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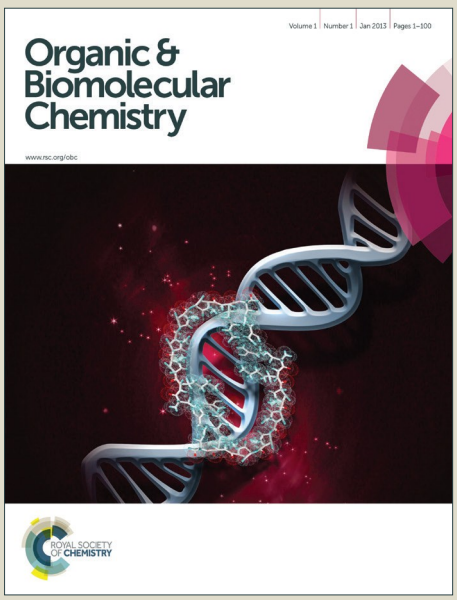

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Aggregation of Inorganic NPs Mediated by Biomimetic Oligomers

Hagar Tigger-Zaborov and Galia Maayan*

Abstract

Assemblies of nanoparticles (NPs) have been broadly used for the construction of materials with unique spectroscopic and chiral properties for applications in various scientific disciplines such as sensing, bio-nanotechnology and medicine. Mediating the aggregation of NPs by synthetic biomimetic oligomers, namely, DNA, PNA, peptides and peptide mimics, rather than by small organic molecules has shown to produce interesting supramolecular structures and enable the combination between the biocompatibility of the mediators and the NPs spectroscopic properties. Yet, the key of using this powerful approach for designing new functional materials is to understand the aggregation patterns of NPs by biopolymers and biomimetic oligomers. Herein we describe the important developments in this field, from early studies to recent work with an emphasis on synthetic methods and tools for controlled assembly of metal NPs by biomimetic polymers and oligomers.

1. Introduction

1.1. Definition and Preparation of Nanoparticles

NPs are assemblies of atoms with overall dimensions of less than 100 nm.[1] Although research on NPs have gained allot of momentum in the last few decades, NPs have been used all throughout history.[2] One of the oldest evidenced for it is their use by artistes in Mesopotamia twelve centuries ago to light up dishes.[2] Metal(0) NPs were also used in medieval times for glass staining, due to their unique optical properties.[2] These properties, however, were only addressed scientifically for the first time by M. Faraday in 1857.[3] Since then, NPs have been implanted in a variety of disciplines due to their vast applications in many fields, such as medicine,[4] electronic storage systems,[5] biotechnology,[4] and sensing.[6, 7] The development towards these applications is related to the NPs physical and chemical properties, which are different from those observed in the bulk. Due to their small size, NPs possess a high surface area.[1] One example is the case of cuboctahedronic Pt(0) NPs, in which about 40% of all the atoms in a particle are located at the surface layer.[8] The high surface area results in a dramatic change in the electronic and optical properties of NPs compared to the bulk materials, leading to wide possibilities of applications.[1, 9] The applicability of

NPs depends on the strategy employed for their stabilization; bare NPs tend to get stabilized either by absorption of molecules from the surroundings or by lowering the surface area through agglomeration (precipitation). In order to avoid the later, NPs have to be stabilized by external cupping agents.[9, 10] The common stabilizers are divided to three groups, according to their interactions with the NPs: electrostatic, steric, and electro-steric.[10] Common ligands contain functional groups such as thiols,[11, 12] amines,[13-15] and phosphines,[16] which interact with the NPs surface and can often be introduced within biomolecules.[17, 18] Small molecules such as trisodium-citrate,[15, 19] ethylene glycole,[20] resorcinol,[21] phenantroline,[14, 15] as well as macromolecules such as polymers,[22] peptides,[18, 23, 24] DNA,[17, 25] peptide mimics,[15, 26] and others[27] have been successfully used as stabilizers for metal NPs.

There are two approaches for NPs synthesis: the top-down approach, in which a bulk material is cut down to the nanoscale particles, and the bottom-up approach, which is associated with constructing of nanostructures by chemical synthesis, using single atoms, molecules or ions as precursors.[18,28] The first approach offers precise control over the size and shape of NPs, however it is time consuming. The second approach involves chemical synthesis of NPs, which is typically influenced by the reactants concentration, the solvent type, the reaction time and especially by the presence of a stabilizing agent. There are two common chemical methods for NPs synthesis. The first method is the *indirect synthesis*, in which a known reduction protocol that includes the use of labile ligands (e.g. citrate,[15, 19] ethylene glycol[20]) stable under the reaction conditions, is followed by their displacement by unique ligands, (e.g. biomimetic molecules), that are typically unstable under the reaction conditions.[15, 17] The second approach is the *direct synthesis*, in which the NPs are stabilized directly by the unique ligands under the appropriate reaction conditions. The ligand used for stabilization can also serve as the reduction agent in the synthesis of the NPs from the corresponding metal ions precursor(s).[29] In the last two decades, numerous synthetic protocols for the stabilization and aggregation of metal NPs with biomolecules have been reported for a variety of metal NPs, such as Ag(0),[15, 29, 30] Au(0),[17, 23] PtCo(0),[31] Fe(0),[50 32] Cu(0),[11] and more[2, 7, 33].

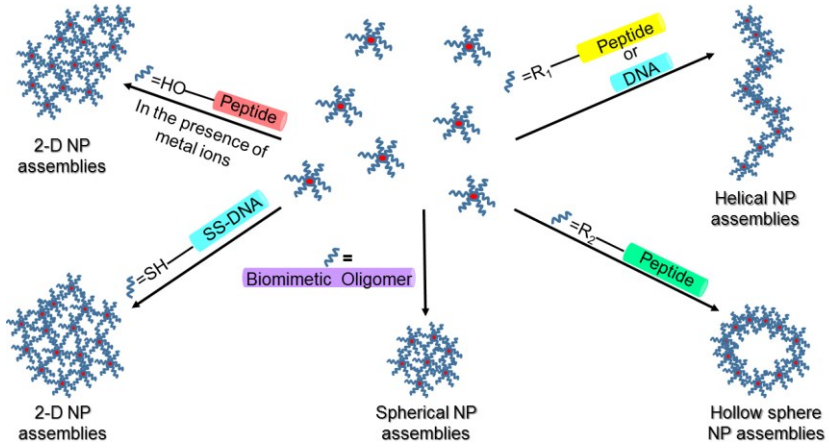
1.2. Controlled Assembly of Nanoparticles

Organization of individual NPs into defined assemblies, also known as controlled aggregation of NPs was investigated extensively in the last three decades due to the unique spectroscopic properties of the assemblies, which arise from their arrangements. Such assemblies

enable interparticle interactions as well as the creation of interfacial or interparticle binding sites for molecular interaction or recognition, which are the basis of chemical sensing.[7] For example, the sorption of volatile organic compounds (VOCs) on NPs assemblies leads to a change in electron hopping or tunneling properties depending upon the particle size and the inter-particle distance.[7, 34] Similarly, NPs assemblies are used as probes for the separation and detection of DNAs, proteins and VOCs via interparticle amplification of optical and surface-enhanced Raman scattering (SERS) signals.[6,7] In general, aggregation of NPs is induced by intermolecular interactions that origin from the intramolecular attraction forces within the ligands and from the surrounding media.[35-37] Depending on the chosen ligand, it is possible to modify the arrangements of the assemblies, namely their size, shape and morphology, and control the inter-particle distance within the assemblies. [23, 31, 38-40] Ensembles containing both metal NPs and organic molecules are of special interest because they display the properties of both components, which may lead to unique applications.[34] These ensembles and their applications have been reviewed elswehere focusing mainly on the assembley process and its dynamics, assembly by small organic molecules and synthetic polymers, and the function of the NPs assemblies.[4, 5, 41-43] Herein, we intend to review synthetic methods for NPs assembly mediated by biomimetic polymers and oligomers, namely DNA, PNA and peptidomimetics. Moreover, we believe that in order to be able to get insights into the NPs aggregation process as well as to control the properties of the final NPs assemblies towards specific applications, there is a need to develop a synthetic tool box for the assembly of NPs. Hence, this review will focus on various modifications that were systematically applied on the biomimetic polymers and oligomers mentioned above towards controlling the final size, shape and morphology of the NPs assemblies (Scheme 1).

