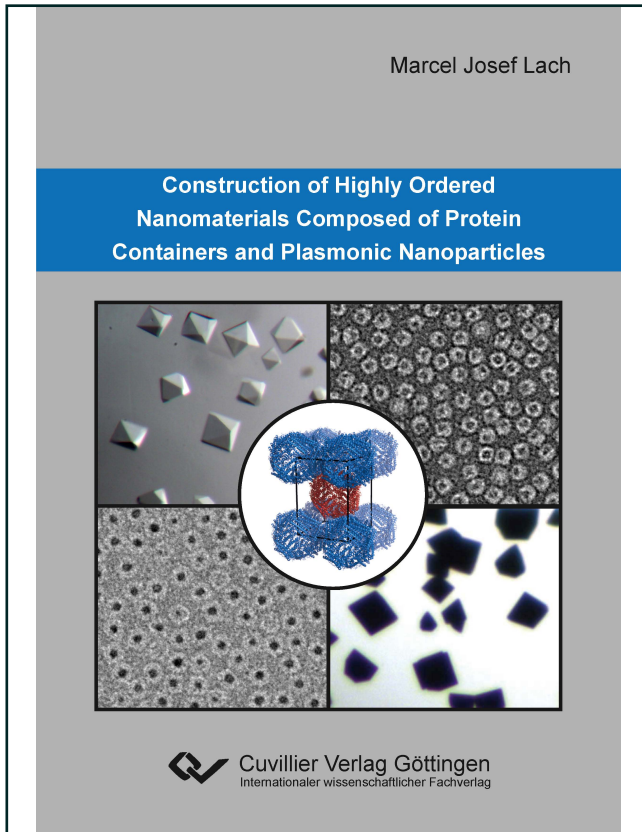




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**Construction of Highly Ordered Nanomaterials Composed of Protein Containers and Plasmonic Nanoparticles**



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# 1 Introduction

Nanoparticles have become the focus of much attention due to their remarkable physical and chemical properties, which arise from their small size and high specific surface area.<sup>[1]</sup> They can be employed, among other applications, in catalysis,<sup>[2]</sup> imaging,<sup>[3,4]</sup> data storage<sup>[5]</sup> and drug-delivery<sup>[6]</sup> or for the production of new materials, such as magnetic fluids<sup>[7]</sup> and polymer nanocomposites.<sup>[8,9]</sup> The precise arrangement of nanoparticles in defined structures is a prerequisite for the generation of so-called metamaterials. These materials are envisioned to have emergent electronic, magnetic or optical properties because of the interactions between individual nanoparticles inside the materials.<sup>[10]</sup>

The formation of highly ordered materials requires discrete building blocks that carry the useful function and interact with each other in a structured manner.<sup>[11]</sup> Nanoparticles have remarkable properties, but are difficult to order due to their limited monodisperse size distribution. Control over the particle packing is strongly limited and only small domain sizes are obtained in binary structures.<sup>[12,13]</sup> As a consequence, the development of new methods for nanoparticle assembly are mandatory. Nature has always acted as role-model for the creation of new technologies. For example, the structure of bones has inspired mankind to construct buildings such as the Eiffel tower. In chemistry, the nature's principles to assemble fascinating complexes led to the establishment of supramolecular chemistry, where non-covalent interactions between molecules are utilized to create new materials.<sup>[14]</sup> With these design principles, the design and synthesis of molecular machines was possible.<sup>[15,16]</sup>

Several techniques have been developed to exploit biomolecules for the controlled nanoparticle assembly.<sup>[17,18]</sup> Typically, most work, pioneered by the MIRKIN and GANG groups, has focused on the assembly of oligonucleotide-functionalized nanoparticles into crystalline superlattices.<sup>[19,20]</sup> For instance, complementary DNA molecules were used as linkers between gold nanoparticles to create single-component and binary-component crystals.<sup>[21]</sup> In this way, the organization of various nanoparticle types is enabled to produce binary and ternary assemblies with tailorable crystal lattices and lattice parameters.<sup>[22,23]</sup> However, still several disadvantages exist such as the instability outside the growth medium or the low long-range order due to the polydispersity of the nanoparticles.

