chemical groups that are able to bind to metal ions. The atoms and groups in nucleobases responsible for these interactions are highlighted in red and numbered for clarity.

Recently, nucleotides and nucleic acids have emerged as attractive biomineralization templates. The attractiveness of nucleic acid-derived biomineralization processes stems from several desirable features: (1) nucleic acids interact with metal ions via several different and well-established binding modes^{19–21}; (2) nucleic acids in solution adopt a well-defined three-dimensional shape, termed conformation, which is strongly dependent on the nucleotide sequence and which might provide the necessary microenvironment to confine and control a mineralization event; (3) nucleic acid libraries consisting of millions of different sequences can be generated more efficiently and cost effectively than polypeptide sequences via the use of powerful synthetic, enzymatic and *in vitro* evolutionary techniques, such as the so-called SELEX process²², which will be described later; (4) the recognition properties and chemical reactivity of nucleic acids can be easily expanded by incorporating synthetic nucleotides endowed with custom functional groups through chemical or enzymatic procedures²³.

METAL–NUCLEIC ACID INTERACTIONS

The initiating event of every biomineralization process is the formation of a precursor complex via specific interactions between a metal or metal ion and a biotemplate. The phosphate group, as well as a number of other functionalities present in nucleic acids and their constituent nucleotides, interact extensively with metal ions (Fig. 1). Two different modes of interaction are possible and the extent of each is determined by the type of metal (Table 1).

At physiological pH, the negatively charged phosphate backbone interacts extensively with positively charged metal cations^{20,24,25,26,27} through simple electrostatic attraction. Alternatively, empty orbitals of the metal cations can accept electrons from the phosphate oxygen or from the nucleobase moieties forming coordination complexes²⁸. These are the primary types of interaction for alkali, alkali earth metals and a few transition metal ions (Table 1).

For the nucleobases guanine (G) and adenine (A), N1 and N7 are the favoured binding sites, with O6 of G playing a secondary role in metal coordination. Although N7 is generally considered to be the preferred metal ion binding position, recent reports indicate

that at basic pH, N1 becomes the favoured coordination site²⁰. For the nucleobases cytosine (C), thymine (T) and uracil (U), metal ion binding occurs primarily at N3, although this is also highly pH dependent²⁰. At neutral pH, the order of stability for nucleobase complexes with transition metal ions has been determined as N7/O6(G) > N3(C) > N7(A) > N1(A) > N3(A,G)^{29,21}, with T and U giving no significant complexation through base coordination. With the exception of Ag⁺, Hg²⁺ and Pt²⁺, which complex exclusively to nucleobases, most metals form mixed complexes, where the metal ions interact simultaneously with both the nucleobase and the nucleic acid backbone (Table 1).

It is important to point out that, although the type of metal–nucleic acid binding is determined by the nature of the metal ion, the strength of this interaction is dependent on other factors such as the solvent or the ionic strength. The experimental conditions must therefore be considered when predicting the mode of interaction.

MECHANISM OF NANOPARTICLE GROWTH

Although a general model for nucleic acid/nucleotide templated NP growth has not been unequivocally identified, a mechanism can be postulated based on the accepted mechanism of NP formation² and on findings by Hinds *et al.*³⁰ according to the following steps:

- Step 1: Initiation. Nucleic acid/nucleotide ligands bind metal cations forming a NP precursor and providing the necessary microenvironment for triggering the nucleation event. Both metal–phosphate and metal–nucleotide interactions are crucial in this initial step.
- Step 2: Growth. Small clusters produced during the initiation step are coated with a negatively charged layer, known as capping, of nucleic acid/nucleotide molecules. The small clusters tend to lose their capping through a thermodynamically more favourable aggregation into larger clusters carrying a larger number of capping molecules on their surface. The tendency towards cluster aggregation is counterbalanced by the electrostatic barrier formed by the nucleotide/nucleic acid cap that must be overcome in order to bring the clusters into close proximity to aggregate.