The use of synthetic biopolymers, biomolecules and biomimetic polymers/oligomers/molecules as mediators for NPs assembly offers two major advantages over small organic molecules or synthetic polymers. The first advantage is the versatility of these molecules regarding their sequence, structure and their ability to have intermolecular interactions (e.g. by unique cross linking) enables their precise tuning towards better control over the size, shape and morphology, and consequentially over the functionality of the final aggregated products.[44] Thus, the properties of NPs assemblies can be controlled by tailoring the stabilizing ligand molecules to interact by hydrophobic and/or hydrophilic interactions,[35, 45] hydrogen bonding,[39, 45] л-л stacking,[45] and DNA sticky ends.[17] The second advantage is the capability to combine between the

biocompatibility of the mediator and NPs properties.[15, 31] In addition, due to the influence of the surrounding medium (e.g. the reaction solvent,[45] the pH,[45, 46] and heavy metal ions content,[47, 48]) on biomimetic polymers and oligomers, modifying these parameters may impact their ability and/or mode of action as mediator for NPs assembly. These two advantages as mentioned above, provided a powerful approach for designing sensors,[34] developing gene carriers[49] and enhancing spectroscopic signals.[6]



Scheme 1. Graphical summary of synthetic methods and oligomers types discussed in this review.

1.3. Optical Properties of NPs, NPs Assemblies and Related Applications

One of the most common applications for NPs assemblies is sensing, which is directly related to their unique optical properties. In general, the optical properties of NPs are derived from the plasmons phenomena, which was documented for the first time by Faraday in 1857.[3] In 1908, Mie published a paper describing how to calculate light scattering by small spherical particles using Maxwell's equations. These calculations provided an explanation for the observation that the color of Au(0) NPs changes as their diameter changes.[50] These results were later interpreted in terms of surface plasmons.[51]

Plasmons are free electrons present in the metallic NP surface that are coupled to generate collective oscillations in the presence of electromagnetic radiation.[7, 52] These oscillations reach resonance in different wavelengths depending on the nanostructure size,[7, 52] identity,[7, 52] and the

dialectic constant of the medium.[7, 52] When electric radiation applies force on metallic NPs, the plasmons oscillate collectively.[7, 52] At a certain frequency, these oscillations will be in resonance with the incident light, which is known as the localized surface plasmon resonance (LSPR).[52] NPs that support LSPRs are those of Ag(0), Au(0) and Cu(0).[7, 52] When the NPs assembly process occurs, the distances between the NPs are decreased according to the reaction conditions leading to a unique optical activity of the NPs assembly, different from the optical activity of the well-dispersed NPs.[7] Applying an electromagnetic radiation on NPs assemblies, results in LSPRs coupling and red shifted absorbance band(s) (Fig. 1).[7] In addition, a massive and highly localized radiation enhancement is generated between the individual NPs, commonly known as the hot-spots.[7] Thus, controlling the gap width between the NPs is highly important for tuning the spectroscopic properties of the assemblies towards applications in sensing.[7] Moreover, the LSPR absorbance is dependent on the gap width; SPR absorbance of NPs dimers with a 2 nm gap width is located at about 500 nm, while a 10 nm gap width result in an absorbance band at 450 nm as demonstrate in Fig. 1.[7] Most of these changes can be observed by the human eye.

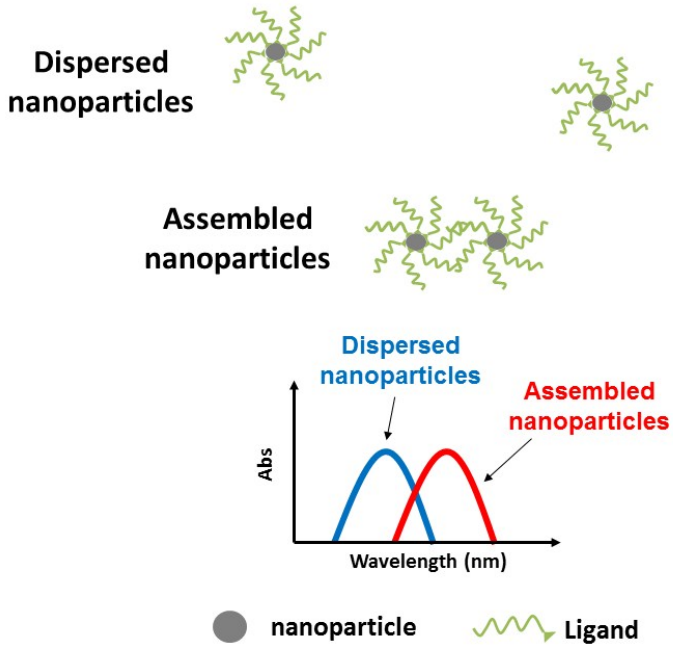


Fig. 1 Schematic representation of NPs aggregation and its effect on plasmonic absorbance.

Due to these properties, NPs-plasmon based applications were developed, some of them are presented as follows. *(i)* *colorimetric sensing*. This method,[47, 53,54] includes detection of the assembly

mediator, which its presence can be visually observed. The assembly mediators can be either different metal ions,[53] oligonucleotids (in case of DNA mediated NPs),[17] Peptide Nucleic Acids,[55] pH,[46] or solvent[56]. *(ii) Surface Enhancd Raman Spectroscopy (SERS)* Raman spectroscopy is a technique that enables the detection of molecular vibrations. However, this method known for its relatively low signal, therefore, signal enhancement is needed for detection of compounds at low concentrations.[7] SERS answers this requirement; the presence of noble metal NPs assemblies within the analyzed solution enhances the intensity and sensitivity of the Raman spectroscopy.[7] This effect is a result of surface plasmons that can transfer energy to the analyzed molecule, through the associated electric field formation (hot-spots). In addition, the location of these hotspots, in which the electromagnetic field was concentrated, is largely determined by the spatial arrangements of nanoparticles and their shapes.[6, 57]

A different plasmonic related NPs property is chirality. Chiral NPs can be observed by Circular Dichroism (CD) spectroscopy analysis along with the plasmonic wavelength of the NPs. This phenomena can be observed in several cases: (1) when non-chiral NP interacts with chiral molecules,[58] (2) when achiral NPs arranged in a chiral configuration,[33, 58] and (3) when a chiral shape of NP is created.[58] Chiral NPs can be prepared by several approaches; two will be discussed here. The first approach involves chiral arrangement of NPs.[58, 59] One example is by using DNA Origami method,[33] in which the NPs are modified with specific oligonucleotides that are hybridized to a chiral structure. The second approach involves chiral stabilizers in two synthetic methods; the first is a direct placement of NPs on chiral configuration, namely synthesis of NPs directly in presence of chiral stabilizer, e.g. helices of peptide,[39] DNA,[29] and more[60]. The second method refers to the indirect approach of NPs synthesis, namely the replacement of achiral labile stabilizers of the NPs by chiral ones. In addition, NPs aggregates can also form chiral superstructures that have an effect on the plasmonic CD signal.

2. Biomolecules and Biomimetic Oligomers as Mediators for Nanoparticle Assembly

2.1 DNA as a Mediator

Oligonucleotides are useful NP stabilizers and function successfully as mediators for NPs assembly under biotic conditions. In order to allow efficient interactions between the oligonucleotides and the NPs, several different modifications can easily be done by the incorporation

of various (NPs) linker groups to the oligonucleotides. Moreover, the DNA's base-pairing interactions can also provide a variety of aggregation programming capabilities.

In 1996, Alivisatos and co-workers created a discrete array of Au(0) NPs, by using a DNA mold.[25] The assemblies were generated in two steps: first, phosphine and N-propylaminaleimide mediated Au(0) NPs were prepared. Thereafter, the NPs were integrated within sulfhydryl groups (by coupling to the N-propylaminaleimide groups) located on the 3' or 5' terminus positions of an 18 bases oligonucleotide (shown in Fig. 2). These NP-oligonucleotide hybrids were combined with the long corresponded 37 or 56 bases complimentary oligonucleotides to form a DNA scaffold, under biotic conditions, leading to a successful synthesis of parallel dimers and trimmers. This approach was expended to the construction of larger Au(0) NPs assemblies in 1999 forming NPs assemblies of homodimeric, heterodimeric, homotrimeric and heterotrimeric nucleotides.[61]

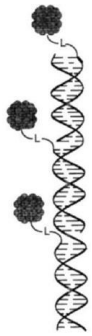


Fig. 2 Parallel trimmer incorporating Au(0) NPs Parallel trimmer. Reprinted with permission from the *Nature* Publishing Group, copyright (1996) from ref 25.

In 1996, Mirkin et al introduced thioled-oligonucleotide cupped Au(0) NPs assemblies.[17] These assemblies were synthesized using the direct approach: primarily, a solution of thioled DNA (3'-HS-TACCGTTG-5') protected Au(0) NPs was mixed with a solution containing 3'-HS-TTTGCTGA-5' mediated Au(0) NPs. Due to the non-complimentary nature of the oligonucleotides, mixing both solutions did not result in any aggregation. Secondly, addition of 12-bases duplex containing eight complimentary sticky ends to the above oligonucleotide-cupped Au(0) NPs mixture and adjusting the solution to DNA hybridization conditions resulted in NPs aggregation (shown in Fig. 3). The assembly process was followed by a color change from dark red to purple leading to an absorbance band located at 700 nm. This aggregation pattern is reversible; increasing the

temperature to 80°C caused a disassociation of the DNA duplex. This method can be used for sequence specific oligonucleotide colorimetric detection, as well as for controlling the distances between the NPs in the assembly towards potential SERS application.