From a materials perspective, proteins are an interesting building block due to their atomically precise defined structure. Moreover, proteins can assemble via non-covalent bonds into multimeric complexes such as protein containers.<sup>[24]</sup> Protein containers are cage-like structures with an empty inner cavity, which can be used as nanoreactor for the synthesis of nanoparticles.<sup>[25]</sup> For example, the protein ferritin<sup>[26]</sup> has been extensively used for the size-constrained synthesis of nanoparticles including metals,<sup>[27-31]</sup> metal oxides<sup>[32-34]</sup> and semiconductor materials.<sup>[35-38]</sup> In previous work within this research group, these nanoparticle-protein container composites were assembled into a highly ordered structure by protein crystallization with the protein scaffold as matrix for the nanoparticle assembly.<sup>[39,40]</sup>

In this work, a novel strategy for the construction of biohybrid materials composed of plasmonic nanomaterials and protein containers is presented. Two oppositely charged ferritin protein containers are loaded with plasmonic nanoparticles using a new encapsulation approach by opening and closing the protein container while the nanoparticles are added. The crystallization of the building blocks leads to large crystalline assemblies with sizes up to hundred micrometers. Moreover, binary nanoparticle superlattices are generated because oppositely charged building blocks are utilized. These two building blocks enable the incorporation of one type of nanoparticle in each building block. Due to stabilization of the protein matrix with cross-linking agents, applications in catalysis or as optical materials are feasible.

## 2 Theoretical background

### 2.1 Plasmonic nanoparticles

The term nanoparticle is used for the associations of atoms with a dimension in the range of 1 nm to 100 nm, which classifies them between molecules and bulk materials.<sup>[41]</sup> The smaller size compared to bulk materials leads to novel properties that differ from the corresponding bulk material. These properties can be attributed to three different effects. First, the small nanoparticle size enhances surface-dependent properties because of the higher surface to volume ratio. A typical example is the higher catalytic activity due to increased number of catalytic active centers in comparison to the bulk material.<sup>[42]</sup> Second, size-dependent particle properties can emerge such as interference effects in photonic crystal<sup>[43]</sup> or superparamagnetic properties of Fe<sub>3</sub>O<sub>4</sub> nanoparticles.<sup>[44]</sup> Third, size-dependent quantum effects occur caused by the influence of the nanoparticle size on the electronic structure. The transition from an atom with defined energy levels to the dispersed bands of collective ensembles of atoms leads to novel properties. For example, the position of the localized surface plasmon resonance (LSPR) of gold nanoparticles is redshifted with increasing nanoparticle size<sup>[45]</sup> or the luminescence of quantum dots is altered.<sup>[46]</sup> Plasmonic nanoparticles are metallic nanoparticles usually composed of noble metal atoms such as silver or gold. These particles are of high interest for the generation of novel nanomaterials because of their interesting size-dependent quantum effects. Structures based on plasmonic nanoparticles have potential for application in the construction of metamaterials with negative refractive index<sup>[47]</sup> and light-harvesting materials, such as in photovoltaics,<sup>[48]</sup> or sensing of biological analytes.<sup>[49]</sup>

#### 2.1.1 Synthesis and functionalization

The application of nanoparticles in materials is strongly connected to the synthesis of nanoparticles in the favored size range. The requirements for the synthesis are a monodisperse size distribution, suppression of agglomeration processes and a specific control over the surface functionality. In general, nanoparticles can be obtained by two fundamental strategies: “top-down” or “bottom-up” approach. In the “top-down” approach, bulk material is minced to smaller particles for example in mechanical milling<sup>[50]</sup> or laser-ablation technique.<sup>[51]</sup> Industrially, “top-down” methods are favored due to the economical production of large amounts of nanoparticles. However, the nanoparticle

size is limited to nanoparticles larger than 50 nm and a wide size distribution is obtained. In the “bottom-up” approach, nanoparticles are constructed from small building blocks such as atoms or molecules, including for example chemical reduction methods,<sup>[52,53]</sup> laser-induced-assembly<sup>[54]</sup> or colloidal aggregation.<sup>[55]</sup> In this case, the size of the nanoparticle and the agglomeration behavior are effectively controlled by stabilizing the reactive surface with ligands. Moreover, by the right choice of ligands the form and shape of the nanoparticles can be controlled in various geometries such as spheres, rods, cubes, triangles, prisms, cages and stars.<sup>[56–60]</sup>