Table 1 Nucleic acids can bind metal ions through different types of interactions. Often, more than one interaction is responsible for metal binding. It is possible to define generic guidelines which might be of help in directing the choice of metals to be used in the synthesis of inorganic NPs templated by nucleic acids or nucleotides. This table illustrates the most common binding sites of nucleic acids for representative examples of mono- and divalent metal ions. The ions are listed in increasing order of binding strength or in increasing order of affinity for the base (adapted from ref. 19).

Binding Site	Metal Ion
Phosphate	Li ⁺ , Na ⁺ , K ⁺ , Rb ⁺ , Cs ⁺ , Mg ²⁺ , Ca ²⁺ , Sr ²⁺ , Ba ²⁺ , trivalent lanthanides
Phosphate and base	Co ²⁺ =Ni ²⁺ , Fe ^{2+*} , Mn ²⁺ , Zn ²⁺ , Cd ²⁺ , Pb ²⁺ , Cu ²⁺
Base	Ag ⁺ , Hg ²⁺ , Pt ²⁺

*Approximate position of Fe²⁺ is extrapolated from ref. 26.

- Step 3: Termination. The nucleic acid/nucleotide capping process provides a termination point in NP growth by acting as a stabilizing electrostatic shell. Upon reaching a critical size, the electrostatic barrier for bringing clusters into close proximity is too large to be overcome, preventing further aggregation.
- Step 4: Passivation. Like other capping molecules, nucleic acid/nucleotide molecules serve to suppress defect states on the surface of semiconductor NPs by saturating empty orbitals and dangling bonds resulting in improved physico-chemical characteristics (for example, narrower emission bands and higher quantum yields).
- Step 5: Solubilization. Nucleic acid/nucleotide ligands ensure that NPs remain dispersed in aqueous solution.

Recently, Feldheim and Eaton proposed an alternative mechanism for the biomolecule-mediated crystal formation³¹. The steps involved in their hypothesis are the same as the ones illustrated above, but they highlight the role of a generic biomolecule by proposing a kinetic growth model that might help in defining how the biomolecule–inorganic phase interaction affects the formation of a crystal.

NUCLEOTIDE-MEDIATED SYNTHESIS OF INORGANIC NANOPARTICLES

The preparation of NPs can be achieved not only by nucleic acid templates, but also by the very building blocks that are used to synthesize nucleic acids: nucleosides and nucleotides. Nucleosides consist of a nucleobase (A, T, G or C) and a sugar (ribose for RNA and 2'-deoxyribose for DNA) ring (Fig. 1). Nucleotide triphosphates (NTPs) are triphosphorylated versions of nucleosides. It is currently assumed that NTP-templated NP formation proceeds via a mechanism similar to nucleic acid-templated methods (see previous section), resulting in NPs that are covered by a protective NTP outer layer or cap.

Kumar^{32–35} and Green³⁶ first reported the use of nucleosides, respectively, as capping agents and templates for the preparation of CdS nanoparticles. It was initially thought that the nucleobase of NTP templates would result in a coating through which the NPs could interact with biomolecules. Instead, Fourier transform infrared spectroscopy revealed that the nucleobase interacted directly with the CdS, thus preventing subsequent interaction with the surrounding environment. Structural studies conducted by both the Kelley and Green groups, revealed the salient chemical features of nucleotides involved in CdS NP formation^{30,36–38}. Of the natural and unnatural NTPs tested, guanosine triphosphate (GTP) was the most effective at stabilizing emissive CdS NPs.

A general trend emerged from these studies, which identified the nucleobase N7 as a critical functionality in CdS NP formation (Fig. 2). Although the N2 amino function of GTP appears to be influential in altering the emissive properties of CdS NPs, it was shown not to be critical for CdS formation. This is in direct contrast to a comparative study with PbS NP formation, where the N2 amino function of GTP was crucial for their formation, most likely owing to a different mechanism of surface interaction³⁰.