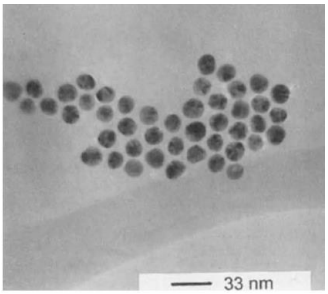


Fig. 3 TEM micrograph of polymeric DNA-Au(0) NPs. Reprinted with permission from the Nature Publishing Group, copyright (1996) from ref 17.

DNA mediated Au(0) NPs can be also assembled by the presence of metal ions in a nano-molar concentration, as demonstrate by Lee and Mirkin in 2007.[53] In this study, two types of DNA mediated 15 nm Au(0) NPs were synthesized by the indirect approach; type A was passivated with 5'HS-$C_{10}A_{10}TA_{10}$-3' and type B was cupped with 5'HS-$C_{10}T_{10}TT_{10}$-3', which are complementary except for a single thymidine–thymidine mismatch. Combining type A and B under biotic conditions produced a red solution of NPs assembly, which exhibit sharp melting transitions, Tm, at 46°C. Addition of Hg^{+2} ions to the system, resulted in selective coordination of Hg^{+2} ions to the bases that created a T–T mismatch, thus raising the Tm, accompanied by a color change to purple (Fig. 4). The changes in the Tm were monitored as a function of Hg^{+2} ions concentration; for example, addition of 2.0 µM Hg^{+2} ions, resulted in Tm of 56°C. Additional experiments showed that each increase of 1 µM in concentration results in an increase of ~5°C in Tm. The system's detection limit is approximately 20 ppb of Hg^{+2} ions and it is highly selective for the detection of Hg^{+2}, as experiments done with other metal ions did not lead to an increase in the Tm. The colorimetric sensor assembly's sensitivity, selectivity, and quantitative capabilities origins from the combination between the oligonucleotides sequence, which designed for selective binding for Hg^{+2} ions and the Au(0) NPs, which are the actual detection agents.[53]

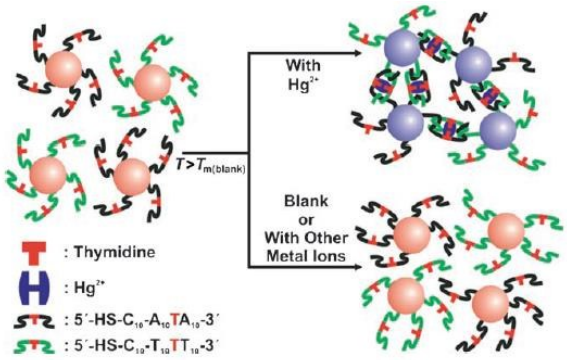


Fig. 4 Colorimetric detection of mercuric ion using DNA–Au(0) NPs. Reprinted with permission from John Wiley and Sons, copyright (1997) from ref 53.

The formation of Ag(0) NPs assemblies can be also mediated with oligonucleotides that were attached to a cyclic disulfide linker group (DSP), as demonstrated by the group of Mirkin in 2007 (Fig. 5).[30] A typical experiment included the synthesis of 31 nm Ag(0) NPs by the indirect approach. Two types of Ag(0) NPs were synthesized, Type A, which was mediated with 5'-$(DSP)_3$-A_{10}ATTATCACT-3', and Type B, which was cupped with 5' $(DSP)_3$-A_{10}AGTGATAAT-3'. Both solutions resulted in a yellow color. The assembly process, in which equal amounts of both Ag(0) NPs solutions were mixed at room temperature, was carried out under high salt content and phosphate buffer. This experiment resulted in pale red colored solution and a shift in the UV-Vis spectrum, from 400 nm to 560 nm. Analogues to previous research done with Au(0) NPs,[17] this process is also reversible. The Tm of the aggregate's solution is dependent on its NaCl concentration; for example, 0.15 M and 0.70 M solutions showed Tm data at 45°C or 60°C respectively. This approach can be used to tune the distances between the NPs, towards the construction of NPs assemblies suitable for SERS applications.

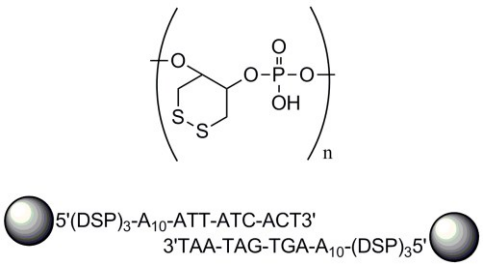


Fig. 5 Oligonucleotide sequnce and stucture of DSP.

In 2008, Köhler and Fritzsche prepared bimetallic aggregates, combining Au(0) and Ag(0) NPs by using the DNA hybridization method, which is based on DNA sticky ends, as before.[62] In this work, a 14 nm Au(0) NPs were synthesized by the indirect approach, and were passivated by 5'-SH-TGCT$_4$ATCGGGCGGAATG-3'. Separately, 23 nm Ag(0) NPs were passivated by a complimentary oligonucleotide (5'-SH-CATTCCGCCGATA$_4$GCA-3'). Mixing both NPs solutions under DNA hybridization conditions produced bimetallic assemblies as shown in Fig. 6. The ratio between the different NPs in the assembly products depended on the ration between the two NPs solutions. Notably, these assemblies were not uniform; in some cases, the Au(0) NPs did not fully cover the Ag(0) NPs, some triangular shaped Au(0) NPs were formed in addition to the spherical ones and it was not clear whether the short rod-like assembly of Au(0) NPs is a part of the whole assembly or whether it is a consequence of a different assembly process that does not involve the Ag(0) NPs. However, such approach also enables the combination of bimetallic assemblies to be applied in the formation of trimetallic or quadrometallic NPs aggregates such as pyramids.

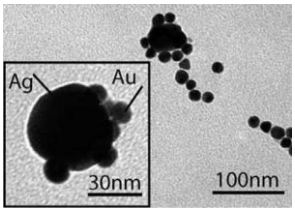


Fig. 6 TEM micrograph of DNA-cupped Ag(0) and Au(0) NPs aggregates. Reprinted with permission from John Wiley and Sons, copyright (2008) from ref 62.

In 2012 Kotov and collaborators prepared tetrahedral nanostructures containing four different NPs on the edges of a pyramid.[33] In order to construct pyramid shaped NPs assemblies, different oligonucleotides bearing a hexyl-thiol linker at their 5th end were used to mediate the assembly of NPs. Thus, several types of NPs were prepared: a 10 nm, 15 nm and 25 nm Au(0) NPs, 10 nm Ag(0) NPs and 10 nm Cd-Se(0) core with Zn-S(0) shell quantum dots (QDs). The NPs were assembled to pyramids using DNA, under DNA hybridization conditions, in two-steps; first, NP dimers were prepared by hybridizing two complementary NP−oligonucleotides complexes, and second, in order to assemble the final pyramid, additional hybridization of the two NP dimers was performed. Some of the resulted pyramids were chiral, due to the presence of different NPs in each

edge. Their chirality was monitored by Circular Dichroism (CD) spectroscopy, which can detect the absorbance of such NPs due to their plasmonic wavelength. Six different types of pyramids arrangement were synthesized, by changing the geometrical parameters, the NPs size and the metal based-NPs. The pyramid types that were obtained were the following: *type 1* containing all four 10 nm Au(0) NPs, *type 2* containing three 15 nm Au(0) NPs with 25 nm Au(0) NPs, *type 3* containing two 15 nm Au(0) NPs with two QDs, and *type 4* containing 15 nm Au(0) NP with 25 nm Au(0) NP and two QDs. Finally, *S - type 5* and *R - type 6* containing enantiomers of 10 nm Au(0) NPs were obtained in addition to QD and Ag(0) NPs-based pyramids. The CD spectra and the TEM micrographs of the ensemble pyramids of types 6 and 5 are shown in Fig. 7. In the context of CD analysis of NPs it is important to note that this phenomena can be observed in the following occasions: (i) when non-chiral NPs interact with chiral molecules,[58] (ii) when the NPs are arranged in a chiral configuration,[55] and (iii) when the NPs formed have a chiral shape.[58, 59]

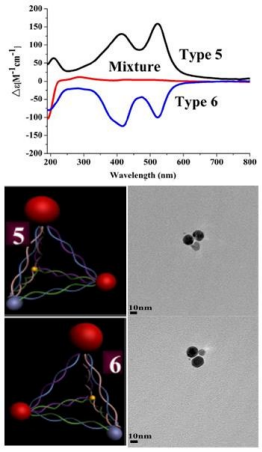


Fig. 7 TEM micrograph and CD spectra of self-assembled pyramids; S (type 5) and R (type 6) enantiomers. Reprinted with permission from American Chemical Society, copyright (2012) from ref 33.