In 1951 the synthesis of gold nanoparticles (AuNP) was pioneered by Turkevich:<sup>[61]</sup> Tetrachloroauric acid was reduced with sodium citrate as a reducing and stabilizing agent to obtain AuNPs in aqueous solution. Over time, syntheses were improved by the variation of the solvent and ligands to obtain different types of AuNPs.<sup>[62]</sup> Moreover, the results were transferred to synthesize other plasmonic nanoparticles such as silver nanoparticles (AgNP).<sup>[63]</sup> Interestingly, the general mechanism of nanoparticle formation remains the same and can be subdivided into three steps: nucleation, nanoparticle growth and formation of size distribution.<sup>[64]</sup> The nucleation describes the formation of nuclei that can act as template for crystal growth. Considering the thermodynamics of the particle formation, the total free energy  $\Delta G$  is defined as the sum of the surface free energy  $\gamma$  and the free energy of the bulk crystal  $\Delta G_v$ . For a spherical particle of radius  $r$ , the total free energy is described according to equation 2.1.:<sup>[64]</sup>

$$\Delta G = 4\pi r^2 \gamma + \frac{4}{3} \pi r^3 \Delta G_v \quad (2.1)$$

The bulk crystal free energy  $\Delta G_v$  in turn, depends on Boltzmann’s constant  $k_B$ , the temperature  $T$ , the supersaturation of the solution  $S$  and its molar volume  $v$ :

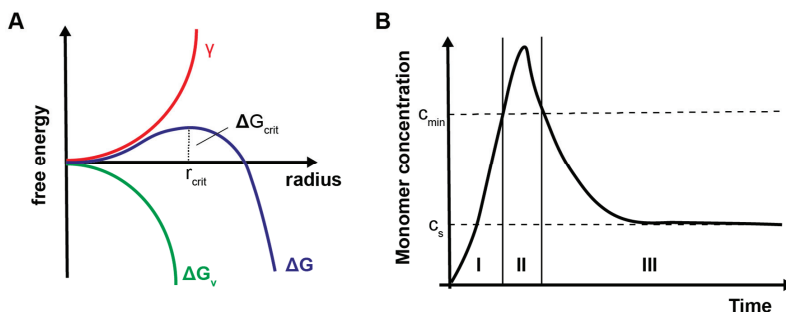
$$\Delta G_v = \frac{-k_B T \ln(S)}{v} \quad (2.2)$$

A critical supersaturation is needed to initiate the particle formation. Since the surface energy always adopts a positive value and the crystal energy a negative value, it is possible to find a maximum free energy (Figure 2.1A). By differentiating  $\Delta G$  with respect to the particle radius and setting it to 0, the critical radius  $r_{\text{crit}}$  with the maximum free energy is obtained:

$$r_{\text{crit}} = -\frac{2\gamma}{\Delta G_v} \quad (2.3)$$

$$\Delta G_{\text{crit}} = \frac{4}{3} \pi \gamma r_{\text{crit}}^2 \quad (2.4)$$

The critical radius represents the minimum size that a particle needs to reach to avoid redissolving in solution, whereas the critical free energy is equivalent to the activation energy for nuclei/particle formation. After the nuclei are formed, the nuclei grow to form the nanoparticle. The growth of nanoparticles depends on the monomer diffusion to the surface and the reaction of the monomers on the surface. The first mechanism for nucleation and growth of nanoparticle was described by the LaMer burst nucleation mechanism (Figure 2.1B).<sup>[65,66]</sup>



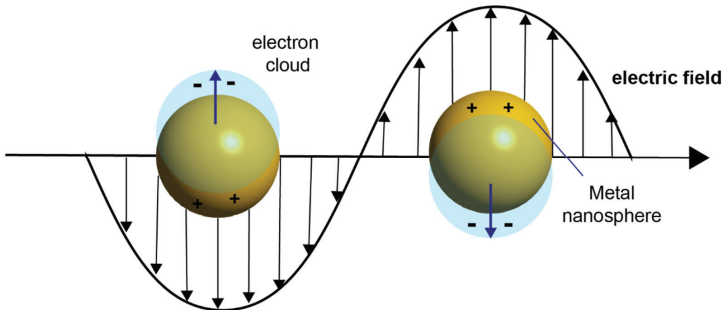
**Figure 2.1: Nanoparticle nucleation and growth.** (A) Free energy diagram for nucleation. (B) Classic LaMer mechanism for nanoparticle growth.<sup>[66]</sup>