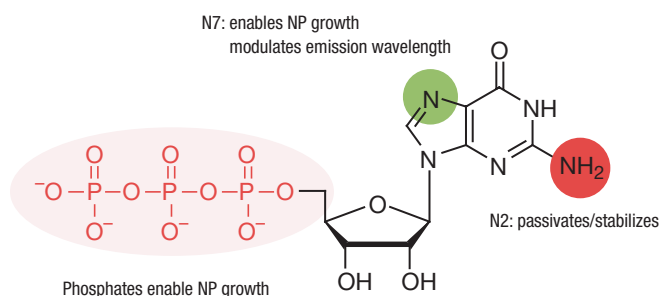


Figure 2 Each chemical moiety of a nucleotide plays a specific role in directing the templated synthesis of inorganic NPs. Here, the proposed roles of chemical functionalities present in guanosine triphosphate (GTP) in the GTP-templated synthesis of PbS NPs are illustrated. The triphosphate group and N7 were shown to be essential to achieve NP formation, and N7 was shown to play a role in modulating the emission properties of the ensuing NPs. The role of N2 is dependent on the type of NP synthesized, and was shown to be essential for PbS formation, but not for CdS formation, where its presence or absence simply altered the emissive properties. Reproduced with permission from ref. 30. Copyright (2006) ACS.

Another consistent trend for both NTP-templated CdS and PbS NP formation was that the triphosphate moiety appeared to be essential for successful NP growth (Fig. 2). This was revealed when diphosphate, monophosphate and nucleoside G analogues imparted reduced or unsuccessful NP formation. The importance of both the triphosphate and nucleobase in the templated synthesis of NPs has been established, but the role of the sugar portion (Fig. 1) of NTPs is still unclear.

Variations in pH have been shown to affect both the formation of NPs in the presence of NTPs and their emissive properties. Interestingly, in all cases except GTP, the emissive properties of the CdS NPs produced at pH 10 increased by at least an order of magnitude, whereas for GTP, the emissive properties slightly decreased. These results indicate that the surface of the CdS NPs is probably covered by a Cd(OH)₂ shell in all cases but GTP³⁴. This finding is consistent with G binding very strongly to cadmium and preventing any further surface reaction.

Microscopic studies revealed large deviations in both the size and shape of NPs templated by different NTPs. GTP-templated CdS NPs produced under different conditions exhibited the least variability^{37,38}, whereas ATP produced large particles accompanied by a large size distribution. However, when synthesized under basic conditions CdS NPs revealed different size dependences as a function of the nucleobase. For instance, similarly sized particles were obtained with the ATP and GTP ligands, although with large shape variations, whereas TTP and CTP ligands produced much larger CdS NPs. The formation of Au nanostructures templated by ATP has also been recently reported³⁹. Furthermore it has