The ability to tune and control the positions of NPs in an assembly opened a wide door for the formation of linear assemblies, as demonstrated in 2010, by Zuckermann and Bokor.[63] Their research focused on using DNA for the organization of Au(0) NPs with different sizes in a linear structure with controlled spacing, by a DNA scuffled origami method.[63] First, a long cyclic oligonucleotide was hybridizes with designed single-strands to form a triangular DNA template with different binding sites on one side of its surface (which contained DNA sticky-ends). Second, different Au(0) NPs (15, 10,

and 5 nm) covered by the corresponding thiolated complementary DNA strands, were assembled to the designed position of the DNA mold through complementary hybridizations. This procedure is shown on Fig. 8. The spacing between the NPs was controlled by the position of these sticky-ends. Each Au(0) NP (15, 10, and 5 nm) was bounded by three DNA linkages to the DNA template.

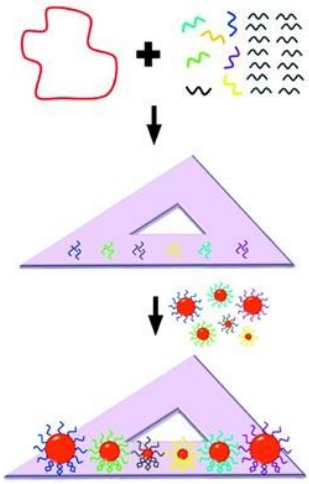


Fig. 8 Schematic representation of the NPs assembly formed from six Au(0) NPs on a triangular DNA mold, attached through DNA hybridization. Reprinted with permission from American Chemical Society, copyright (2010) from ref 63.

The DNA origami method as described above, was also applied for the construction of helical Au(0) NPs assemblies, based on DNA 24-helix in a research published by Kuzyk, Govorov and Liedl in 2012.[64] The helical assemblies were generated on a surface of DNA origami 24-helix packages, which includes 15-nucleotide-long single-stranded extensions of oligonucleotides. The formed helix assemblies had a diameter of 34 nm and a helical pitch of 57 nm. This mold was used for the assembly of nine Au(0) NPs, each having a diameter of 10 nm, which were passivated by complementary thioled-DNA strands, as shown in Fig. 9. The optical activity of these assemblies was confirmed by their CD spectra showing an absorbance band at 545 nm, which is a typical LSPR peak corresponding for assembled Au(0) NPs. In this case, the chirality was originated from the helical arrangement of the Au(0) NPs.

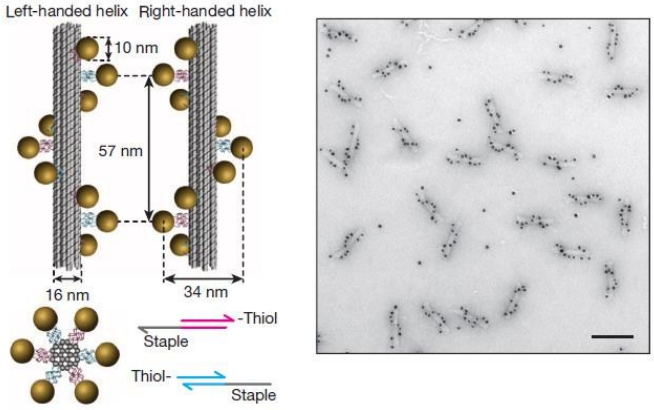


Fig. 9 Assembly of DNA origami Au(0) NPs helices. Reprinted with permission from the Nature Publishing Group, copyright (2012) from ref 64.

The DNA origami approach was further developed for crystalline DNA-Au(0) NPs assemblies synthesis as demonstrated in a study that was carried out by Mirkin and collaborators.[65] As mentioned above, the DNA origami approach involves pre-tuned DNA interactions leading to the formation of superlattices with different symmetries. The crystalline assembly approach relies on oligonucleotides that are designed to hybridize with sequence specific DNA sticky ends in order to mediate the assembly of NPs. These approach resulted in a simple cubic superlattice of Au(0) NPs (a schematic representation is shown in Fig. 10). In order to create other superlattices with several different symmetries, such as simple hexagonal and graphite-type, spherical nucleic acid (SNA) NPs conjugates were used. The SNAs synthesis included the formation of a crosslinking alkyne-modified DNA on the Au(0) NP surface and subsequent dissolution of the Au(0) NPs mold to create hollow spherical NPs with a layer of densely packed, oriented nucleic acids. The SNA can be used as three-dimensional spacers that occupy specific lattice positions based on their DNA sequence.

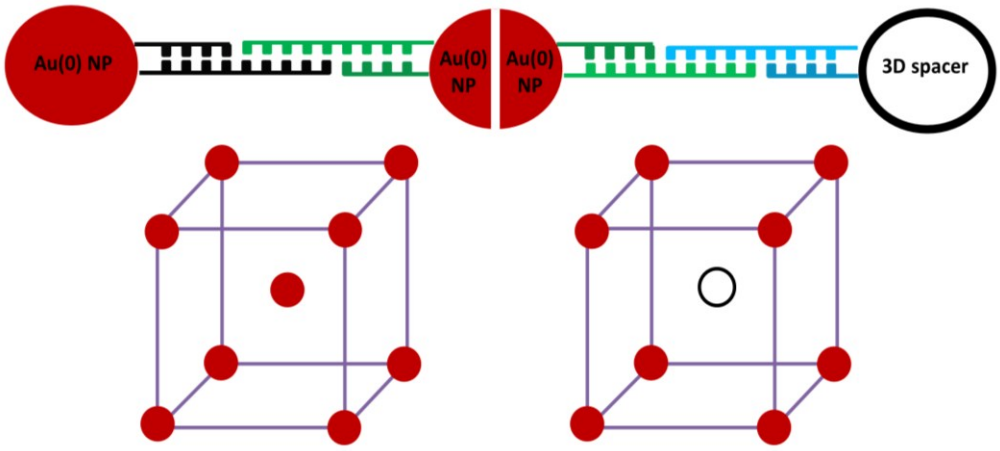


Fig. 10 Schematic representation of a simple cubic lattice formed by Au(0) NPs and complimentary DNA sticky ends (left) and a 3D spacer (SNA) incorporated in a simple cubic lattice formed by Au(0) NPs (right).

Overall we can see that the use of DNA as a mediator for the assembly of NPs can be controlled by a careful design of the oligonucleotide strands and their sequence modifications. The use of specific DNA base-pairing can lead to 2-D, chiral, helical and even crystalline NPs assemblies. The rigid base paring of DNA provides opportunities to construct a variety of NPs assembly geometries towards specific application development. However, the stabilization and aggregation of NPs by DNA often requires synthetic modifications such as the incorporation of special linkers. These modifications may change the properties of the oligonucleotide mediators, thus influencing their final applications. Moreover, the use of DNA as a mediator and of the NPs assemblies mediated by DNA is limited due to the specific conditions in which DNA base paring occurs, namely only at neutral pH, under specific salt content, and below the Tm.

2.2. Peptide Nucleic Acids as Mediators

Peptide nucleic acids (PNAs) are synthetic homologs of nucleic acids in which the phosphate–sugar polynucleotide backbone is replaced by a flexible pseudo-peptide polymer.[90] This structure gives PNAs the capacity to hybridize with high affinity and specificity to complementary sequences of DNA and RNA, following Watson-Crick base pairing rules.[66, 67] The advantages of PNAs include better stability than DNA duplexes, especially for short sequences, and superior mismatch sensitivity.[67, 68] In addition, unlike the sugar-phosphate scaffolds, the polyamide one can be easily modified, thus allowing to tune the properties of the PNA molecule for example, by using

other amino acids such as glycine.[67] In 2003, Chakrabarti and Klibanov discovered that the hybridization of PNA or thioled-DNA modified Au(0) NPs for assembly formation is possible.[69] First, exanimation of the stability of PNA cupped Au(0) NPs indicated that hydrophobic interactions decrease the NPs stability while high charge density (charged PNAs) enhance the stability of the NPs. Assembly of PNA cupped Au(0) NPs was directed by a complimentary PNA linker strands under low salt concentration. The procedure included freezing the mixture at -78^{o}C for an hour, followed by thawing to room temperature. For example, Au(0) NPs which were mediated by Ac-Cys-O_5-TTTCTTCGGACTTGTAAC-Glu-am (Ac – acetyl, am – amide, O_n refers to an n-mer of the linker unit -$NHCH_2$-O-CH_2-CH_2-O-CH_2-CO-), were assembled with Au(0) NPs, which were mediated by Ac-GTCAGTGCTACTO-Glu-Glu-Glu-Cys-am via the complimentary DNA, Ac-CACTGACTGTTACAA-am. The assemblies are shown in Fig. 11. These NPs exhibited change in the SPR from 520 mn to 529 nm. However, these NPs assemblies precipitated after five days. The requirement of low temperatures to facilitate assembly of the PNA system indicates that the electrostatic barrier for NPs approach to each other is high. Freezing may initiate assembly by forcing the particles into close proximity, overcoming the initial barrier to the formation of aggregate nuclei.