Within the LaMer mechanism, the reaction progress is subdivided into three phases. Initially, (I) the precursor is converted and the concentration of free atoms increases until the saturation concentration ( $c_s$ ) is exceeded forming a supersaturated solution. When the so-called critical supersaturation concentration ( $c_{min}$ ) is reached (II), burst nucleation events occur. Nuclei are simultaneously formed and the concentration of free atoms is drastically reduced. After lowering  $c_{min}$ , no further nucleation occurs and (III) the nuclei growth to nanoparticle starts until the concentration of free atoms reaches  $c_s$  again. The formation of size distribution happens after the consumption of available free atoms. In general, two different types of mechanism are described. Smaller particles are dissolved due to their unfavorable surface to bulk energy ratio and allow larger nanoparticles to grow even more (Ostwald ripening).<sup>[67]</sup> Coalescence of particles occurs when two particles of arbitrary size merge and form a larger particle with inconsistent crystal domains.<sup>[68]</sup> Ideally, particles would grow unhindered and form bulk material in the end, if enough free atoms were available. Therefore, the particle growth needs to be stopped by deactivating their surface with ligands.<sup>[69]</sup>

The surface functionalization is crucial for the preparation of nanoparticles for potential applications because it controls their interaction with the environment.<sup>[70]</sup> Hence, the solubility,<sup>[71]</sup> colloidal stability,<sup>[72]</sup> or binding affinity<sup>[73]</sup> are affected. In general, the choice of ligands is predetermined by the solvent used for the nanoparticle synthesis. In apolar organic solvents, the nanoparticle surface is covered by hydrophobic ligands to prevent the aggregation of the nanoparticles. Here, ligand molecules with long or bulky hydrocarbon chains are used to ensure steric repulsion between nanoparticles. In polar or aqueous solutions, electrostatic repulsion plays a crucial role for the nanoparticle stabilization. In contrast to the steric repulsion, the electrostatic repulsion is highly affected by external influences. High salt concentration can shield the ligand electric field so that nanoparticles can come closer to each other until the attractive forces cause the particle agglomeration.<sup>[74]</sup> Moreover, the surface charge depends on the pH value, and thus influences the nanoparticle interaction between each other. Consequently, the steric stabilization is much more resistant than electrostatic stabilization. One requirement for the ligand molecule is that it binds to the particle surface by some attractive interactions such as chemisorption, electrostatic attraction, or hydrophobic interaction. Usually, binding to the surface is provided by a head group of the ligand molecule for example thiol,<sup>[75]</sup> amine<sup>[76]</sup> or phosphine<sup>[77]</sup> atoms. However, the inorganic surface and the electron donating-group may undergo a dynamic binding and desorption process. These dynamics enable the possibility to replace the attached ligands with others. Since many nanoparticle syntheses are performed in organic solvents, even a phase transfer is possible with a ligand exchange reaction to obtain water-soluble nanoparticles. Thiol ligands are commonly used for ligand exchange reactions because the thiol group possess the highest binding affinity to noble metal surfaces.<sup>[78]</sup> For example, AuNPs stabilized with phosphine ligands<sup>[79]</sup> as well as amine ligands<sup>[80]</sup> were transferred to aqueous solution by replacing the ligand shell with thiol-based ligands. Moreover, a mixture of different ligand molecules were employed to obtain so-called Janus nanoparticles.<sup>[81]</sup> The ligand exchange in the same solution can be challenging especially for polar ligands. An exchange from a positively charged ligand to a negatively charged ligand or vice versa can lead to unwanted aggregation of nanoparticles because of the detrimental electrostatic interaction between the two ligands. A two-step phase transfer including a phase-transfer to uncharged hydrophobic ligands can simplify the surface functionalization.<sup>[82]</sup>

## 2.1.2 Plasmonic nanoparticle properties and applications

Plasmonic nanoparticles can be distinguished from other nanoparticles such as metal oxide nanoparticle or carbon-based nanoparticles by their unique optical properties.<sup>[83]</sup> Since the antiquity, optical properties of colloidal gold were incorporated in materials for example in ceramics as ruby glass. However, in 1857 Faraday was the first to attribute these fascinating behavior to the optical properties of colloidal gold.<sup>[84]</sup> He reported the formation of a deep red solution of colloidal gold by reduction of chloroaurate and observed reversible color changes of thin films upon mechanical compression. The optical properties of plasmonic nanoparticles originates from the local surface plasmon resonance (LSPR). Plasmon resonance is an optical phenomenon arising from collective oscillation of the electron cloud in a metal when conduction electrons are disturbed from their equilibrium position (Figure 2.2).<sup>[62]</sup>