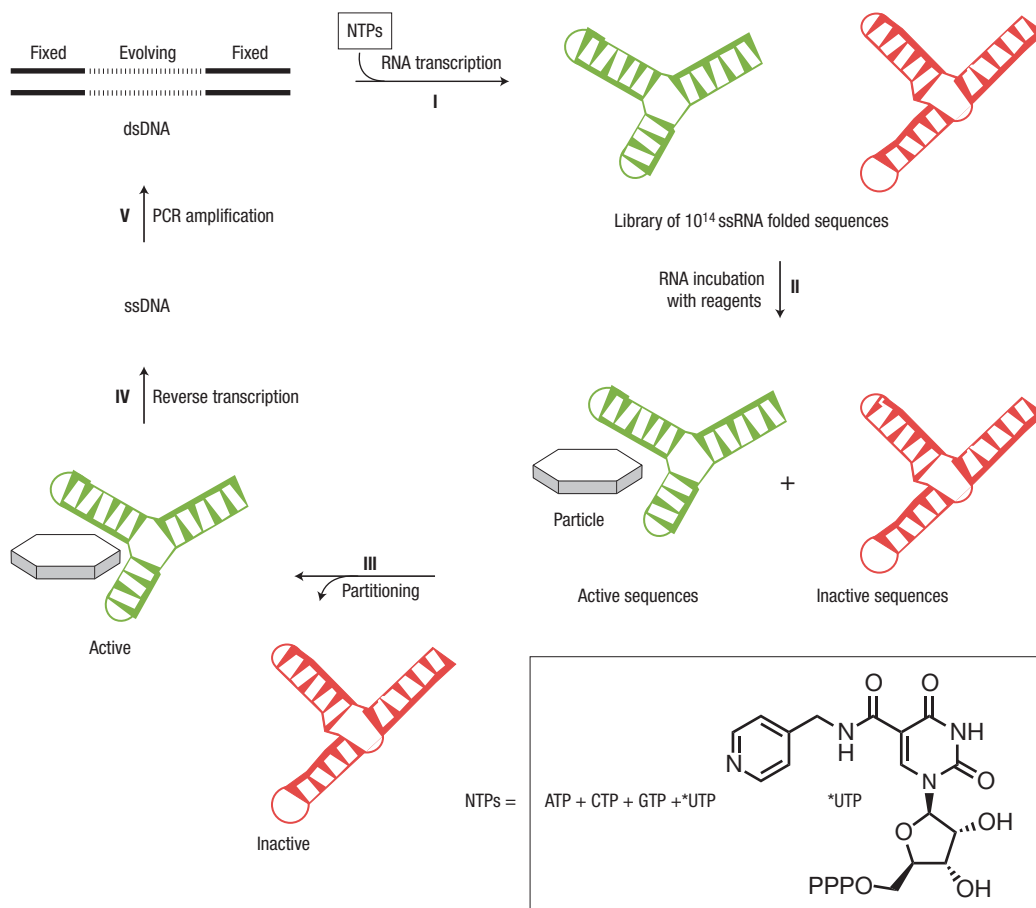


Figure 3 Nucleic acid sequences are able to recognize or catalyse the formation of NPs with specific morphologies. These NPs can be selected through powerful methods based on evolutionary selection. This schematic depiction illustrates one such method — the systematic evolution of ligands by exponential enrichment (SELEX) process — applied to the selection of RNA sequences catalysing the formation of Pd NPs. This process starts from a library of DNA sequences containing a stretch of randomized nucleotides in all possible permutations. Transcription of the DNA library (I) generates an RNA library consisting of 10¹⁴ unique RNA sequences, which fold in unique shapes that are strictly related to their constituting nucleotide sequence (UTP* is a modified nucleotide — see text). The RNA library is then screened against independently synthesized hexagonal Pd NPs (II). The RNA sequences that bind the Pd NPs are separated (III) and reverse transcribed into a more focused DNA library (IV). The new DNA library is amplified through the polymerase chain reaction (V), and the steps are repeated generating an even more focused DNA library. After a number of cycles only the strongest binding RNA sequences (and DNA library encoding them) are left and identified by sequencing. Reproduced with permission from ref. 60. Copyright (2004) AAAS.

been demonstrated that the size of Au NPs can be modulated by stabilizing them with different nucleotides⁴⁰.

NUCLEIC ACIDS AS BIOMINERALIZATION TEMPLATES

The first known report of nucleic acids being used as templates for NP synthesis was that of Coffer and co-workers, who discovered that DNA sequences stabilized CdS NPs and affected their photoluminescence and aqueous solubility^{41–46}. In these studies, it was evident that the physico-chemical, morphological and photophysical characteristics of the NPs were critically dependent on the nucleic acid composition⁴⁴. DNA sequences comprising a high A content resulted in the stabilization of smaller NPs, whereas larger NPs were obtained using polyG, polyC and polyU sequences. This result was substantiated by the synthesis of smaller CdS NPs using *Clostridium perfringens* DNA templates, which have a higher AT content (74% AT) compared with the larger NPs synthesized with either calf thymus or *Escherichia coli* DNA (50% AT). The fluorescence characteristics were also affected by the DNA composition. Whereas CdS NPs synthesized in the presence of polyA templates yielded NPs exhibiting an emission maximum

near 520 nm, CdS NPs produced with a lower A content resulted in a significantly redshifted emission maximum (600–620 nm)⁴⁴.