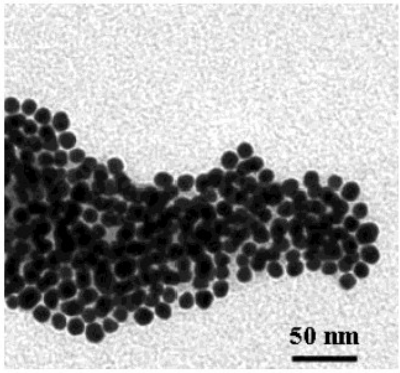


Fig. 11 PNA mediated Au(0) NP assembles. Reprinted with permission from American Chemical Society, copyright (2013) from ref 69.

In 2009, Schatz, Mirkin, Nguyen and coworkers investigated the stability and Tm of DNA-PNA modified Au(0) NPs, (Fig. 12).[70] Because PNA mediated Ag(0) NPs exhibit low stability and low water solubility in the absence of surfactants or charged amino acids, the Ag(0) NPs were mediated with DNA by the indirect approach. The aggregation was carried out in the presence of complimentary PNA linkers in a PBS buffer (0-0.5 M NaCl, 10 mM phosphate, pH = 7.1). In order

to monitor changes in the Tm, this mixture was heated to 55ºC for 10 minutes, and was allowed to equilibrate at room temperature. Tm monitoring revealed interesting trends. First, DNA modified Ag(0) NPs, which were assembled by a PNA linker, exhibited sharp Tm, in spite of the uncharged state of the PNA linker. Second, a reversed dependence of the PNA-linked NPs Tm with regard to salt content, was observed, in contrast to this dependence using the free DNA-PNA duplexes. According to the authors, this reversed dependence could be attributed to the condensed ion clouds associated with closely spaced PNA:DNA duplexes within the aggregates.

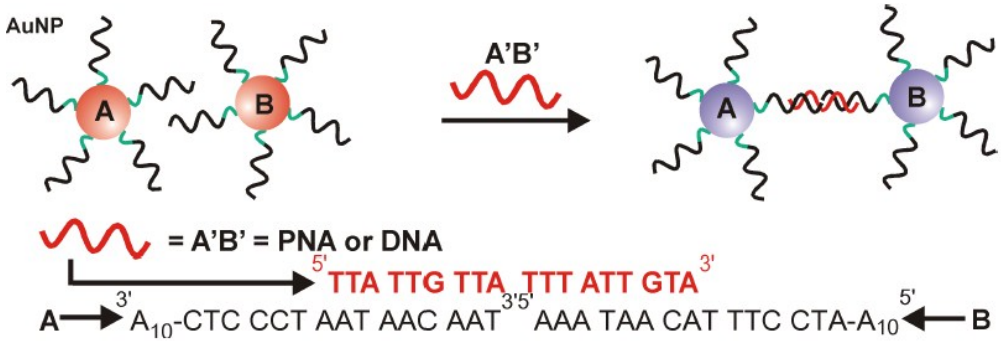


Fig. 12 Schematic repredentation of DNA-modified Au(0) NPs linked with complementary PNA or DNA. Reprinted with permission from John Wiley and Sons, copyright (2009) from ref 70.

Most of the DNA/PNA-NPs assemblies that are reported in the literature were prepared by an interparticle cross-linking aggregation mechanism. Another type of assembly mechanism is aggregation without hybridization, known as the non-crosslinking mechanism. For example, citrate anions mediated Au(0) or Au(0) NPs undergo aggregation in the presence of PNA molecules. This mechanism was used for the colorimetric detection of DNA fragments, by Kanjanawarut and Su in 2009.[71] The dispersion by PNA-DNA duplexes points out that PNAs can bind citrate ions coated metal(0) NPs and change the NPs surface properties.[72] This phenomena originates from the strong PNA-metal(0) NPs interactions, involving both the nucleobases and the peptide backbones.[72] In addition, the adsorption of PNA molecules around the citrate mediated NPs, either displaces weakly bound citrate ions, or shields the citrate ions charge. Both possibilities lead to the loss of charge repulsion and therefore particle aggregation.[71] This aggregation could be enhanced by the presence of positive charges from the *N*-terminal amines, which are located on the PNA, at neutral pH (positive charged amino group toward the negatively charged Au(0)), as similarly reported for

peptide-Au(0) NPs interactions. In contrast, when the above aggregation is followed by the addition of the complementary DNA strand, a PNA-DNA complex is formed and the NPs solution becomes dispersed (Fig. 13). This could be attributed to the negative phosphate backbone of the DNA strands, which restores the charge repulsion between the NPs, leading to their re-dispersion. For colorimetric detection of DNA fragments, citrate mediated 13.2 nm Au(0) NPs were synthesized by reducing Au(0) or Ag(0) salts with sodium borohydride. The aggregation was induced by the presence of PNA molecules. Addition of complimentary DNA to the system, inverted the aggregation tendency (Fig. 13).[71] In case of citrate mediated Au(0) NPs, addition of PNA resulted in a color change from red to purple, which accompanied by a red shifted absorbance band (from 520 nm to 600 nm). In addition, when a PNA–DNA duplex was added, the Au(0) NPs did not assemble. Moreover, when the solution contained a mixture of un-hybridized PNA and non-complementary DNA sequence, the particles solution underwent a certain extent of aggregation, showing a color change to purple and the appearance of an absorption band at about 600 nm. The spectrum detection limit for the obtained ratio DNA/PNA was 0.05 and the visual detection limit was 0.1 mM. This lower detection limit was attributed to the extraordinary coagulating property of the PNAs probes.

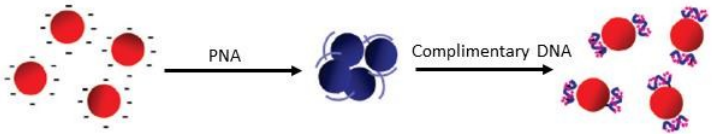


Fig. 13 Aggregation of citrate mediated NPs by PNA's and the assembly separation by addition of complimentary DNA. Reprinted with permission from American Chemical Society, copyright (2009) from ref 71.

Overall, PNA oligomers prove to be very good candidates for mediating the assembly of NPs, mainly because their sequence and properties can be easily tuned. This versatility is a great advantage for application development. If the assembly process utilizes base pairing, however, the synthesis requires very low temperature(s) and the final product(s) are unstable in ambient conditions and for time periods of more than a few days. This problem could be solved by using DNA-PNA base paring. In these cases, neutral pH and presence of salts are necessary for aggregation. An alternative assembly method, which does not involve base pairing, but rather involves reducing of the repulsion forces between the NPs was recently developed. Despite these advances, there currently only a few examples of PNA oligomers as mediators for NPs assemblies. We feel that this field could be broadly explored, if facile assembly processes and ways to increase the stability of the assembly products will be further developed.

2.3 Peptides as Mediators

Peptides can mediate NPs assembly successfully,[18] due to efficient interactions between amino acids, which include NPs binding-ligands, such as Tyrosine, Serine, and Cystatin. Furthermore, mediating NPs assembly with peptides allows tuning of the assembly process due to the possibility to control the peptide sequence by using various natural[73] and synthetic[74] amino acids. Moreover, in comparison to DNA and PNA, peptides are capable to successfully generate NPs assembly under varied conditions.[18, 48] Tuning NPs aggregation by a pre-assembled peptides mold was applied in the research group of Stupp in 2004,[54] demonstrating the production of cylindrical nano-fibers assemblies from CdS(0) NPs.[54] The peptide template was prepared from a peptide-amphiphile (**PA**, Fig. 14),[75] which contained the following four parts: (i) long hydrophobic tail in order to convert the peptide to amphiphilic, (ii) four cysteine residues in a row expected to form disulfide bonds when oxidized, (iii) flexible linker of three glycines, which function as the hydrophilic part, and (iv) a single phosphorylated serine, which coordinates strongly with Cd^{+2} in order to create to preferred CdS(0) NPs nucleation site. Due to the amphiphilic structure of **PA**, it assembles into 6-8 nm cylindrical nano-fibers under acidic conditions.[54] The fiber-like assemblies were prepared with diluted aqueous suspensions of **PA** fibers, which were combined with aqueous solution of $Cd(NO_3)_2{\cdot}4H_2O$. This mixture was then exposed to hydrogen sulfide (H_2S) gas resulting in CdS(0) nanofibers. Increasing the Cd^{+2} ions:**PA** molar ratios moderately led to thicker cover-layer; the NPs growth was monitored by UV-Vis spectroscopy. For example, when the ratio of Cd^{+2}:**PA** was 2.4:1, an absorbance at 476 nm was observed, corresponding for CdS(0) NPs with a diameter of 4 nm. Multiply the Cd^{+2} ions amount by 10 to 24:1 Cd^{+2} ions:**PA** ratio (Fig. 14),[72] resulted in full cover of the PA nanofibers with continues layer of CdS(0) grains in size of 7-5 nm.