**Figure 2.2: Schematic illustration of the local surface plasmon resonance.**<sup>[85]</sup>

In detail, an incoming electromagnetic wave (light) will interact with the particle and immediately induce the conduction electrons to move from the equilibrium position. The small size of the particle prevents the free movement of the charge, as it would be possible in the bulk material. Thus, the electrons accumulate on the particle boundary. Coulomb attraction between the positively charged ions in the metal lattice and the negatively charged electrons at the particle boundary forces the electrons back to the equilibrium position, leading to an oscillation of the free electrons. When the oscillation frequency is approximately the same as the incident light, a resonance condition is reached, resulting in one plasmon resonance peak. Importantly, the position of the plasmon resonance peak is affected by the type of metal,<sup>[86]</sup> surrounding dielectric medium<sup>[87,88]</sup> and the electromagnetic coupling of the nanoparticles.<sup>[89,90]</sup> Any variation in the shape or size of the particle can alter the surface polarization.<sup>[91]</sup> The interaction of



the electromagnetic wave with the nanoparticle can be understood by solving Maxwell's equation. The Mie theory enables the calculation of extinction spectra but it is limited to spherical particles of diverse sizes.<sup>[92]</sup>

The great interest in plasmonic nanoparticles is driven by their potential applications. Plasmonic nanoparticles can compress light signals down to the nanoscale. They can form the basis of novel nanoscopic optical devices for light concentration and guiding in plasmon optics. For example, the change in LSPR peak position and intensity can be used for optical sensing.<sup>[93]</sup> Due to the dependency of the LSPR peak position on the refractive index, molecular binding events can be monitored. For example, the detection of human immunodeficiency virus (HIV) was possible by the alteration of refractive index caused by the adsorption on the nanoparticle surface.<sup>[94]</sup> Moreover, the same concept can be utilized to perform immunoassays without additional labelling material.<sup>[95]</sup> As an alternative approach, the plasmon coupling between single nanoparticles can be exploited to report conformational changes and intramolecular distances of single biomolecules similar to Förster resonance energy transfer (FRET).<sup>[96]</sup> Furthermore, molecular rulers based on fluorophores suffer from low and fluctuating signal intensities, limited observation time due to photobleaching and an upper distance limit of approximately 10 nm. In contrast, plasmonic nanoparticle-based molecular rulers do not bleach and larger distances can be measured. Another feature of plasmonic nanoparticles is the enhancement of the local electromagnetic field by the LSPR. This effect is utilized in surface enhanced Raman spectroscopy (SERS) to improve Raman scattering cross section. Enhancement factors of  $10^4$  to  $10^{11}$  are obtained.<sup>[97]</sup> More importantly, plasmonic nanoparticles can be utilized for the transport of optical signals in plasmonic waveguides due to the ability to manipulate electromagnetic signals below the diffraction limit of light.<sup>[98]</sup> A significant prerequisite for this application is the close and precise positioning of the plasmonic nanoparticles to enable strong near-field coupling between adjacent nanoparticles.<sup>[99]</sup> In this way, highly ordered structures of plasmonic nanoparticles can guide electromagnetic energy by converting optical modes into non-radiating surface plasmons.<sup>[100]</sup> Interestingly, this property can be exploited for optical devices in computing and informational technology.<sup>[101]</sup> According to Moore's law the development of fast electronic devices increased. However, the current electrical interconnection based on silicon-derived components reaches their limit. Electrons as information carrier cause signal delay and thermal issues, preventing significant increase in processor speed. In contrast, optical devices

possess a large data-carrying capacity (bandwidth) by using light as efficient information carrier.<sup>[102]</sup> On the one hand, an increased information density is achieved using several frequencies simultaneously.<sup>[103]</sup> On the other hand, the same scaling of the devices as in electronics is hampered by the fundamental laws of diffraction, complicating the interconnection with other electronic devices and thus the application.<sup>[101]</sup> Although optical devices are used for data transmission for example in optical fiber cables,<sup>[104]</sup> the shift in computing from conventional devices based on electrons as information carrier to optical devices remains challenging.

The common feature of all optical devices is the precise organization of the plasmonic nanoparticles into highly ordered materials to enable strong near-field coupling between neighboring nanoparticles. In the next chapter, general methods for the formation of nanoparticle superlattices are presented.