Besides sequence and composition, other variables play a role in templating the synthesis of NPs. For instance, in order to explore the influence of oligonucleotide length on NP synthesis, Berti *et al.* have recently reported that the size and dispersity of CdS NPs can be modulated by templating their formation with oligoguanines (oligoG) of different lengths⁴⁷. Interestingly, longer oligoGs, such as the 15- or 20-base oligonucleotides, catalysed the formation of smaller NPs and generated more monodispersed populations. Conversely, shorter ones (5- and 10-base oligonucleotides) resulted in the formation of larger NPs characterized by higher polydispersity. Although the reasons for this observed trend are still not clear, it can be speculated that the higher complexing capacity for Cd²⁺ of the longer oligoGs is essential in providing a more confined templating environment, resulting in tighter interactions between oligonucleotide and ionic precursor and consequently smaller NPs and less dispersed populations. In a similar study it was shown that the photophysical properties of CdS NP templated by oligonucleotides of homogeneous composition were dependent on the nucleotide⁴⁸.

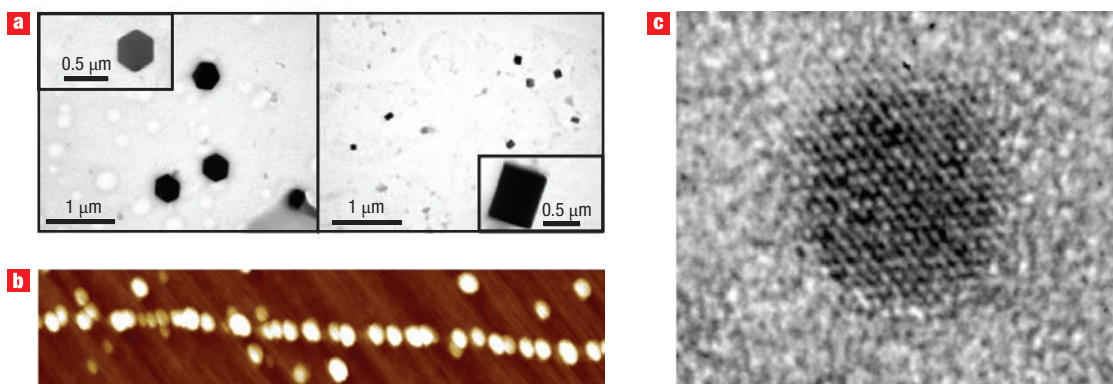


Figure 4 The use of nucleic acids as templates for the growth of inorganic NPs has proven successful for the synthesis of several nanomaterials. This figure illustrates representative examples of nucleic acid-templated nanomaterials and highlights the degree of control that can be achieved by this strategy. **a**, Hexagonal (left) or cubic (right) Pd NPs were obtained by RNA-templated synthesis selected by the SELEX *in vitro* evolutionary method. Reproduced with permission from ref. 61. Copyright (2005) ACS. **b**, Ag-core/Ni-shell NPs templated by λ -phage DNA via photoreduction/electroless deposition⁵⁸. **c**, Infrared-emitting PbS NPs templated by calf thymus DNA showing a high degree of crystallinity. Reproduced with permission from ref. 65. Copyright (2005) Wiley.

Backbone modification was explored as a NP tuning element by Jiang *et al.* who investigated CdS NP synthesis using oligoG₁₀ phosphorothioate templates⁴⁹. The phosphorothioate group is a standard backbone modification where a P=O bond is substituted for a P=S bond, resulting in nucleic acid sequences characterized by an increase in hydrophobicity and higher resistance to hydrolysis. In this example, the sulphur atom of the phosphorothioate moiety provided a strong additional interaction with Cd²⁺ increasing the affinity of this ion for the oligonucleotide backbone. This interaction resulted in the disruption of the characteristic intermolecular hydrogen bonding between oligoG₁₀ molecules while providing a nucleation centre. On addition of S²⁻, 2-nm CdS NPs templated by oligoG₁₀ were formed and the coordination site switched from the phosphorothioate moiety to the guanine base.