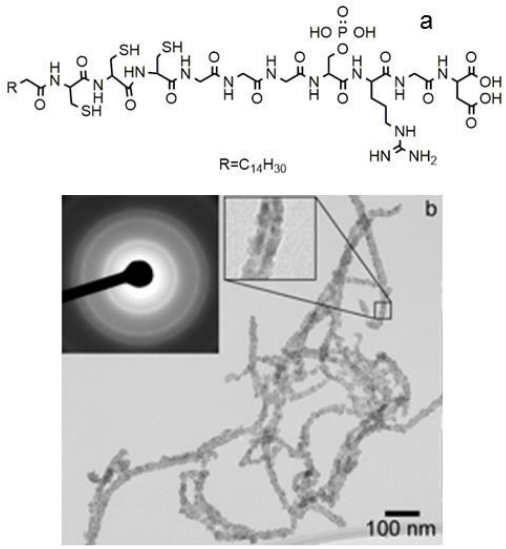


Fig. 14 Sequence of peptide-amphiphile **PA** (a) and TEM micrographs of CdS(0) NPs assembled on **PA** template in molar ratio of Cd^{2+}:**PA**-24:1. Left inset shows an electron diffraction pattern, which is corresponding to the CdS(0). Reprinted with permission from American Chemical Society, copyright (2004) from ref 54.

The controlled aggregation of Au(0) NPs by structural changes in a peptide (folding) as well as peptides dimerization in the presence of Zn^{+2} were studied by Liedberg and collaborators in 2008.[35] In this work, a 42 monomers long peptide, rich in glutamic acid, which can fold into helix–loop–helix, was synthesized. This peptide folds into a four-helix bundle upon dimerization. Both dimerization and folding can occur in acidic pH conditions, and are driven by the creation of a hydrophobic core. This core is formed by the hydrophobic faces of the amphiphilic helices, or in presence of Zn^{+2} ions (Fig. 15). Attaching the peptide to Au(0) NPs was done by the indirect approach; a 13 nm Au(0) NPs were prepared by the reduction of $HAuCl_4$ in the presence of citrate ions, followed by mixing the peptide solution in citric buffer at pH 6.0 with the as-prepared Au(0) NPs. The peptides leftovers were removed by centrifuge and the Au(0) NPs were re-dispersed in Tris buffer. In order to study the folding effect of Zn^{+2} ions, the peptides were kept in Zn^{+2} solution, at pH = 7.0 for 60 minutes. Addition of Zn^{+2} to the NPs solution resulted in NPs aggregation, as a consequence of peptides dimerization under 2 mM or higher Zn^{+2} concentrations. The aggregation, which origins from a dimerization between peptides immobilized on separate particles, was induced by the coordination of Zn^{+2} ions to the carboxylic acids residues. The aggregation process was

reflected in an immediate red shift of the LSPR peak from 523 to 545 nm. In addition, the aggregation process was reversible via the removal of the Zn^{+2} ions with EDTA.

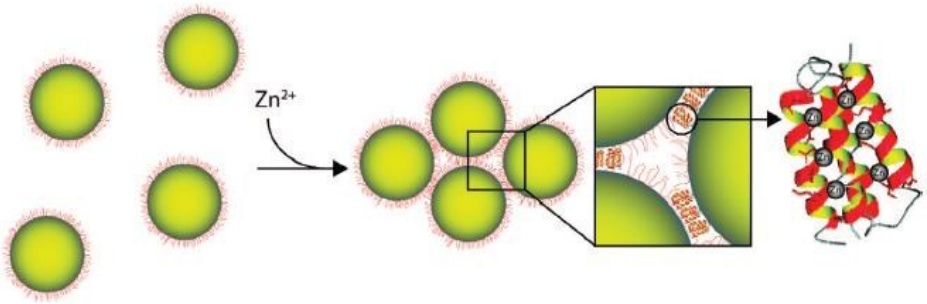


Fig. 15 The binding of Zn^{2+} by peptideimmobilized on Au(0) NPs induces dimerization and folding between peptides located on separate particles resulting in particle aggregation. Reprinted with permission from American Chemical Society, copyright (2008) from ref 35.

Peptides incorporating tyrosine can function as reduction agents in the synthesis of Au(0) and Ag(0) NPs, as was demonstrated by Mandal et al in 2006.[24] The reduction mechanism includes electron transfer from the tyrosinate ion to the metal ion under basic conditions, through the formation of a radical tyrosyl, which eventually converts into its dityrosine form.[24] The synthesis of Au(0) NPs by the short peptide **NH_2-LBY-COO-Me** (B refers to 2-Aminoisobutyric acid, see Fig. 16) under basic conditions, included the addition of the peptide solution in methanol to a solution of $HAuCl_4$ in basic aqueous solution. The pH was adjusted to 11 by sodium hydroxide solution. The alkaline conditions hydrolyzed the COO-Me to sodium carboxylate.[24] This red NPs solution has been successfully used as a colorimetric sensor for low concentrations of metal ions, such as Hg^{+2},[47] Pb^{+2},Cd^{+2}, Cu^{+2},and Zn^{+2}.[48] In all cases, the presence of metal ions created chelate complexes, which derived the self-assembly of peptide-Au(0) NPs, generating a purple solution (see Fig. 17). The aggregates formation was shown to be reversible; addition of EDTA solution reverses the colorimetric response. In 2007, the same research group also explored the pH dependence of the Au(0) NPs system.[46,48] Under alkaline conditions, the NPs were well dispersed, due to repulsion forces between the carboxylate ions, and the solution color was red. Adjustment of the pH to lower values did not lead to any aggregation. At pH = 4, a network of nanochains was formed. Further pH decrease to 2.5 resulted in random shaped NPs assemblies, and between pH = 4 to 2.5 a mixed trend was observed. The solutions colors were changed from red to violet. The aggregation trend can be explained by the pKa values of the carboxylate external edge of the peptide; above its pKa the

COONa is the favorable form. Below the pKa, the protonated structure is dominant, leading to the creation of H-bonds between two carboxylic acids and aggregation enhancement.[46]

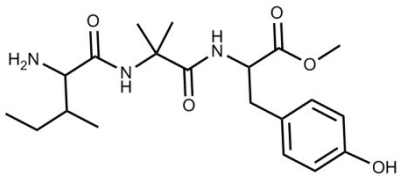


Fig. 16 NH_2-LBY-COO-Me peptide, for Au(0) NPs stabilization.

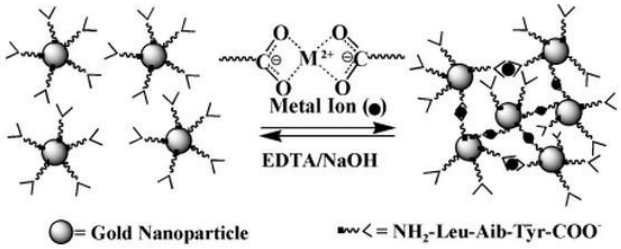


Fig. 17 The Au(0) NPs-carboxylated peptides and their aggregation upon addition of heavy metal ions via chelate coordination. Reprinted with permission from John Wiley and Sons, copyright (2008) from ref 48.

A good example for the influence of hydrophobic interactions on self-assembled Au(0) NPs was demonstrated by Rossi and coworkers using the L-amino acid sequence AYSSGAPPMPPF (**PEP**,[76] Fig. 18). **PEP** was identified from a phage peptide library, showing a unique capability to reduce Au^{+3} to Au(0) by tyrosine, and to stabilize Au(0) NPs.[76]

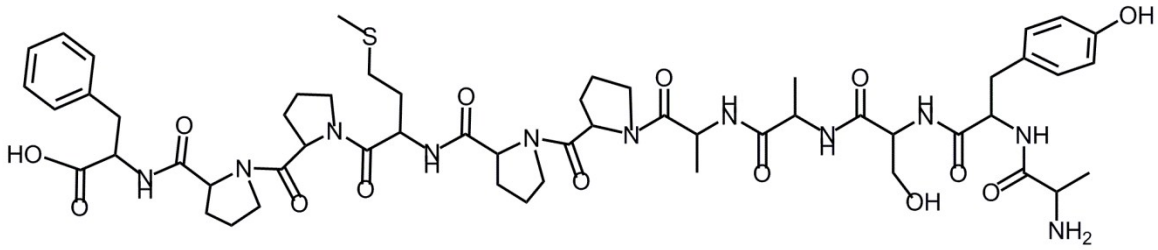


Fig. 18 schematic illustration of **PEP** peptide.