## **2.2 Formation of nanoparticle superlattices**

For the assembly of nanoparticles, single particle manipulations are not feasible since typical lithographic techniques cannot manipulate small nanoparticles for the creation of complex three-dimensional arrays.<sup>[105]</sup> In contrast, self-assembly of nanoparticles into colloidal, crystalline systems is seen as a promising strategy.<sup>[12]</sup> Self-assembly can yield structures with unrivalled precision in positioning of individual particles and is able to simplify the generation of metamaterials considerably.<sup>[13]</sup> In the following section, current approaches for self-assembly of nanoscale building blocks are highlighted.

### **2.2.1 Self-assembly with organic ligands**

Self-assembly describes the spontaneous organization of building blocks into highly ordered structures by direct interactions or indirectly using a template. The obtained structures are modulated by carefully choosing and constructing the building block. In general, ligand-stabilized nanoparticles can be considered as soft spheres with an effective hard sphere assigned to the inorganic core. The inorganic core provides interesting properties for the material, and the ligand shell is responsible for the interaction between building blocks. These interactions can be altered chemically (surface functionalization) or with external stimuli (pH, temperature, solvent polarity). This way, the structure can be controlled by manipulation of the guiding forces for the assembly such as hydrophobic interactions, hydrogen bonding or molecular dipole-interactions.<sup>[106]</sup>

Spherical nanoparticles with a narrow monomodal size distribution can be easily ordered directly after the synthesis, when the solvent is allowed to evaporate in a controlled fashion. Monolayer films are formed in cubic close-packed or hexagonal close-packed structures.<sup>[107,108]</sup> Moreover, nanoparticles with a bimodal size distribution can organize into two-dimensional arrays.<sup>[109]</sup> Due to the effective hard sphere of nanoparticles, the formation of binary structures should proceed similar to conventional inorganic salt. In inorganic salts, the structure of two different atoms with atomic radii  $r_A$  and  $r_B$  depends mainly on the ratio of the radii ( $r_A/r_B$ ) and the ratio of atoms ( $n_A/n_B$ ).<sup>[110]</sup> Several calculations were performed to identify preliminary ranges of size and number ratio to lead to specific structures.<sup>[111,112]</sup> For example, when  $n_A/n_B = 1$  and  $r_A/r_B = 1$ , an AB structure analogous to CsCl is expected. Alternatively, when  $n_A/n_B = 1$  and  $0.27 \leq r_A/r_B \leq 0.425$ , an AB structure analogous to NaCl is expected. Kiely *et al.* showed that a mixture of differently sized AuNP and AgNP forms monolayer arrays with an AB<sub>2</sub> structure.<sup>[113]</sup> Importantly, the formed structure agrees with the expected AB<sub>2</sub> structure similar to an AIB<sub>2</sub> alloy structure. The distance between the inorganic cores is predetermined by the ligand shell thickness. In a next step, the formation of binary structures has been extended from monolayer arrays to binary nanoparticle crystals. For instance, the use of specific size ratios between PbSe semiconductor quantum dots and Fe<sub>2</sub>O<sub>3</sub> magnetic nanoparticles directs the assembly to AB<sub>13</sub> or AB<sub>2</sub> structures.<sup>[114]</sup> In the AB<sub>13</sub> structure, the larger Fe<sub>2</sub>O<sub>3</sub> nanoparticles form a cubic lattice with an icosahedron of smaller PbSe quantum dots in the middle isostructural to NaZn<sub>13</sub>. In contrast, the AB<sub>2</sub> structure is composed of a hexagonal unit cell isostructural to AIB<sub>2</sub>. Single domains of AB<sub>13</sub> and AB<sub>2</sub> were formed in the size range from 0.16  $\mu\text{m}^2$  to 2  $\mu\text{m}^2$  separated by pure nanoparticle superlattices. Furthermore, the assembly of iron and gold nanoparticles to a three dimensional AB nanoparticle superlattice was performed.<sup>[115]</sup> Again, domains of pure and binary nanoparticle superlattices appear side by side. In all these materials, the control over the particle packing is strongly limited because of the isotropic ligand shell.

In an extension of this approach, a colloidal model system with oppositely charged nanoparticles is generated to form binary ionic crystals.<sup>[116]</sup> Grzybowski *et al.* showed the formation of a binary nanoparticle superlattice with a crystal size up to 3  $\mu\text{m}$  using oppositely charged AuNP and AgNP.<sup>[117]</sup> The surface is charged by functionalization with alkane thiols with either carboxy or tertiary amine groups. After assembly, a diamond-like lattice was formed. Each NP is surrounded by four other nanoparticles,