This phosphate-to-base coordination switch was also observed by Jin and co-workers who used salmon sperm DNA as the corresponding template for the synthesis of CdS NPs⁵⁰. The growth of CuS NPs with diameters of less than 10 nm have also been observed both in solution and on a substrate, using the genomic DNA of bacteriophage λ — a common virus — as a template. TEM imaging confirmed NP crystallinity. However no details were provided concerning the sequence or composition dependence of NP formation⁵¹.

Metal NPs have been formed using DNA templates and this topic has been reviewed elsewhere^{52,53}. An interesting example was that of Petty *et al.*, which showed that very small silver clusters synthesized by controlling the stoichiometry of complexation with short ssDNA sequences exhibit intense fluorescence⁵⁴. In a similar study, fluorescent silver nanoclusters were grown on oligoC₁₂ sequences and the metal binding was confirmed to occur at cytosine N3 in a pH-dependent fashion⁵⁵. In another recent investigation, silver NPs grown on dsDNA displayed chiral optical properties, most likely arising from the intrinsic polynucleotide helical chirality⁵⁶. It was speculated that this chirality stemmed from the induction of asymmetric growth because it was not displayed in silver nanoparticles that were grown in solution and later complexed onto dsDNA. Berti *et al.* demonstrated that DNA is not just a passive structural biomineralization template and that it can play an active role in NP synthesis. Exploiting the light-harvesting properties of DNA, it was demonstrated that photoinduced reduction of pre-complexed Ag⁺ atoms led to formation of small Ag nanoparticles⁵⁷, which were further grown under DNA control

by electroless addition of Ni (ref. 58) (Fig. 4b). Pd-core, Ni-shell NPs were prepared similarly⁵⁹.

RNA is a biopolymer closely related to DNA, from which it differs only by the presence of a hydroxyl group on the ribose sugar ring (see Fig. 1). RNA has been demonstrated to be an outstanding biotemplate as it adopts stable and well defined shapes which are highly sequence-dependent. In a seminal publication, Eaton and co-workers have exploited *in vitro* evolutionary techniques to select RNA sequences that have the ability to nucleate and stabilize the formation of hexagonal Pd nanoparticles⁶⁰ (Fig. 3). In their study, 10¹⁴ random RNA sequences containing the unnatural base 5-(4-pyridylmethyl)-uridine were generated and exposed to a source of Pd⁰. RNA sequences that either nucleated Pd nanoparticles and remained bound to them, or RNA sequences binding to spontaneously formed NPs were subjected to evolutionary selection. After eight cycles, Pd NPs of mainly hexagonal shape were formed, with a minority of cube and rod shaped NPs. It was suggested that a single RNA sequence can potentially catalyse the formation of Pd NPs and that the sequence is directly related to the rate of formation and to the final shape⁶¹ (Fig. 4a). RNA sequences responsible for nucleating hexagonal and cubic Pd NPs were also immobilized on an Au surface, showing that their NP templating activity persisted even when immobilized onto a substrate⁶². A dilution series of RNA sequences on the surface further reinforced the hypothesis that one RNA molecule might be enough to nucleate NPs. RNA sequences have also been shown to direct the formation of fluorescent NPs. For instance, synthesis of CdS in the presence of *E. coli* transfer RNA — a type of RNA involved in protein biosynthesis — yielded highly soluble and fluorescent NPs, although no indication of sequence dependence was reported⁶³. In another study, yeast RNA was shown to template the synthesis of PbS NPs and their photophysical characteristics were shown to be dependent on the experimental conditions used⁶⁴. Interestingly, PbS NPs templated by DNA (Fig. 4c) showed an emission maximum at 1,100 nm (ref. 65) whereas PbS NPs templated by RNA showed an emission in the visible region, around 675 nm (ref. 64).