The **PEP** structure contains hydrophobic, hydrophilic and rigid parts (which include prolines). In addition, the amino acid sequence AYSS is known in its ability to form β-sheets. In fact, mediating **PEP** with Au(0) NPs in the presence of HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) in water, resulted in well-dispersed Au(0) NPs, due to **PEP**'s

dominant hydrophilic part.[76] Rossi and coworkers added a variety of hydrophobic additives at the *N*-terminus of **PEP** and mediated it with Au(0) NPs. Later on, they investigated the aggregation patterns of this system in various conditions.[23, 77] The main conclusions from these sets of experiments were drawn by analyzing the aggregation patterns that resulted from the hydrophobic additive identity and length, the reactions temperature, and the reactions time. It also should be noted that the environment includes HEPES buffer and triethylammonium acetate (TEAA), both can function as weak stabilizers in addition to the peptide, HEPES buffer can also reduce Au^{+3} to Au(0), and the peptide can assemble without the NPs addition. In order to avoid pre-mold processes, the peptides solution was filtered before it was reacted with the NPs. The NPs synthesis involved the combination of peptide solution and $HAuCl_4$ in TEAA and HEPES, at room temperature for 30 minutes. The studies about the influence of the hydrocarbon chain length on the aggregation pattern are summarized in Fig. 19. The first conclusion was that the long hydrocarbon chain increased the strength of the hydrophobic interactions. Therefore, in case of short chains or in the absence of such hydrophobic chain (**C_6-PEP** or **PEP**) the aggregation was poor or didn't occurr.[78] The addition of a longer chain (**C_9-PEP**) resulted in random aggregation; apparently nine carbons do not create enough hydrophobic interactions in order to form arranged aggregation.[7] In compression, the use of an even longer chain, which produced **C_{12}-PEP**, resulted in highly ordered double helixes sieves, in a micrometer length (Fig. 19).[23] Next, the influence of sequence modifications to **C_6-PEP** was studied, aiming to enhance the β-sheets factor, in order to create an ordered aggregation pattern.[7] For this purpose, two additional alanine residues were attached to the *N*-terminus of **C_6-PEP** to **C_6-AA-PEP**.[78] Incubating **C_6-AA-PEP** with aureate salt as demonstrated previously, resulted in a light-purple solution, indicating the formation of spherical Au(0) NPs assemblies, as shown in Fig. 20.[78]

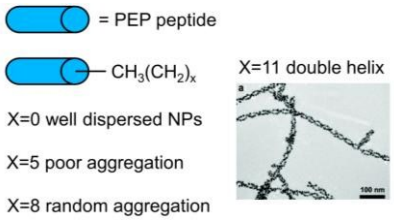


Fig. 19 PEP modified with hydrocarbon chain in different lengths; the TEM micrograph represent highly ordered double helixes sieves, in a micrometer length. Reprinted with permission from American Chemical Society, copyright (2008) from ref 23.

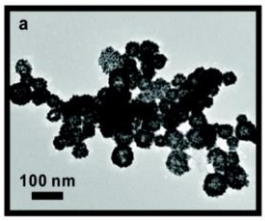


Fig. 20 Spherical Au(0) NPs - **C6-AA-PEP** superstructures. Reprinted with permission from American Chemical Society, copyright (2010) from ref 78.

In 2013, the same research group reported the preparation of hollow spherical superstructures from platinum-cobalt NPs that were formed by the interactions with peptides. The peptide sequence that was used in this research, HYPTLPLGSSTY (**PEP$_{CO}$**),[31] was previously isolated also by Naik et. al. from a phage peptide library, and proved as efficient PtCo NPs cupping ligand.[79] In this study, the binding peptide was modified with BP ($C_{12}H_9CO$, biphenyl) at its *N*-terminus, forming **BP-PEP$_{CO}$** (Fig. 21) in order to induce NPs assembly. The peptide modification was followed by formation of PtCo(0) NPs hollow spherical superstructures in the presence of HEPES buffer (see Fig. 22).

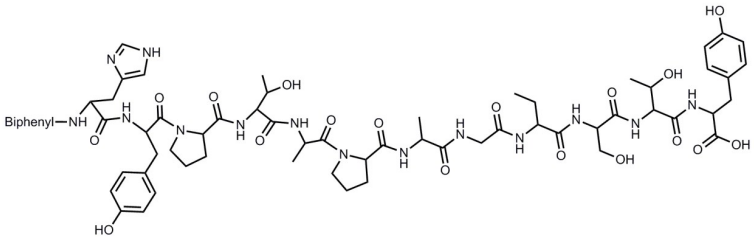


Fig. 21 Structure of **BP-PEP$_{CO}$** peptide.

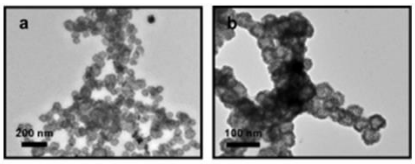


Fig. 22: TEM micrographs of **BP-PEP$_{CO}$** mediate PtCo NPs assembles to hollow nanospheres. Reprinted with permission from John Wiley and Sons copyright (2013) from ref 79.

In another work from the group of Rossi, the optical activity of unique peptides-NPs assemblies was investigated.[39] These assemblies were based on the interactions between chiral double-helical peptides and Au(0) NPs. The chirality of these aggregates can be tuned by modifying their structure and composition. L-amino acids were used for the synthesis of **C_{12}-L-PEP** peptide. This peptide assembles into a left-handed helix, having a regular pitch of about 84 nm in presence of HEPES buffer. In addition, the right-handed helix, **C_{12}-D-PEP** was also synthesized using D-amino acids exhibiting the opposite optical activity. The Au(0)-peptide left and right helix aggregates were both prepared by the indirect approach; initially, triethylammonium acetate (TEAA) buffer at pH = 7.0 was treated with chloroauric acid ($HAuCl_4$) and the mixture was kept unstirred for 10 minutes at room temperature. Then, the peptides **C_{12}-L-PEP** or **C_{12}-L-PEP** were dissolved in HEPES and citrate buffers (pH = 7.3) and the peptide solution was kept at room temperature for 30 minutes. In the next step, the Au(0) ions solution was added to the peptide solution, stirred for a few seconds and the mixture was kept undisturbed at room temperature. Au(0) NPs assembled into double helices were formed after about an hour as observed by TEM analysis. This procedure resulted in spherical Au(0) NPs with a diameter of about 5.7 nm assembled into left-handed helices and spherical Au(0) NPs with a diameter of about 5.2 nm assembled into right-handed helices. Both left handed and right handed helices exhibited a pitch of about 82 nm, an inter-helical distance of about 7.5 nm and an inter-particle distance of about 1.7 nm, with a final length of about 1 μm. This solution was incubated at room temperature for a day. During the incubation, the Au(0) NPs diameter grew to about 8 nm while the inter-helical distance and the inter-particle distance decreased to about 6.5 nm and 1.4 nm respectively, and the pitch distances of the helices remind approximately the same. The left and right-handed double helices exhibited a negative and positive CD, respectively, showing absorbance bands at about 220 nm and 562 nm. Treating this solution with an additional aqueous solution of $HAuCl_4$ resulted in helices with larger Au(0) NPs size (about 10.5 nm for left-handed helices and 10.7 nm for right-handed helices) and smaller interparticle distances. In addition, the CD

spectrum exhibited increased intensity and red shifting to about 600 nm for both the left-handed helices and the right-handed helices.

Zuckermann et al. functionalized peptide nano-fibers in order to produce a mold using divalent metal ion coordination for producing a NPs fiber like aggregats.[80] The peptide **GNNQQNY–NH_2** (Fig. 23) was chosen, due to its side-chains, which have no propensity to coordinate metal ions, and, the amides from N and Q stabilizes the nano-fiber through side-chain hydrogen bonds between peptide chains.[81, 82] N-(S-1-carboxyethyl) glycine moiety was attached to the *N*-terminus of this peptide to function as a metal ion coordination site at the nano-fiber surface. In addition, one or two amino-hexanoic acid linkers were incorporated in order to separate the functional moiety of this terminal group from the structural body of the peptide (Fig. 23). The peptides metal ions nano-fibers were synthesized by mixing aqueous solution of peptide, NaOH, Tris-HCl (pH = 7.5), NaCl and metal chloride (this assembly is reversible by adding EDTA). Fiber aggregation was observed in the presence of Cu^{+2} and Ni^{+2} for the peptide, due to the flexible linkers. The next step was the growth of metal NPs on the nano-fiber-metal ion mold, the NPs growth includes addition of sodium borohydride and incubation of 30 minutes. This procedure result in fibers that assembled from the peptide; Cu(0) NPs in 4.1 nm diameter, and Ni(0) NPs in 1.8 nm diameter.