Peptide nucleic acid (PNA) has also been investigated as a templating structure. PNA is a synthetic polymer, related to DNA and RNA, where the phosphodiester bond connecting the constituent nucleotides has been substituted for a peptidic (amide) linkage. Although the self-recognition properties and sequence-dependent shape typical of nucleic acids are retained in PNA, these synthetic

molecules are characterized by high resistance to enzymatic and chemical degradation and by the absence of electrostatic charges on the backbone. In a recent example by Wang *et al.* it was reported that chains of Pt nanoparticles were formed by chemical reduction of a Pt-precursor complex pre-bound on a PNA template. The dimension of the resulting Pt NPs could also be tuned by controlling the reduction time⁶⁶. The formation of NPs on a PNA template would seem to minimize the role that phosphates might play in templating nucleic acid sequences as PNA is devoid of a complexing backbone. Although the exact mechanism of nucleic acid-templated NP formation is still unclear, theoretical simulations of nucleic acid-templated Pt NP formation have shown that a Pt–Pt bond forms as the result of proximal interactions of two Pt complexes — one bound to the biopolymer and one free in solution^{67,68}. The formation of the Pt–Pt bond is concomitant to the release of a water molecule and this process is apparently favoured by strong donor ligands, such as purine bases. Although there is no evidence to support it, this relationship might also be valid for other metallic precursors.

OUTLOOK

Nucleic acid/nucleotide-templated NP synthesis is a young and rapidly evolving field. The ability to tune the size and physico-chemical properties of NPs according to nucleic acid structure, composition and sequence holds enormous potential for the rational design of NPs⁶⁹. As the Eaton^{60–62} and Kelley^{30,38,63} groups have shown, the properties of NPs can be fine tuned according to the constituent nucleotide or nucleotide triphosphate building blocks. The ability to synthesize and screen libraries of modified nucleotides holds considerable promise for the synthesis and selection of non-natural sequences that might lead to the synthesis of NPs with novel properties.

Another advantage of nucleic acids as ligands is the use of *in vitro* evolutionary techniques to identify suitable templating sequences⁷⁰. It is also envisaged that the use of unnatural nucleotides incorporating reactive chemical groups would allow further augmentation of functionality at various checkpoints in the molecular evolution process, thereby providing further modulation of metal–nucleic acid interactions. The potential of such reactive moieties has been elegantly demonstrated by the incorporation of alkyne functions into DNA strands using the polymerase chain reaction. The power of this method was highlighted by the ability of modified DNA strands to selectively direct silver deposition in the presence of naturally occurring DNA strands^{71–73}.

One area of NP research that is particularly challenging is the ability to produce NPs that are both biocompatible and are outfitted with a defined set of recognition motifs for bioconjugation. Current methods rely on stoichiometric control by statistical methods. However such techniques are complicated by tedious separations for selecting the desired functionalization ratios. As the Eaton group suggested, a single RNA molecule seemed sufficient to template Pd NPs^{61,62}. It would therefore be feasible to generate bifunctional RNA modules where one end templates the synthesis of NPs while the other interacts with the surrounding environment without interfering with the NP surface.

The lack of a fundamental understanding of the underlying mechanisms of nucleic acid/nucleotide templated NPs synthesis is currently an issue⁷⁴. It is both desirable and foreseeable that a great deal of effort will be put towards the elucidation of the templating mechanism. To this extent, a systematic investigation of the role that each component of the template (sequence, composition, length, concentration, and so on) has in NP synthesis would be an important undertaking in order to provide a set of rules that will assist in programming the synthesis of NPs. Other important questions that need to be addressed are the role of metal ion–ligand bond strength in defining the outcome of the templated NP synthesis and the effect of the nucleobase on the size and photophysical properties of templated

NPs. A concerted multidisciplinary effort involving chemistry, biology and physics will most likely be required in order to fully explore the potential of nucleic acids/nucleotide-templated NP synthesis.

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