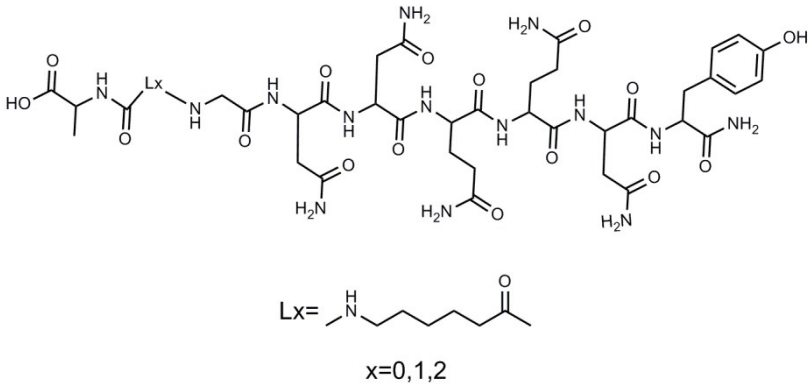


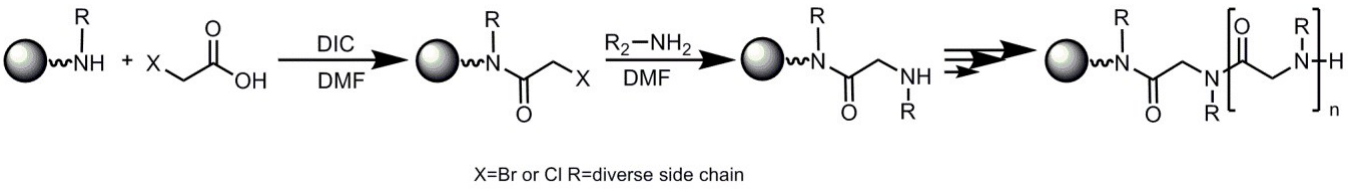
Fig. 23 The chemical structure of **GNNQQNY–NH_2** peptide and its additives.

Overall, peptides can be used as highly efficient mediators for the assembly of NPs due to their high versatility and sequence specificity. Hence, precise control over the final geometry of the assembly products can be achieved via preliminary design of the peptides and their modifications. The above examples demonstrate that the final shape of the assembly products can be altered simply by changing the length of an attached hydrocarbon chain and/or its chemical properties by replacing it by aromatic or

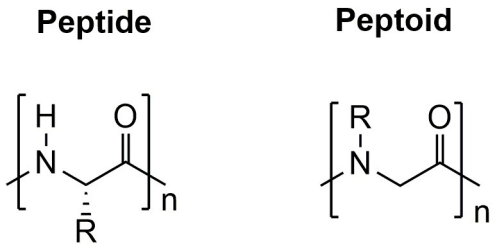
hydrophilic residues. In other cases, control over the peptides structure (e.g. by inducing folding) and/or reaction conditions such as pH and metal ions content can also lead to distinct assemblies. However, similar to DNA, the use of peptides as mediators is limited by the stability of the peptides themselves as well as of the assembly products, in various reaction conditions such as pH, temperatures, salt content, solvents and the presence of organic reagents. These factors might also limit the scope of applications possible for these NPs assemblies.

2.4 *N*-Substituted Glycine Oligomers – Peptoids – as Mediators:

Currently we are aware of only one type of peptidomimetics that were used for the stabilization of NPs and for the assembly of NPs. These peptidomimetics are called "peptoids" and they are *N*-substituted glycine oligomers that were developed in the early 1990s for combinatorial drug discovery.[83-85] Peptoids can be synthesized from primary amines in a straightforward solid phase synthesis by the efficient sub-monomer protocol (Scheme 2). This leads to diverse sequences due to the use of innumerable commercial and/or readily available primary amines. Thus, it enables the incorporation of various functional groups at specified *N*-positions without using protection and de-protection steps as in peptide synthesis.[83, 85] As a result, peptoid's side chains are appended to the amide nitrogen instead of to the α-carbon (Scheme 3), which produce achiral backbones and diminish the ability for hydrogen bonding.[2] However, peptoids sequences incorporating bulky chiral side chains, can form stable helices in aqueous and in organic solvents, similar to polyproline type helices.[83, 84] Moreover, the number of chiral centers, the helical secondary structure content, and other physico-chemical attributes can be tuned in a precise manner through introduction of different side chains at various positions.[83,86] All of these properties result in substantial conformational flexibility in the main chains and the side groups.



Scheme 2. The submonomer protocol.



Scheme 3. Peptide and peptoid structures.

The use of peptoids as capping agents for NPs, can be fruitful, mainly because peptoids exhibit stability in a wide range of pH and temperature conditions and are inert to the presence of many organic reagents.[83] Peptoids can also be easily generated to be compatible with various biotic and abiotic enviroments,[83, 87] and a wide range of solvents.[15, 83] Thus, peptoids represent a unique opportunity for functionalizing and assembly of inorganic NPs towards a variety of applications.

Peptoid research have developed significantly in the last two decays, however, only recently, peptoids have been used as metal-NPs stabilizers and only two studies have been published so far.[15, 26] In 2011, Zuckermann and Robinson investigated the stabilization of Au(0) NPs by using peptoids as stabilizers, under high concentration of salts.[26] Neither self-assembly nor controlled aggregation have been reported for these NPs. In the same year, spherical assemblies generated from citrate stabilized Ag(0) NPs (Fig. 24A) and a metal-binding peptoid heptamer have been reported by Maayan and Liu (Fig. 24).[15] The peptoid structure included six S-1-Phenylethylamine side chains and one 1, 10-phenanthroline ligand, which functions as a linker to Ag(0) NPs, at the peptoid's *N*-terminus (peptoid **PHP**, Fig. 24B). The formation of these spherical assemblies has been obtained only in pH = 3.5. In addition, the chirality of these assemblies was not explored.

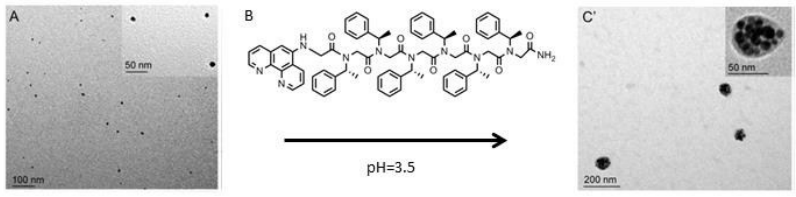


Fig. 24 (A) dispersed citrate-stabilized Ag(0) NPs; (B) **PHP** peptoid; (C') discrete spherical assemblies of **PHP** - capped Ag(0) NPs. Reproduced with permission from John Wiley and Sons, copyright (2011) from ref 15.

3. Conclusions and Outlook

In conclusion, mediating the controlled aggregation of metal NPs by biological and biomimetic oligomers offers versatile options for the formation of new functional nanomaterials. In addition, the capability to control the final morphology of the NPs assemblies is important for the production of materials with specific physical properties and may play a role in their biological functionalities and other possible applications. Despite these advantages, the construction of natural polymers, synthetic peptides and DNA as mediators for NPs assembly, and the use of such assemblies as functional materials are still quite challenging. In the case of natural polymers and synthetic peptides/DNA, their limited stability regarding variations in pH, temperatures, salt content, solvents and the presence of organic reagents, restricts the aggregates functionality.[48] In the case of peptides, only twenty amino acids are available,[49] while artificial amino acids may involve complicated synthetic protocols. Therefore, the control over the aggregation patterns by using natural and synthetic polymers and peptides is still difficult to achieve, not fully understood and limited in applications. Thus, the use of synthetic biomimetic oligomers as mediators for NPs assembly might enable to overcome these limitations providing new insights into the NPs aggregation processes as well as new functional NPs assemblies.

The use biomimetic oligomers can provide a convenient rote to preserve the biocompatibility and secondary structures of peptides, DNA, and PNA, but in a wide range of pH and temperature conditions, and in the presence of various solvents and reagents. Moreover, understanding the assembly process as well as control over size and shape, might be possible simply by systematic changes to the sequences and structures of versatile and easily produced peptidomimetic oligomers, e.g. peptoids. This will hopefully lead to more applications, that until now were only demonstrated by NPs assemblies mediated via small organic molecules, especially SERS and other spectroscopic detection methods. Such applications, which are still lacking in the field described in this review, and other applications such as ones in medicine, chiral sensing and catalysis, might be possible after a strong basis of understanding NPs aggregation by biomimetic oligomers will be fully developed. Peptoids, for example, that can fold into stable secondary structures, and exhibit high sequence specificity, can be used to form peptoid-NPs assemblies that might be further exploited for biological and optical purposes.[88-91]

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